

EAPR 2014 Brussels

19<sup>th</sup> Triennial Conference of the European Association for Potato Research 6 to 11 July 2014

# ABSTRACTS BOOK (appendix)

# **Proceedings of the Conference**

Full texts of abstracts submitted as ORAL or POSTER presentations

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Proceedings of the Conference

Full texts of abstracts submitted as ORAL or POSTER presentation

**<u>Editors</u>**: Jean-Pierre **GOFFART**, Jean-Louis **ROLOT** (CRA-W, Belgium)

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#### Texts of ABSTRACTS submitted as ORAL or POSTER

Abstracts assigned to <u>ORAL</u> sessions (scientific parallel sessions or workshops) are <u>ordered from 1 to 131 bis</u>, and grouped into thematic parallel scientific sessions as scheduled in the detailed scientific program of the Abstracts book.

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Session 2 - Fungi/Bacteria (abs. 7 to 12)

Session 3 – Global food security (abs. 13 to 18)

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# **ORAL PRESENTATIONS**

(Parallel scientific sessions and Workshops)

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#### Session 1 (OP) - GENOMICS (1)

1

APPLICATION OF GENOTYPING-BY-SEQUENCING FOR IDENTIFYING SNP VARIATION AND PERFORMING GENOME-WIDE ASSOCIATION STUDIES IN A TETRAPLOID POTATO ASSOCIATION PANEL

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Sequencing of the potato genome has opened up new vistas for potato genetics and breeding. Further conversion of the sequenced genome into well-structured, oriented and annotated chromosome-scale pseudomolecules has made potato more amenable to modern genomic approaches such as sequencedbased genotyping. Genome-wide association studies (GWAS) are feasible and are gaining importance for studying the genetics of natural variation and traits of agricultural importance. GWAS offers significant advantages, such as increased mapping resolution, increased numbers of segregating traits, and greater allelic diversity than traditional mapping using bi-parental crosses. For a robust GWAS analysis, presence of high-throughput and marker-dense genotyping platforms are preferable. Previously, GWAS has been applied to potato using both more traditional (e.g. SSRs and AFLPs) as well as contemporary, sequencebased (e.g. SNPs) marker types. We are exploiting the latest high-throughput Next Generation Sequencing (NGS) technologies for performing GWAS in potato by employing a 'Genotyping-by-Sequencing' (GBS) approach. GBS offers advantages of simultaneous genotyping as well marker discovery and alleviates issues arising from factors such as ascertainment bias. Our Association Panel comprises 350 diverse autotetraploid potato cultivars which have been Illumina-sequenced at a reduced complexity level coupled with indexed sample multiplexing. The panel has also been genotyped using the SolCAP 8k Infinium array for the initial assessment of population stratification and Linkage Disequilibrium (LD). The selected germplasm has been phenotyped in two environments over two growing seasons. Potato, a highly heterozygous and tetraploid crop, brings many challenges for the downstream analysis of GBS data as well as GWAS analysis. Insights from these efforts will be presented.

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# A COMPLEX DISEASE RESISTANCE LOCUS ON POTATO AND TOMATO CHROMOSOME 4 EXHIBITS A CONSERVED STRUCTURE DISPLAYING DIFFERENT RATES OF EVOLUTION IN DIFFERENT LINEAGES

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#### Introduction

In plant genomes, NB-LRR based resistance (R) genes tend to occur as clusters of variable size in a relatively small number of regions. The R-gene sequences mostly differentiate by accumulating point mutations and gene conversion events. Potato and tomato chromosome 4 harbours a syntenic R-gene locus (known as the R2 locus in potato) that has mainly been examined in central American/Mexican wild potato species on the basis of its contribution to resistance to late blight, caused by the oomycete pathogen *Phytophthora infestans*. Evidence to date indicates the occurrence of a fast evolutionary mode characterized by gene conversion events at the locus in these genotypes. The recent sequencing of the potato and tomato genomes has afforded the opportunity to examine the evolutionary mode of this locus in a different lineage, of South American origin, where co-evolution with diverse and aggressive isolates of the late blight pathogen may not have been as significant a driving force.

#### **Methods and Results**

A physical map of the R2 locus (representative of the Group *Phureja* lineage) was developed for three *S. tuberosum* genotypes and used to identify the tomato syntenic sequence. Functional annotation of the locus revealed the presence of numerous resistance gene homologs (RGHs) belonging to the R2 gene family (R2GHs) organized into several discrete clusters, three of which were conserved across *S. tuberosum* and tomato. The locus has expanded in *S. tuberosum* Group *Phureja* and footprints of recent duplication events can be identified between five sets of potato paralogs. Phylogenetic analysis showed clear orthologous relationships between *S. tuberosum* Group *Phureja* R2GHs but not in R2GHs cloned from *Solanum* wild species. This study confirmed that, in contrast to the wild species R2GHs, which have evolved through extensive sequence exchanges between paralogs, gene conversion was not a major force for differentiation in *S. tuberosum* Group *Phureja* R2GHs, and orthologous relationships have been maintained via a slow accumulation of point mutations in these genotypes. In addition, as well as the expected level of positive selection in the LRR regions, members of the R2 gene family from both lineages share significant levels of positive selection in codons at the 5' end.

#### Discussion

Comparative analysis reveals a conserved tripartite structure at the R2 locus predating speciation of potato and tomato. *S. tuberosum* Group *Phureja* and *S. lycopersicum* R2GHs evolved mostly through duplication and deletion events, followed by gradual accumulation of mutations. This slow evolutionary rate unequivocally delineated the orthologous relationships between R2GHs in *S. tuberosum* genotypes. Conversely, the numerous gene conversion events detected in R2GHs from *Solanum* wild species conceal mutual orthologous relationships.

#### **Conclusions**

We conclude that different selective forces shaped the evolution of the R2 locus and that co-evolution with a pathogen steered selection on different evolutionary paths.

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#### ALLELE-SPECIFIC MOLECULAR MARKERS FOR THE POTATO'S DISEASE RESISTANCE GENES **VIA NEXT GENERATION SEQUENCING**

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Potato breeding and selection using molecular markers is challenging due to the large number of alleles per genetic locus and the autotetraploid mode of inheritance. Next generation sequencing together with accurate phenotyping offers a way to generate exhaustive genetic markers at fast speed and low cost. Currently, we aim to index the (nearly entire) diversity of all major resistance genes (R genes) of the cultivated potato (S. tuberosum) genepool.

The goals of the project are to explore and catalog nearly all diverse R alleles (R haplotypes) conferring disease and pest resistance, at the genetic loci of R genes belonging to the NBS-type meta-family in Solanum (with emphasis on the common potato), to determine in a large-scale approach R allele fragments by their association with the resistance phenotype, to explore evolutionary, structural, and diversification aspects of the plant R genes and to set up a method for the development of molecular tools (markers; sets of PCR primers) that can be used in research and by plant breeders, for fast tracking of R alleles conferring resistance to pathogens and pests in Solanum.

We sequenced the NB-LR disease resistance genomes of 96 old and modern potato varieties. For this study the potato samples were kindly provided by potato breeders and gene banks. Primers for the NBS profiling were newly developed and tested. All NBS containing sequencing from the DM reference genome (Jupe et al. 2012) were aligned to the p loop motif, the kinase 2 domain and the GLPL domain and degenerated primer were designed on the sequence homology. In total six primers tagging those specific parts of resistance genes were employed. Amplification products were subjected to HiSeq (Illumina) sequencing at GATC Biotech (Konstanz, Germany).

The sequences were analysed using standard tools for sequence analysis and then aligned to the potato reference sequence (PGSC\_DM\_v4.03) using NextGenMap 0.4.4 (Sedlazeck et al. 2013). The R genome sequences and the results of our bioinformatics analyses will be available online.

To prove our concept we used a mapping population segregating for a new PVY resistance. The mapping population consist of 250 progenies and the parents were subjected to NBS sequencing. To localize the resistance gene on the genetic linkage map, microsatellite markers were applied on the population. The raw linkage map was calculated using TetraploidMap. The PVY resistance was linked to SSR markers from chromosome 9. For chromosome 9 65 NBS containing sequences are described (Jupe et al. 2013). The parent NBS sequences were search for SNPs among the parents. Based on the SNPs present in the resistant parent primers were designed. Several markers based on the NGS data were tested and all markers were placed on chromosome 9 in the right order according to the reference genome. Until now no linked marker to the PVY resistance could be found. New primer for different position on chromosome 9 will be further tested.

#### Acknowledgments

The project has been financed by FWF, Austria, and the Government of Lower Austria ('SolaRindex', TRP146). Development of potato virus resistance markers is kindly financed by the AVIMA research project (German Federal Ministry of Education and Research.).

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# CYTOPLASM TYPES IN THE EUROPEAN POTATO GENE POOL AND THEIR RELATIONSHIP TO COMPLEX AGRONOMIC TRAITS

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Maternally inherited cytoplasmic markers are useful to evaluate cytoplasmic genome diversity and origin. Hosaka and Sanetomo (2012) developed five DNA markers, by which six types of potato cytoplasms can be distinguished (T, D, P, A, M, and W). Using these markers, the cytoplasm type of 792 tetraploid potato genotypes was determined, which included European varieties and German breeding lines. Cytoplasm types T, D, P, A, M and W were assigned to 417 (52.7%), 246 (31.1%), 0 (0%), 5 (0.6%), 4 (0.5%) and 120 (15.2%) and genotypes, respectively. 110 of 120 W cytoplasm types were the W/v sub-type of S. stoloniferum. Compared with the Japanese potato gene pool, the frequency of T type cytoplasm (the most prevalent type in S. tuberosum ssp. tuberosum) was lower, whereas the D (introduced from S. demissum) and W/v type cytoplasms were surprisingly more frequent. This indicates that the cytoplasms derived from S. demissum and S. stoloniferum were frequently used in German potato breeding programs, probably due to late blight and PVY resistances introgressed from these species. These cytoplasms are known to cause male sterility in S. tuberosum nuclear genome background, so these breeding materials can only be used as maternal parents. On the other hand, P (corresponding to S. phureja cytoplasm), A (the most prevalent type in S. tuberosum ssp. andigena) and M (ancestral types of Andean cultivated potatoes) cytoplasm types were very rare, indicating that very limited cytoplasmic diversity has been utilized in Europe.

To investigate the association of specific cytoplasm types with agronomic characters, ANOVA analysis and Tukey HSD test were performed in 4 association mapping populations (BRUISE205, CHIPS-ALL, PIN184 and SUGAR40), which have been phenotyped for several complex agronomic traits (Li et al. 2008, Pajerowska et al. 2009, Urbany et al. 2011, Fischer et al. 2013). The T cytoplasm was always associated with earlier maturity, while W cytoplasm was associated with later maturity. In BRUISE205, genotypes with W/y cytoplasm showed significantly higher bruising susceptibility and higher tuber yield compared to those with T cytoplasm. Tuber starch content was on average higher in genotypes with W/y cytoplasm compared to all other cytoplasm types. Different cytoplasms did not show significant association with processing quality traits (chip color, reducing sugar content in response to cold storage). In the PIN184 population phenotyped for late blight resistance and maturity, the relative area under disease progress curve (rAUDPC) and maturity corrected resistance (MCR) were strongly associated with cytoplasm type. In particular, genotypes with M cytoplasm showed lower MCR values (increased resistance to late blight) compared with the other cytoplasms. Genotypes with D cytoplasm also showed lower MCR and rAUDPC values than those with T and W cytoplasm. These results strongly indicate that late blight resistance is controlled not only by the nuclear but also by the cytoplasmic genome, although it might be possible that the S. demissum cytoplasm was exclusively transmitted together with late blight resistance because of cytoplasmic male sterility. Our study suggests that the cytoplasm type is associated with several important traits and should be considered in marker-assisted breeding schemes of potato.

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# EQTL ANALYSIS AND NETWORK INFERENCE OF A DROUGHT STRESSED DIPLOID POTATO MAPPING POPULATION

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#### Introduction

Drought is one of the most devastating environmental stresses, affecting crop yield worldwide. Potato uses water efficiently for food production, but is sensitive to drought stress. Discovery of genetic factors that contribute to drought tolerance in potato will facilitate the breeding of tolerant cultivars.

We previously evaluated the CxE diploid mapping population for drought tolerance, and found an important QTL locus on Chromosome 5. We analysed the full transcriptome in 94 individuals of this population and used the dataset for eQTL analysis and search for candidate genes.

#### Methods

94 progeny of the CxE mapping population were grown in the greenhouse from tubers in pots. At average stolon initiation stage watering was stopped, and RNA isolated 4 days later when wilting signs were visible. The plants were extensively phenotyped. RNA of each genotype was hybridised to the POCI potato oligo array [2]. The data was analysed in Genstat (15th ed.) and with bioinformatics tools like R, BLAST2Go, OmicsFusion, and an inference algorithm applied through the GenePattern95 DREAM network inference platform (http://dream.broadinstitute.org/gp/pages/index.jsf) [3].

#### Results

A PCA with all expression data showed a distinct group of drought-stressed genotypes that also had relatively high yields. One of the genes expressed in this group was a dehydrin (TAS14 homolog). Several possible candidates for root and shoot traits were identified with Omics Fusion, including a MnSOD gene implicated in oxidative stress tolerance.

eQTLs were mapped for the expression datasets of control, drought-stressed, and drought vs control plants. The most remarkable result is a peak of eQTLs clustering on a single eQTL hotspot on Chromosome 5, close to but not co-localizing with the maturity locus. By network inference analysis a particular transcription factor was found to be a central node to the regulation of the genes with eQTLs in this hotspot. This transcription factor is an NF-YC homolog. Also, an ethylene response factor (ER24) appears to be directing the Heat Shock Protein response in the drought-stressed potatoes.

#### **Discussion**

Expression of a large number of genes that display an early response to drought treatment of the CxE population is linked to the decrease in expression of the transcription factor NF-YC4. NF-Y factors are thought to act in trimers as transcriptional regulators of many important processes in plants, and NF-YC4 located on Chromosome 5 may be the molecular switch driving the drought response. Studies are ongoing to further characterize this gene as well as TAS14 and ER24, and their roles in the drought response and drought tolerance in potato.

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# COMPARISON AND INTEGRATION OF EXISTING FUNCTIONAL ANNOTATION PIPELINES FOR NON-MODEL ORGANISMS – IMPROVING FUNCTIONAL ANNOTATION OF THE POTATO GENOME

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#### Introduction

For a non-model organism, such as potato, little functional information on individual genes or proteins exists, even if the genome sequence is available. Comprehensive and accurate pipelines that utilize sequence-based analysis, literature information and available 'omics' data are highly desirable. To date some pipelines for functional analysis have emerged, however, the research community encounters little guidance on how well they perform. As part of the AllBio initiative (www.allbioinformatics.eu), we aimed to evaluate, compare, and integrate extant annotations pipelines to improve the functional annotation of potato genes. The potato genome sequence was released in 2011 by PGSC, and later when the tomato genome was presented an updated set of gene prediction models (so called ITAGs) was proposed.

#### Results

Based on either the PGSC or ITAG gene models the following annotation pipelines were compared: Trinotate-HMM, Trinotate-BLAST, OrthoMCL, BLAST2GO, Phytosome and BioMart. BioMart and OrthoMCL provided the highest number of annotated genes (ca 75%), and number of Gene Ontology (GO) terms per gene (ca 7). Jaccard scores were used for pair-wise comparison of the predicted annotations between pipelines both in terms of the degree of absolute overlap of GO terms and similarity weighing in relatedness of associated GO terms. Overall, there was low similarity between annotation pipelines (average of 0.21 in PGSC out of 1 indicating identical composition). As could be expected, the similarity was higher when the relatedness of predicted GO terms was considered (average of 0.33). In order to determine and compare the accuracy of the annotation pipelines two independent approaches were used: (1) a gold standard consisting of ca 1000 gene-GO term pairs assembled manually by us based on published functional studies or derived from MetaCyc, (2) gene co-expression data based on whole-genome microarray data across 326 conditions. The latter assumes that a given set of genes predicted to be associated to a GO term according to a specific annotation pipeline, has a significantly higher level of co-expression than expected in a random group of genes. We show that while all pipelines provide much better intra-process co-expression than expected by chance, there are large differences among the methods. Based on the gold standard we calculated the precision and recall (F-measure) of GO terms for each pipeline. BioMart with an F-measure of 0.6 performed best. Granted the differences between annotation pipelines, we constructed an algorithm to reconcile predictions of the pipelines. This merged annotation covered more genes and increased the number of significant GO terms ca 1.5-fold compared to the single pipelines. This improvement did not come at the expense of accuracy; i.e. the Fmeasure increased to 0.8.

#### **Conclusions and perspectives**

We show that the annotation predictions of existent annotations pipelines of genes are markedly different. We therefore introduce a merged annotation of the different pipelines, which outperformed the single tested pipelines both regarding coverage and accuracy. Our methodology generates a robust and automated framework for gene annotation also applicable to other non-model species. The new functional annotations of the potato genome will be made publicly available together with tools for implementing the methodology with additional pipelines and other sequenced organisms in the form of an open source R code.

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# EPIDEMIOLOGY AND CONTROL OF POTATO POWDER SCAB CAUSED BY SPONGOSPORA SUBTERRANEA

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Powdery scab (PS), caused by Spongospora subterranea f. sp. subterranean (Sss), occurs in Israel both in the spring and fall-winter seasons. The disease became prevalent during recent years in Israel, although conditions such as lack of waterlogged soils and relatively high soil temperature (during the spring season) are not favourable for Sss infection. The increasing economic damage caused by PS during the recent years, emphasize the need for efficient soil and seed tuber treatment to prevent the spread and establishment of the disease under hot climate growth conditions. Seed-borne inoculum arrives each year through certified seed lots imported from Northern Europe. During the last decade 23% of the seed lots were contaminated with PS (15% of them less than 5% tuber area covered). The main objectives of the present study were: to evaluate pre-planting soil treatments and the efficacy of seed treatments on disease incidence and severity; to study the effect of planting date on disease development and the quality of the daughter tubers; to screen commercial potato cultivars for susceptibility to the disease; to evaluate the role of dust storms in spreading the pathogen; to evaluate the role of latent infection in seed tubers. Several field trials were conducted between 2005-2013 to evaluate soil and seed treatments. Soil fumigation either with metam sodium (51% a.i. at dose of 430 or 620 l/ha) or with chloropicrin (200 and 400 l/ha), significantly reduced the incidence and severity of powdery scab on daughter tubers. Soil treatments with fluazinam were not effective as the soil fumigation. Various agents for seed tuber treatments were evaluated in field trials, amongst them, mancozeb, fludioxonil and fluazinam were effective in reducing PS incidence on daughter tubers. The susceptibility of major potato cultivars to PS was tested in several field trial carried out on naturally infested soil. Annabelle, Mozart and Nicola were the most susceptible cultivars, whereas Valor, Rosanna and Winston were the most tolerant. Latent infection of seed tubers was observed and the potential spread of Sss was demonstrated. Both factors may have an important role in the epidemiology of the disease and its management.

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# EARLY BLIGHT DIAGNOSTICS IN POTATO: THE ROLE OF *ALTERNARIA SOLANI*, *A. ALTERNATA* AND DAMAGE BY OZONE

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In recent years early blight in potato received more attention. This has been ascribed to climate changes but more so to increasingly specific treatments of late blight, allowing early blight to proliferate. With the introduction of integrative pest management (IPM), farmers are increasingly required to be able to show which fungicides they apply and for which reason. Therefore, proper diagnosis of early blight is required in order to prevent unnecessary applications of fungicides specifically applied to this disease.

In this study we demonstrate that early blight is not easy to recognize. From over 9000 leaf spots analysed as potential early blight in 2009-2013, only approximately 20% actually contained *Alternaria solani*. Additionally, we found many lesions on leaves that contained no fungi at all, making us question the role of *Alternaria alternata* as a pathogen.

In lab- and fieldtrials we tested the pathogenic properties of both *Alternaria solani* and *A. alternata*. In the laboratory trial we applied solutions containing spores (10.000/ml) and mycelium of multiple isolates obtained in recent years to leaves of eight cultivars. To make it as easy as possible for the fungus to infect, half of the leaves were wounded. After 8 days, lesions were measured. On all cultivars, only the isolates of *A. solani* were able to produce necrotic spots. *A. alternata* was unable to cause any spots, even when leaves were wounded.

In the field trial, eight cultivars were planted and inoculated with a cocktail of *A. solani* isolates, a cocktail of *A. alternata* isolates or just water. The trial was performed *in triplo*. Similar to the trial in the lab, only addition of *A. solani* caused development of lesions. While many lesions also appeared in the untreated and *A. alternata*-treated plots, there was no significant increase of *A. alternata* in these spots. Finally, statistical tests performed on our database shows that both *Alternaria* species appear independent of each other, suggesting they are not competitors. This further increases the idea that *A. alternata* is not a pathogen.

A. alternata is known to be present in high concentrations in the air and randomly drops into lesions on potato and lives there as a saprophytic fungus. This does leave open the question of what caused the large amount (80%) of lesions observed in the last five years.

Ozone damage is widely known to be damaging to all kinds of crops, including potato [1]. However, agricultural practice in the Netherlands is largely unaware of this problem. As an eye-opener to both the farmers as well as the industry, we also performed small and simple trials where we artificially applied ozone to several popular cultivars known to suffer from ozone damage. High amounts of ozone caused wilting within an hour and dying off within a day, demonstrating in an exaggerated way how damaging ozone is. Lower, more naturally occurring doses, produced symptoms similar to early blight.

Altogether, this shows diagnosis of early blight can prove to be difficult, resulting in many unnecessary applications of fungicides.

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#### RESISTANCE TO ALTERNARIA SOLANI IN POTATO

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#### Introduction

Alternaria solani, is an asexual plant pathogenic species that causes early blight of potato (Solanum tuberosum L.), and other members of the Solanaceae family. The disease can result in large crop losses in many potato and tomato producing areas worldwide. Presently, fungicide application is the main control practice adopted but environmental and social concerns require a reduction in fungicide usage. Therefore, more efforts to breed for improved resistance to this disease would be eligible. Early blight resistance has been evaluated in potato germplasm by a number of researchers and it has been observed that early maturing cultivars are generally more susceptible than late maturing cultivars. Furthermore, plant age and leaf age are important for resistance since susceptibility increases as plants grow older and older leaves are more susceptible. Field scoring has been the main procedure in most screenings. However, results can be affected by uncontrollable environmental conditions and the presence of other pathogens. Therefore, reliable greenhouse scoring methods would be feasible for screening breeding material.

Resistance to *Alternaria* in potato appear to be of quantitative nature, i.e. no major gene for resistance has been identified. There is a need to understand the importance of different plant defence mechanism against various pathogens. Plant defence depend on signalling cascades that are regulated by molecules such as jasmonic acid (JA) and salicylic acid (SA). In general, defence responses depend on specific plant-pathogen interactions and little is known about the interactions between potato and *Alternaria*.

#### **Materials and Methods**

We have studied the level of resistance in potato germplasm in greenhouse. In addition, the degree of tuber resistance was investigated for the same germplasm.

To understand the importance of different signalling transduction pathways for *A. solani* resistance in potato we study transgenic plants compromised in SA (NahG mutants) and/or JA (OPR3 mutants) metabolism in comparison with wild type plants.

#### **Results and Conclusions**

Cultivars and breeding clones differed significantly in resistance both in leaves and tubers. However, tuber resistance did not correlate to leaf resistance. For leaf resistance we found a good correlation between field assay and inoculation of intact plants but a poor correlation between detached leaf assays and inoculation of intact plants or field experiment. Therefore detached leaf assays are not suitable for resistance tests [1].

In general, SA signalling pathways are coupled to resistance to biotrophs or hemibiotrophs, whereas JA responses are coupled to resistance against necrotrophs. However, unexpectedly we found that the NahG's clones were more susceptible than the wild type clone, while the level of resistance in the OPR3 mutants did not differ from the wild type. This indicates that SA enhances resistance against *A. solani*, which also have been observed in tomato against *A. alternata* [2]. The latest results of the studies will be presented.

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# DETACHED-LEAF EVALUATIONS OF POTATO CLONES FOR RESISTANCE TO ALTERNARIA SOLANI

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Early blight disease, caused by Alternaria solani, is a serious disease of potato foliage and tubers that occurs in most potato-growing regions world-wide. Developing new potato cultivars with resistance to early blight may reduce losses in the field and in storage, and lessen the need for fungicide applications. In this study, A total of 229 clones, derived from 13 different hybrid families were evaluated for resistance to A. solani in detached leaf tests. Fully developed leaflets were detached from the middle part of the 6- to 12-weekold field-grown potato plants. Three detached leaflets of each clone were inoculated with 5-mm agar plugs of 1-week-old colonies of A. solani grown on tomato juice agar medium. Treated leaflets were placed on moist, sterile filter paper in a 90 mm covered Petri dish. Another leaflets were inoculated as a control with plain agar plugs. Leaflets were incubated in moist chambers at 20±2 °C for 7 days before measurements were taken. Significant differences were found among families, and within families (P < 0.05). Out of 229 clones,115 were highly resistant (not show any symptoms of infection). For instance, clones, A2/11 and A2/132 derived from MF-1 X TS-4 hybrid family were very sensible to A solani, while the clones A2/120, A2/179 and A2/109 were found highly resistant to the pathogen. Similarly, the clones A3/20,A3/303, A3/117, T3/36, and A3/55 derived from Serrana x TS-9 hybrid family were very sensible to A solani while the clones A3/4, A3/66, A3/74, and A3/284 were found highly resistant to the pathogen. These results suggest that these potato clones are worthy of use in breeding for early blight resistance.

# INOCULATION OF A COVER CROP WITH RHIZOPHAGUS IRREGULARIS AND TRICHODERMA HARZIANUM PRIOR TO POTATO PLANTATION INCREASES POTATO YIELD

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Arbuscular mycorrhizal fungi (AMF) and *Trichoderma* spp. are plant beneficial microorganisms present in the rhizosphere. A field experiment was conducted to investigate their benefits on potato crop under chemical or non-chemical weed and disease management practices. *Rhizophagus irregularis* MUCL 41833 and *T. harzianum* MUCL 29707 were introduced in the field via the inoculation of *Medicago sativa* (used as cover crop prior to potato plantation) or via the direct inoculation of potato tubers at plantation. Inoculating cover crop prior to potato plantation significantly increased potato yield by 13 % for non-chemical and chemical managed soils confounded. Proportion of marketable tubers (caliber > 30 mm) was significantly higher for both types of inoculation strategies. In addition, significant positive correlation was observed between total root colonization by arbuscular mycorrhizal fungi and yield. These results indicated that agricultural practices such as cover crop inoculation prior to potato plantation may be a good strategy to increase root colonization by arbuscular mycorrhizal fungi and subsequent increase of potato yield. Further long–term studies are necessary to investigate agricultural practices that may enhance root colonization and crop yield.

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# DIVERSITY OF BACTERIAL ENDOPHYTES ISOLATED FROM POTATO PLANTS OF DIFFERENT CULTIVARS IS SPAIN, WITH POTENTIAL AS BIOFERTILIZERS

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Potato (*Solanum tuberosum* L.) is the first global culture outside the group of cereals, playing a key role in human consumption. The potato crop is therefore of great importance, however, despite the success in potato production worldwide the legal limitations in the use of chemicals and pesticides rise the need of development of culture techniques that prevent environmental damage. For integrated production schemes, the use of microorganisms as biofertilizers for plant crops is an alternative environmentally friendly preserving yield, since some of these organisms have a biostimulant effect through diverse mechanisms. In this sense, the endophytic bacteria may be good candidates in the formulation of biofertilizers by establishing close interaction with plants, allowing them to exert these effects in more success. Although a few papers on the diversity of potato endophytic bacteria are available [1, 2, 3], knowledge on this field is still scarce. In this work we analysed the phylogenetic diversity of endophytic bacteria of potato plants from different cultivars growing in two soils subjected to integrated production systems in Northern Spain. The isolation of endophytes was performed by surface disinfection followed by crushing and maceration of potato tissues in PBS, and after streaking in petri dishes with TSA medium. The phylogenetic diversity was assessed by amplification and sequencing of the 16S rRNA gene and the sequences obtained were compared with those from EzTaxon-e server.

The results showed a very high genetic diversity among the isolates, and about 25 different genera within 12 families of bacteria were identified. Most endophytic strains isolated from *Solanum tuberosum* roots and stems were Gram positive of low G+C content classified within the Firmicutes and belonged to the genera *Bacillus*, *Bhargavaea*, *Lysinibacillus*, *Paenibacillus*, *Oceanobacillus*, and *Terribacillus*. Also, other gram positive bacteria within class Actinobacteria were found as well as gram negative bacteria belonging to the alpha, beta and gamma classes of Proteobacteria. Finally, *Flavobaceria* of phylum *Bacteroidetes* was also detected. Four genera are reported for the first time in the inner of potato plants in this work to our knowledge, and according to the 16S rRNA gene sequence divergence, several putatively novel species of bacteria were detected in this work. The possible mechanisms for plant growth-promotion ability of these strains are being currently investigated in order to select good candidates to be used as inoculants for biofertilizers formulations targeting potato crops.

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#### Session 3 (OP) - POTATO AND GLOBAL FOOD SECURITY

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# IMPROVING SEED-TO-WARE, WARE AND KNOWLEDGE CHAINS IN POTATO CULTIVATION IN EASTERN AFRICA

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Potato is becoming increasingly popular in Eastern Africa. It is widely grown by millions of farmers replacing traditional root and tuber crops. Potato is often grown by poor farmers as hunger breaker as it has a short cycle and can be harvested before cereal crops have matured. Certain areas are suitable for production of quality-declared seed for local use or export. Ware potato can be marketed and processed provided suitable storage and processing facilities are available. Many European countries are eager to invest in Eastern Africa.

Potato production in Eastern Africa, however, is still facing many constraints. Some constraints are institutional, e.g., feminisation of agriculture, weak seed systems, poor access to agricultural inputs and credit, poor infrastructure limiting transport, and less developed knowledge chains. Other constraints are economic, e.g., poor incentive of producing high-quality seed or ware, small difference in price between seed and ware, and high costs of fertilizers and fungicides. Moreover, for the poorest farmers it is difficult to become 'adult' players in the value chains. Their production levels are low because of small farm sizes and because technology development is often under-addressing their problems and potentials.

Although in Eastern Africa most seed potato production is still based on farmer-saved seed, most countries produce basic seed, often based on a combination of tissue culture and minituber production on hydroponics. Incipient formal systems or alternative systems bridging the gap between farmer-saved seed and formal seed are developing. A strong driver is the continued interest in new cultivars, with high quality combined with resistance against major diseases. There are also promising developments in the informal seed sector: seed supply improved over recent years by scaling up diffuse light storage and producing seed tubers through positive selection. Positive selection is a simple, robust technique with a large immediate effect; it might even reverse the process of degeneration over several generations of traditional production of seed tubers, e.g., by lowering the virus load. Unfortunately, seed potato production in Eastern Africa is not only suffering from viruses; bacterial wilt, late blight and physiological age are also major constraints. Bacterial wilt is indigenous in many areas of Eastern Africa and very hard to control. Late blight can occur very early in the growing season, with a continuously high inoculum pressure high. Getting seed in the proper physiological age in regions with several growing seasons per calendar year is also a major challenge.

For ware the main problems are the lack of proper storage facilities as the diffuse light stores are only suitable for seed. Processing is becoming more popular and this could create the necessary pull to bring the entire chain to a higher level of added value.

Future research of our consortium aims at investigating further how to improve seed-to-ware chains, value chains and knowledge chains by increasing output, efficiency, quality, food security and safety, involving breeders, seed companies, (basic) seed suppliers, intermediaries and ware growers, extensionists, researchers and universities. We aim at supporting the development of seed systems and variety improvement, processing plants, a community-based control strategy for bacterial wilt and functional networks of knowledge transfer.

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# CIP'S POTATO STRATEGY FOR AFRICA: IMPROVING LIVELIHOODS OF POTATO FARMERS IN AFRICA BY TACKLING DETERIORATED SEED QUALITY THROUGH AN INTEGRATED APPROACH

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Potato is an ideal crop for smallholder farmers in African highlands, as it yields more calories per unit area with a short growing cycle, and is nutritious, making potato an important food security and cash crop. However, yields in Sub-Saharan Africa (SSA) range from 6-10 t ha<sup>-1</sup>, far below attainable yields of 25-35t ha<sup>-1</sup>. Demand is increasing for potato in SSA, but the trend is to increase the area under production rather than tackle productivity constraints. Although the solution is to increase productivity, the major bottleneck is limited access to quality seed of suitable varieties, which limits yields, food availability, and incomes. Seed potato production systems in most SSA countries have traditionally been the sole responsibility of national programs, and the systems have largely been operating under limited human capacity and facilities without a functioning quality control system, although seed regulations exist in most of the countries. Consequently, farmers use unmarketable small potatoes for planting, which are generally of poor health status, causing diseases accumulation and spread in farmer-saved seed stocks. Furthermore, advances in breeding often bypass farmers' due to the inability to access quality seed of these varieties. which largely undermines investments and innovations in breeding. CIP has proven experience from interventions in six African countries of a strategy to improve seed qualities and seed production. The strategy integrates Rapid Multiplication Technologies, such as aeroponics, to increase early multiplication rates to reduce the number of field multiplications from five to three generations under the "3 Generations" approach and includes scaling-up and out decentralized seed production, implementing quality control by using Quality Declared Planting Material (QDPM) standards, an extensive awareness building campaign, distribution in small seed packages to increase accessibility to quality seed, and training farmers to maintain seed quality on farm. In Ethiopia where there is no functional seed certification, piloting QDPM seed quality control, which is based solely on visual inspection, resulted in significantly better health status of the crop and doubled yields compared with farmers saved seed, illustrating the potential of using substandards in developing countries rather than relying on expensive and malfunctioning certification schemes alone [1]. Through project interventions, CIP successfully supported seed production systems in eight African countries. Key to the success was/is the strategic partnering between the private and public sectors along the seed value chain. In Kenya for instance, the production of certified seed rose from 250t in 2009 to about 4000t in 2013, predominately produced by private seed producers. Additionally, further multiplication by decentralized multipliers provided another 5000t of improved seed qualities. The presented paper gives an overview and comparative analysis from experiences from seed interventions in SSA of the past five years and the strategy for the next decade.

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#### POTATO IN CEREAL BASED SYSTEM TO MEET FOOD SECURITY IN SOUTH ASIA

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#### Introduction

South Asia was last reported at 69.86% living in rural area in 2010, according to a World Bank report published in 2012. The prediction by the French Institute of Demographic Studies (INED) is that India alone will succeed China in population by 2050 with a population of 1.6 billion which would give South Asia the highest global population. Malnutrition is still a major problem in South-Asia despite economic growth and reduction in poverty as about half of the children below five are stunted (47% in 2008 according to WDI).

#### Potato in Sub-tropical Lowlands of South Asia (SA)

Potato is an important food and cash crop in the subtropical lowlands of the Indo-Gangetic Plains (IGP) cultivated in the winter under short day. It is a key rotation crop in cereal systems. India has the largest area under potato: 1.9 million hectares followed by Bangladesh: 0.534 million hectares and Pakistan 0.185 million hectares (FAO Stat.2012). Accelerated breeding schemes, improved seed delivery, diversification of value chains, and ecological management practices in region can enhance productivity and incomes in a sustainable and equitable manner. This could be accomplished through use of short maturing potato that in extensive cereal-based systems of South Asia.

#### Constraints to wide adoption of potato

The private sector can play a key role in the scaling up of improved varieties and practice in SA. One of the aspects that make the value chain disarticulated is a poorly developed processing sector that could operate in preventing gluts and stabilizing market prices. Many countries cannot develop their own sustainable seed program based on locally developed varieties due to high dependency on imported seed and lack of trained manpower and infrastructure.

#### **Targeting systems**

In Eastern IGP, the farmers practicing boro (summer) rice after potato, do not get potential yields of boro rice or potato or both because; i) harvesting of potato at full maturity delays transplanting of boro rice reducing yield of boro rice, ii) early harvesting of potato for timely transplanting of boro rice reduces the potato yield, iii) the water requirement of boro rice is increased by delayed transplanting of boro rice, iv) the boro rice planted after potato remains in the field during summer and becomes more prone to abiotic and biotic stresses and natural calamities. The lack of heat tolerant varieties has limited wheat-potato area in North-IGP. The development of promising early maturing clones/varieties to heat tolerance can meet new opportunities of potato in the rice/wheat system.

#### CIP-Research and Development Strategy for SA

- 1. Advances in breeding technology for abiotic and biotic stresses and earliness
- 2. Enhance the processed potatoes from today's 3-6% in the SA region to 20% by 2020.
- 3. Participatory variety selection to accelerate the release varieties of stakeholder choice and adapted to system.
- 4. Regional networking to facilitate the exchange of information and material. Capacity building: Create regional training hubs for NARS, farmers associations, and NGOs
- 5. Increasing engagement of private sector in processing and seed production sectors.
- 6. Increasing women's role in project design and targeting, evaluation and dissemination. World Development Indicators (WDI): <a href="http://databank.worldbank.org">http://databank.worldbank.org</a>.

#### RISK ASSESSMENT OF POST-HARVEST ILLUMINATED POTATO TUBERS

#### RISK ASSESSMENT OF POST-HARVEST ILLUMINATED POTATO TUBERS

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The potato value-chain of potatoes includes several critical treatments until consumer offering. Despite of most careful handlings tuber stress reactions cannot be excluded. Several transport belts with free falling steps may attack tuber quality, known as harvest injuries. Mechanical impact is also given by washing, polishing and packaging the potato. Finally, artificial illumination may take place, e.g. at the point of sale. These stress factors will have an influence upon glycoalkaloid (SGA) de-novo synthesis, a group of secondary plant compounds involved in the plant defense strategy of *Solanaceae*.

To find out any positive or negative effects on tuber quality, mechanical inputs were simulated by a shaking desk (1 min, 30 mm stroke, 290 strokes min<sup>-1</sup>) using cold tubers (4 °C). A short term illumination (3 weeks) took place in a room with fluorescent lamps (tuber surface: 1.3 and 15.6  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup> at 555 nm, respectively) and in a growth chamber with metal halid lamps (32.5  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup> at 555 nm). Potato tubers of two (mechanical input) and three (illumination) varieties, respectively, grown at four different locations in Germany, were treated just after lifting and after six months storage period of +8°C at 90 % rel. humidity. Peel colour was measured with a colorimeter. Then the illuminated side of tubers were separated into peel and mark, lyophilized and ground.  $\alpha$ -Solanine and  $\alpha$ -Chaconine were cleaned up by solid phase extraction (SPE) and determined quantitatively by HPLC UV-detection.

Mechanically treated tubers induced a de-novo synthesis of glycoalkaloids. One week after treatment, glycoalkaloid content of the two varieties investigated was increased of about 20 and 45 %, respectively. Illumination induced chlorophyll and glycoalkaloid content, both. Greening was in relation to light intensity, but stored tubers had less greening effect. Glycoalkaloids did not increase at the variant with low light intensity, neither at lifting nor after storage, but variants with higher light intensity did so. After lifting, the variant with highest light intensity exceeded the safety level of 200 mg kg<sup>-1</sup> fresh matter, but not after storage. Glycoalkaloids could also be detected in peeled tubers, but concentration was much lower than in non-peeled potatoes. In any case, the general trend in combination with illumination was the same Investigated potato varieties had individual SGA-levels, but the overall trend was the same. Illumination of stored tubers induced sprouting with very high glycoalkaloid levels also in very small sprouts.

Correlations between tuber greening and glycoalkaloid enrichment gave no consistent results. In some cases correlation coefficient reached values up to r = 0.7, but most values were below r = 0.4. Therefore, no clear interaction of both pathways can be deduced.

Glycoalkaloids in potato tubers can be synthesized also post-harvest. Two stress factors were identified (mechanical and light stress), which should be reduced as much as possible. Consumer offering of fresh potatoes seems to be the most critical point. Potential solutions against illumination have already been developed, but consumers have to accept those alternative offering.

# POTATO YIELD GAP ANALYSIS IN SUB-SAHARAN AFRICA THROUGH PARTICIPATORY MODELING: OPTIMIZING THE VALUE OF HISTORICAL BREEDING TRIAL DATA

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The yield gap, defined as the mathematical difference between the potential yield and the average farmers' yield over some specified spatial and temporal scale [1, 2], is an important concept to estimate to what extent the production could be increased if all factors are controlled and non-limiting. This information is well documented for cereals but a lot still needs to be done for other commodities like potato, the most important non-cereal food in the world. A challenge in this endeavor is the scalability of the analysis as data are in most cases scarce in developing countries especially in Sub-Saharan Africa (SSA). To this end, scientists recommended using simulation models [1] but again their parameterization is at times a nightmare.

It is in this context that a regional study has been conducted in order to estimate the potato yield gaps. Scientists from West Africa (Nigeria), Eastern and Central Africa (Cameroon, Burundi, Rwanda, Kenya, Uganda, Tanzania, Democratic Republic of Congo and Ethiopia), and Southern Africa (Angola, Malawi, Madagascar and Mozambique) participated in the study. Having a wide knowledge of the crop was one of the pre-requisites to attend the workshops as the study was based on historical field data in most cases with missing parameters that had to be estimated using technological tools but with validation by field experts. The experience on the crop ranged from 8 to more than 31 years for 67% of the participants. This was the main driver of the workshops as field data for modeling purposes are seldom complete in most developing countries in general and SSA in particular.

The first task was to get the scientists acquainted with key concepts like systems research and yield gap analysis, and the tools to be used in the exercise. This was achieved in two workshops respectively held in Nairobi, Kenya and then Addis Ababa, Ethiopia. The big challenge was to estimate the parameters to feed the *Solanum* model developed by CIP and downloadable at:

http://inrm.cip.cgiar.org/home/downmod.htm . To this end, a Parameter Estimator routine was developed, but the expert's opinion was the driver to achieve reliable values prior to simulations.

This study was conducted on 12 potato genotypes – all from the International Potato Center except two-that have been evaluated in different breeding Programs. By the end of the second workshop, all the participants who had brought their data completed the simulations. Regardless of the genotypes, seasons and sites, those yields turned to be 50.6 t/ha for potential yield, 28.6 t/ha on-station yield, 8.2 t/ha as average farmers' yield and 42 t/ha for the overall yield gap. Boxplots generated show clearly that yield gaps in SSA are superior to yields normally obtained on-station. With less than 10 t/ha as average farmers' yield, there is a high potential in SSA to increase production if investments are made to optimize the defining production factors and manage properly limiting and reducing ones. Nevertheless, the study conducted was site-specific. Therefore, the community of practice initiated during the workshops decided to extend the study to special analysis. This will be achieved through an initiative called "Climate-Smart Potato in SSA" conceived by the same community of practice.

# TOWARDS ON-LINE ESTIMATION OF DRY MATTER IN UNPEELED POTATO TUBERS USING NEAR-INFRARED (NIR) INTERACTANCE

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Variation in raw material quality is a huge challenge for the potato industry. A lorry load (typically 30 metric tons) of potato tubers is notoriously heterogeneous, and for industrial processing differences in e.g. dry matter content may add significant costs related to product quality flaws and waste quantities. Today, the raw material quality is only partly controlled and not always systematically monitored prior to processing. In order to assess dry matter, the common methods used include the underwater weight and graduation in saline solutions. Both methods yield reliable values for dry matter content, but there are severe drawbacks. The underwater weight is only able to assess batch averages and the saline solutions are highly corrosive, hence frequent maintenance of equipment is required. The need for improved technology for dry matter estimation is thus obvious. Near-Infrared (NIR) spectroscopy has previously been presented as an approach for estimation of dry matter and starch contents in rather homogeneous samples of potato mash and potato slices [1, 2]. However, to be useful in an industrial application the method needs to be able to rapidly assess potato tubers moving on a conveyor belt. The authors have previously shown that the principle of NIR interactance can be used for assessing dry matter in stationary samples.[3] In a recent study, we investigated the possibilities of using NIR interactance as an approach to rapidly determine the dry matter content of potatoes on a conveyor belt. The samples were measured both stationary and while in motion. NIR interactance was shown to be an effective way of measuring whole unpeeled potato tubers along a conveyor belt. In this presentation, these results will be discussed along with the potential of measuring heterogeneity within each tuber. The technology used allows for up-scaling, hence increasing the speed and through-put of the analyses. The technology will allow for both total batch average and batch variation to be calculated. Combined with sorting solutions it might result in tools for obtaining more homogeneous raw material in the future.

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#### **SESSION 4 (OP) - SUSTAINABLE POTATO PRODUCTION**

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#### **GLOBAL SUSTAINABILITY ISSUES IN POTATO PRODUCTION**

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Potato and its derivatives increasingly become globally traded products. Potatoes grown and processed in e.g. Belgium are transported to far-away destinies such as Chile and Autralia ans seed potatoes from the Netherlands are shipped to all continents. The northern North American harbours ship fresh and frozen potatoes to many countries of the Pacific Rim. Commercial companies more and more want to quantify the environmental footprints such as the efficiency of the use of land and water, greenhouse gas emissions and the risks of eutrophication and contamination of the environment with pesticides. This to satisfy the demand of the customer. This paper reviews how quantitative model based approaches allow a rapid appraisal of the footprints of potato crops in different production environments. A crop growth model LINTUL - using temperature for development and light interception for growth allows the calculation of attainable yields and crop water needed as a bench mark of which actual yields and water supply. To measure the CO<sub>2</sub> footprint use is made of the Cool farm Tool (app.coolfarmtool.org) that distinguishes emissions from chemical inputs, field operations, irrigation, storage and transport. We recently published in Potato Research how from various sources global maps with grid cells of circa 8600 ha (near the equator) were drawn representing potato harvested area, potato fresh tuber yield (land use efficiency), slopes (risks of erosion), precipitation deficit (risks of depletion of fresh water resources through irrigation). average daily maximum temperature throughout the season (risks of occurrence of pest epidemics and emission of pesticides). Hotspots for erosion are the slopes of the mountains in the Andes, African Rift, Southern China and volcanic areas in southern China, and the island countries in South East Asia. Fresh water availability may become limited in the East of North America, northern India and China. Risks of insects are increased in continental hot summer climates and short spring crops with high temperatures towards harvest. Late blight is a threat in all humid areas such as maritime Europe, equatorial tropical highlands and the humid western Pacific Ring. In another recently published paper a wide variation of potato growing environments in Chile was analysed and from it directions for research and development were derived. This paper discusses the sustainable long-term and long-range sourcing of potato globally as well as repercussions of trends such as globalization and climate change; the latter being relative favourable for the root and tuber crop potato compared to grain crops.

# FACTORS AFFECTING YIELDS FROM INTENSIVELY MANAGED POTATO CROPS IN NEW ZEALAND

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#### Introduction

Current potato yields in New Zealand are becoming uneconomic at 50-60 t/ha in spite of high input crop management. Modelling shows that yields of 90 t/ha are possible, suggesting a large gap between actual and potential yields. A survey of processed potato crops was carried out in Canterbury during the 2012/13 growing season which aimed to identify factors limiting yields.

#### **Materials and Methods**

Eleven commercial crops were monitored. Pre-planting soil samples from the fields were assayed for DNA of important soil-borne pathogens. Fertilizer trials were established in four of the crops, where treatments of nitrogen (N), phosphorus (P) and potassium (K) were applied (grower rate and double grower rate). The crops were planted with the cultivars 'Russet Burbank' or 'Innovator' in fields with either no potatoes in previous rotations or potatoes in the last ten years. Every 10-14 days, plants were sampled from a defined area in each crop. These plants were assessed for disease incidence and severity, and other potentially yield-limiting factors (including soil quality). Individual plants in the crop (either "healthy" or affected by particular factors) were also identified, and used for yield comparisons. A crop growth model based on local radiation, temperature and soil data was used to compare potential yield with individual plant yield and whole crop commercial yield.

#### Results

Commercial yields from the crops were between 49 and 66 t/ha, 20-42 t/ha less than potential yield. Current grower N, P or K rates were not yield-limiting. Foliar diseases were controlled with prophylactic fungicide applications until late in the season, but four crops had shortened canopy duration due to early blight. Pre-planting soil assays identified pathogen DNA in ten of the fields, and large amounts of DNA of *Rhizoctonia solani* AG2-1, *Spongospora subterranea* and *Colletotrichum coccodes* were detected in some of the fields. *Rhizoctonia* stem canker, in varying incidence and severity, was observed on plants from all crops. *Spongospora* root galls were found in six of the crops, and in five of these, soil compaction was also likely to have been yield-limiting. Individual yields from "healthy" plants were equivalent to 91 t/ha, and yields from "diseased" plants in compacted soil were equivalent to 26 t/ha. Other yield-limiting factors were uneven emergence (one crop), inefficient irrigation (two crops) and weed infestation (two crops).

#### **Conclusion and perspectives**

Rhizoctonia stem canker and Spongospora root galls were the most prevalent soilborne diseases. Moreover, soil compaction was likely to exacerbate the effects of soil-borne diseases. These two diseases are well-recognised as potential yield-limiting factors (1, 2). Weeds, inefficient irrigation and poor quality seed tubers may also be important.

An additional trial has been established to quantify the effects of soil-borne diseases on potato productivity, using pesticides with a range of efficacies on a variety of soil-borne pathogens. Results from this trial will also be presented in this paper.

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# MERINOVA -'METEOROLOGICAL RISKS AS DRIVERS OF ENVIRONMENTAL INNOVATION IN AGRO-ECOSYSTEMS MANAGEMENT': VULNERABILITY AND RISK MAPS FOR POTATO IN BELGIUM

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Devastating weather-related events recorded in recent years have captured the interest of the general public in Belgium. In August 2003, Europe recorded its worst unprecedented heat wave with record breaking temperatures surpassing 40°C in Belgium. Severe prolonged droughts hit the 2007 and 2011 spring seasons causing severe crop damage. In May 2009, storms with lightning and hail resulted in devastating destruction across the country. In November 2010, excessive rainfall of up to 90 mm during 3 days triggered the worst flooding in 50 years. All of these natural hazards were caused by "severe" or "extreme" weather events. Since more than half of the Belgian territory is managed by the agricultural sector, extreme and severe weather events have significant impacts on agro-ecosystems, their functions and services. These extreme events are likely to increase under climate change. Current knowledge gaps related to the frequency and magnitude of extreme events and the resulting response of agro-ecosystems need to be addressed in conjunction with a clearer assessment of their vulnerability, resilience and adaptive possibilities.

The BELSPO funded project 'MERINOVA' deals with risks associated with extreme weather phenomena and with risks of biological origin such as pests and diseases. The major objectives of the proposed project are to characterize extreme meteorological events, assess the impact on Belgian agroecosystems, characterize their vulnerability and resilience to these events, and explore innovative adaptation options to agricultural risk management.

The present work aims at presenting the results of the case study focused on potato cropping and will mainly concern the vulnerability and risk maps definition. Vulnerability refers to the susceptibility of an agro-ecosystem to a hazard and the prevailing conditions, including physical, socio-economic and political factors that adversely affect its ability to respond to hazards or disaster events. In this context, a large collection of spatial data sets (land use and cover, soil, topographic, anthropogenic maps, etc.) has been collected at Belgian territory level. These data sets have been combined using a multi-criteria analysis within a specific geospatial database architecture defined to support the production of vulnerability maps. Vulnerability has been assessed at socio-economic (e.g. yield and income loss), environmental (e.g. soil heritage deterioration, groundwater and rivers pollution) and societal (e.g. damages to private and public infrastructures) level. Risk maps were produced by combining vulnerability with spatial information on the likely frequency and magnitude of extreme meteorological events (return period maps). Return period maps have been assessed by means of probability density functions for key periods during the growing season of the crop: waterlogging at planting and harvest, droughts during the growing season.

These vulnerability and risk maps represent a valuable source of information for public authorities to analyze the impacts of an exceptional event (understanding, monitoring and evaluating) and to understand the interaction with environmental, social and economic factors which ease the emergence of innovative adaptation strategies.

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#### RESOURCE USE EFFICIENCIES OF SOUTH AFRICAN POTATO PRODUCTION SYSTEMS

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#### Introduction

Potatoes are produced in 16 geographical regions of South Africa, differing in climate, soil, production practices and distance to markets. These factors affect the amount of input resources required to produce a ton of potatoes, and therefore, the carbon (C), land, water, and nutrient use efficiencies, which indicate sustainability of production. The objective of this study was to assess and benchmark South African potato production areas regarding the use of land, water, nutrients and energy to produce potatoes. The outcome of the study will allow exploitation of conditions to make production systems more efficient and to ensure economic and environmental sustainability.

#### Materials and methods

Farmer surveys were conducted in all potato production regions by interviewing at least three farmers for each production system present (e.g. seed, table, processing, rainfed, irrigation). The collected information enabled calculation of land, water and C-footprints. For the latter, the Cool Farm Tool-Potato [1] was used. Footprints were calculated for farm gate and factory/retail point. Potential crop yields per region and system were determined with the LINTUL-POTATO crop growth model [2]. Variability in yield gap (difference between potential and actual yield) was subsequently used to identify yield limiting factors.

#### **Results and Discussion**

The total amount of CO<sub>2</sub>-equivalent greenhouse gases produced per ton of fresh potato ranged from approximately 150 kg/t (rain fed production) to 300 kg/t (high input and irrigation). Rain fed systems had a low land use efficiency (yield), but achieved a high C use efficiency. Fertilizers, irrigation and grading / storage are the greatest contributors to C-footprints. Energy for pumping was not only related to the amount of water applied, but also to the depth and distance of pumping. Long distance travel of produce to retail points contributes substantially to the C-footprints in some regions. Water and nutrient footprints differed vastly between regions and were not directly proportional to the water requirements and yields achieved. Actual yields ranged between 25 and 85% of potential yield, suggesting that significant improvements are possible by improving management practices.

#### **Conclusions**

The study revealed that  $CO_2$  emissions associated with irrigation activities are a major contributor to the total C-footprint, giving irrigated systems a considerably higher C-footprint per t of fresh produce than rain fed systems in South Africa or elsewhere [1]. Large variability in resource use efficiencies and yield gap between farms, systems and regions suggests there is considerable room for improving resource use efficiencies to ensure more sustainable potato production.

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#### MONITORING RESIDUAL NITROGEN IN POTATO CROP

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#### Introduction

In order to obtain water quality objectives set by the European nitrate directive, the 'Coordination centre for extension services for sustainable fertilization' (CVBB) was founded in 2012. The mission of CVBB is to support farmers in their fertilizing management. It is known that vegetable and potato growers have difficulties to prevent nitrate-N leaching during autumn and winter. Residual soil nitrate-N is monitored by the authorities in 0-90 cm between October 1st and November 15th. Farmers get consequences for exceeding a threshold. This threshold depends on the water quality in the region, the soil type and the cultivated crop.

#### Materials and methods

In 2012 a detailed analysis of nitrate-N residues was done on 536 fields in Flanders with a wide range of crops (110 analyses on potatoes). The aim of the project was to check the influence of fertilizing management, soil type and weather conditions on nitrate-N leaching during autumn. 135 Farmers in high risk regions of Flanders, choose 4 fields managed according to good agricultural practice. They were asked to fertilize on the base of a soil analysis prior to the crop. Organic manure was analyzed and total amount of nitrogen was adjusted to the needs of the crop according to N-advice (soil sample). At the end of the season, residual nitrogen was monitored in 0-90cm. At three times during the sampling period (Oct 1 – Nov 15), soil samples were taken and analyzed.

#### Results

As expected, the results of the monitoring program 2012 showed residual nitrogen exceeding the threshold for vegetables and potato crop. In potato crops residual nitrogen may not exceed 90 kg NO3-N/ha in low risk regions and 85 kg NO3-N/ha in high risk regions. Although nitrogen fertilizing occurred according N-advice, mean nitrate-N content found in monitored potato fields was exceeding 90 kg NO3-N/ha. The nitrate-N content in the 0-90 cm soil profile did not significantly change between October 1st and November 15th. However, more residual nitrogen was found in fields sampled after potatoes had been harvested. Increasing mineralization after soil lifting increased total amount of residual nitrate-N.

#### **Conclusion and perspectives**

Fertilizing potato crops according to the needs of the plant, did not allow all farmers to reduce residual soil nitrate below the threshold. Yield, weather and time of sampling affected residual nitrate-N.

# USE OF MICRO-DAMS IN POTATO FURROWS TO REDUCE EROSION AND RUOFF AND MINIMISE SURFACE WATER CONTAMINATION THROUGH PESTICIDES.

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In hilly areas, potato fields are very sensitive to erosion and runoff as this crop is always planted in the direction of the slope (make harvest possible), the infiltration surface between the ridges is limited and the crop covers the soil only very late. Especially in erosion sensitive areas erosion and runoff are major sources of surface water contamination in addition to point source pollution. On its demonstration farm for sustainable agriculture in Huldenberg (Belgium), Bayer CropScience has set up a research project in collaboration with CRA-W and ULG - Gbx AGROBIOTECH on the application of micro-dams in potato furrows [1].

In a sandy loam soil with a slope of more than 3% a trial was installed to compare the effects of in-furrow micro-dams with a non-treated plot of 5 ridges over a length of 30 m. We created micro-dams with the French Cottard Barbutte equipment. Containers were installed for collecting runoff and the eroded sediment over the whole growing season. The water samples and the extracted sediment were analysed by LC-MS/MS for residues of the applied plant protection products.

After some storms with heavy rainfall in summer, we measured big differences in erosion and runoff quantities between the treated and the non-treated plot. With the micro-dams a runoff reduction of at least 50% was detected. The global runoff coefficient, used as an indicator for the runoff capacity of a soil in surface hydrology, is reduced at least by half over the whole period of measurement (12,1% non-treated vs 6,1% treated). The soil humidity increased with 10–15% for the overall profile to a depth of 80 cm due to a better rainfall infiltration in the soil in the parcel with micro-dams.

The total amount of exported sediment in the field with micro-dams was limited at 3 tons/ha compared with >9 tons in the non-treated area. This means a reduction of sediment loss by at least 66%.

Analysis of the water and the extracted sediment samples shows also interesting differences in the exported amount of PPP. In the trial plot without micro-dams the total quantity of exported active ingredients by erosion was >1,6% of the total amount of applied herbicides compared to >0,7% runoff. This clearly indicates that erosion can be a significant factor in surface water contamination besides the runoff effects.

The use of micro-dams gives a reduction of at least 97% for the exportation of active ingredient by erosion and at least 87% for runoff. Depending on the specific characteristics of the active ingredients, such as water solubility and soil adsorption coefficient, big differences in exported quantities between A.I.s were measured.

In general, the application of micro-dams in potatoes in erosion sensitive areas particularly has an effect on erosion and runoff. This is an important agricultural application technique to avoid loss of fertile soil and surface water contamination by nutrients and PPP. Even in regions with a slope starting at 1% it will be interesting to apply this technology. Current experiences in practice fields are very positive and well received by the farmers. This application is recently introduced as an element of the national action plans and mentioned in the IPM legislation in Belgium.

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# DURABLE CISGENIC RESISTANCE TO *PHYTOPHTHORA INFESTANS* IN POTATO AND PERSPECTIVES FOR APPLICATIONS IN EUROPE

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A promising strategy to combat potato late blight disease is to combine multiple resistance genes into one genotype to build a durable resistance. Resistance genes from wild relatives can be introduced by breeding or by transformation. Single resistance genes are not durable because mutant pathogens that avoid recognition will easily be selected.

Genetic engineering is a straightforward method to introduce a combination of different natural resistance genes into a potato cultivar. Since these genes can also be introduced via classical breeding, the resulting potato plants are called cisgenic. This is in contrast to the so-called transgenic potatoes that have received DNA from non-crossable species.

Three R genes (Rpi), Rpi-sto1 (Solanum stoloniferum), Rpi-vnt1.1 (S. venturii) and Rpi-blb3 (S. bulbocastanum) were cloned and transformed separately or as a combination into the susceptible cultivar Désirée. The transformed lines were screened for late blight resistance using a detached leaf assay, and they were also evaluated for true to type performance in the greenhouse. Selected lines were tested in field trials in The Netherlands and Belgium in 2011 and 2012 in comparison with the susceptible parent Désirée, and other susceptible and resistant cultivars. In both years the plots were not treated with fungicides against Phytophthora infestans. In contrast to the summer of 2011, the summer of 2012 was very humid resulting in a high natural disease pressure. Nevertheless the two seasons showed similar results with clear differences between the susceptible reference lines and the genetically modified resistant lines.

Currently, about twenty Rpi genes have been mapped or cloned and more will follow. Therefore a collection of double or triple resistant cultivars can be generated that have the potential to make potato cultivation much more sustainable. Although society may find cisgenic plants less controversial than transgenic plants, the actual implementation of cisgenic potato plants will largely depend on their handling by the European regulatory system.

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### DEVELOPMENT OF LATE BLIGHT RESISTANT POTATO BIOTECH VARIETIES FOR SUB-SAHARAN AFRICA

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Phytophthora infestans remains the most devastating potato pathogen worldwide. In developing countries it causes annual losses of up to 2.75 billion USD, excluding the cost of pesticides. Host plant resistance mediated by single R genes has been rapidly overcome by P. infestans on many occasions. However, durable resistance might be achieved through genetic transformation of a potato variety with different combinations of R genes from Solanum wild species. The RB, Rpi-blb2 (isolated from Solanum bulbocastanum) and the Rpi-vnt1.1 (isolated from S. venturii) genes are being transferred into the susceptible variety Desiree and other varieties grown in sub-Saharan Africa (SSA). Close to 600 transgenic events (TE) were produced from Desiree with either a single R gene (251) or the 3R gene stack (331). TE with high levels of resistance to P. infestans were identified by in-vitro and whole-plant assays. Fewer than 10% of the TE with either a single R gene or the 3R gene stack were found to be highly resistant. A hypersensitive reaction to specific isolates and extreme resistance was observed for 7 out of 64 RB-TE, 4 out of 117 Rpi-blb2-TE, 1 out of 25 Rpi-vnt1.1-TE, and 16 out of 162 3R-TE. Varieties from SSA have been identified as candidates for transformation, with priority given to three: Shangi (rapidly increasing adoption in Kenya), Tigoni (well established variety in Kenya), and Cruza 148 (grown in the Lake Kivu region, especially in Burundi and Rwanda). After greenhouse screening of all existing TE, the top 10 highly resistant 3R-TE will be evaluated in confined field trials. This will be performed during several cropping seasons to monitor resistance stability and dynamics of the pathogen population.

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# MARKER DEVELOPMENT AND CLONING OF RESISTANCE GENES AGAINST PHYTOPHTHORA INFESTANS IN POTATO CLONE SW93-1015

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Potato, the world's fourth largest crop, is despite more than a century of resistance breeding still severely hit by late blight, causing billion dollar losses annually [1]. The SW93-1015 potato clone has an efficient resistance against *P. infestans*, both under field conditions and in the laboratory [2].

We took a classic genetic approach in order to investigate the genetic basis for this resistance in SW93-1015 and combined that with RNA-seq analysis to generate a molecular understanding of the defense mechanism in this clone. We analyzed *P. infestans* resistance of 76 F1 potato progenies from two individual crosses of SW93-1015 with the susceptible potato clone Desiree. Approximately 50% of the clones from the two crossing populations were resistant against *P. infestans*. We tested two resistant SW93-1015 progenies for HR response to in planta expression of the effectors AVR3KI, AVRVNT1 and AVR2. We found HR reaction to AVR2 in both of these progenies indicating that they may contain an R2-like *Phytophthora* resistance protein sequence.

By RNA-seq analyses of 34 clones from the crossing population we made a DNA marker for the resistance locus. We cloned and sequenced ten different R2GHs from SW93-1015.

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#### BREEDING FOR LATE BLIGHT RESISTANCE IN BELGIUM

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Since 2005, a potato breeding program kicked-off at CRA-W. The objective of this program is to obtain durable resistance to late blight (*Phytophthora infestans*) and to release Belgian varieties.

Since then, 40 000 clones obtained from 120 crossings have been assessed, in joint venture with the National Institute of Research and Development for Potato and Sugar Beet (Brasov, Romania), the Industrial Crops Research Centre (Bologna, Italy), and a private partner, a seed producer and exporter. Following a classical breeding scheme, the assessment of the clones consists of observing the plants and their tuber formation for the first four years. Later, field trials are planted for the assessment of the agronomic and cultural value of the clones (yield, culinary and technological value, late blight resistance), on several locations for the more advanced clones, in Belgium and abroad. Besides, despite its modest size, the program produces its first results: 6 clones are now available for development by private partners, and a first submission to the Belgian catalogue has been introduced.

Finally, a research project started in 2013 and will lead to intensify the breeding program, by using wider genetic resources (wild species of *Solanum*), by marker assisted selection and cis-genesis.

#### A CISGENIC POTATO LATE BLIGHT RESISTANCE BREEDING STRATEGY

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Late blight, caused by the oomycete Phytophthora infestans, is the biggest threat to potato (Solanum tuberosum) production worldwide. Resistance (R) gene stacking is a defence strategy that is successfully applied by wild relatives of potato and it is the current challenge for breeders to introgress multiple new late blight R genes from related Solanum species into their cultivars. Introgression using classical breeding strategies requires multiple decades of backcrossing and often linkage drag is hard or impossible to remove. Especially for a rapidly changing disease as late blight, a higher speed of introgression is required. The cisgenesis principle that was recently postulated provides a natural framework to transfer genes between crossable species using biotechnology. This way, genes from the existing breeding pool can rapidly and accurately be transferred to crops. Several cloned late blight R genes are already available for such a cisgenic breeding strategy. Also the cloning of several novel late blight R genes from crossable Solanum species will be reported. The complementarity of these R genes was assessed using their recognition specificity and performance in field trials. Next, multiple late blight R genes were introduced into well-established cultivars using markerfree transformation. The PCR positive regenerants were tested for the appropriate expression of all introduced R genes. Successively, the cisqenic plants are validated for their late blight resistance in greenhouse and field conditions. It was found that our cisgenic varieties show excellent late blight resistance at multiple geographic locations. Finally, using multiple years of field trials we have gathered evidence that R gene stacking indeed contributes to durability, thereby providing a highly feasible solution to the late blight problem.

# Session 6 (OP) POST-HARVEST and STORAGE

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## IMPROVING EFFICIENCY OF POTATO STORE OPERATION IN GREAT BRITAIN

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This paper describes work commissioned by AHDB Potato Council, following a review by Kneeshaw (2006), to investigate how the efficiency of energy use is affected by a range of storage conditions.

Initial work (Cunnington et al, 2011) provided baseline data but recent assessments included air leakage; air movement efficiency; humidification; insulation; refrigeration efficiency and temperature uniformity. Uncontrolled air leakage in potato stores leads to higher energy use. Leaks occur around doors, louvres and construction joints. Stores were tested using Retrotec pressure systems [Stroma, Castleford, UK] and showed that air leakage is responsible for a large amount of energy use. This was c37% of the store's total energy consumption for those supplying fresh market and c55% in processing stores.

Refrigeration systems tested had COPs of 1.6-3.9, varying c. 250% from the best to worst. Some improvements that may be made include:

- Optimisation of condenser and thermostat settings.
- Better maintenance of refrigerant charge.
- Adoption of VSDs, sub-cooling and heat recovery.
- Upgrading of condensers to EC fan units.

Improving insulation reduces the need for heating or cooling as it cuts heat transmission through a structure. Simple improvements (eg by adding 50 mm of polyurethane spray foam to a store with 50 mm spray foam initially) resulted in savings c11%. Increasing composite panel thickness 50% to 120 mm gave a more modest 6% saving, while upgrading from 100 mm to 150 mm of EEP board resulted in a 7.6% saving.

Box and bulk stores have different ventilation and air movement needs, especially as most box stores in GB are not positively ventilated. In a bulk store, air volume and pressure drops affect energy use. In contrast, for an overhead-throw box store, air volume and discharge speed (determining the distance air travels) are key. For some box stores, while the volume of air delivered met guidelines, air speed/distribution was not satisfactory. In some cases, low velocity led to poor air mixing whereas in other stores air volume in parts of the store was too high. Overall, systems were good for managing early crop condition, but over-ventilated for much of the holding period. Use of VSD fans as an energy-saving measure would mitigate against any losses caused by excess air.

Temperature uniformity in box stores was also evaluated; it was inconsistent due to short circuiting and poor mixing. Using 'air divider' curtains was shown to reduce short circuits and increase airflow to the pallet slots but local variation was still evident.

Humidification provided extended hours of (adiabatic) ambient ventilation, but was unable to deliver its full theoretical potential. Despite this, the value of adiabatic cooling for closer control of temperature (especially after store loading in mild conditions) was able to be demonstrated.

This project has prompted a new 'StoreCheck' audit for potato stores, a service offered by SBCSR, in collaboration with Farm Energy.

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# IMPROVING THE USE OF SPEARMINT OIL, A NATURAL SPROUTS INHIBITOR: FROM HOTFOGGING TO VAPOUR APPLICATIONS

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### Introduction

Sprouting is a physiological natural evolution for potato tubers in storage which mainly requires the use of chemical sprouts inhibitors. In order to reduce the risk of residues in tubers, the development of new products integrates better profile than CIPC. As for traditional (Regulation (EC) n° 1107/2009) than for organic farming (Regulation (CE) n° 2092/91), spearmint oil is now registered in Europe and can be applied through the commercial product Biox M® in a few countries (BE, ES, FR, IT, NL, SE, UK). The study explores the ways for improving its application with classical hot-fogging technique but also through an innovative vaporization of essential oil.

### **Materials and Methods**

Experiments were carried over several years in the cells of the ARVALIS-Institut du vegetal Experimental Center of Villers-Saint-Christophe, in North of France. The trials were first oriented in the evaluation and optimization of the product applied by hot-fogging techniques. This was done in pilot cells with four to six replicates of different varieties and notations done through the duration of storage. Then the vapor efficiency was tested in a small scale design using 40 kg boxes of tubers covered with a thick veil of nonwoven fabric (Toptex®) and stored at 7.5°C. In each, were placed vapor generators of corrugated cardboard where regular amount of mint oil was deposited along the storage period. The vapor phase was secondly tested in the pilot cells using the new vaporization design Xedavap® where product is regularly in time supplied on vertical tissues placed in semi-permanent air stream. Efficiency was evaluated as previously described.

### Results

The good results obtained first with timely split hotfogging treatments using Electrofog® equipment contributed to the official registration of Biox M® product in France. The property of the product to induce a quick necrosis of sprouts present on the tubers opened the possibility to optimize the applied dose in refrigerated box store by adjusting the application at the sprout emergence. The measurement done on carvone concentration in the ambiance of the cells showed a very strong vaporization of the essential oil in correlation with the dose applied, growing till 50% saturation.

The experiments done in small boxes showed the good natural volatility of spearmint oil in comparison with eugenol, a natural compound extracted from clove oil (Eugenia caryophyllata), used as sprout suppressant in the USA. This easier vaporization promoted much better efficiency as sprout inhibitor for spearmint oil. In a larger scale, using the industrial "vaporizator" Xedavap® in pilot cells at 5°C, reproducing commercial refrigerated box store, a necrosis effect on meristems have been observed on sprouts when applying vapors of spearmint oil at high rate. The first observations recently done in this storage configuration with the same equipment but used in daily low dose diffusion show a possibility to obtain good sprout control during the whole season.

# **Conclusion and perspectives**

Spearmint oil appears like an effective natural sprout suppressant for which the technique of application (hot-fogging, vaporization) can induce better adaptation to the type of storage and its commercial management. The new Xedavap® equipment, working at ambient temperature, gives an opportunity to prevent also fire risk during the treatment.

### SPROUT CONTROL OF PROCESSING VARIETIES BY ETHYLENE

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CIPC is currently the the dominant storage sprout suppressant available for the GB processing industry but there are threats to its continued use and alternative sprout suppressants are urgently required. One potential alternative is ethylene which Prange *et al.* (1998) found reduced sprout length when continuously applied to potatoes during storage at 4 ppm. Ethylene is now used in GB as a residue free alternative to CIPC for cold stored, pre-packed potatoes. Although ethylene is a very effective suppressant for some cultivars under some storage conditions, not all commercial cultivars respond to ethylene sufficiently well for it to be commercially viable alternative and, in particular, ethylene can result in darker fry colours with commercial important consequences.

Overcoming these constraints to the uptake of alternatives to CIPC by the processing industry are important objectives for the Potato Council and our recent research is described in this presentation.

The undesired effect on fry colours can be mitigated, at least partially, by pre-treatment with 1-methylcyclopropene (1-MCP, Prange *et al.* 2005). This compound competitively inhibits ethylene receptors and prevents signal transduction of an ethylene response. Trials including 1-MCP treatment and its effects are described.

An empirical study of how current and emerging GB processing varieties respond to ethylene, with or without other sprout suppressants, is providing information on potential future sprout suppression strategies for these varieties.

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THE USE OF BIOX CM12, A MIX OF SPEARMINT (MENTA SPICATA) AND CLOVER (SYZYGIUM AROMATICUM) ESSENTIAL OILS, TO CONTROL POTATO SPROUTING OF SEED POTATOES STORED AT 8°C.

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#### Introduction

Some essential oils extracted from plants are proved effective as potato sprouts suppressant through the action of their volatile aromatic compounds [1]. It is the case for the mint oil extracted from spearmint (a.i, R-Carvone) and clover oil (a.i, Eugenol). The mode of action of these ingredients is to physically damage the developing sprouts by burning their apex when treated [2]. None of these active substances are authorized to be used on potato seeds because the risk they put on the sprouts development.

Nevertheless, we tested the use of BIOX CM12, a potato sprout suppressant from XEDA international containing 70% of spearmint and 30% of clove essential oils, in a seed potato storage system where the storage temperature was elevated at 8°C and we compared it with the classical system of storage at 3°C without treatment. We were particularly attentive to check the possible negative effects of the system on the capacity of the treated tubers to give normal productions in field.

### Material and methods.

Two trials were conducted respectively in 2012 and 2013. In 2012, BIOX CM12 was applied one time per week using a thermal fogging technique. In 2013, the mode of application was changed due to the observation of phytotoxicity on some of the cultivars taking part in the 2012 experiment. BIOX CM12 was applied daily using a forced cold vaporization technique. In 2012, 13 varieties were included in the trial and in 2013, only 8 among which those having showed toxicity problems in 2012. Field trials were conducted in a split-plot design in 4 replications, with experimental units of two lines of 5 tubers. The observations made were: (i) sprouts development after the storage period, (ii) field emergence, (iii) stems number, (iv) yield, (v) tubers number per plant, (vi) dry matter.

# **Results**

Results have shown that the use of BIOX CM12 can effectively be phytotoxic for the tuber ability to germinate in a good way after treatment and, consequently, affects the field behavior, if applied with the thermofumigation technique (weekly doses of 10 to 70 ml per stored ton). This phytotoxicity is however variety dependant. It concerns essentially early varieties (Ukama, Première, Anosta, Monalisa) while midlate and late varieties are not impacted (Bintje, Spunta, Cara, Kennebec, Nicola, Marfona, Tebina and Désirée).

The use of a forced cold vaporization technique in the 2013 trial (daily doses of 1.5 ml per stored ton) solved all the phytotoxicity problems leading to a normal behavior in field without any significant differences in the measured parameters when compared to the untreated seeds stored at 3°C. The level of sprouting control during the storage period was a little more less in the cell at 8°C, but quite very acceptable.

### **Conclusions**

The choice of a soft technique for BIOX CM12 application, i.e forced cold vaporization of daily low doses of the product, gave a good control of sprouting when seed tubers were stored at 8°C and proved also that it did not impact the ability of each of the tested varieties to sprout correctly after storage and give correct yields.

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### HARNESSING POTATO RESPIRATION TO DRIVE THE STORE VENTILATION SYSTEM

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Potato storage in the Central Highlands of Afghanistan epitomize that in many high-elevation, temperate, less-developed countries. Tubers are placed in large pits and directly covered with earth to protect them from frost. Overnight temperatures frequently fall to -20°C to -30°C at night during January and February, and rise to just above 0°C on most days. Significant losses occur within four months of harvest (October to January) and average 40% in Spring, when total losses are frequent. Seed derived from the remaining tubers is of poor quality.

A simple, low-cost modification, costing <\$10, to the traditional pit is described. A vertical pipe (inlet vent) was connected to a horizontal vent the length of the pit floor. Heat generated by respiration in the potato stack was trapped above the stack. When the trapped air was rapidly exhausted, cool, outside ambient air was drawn-in through the inlet vent and throughout the stack. Temperature and humidity within the store were monitored by exposing a shiny metallic surface to the exhausting air; signs of condensation indicating that excess heat and moisture were present.

Improved storage concepts were discussed with household members in six villages each in two districts (Chaghcharan and Lal) of Ghor Province. Male and female members from each household were included in all activities, since each has specific roles in potato cultivation and storage. Modified demonstration stores were constructed in each village and 954 households stored a weighed sample of their crop over two seasons (Season 1, Oct 2011 – May 2012 and Season 2, Oct 2012 – May 2013). Farmers were also encouraged to improve their harvest and post-harvest sorting and handling practices.

The safe storage period was extended from four months to at least seven months. In Season 1, storage losses by weight after seven months were reduced from 28% (range 18% - 39%) to 5% (3% - 13%). In Season 2, following further store modifications, losses were reduced from 32% to 3% (1% - 6%). Crop yields in comparative yield trials, farmers' saved seed vs improved-store saved seed, showed average yield increases in Season 1 of 47% (P=<0.05; n=26) in Chaghcharan and 15% in Lal (NS; n=46), where conditions are more favourable for potato cultivation and production levels are higher. Yields were not measured in Season 2 due to a severe dry spell, although rate and % emergence were notably higher with seed from the improved stores.

In addition to the direct outcome of reduced losses, the ability of farmers to store the crop throughout the winter relieved chronic spring-time food insecurity in the region and allowed farmers to profit from higher spring potato prices (US38¢ in October 2011 to US70¢ in April 2012) with increased flexibility in the timing of marketing.

A survey was conducted in May 2013 to ascertain household opinions of the improved practices. Among both men and women (n=267), 93% responded that flexibility in storing, consuming and marketing potatoes as their reason for adoption of the practices; 70% cited reduced seed storage losses and 56% cited that stores were simple to operate. Uses of the extra production or income were for home consumption, 99% (n=153); sale for purchase of wheat or other foodstuffs, 71%; purchase of consumables, particularly clothing, 69%; and school fees, 36%.

Uptake appears rapid, including in neighbouring communities. An uptake survey will be conducted when the October 2014 harvest is in store.

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## CRISP PROCESSING INCLUDING LEACHING STRATEGIES - HEALTHY AND QUALITY ASPECTS

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Potato crisp producers offer a well accepted product with long shelf-life. Therefore, this potato product is produced and distributed worldwide, but food nutritionists often criticize the relative high fat content (up to 40 %), and (since few years) toxicologists point out the acrylamide (aa) level, which may exceed 1000  $\mu$ g kg<sup>-1</sup>.

A frying experiment with two potato varieties, stored for 6 months either at +4 or +8 °C, was conducted to explore healthy and quality aspects, both. Specifically, a leaching step in front of deep-fat frying of thinly sliced potatoes was tested with view to fat content and acrylamide reduction in the final product. After mechanically peeling tubers were sliced with a commercial slicer. Leaching was in a continuously running blancher (9.2 min, temperature from room temperature up to 80 °C). Slices were fried in a restaurant like fryer with 30 I oil bath (peanut oil). Fat content was determined after Soxhlet extraction, reducing sugars were measured with an enzymatic test kit (Megazyme). Acrylamide was determined by GC-MS. Pectin and esterification degree were calculated after cuprizon-reaction of previously isolated cell wall material. Leaching in front of frying significantly lowered the concentration of reducing sugar in relation to water temperature. Parallel to that, acrylamide concentration had a downward trend. Despite of a very high acrylamide level of the control sample, high temperature leaching resulted in aa-concentrations below the official guidance value of the European Union (1000  $\mu$ g kg<sup>-1</sup>). Correlation coefficient of both parameters was r = 0.92 and r = 0.77, respectively.

During frying integrity of the potato cells determines velocity of water loss and fat uptake. One of the most relevant compounds is polygalacturonic acid (cell wall based) and its degradation by an enzymatic reaction of pectin methyl esterase (PME). Thereby, PME reacts as a key enzyme, which opens gates for other enzymatic reactions, e.g. polygalacturonase (PG). In the experiment increasing leaching temperatures up to 60 °C rose PME-activity. Above that temperature a sharp decrease was detected.

Concurrently, PME de-esterified pectin and cell wall stability dropped down with a loss of textural force. Total esterification was stable until gelatinization temperature. After that, a remarkable decrease occurred independent to variety and storage condition. The cell wall loosened up and fat moved inside resulting in an increased fat content. Both varieties independent to storage regime pointed out a linear increase of fat (from 33 to 42 and from 36 to 43 % dry matter, respectively).

The processing contaminant acrylamide could be reduced significantly by an additional leaching step in front of frying. At the same time, fat content increased indicating a principal conflict between health and quality related aspects. A low to medium temperature leaching step could be the compromise. In addition, a defatting procedure could be implemented to improve food quality.

### **ENHANCING CAROTENOID LEVELS IN POTATO TUBERS**

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### Introduction

The carotenoid content of potato tubers is a key nutritional and quality trait. In potato there are wide variations in tuber carotenoid accumulation levels. White-fleshed tubers contain low levels of carotenoid however considerably higher levels accumulate in yellow/orange fleshed tuber varieties [1]. Transgenic studies have demonstrated that tubers have the capacity to accumulate nutritionally significant levels of carotenoids for example, [2]. We have used a range of techniques to discover the genes that underpin natural variation in potato tuber carotenoid content. Two major Quantitative Trait Loci affecting overall tuber carotenoid content were identified and transcriptomic approaches have identified additional candidate genes. To address the nature of the organelle in which carotenoids accumulate, localisation of fluorescently tagged carotenoid enzymes has been determined using confocal microscopy.

## **Materials and Methods**

Transgenic lines were generated in which the levels of enzymes of carotenoid biosynthesis and turnover have been manipulated. Potato populations that segregate for tuber carotenoid content have been developed, and QTL analysis has been employed to determine the genetic architecture of the trait. Transcriptomic analysis using a potato microarray has been used to determine patterns of gene expression associated with the accumulation of different carotenoids. Confocal microscopy of RFP-tagged carotenoid biosynthetic enzymes has been employed to gain insights into the sub-cellular site of carotenoid biosynthesis.

## **Results**

Gene stacking approaches, using a combination of transgenes and appropriate parental genotypes have been employed to engineer significant levels of the high value carotenoid astaxanthin. Two major QTL, affecting tuber carotenoid content were identified on chromosomes 3 and 9. Whereas a known biosynthetic gene was shown to underpin the QTL on chromosome 3 (crtR-b2), no known biosynthetic gene maps to the chromosome 9 QTL. A genetical genomics approach was used to identify candidate genes for this QTL. Functional analysis of carotenoid cleavage dioxygenases (CCD4 and CCD8) demonstrated the impacts of these enzymes on tuber carotenoid content and also in tuber development. Transgenic lines in which carotenoid synthesis related enzymes have been tagged with RFP have been developed. The localisation of the carotenoid biosynthetic enzymes was revealed by this analysis, with different locations for phytoene synthase and  $\beta$ -carotene hydroxylase.

# **Conclusions and Perspectives**

Using transgenic approaches it is possible to engineer potato tuber carotenoid content to provide nutritionally significant levels of carotenoids including b-carotene, lutein and astaxanthin. Carotenoid turnover is a significant factor in the levels of tuber carotenoids and derived metabolites have a major influence on the tuber life-cycle. The genetic architecture of the tuber carotenoid trait is complex and key regulators remain to be determined.

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# EXPLORING BIODIVERSITY TO INTRODUCE NUTRITIONAL QUALITY CRITERIA IN POTATO BREEDING PROGRAM

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The potato cultivars currently grown in Colombia have been selected seeking higher yield, resistance to late blight and other diseases, adapted to local conditions, with color and shape attending market demands and suitable for processing. However, in spite of severe nutritional problems of Colombian vulnerable population, nutritional quality criterion has been neglected. Therefore, we are focusing our research to generate new potato cultivars taking into account: i) nutritional and functional food contents, ii) disease resistance, iii) acceptance by producers and consumers, iv) Colombian potato biodiversity. Our objective was to characterize biodiversity regarding nutritional quality in potato tubers. The plant material employed was Solanum tuberosum group Phureja belonging to Colombian Core Collection (CCC), 95 individuals; commercial phureja genotypes (VC), six; breeding advanced clones (CA), collected potato local varieties (LV) and accessions from the German Germplasm Bank (IPK). These materials were harvested and tubers were washed, cooked and lyophilized. Macronutrient content was determined: Moisture values were found in a range of 74.1% to 82.1%; fat content range was between 0.011% and 0.104%; protein 0.711% to 2.843 % and ash of 0.569% to 1.53%. Total carbohydrates varied from 8.295% to 33.32%; total dietary fiber between 2.533% and 6.39%. Contents of iron and zinc were evaluated by the method of induction coupled plasma emission spectrometry in 109 clones of potato with skin. The iron content in CA varied from 18.0 to 26.0 ppm, in CCC from 11.0 to 49.0 ppm and in VC between 15.0 and 24.0 ppm. Zinc content for CA ranged from 12.0 to 17.0 ppm; for CCC 9.8 to 47.0 ppm and for VC from 10.0 to 19.0 ppm. Phenolic compounds were quantified by the external standard method UHPL coupled to a diode array detector. Quantity of hidroxycinnamic acids (non-ACN-HCA-LC) was expressed as mg/100 g potato dry weight (DW). Non-ACN-HCA-LC content was calculated from the total area of chromatographic profiles at 320 nm and expressed in relative units. Hydroxycinnamic acid-like compounds (HCA-LC) were the main phenolic compounds in quantity and diversity present in potato tubers. Chlorogenic acid (ChA), their isomers neo-ChA and crypto-ChA and caffeic acid (CaA) were the main contributors in quantity to the non-ACN-HCA-LC. ChA ranged from 79.2±29.0 to 440.2±111.5 mg/100 g DW and they were by far the most abundant non-ACN-HCA-LC, followed by crypto-ChA, 7.3±8.2 to 101.6±14.3 mg/100 g DW. CaA, 0.9±1.1 to 10.4±2.2 mg/100 g DW and neo-ChA, 3.4x±1.2 to 30.1±7.8 mg/100 g DW were less abundant in all genotypes. The high coefficient of variation (CV) found for ChA when the biological replicates were analysed ranged from 4 to 75% contrasts with the good reproducibility of the chromatographic method where CV was lower than 4% within and between days. Accurate phenotyping characterization allowed us to find significant diversity in micro and macro nutrients in the genotypes evaluated, also phenolic compounds are abundant and present a wide spectrum of variation. These results give the basis to incorporate in the potato breeding program criteria on nutritional quality and they will be analyzed regarding the nutritional status of the target population.

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# GENOTYPIC AND ENVIRONMENTAL VARIATION IN POTATO FLOUR RHEOLOGICAL PROPERTIES AND THEIR ASSOCIATION WITH DIETARY CARBOHYDRATE PROFILES

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Potato is one of the most common starchy foods, and its popularity has been increasing in some parts of the world. In western countries, recent trends for "health and wellness" products has driven interest in exploring avenues developing high quality potatoes with enhanced nutritional qualities to meet consumer demands. We have previously demonstrated genotypic variation and the influence of environment in carbohydrate profiles/composition in relation to dietary fibre and starch composition in advanced breeding clones [1, 2]. In this study we investigated the variation in rheological properties in genotypes previously characterized for dietary carbohydrate profiles. The pasting characteristic of potato flour was determined using a CVO Rheometer (Bohlin Instruments). Rheological properties such as peak viscosity; breakdown, final viscosity, and setback were analyzed. The results indicate genetic and environmental effects in advanced breeding clones. Some associations were noted between flour pasting characteristics and dietary carbohydrates profiles. Setback appeared to be correlated with resistant starch content suggesting that it could be used to select lines with improved nutritional profile. Improvement in carbohydrate characteristics of interest also requires rapid screening methods to practise selection over a number of vegetative generations for clones with desirable traits. The application of near infrared spectroscopy (NIRS) offers tremendous potential in that regard.

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# SUPPORTING IRON AND ZINC POTATO BIOFORTIFICATION THROUGH XRF FAST SCREENING TECHNOLOGY

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### Introduction

Micronutrient malnutrition diminishes the health and productivity of over half of the world's population, impacting primarily on the well-being of women, infants and children. Biofortification is the process of breeding new varieties of staple food crops with increased mineral and vitamin concentration [1]. Potato is the world's third most important food crop and its production is expected to double in the next several years. Already an important source of energy, vitamins, minerals, and protein of high biological value, its significant, heritable variation for micronutrient concentration, low concentration of phytates, and high vitamin C make it a promising crop for biofortification [2]. While population improvement is under way at the International Potato Center (CIP) to develop biofortified varieties as components of the diet in targeted African and Asian countries, a concept of improved variety mixtures with complementary nutritional characteristics is under study in the Andes where diversity of landrace potatoes is high. X-ray fluorescence spectrometry (XRF) technology can offer research breeding programs potential for estimating iron (Fe) and zinc (Zn) concentration of thousands of genotypes within relatively short time-frames (e.g., for selection purposes between harvest and planting the next season) and at low cost [3].

### **Materials and Methods**

For calibration development and validation procedure, a collection of in total 113 potato samples with large variation for Fe and Zn concentration determined by the reference method inductively-coupled plasma-optical emission spectroscopy (ICP-OES) was obtained from six locations in Peru: Huancayo, La Victoria, La Molina, Huanta, Pataz and Arequipa. For calibration development 68 samples were used and the remaining 45 samples not included in the calibration data set were used for validation of developed calibration model. All samples were peeled, freeze dried and milled before the analysis.

### Results

The 68 calibration samples ranged from 8 to 36 mg/kg for Fe and 5 to 35 mg/kg for Zn. The standard errors of the calibration developed (SEC) were 2.68 mg/kg for Fe and 1.90 mg/kg for Zn with R<sup>2</sup> of 0.87 and 0.93, respectively (Table 1). In validation, the standard errors of prediction (SEP) were 2.03 mg/kg for Fe and 1.47 mg/kg for Zn with R<sup>2</sup> of 0.86 and 0.95, respectively (Table 1).

# **Conclusion and Perspectives**

XRF calibrations developed to estimate Fe and Zn allow accurate, fast and cost-effective analysis of Fe and Zn in potato samples. They provide a new, more rapid and economical way to estimate mineral concentrations for timely decision-making in the course of breeding potatoes for increased micronutrient density.

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**Table 1:** Variation of concentrations and calibration and validation statistics for XRF analysis for Fe and Zn concentrations in freeze dried and milled Potato samples

Trait		Rangel	CP (mg/kg)	XRF-Calibration			Rangel	CP (mg/kg)	XRF-Validation	
	N	min	max	R <sup>2</sup>	SEC	N	min	max	R <sup>2</sup>	SEP
Iron	68	8	36	0.87	2.68	45	8	30	0.86	2.03
Zinc	68	5	35	0.93	1.90	45	7	33	0.95	1.47

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# MARKETING OF FRESH POTATO AND MANAGERIAL ASPECTS IN THE SUPPLY CHAIN ON MEETING THE CHALLENGES FROM A CHANGING DEMAND

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### Introduction

Over the last decades, fresh potato consumption has dropped significantly in most European countries, as well as in North America. This paper works on the example of the Swedish market where, traditionally, the fresh potato has been a main carbohydrate source, but increasingly replaced by substitutes. Swedish fresh potato consumption has declined from nearly 90 kg per person and year in 1960 to 43 kg per person in 2010, while pasta, rice, bread and prepared potato products, have increased [1]. Suggestions made to elucidate this change include explanations related to health and nutrition aspects, increased demand for convenience and fast food, and a changing work force structure.

This paper aims at explaining the reasons behind this changing consumption, and the managerial implications in the supply chain on responding to it. As producers and other actors in the supply chain are under pressure, the need for aligning with consumer wants and demands increases. This calls for careful strategic positioning on the market.

### **Materials and Methods**

Studies performed at the Swedish University of Agricultural Sciences over the last ten years have focused on understanding consumer behaviour related to potato consumption. These include a focus group study with six groups in different life-stages, and a consumer survey of 300 respondents. In addition, semi-structured interviews were made with eleven key actors in the Swedish supply chain for fresh table potatoes, with focus on managerial perspectives on responding to the described market changes.

### Results

The focus group study revealed seven factors decisive to consumer choice of potatoes. These were factors related to convenience and preparation; health; information and packaging; sensory appeal; price; habit and familiarity; and factors related to sustainability and ethics. The findings indicate that convenience appears to be the most important factor in consumer choice, whereas health related issues seem to be subordinate. The consumer survey showed that attributes related to sensory properties, origin and variety in supply may be important marketing arguments.

The interviews with managers in the supply chain showed that the industry is aware of a need for differentiation. However, the industry's perceived obstacles were not always in line with actual consumer statements. For example, some managers pronounced health as an explanatory factor, while consumers considered it to be of less importance. Thus, it appears that there is a gap between managers' view on consumer demand and consumers' statements.

## **Conclusion and Perspectives**

Decreasing demand for fresh potato can be described by consumer wants, which are not met with the present supply of products on the market. The supply chain needs to adjust to new market conditions to gain competitiveness in relation to the alternatives. In the perspective of strategic positioning, differentiation, and focus on convenience, could be one way to compete, as well as highlighting some unique features of the fresh table potato, such as sensory appeal and variation. Theoretical and practical implications are discussed.

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### CONSTRAINTS TO COMMERCIALIZING POTATO IN KENYA

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Although potato is an important source of food and income in Kenya, poor yields and erratic returns have constrained profitability and expansion of the crop. The objective of this study was to describe the potato production system, identify constraints, and determine levels of use of inputs; and the profitability of potato as a business in 4 out of the 10 most important potato producing Counties. We used multistage sampling to select 390 farmers from 4 of the 10 most important potato producing Counties. A questionnaire was used to collect information from farmers and means were separated using ANOVA. Results showed that farming was the main source of income for both husband and wife in at least 66% of households and these where fairly young families with the household heads aged 45 years; having 15years of experience in potato production, and having had 13 years of schooling-with significant differences across regions. Potatoes were grown principally by small-holder farmers twice a year on about 0.92 acres in the March/May season and 0.87 acres in the September/October season with significant differences across regions. There was uniformity in variety choice with Changi being predominant in three out of the four Counties. Productivity was at a sub-optimal level with yields of 5.6tons/ha compared to an African average of 10.8tons/ha (CIP 2007) - with significant regional differences. The low productivity can be partially explained by the low input use (seed and fertilizer) which was well below what was recommended by the Government extension officers. The quality of the seed used was of doubtful quality as it was not certified seed but sourced mainly from own harvest, from neighbors or from nearby primary markets. Despite all these shortcomings potato production gave farmers a gross income of about \$1240 in the March/May season and \$1173 in the Oct/Sept and this was above the UN poverty level of \$1.5 per day (or approx. \$270 per season of 6 months) -with that income differing significantly across regions.

Potato thus has the potential to significantly impact the food and income situations of small-holder farmers in Kenya if the identified constraints can be addressed in socially and economically acceptable ways.

# FARMERS' CULTURAL AND AGRONOMIC PRACTICES USED IN SMALL SCALE POTATO (SOLANUM TUBEROSUM L) PRODUCTION SYSTEM IN KENYA

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Potato is a very important crop in Kenya. It is both a staple food and a cash crop for many rural families and ranks as the second most important food crop after maize. Potato is grown by over 800,000 households [1]. However, the potato industry in Kenya has faced a number of challenges that include, Low vields, high pest and disease incidences, lack of adequate suitable varieties, improper agronomic practices, ineffective and inappropriate disease control measures[2]. A field survey was conducted in four major potato growing areas in Kenya (Kiambu, Nyandarua, Meru and Molo Counties) during January and February, 2008 (short rains crop) and June 2008 (long rains crop) to determine the occurrence of predators, parasitoids and pathogens of the aphids Myzus persicae Sulzer and Aphis gossypii Glover in potato crops. In each of the four areas, 30 potato farms distributed in different parts of the survey area were selected at random for the surveys. At each farm, the survey started with the collection of basic data about the farm using a survey questionnaire. The data collected and recorded included physical and geographical location of the farm, GPS coordinates and data on cultural and agronomic practices on the farm e.g. potato variety grown, pesticide use, use of irrigation on the farm and other crops grown on the farm. This was used to correlate the occurrence or lack of occurrence of some insect species with the practices on the farm or the geographical positioning of the farms. This paper presents results of the responses by farmers on the different cultural and agronomic practices used on the small scale potato farms in Kenya. Over 90% of farmers do not use irrigation and depend on natural rainfall. Majority (over 80 %) spray their crop against late blight disease but very few (less than 40%) spray to control insect pests. Over 75% use fertilizers at less than the recommended rates or only use manure or none at all. Very few (less than 50 %) practice dehaulming as recommended before harvesting their potato crop. The results show that most farmers do not practice potato cultural and agronomic practices as recommended. This could contribute to the low yields experienced at small scale potato farms (less than 10MT/ha) as compared to the potential yields of over 40MT/ha obtained at the potato research stations [3]. It is therefore recommended to train farmers on the importance of following the recommended cultural and agronomic practices in order to realize better yields at their farms.

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### **BRINGING INNATE POTATOES TO MARKET**

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Innate™ technology is a patented plant biotechnology process that works with a plant's own genes to enhance desirable traits and to decrease less desirable traits. Simplot's first application of Innate™ Technology includes the following traits: 1) reduced black spot bruising through PPO silencing; 2) reduced free asparagine levels. The first petition submitted to the USDA for regulatory review includes these traits in the following varieties: Russet Burbank, Ranger Russet, and Atlantic. Larger trials with these products were grown under commercial growing conditions in 2012 and 2013. Regulatory field trials with the Snowden variety, improved with these same traits, began in 2012 with a petition submission in late 2013. Future traits include Late Blight resistance, Invertase silencing, and more. In total, Innate™ technology encompasses more than a dozen patents granted and others currently pending in the areas of: precise breeding, gene silencing, promoter-based gene silencing and transformation methods. Commercial sales of Innate™ potatoes are expected to begin with the 2014 fall crop. Consumer research reveals favorable attitudes toward Innate™ potatoes.

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# PHYTOPHTHORA MANAGEMENT IN POTATO CULTIVATION - AN EXAMPLE OF A COMPLEX SOCIAL-ECOLOGICAL SYSTEM

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Phytophthora infestans (late blight) is one of the most important diseases in potato (Solanum tuberosum) production and is responsible for major losses in yield. Because of its short life cycle and spore dispersal by wind, a late blight epidemic can spread over large regions. Furthermore it can reproduce sexually which increases its virulence. We present a research project that will focus on the Netherlands, which has a very high potato density and combined with climatic conditions favourable for late blight, frequently experiences high pressure of the disease. A major element in prevention of plant disease is the use of resistant cultivars. Recent research insights in the spatial epidemiology of Phytophthora show that advances in reduction of the disease pressure can be achieved by spatial arrangement of resistant cultivars at field to regional scales [1]. Resistant cultivars are developed by private companies that aim for rapid widespread market penetration. Unfortunately, when resistant cultivars came to be more widely used, the selection of Phytophthora strains with compatible virulence genes increased and as a result resistance of several genes has been broken. Additional resistance management measures are needed to protect resistance genes in new varieties, which are being produced at great public and private cost. In this project the potato-Phytophthora host-pathogen system is analysed as a model system to study management and governance of crop-disease interactions. Data was collected by in-depth interviews with farmers, breeders and experts to identify current Phytophthora management strategies and the factors involved in decision making. This was combined with information derived from literature on farmers' decision making and Phytophthora epidemiology. It was found that the use of resistant cultivars in Phytophthora management is strongly related to the application of fungicides and the removal of infection sources. Both have a large effect on Phytophthora epidemiology and the overall infection pressure on resistant cultivars. Also the availability of fungicides strongly reduces the need to use resistant cultivars in crop protection. Furthermore, farmers, traders as well as breeders seem reluctant to embark on spatial strategies for fear of transaction costs and reduced autonomy in cultivar choice in a context where potato traders and packers are dominating cultivar requirements to a large extent. Since the use and effectiveness of resistant cultivars in *Phytophthora* management depends on interactions between people through social networks, as well as between plants and pathogens through disease epidemiology, it represents a social-ecological system. Such systems are driven by social and biophysical processes that interrelate and interact across multiple temporal and spatial scales and are characterized by nonlinear dynamics, self-organisation and feedback mechanisms [2]. Agent-based models have been recognized as highly suitable to represent the behaviour of individuals and groups within an environment in which biophysical processes occur and would therefore be a good tool to analyse the system dynamics.

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# DIAGNOSTIC DNA MARKERS FOR COMPLEX TUBER QUALITY TRAITS: IDENTIFICATION, VALIDATION AND USE IN BREEDING PROGRAMS.

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Tuber yield, starch content, starch yield, chip color and susceptibility to bruising are complex traits that are important for industrial uses and food processing. Complex traits are controlled by multiple genetic and environmental factors. Efficient selection of cultivars with superior tuber quality is therefore hampered by the fact that phenotypic selection requires multiple year and location trials. Application of DNA-based markers early in the breeding cycle, which are diagnostic for superior or inferior trait alleles, will increase the precision of selecting parents and progeny, thereby reducing the number of cultivars to be evaluated in field trials (precision breeding) [1]. Association mapping in populations of varieties and breeding clones, using as markers DNA variants in genes functional in starch-sugar interconversion identified, among others, alleles of invertases, starch phosphorylases and ADP-glucose pyrophosphorylase (large subunit) that are positively or negatively associated with tuber quality traits. Based on comparative proteomics, novel candidate genes were identified such as a leucine aminopeptidase (Lap), lipase III and protease inhibitors [2, 3]. Single nucleotide polymorphisms (SNPs) in Lap showed strong association with processing quality [2]. Several marker-trait associations were validated in a panel of 40 varieties that were selected for having superior or inferior processing quality and were evaluated for reducing sugar content during cold storage. As another form of validation we performed marker-assisted selection (MAS) in tetraploid breeding populations, using various combinations of allele specific markers derived from candidate genes that were associated with tuber quality traits [4]. To facilitate MAS, user friendly PCR assays were developed for specific candidate gene alleles based on diagnostic SNPs. In a multi-parental population of advanced breeding clones, genotypes selected for combining five positive marker alleles performed on average better than genotypes selected for combining the corresponding negative alleles. When tested individually, seven of eight markers showed an effect on at least one quality trait. The direction of effect was as expected. Combinations of two to three marker alleles were identified that significantly improved average chip quality after cold storage and tuber starch content [4]. In the combination of candidate gene association mapping and comparative proteomics successfully identified a first generation of diagnostic DNA markers for tuber quality traits, which can further explored breeding programs.

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# COMBINING PLANT GENETIC RESOURCES AND MOLECULAR MARKER TECHNIQUES TO SELECT POTATOES WITH OPTIMALLY UTILIZABLE BIOMASS

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### Introduction

The IPK Potato collections at Gross Luesewitz (GLKS) of the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) maintain genetic resources of potato in form of tubers, in vitro and seed material from several decades of potato breeding. Also, approximately 1,300 genotypes are cryopreserved under liquid nitrogen in the main institute at Gatersleben. Currently, about 6,100 different potato accessions are maintained at GLKS, which are divided into three groups; cultivated potatoes (KKS, about 2,700 genotypes), Southern and Central American material (AKS, 550 genotypes) and wild species (WKS, 2,850 populations of in total 140 botanical species) .

In a project funded by BMEL (Federal Ministry of Food and Agriculture)/ FNR (Fachagentur Nachwachsende Rohstoffe e.V.) the potential of biomass production from potatoes is investigated by combining the trait high yield with high starch content, long tuber storability and high level of late blight (*Phytophthora infestans* (Mont.) de Bary) resistance. After analysis of the population structure on genebank material and recent high starch cultivars using microsatellite markers, IPK focuses on associations between dry matter yield, storage stability and molecular markers.

### **Material & Methods**

In greenhouse and field experiments 27 high starch cultivars, 32 accessions of *S. pinnatisectum*, a wild *Solanum* species (known for its high tuber starch content), 21 accessions of *S. stenotomum* (known for its tuber dormancy) as well as 128 genotypes from selfing progenies obtained from high starch cultivars were cultivated, together with appr. 700 old cultivars and landraces from the GLKS. The storage stability, starch content, using a non-invasive balance system, and tuber yield were determined. Due to the small tuber size of the wild Solanum species a non-invasive technique to measure the starch content of microsamples had to be established in cooperation with JKI Gross Luesewitz. The storage stability was determined after five to eight month storage at 4°C and a relative humidity of 80% evaluating tuber dormancy by using a scale from 1 (low dormancy) to 9 (high dormancy).

#### Results

Results obtained after the first two years indicate a high potential of starch content especially in genotypes of *S. pinnatisectum* with up to 36.4%. Starch content in selfing progenies constitutes up to 30% and in cultivars nearly 28 %.

Tuber dormancy was highest in S. pinnatisectum with 90% of the genotypes still dormant after seven month of storage. In selfing progenies 17% of the genotypes were dormant after five months of storage, but only 8% in *S. stenotomum*. In the cultivated GLKS potatoes, dormancy was 5% after eight months of storage.

# **Conclusion and Perspectives**

Results reveal a high variation within the analyzed material regarding starch content and dormancy. Detecting and utilizing associations of these and other phenotypic traits to molecular markers will be employed for a better genotyping of IPK's potato resources. The respective data will be made publicly available via IPK's genebank information system, GBIS, and facilitate the access of breeders and researchers to genebank material.

# EFFICIENCY OF GENOMIC SELECTION FOR YIELD, MERCEOLOGICAL, AND NUTRITIONAL QUALITY TRAITS IN HUNDRED THIRTY-NINE CULTIVATED POTATO GENOTYPES

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Potato (Solanum tuberosum L.) is the third most important food crop and the most widely grown noncereal crop worldwide. In order to meet the increasing food needs, it is critical to increase genetic gain across cultivated plant species. DNA technology heralded molecular marker-aided breeding and boosted productivity in several crops, but applications in cultivated potato proved more difficult mainly due to complex genetics of this crop characterized by high allelic variation caused by the autotetraploid (2n = 4x)= 48) nature and its tetrasomic inheritance [1]. The high level of heterozygosity and the sensitivity to inbreeding depression also stymied classical potato improvement. Genomic selection (GS) demonstrated superior results in several crops [2], and can become the most cost-effective breeding tool in potato. GS differs from approaches heretofore implemented as it combines historical crop performance and whole genome molecular marker systems to predict genetic merit upon which the success for a potential new cultivar is assessed before it is field tested. This is expected to shorten the breeding cycle and time to cultivar development, reduce the cost of current breeding programs, and help avoid committing resources on plant materials that won't meet the breeding and market standards. This work aimed at evaluating the potential of GS in predicting total yield and its components, and merceological and nutritional traits in the VCU potato trial conducted in 2013 at CRA-CIN, in Italy. Hundred thirty-nine cultivated potato genotypes from several EU private breeding companies and CRA-CIN breeding program were scored for 11 traits, and whole genome genotyped for 45000 diversity array technology (DArT) markers. Three GS models (Table 1), Bayesian LASSO (BL), genomic best linear unbiased prediction (G-BLUP), and reproducing kernel Hilbert space (RKHS), were evaluated using DArT marker information and a 10-folds crossvalidated accuracy (r) of genomic estimated breeding values. Genetic diversity was assessed with genomic inbreeding and genomic kinship coefficients. The working population displayed no relevant genetic sub-structures and was made up of genetically unrelated genotypes with low levels of inbreeding. The mean and third quartile values were -0.007 and 0.011, and -0.007 and 0.018, respectively, for genomic kinship and genomic inbreeding coefficients. Broad-sense heritability was high (H2>= 0.90) for all traits. BL and G-BLUP outperformed RKHS (r = 0.40 vs. 0.20) and predicted total yield (r >=0.40) even better than in some other important crops like wheat [2]. The predicting ability was very high (r > 0.60) for the carotenoids (\*b value, BVL) and tuber dry matter (TDM), good for total yield (TYLD) and all 5 tuber grades evaluated (in mm and the order given, D45, 35-45; D35, <35; D80, 70-80; DXL, >80; and D55, 45-55) but grade 55-70 (D70). GS accuracy was poor for the number of tubers (NTB) and the number of stems (NTM) per plant. Clearly, the results of this study show that GS can effectively sustain potato breeding, particularly in selection of elite clones for total yield, and merceological and nutritional quality traits.

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Table 1. Predicting ability of 3 DArT-based GS models in 139 potato genotypes (abbreviations in-text explained)

GS Models	TYLD	NTB	NTM	TDM	BVL	D35	D45	D55	D70	D80	DXL	MEAN r
BL	0.40	0.22	0.05	0.67	0.72	0.30	0.42	0.28	0.12	0.28	0.34	0.35
G-BLUP	0.43	0.23	0.07	0.68	0.72	0.40	0.44	0.34	0.22	0.38	0.36	0.39
RKHS	0.22	0.12	-0.01	0.57	0.64	0.19	0.21	0.10	-0.05	0.22	0.17	0.22
$\mathbf{H}^2$	0.87	0.95	0.97	0.99	0.99	0.97	0.97	0.98	0.95	0.97	0.95	0.96

# CHALLENGES AND WAY-FORWARD IN SELECTION OF SUPERIOR PARENTS, CROSSES AND CLONES IN POTATO BREEDING

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### Introduction

Two basic steps in any potato breeding programme are to identify 1) superior parents and cross combinations to produce segregating progenies and 2) select superior clones from the progenies generated. However, choice of parents and cross combinations, and superior clones from the segregating progeny has always puzzled the potato breeders. This paper reports the status and the constraints encountered in potato breeding, and the way-forward to improve the efficiency of selection.

Combining Ability: Information on combining ability of parents can help breeders to decide their hybridization strategy. However, this requires making a number of test-crosses in which selected females are crossed with a random sample of testers in a specific pattern based on the design used. Matings based on selected individual single testers (top-cross) resulted in GCA estimates similar to those based on a number of testers (1). However, literature is full of contradictory results on relative importance of GCA and SCA for various characters. Thus, the information on combining ability needs to be sought afresh for identifying promising parents.

Progeny Test: Reports showed that these tests are effective in selection of superior cross combinations for a number of characters. Progeny tests in early generation are reported to be effective even for identifying parents that produce broadly adapted progeny (2).

Mid-parent and Mid-self-Values: In the absence of progeny means, mid-parent or mid-self-values can be used at the start of the breeding program to predict the mean performance of crosses. However, their effectiveness is reported to be low.

Genetic Diversity of Parents: Literature survey showed that genetic divergence though of use in identifying parents for exploiting heterosis in progeny, has only moderate effectiveness and that too when  $G \times E$  interaction is taken care of.

Selection of superior clones: A large number of reports showed that in early generations for selection of superior clones from the segregating progeny, the selection pressure needs to be nil to low, and only very poor types should be rejected (negative selection) in the first three generations for traits like tuber yield and its components. Positive selection can be practiced for qualitative characters and disease/pest resistance in early generations itself depending upon the stability of expression of the character(s) concerned. The production of microtubers in sufficient numbers can help in early large scale trials and reduce the time required in development of potato varieties

# Conclusion

The various strategies for selection of superior parents and cross-combinations in potato breeding programmes need to be used in combination, as none of these at its own are efficient enough to lead to reliable results, although the progeny test is a good method to select for superior parents and cross combinations

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# DEVELOPMENT AND APPLICATION OF A 20K SNP ARRAY TO CHARACTERISE THE TETRAPLOID GENE POOL OF POTATO (SOLANUM TUBEROSUM)

P. Vos 1, M.J. Paulo 2, F.A. van Eeuwijk 2, R.G.F. Visser 1, H.J. van Eck 1

A non-redundant subset (15138 SNPs) of the ~129000 SNPs identified by (Uitdewilligen et al, 2013) and 4454 SolCap markers from (Hamilton et al, 2011) were combined on a 20k Infinium SNP array for genotyping a total of ~2400 tetraploid genotypes of which 538 were used for this study to characterise the gene pool of potato. Genotypes from different countries and different market segments complemented with heirloom cultivars and important progenitors were used to perform several experiments. An important parameter of this array is the ability to review the introgression breeding over the last 70 years. Furthermore LD-decay, population structure and the effect of breeding on allele frequency changes over time and for different market segments were analysed.

Major results are that 3219 SNPs present on the array are the result of newly introduced alleles, from the 1940-ties onwards, most likely originating from wild species,. Only few of this "new" SNPs reach high allele frequencies in modern potato cultivars. The majority is therefore assumed to be linkage drag, not adding beneficial traits to the gene pool. The variation originating from heirloom cultivars is for 99% still present in modern cultivars.

This is an intriguing observation for two reasons. First it contradicts the suggested genetic erosion caused by modern breeding efforts. Second, it may help to understand the lack of genetic gains over a century of breeding. Linkage disequilibrium (LD) between SNP loci was analysed. The LD in tetraploid potato decays to background level at 1-2 mega bases on average. However stretches with longer LD are present in within population structure groups and in introgression segments.

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# EARLY SELECTION OF POTATO CLONES FOR TUBER DORMANCY AND APICAL DOMINANCE PERIOD

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Potato is the most important horticultural crop in Brazil. Considering the different growing conditions, potato is planted and harvest all year around. In some climate conditions, two crop seasons are possible. but require potato varieties with early vine maturity and short tuber dormancy. In this case, tuber dormancy is a key factor, since leads to major yield losses, because of low plant density. On the other hand, potato varieties for one season crop should have long dormancy, because of sprouting leads to quality losses of stored tubers. Since dormancy and control of tuber sprouting are important objectives for many potato-breeding programs, the development of an early selection method for dormancy period is critical. The objective was to develop an early selection method to categorize potato clones based upon tuber dormancy and apical dominancy level. The experiments were carried out between 2010 and 2013 with two independent sets of tuber families, one to develop and other to validate the innovative method. The tuber families were produced, as usually, in a soilless system in a screen house of the breeding program during autumn/winter season. After harvest, minitubers were sprayed with a 30 mg L-1 solution of gibberellic acid and storage at 20oC until dormancy breaking. The clones were separated in groups every 15 days based upon the number of days until sprouting. One set of tuber families referred to the evaluation of four groups (tubers sprouted until 45, 60, 75 and 90 days) with 982 clones, and another one of five groups (tubers sprouted until 30, 45, 60, 75 and 90 days) with 1076 clones. Tubers were then planted in the field during the summer season of template conditions to compare the groups of clones in terms of dormancy and dominance breaking period. The experiment was a random design with four replications. The dormancy and apical dominance breaking were considered when counted respectively one or two 2mm-sprout per tuber. In both sets of clones from different tuber families, the number of days until minituber sprouting used to group the clones was directly related to the dormancy and apical dominance period of field produced tubers. Further clonal generations of selection are under evaluation and the intellectual property right was request in Brazil (Application PI number 22110946768). This earlygeneration selection method has the advantage of identifying clones at the tuber family stage and makes possible to separate clones in different groups for both dormancy and apical dominancy period to fit the objectives of each breeding program.

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# THE PHYTOPHTHORA INFESTANS POPULATION IN NORTHERN IRELAND: A CLONAL POPULATION UNDERGOING CHANGE

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Late blight remains potentially the most serious disease of potato crops in Northern Ireland. Changes in the population of the causal pathogen *Phytophthora infestans* and their implications for its control have been investigated in Belfast since the 1980s. Between 2008 and 2011, an all-Ireland population study was carried out, which showed dramatic changes in the occurrence of genotypes including the aggressive Blue 13 (13\_A2). This was first identified in Northern Ireland in 2007, increased to dominate the population in 2009 and 2010, then declined in 2011. In 2012-13 this work was continued in Northern Ireland, in 2013 as part of a new all-Ireland project MonPESC (Monitoring Pathogen Evolution for Sustainable Cropping, funded by the Republic of Ireland's Dept. of Agriculture, Food & the Marine through their Research Stimulus Fund).

The 2012 season was very conducive to late blight, but warm, dry weather in summer 2013 resulted in fewer outbreaks. In 2012 nearly 100 isolates of *P. infestans* were obtained from 34 crops representing all potato-growing regions of Northern Ireland and these were characterised for metalaxyl resistance, mating type and Pep allozyme genotype. The marked decline in the incidence of the A2 mating type between 2010 and 2011 (from over 70% to 10%) was partially reversed: 37% of isolates proved to be A2. All A2 isolates were metalaxyl-resistant and the vast majority was from the south and east of Northern Ireland (Cos. Down and Antrim). Overall, 62% of isolates were metalaxyl-resistant. All A2 isolates were Pep 96/96 and SSR analysis of three showed that they were Blue 13 (13\_A2). All but two A1 isolates were Pep 100/100; these two, which were Pep 96/96, were shown to belong to the Pink 6 (6\_A1) genotype by SSR (the first finding in Ireland since 2009), whereas the other A1 isolates analysed by SSR were all 8 A1.

In 2013, c. 50 isolates have been obtained from 12 potato crops across Northern Ireland; characterisation of these is in progress. So far of 48 tested for metalaxyl resistance, over 80% have proved to be metalaxyl-sensitive and of 47 isolates characterised for mating type, all proved to be A1. The environmental conditions in 2013 have apparently not favoured the Blue 13 genotype, resulting in an even greater decline than occurred in 2011. However, whereas in Great Britain declines in Blue 13 have been associated with a concomitant increase in the A1 genotype Pink 6, preliminary evidence suggests that in Northern Ireland Blue 13 has been replaced by older A1 genotypes. The highly clonal population of *P. infestans* in Northern Ireland is undergoing regular upheavals which may be related to a combination of environmental factors and founder effects.

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# AGGRESSIVENESS AND GENETIC STRUCTURE OF FRENCH POPULATIONS OF *PHYTOPHTHORA INFESTANS* FROM 2001 TO 2008. ARE THE MOST AGGRESSIVE ISOLATES THE FITTEST?

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The oomycete *Phytophthora infestans*, the causal agent of late blight, is one of the major pathogens of potato. This biotroph parasite infects leaves, stems and tubers of its host. *P. infestans* is a heterothallic species with two different mating types designated A1 and A2. In France, although the presence of both mating types is observed, populations are largely clonal with only rare sexual events [1]. Its life cycle is mainly aerial and can be separated in two steps: an epidemic step during the culture period of the host and a survival step between two successive epidemics. French *P. infestans* isolates are therefore thought to overwinter as asexual mycelium surviving in blight-affected tubers.

The development of molecular markers, such as simple sequence repeats (SSR), provide a powerful tool to study *P.infestans* population diversity. Recent studies have related drastic and frequent changes of the genotype structure of *P. infestans* populations in European countries over the last decade [1, 2]. These studies have notably showed the emergence of a new lineage, named as "13\_A2", which rapidly displaced other lineages from 2006. The processes involved in these changes are still not clearly established but some authors suggest that a higher aggressiveness (quantitative component of the pathogenicity) could permit isolates to be selected in populations [2, 3]. Nevertheless, if the most aggressive isolates are favored during the epidemics [4], they could not necessarily be the fittest when the whole complete cycle is considered. For instance, it was showed that isolates with a high aggressiveness level experienced a low transmission between two successive epidemics [5].

The objective of the present study was to investigate the evolution of genetic structure of French P. infestans populations and to determine if this evolution could be linked with the aggressiveness level of isolates. We have used and completed a dataset of isolates collected in different French regions from 2001 to 2008. These isolates were genotyped with 10 SSR markers and characterized for mating type and aggressiveness on potato (cv. Bintje).

Our results showed that several changes appeared in the genetic structure of French P.infestans populations during the survey period, as in other European countries. Moreover our study indicated that the predominance of some multilocus isolates was generally not linked with a high aggressiveness level.

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# TRANSCRIPTOME AND SECRETOME ANALYSIS IN RESPONSE TO PHOSPHITE TREATMENT IN POTATO AND EFFECT ON LATE BLIGHT DEVELOPMENT IN FIELD TRIALS

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### Introduction

Potato late blight disease caused by the oomycete *Phytophthora infestans* is the most severely damaging disease of the potato crop. Breeding for resistant varieties using traditional breeding methods has been time consuming and labor intensive. The predominant method to control for late blight disease has been through frequent use of fungicides. However, there is a need to develop alternative methods to control late blight disease due to the overall harmful impact of continual fungicide spray and high costs incurred due to spraying [1]. Phosphites (Phi) are increasingly used in controlling oomycete pathogens [2]. We have investigated the effect of Phi on gene expression and secreted proteins in potato leaflets grown in a controlled environment and the potential of using Phi against late blight in field trials.

## **Materials and Methods**

For transcriptomic and proteomic studies leaflets from Phi (36mM) and water treated potato plants (cv. Desiree) were harvested 3, 6, 11, 24, 48 and 120 h after foliar-spray, RNA was extracted and wholegenome expression analysis was performed by microarrays (Agilent JHI *Solanum tuberosum* 60k v1). Label-free, quantitative mass spectrometric analysis of proteins secreted 48 hours after Phi treatment was performed. Detached leaf assays were performed at all the time points. In three years field trials we investigated the effect of Phi on late blight in four potato cultivars. Plots of 30 m2 each was treated with Phi with different concentrations every week during the growth season. The effect of Phi was compared with the fungicides Shirlan and Ranman Top and also with combinations of Phi and the fungicides. The level of infection was scored weekly until defoliation.

# **Results and Conclusions**

Phi had a rapid and transient effect on the transcriptome, with a response 3 hours after treatment. Interestingly, this effect lasted only until 11 hours after treatment, whereas resistance was observed throughout all the time points tested in the detached leaf assay. Transcriptome data analysis revealed that transcripts associated with defense, wounding, and oxidative stress constituted the core of Phi responsive transcripts, indicating an activation of a general stress response. 67 secretome proteins predominantly associated with cell wall processes and defense changed in abundance 48 hours after treatment. Phosphate levels were unaffected by Phi treatment.

In the field we found a dose dependent effect of Phi on the rate of late blight development. Using Phi alone the effect was not as good as fungicides, although in two of the cultivars the effect was almost as good as treatment with Shirlan. However, using Phi in combination with a reduced dose (50% and 25% of recommended) fungicide gave at least as good and sometimes better control than using fungicide alone. Therefore, we believe that Phi could be part of a treatment strategy against late blight that reduces the demand of fungicides. In addition, Phi decreased the development of tuber blight after storage.

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# CONTROL STRATEGIES AGAINST PRIMARY *PHYTOPHTHORA* INFECTIONS IN CONVENTIONAL AND ORGANIC POTATO FARMING

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Potato late blight epidemics caused by *P. infestans* often originate from infested seed potatoes remaining unnoticed during storage and brought to the fields at planting. PCR data show that ca 10% of the seed potatoes are latently infected with *P. infestans*, i.e. do not show symptoms but carry the pathogen. In the field, when soils are moist and temperatures suitable, primary stem infections can develop from these tubers. They will then serve as starting points for subsequent *Phytophthora* epidemics after the pathogen has grown up the stem. This study investigated different ways to reduce primary stem infections in conventional and organic potato production by fungicides applied on foliage or tuber.

Field tests were carried out using a double setting technique, with one healthy and one artificially infected tuber planted on the same position. The artificially infected tuber served as a source of inoculum for the neighbouring healthy tuber, facilitating the development of stem infections. In case of the tuber treatment tests, the healthy tuber received a fungicide dressing, while the infected tuber remained untreated. In case of the foliage treatment tests none of the two tubers was treated. The tests were set up at two sites in S Germany in 2011 or 2012.

Our field trials have shown that in conventional farming primary infections can only be effectively controlled by the application of systemic fungicides approximately one week ahead of the first stem blight symptoms, as these inhibit the growth of the pathogen inside the plant. An alternative or additional control measure is the use of seed dressings that can also reduce primary infection rates in the field. In our tests, Cu hydroxide and cymoxanil dressings had a significant effect on *P. infestans* primary stem infections when applied with planting in spring. Such contact/ locally systemic fungicides are effective against Phytophthora propagules on the tuber surface or in the surrounding soil. They also can protect neighbouring tubers from inoculum transmitted in the soil.

In organic farming, postponing the onset of infections originating from infected seed tubers is even more important, as systemic fungicides for control are not available. Therefore, we tested seed dressings with Cu and other products as means to reduce primary stem infections. However, although artificially infected seed tubers were used, incidence of stem blight was low in these trials, most likely due to dry weather conditions during spring. Therefore, no data on the effect of the seed dressing on stem infections are available. However, when (secondary) leaf infection data were considered, an effect of several treatments on the degree of leaf infections could be observed. In addition, alternative products for foliar applications in organic farming were tested as part of a management strategy to reduce the extent of secondary leaf infections, and to minimise the deposition of sporangial inoculum on the soil and the crop. Several leaf and potted plant assays have revealed promising candidate substances for potential use in the field.

In both conventional and organic potato farming, the use of leaf fungicides and seed treatments might be able to achieve a further retardation of late blight epidemics and thus might help to better control the disease and its impact on potato yield. In organic farming, this strategy might further reduce Cu input and help to produce disease free seed tubers.

# THE NOVEL ACTIVE SUBSTANCE COS-OGA PROTECTS POTATO AGAINST LATE BLIGHT THROUGH SALICYLIC ACID-DEPENDENT DEFENSE REACTIONS

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Potato is one of the most important culture in Europe with more than six millions Ha cultivated in 2011. Annual losses and control costs caused by *Phythophthora infestans*, the late blight agent, are assessed at more than one billion euros per year. *P. infestans* possesses a strong adaptability and the susceptible cultivar Bintje continues to be dominant as resistant cultivars struggle to enter the market. The disease in essentially controlled by considerable amounts of chemical fungicides and environmental considerations completed by European regulation framework on plant protection products foster research for new alternatives.

Plants possess an innate imunity that offers the opportunity to develop new efficient plant protection tools. Indeed, the so-called elicitors are at the forefront of the plant defense system that comprises both non-self conserved microbial signatures often called microbe-associated molecular patterns (MAMP) and self molecules called damage-associated molecular patterns (DAMP) released from the plant host by wounding or enzymatic degradation. Together they form pathogen-associated molecular patterns (PAMP) that are recognized by pattern recognition receptors mostly localized to the plasma-membrane. The recognition at the level of the plasmalemma induces PAMP-triggered immunity (PTI), as opposed to effector-triggered immunity that relies on interaction between the products of plant R genes and pathogen effectors injected into the cytoplasm.

COS-OGA is a new active substance for plant protection that stimulates PTI. The product is currently following the European registration process under EC Regulation No 1107/2009 for use on cucurbits against powdery mildew. The elicitor contains chitosan oligomers (COS) associated with pectin-derived oligogalacturonides (OGA). The COS MAMP combines with the OGA DAMP and thereby mimicks the interaction between a plant and a fungal pathogen. Together they form an oligosaccharidic complex formed by COS and OGA with a supramolecular conformation stabilized by calcium.

The plant defense stimulation was investigated on solanaceae after several foliar sprayings of COS-OGA. A proteomic study on leaves revealed that the elicitor treatment led to overexpression of pathogenesis-related proteins, heat shock proteins, proteins involved in DNA/RNA remodeling and proteins involved in energy metabolism and in photosynthesis. The expression of a selection of genes related to plant defense was also followed by quantitative RT-PCR. Transcripts coding for jasmonic acid and ethylene-responsive genes were not affected by COS-OGA but transcripts of salicylic acid (SA)-associated genes were significantly up-regulated. SA quantification showed an increase of free SA level in leaves proportional to the number of COS-OGA applications. These results suggest a SAR-like mechanism of action of the oligosaccharide complex. We also studied COS-OGA efficacy in the protection of potato against late blight. The elicitor was effective and slight modifications of its standard formulation seemed to considerably increase the efficacy of the elicitor to control late blight.

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## PROTECTIVE EFFECT OF THE ELICITOR COS-OGA AGAINST POTATO LATE BLIGHT

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Potato is one of the most heavily pesticide-treated crops in Wallonia, mainly in efforts to combat late blight caused by the pathogen Phytophthora infestans. Within the context of a European Union (EU) directive (2009/128/EC), focusing on the sustainable use of pesticides, and an EU regulation (1107/2009), which gives priority to molecules that do not endanger human or animal health or adversely affect the environment, an alternative to pesticides for the control of late blight disease needs to be developed. COS-OGA (chitosan oligomers - oligogalacturonides), a new elicitor developed by the University of Namur, has been reported to stimulate natural plant defences and to control diseases in cucurbit, grapevine and tomato. In this study, we looked at the effect of COS-OGA on P. infestans. Assays were conducted under controlled conditions whereby plants were elicited with COS-OGA before inoculation with P. infestans. The disease was assessed by leaf infection analysis, followed by quantitative reverse transcription - polymerase chain reaction (RT-PCR) analysis of the expression of genes involved in plant defence. COS-OGA elicitation accounted for a reduction of up to 57% of the leaf infection 7 days after inoculation. This protection was correlated with inoculum pressure. The gene expression analyses showed a higher expression of pathogenesis-related genes (PR1 and PR2) on plants elicited with COS-OGA. These results indicated the systemic acquired resistance-like mechanisms of action of COS-OGA and confirmed the results obtained by the University of Namur team with proteomic and transcriptomic tests. This study supports the use of the COS-OGA elicitor in the integrated pest management of potato crops.

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# EFFECT OF VARIETAL DIFFERENCE IN ROOT SYSTEM ON SOIL WATER ABSORPTION IN FOUR POTATO VARIETIES WITH DIFFERENT ROOT MASS

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#### Introduction

Potato (*Solanum tuberosum* L.) is very sensitive to drought, because of its shallow and sparse root system. To improve its drought tolerance, we bred Konyu varieties with large root mass using the criteria of large root mass in plow layer and high tuber starch yield in 2007. Under soil water stress condition, Konyu varieties had higher tuber yield compared to commercial varieties. Although this result indicated importance of root system improvement to increase drought tolerance, contributions of root mass increase on soil water absorption and plant physiological characters are still not clear. In the present study, we investigated relationships of varietal difference in root vertical root distribution with water absorption at different soil depths.

### **Materials and Methods**

Konyu-1, Konyu-2 and Konyu-4, and a check variety Konafubuki (a parent of Konyu varieties) were cultivated in fields for 4 years (2008-2011). The planting was conducted in May 6-18. In middle June, after ridging rows, two poly-shelters (each 6.3×25 m) were set up to prevent rainfall in the droughted field. At the same time, furrow irrigation tubes were set up in the irrigated field of rain-fed condition to maintain soil water potential above -60 kPa. In early August (S2: maximum shoot growth stage of Konyu-4 and Konafubuki) and in late August (S3: maximum shoot growth stage of Konyu-1 and Konyu-2), root length density (RLD) was measured at three soil depths of 20, 60 and 100 cm from ridge surface with core sampling method. Soil matric potential (ψsoil) was measured with MPS-1 (Decagon Device, USA) at 60 and 100 cm soil depths for Konyu-1 and Konafubuki in 2008 (no replication) and 2009 (3 replications), and at 20, 60 and 100cm soil depths for all varieties in 2010 and 2011 (4 replications).

#### Results

Consistently over 4 years, the RLD at 20 cm soil depth was significantly higher in Konyu varieties than in Konafubuki regardless of soil water treatment at S2. Thereafter, the RLD at 60 and 100 cm soil depths increased in Konyu varieties, while not in Konafubuki, resulting in much higher RLD in Konyu varieties than in Konafubuki at all soil depths at S3. The result indicated that a superior deep rooting ability of Konyu varieties was derived from longer duration of root extension to deeper soil depth, especially in droughted field.

The decrease of  $\psi$ soil started later at deeper soil depths. In addition, the  $\psi$ soil was significantly lower in droughted field than in irrigated field at each soil depth. Varietal difference in  $\psi$ soil became clear since S2. The  $\psi$ soil was lower in Konafubuki than in Konyu varieties at 20 cm soil depth, while contrary at 60 and 100 cm soil depths. A significantly negative correlation was found between  $\psi$ soil and RLD at 100 cm soil depth at S2 (r = -0.88, P<0.01) and S3 (r = -0.82, P<0.01), suggesting the importance of higher RLD for water absorption from deeper soil depth.

### **Conclusion and Perspectives**

Compared to Konafubuki, Konyu varieties attained larger RLD in deep soil layer due to longer duration of root extension and resulted in superior soil water absorption from deeper soil layer. These result clearly indicated the importance of deep rooting to enhance drought tolerance in potato crop.

# EFFECTS OF DAY LENGTH AND SEED TUBER AGE ON THE DIFFERENCE IN ROOT MASS BETWEEN TWO POTATO VARIETIES WITH SAME MATURITY

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## Introduction

Larger root mass is important for drought tolerance in potato. We bred Konyu cultivars with large root mass and high tuber yield potential [1]. In this study we examined the effects of day length and seed tuber age on root mass with two cultivars of same maturity class under artificial climate condition.

#### **Materials and Methods**

Two levels of day length (SD:8 hrs., LD:16 hrs.) and three levels of seed tuber age (Age1: 11 days, Age2: 25 days, Age3: 41 days under pre-sprouting in a room at 22 degree Celsius of temperature) were examined for two cultivars of late maturity class (Konyu-4: large root mass, Konafubuki: small root mass). Each four plants were grown in pots (22 cm in height, 4.5 liter) under two artificial climate rooms with 23 (day) and 12 (night) degree Celsius of air temperature, 65 % of relative humidity and 500 (SD and LD for 8 hrs.) plus 100 (LD for 8 hrs.) micro mole per square meter per sec. of photosynthetic active radiation. At 35 days after sprouting, dry weight of plant organs (shoot, root and tuber) were recorded. To evaluate the treatment effects, ANOVA was calculated with analysis of combined experiments [2] using day length and seed tuber age as main plot, and variety as subplot with 4 replications.

#### Results

Root dry weight (DW) was larger in LD than in SD, and the largest at Age2 and the smallest at Age3. The effect of treatment was much stronger for day length than for seed tuber age. There is a significant interaction between two treatments, resulting in less difference in root DW due to seed tuber age under SD relative to LD. Although main effect of variety on root DW was highly significant, significant interactions were also found between day length and variety, between seed tuber age and variety, and between three factors. Two varieties showed a large difference in root DW under LD, while similar root DW under SD. The varietal difference was smaller at Age3 than at Age1 and Age2, and the largest at Age2 of LD and the smallest at Age3 of SD. These differences in root DW between treatments and varieties were mainly due to those in dry matter partition ratio (DR) to root. There was a highly significant correlation between DW and DR of root for two varieties (r=0.971, p<0.001). Additionally, a highly significant negative correlation was found between root DR and tuber DR for two varieties (r=--0.968, p<0.001), indicating that the effect of day length and seed tuber age on the varietal difference in root DW occurred due to the change in dry matter partition ratio throughout plant, especially between root and tuber.

# **Conclusions and Perspectives**

The present results indicate that a genotypic difference in root mass will be changed strongly by day length and modestly by seed tuber age. A selection of desired genotypes in relation to root mass must be done after due consideration on day length of location and season, and used seed tuber age in cultivation.

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# IS THE DISCRIMINATION AGAINST 13C IN LEAFLETS AND TUBERS AN APPROPRIATE TRAIT TO DETERMINE GENOTYPIC DIFFERENCES RELATED TO DROUGHT TOLERANCE IN POTATO?

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Drought tolerance selection through phenotyping entails prioritizing plant traits that synthesize and integrate critical physiological processes occurring during the crop's growth. Potato studies where discrimination against  $^{13}$ C ( $\Delta$ ) in leaflets ( $\Delta_{leaflet}$ ) and tubers ( $\Delta_{tuber}$ ) were monitored, concluded that  $\Delta_{leaflet}$  is not an appropriate trait for screening tolerance to mild water stress [1], and that  $\Delta_{tuber}$  differences do not reflect final yield [2]. The present study was designed to revisit these findings, comparing the  $\Delta_{\text{leaflet}}$  and  $\Delta_{\text{tuber}}$  throughout the phenology of two advanced varieties with acceptable yield under water limiting conditions (UNICA, CIP N°392797.22 and Sarnav, CIP N°397077.16) and contrasted with a cultivar commonly tested in carbon isotope studies (Désirée). The drought treatment consisted of a deficit irrigation with 50% of field capacity, which was established after tuber initiation onset (TIO). The control plants were watered until the soil reached field capacity. Six sequential harvests were carried out to assess  $\Delta$  in dry biomass of leaflets and tubers. Prior to each harvest, gas exchange was measured in leaflets. The variety Sarnav showed the higher final tuber dry biomass (75.96±1.96 g plant<sup>-1</sup>) under drought as well as the maximum tuber bulking (1.65±0.05 g day<sup>-1</sup>) under control conditions. The average difference control-drought, for both  $\Delta_{\text{leaflet}}$  and  $\Delta_{\text{tuber}}$ , was positively correlated with the drought tolerance index (DTI) [3]. DTI ranking among genotypes was Sarnav > Unica > Désirée. Despite Sarnav's higher average stomatal conductance (242.4 $\pm$ 15.4 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) and lower intrinsic water use efficiency (A/ $g_s$ ; 79.3±5.4 µmol/mol), low  $\Delta_{leaflet}$  (20.3±0.17‰) was evidenced under control treatment, attributed to a larger photosynthetic capacity [4]. Sarnav showed the lowest Δ<sub>tuber</sub> (15.8±0.17‰) of the three varieties under drought conditions, suggesting a more extensive use of carbon products synthesized in leaves for drought tolerant mechanism, thus confirming previous findings for this variety [5]. Due to the strong negative correlation found between  $\Delta$  and A/ $g_s$  (r<sub>Pearson</sub> between -0.77 and -0.89), the use of  $\Delta$  as a method for screening drought tolerance in advanced potato genotypes seems warranted. We recommend sampling for Δ analyses before senescence i.e. between 30 and 60 days after TIO, or 350 and 700 °C days of accumulated thermal time after TIO.

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# RELATIONSHIP OF WUE, YIELD AND DROUGHT TOLERANCE IN POTATO (SOLANUM TUBEROSUM L.) UNDER CONTROLLED CONDITIONS

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Potato (*Solanum tuberosum* L.) is a drought-sensitive crop due to a sparse and shallow root system that needs soil water to be maintained constantly for maximum yield and quality. Understanding the relative responses of different potato genotypes to drought stress can ensure efficient potato yield and minimize quality losses due to water shortage. Water use efficiency (WUE), allows for increased plant production per unit water used ("more crop per drop") and is often considered an important determinant of yield under drought stress.

We performed a controlled greenhouse experiment to determine the responses to water deficit (WD) of fifteen (15) potato genotypes from CIP's advanced breeding population. Throughout the experiment, we measured a large number of morphological and physiological parameters such as chlorophyll content (SPAD), relative water content, height, leaf area, transpiration rate, and yield, among others. To rank the genotypes as tolerant, we used the transpiration rate and the number of days needed for the plants to deplete water from soil.

Our results showed that four genotypes, CIP397077.16, CIP398208.620, CIP720088, CIP392797.22, were able to maintain a high WUE compared to the control plants, as well as to achieve higher harvest index under WD conditions. Regression analysis between biomass and total transpiration (R<sup>2</sup>= 0.8409) also showed these four genotypes as good candidate for drought tolerance.

This study showed that the relationship between yield and WUE deserves more attention as it may help to increase food production by helping to identify potato genotypes able to use water more efficiently under limited conditions.

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# THE EFFECT OF LIGHT SPECTRAL QUALITY ON POST-CRYOPRESERVATION SURVIVAL AND REGENERATION OF POTATO (SOLANUM TUBEROSUM L.) SHOOT TIPS

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Cryopreservation, the storage of plant genetic resources on ultra-low temperature, has become the most promising conservation method for long-term purposes. The material is usually stored on the temperature of liquid nitrogen (-196°C) or in its vapour so that the viability is maintained and growth can be resumed. On such a low temperatures all metabolic processes are disabled and thus the storage time is unlimited. Up to date, valid cryopreservation protocols are available for numerous species and routine cryopreservation is carried out in potato genebanks [1]. However, species- or cultivar-specificity is still a major problem affecting survival and regeneration of cryostored material.

It is well known that physiological factors before and after cryopreservation are crucial for the cryopreservation success [2]. Therefore we studied the effect of different light spectral qualities on the survival and regeneration of potato shoot tips after cryopreservation. In vitro plants of five potato varieties 'Varajane kollane', 'Maret', 'Bintje', 'Desireé' and 'Anti' were prepared for the experiments. Before cryopreservation all the plants were stored under cool white fluorescent lamps. After cryopreservation, the plants were let to regenerate in six different light qualities: cool white fluorescent, warm white, blue LEDs, red LEDs, red-blue mixture with 90 % red and 10 % of blue without and with far-red LED lights. The results were recorded after six weeks of thawing.

Light spectral quality affected significantly the survival and regeneration of all varieties. The highest regeneration was observed in the mixture of red and blue LEDs. This effect was observed for all the varieties used in this study. The lowest regeneration was observed mainly in red light but not for all varieties tested. The results show that the regeneration of potato shoot tips after cryopreservation can be significantly affected by light spectral quality.

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# ROLE OF PROLINE IN THE RESISTANCE OF BLUE POTATOES TO ABIOTIC STRESS

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#### Introduction

The Andean potatoes grow in conditions of frost, drought, high irradiation and salinity, which are the normal types of weather in the region and the origin centre of this crop. The *Solanum* species have different behaviours in presence of abiotic stresses, ranging from susceptible to tolerant and resistant ones, depending on their genetic patrimony.

The wild potatoes give us a very nice opportunity to understand and to know better their elegant and efficient mechanism to grow and develop under drought and frost stresses.

### Material and methods

We have followed the capacity of wild (blue and yellow) and cultivated potatoes to adjust their osmotic regulation under three levels of salinity stress and their proline accumulation. The *Solanum* species used for this study were *Solanum juzepczukii*, *Solanum curtilobum*, *Solanum goniocalix*, *Solanum andigena* and *Solanum tuberosum L*.

By other hand, we studied the effect of the pyrroline-5-carboxylate-synthetase introduced on *Solanum tuberosum* for resistance to salinity. We have used Southern and PCR to confirm the positive transformed potatoes.

# Results

The proline accumulation has a positive correlation with resistance to drought, salinity and frost stresses. The *Solanum juzepczukii* and *Solanum curtilobum* showed more resistance to salinity, drought and frost stresses than *S. andigena* and *Solanum tuberosum*.

### **Conclusions and perspectives**

The genetic potentiality of wild potatoes as a source of resistant genes for abiotic stresses is very important for its quality and its quantity. Their utilization in breeding programs is well known and the use of its potentiality for the human needs is in our hands for its utilization in all the regions around the world, even the most difficult ones to grow potatoes.

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RECENT ADVANCES IN UNDERSTANDING ENVIRONMENTAL AND MANAGEMENT FACTORS AFFECTING SPREAD OF POTATO VIRUS Y IN COMMERCIAL POTATO FIELDS OF NEW BRUNSWICK, CANADA

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The spread of Potato virus Y (PVY) is a major issue in many potato growing areas around the world. Earlier work by our group (1,2) had identified widely varying rates of on-farm PVY spread in commercial fields in the main potato growing region of New Brunswick, Canada, and several management and environmental factors that may affect that spread. For this study, we sought to create a general model and suite of best management practice (BMP) recommendations by including many fields with widely different management practices, over four crop seasons with varying inoculum, vector and environmental conditions. PVY spread was monitored in 42 fields, between 2010 and 2013. In each field, 100-110 virusfree plants were identified after emergence; marked plants were monitored for PVY infection with midseason leaf tests and tuber test after harvesting. PVY spread to initially virus-free plants ranged from 0% to 76% across the 42 fields. The PVY spread in each field was compared to detailed records of field management, rates of seed-borne PVY for each field, local aphid abundance and weather conditions during the growing season. A logistic regression model was constructed to estimate PVY spread based on seven PVY inoculum, aphid, climate and management parameters. The single strongest factor explaining on-farm PVY spread was the number of insecticide-supplemented mineral oil sprays applied through the season, with mineral oil alone causing a substantially weaker reduction in spread. Seed-borne PVY, earlyseason aphid abundance, high June temperatures and low numbers of days with rain in June were all significantly associated with increased PVY spread. This regression model, using only data available by the middle of the growing season at least 6 weeks before harvest, is strongly predictive of PVY status in harvested tubers (actual vs. prediction  $r^2 = 0.84$ ). Across the four years, 20 of 42 study fields would have exceeded the new government regulatory threshold of 5% PVY for commercial seed planting, and the developed model correctly predicted whether the harvested crop would exceed that threshold or not in over 90% of the study fields. Over the past four years included in this study, on-farm PVY spread on average has been declining, coincident with increased use of foliar-spray insecticide, later planting of the crop and shorter times between planting and first oil and insecticide sprays. Based on these observations and the statistical analysis in this multi-year field study, recommendations for best management practices to reduce PVY spread have been produced.

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## PVYN PREVALENCE IN POTATO CROPS: IMPACT OF STRAIN COMPETITION AND DIFFERENTIAL ABILITY TO OVERCOME PLANT RESISTANCE MECHANISMS.

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## Introduction

Potato virus Y (PVY) is the most important viral pathogen affecting potato crops worldwide [1]. PVY is transmitted between plants in a non-persistent manner by several aphid species, including many that do not colonise potato plants. PVY exists as a complex of strains that can be distinguished according to their biology, serology and genome composition. While virus incidence is low in Scottish seed potato crops, PVY has recently become the most prevalent virus. A drift in the PVY population structure from PVYO, PVYC to PVYN to recombinant PVYNTN strains (N-Tuber Necrosis) that can cause Potato Tuber Necrotic Ringspot Disease (PTNRD) has also been observed in Scotland and worldwide [2]. We studied the molecular nature and epidemiology of PVYN isolates to gain a better understanding of factors that are driving their prevalence

## **Materials and Methods**

Biological characterization of PVY isolates was undertaken by assessing symptoms elicited on indicator plants. Genome sequencing of PVYN isolates collected from potato fields, phylogenetic analysis and field infection and transmission studies were as previously described [3].

#### Results

The molecular diversity of field isolates of PVYN serotypes indicates that they belong predominantly to the European (EU)-NTN (PVYEU-NTN) molecular group. Biological characterisation classifies them as belonging to the PVYNTN strain group with only one isolate identified as a PVYE strain, i.e. failing to elicit vein necrosis in tobacco. All the isolates tested triggered different degrees of severity in foliar symptoms and PTNRD symptoms on tubers of susceptible potato cultivars. Transmission in field trials of selected PVY isolates belonging to the three main molecular groups (PVYO, PVYEU-NTN, PVYNA-NTN) resulted in a significantly higher incidence of PVYEU-NTN in tuber progeny. Virus incidence in plants infected at different times after emergence, revealed a higher capacity of PVYEU-NTN to infect older plants in comparison to PVYO.

#### **Conclusion and Perspectives**

The recombinant PVYNTN strain, belonging to the EU-NTN molecular group, has become the prevalent PVY strain in field populations in Scottish seed potatoes. Our results show that PVYEU-NTN has an ability to out-compete other PVY strains by virtue of higher transmission rates and a greater ability to overcome mature plant resistance mechanisms. Further characterization of these different PVY molecular variants by assessing their replication rates and both local and systemic movement will help to identify the key mechanisms driving PVY population dynamics in our environment.

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## WITHIN-FIELD SPREAD OF TWO STRAINS OF POTATO VIRUS Y (PVY)

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### Introduction

Potato virus Y (PVY) is economically the most detrimental viral disease affecting seed potato production in Europe [1] PVY is transmitted in a non-persistant manner by several species of aphids [2]. Within-field PVY spread is affected by different factors such as cultivar susceptibility [3]; vectors pressure [4]; mineral oil treatments [5]; mature plant resistance [6] and haulm killing date [7]. This study will focus on plant-pathogen interactions affecting within-field PVY spread.

### **Material and Methods**

A two-years field trial was conducted in Switzerland to assess the spread of two PVY isolates from two different strains (PVYNTN and PVYN-WI) on two different cultivars (Charlotte and Nicola). No mineral oil and no insecticide was sprayed during the season and flying aphids were captured at weekly intervals to evaluate the risk of PVY transmission. Leaf samples taken at two time points during the growing season as well as pre-haulm killing and pre-harvest samples were analyzed by DAS-ELISA to evaluate the rate of PVY spread from emergence until harvest.

#### Results

Peak of aphid flight arrived around 20 days later the second year of experiment but the main PVY infections appeared at the same time for both year. Over time aphid species distribution could explain this phenomenon. Overall, cv. Nicola was more susceptible than cv. Charlotte and the PVYN-WI isolate was spreading at a higher rate than the PVYNTN isolate. Nevertheless, many interactions between year and isolate and year and cultivar were statistically significant.

### **Conclusions and perspectives**

This study shows that two PVY isolates from different PVY strains can spread at different rates under the same agro-climatic conditions. The next step would be to determine if, within the same PVY strain, different isolates could spread differently.

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# RELIABILITY OF POTATO VIRUS Y (PVY) DETECTION IN POTATO TUBERS SAMPLED AT DIFFERENT PHYSIOLOGICAL STAGES

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Potato potyvirus Y (PVY), the most prevalent virus in potato growing regions, is responsible for 60 to 80% of viral infections on seed potato crops. Because of both yield losses and degradation of the tuber quality due to the necrotic ringspot disease, PVY is considered as an economical threat for the potato industry. To preserve environment and potato crops, a seed certification procedure has been defined to reduce the proportion of viral-infected tuber in the produce seed potatoes. In France, a step of this certification scheme consists in the checking of the sanitary quality of the produced progeny using serological tests applied on sprouted tubers. However, this procedure is time consuming and leads to significant costs. Therefore, with the advances in both knowledge of PVY and the development of new diagnostic technologies, the challenge for virus testing laboratories is to design new procedure(s) allowing PVY detection in potato tubers. The objectives of the presented work were i) to compare the reliability of PVY detection of five serological- or molecular-based techniques applied on potato tubers at different physiological stages (i.e. at 0 [harvest time], 1 and 3 months of storage at 20°C) and ii) to define the limit of tuber bulking allowing a reliable PVY detection of one infected tuber mixed with a bulk of healthy samples. The rates of PVY detection obtained with the different methods were compared with the detection rates determined using the standard seed certification procedure. The results obtained in both artificial and natural conditions shown that the real-time RT-PCR was the most sensitive and suitable tool for early detection of PVY in infected samples regardless of the physiological conditions of the tubers and the number of bulked tubers.

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# EFFECT OF SOME COMBINED THERAPIES ON PVY AND PVX INFECTED POTATO PLANTLETS (CV. ROCLAS)

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### Introduction

Elimination of PVY and PVX from potato supply is essential for seed potato production. Also, in this study, the efficiency of some techniques (chemotherapy, treatments with *Satureja hortensis* oils, hydrogen peroxide, ascorbic acid, electrotherapy) in decreasing the infection level of PVY and PVX infected plants and producing virus-free plants (cultivar Roclas) was evaluated. At the same time, the behavior of the treated plants and their chlorophyll content were researched.

## **Materials and Methods**

Plantlets (variety Roclas) obtained from PVY (PVYo) and PVX infected material (mechanically inoculated) were used in the experiments. Electrotherapy was applied in 4 variants: the infected plantlets were exposed to either 50 or 100 miliampers (mA), for 10 and 20 minutes, washed, divided into single node cuttings and multiplied in vitro. Chemotherapy was undertaken with ribavirin (RBV) and oseltamivir (OSMV) (RBV 40 mg  $\Gamma^1$  + OSMV 40mg  $\Gamma^1$ ). Solanum tuberosum L. plantlets regenerated were removed from the culture medium, acclimated in green house and with Satureja hortensis essential oils suspensions,  $H_2O_2$  1mM pH 5.6, ascorbic acid 3mM pH 5.6 treated (spraying twice weekly). The survivor plants were indexed (DAS ELISA). The variant leading to high rates of both virus elimination and plant regeneration was estimate using the Therapy Efficiency Index (TEI) [1, 2]. For chlorophyll content determination of regenerated healthy plants a portable SPAD 502 (Chlorophyll Meter) was used.

## Results

Electrotherapy (100 mA, 10minutes) applied to infected plantlets, chemotherapy (RBV40mg I<sup>-1</sup> OSMV40mg I<sup>-1</sup>), *Satureja hortensis* EOs essential oils treatments of acclimatized plants led to the highest rate of virus eradication, the maximum values of the therapy efficiency. Satureja hortensis EOs and hydrogen peroxide (1mM) or ascorbic acid (3mM) treatments of acclimatized plants increased the therapy efficiency index (TEI) in all the variants, having beneficial effects on the plants obtained by chemotherapy from PVY and PVX infected potato plants. This effect was stronger when the therapies were applied to material infected with PVX. Regarding the chlorophyll content, all the regenerated plants from infected material treated 10minutes with electric current (100mA), had higher values than the negative control (healthy plantlets untreated).

## **Conclusions and Perspectives**

This preliminary study revealed that applying combination of electrotherapy (100 mA, 10minutes) chemotherapy (40mg/l RBV + 40mg/l OSMV), followed by treatments with *Satureja hortensis* essential oils of acclimatized plants could have beneficial effects on PVX and PVY elimination from potato plant tissues. Some elements remain to be tested and/or improved in the future: the phytotoxicity of the treatments has to be verify; to define the efficiency of the treatments with bulked samples is required.

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# LESIONS IN COMPATIBLE AND INCOMPATIBLE POTATO - POTATO VIRUS Y INTERACTION: MORPHOLOGY AND GENE EXPRESSION

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#### Introduction

Potato virus Y (PVY) is of extreme economic importance as it is responsible for yearly losses in production of crops, and thus the subjects of investigation in many research groups all over the world. The tuber necrotic strain of Potato virus Y (PVY<sup>NTN</sup>) causes potato tuber necrotic ringspot disease (PTNRD) in sensitive potato (*Solanum tuberosum* L.) cultivars that is responsible for great losses in crop industry. Sensitive cultivars of potato infected with PVY<sup>NTN</sup> show growth inhibition, faster senescence and leaf drop, chlorotic ringspots and/or spot necrosis on inoculated leaves, crinkles and mosaics on systemic infected leaves and necrotic ring spots on tubers. Viruses from PVY<sup>N-Wi</sup> group can also cause severe symptoms on potato. Symptom development and their severity depend on the isolate of PVY, potato cultivar, environmental conditions and other factors. Besides, virus-infected leaf tissues comprise a heterogeneous mixture of cells at different stages of infection and spatial and temporal relationships between sites of virus accumulation and the accompanying host responses are not well defined.

#### **Materials and Methods**

In our studies, we analysed lesion development in PVY-inoculated leaves of two genotypes: a resistant cv. Rywal and a susceptible NahG-Rywal, compromised in salicylic acid signalling, after the infection with two isolates of Potato virus Y, PVY<sup>NTN</sup> and PVY<sup>N-Wi</sup>. Spatio temporal analyses were performed on the macroscopic, microscopic and molecular (gene expression) levels.

#### Results

Although lesions were formed on the inoculated leaves of both potato genotypes, their number and size differed in the two genotypes. On the ultrastructural level cells in the lesions of both genotypes show features of vacuolar cell death with organelles deformation and shrunken protoplasts. Presence of viral RNA and expression of 20 genes involved in primary metabolism, redox processes, hormonal signalling, silencing and defence responses were analysed in leaf tissue in the centre of the lesion and 3 distal leaf pieces. While there was most of viral RNA in the centre of the lesion in both genotypes, the expression pattern of genes changed with distance from the centre of the lesion and in some cases the response was different in the two genotypes.

### **Conclusion and perspectives**

Understanding the processes at the point of the viral entry can help us identify key components involved in plant resistance responses.

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## MAPPING AND CHARACTERISATION OF BROAD SPECTRUM RESISTANCE TO POTATO VIRUS Y IN SOLANUM PHUREJA

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Potato virus Y (PVY), the type species of the Genus Potyvirus, is the most important viral pathogen of potato worldwide and is economically damaging in related crops such as pepper, tomato and tobacco. Potyviruses including PVY are spread, rapidly by aphids, often being transmitted before insecticides repel or kill the vector. Host resistance is the most effective way to control PVY and a few sources of dominant resistance have been reported in wild species of potato; these are Rysto from *Solanum stoloniferum*, Ryadg from *S. tuberosum* spp. *andigena* and Rychc from *S. chacoense*, and they have been mapped to the potato chromosomes XII, XI and IX, respectively. To date, none of these resistance genes have been precisely mapped or cloned.

The James Hutton Institute, Dundee curates a large germplasm collection of wild and cultivated potato species and accessions. We have previously reported the identification of accessions resistant to PVY (strains PVYo, PVYC, PVYN and PVYNTN) and to Potato virus A. PCR markers published for Rysto and Ryadg did not associate with resistant plants suggesting that the *Phureja* resistance is different to the previously known resistances (1). Further genotype analysis of segregating populations using molecular markers and DArT methodology has mapped the PVY resistance to a region in the lower half of chromosome IX, a location not previously associated with any known virus resistances. However, more detailed analysis suggests that resistance is influenced by a second locus in chromosome IV. We found that resistance in some clones was non-functional when the inoculated plants were maintained at 28°C whereas it was maintained in others. The mechanism of resistance was studied by conducting comparative transcriptomic analysis of infected plants at 22°C and 28°C. In addition, a GFP-tagged PVY clone was used to study the early stages of virus replication and movement in *Phureja* resistant plants compared with plants containing Rysto. The results of these studies will be presented.

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## GENETIC DIVERSITY OF POTATO VIRUS Y (PVY) IN NEW BRUNSWICK AND CHARACTERIZATION OF ISOLATES BELONGING TO PVYO STRAIN GROUP

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Potato virus Y (PVY) has reemerged as a major problem in seed potato production in North America. It has been suggested that a change in genetic composition of PVY has contributed to high PVY incidences. To reveal the genetic diversity of PVY in seed-lot potatoes in New Brunswick (NB), Canada, 2000 tubers from 11 cultivars of 20 seed-lots were tested by multiplex RT-PCR, serological and biological assays. Results indicated that PVYO was the predominant strain in the province. However, recombinant strains, namely PVYN:O and European (Eu)-PVYNTN, were also widespread in NB. PVYN:O was identified in 13 of the 20 seed-lots while Eu-PVYNTN was identified in 11 of the 20 seed-lots. Three PVYO variant groups, i.e., PVYO-Oz/-FL, PVYO-139/-RB, and PVYO-SASA-110-like/uncharacterized PVYO type, were identified. To further unveil the properties of various PVYO isolates collected in NB, reprehensive isolates including FL, RB, A28, K6 and NB7 were subjected to sero-, geno- and patho-typing with ELISA, RT-PCR and bioassays. As expected, all isolates but NB7 reacted to PVYO-antibody only, showed a PVYOspecific band in a RT-PCR assay, and induced mosaic on tobacco plants. NB7 not only reacted to PVYOantibody but also reacted to PVYN-antibody 1F5, indicating that it belongs to a PVYO-group termed PVYO-O5, an isolate group has been reported in some parts in USA. Sequence analysis of the complete genome revealed that NB7 was closely related to other O5 isolates. Pathogenetic analysis using potato cv Jemseg unveiled that all isolates incited local and systemic lesions on the plants, but with varying degrees of severity. Further pathological analysis was carried out on Physalis floridana. Previously we have demonstrated that PVYO-CP gene plays a pivotal role in induction of leaf necrosis/leaf drop and plant death in P. floridana. As expected, all isolates incited necrotic responses on the plants. However, unlike other isolate, NB7 did not cause plant death on the plants.

## MUTATIONS IN THE HCPRO PROTEIN OF POTATO VIRUS Y-O ALLOW THE VIRUS TO OVERCOME RESISTANCE CONFERRED BY THE GENE NY IN POTATO

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### Introduction

Potato virus Y (PVY; genus Potyvirus) is the most economically damaging and widely distributed virus in potato. Aphids transmit PVY in the field, which can be controlled by growing resistant cultivars. The gene Nytbr from *S. tuberosum* resides in potato chromosome IV. It is common in potato cultivars and recognizes the 'ordinary' strains of PVY forming the strain group PVYO. Recognition is believed to occur on a gene-for-gene basis and results in a hypersensitive response (HR) at the site of infection. Consequently, movement of PVYO to other parts of the plant is blocked. However, members of the strain group PVYN and the tuber necrosis-inducing variants of PVYN (designated as PVYN-NTN) overcome Nytbr. PVYN-NTN has become common worldwide in the potato-growing areas and causes heavy yield losses in sensitive potato cultivars. There are also other, less common strain groups of PVY (e.g., PVYN recognized by the gene Nctbr) that overcome Ny [1]. Accurate molecular detection of the PVY strains overcoming Ny would be very useful but requires that the genetic determinants of PVY recognized by Ny are identified, which was the aim of this study.

### **Materials and Methods**

An infectious clone of PVY (strain PVYN-605) was kindly provided by Prof. Edgar Maiss, University of Hannover, Germany. The genomic region of PVYN that allows the virus to overcome Nytbr was mapped by replacing the 5'-proximal part of the infectious PVYN clone with the corresponding region of PVYOUK. The resulting virus chimera (5'UTRO5) was used to prepare nine additional PVY chimeras and mutants to narrow down the genomic region of PVYN required to overcome Nytbr. PVY strains, chimeras and mutants were inoculated to three standard differential potato cultivars whose resistance genes and responses to PVY strains are well-characterized: Pentland Crown (Nytbr), Pentland Ivory (Nctbr, Nytbr), and King Edward (Nctbr).

### Results

The viral determinants necessary and sufficient to overcome Nytbr were found to reside within the helper component proteinase (HCpro) residues 227–327. This region was compared in 14 and 31 strains of PVYO and PVYN, respectively, whose strain groups have been determined. Eight residues and the modelled three-dimensional conformation of this HC-Pro region distinguish PVYN from PVYO strains. The PVYO signature comprising the eight key residues of HC-Pro also differed from PVYC and PVYZ.

## **Conclusions and Perspectives**

Our results show that Nytbr recognizes a central region of the HCpro protein in PVYO [2], whereas Nctbr recognizes the C-proximal part of HCpro in PVYC [3]. The results suggested a structure-function relationship in recognition of PVYO HC-Pro by Nytbr. The identified HC-Pro amino acid signatures specific to PVYO and PVYN will facilitate identification of PVY strains overcoming Nytbr.

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## EVALUATION OF THREE MULTIPLEX REAL-TIME RT-PCR METHODS FOR VIRUS DETECTION FROM DORMANT POTATO TUBERS

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### Introduction

For seed certification and the phytosanitary controls the potatoes have to be checked for different viruses. Although ELISA tests are routinely used in many laboratories for this purpose, this technique needs the dormant potato tubers, which are sampled for the control, to be sprouted to rise the titer of viruses above its detection limit, thereby increasing the cost and the time needed for the analysis. Multiplex Real-Time RT-PCR now allows detecting up to five viruses simultaneously directly from dormant potato tubers. In this study, three multiplex Real-Time RT-PCR methods, one pentaplex based on EvaGreen ® dye and two Taqman ® quadruplexes based on new primers and probes combinations, have been tested. The two best methods were compared to the ELISA standard method.

#### **Materials and Methods**

RNAs extracted from leaves and dormant tubers of potatoes were analyzed by one-step Real-Time RT-PCR with three different sets of primers and probes: two sets, consisting of new combinations of primers and probes previously developed by Boonham et al. [1], Agindotan et al. [2] and Singh et al. [3], for the detection of PVY, PVX, PLRV and PVA; and one set, developed by Cheng et al. [4], for the detection of PVY, PVX, PLRV, PVA and PVS. After a selection based on interaction studies and in silico analysis, the two best methods were then compared with an ELISA standard procedure on dormant tubers and on the corresponding fully developed plants respectively.

## **Results**

Comparison of the three Real-Time RT-PCRs with one another showed that they all met their theoretical specifications amplifying the four or five expected viruses with a good sensitivity and repeatability. The interaction studies however, allowed discarding one Taqman RT-PCR because of interaction problems. Similar results were obtained in the comparison of the best Taqman and the EvaGreen detection methods with the ELISA standard method.

### Conclusion and perspectives

These preliminary results showed that, although thresholds have to be further defined, the two selected Real-Time RT-PCRs allow the efficient detection of PVY, PVX, PLRV, PVA (and PVS) from potato leaves and dormant tubers. One of the two Taqman techniques was discarded because of its lack of sensitivity for PLRV in multiple infections and the comparison with the reference ELISA method showed similar results. Despite the fact that additional validation tests have to be carried out, these two selected methods can potentially be used for the potato seed certification and the phytosanitary controls.

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## MICRO-EVOLUTION OF POTATO VIRUS Y THROUGH DEEP SEQUENCING - TESTING THE ROLE OF THE HOST PLANT ON THE VARIABILITY OF THE VIRUS GENOME

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Potato virus Y (PVY) is a major pathogen of potato crops worldwide. Genomic recombination events between viral strains gave rise to multiple PVY variants that are considered as different viral types. The potential impact of the host on the evolution and variation of the PVY genome is however still unknown. Our study aimed at assessing the possible influence of three potato varieties (Bintje, Kennebec and Lady Rosetta) or tobacco on the genome variability of a Belgian N-NTN strain of PVY. PVY-inoculated potatoes were left for 42 days in a controlled chamber before extraction of the total RNAs.

For each PVY-infected potato variety, tobacco as well as the inoculum (original strain) and experimental controls (transcripts from plasmid clones), three targets were amplified by RT-PCR, two covering the coding sequence of the protein HcPro (Hc1 and Hc2) and one for the N-terminal coding sequence of the coat protein (CP). Amplicons were sent for 454 deep sequencing (Beckman Coulter Genomics, USA). More than 500.000 different sequences were generated, sorted and aligned using bioinformatics tools. The results analysis and implications in terms of understanding the PVY micro-evolution under different varietal contexts will be discussed.

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RESISTANCE GENE ENRICHMENT SEQUENCING (RENSEQ) A METHOD FOR RAPID MAPPING OF RESISTANCE LOCI IN SEGREGATING POPULATIONS AND THE NB-LRR GENE FAMILY DISCOVERY AND ANNOTATION

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### Introduction

Plant pathogens can evolve to overcome new resistance (R) genes introduced into crop varieties by plant breeders. This makes resistance breeding a challenging task involving constant recruitment of new R genes, mostly belonging to the NB-LRR gene family. Although whole genome shotgun sequencing has become affordable, and many techniques for NGS-based mapping have been developed, it remains challenging to identify the full NB-LRR gene repertoire and to detect polymorphism directly in R gene alleles. We therefore established a method for R gene enrichment and sequencing (RenSeq), which allows detailed study of the NB-LRR gene family in *Solanum* species [1].

## **Materials and Methods**

Because we study diseases of tomato and potato, we designed Agilent SureSelect 120-mer RNA probes against the annotated NB-LRR complement from the reference genomes of these crops. Target enrichment of tomato and potato NB-LRR R gene homologs followed by Illumina or PacBio sequencing of gDNA and cDNA from parental lines and bulked resistant or susceptible populations generates over 200x read depth for all members of this gene family.

### Results

We successfully applied RenSeq to several sequenced *Solanaceae* model and crop plants, including the reference genomes of potato (clone DM) and tomato (*S. lycopersicum* Heinz 1706 and *S. pimpinellifolium* LA1598). Analysis of the RenSeq data increased the number of NB-LRRs from 438 to 755 in DM, as well as corrected the number to 334 in Heinz 1706. The majority of these identified R gene loci reside in poorly- or previous un-annotated regions of the genome. Additionally, we were able to correct wrongly assembled gene models, using long MiSeq 250bp reads.

We further developed a methodology that applies RenSeq to rapidly identify molecular markers that cosegregate with disease resistance. We were able to apply this method to successfully identify markers cosegregating with resistance genes towards the late blight pathogen *Phytophthora infestans* in several diploid *Solanum* species and also the tetraploid fresh market potato cultivar 'Sarpo Mira'. We further use RenSeq on cDNA and are able to reduce the number of candidate R genes in resistant species by around 50%. Using long MiSeq and PacBio reads we have developed a pipeline to *de novo* assemble NB-LRR complement of yet un-sequenced genomes.

## **Conclusion and perspectives**

We anticipate that carrying out RenSeq on other assembled plant genomes would increase the number of annotated NB-LRR sequences and will enable more targeted and specific resistance breeding strategies. While RenSeq on bulked resistant and bulked susceptible plants leads to the identification of NB-LRR gene alleles that co-segregate with a resistance phenotype, cDNA RenSeq limits the number of R gene candidates to only those that are expressed. A combination of these methods will greatly accelerate the recruitment of natural resistance gene biodiversity for crop improvement.

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### MOLECULAR INVESTIGATION OF IPK'S POTATO COLLECTIONS AT A LARGE SCALE

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### Introduction

The IPK Genebank harbours more than 3,200 cultivated potato accessions (cultivars, landraces, breeding material) originating from 65 countries and five continents. The collection shows a very high diversity, e.g. phenotypically concerning variation in tuber shape, skin colour, flesh colour, but also variation in maturity and utilisation type. The collection contains cultivars described as early as 1760 up to the 21st century. However, despite the age of some of the entries, their genotypic characterization is rather suboptimal. In recent years several studies were conducted to characterize present potato cultivars and landraces on a molecular level (e.g. [1, 2]). In order to make the IPK Genebank entries comparable to modern potato varieties and to provide breeders and researchers with more background on our plant genetic resources (PGR) material, IPK aimed to fingerprint its entire clonally propagated collections using microsatellite markers.

#### **Materials and Methods**

DNA was extracted from freeze dried leaf material following a modified protocol of [3]. Amplification was carried out using a set of 20 microsatellite markers grouped into five multiplexes using the Qiagen Multiplex PCR Kit (Qiagen, Germany). Microsatellites were chosen using the following criteria: each linkage group had to be represented at least once, the polymorphic information content (PIC) had to be high, and some markers should have been used in previous studies [1, 2] to ensure comparability between different studies. Fragment separation was conducted on an ABI377 (Life Technologies, USA) and final analysis was carried out in BioNumerics 7.1 (Applied Maths, Belgium).

### Results

Although currently still ongoing, the study already permitted the detection of duplicate accessions, conclusions on geographic clusters and on the diversity maintained at GLKS. More detailed results will be presented at the EAPR conference.

### Conclusion and perspectives

The mission of genebanks is to preserve crop plants and their wild relatives for the future. Besides collecting and maintaining them, the evaluation and documentation of PGR are a major task of genebanks. In recent years several studies were carried out on subsets of IPK's potato accessions. However, this is the first study on the entire vegetatively propagated GLKS material. It will help in the long run to improve the genebank management by finding and removing duplicates, monitoring diversity and providing more information to genebank users. It also will allow the combination of phenotypic with molecular data and thus give a thorough insight into our collections.

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GRAPHICAL GENOTYPING AS A METHOD TO IDENTIFY IDENTITY-BY- DESCENT OF SNP ALLELES IN A SMALL CULTIVAR PANEL ALLOWED MAPPING OF A PVY RESISTANCE GENE FROM S. STOLONIFERUM CPC 2093 ON CHROMOSOME 11.

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### Introduction

Resistance genes against PVY have been introgressed in potato cultivars from various wild potato species such as the Ry-sto gene on chromosome 12 from *S. stoloniferum* [1] and the Ry-adg gene on chromosome 11 from *S.* andigena [2]. However, Brignetti et al [3] reported earlier than Song et al [1] on the localisation of a PVY resistance from *S. stoloniferum* on chromosome 11, but their work has not received much follow up and their locus name Ry sto has remained confusing since then. Clone F87084 descended from CPC 2093 [4].

Sante is a PVY resistant cultivar also descending from *S. stoloniferum* CPC 2093 but the markers developed by Song et al [5] are not successful to identify the PVY resistance of this clone and other Dutch PVY resistant cultivars.

#### **Materials and Methods**

Next generation sequencing of a panel of 83 tetraploid cultivars and progenitors including Eos, Festien and Y 66-13-636 resulted in the genotyping of 129,156 sequence variants [6]. Sante was not included, but the susceptible parent Kartel was included. Using filters in a .xls file on the 83 cultivars the 129156 rows could be reduced as follows. Specific alleles should be present in Festien and Y 66-13-636 and nulliplex genotypes were selected in columns representing susceptible material.

## Results

Selection of SNPs according to three criteria (1) low allele frequency, (2) present in resistant material, and (3) absent in susceptible clones resulted in patterns of the 535 remaining SNPs in a specific pattern. This pattern is reminiscent of the patterns observed during recombinant analysis in biparental populations. However, the cultivar panel is comprised of highly diverse material. If any haplotypes are shared between members of the cultivar panel, then this could suggest identity-by-descent for those shared haplotypes. We conclude that graphical genotyping is not only suitable to map loci in biparental mapping populations, but also in panels of cultivars hardly related. The graphical genotyping patterns observed here suggest the presence of specific haplotypes uniquely tagged by series of haplotype specific SNPs. Further analysis showed one haploblock shared by Eos, Festien and Y 66-13-636, because all SNP loci had genomic coordinates within a 500 kb interval on superscaffold PGSC0003DMB000000148:284162..814554 [7]. This genomic position is only at 400 kb distance of the well-known R-gene cluster that includes R-genes homologous to TMV resistance gene N.

### Conclusion

We have mapped a locus involved in PVY resistance derived from CPC2093. Analysis of the pedigrees with our pedigree database [8] suggest that the material used by Brignetti et al [3] and the widely used resistance donor Y 66-13-636 could be identical-by-descent. This source of PVY resistance is not associated with male sterility, whereas the resistance analysed by Song et al [5] is maternally transmitted.

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## EXPRESSIONAL ANALYSIS OF *PHYTOPHTHORA INFESTANS* INDUCED RESISTANCE RESPONSE GENES IN POTATO

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Information on the molecular genetic background of biotic stress response is accumulating rapidly. Highthroughput analyzing methods, like next generation sequencing (NGS) enable the real time profiling of whole genome transcripts. In this study, we analyzed the expressional profile of biotic stress response genes which showed transcript number increase in potato after inoculation with *Phytophthora infestans*. Quantitation of the transcripts was done by qPCR. Genes for analysis were chosen from an NGS generated transcriptome (TC) dataset that was established from the potato cultivar White Lady. This cultivar has high tolerance to P. infestans races presently widespread in Hungary. From among the more than 38 thousand transcriptomes of the TC dataset biotic stress response genes were chosen for quantitation according to heat map analysis and according to the RPKM (reads per kilobase per million mapped reads) value, which latter indicates the pathogen inoculation induced changes in the copy number of a transcriptome. Inoculation with the H12/10 P. infestans isolate (containing avr1, 3, 4, 7, 10 and 11) was done on leaves of developed White Lady plants obtained from pathogen-free in vitro plants. Gene expression was tested in the following time points: just before infection (for control), then 1, 4, 17, 24, 31, 48 and 65 hours post inoculation (hpi). In total five different protease inhibitors, four genes belonging to the reactive oxygen species (ROS), three pathogenesis related protein (PR) genes, seven NBS-LRR type P. infestans resistance genes and one plant immune receptor gene was analyzed by qPCR. The beta-tubulin gene was applied as housekeeping gene in the analysis.

All the tested genes belong to gene families which according to the literature play important role in the pathogen induced hypersensitive response (HR). In our TC dataset different number of genes belong to these families (Fig.1) from which in this study only those with different type of homologs and high copy number increase were quantitated. The expressional profile of each gene was different in the tested time interval and results will be summarized and discussed here.

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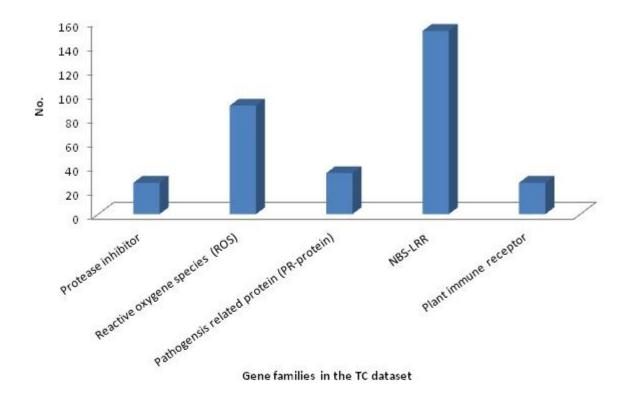


Fig.1. Number of gene homologs belonging to different gene families involved in resistance response

# GENETIC, CYTOGENETIC AND GENOMIC TOOLS TO ASSIST THE USE OF A WILD RELATIVE (SOLANUM COMMERSONII) IN POTATO BREEDING.

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Solanum commersonii (2x=24, 1EBN), a wild species native to Uruguay, is one of the diverse genetic resources in the potato tertiary gene pool. It has attracted the attention of breeders due to its unique resistance to bacterial wilt (Ralstonia solanacearum) and frost tolerance. The main objective of this study is to explore the use of this species by reducing major bottlenecks in introgressive hybridisation breeding through advanced genetic and genomic approaches. To circumvent postzygotic incompatibility and avoid ploidy level manipulations we exploited the production of unreduced (2n) female gametes. S. tuberosum Group Phureja (2x=24, 2EBN) was used as pollinator parent in the first cross. The 3x hybrids obtained were crossed to S. tuberosum Group Tuberosum (4x=48, 4EBN) to produce 5x BC1 individuals. BC2 and BC3 families were obtained by backcrossing those to different Group Tuberosum genotypes. All materials were characterized for resistance to R. solanacearum under controlled conditions and for morphological and agronomical traits in field trials. Cases of remaining aneuploidy were assessed. A detailed description of meiosis was made to evaluate homeologous recombination. Pairing behaviour at diakinesis of the 3x hybrids was studied and quantified by Fluorescent in situ Hybridization (FISH) using combinations of chromosome-specific BAC probes. This same approach is being applied to pachytene complements of S. commersonii to analyze collinearity with S. tuberosum. Additionally, we performed GISH on complements of the hybrids and backcrosses using S. commersonii as probe. A large variation in combining ability was observed in BC2 genotypes through progeny evaluation. Segregation among genotypes was observed for all traits in advanced backcrosses, allowing selecting for bacterial wilt resistance, tuber yield and commercial quality. Segregation patterns suggest that bacterial wilt resistance could be controlled by several genes. Triploid hybrids showed nearly-autotriploid meiotic behaviour, forming up to 12 trivalents indicating high pairing affinity. In these hybrids, chromosomes identified by FISH with potato BACs as probes appear both in III and II+I configurations at diakinesis. The position of BAC signals and loop formation in pachytene trivalents revealed rearrangements between some homeologues. In the backcrosses we observed multiple pairing at pachytene and complex multivalent formation at diakinesis. Even under highly specific conditions, genome painting did not discriminate chromosomes from different species, suggesting little divergence in repetitive sequences. The lack of genome differentiation facilitates homeologous pairing and recombination, but rearrangements may prevent introgression of specific chromosome regions. Comparative cytogenetic mapping and the evaluation of synteny among these species by high-resolution BAC-FISH mapping will help anticipate potential drawbacks like linkage drag. Sequencing of the S. commersonii genome is underway, coupled with optical mapping of stretched DNA in nanochannels to achieve accurate de novo assembly of the genome and detection of structural variations. This will be the first high quality reference genome of a potato wild relative. It will be available for genomic comparisons with other Solanum sequences and it can be a very valuable breeding tool. The genetic materials developed in this project might be a very valuable contribution to the available germplasm of cultivated potato.

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### AN1 IS HIGHLY VARIABLE AND INTERACTS WITH STJAF13 TO COLOUR POTATO LEAVES

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Anthocyanin accumulation is controlled at the transcriptional level by the ternary complex MBW. In this complex, the affinity between MYB and cis-elements of the target gene may be partly influenced by the bHLH partners and, consequently, by the physiological event that it can regulate [1]. An1 is a key gene in potato tissue pigmentation and codifies for a R2R3MYB [2]. Anthocyanins are important not only for tuber quality characteristics but also because the presence of high anthocyanin content in tissues exposed to stress conditions could be an important advantage for plant resistance. In fact, in the leaves these pigments can act as UV-B filters, protect DNA from oxidative damage and defend plants thanks to their antimicrobial activity and feeding deterrents [3]. The aim of our work was to investigate an1 sequence variability in cultivated varieties and to identify the main factors regulating anthocyanin gene expression in potato leaves. We found high variability in both coding and non coding sequences of an1 and, in particular, seven polymorphisms that could cause single amino-acid substitutions in the predicted protein. These may have potentially functional effects, producing structural protein modifications and, consequently, alteration in the stability of the complex with DNA or with other transcription factors. Expression analysis confirmed that an1 is involved in anthocyanin production in leaves. Genotypes with high content of anthocyanins in leaves had the highest number of copies of an1 mRNA as well as the highest number of copies of mRNA of relevant structural genes involved in the biosynthetic anthocyanin pathway. We found a strong association of StJAF13 expression and anthocyanin production in leaves too. An1/StJAF13 interaction was further verified by yeast two hybrid experiments. They confirmed that StJAF13 can physically interact with an1 only when its N-terminal part is present. The interaction of StJAF13 and an1 was confirmed in vivo using BiFC, that also showed a nuclear localization of the interaction. Finally, the effect of an1 and StJAF13 complex was investigated by their over-expression in tobacco. Transgenic plants showed differential phenotypes depending on the transgenes inserted (an1 and StJAF13 as single genes or their combination). Our results may provide the basis to identify genes responsible for anthocyanin biosynthesis, facilitating the selection of progeny with high level of anthocyanins in leaves.

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## CAN AN UNDERSTANDING OF SIGNATURE VOLATILE COMPOUNDS EMITTED BY RHIZOBACTERIA BE USED FOR CONTROL OF DISEASE IN POTATO?

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The problem of how to feed an ever increasing human population, in a sustainable fashion, is probably one of the most intractable issues facing humankind today. The widespread use of expensive agrichemicals for the control of disease not only reduces biodiversity in the soil but may also eventually lead to the development of pathogen resistance if overused. In Europe, the array of agrichemicals on the market is being reduced, in part due to heightened consumer sensitivities with respect to product safety. For agriculture to become sustainable, plant disease-control strategies must become more ecologically friendly with lower inputs. A biological approach which includes the use of beneficial microbes offers an alternative and more sustainable solution. It has been proposed as an alternative to chemical-based approaches for the suppression of plant pathogens and the control of plant diseases in an integrated pest management system. In recent years, the plant pathogen-suppressing action of rhizobacterial volatiles has attracted attention with regard to biological control applications. The beneficial microbes, in the absence of physical contact with plants, release a wide range of volatile organic compounds (VOCs), which may be one mechanism for how these bacteria influence plant health and induce systemic resistance (ISR). Our research project VALORAM 'Valorizing Andean microbial diversity through sustainable intensification of potato-based farming systems' aims at exploring the existing large biodiversity of soil micro-organisms of Andean countries for the development of alternative, efficient technologies and crop management practices to improve the sustainability, food security, environmental protection and productivity of Andean cropping systems, benefiting rural farming households. During the course of our research in VALORAM, laboratory and field-based studies were carried out in order to examine the plant growth promotion and disease suppression potential of rhizobacteria that had been isolated from the rhizosphere of potato in the central Andean highlands. Of ~670 rhizobacterial isolates, ~30 isolates showed antagonistic activity against R.solani and P.infestans on plate assays. Many of the isolates were positive in a number of functional assays including indole acetic acid (IAA) production, 1aminocyclopropane-1-carboxylate (ACC) deaminase activity, phosphate solubilisation, ammonia and HCN production. During growth-room experiments, 23 isolates were associated with growth promotion and/or disease suppression. Gas chromatography coupled with mass spectrometry (GC/MS) was used to detect VOCs produced by rhizobacteria. Volatile analysis of the rhizobacterial strains revealed a range of volatile organic compounds including 2,3-butanediol which is known to be involved in plant growth promotion and in induced systemic resistance. A number of antifungal compounds were detected including 2-hexen-1-ol. In split plate assays, we found that pure synthetic 2-hexen-1-ol can also inhibit R.solani. Field trials were carried out with a selection of these rhizobacterial strains in their respective countries of origin i.e Bolivia, Ecuador and Peru. Of the isolates deployed in the field, some showed a significant response in terms of disease suppression and plant-growth promotion. We suggest that this approach, using appropriate deployment strategies, may offer promise as part of a low-input integrated pest management system.

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## ANTIBIOSIS-MEDIATED BIOCONTROL AGAINST THE POTATO PATHOGENS PECTOBACTERIUM AND DICKEYA

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### Introduction

The potato blackleg and soft-rot diseases caused by the pectinolytic enterobacteria *Pectobacterium* and *Dickeya* are among the major causes for seed potatoes rejecting and downgrading in France and Europe. Currently, there is no effective method to control these diseases and no resistant cultivar. Different biocontrol approaches have been developed since several years. The first aims to target the quorumsensing regulation of the virulence factors in Pectobacterium [1, 2, 3]. A biocontrol *Rhodococcus erythropolis* strain R138 is under evaluation for its survival and blackleg protection in field experiments (see poster of Beury-Cirou et al., EAPR 2014). The second strategy, which is presented here, aims to develop a bio-protection strategy targeting antibiosis for both *Pectobacterium* and *Dickeya* including the new species *D. solani* [4].

## **Materials and Methods**

A large library of bacterial isolates were collected from different environmental samples (healthy tubers, soil, plants or rotted tubers, blackleg expressing plants or contaminated soil). They were screened for antibiosis activity against four pectinolytic strains *P. atrosepticum* CFBP6276, *P. carotovorum* subsp. *carotovorum* 98-1, *D. dianthicola* RNS04-9 and *D. solani* RNS08.23.3.1A. Then, the identified isolates were tested for their ability to inhibit the pathogens in a liquid medium and to reduce soft rot symptoms on tubers. Finally, greenhouse assays were conducted in the presence of *D. dianthicola* RNS04-9.

#### Results

After the screening of 10,000 bacterial isolates, 96 of them were retained because of an efficient antibiosis activity against *Pectobacterium* and *Dickeya*. Then 6 bacterial isolates, which induced no symptoms on potato tubers, were deeply characterized using a genomic approach. They belong to the *Pseudomonas* and *Bacillus* genera. Blackleg incidence of the bacterial antagonists was measured in two different soils in the presence of *Dickeya*. A reduction of blackleg incidence was observed with a combination of the antagonists. Greenhouse assays will be performed again to consolidate the results.

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Virulence test (N=10)



Pa CFBP 6276 alone



P. a CFBP 6276 + Antagonist n°4



P. a CFBP 6276 + Antagonist n°5



P. a CFBP 6276 + Antagonist n°6

# APPLICATION OF RHIZOBACTERIA INOCULANTS IN BIOCONTROL OF BACTERIAL WILT (RALSTONIA SOLANACEARUM) IN POTATO (SOLANUM TUBEROSUM) PRODUCTION

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Bacterial wilt is an important soil borne disease that threatens potato production. It is difficult to control due to the large number of hosts, high genetic and phenotypic variability of the pathogen, systemic localization of the pathogen, and lack of chemical control. Use of antagonistic microorganisms is critical due to their role in suppressing pathogenic infection. The objective of this study was to evaluate the effectiveness of three rhizobacteria isolates in suppressing of bacterial wilt on potato (cv. Tigoni and clone 387164.4). Potatoes were planted under controlled condition in complete randomized block design with 11 treatments: Azoctobacter, Bacillus and Pseudomonas as single, double and triplicate combinations, three organic amendments of poultry, cattle and compost manure and untreated control. Except for single Azoctobacter treatment all the microbial treatments and poultry manure suppressed bacterial wilt infection, reducing area under the disease progress curve by 13-40 compared to control with 53. Triple inoculation of Pseudomonas+Bacillus+Azoctobacter and duo inoculants of Bacillus+ Azoctobacter and Pseudomonas+ Azoctobacter had significantly (P<0.05) higher tuber weight of 362-418g compared to control with 143g. Apparently healthy tubers from Pseudomonas+Bacillus+Azoctobacter in clone 387164.4 were free from latent infection. There was however, no effect of the microbial inoculants and organic amendments on small and medium tuber grades and on tuber phosphorus content. These microbial inoculants were effective as biocontrols under controlled condition but more studies need to be undertaken to ascertain their effectiveness.

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## THE EFFECT OF TUBER PRE-PLANTING THERMAL TREATMENTS AND HUMIC PREPARATION 'RUPONICS' ON POTATO TUBER DISEASES

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Bacterial and fungal tuber diseases are major threats to potato industry. Therefore treatments or factors that increase the occurrence of tuber diseases should be avoided while growing potato. We aimed to investigate how different tuber pre-planting treatments such as pre-sprouting and thermal shock and different used humic preparation (HP) Ruponics quantities are affecting the tuber disease occurrence such as common scab (*Streptomyces* spp.), silver scurf (*Helminthosporium solani*), dry rot (*Fusarium* spp.) and soft rot (*Erwinia* spp.).

The field trial with the potato cultivar 'Laura' (middle-maturing, bred in Germany) was conducted in 2011. Seed tuber pre-planting treatments were as follows: 1) T0 – non treated (control). Tubers were planted directly from storage; 2) TS – thermal shock. Seed tubers were kept 5 days in a room with a temperature of 30°C and 2 days in a room with a temperature of 12°C; 3) PS – pre-sprouting. Seed tubers were kept 26 days before planting in a room with a temperature of 15°C and 10 days in a room with a temperature of 12°C. The used HP quantities were as follows: 1) HP0 – without HP (control); 2) HP25 – HP (25 I ha<sup>-1</sup>) is sprayed on the surface of the soil before planting the tubers; 3) HP50 – HP (50 I ha<sup>-1</sup>) is sprayed on the surface of the soil before planting the tubers. In October 2010 all the treatments received composted cattle manure 50 t ha<sup>-1</sup> (N217P103K193 kg ha<sup>-1</sup>) and mineral fertilizers during the potato planting with the amount of N30P13K48 kg ha<sup>-1</sup>.

The effect of tuber pre-planting treatments on tuber disease occurrence:

- The tuber pre-planting treatments had a non-significant influence on the occurrence of potato common scab. Overall 16.3 (T0) to 19.7 (TS) percent of the yields were infected with common scab.
- TS decreased significantly the occurrence of dry rot having no yield infected compared to T0 and PS treatments which both had 0.7% of the tubers infected.
- To had significantly less tubers infected with silver scurf compared to TS and non-significantly less tubers infected compared to PS. Overall 3.0–9.3% of the tubers were infected with silver scurf. The effect of HP quantities on tuber disease occurrence:
- The use of HP increased significantly the occurrence of common scab. About 10 (HP0) to 21.7 (HP25) percent of the tubers were infected with common scab.
- The use of HP25 decreased significantly the dry rot infections. HP0 and HP50 both caused some problems with dry rot having 0.7% of all the tubers infected with the disease.
- HP had non-significant influence on silver scurf infections which ranged between 5.3 (HP25) and 6.0 (HP0, HP50) percent of the overall yield.
   No dry rot lesions were found.

The results after one-year experiment revealed that tuber physiological ageing with higher temperatures may have a positive effect against tuber dry rot occurrence, because the rapid healing is promoted by raising the temperatures. As HP Ruponics is a liquid extract from composted cattle manure the common scab incidences were higher when the HP was used. However HP25 helped to decrease the dry rot incidences.

## HIGH-THROUGHPUT SCREENING OF RHIZOBACTERIA WITH ANTAGONISTIC AND ELICITATION ACTIVITIES FOR POTATO PROTECTION.

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The increasing acreage of organic crops altogether with policies more restrictive regarding the number and quantities of pesticides available for agriculture stimulate research of new strategies for crop protection in both organic as well as integrated pest management applied to potato crops.

A high throughput screening method was used to select high potential bacterial strain to be used as growth promoting rhizobacteria for putative application as biopesticide.

More than 2600 bacterial strains of Pseudomonas spp. and Bacillus spp. have been collected in fifteen soils, composts and potato plants in the Waloon region. Their antagonist activity was assessed in vitro against three pathogens of potato: *Phytophthora infestans*, *Fusarium solani* and *Pectobacterium carotovorum*. On the 2600 selected strains, 45 Bacillus spp. and 15 Pseudomonas spp. showed direct activity against one or some of the pathogens. Such strains were further tested for their eliciting abilities. Mutant Arabidopsis were used to make a first selection between the bacterial strains: the reporter GUS fused with an Arabidopsis plant defensin gene was used to indicate the elicitation of induced systemic resistance by the tested bacteria. The Arabidopsis with the higher GUS-expression were selected and expression of selected Arabidopsis genes involved in ISR was followed by RT-qPCR. Finally, the nine best ISR-inducing bacteria were selected and further evaluated under greenhouse condition. Possibilities for large scale applications as well as putative side effects will be discussed.

### AGROBODY-MEDIATED CROP PROTECTION

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AgroSavfe employs its proprietary Agrobody <sup>™</sup> technology platform to develop superior crop protection products, based on active ingredients (a.i.) with proven efficacy and Agrobodies <sup>™</sup> as formulation agents. Agrobodies <sup>™</sup> are derived from camelid antibodies and can be generated against virtually any target, to which they bind with high affinity and specificity. Agrobodies <sup>™</sup> directed against crop produce, leaves, seeds, pests or particular structures thereof enable targeted delivery and retention of the a.i. at or near its site of action. Targeted delivery and improved retention of Agrobody <sup>™</sup>-based crop protection products allow for reduced application dosage and for extended performance with reduced application frequencies.

Control of potato late blight, caused by the water mold *Phytophthora infestans*, requires maintaining an effective dose of fungicide on the plant surface. Therefore, there is a need for weekly preventive fungicide spraying during the entire growing season, resulting in the application of large quantities of fungicides [1]. However, retention of foliar applied pesticides depends on the characteristics of the spray, the spray deposits and the leaf surface, on the formulation of the pesticide, water solubility of the a.i. and on environmental factors, of which rainfall has been reported as having the greatest effect upon the residual activity. The reduction in efficacy of the pesticide depends on the quantity and intensity of rain, the time between application and rainfall and the resistance of the pesticide to rainwash [2] [3].

AgroSavfe is aiming to extend fungicidal activity by improving rainfastness of fungicides formulated with Agrobody ™ binding technology. Therefore a series of Agrobodies™ have been generated and selected that exhibit strong binding to potato leaves. Such Agrobodies™ have been coupled to microcapsules, containing registered a.i. with proven activity against *P. infestans*. To study the retention behavior, the encapsulated a.i. coated with Agrobodies™ (Agrocapsule) have been sprayed on isolated leaves and on potato pot plants and different rain volumes have been applied using a rainfall simulator. Several Agrocapsules have shown an increasing retention to wash off at increasing rain volumes.

In conclusion, retention of crop protection products on potato plants can be improved with an Agrobody <sup>TM</sup> based formulation. Such improved retention would allow extending the activity of a fungicide so that the interval between fungicide applications can be extended whereby overall use of fungicide is reduced and costs and time, associated with each application, are drastically decreased.

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## IN ROW APPLICATION OF NITROGEN IN WARE POTATOES

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### Introduction

Residual soil nitrate after the harvest of potatoes is often too high. Nitrogen (N) efficiency of potatoes is low. This is among other things due to the poorly developed root system of the plants. N uptake by potatoes from soil layers deeper than 60 cm is poor and plant roots in adjacent rows overlap barely. By means of fertilization in the row, the fertilizer is better positioned, close to the seed tubers. This technique should allow reducing the total dose of applied nitrogen, a better utilization of the given nitrate and a lower residual soil nitrate while maintaining yield and quality.

In the framework of the project "Bring N to the potato to utilize N more efficient" (financed by the Flemish Government), 11 field trials were set up in 2012 and 2013. On these fields, the use of fertilization in the row in potatoes was demonstrated.

## Trial setup

Depending on availability, very different machines were used with solid or liquid N fertilizer. The majority of the row application occurred in one passage together with planting and ridging. The latter is a common practice in Belgium, especially in sandy, sandy loam and loam soils. In clay soils planting and ridging form 2 separate passages. In this case N was applied in the row just before or during ridging.

The applied N-dose depended on the field and was based on soil sampling on each field prior to planting. On all fields the fully advised N-dose was applied as well broadcast as well in the row. These modalities were compared with application of only 80% of the advised N-dose at planting and with a split application of 80% N at planting in the row and the remaining 20% broadcast a few weeks after planting. At harvest yield, grading and dry matter content of the potatoes were determined as well as the residual soil nitrate (up to 90 cm depth).

### Results

After two years of trials first conclusions can be drawn. Use of the full advised N-dose at planting in the row or broadcast showed little difference in terms of total yield, gross grading, dry matter content of the tubers and residual soil nitrate. Applying 80% of the N-dose in the row at planting supplemented with 20% N after emergence resulted in a slightly higher yield, but also in a clearly lower soil nitrate after harvest. Lower soil nitrate was also obtained when only 80% of the advised N-dose was applied in the row, but in this treatment also total yield showed a decline and tubers showed a finer grading. So from our field trials, we could not conclude that it's possible to save 20% nitrogen by row application.

# NUTRIENT LEGISLATION VERSUS FERTILISATION PRACTICES IN POTATO FIELDS IN FLANDERS, BELGIUM

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In Flanders, the Manure Decree was issued to reduce water pollution by nitrates and phosphates from agricultural origin and to prevent future pollution. Flanders has a long tradition in livestock breeding and in the application of animal manure, resulting in intensive nitrate leaching and poor water quality. In the Manure Decree several measures in relation to fertilisation, like fertilisation standards are set out. The fertilisation standards limit the amount of nitrogen and phosphate which can be applied. Also, Flanders has translated the norm of 50 mg  $NO_3$ -N/l in the surface and groundwater in a limit for residual nitrate in the soil. At the end of the growing season (1/10-15/11) the residual nitrate (in kg  $NO_3$ -N/ha) (0-90 cm) is determined.

In this paper the area of tension between nutrient legislation on one hand and fertilisation practices in potato on the other hand will be illustrated based on field research and practical examples. The focus will be on the importance of adequate crop and parcel specific fertilisation advices for N and P and the optimal use of manure in fertilisation practices.

For potato, a maximum of 65 kg  $P_2O_5$  /ha.year and a total of 190-210 kg effective N/ha.year can applied. For N only 170 kg/ha.year can applied as manure. As a consequence of these strict regulations and in order to maximise the potato yield with respect for the environment, it is of great importance to fertilise judiciously based on a soil analysis.

An optimal P-fertilisation is crop specific and parcel specific. The Soil Service of Belgium (SSB formulates each year thousands of fertilization recommendations based on soil analyses. Soil samples are taken in the ploughing layer (0-23 cm) in order to determine the overall soil fertility (pH, C, P, K, Mg, Ca). Liming and fertilization recommendations are then calculated by the BEMEX expert system. The majority of potato parcels in Flanders has a rather high to very high phosphorus content. On average a phosphorus fertilisation advice of 40-70 kg  $P_2O_5$ /ha is given for these potato parcels.

In relation to nitrogen, potatoes have a high nitrogen need but a low uptake efficiency. This low uptake efficiency in combination with high nitrogen fertilisation often results in a high residual nitrate. In 2012 the residual nitrate exceeded the limit in more than 60% of the sampled potato parcels. In order to control this residual nitrate an adequate nitrogen fertilisation is primordial. In Flanders many nitrogen fertilisation advices are calculated based on the N-INDEX. The N-INDEX is defined as the amount of nitrogen that will be available to the crop during the growing season. Figure 1 shows the average mineral N content (0-90 cm) in potato parcels at the beginning of the growing season and the corresponding fertilisation advice.

Finally, as the application of manure is a definit part of fertilisation management in Flanders, manure should be applied optimaly. Most farmers calculate the amount to be used based on average compositions. However manure analyses show a wide variety in nitrogen content. For example, 20 tons of pig slurry contains 180-220 kg N on average, but analyses by SBB show that the N-content can vary between 40 and more than 300 kg N per 20 tons. The importance of a manure analysis and its impact on the applied amount of N and P and on the residual nitrate will be illustrated.

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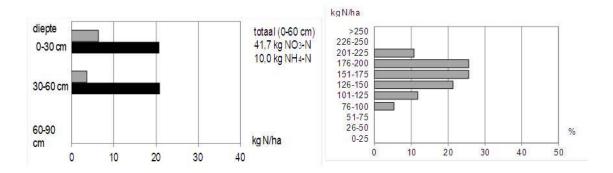


Figure 1: Average mineral N content in the soil (0-60 cm) on parcels with organic fertilisation and green manure (sampled between 1/1/2008 and 15/3/2008) left); Distribution of the corresponding N-fertilisation advices for potato (right).

### PHOSPHORUS AND POTASSIUM FERTILIZATION OF POTATO IN FRANCE: A REVIEW

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### Introduction

Potato has always been one of the crops which are fertilized with the largest amounts of P and K in France. This situation is due to its sensitivity to PK deficiencies which could impact both yield and tuber quality. Nevertheless, several indications lead us to think that optimum PK applications will be more difficult to achieve in the future. Among them, there are the progressive rising of fertilizer prices, the question of P availability at mid-term, and the impact, on soil PK availability, of unbroken PK application decrease on the other crops in the rotation. To face these challenges, we thought it was time to make a review on the PK fertilization management in France (including the review of scientific results supporting it) to identify the way of progress to guarantee an optimal PK nutrition of potato in the future.

## PK rate calculation and application methods

Since 1993, French agronomists have used a method, so called "COMIFER method", based on four criteria to calculate PK fertilizer rate [1]. This tool was parameterized on one hundred long term trials carried out during 70's and 80's, and updated in 2009. The first criterion is the yield crop sensitivity. Potato had been classified as high sensitive crop to K and P. A review of data available showed that, if this classification is justified for K, it could be discussed for P. The importance of K is reinforced by its impact on tuber quality. The second criterion is the soil PK availability. The last two criteria are recent fertilization practices and the management of previous crop residues. The specificity of this method is to link PK application rate to PK amount harvested using a multiplying factor. While avoiding yield loss, the calculation leads also to an increase or a decrease of PK soil content depending on which side the soil analysis is, compared to a threshold parameterized for each soil type. The method could be used both in a tactic way (avoiding yield loss one year) and in a strategic way (maintaining PK availability in soil). Aside of application rate calculation, we also reviewed the data available in France and other countries with similar climate, regarding 1) impacts of fertilizer type on tuber yield and quality and 2) the general impact of date of fertilizer application (including localization at planting).

### Soil PK availability forecasting

Using "COMIFER method", soil analysis databases and relations between PK exports and soil analyses evolution, we were able to make a cartographic diagnosis of PK deficiency risks for high sensitive crops like potato, and some forecasts on its evolution under different agricultural practice scenarios. In the intensive production area of northern France, it appears that the current situation is rather comfortable and could allow some PK application decrease. Nevertheless, a 20 and 40 years forecasts under no PK application or "COMIFER method" scenarios lead to the increase of problematic situations in specific areas. Although the hypotheses supporting this study are questionable, it delivers some indication on foreseeable futures.

### Conclusion

The reviewing of data available to assess the best PK fertilization practices will help us to identify the right tools to face the foreseeable problems according to the evolution of PK availability for farmers in the future.

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### RESPONSE OF TWO POTATO CULTIVARS TO NITROGEN FERTILIZATION IN SWITZERLAND

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Potatoes require high nitrogen (N) fertilizer rates because of their poor N efficiency. Previous studies have reported large variations in N fertilizer use efficiency (NUE) among potato cultivars. A three-year field experiment was conducted by the Swiss Research Station Agroscope ACW from 2009 to 2011 to investigate the yield and quality response of two commercial potato cultivars, Bintje and Laura, to N fertilization. Five doses of ammonium nitrate were tested: 0, 80, 120, 160 and 200 kg N ha-1 applied in one to three applications. Leaf and root growths, total yield, starch content and tuber sizes were measured annually. In 2011, the total N uptake and the soil mineral N content were also measured during the growing season and at harvest.

The study showed that N fertilization had a positive effect on yield and on the percentage of large tubers (>70mm), and a negative effect on starch content. Both cultivars presented the same potential yield, although the optimum N fertilizer rates were 155 and 94 kg N ha-1, respectively, for Laura and Bintje cultivars. Laura's yield was more affected by nitrogen fertilization deficiency but also more responsive to the late N fertilizer application. At harvest, both cultivars had similar N uptake efficiency (NUpE) and N utilization efficiency (NUtE). However, they differed with respect to N uptake dynamics. N uptake was slower for Laura than Bintje due to a longer time period required for the development of the root system. Thus, Laura had a lower NUpE than Bintje 65 days after planting (dap) which resulted thereafter in a weaker NUtE at 80 dap.

The results provide useful recommendations for improvement of N fertilization practices (e.g. rate and time of application) of these two cultivars in Swiss conditions.

## SILICA FERTILIZATION OF POTATO TO IMPROVE TUBER QUALITY UNDER CHANGING CLIMATE

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## Introduction

Silica (Si) is an abundant element in the soil and is considered non-essential nutrient for agriculture. However, soil silicon dioxide is of low availability to the plants, and field data point at the beneficial effects of Si fertilization in the form of silicic acid on plant resistance against biotic and abiotic stresses. The mechanism of resistance is unknown yet, and it was suggested that Si enhances the endogenic defense response of the plant. It was also suggested that Si reinforces cell walls by enhancing the synthesis of lignin and suberin. The latter is a macromolecule consisting of lignin-like aromatic domain and cutin-like aliphatic domain.

Potato skin protects the tuber against water loss, pathogen invasion and mechanical wounding. Potatoes grown in regions characterized by hot climates are prone to skin russeting which result with decreased tuber quality in storage and a reduction in tuber marketability [1,2]. As potato skin is made of lignin and suberin, the possible contribution of Si to improve skin quality was tested. The effect of Si fertilization on potato drought tolerance was also monitored.

## **Materials and Methods**

Plants of *Solanum tuberosum* L. Winston were grown in a greenhouse in 50L pots filled with perlite. Plants were irrigated with nutrient solution and Si treatment was applied by manual application of 100ppm Si solution (Sodium silicate) to the pots. At eight and eleven weeks post sprout emergence tissue samples were collected: leaves, stems, roots, stolons, tuber peel and tuber flesh. Drought treatment was applied by disconnecting the irrigation until leaf wilting. Samples as above were collected two days after reconnecting the irrigation. Tissue samples were used for Si determination, RNA extraction, and gene expression by quantitative PCR, and histological studies by light, UV and Raman microscopies.

### **Results**

The gene coding for Si transporter was identified in the potato genome and its cDNA (StSi) was isolated, showing high homology to known Si transporters and conservation of amino acid domains characteristic for aquaporins. StSi transcripts were detected only in root and leaf tissues and their level was increased two folds in plants fertilized with sodium silicate. The expression of StSi in roots was not affected by drought treatment; however its expression in leaves was about five folds higher in plants that were fertilized with Si and also exposed to drought stress compared to controls.

High level of the Si mineral was detected in the tuber peel of Si fertilized potatoes which was also accompanied with increased dry matter and alteration in skin morphology. No change was detected in skin constituents as monitored by Raman microscopy; however up-regulation of suberin specific genes was monitored in Si treated samples. No Si mineral was detected in tuber flesh and no change in tuber flesh dry matter was detected in Si treated, compared to control plants.

## **Conclusions and Perspectives**

Potato accumulates low levels of Si, probably due to low density of Si transporters and lack of transporters in the tuber. Future goal would be to genetically manipulate Si uptake to improve tuber quality and potato ability to overcome stresses.

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# STRATEGIES FOR N-FERTILIZER APPLICATION FOR CHIPPING POTATO TO MINIMIZE NITROGEN LOSSES IN FLORIDA

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Potato is cultivated in Florida during winter and spring and it is considered a high value crop, which typically requires considerable amounts of nitrogen fertilizer to minimize the risk of yield reductions due to nutrient deficiencies. Most of the production areas in Florida rely on seepage irrigation, which is the management of the water table to a depth just below the plant root zone by either adding or removing water from the field depending on the target water table level. One of the challenges of seepage is the nutrient management because during rainfall events, the fields need to be drained quickly to accommodate the volume of rainfall avoiding flooding conditions. Since N is very mobile in the soil and an important component in the potato plant's biological processes, it is critical to determine an optimal Nfertilizer rate and timing of application that maximizes potato production, quality and reduce N losses. The objective of this study was to evaluate the effect of varying levels of N fertilization and application timing to determine the most efficient strategy to increase N use efficiency and reduce N losses. In particular, this study focused on the effect of different N-rate applications at the plant emergence stage (EMN) and sidedress at the beginning of development growth stage the effects (SDN). Four N-fertilizer rates were applied for EMN: 0, 56, 112, 168 kg/ha and two N-rates applied at SDN: 56 and 112 kg/ha, the N-fertilizer rates were compared using a factorial treatment design with four replicates. Total N-fertilizer applied ranged from 112 to 336 kg/ha. Data were collected in three consecutive years for potato variety 'Atlantic' with three farms participating in the study and treatments arranged in a randomized complete block design within each farm. Repeated measurements of soil and plant tissue were taken at specified stages to monitor changes over the course of the growing season. The effects of EMN and SDN on soil N levels, plant biomass, plant N content, and potato yield were quantified. Applied N-rates and rainfall significantly affected the levels of inorganic N in the soil. Total rainfall during the potato season occurred in 2010 was 443 mm compared to 262 mm in 2011; and 165 mm in 2012 season. There was a quadratic response of increase of N-fertilizer rates at EMN in the soil N. However, analysis of tissue data showed that higher EMN rates did not increase plant N content and the application of 112 kg/ha of N at SDN slightly increased plant N content. The maximum daily N uptake happened between 55 to 70 days after planting which coincided with flowering stage and beginning of the tuber bulking stage, the peak of daily N uptake ranged from 3 to 5 kg/ha.day. The average yield ranged from 15 to 33 Mg/ha in 2010; 29 to 41 Mg/ha in 2011 and 33 to 39 Mg/ha in 2012. There was an increase in yield due to the EMN only in 2010 as a response to the large precipitation. There was no increase in yield or plant N uptake with application of Nrate above 56 kg/ha at SDN. The lack of response to N-fertilizer rates may be attributed to the insufficient time for the crop to respond to the SDN application. Potatoes were harvested around 100 days after planting. There was an increased concentration of soil N, which was associated with higher N-treatments however this was not correlated to an increase in potato yield. At harvest, residual soil N ranged from 5 to 40 mg/kg with respect to increasing N application.

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# AGRICULTURAL AND AGRO-INDUSTRIAL AMENDMENTS FOR THE MANAGEMENT OF POTATO CYST NEMATODES (GLOBODERA ROSTOCHIENSIS AND G. PALLIDA)

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Potato cyst nematodes (PCN), Globodera rostochiensis and G. pallida are the most economically important nematode pests of potato in Europe causing an annual loss of approximately €220 million. PCN are managed by an integrated management approach including the use of crop rotation, nematicides and resistance. However, nematicides are costly and their use generates environmental concern. Resistant cultivars are not always responding to expected quality and high yielding cultivars resistant to G. pallida are limited in number. Therefore, the development of alternative methods for managing PCN is needed. Annually, a large amount of agricultural and agro-industrial waste is produced and available as fertilizer. Application of animal manures has been a traditional control method for plant-parasitic nematodes. New types of composts and organic materials are now available but their effects on PCN are unknown. Therefore, we studied the effect of 10 soil amendments (pig slurry, woody biochar, biochar-blended pig slurry, cattle slurry, farmyard manure, crab shell compost, biochar-blended crab shell compost, nitrogen fertilizer, wood chip compost and biochar-blended wood chip compost) on the survival and the reproduction of both PCN species. These amendments were mixed with a sandy soil and added to 2-literpots (survival test) or 4-liter pots (reproduction test). Non-amended soil was used as a control. Cysts of G. rostochiensis or G. pallida were placed in retrievable nylon bags and added to the soil in each pot. Pots were left outside, exposed to prevailing weather conditions, in a randomized design with 4 replicates. For the survival test, the viability of the cysts content was determined at 8, 12 and 16 weeks after soil infestation (WAI) by visual assessment and by measuring the trehalose content of the eggs. Results showed that exposure of cysts to pig slurry, nitrogen fertilizer and wood chip compost caused a reduction in the viability of eggs of PCN 8 and 16 WAI. The other amendments had no effect on the survival of Globodera species. To determine the effects of amendments on the reproduction of each Globodera species, one potato tuber was planted per pot and left to grow. Sixteen WAI, plants were harvested and nematode reproduction was determined. All amendments significantly reduced the reproduction of both species. Pig slurry caused the greatest reduction in the reproduction rate of PCN followed by wood chip compost and nitrogen fertilizer. Addition of biochar to some of the amendments resulted in more nematode reproduction compared with the application of each amendment alone. Our research demonstrated that application of certain agricultural and agro-industrial products affects cyst survival as well as reproduction when the host is present. These findings are important for managing PCN. The mechanisms involved in nematode suppression by soil amendments will be further investigated.

# LESION NEMATODES: FIELD DAMAGE IN POTATO BY *PRATYLENCHUS PENETRANS*, ITS ASSOCIATION WITH TUBER SYMPTOMS AND ITS SURVIVAL IN STORAGE

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Potatoes in Norway are grown annually in 14,332 ha, yielding 350,000 tonnes, which represent an income of approximately € 62.5 million. For farmers the annual costs of skin blemish diseases imply approximately € 3.5 million [1].

In the summer of 1996 a growth depression in potato (cv. Saturna) suggestive of nematode damage was detected in Grue eastern Norway. Analyses of samples for potato cyst nematodes (*Globodera* spp.) were negative, but demonstrated the occurrence of large number of root lesion nematodes *Pratylenchus* penetrans [2]. Tubers from the field had severe symptoms of common scab *Streptomyces* spp. [2].

Analysis of soil samples from the growth depression yielded large numbers of root lesion nematodes *Pratylenchus penetrans*. Growth was greatly reduced in the centre of the patch, but improved gradually towards margins. Transect- sampling showed plant growth to be negatively correlated with densities of *P. penetrans*. Our data suggests a threshold for damage of lower than 100 *P. penetrans* per 250g of soil. *P. penetrans* was present in roots, underground stems, stolons and tubers. Common scab (*Streptomyces* spp.) occurred frequently in the field [2]. In tubers nematodes occurred in the outermost 0.5 mm of the tissue and frequently inside cross-lesions similar to lesions caused by common scab [2]. The development of symptoms was studied in the greenhouse on potato (cv. Saturna). Compared to non-inoculated controls *P. penetrans* alone did significantly increase the extension and severity of scab-like symptoms on tubers, and this was also recorded as a tendency in the combined nematode-bacterium treatment. It is possible that damage by *Streptomyces* spp. may be confused with symptoms caused by the lesion nematode *P. penetrans* [2].

To study the survival of nematodes, tubers from the field were stored at 4oC, for 20 weeks, and transferred to pots with sterile sand and grown for 3 months. After harvest stolons, tubers and soil were examined for the presence of lesion nematodes. Our study confirms that *P. penetrans* survives storage of seed potatoes, from which new infections may develop [2].

Further studies were conducted after the growing seasons 2008 and 2009, a total of 241 potato lots with symptoms of scab and scurf representing different cultivars and counties were analysed for the incidence blemish diseases [1]. The occurrence of *Pratylenchus* spp were investigated on selected 133 potato lots with scab symptoms. Our studies indicated the occurrence of *Pratylenchus* spp. on potato in 60 % of examined subsamples with common scab symptoms [1]. The relationship between root- lesion nematodes and *Streptomyces* spp. does still not clear and need further studies.

Potato tubers do appear to be an important means for the spread of *Pratylenchus* spp to new areas.

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## LIFE CYCLE OF THE ROOT-KNOT NEMATODES *MELOIDOGYNE CHITWOODI*, *M. FALLAX* AND *M. MINOR* ON POTATO AND CONSEQUENCES FOR DAMAGE DEVELOPMENT.

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The temperate root-knot nematodes Meloidogyne chitwoodi, M. fallax and M. minor are able to reproduce on potato and cause quality damage to the tubers. As tubers are not present at initial penetration of the nematodes into the plant roots, it is the second generation that initiates damage. Knowledge on the life cycle duration and the time at which a second generation is formed is therefore of paramount importance to avoid damaged tubers. To assess the life cycle of M. chitwoodi, M. fallax and M. minor, young potato plants were inoculated with freshly hatched second-stage juveniles (J2). The developmental stages of the root-knot nematodes were recorded at weekly intervals after inoculation until second-generation J2 were detected. For M. chitwoodi and M. fallax the degree-days (DD5, base temperature 5°C) required for completing their life cycle were 555-740 DD5. Between 606 and 727 DD5 were needed for M. minor to complete the life cycle. The host plant status of five potato cultivars (Asterix, Bintie, Nicola, Lady Rosetta and Première) for M. chitwoodi, M. fallax and M. minor was determined by evaluating egg mass formation. The three Meloidogyne species developed less egg masses on cv. Première when compared with the other cultivars but in general high numbers of egg masses were found on all cultivars. It is clear from our results that the production of commercially important potato cultivars can be threatened by M. chitwoodi, M. fallax and M. minor as they complete their life cycle within the average crop cycle and second generations can affect the tubers. Therefore, further spread of these nematodes in agricultural fields should be avoided.

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# ASSESSMENT OF PCR-BASED TOOLS FOR THE SPECIFIC IDENTIFICATION OF THE TEMPERATE *MELOIDOGYNE* SPECIES, *M. CHITWOODI* AND *M. FALLAX*

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## Introduction

Root-knot nematodes belonging to the genus *Meloidogyne* are responsible for damage on many crops worldwide. *Meloidogyne chitwoodi* and *M. fallax* are two regulated species in Europe that can affect potato, carrot, salsify, tomato... Reliable identification tests are highly important for crop inspection and testing, soil monitoring and management of preventive measures in order to limit their introduction and spread in non-affected areas. Meloidogyne species are morphologically similar and it is often difficult to differentiate them on this basis. Molecular tests can easily be performed in routine analysis laboratories and on different stages of the nematode (juvenile, female, male). Therefore, performance criteria of three conventional PCR tests recommended by the EPPO protocol PM7/41 for *Meloidogyne chitwoodi* and *M. fallax* diagnosis were assessed in this study (1).

## **Materials and Methods**

27 populations of nematodes belonging to 10 *Meloidogyne* species were used for the assessment of the different PCR assays. 11 populations of other genera of nematodes were also included to check the specificity of these methods. Samples prepared with variable number of J2 belonging to *M. chitwoodi*, *M. fallax* and *M. hapla* in mixture were also tested to check the ability of the three PCR methods to specifically detect the targeted species.

DNA extraction was performed using lysis and bead beating steps. Three conventional PCR tests were assessed according to the original description: a species-specific PCR [2], a SCAR PCR [3] and the rDNA ITS PCR-RFLP [4].

The performance criteria evaluated for each PCR test were sensitivity, repeatability, reproducibility, specificity and accuracy according to the EPPO standard protocol for test validation (PM7/98, EPPO 2010).

### Results

The three tests were 100% sensitive, specific and accurate for *M. fallax* identification.

For *M. chitwoodi* identification, the Wishart test was less specific but more sensitive (respectively 97% and 100%) than the two others (100% for the specificity and 80 to 92% for the sensitivity). Experiments carried out with mixtures of different proportion of *Meloidogyne* species gave a positive result for all the replicates tested with the Wishart et al. (2000) primer set, whereas the two other sets did not allow the DNA amplification of all the replicates of the J2 mixture of 1 *M. chitwoodi*, 1 *M. fallax* and 8 *M. hapla*.

### Conclusion and perspectives

Based on these results, the Wishart test, which is the most sensitive one, could be recommended for surveys. The SCAR PCR (Zijlstra, 2000) could be used to confirm the positive detection results because of its high specificity. For diagnosis purpose, the PCR-RFLP test could be used to identify the species present in the sample.

The evaluation of these tests should continuous, taking into account any new description of the genetic diversity of the target *Meloidogyne* species. Tests newly published should also be included in this continuous assessment.

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NB: This assessment has been done in collaboration between ANSES-LSV (Plant Health Laboratory) and FN3PT (French Federation of Seed potato Growers) with a grant from the French Ministry of Agriculture (project CASDAR IP 0934)

## GENE TRANSCRIPTION CHANGES DURING COMPATIBLE AND INCOMPATIBLE INFECTIONS OF POTATO BY GLOBODERA ROSTOCHIENSIS

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The golden potato cyst nematode, "Globodera rostochiensis", is one of the most important pests of potato. Several pathotypes of the nematode exist, and they may be controlled by one of several sources of single-gene resistance in potato. Here we report that SW93-1015, a breeding line from the potato breeding program at SLU Alnarp, has phenotypic resistance to the Ro1/4 pathotype of "G. rostochiensis". Reproduction of "G. rostochiensis" pathotype Ro1/4 on SW93-1015 is approximately 5% of reproduction on susceptible on the susceptible cultivar Desiree. To further investigate the interaction between potato cyst nematodes and susceptible and resistant host plants, changes in gene transcription were monitored just after root penetration and during syncytium formation in Desiree and SW93-1015 potato plants infected with Ro1/4 "G. rostochiensis". RNA expression in root tissue was analyzed on a microarray at 0, 8, and 48 h post-infection, corresponding to pre-infection, root penetration, and early syncytium formation. Changes in gene transcription over the process of infection in susceptible and resistant genotypes are discussed. The resistance will be further investigated using a crossing population if Desiree and SW93-1015.

# WHICH RESISTANCE ORIGIN TO EXPLOIT FOR DURABLE RESISTANCE TO THE POTATO CYST NEMATODE GLOBODERA PALLIDA?

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Potato Cyst nematodes (PCN) are one of the major problems for the potato crops. INRA group focuses on the resistance to the cyst nematode *Globodera pallida* and exploit three different resistance sources originating from *Solanum vernei*, *S. sparsipilum* and *S. spegazzinii*. All these species have in common one major QTL located on collinear position on the chromosome V, named GpaV [1-3]. Advanced tetraploid materials from *S. vernei* have been obtained and lledher is the first resistant cultivar registered in ware potato category of the French catalog.

The purpose of this study is first to evaluate the resistance durability of genotypes originating from *S. vernei* which carry the major QTL GpaVvrn but differ for their genetic background. In an experimental nematode populations evolution, we have shown that the nematode populations can adapt to *S. vernei* resistant potato genotypes, and that the resistance durability of these genotypes depends on their genetic background. Moreover we highlighted a trade-off between the adaptation to a resistant potato genotype and the adaptation to another one [4].

In a second step, the *S. sparsipilum* and *S. spegazzinii* resistant genotypes were confronted to the virulent nematode populations selected on lledher. We have shown that the virulent nematode populations were also adapted to *S. spegazzinii* (GpaVspg) resistant genotypes but not to *S. sparsipilum* (GpaVspl) resistant ones, indicating possibility of cross-virulence in the case of *S. spegazzinii* [4] while the *S. sparsipilum* resistance remains still efficient. That means that the nematode populations that are virulent on resistance GpaVvrn do not adapt to all the collinear GpaV resistance loci.

The results presented here could be useful 1) to predict evolution of nematode populations in natural agroecosystem and to identify durable strategies of resistance deployment, 2) to design new genotypes which cumulate different genetic factors in order to increase their durability.

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## APPLICATION OF COMPUTED TOMOGRAPHY TO ASSESS CHANGES IN TUBER VOLUME

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Understanding tuber growth is essential when it comes to define factors responsible for tuber size and tuber size distribution, both highly relevant topics for the potato industry. Nonetheless, assessing changes in tuber volume has posed a significant technical challenge throughout the years given the sensitivity of belowground organs to soil disturbance and the need to distinguish with confidence changes in volume in the range of a tenth of a milliliter.

Computed Tomography (CT) is mostly known by its use in medicine and engineering, however, its application in plant science is growing. Hereby we report the implementation of such a system to measure diel changes in tuber volume and the first results obtained.

Plant material consisted of the commercial variety Nicola and two native Chilean genotypes GLKS 22339 (UACH 1258) and GLKS 22349 (UACH 1279) obtained from the Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (Germany). Tuber growth was assessed during a 24 h-period, with measurements every 3 h. Plants were grown in 10 l pots with a photoperiod of 12 h / 12h day/night, a relative air humidity of 60% and a day/night temperature regime of 24°C / 16°C. A v|tome|x s 240 computed tomograph (GE Sensing and Inspection Technologies, phoenix|x-ray, Germany) was used to analyze plants up to 58 cm height and 60 cm diameter with a maximum of 10 kg weight. Plants were exposed to X-rays for 5.3 min at 160 kV / 1270 µA with a 200 ms illumination time per scan.

Changes in individual tuber volume ranged from 0.01 to 1.42 ml per day. These values were lower than previous reports, probably due to artificial limitation in the number of tubers per plant in past experiments, in which water and sugars had to be distributed among a lower number of tubers. Having plants undisturbed with an unlimited number of tubers growing in parallel also revealed that diel growth in individual tubers does not necessarily follow the sigmoid pattern previously described in literature with higher growth rates during the night, but rather displays more constant growth rates throughout the 24 h period. Additionally, we observed that significant changes in 24h-growth rates occur for the same tubers at different plant age, providing evidence that tubers grow in turns.

CT offers the chance to study in detail growth and development of not only tubers, but different organs that grow belowground in systems closer to natural conditions. Future applications regarding responses of tuber growth to different stresses, as well as agronomic management systems could be possible and will hopefully derive in practical applications for the potato Agro-Food industry.

# INTERACTION OF GENOTYPE, PHOTOPERIOD AND PLANT GROWTH REGULATORS ON POTATO (SOLANUM TUBEROSUM L.) MICROTUBERIZATION

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## Introduction

Photoperiod (PP) and temperature within the growth chamber, nutrition components and plant growth regulators (PGRs) within the media, and genotype influence the microtuberization (MTZ) of potato. BAP has a strong promotive effect on MTZ and constitutes major part of the tuberization stimulus, either alone or in combination with other PGRs (1). CCC is also widely used in tissue culture media to promote microtuber (MT) formation (2). The present research was carried out to determine the effect of BAP and CCC alone or in combination on the MTZ of three potato genotypes using stem segments with nodes under short day and continuous dark conditions.

## **Materials and Methods**

Study was carried out at EAARI, Erzurum, Turkey. Pasinler, Granola and Caspar were used in the study. The concentrations of PGRs were: T0: 8% sucrose (control), T1: 5 mg L<sup>-1</sup> BAP, T2: 500 mg L<sup>-1</sup> CCC, T3: 5 mg L<sup>-1</sup> BAP+500 mg L<sup>-1</sup> CCC. Explants were incubated at two different PP conditions such as short day and continuous dark. Observations were recorded on days to MTZ, the rate of MTZ (%), plantlet fresh weight (mg), total number of MT, total MT weight (mg), total fresh weight of biomass, starch assimilation capacity (SAC, mg), harvest index (HI, %), MT diameter (mm), the number of eyes and dry matter (DM) content of MT (%). A completely randomized design (CRD) was used to evaluate three cultivars, seven plant growth regulator combinations with four replications.

## **Results and Discussion**

MTZ started earlier under continuous dark (51.68 days) than short days (55.3 days), on control treatment (47.37 days) in cv. Pasinler (52.33 days). Results presented showed that although MTZ started earlier under continuous dark, other MT characteristics was more dominant under short days. Highest MTZ was obtained under short days (85.42%) compared to dark (80.83%). Insignificant differences were determined on two different PP conditions when the number of MTs was considered. The maximum number of MTs was recorded from cv. Granola (4.15) on control treatment (5.37). Total MT weight was higher under short day (633.28 mg) than continuous dark (547.75 mg) on control treatment (855 mg) in cv. Caspar (603.55 mg). MT diameter and DM content of MTs were also higher under short days (3.38 mm and 20.68%, respectively) compared to dark (2.75 and 19.92%, respectively).

## **Conclusions**

Findings of the present research revealed that the effect of PGRs on plantlet characteristics studied was variable depending on the genotype and PP. The influence of control treatment including only 8% sucrose and combination of BAP+CCC was more pronounced compared to BAP and CCC used singly. Since the results of this research have pointed out that the appropriate PGRs may be unique for each potato cultivar, further studies should be done to determine the best hormone concentrations for new breeding potato lines. Further research will be extended by investigating the factors affecting dormancy period and ex vitro studies of MTs under greenhouse and field conditions.

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# INVESTIGATING THE EFFECTS OF PHOTOPERIOD AND TEMPERATURE ON POTATO TUBER FORMATION

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## Introduction

Tuberisation in potato is sensitive to environmental factors including day-length, temperature and nitrogen level. The sensitivity of tuber yield to temperature is a serious constraint to production in many areas. Good tuber yield under high temperature is thus increasingly becoming a target for potato breeders. Relatively little is known about the effects of elevated temperature on the molecular and biochemical processes underlying tuber development. Increasing our knowledge of such processes would underpin efforts to develop potatoes with improved yield and quality under a wider range of environmental conditions. The present study aims to characterize the response of potato to fluctuations in day-length or temperature. By comparing germplasm with contrasting day-length requirements for tuberisation (*S. tuberosum* group *Andigena* and *Neotuberosum*) we were able to study the effect of day-length on tuber formation. In addition, the effect of temperature on tuber induction in potato was investigated by comparing plants grown at "normal" (22°C) and "elevated" (30°C) temperatures.

### **Materials and Methods**

Solanum tuberosum Andigena (accession 573), Solanum tuberosum Neo-Tuberosum (accession 369) and Desiree were used to determine the effects of day-length and temperature on tuberisation. Plants were transferred to growth cabinets under controlled conditions of daylength and temperature. Metabolite profiles in tubers and leaves were determined by GC/MS. Sample extraction, derivatisation and quantification by GC/MS were as in [1]. A custom Agilent microarray was designed to the predicted transcripts from assembly v.3.4 of the DM potato genome as described [1] and used to assess transcript profiles. Biochemical analysis included photosynthetic gas exchange measurements, extraction and quantification of redox buffers and tuber labeling experiments.

## **Results**

Potato tuber yield is susceptible to mild temperature stress although there is an increase in net photosynthesis at mildly elevated temperature. Using metabolomic and transcriptomic approaches we demonstrate profound effects in both metabolite profiles and transcript patterns in tubers and leaves, at the elevated temperature. RT-PCR revealed perturbation in the expression of circadian clock transcripts including StSP6A, previously identified as a tuberisation signal. We have also characterised the biochemical and transcriptional responses in two divergent potato genotypes under contrasting day length regimes. New insights into tuberisation and associated processes were revealed, particularly the presence of an additional StSP6A allele that is associated with tuber formation under long day-length conditions.

## **Conclusions and Perspectives**

Our data indicate that potato plants grown at moderately elevated temperatures do not exhibit classic symptoms of abiotic stress but that tuber development responds via a diversity of biochemical and molecular signals. Overall, the data presented in this study highlight the subtle interplay between components of the clock-CONSTANS-StSP6A axis which collectively may interact to fine-tune the timing of tuberisation.

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### THE MECHANISMS DRIVING POTATO CROP YIELD AND GRADE DISTRIBUTION

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Under ideal growing conditions (e. g. optimal moisture, temperature, mineral nutrition and disease control) potato dry matter (DM) yield increases with the amount of intercepted photosynthetic active radiation (PARi). However yield is limited by the leafs ability to convert the PARi into carbohydrates (RUE). Another factor that limits yields is the storage capacity of the tubers (sink). Sink size and the size distribution of the final yield is influenced by the number of tubers per plant and the potential growth of individual tubers. These are influenced by plant density, number of stems, the number of stolons produced in the belowground stems and the induction of tuber initiation (TI). The present work investigates the mechanisms driving potato crop yield and grade distribution among three commercial cultivars ('Bondi', 'Fraser' and 'Russet Burbank').

The three cultivars were grown from October 2011 to May 2012 at Canterbury, New Zealand. The potato seed used had been stored at low temperature (~4°C) for most of the storage period. The crops were assessed for number of above and below-ground stems, total and grade potato (50 g grades) tuber yield and number, canopy ground cover (R/Ro), radiation interception and total radiation use efficiency (RUE; expressed in g tuber DM/MJ total radiation intercepted). The relationship between individual tuber growth and below-ground node position was also measured. The Experiment was a randomised complete block replicated three times.

'Bondi' had the largest tuber fresh weight yield (66 t/ha) and concentrated over 75% of it in the grades >250 g weight class (Figure 1a). 'Fraser' and 'Russet Burbank' both yielded ~51.5 t/ha and had around 75% of their yield in the grades <250 g class but produced 70% and 57%, respectively, more (P<0.01) tubers per m² than 'Bondi'.

'Bondi' also produced the highest yield per stem (179 g) compared to 'Fraser' (63.9 g) and 'Russet Burbank' (85.5 g  $\pm$  11).

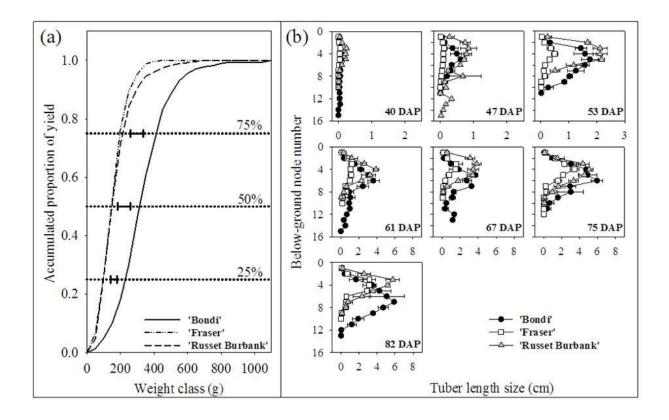
On average the canopy of all three cultivars reached maximum R/Ro (~92%) at ~73 days after planting (DAP) or 500°Cd after crop emergence; (Tb=2°Cd) and remained constant for ~62 days (840°Cd). However, 'Russet Burbank' had the highest rate of canopy senescence (1.6%/day or 0.006 %/°Cd) and 'Fraser' the lowest (0.63%/day or 0.001%/°Cd). Five days before crop desiccation (182 DAP) 'Fraser' had accumulated (2153 MJ/m²) 12% more (P<0.05) total radiation than 'Russet Burbank' with 'Bondi' intermediate. 'Bondi' and 'Russet Burbank' accumulated less (P<0.026) radiation before TI (~200 MJ/m²) than 'Fraser' (375 MJ/m²).

Total crop RUE was higher (P<0.049) for 'Bondi' (1.2) than 'Fraser' (1.0), with 'Russet Burbank' intermediate (1.1).

At 82 DAP the middle node position in the below-ground stems were the most likely (probability >70%) to have initiated a tuber (Figure 1b). At that time 'Bondi' had more tubers in a single stem (~4; nodes 5, 6, 7 and 8) grown to a longer size (e.g. tubers > 4 cm) compared with 'Fraser' (0) and 'Russet Burbank' (~2; nodes 3 and 4).

The results suggest that the potato production was limited by the sink size. The bigger sink in 'Bondi' was attributed to more and larger tubers produced in the middle positioned nodes in the below-ground stem. Future studies could focus on modelling sink size and yield distribution.

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**Figure 1a**: Accumulated proportions of final yield. Bars are LSD; **1b**: Mean length of tubers at different below-ground node number. Node zero represents ground level. Bars are s.e.m.

## THE INFLUENCE OF ETHEPHON ON PLANT GROWTH, YIELD, TUBER SIZE AND SKIN COLOR

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Skin color and appearance are two of the most important factors consumers rely on when making purchasing decisions about specialty potatoes. Plant growth regulators have been evaluated for decades as a means to improve skin color and enhance the appearance of these potatoes in the market. Currently, the only product labeled for this purpose in the USA is the synthetic auxin 2,4dichlorophenoxyacetic acid (2,4-D). Preliminary trials indicate that foliar applications of the growth regulator 2-chloroethylphosphonic acid (ethephon) may be useful for improving skin color of red-skinned cultivars, but relatively little is known about the optimum rate or timing. Several field trials were conducted at the Parma Research and Extension Center during 2011 and 2012 to evaluate the effects of foliar applications of ethephon on plant growth, yield, tuber size and skin color of Red LaSoda (a light redskinned cultivar). Four application rates of ethephon (0, 35, 71, 106, 141g a.i. ha<sup>-1</sup>) were evaluated when applied at flower bud initiation, and again 10 days later (59 and 69 days after planting). Skin color was measured visually and by colorimeter at harvest, and throughout 3 months storage at 4°C. The influence of plant growth stage at the time of application on the response to ethephon (71 g a.i. ha<sup>-1</sup> rate) was also evaluated. Higher rates of ethephon significantly reduced plant height and average tuber size, but did not influence total yield. Ethephon application rate significantly affected tuber skin color, as evidenced by higher visual color ratings, lower L\* values (indicating darker color) and increased chroma (indicating more intense color) when compared to the non-treated check. Differences in skin color at harvest due to ethephon were maintained throughout the duration of storage. The optimum application timing to impact skin color was a relatively narrow window during initial flower development to ten days later, which coincided with the period of tuber initiation. Ethephon is not currently labeled for use in potatoes, but is widely used in other crops to hasten ripening. These results indicate that it might be worthwhile pursuing a label for ethephon application to red-skinned potatoes as an alternative to 2,4-D.

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## IMPROVING THE SELECTION EFFICIENCY IN POTATO BREEDING

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Current potato cultivars suffer from a number of production and quality issues. Commercial cultivar development within Australia used a conventional potato breeding strategy, with outcrossing, then screening of derived lines to identify improved cultivars, although we have investigated methods to make the program more efficient. Implementation of marker-assisted selection (MAS) is highly desirable to increase the efficiency and our initial targets for MAS have been qualitative disease resistances. Two diseases of concern within the Australian potato industry, are potato cyst nematode (PCN) (*G. rostochiensis* Ro1) and Potato virus Y (PVY), so we investigated markers for these diseases, including a potentially diagnostic marker, 57R, for PCN Ro1. As the majority of the target traits for the breeding program are quantitative and controlled by several to a very large number of genes, we have also investigated a quantitative genetic analysis technique used in livestock breeding for these complex traits to develop estimated breeding values (EBVs) for them. The program has now developed a breeding scheme using a combination of MAS, EBVs and conventional screening methods for early generation selection of cultivars with multiple desirable traits. The application of these new techniques, alongside conventional screening will see a significant reduction in the breeding cycle and the cost of breeding, as well as improving genetic gain in a range of traits.

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# MOLECULAR ANALYSES OF THE POTATO COLLECTION MAINTAINED IN THE INRA BRACYSOL BIOLOGICAL RESOURCE CENTER

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The INRA BrACySol Biological Resource Center maintains a large potato collection which is vegetatively propagated. This collection comprises worldwide varieties, wild related species, intra- and interspecific hybrids which include breeding lines that have been proposed to the French breeders, dihaploids and transgenic clones. In order to improve the management of this genetic resources collection and its exploitation in breeding programmes, several molecular analyses have been or are being carried out.

A first study was conducted on a subset of 350 worldwide potato varieties (*Solanum tuberosum* L.), selected to represent the phenotypic diversity that is available in the collection of varieties. Using SSR markers, the genetic diversity and population structure of this subset were examined together with a set of 30 Chiloé Island landraces that are maintained at the experimental station of the Catholic University of Temuco (Chile) [1]. This work confirmed the close genetic proximity of the Chilean *S. tuberosum* populations to the modern potato cultivars, as was previously shown [2]. Structure analyses performed using several methods revealed that a collection which includes modern potato cultivars and Chiloé Island landraces do not present any clear genetic structure. Such genetic resources collection forms therefore an interesting material for developing an association mapping approach.

Two other studies that are underway will be presented. One of these aims at investigating the linkage disequilibrium pattern along the potato genome by analyzing a set of breeding lines with the SolCAP chip. The other study aims at better securing the potato collection by starting with the cryopreservation of some clones and producing a DNA profile of the varieties that are present in the collection.

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# NY-SMIRA GENE CONFERRING HYPERSENSITIVE RESPONSE TO POTATO VIRUS Y IN POTATO CULTIVAR SÁRPO MIRA MAPPED TO THE DISTAL ARM OF THE POTATO CHROMOSOME IX

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#### Introduction

Potato virus Y (PVY, Potyvirus) causes considerable losses in plant production and due to its economic impact it is classified as one of the most important plant viruses worldwide. In potato, the effective method to prevent yield losses is growing resistant cultivars.

The aim of the study was to locate PVY resistance of the potato cv. Sárpo Mira on the potato genetic map and to find effective molecular markers linked to this trait, which will be useful as a selection tool in a breeding process. The PVY resistance of this cultivar is described as very high [1], however the pedigree of Sárpo Mira is unknown and the sources of resistance to PVY remain elusive.

## **Materials and Methods**

A set of plants used in this study consisted of a tetraploid F1 progeny (N=140) obtained from a cross of the resistant cv. Sárpo Mira and PVY-susceptible Maris Piper (SM x MP). Cvs. Rywal containing the Ny-1 gene and Romula containing Ny-2 gene were included as controls. The tests were performed with the PVYNTN isolate 12-94. The plants were tested by whole plant assay and detached leaf assays and inoculated leaves were scored for the presence of necrotic hypersensitive response lesions.

Plants of cv. Sárpo Mira were also tested in conditions of increased infection pressure by graft inoculation. In order to map the gene conferring Sárpo Mira's resistance to PVY, a candidate locus approach was used. It was hypothesized that this gene is localized in one of the hot-spots on chromosome IX, XI or XII where other Ny or Ry genes have been mapped. PCR markers and GoldenGate assay markers were used to define its position.

### Results

Detached leaf, whole plant and grafting assays performed with use of PVYNTN strain showed that cultivar Sárpo Mira reacts to infection with a hypersensitive response and this reaction occurs both at 20 and 28°C. The hypersensitive response to PVYNTN segregated amongst 140 tetraploid progeny of cvs. Sárpo Mira × Maris Piper in a 1: 1 ratio, indicating that it was conferred by a single, dominant gene in simplex. Therefore, it is referred to as Ny-Smira.

The marker Ry186 which was described as linked to the Rychc gene located on potato chromosome IX [2, 3] was found to be linked also to the Ny-Smira gene. This position corresponds to the location of the Rychc and Ny-1 genes for PVY resistance. Marker Ry186 was located approximately 1.4 cM from the Ny-Smira gene. Four GoldenGate assay markers confirmed the position on chromosome IX as the location of the Ny-Smira gene.

## **Conclusions and perspectives**

The study confirmed earlier reports on high Sárpo Mira's resistance to PVY. Our data added genetic evidence that the Ry and Ny genes can be found in the same genomic region and that they may be alleles, originating from different wild potato species.

The marker Ry186 linked to the Ny-Smira gene is stable and simple to use, therefore, can be recommended for selection of PVY resistant progeny of cultivar Sárpo Mira.

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# ON-FARM EVALUATION OF POTATO GENOTYPES FOR YIELD AND LATE BLIGHT RESISTANCE IN MALAWI

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Potato production in Malawi is characterized by low yields with poor quality tubers. To some extent this has been attributed to use of low yielding varieties and poor quality seed tubers. In attempt to address the situation there is a potato variety development program whose overlying objective is to identify high yielding varieties, tolerant to major diseases and insect pests that are also adaptable to local environmental conditions. During the 2012-13 rain fed season, potato clones introduced from breeding programs from International Potato Center (CIP)-Peru, CIP-Kenya and Scotland were evaluated in a uniform yield trial (UYT) for yield and resistance to Late Blight. Two sets of on-farm trials were implemented at Mpalare (Dedza District) and Tsangano (Ntcheu District). The experimental trials were arranged in RCBD with three replications. All the recommended crop management practices were followed in addition to spraying Dithane M45 against Late Blight. Data was collected following the CIP laid procedures.

Results from the first set of trials implemented at both sites, showed significant yield difference (P<0.001) across sites. Mpalare site produced high mean yield of 13.09 t/ha while Tsangano had a mean yield of 6.98 t/ha. There were also significant yield differences (P<0.05) among testing clones at the two sites. The highest yielding clone at Mpalare was Atlantic (15.60 t/ha) which had also the lowest Late Blight infestation of 10%. At Tsangano site, the highest yield was recorded from Red Pontiac (10.86 t/ha). The lower yields at this site may partly be attributed to relatively high incidence of Late Blight which averaged 25%. The second set of trials was implemented at Mpalare site showed significant yield differences (P<0.05) among the clones. The highest and lowest yields recorded from clones Desiree (23.02 t/ha) and Pukara-inia (10.8 t/ha). There was also relatively low Late Blight incidence which averaged 15%. The lowest infestation was recorded from Desiree (10%).

Out of the 12 clones evaluated, 8 clones (Desiree, Pure-inia, Karu-inia, Pehuenche, Pukara-inia, 396033.102, Red Pontiac and Epicure) produced yields of more than 10 t/ha which is the current acceptable benchmark yield. Importantly, almost all the clones produced large sized tubers with more than 75% of total yield comprising of tubers with >35mm diameter. These results confirm the superiority of these clones and suggest their high potential to be released as varieties upon undergoing further multi-location evaluation as well as passing through the variety release committee of Malawi.

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# PHENOTYPING FOR ENHANCED UTILIZATION – IPK'S POTATO GENETIC RESOURCES AS SOURCE FOR NEW AND IMPROVED BREEDING TRAITS

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### Introduction

The Gross Luesewitz potato collections (GLKS) maintain a total of 6,100 accessions, subdivided into three collections: a cultivated potato collection of appr. 2,700 cultivars, land races and breeding lines (KKS), a collection of short day adapted, cultivated genotypes from the Andes and the Equatorial region (AKS, 550 accessions), as well as a collection of more than 130 wild and cultivated species, originating from South and Central America (WKS, 2,900 seed propagated entries).

In order to elucidate the breeding potential of the three GLKS collections and to make them better accessible to potato breeders and researchers, phenotyping for relevant traits has been conducted together with external cooperation partners.

### **Materials**

Resistances of GLKS accessions against *Globodera pallida*, *Phytophthora infestans* and *Synchytium endobioticum*, as well tuber quality traits were assessed in cooperation with Rostock Plant Protection Agency at LALLF M-V, Julius Kühn-Institut Gross Luesewitz, Julius Kühn-Institut Kleinmachnow and STZ/University of Rostock, respectively. These evaluations, partially continuing since more than ten years, covered up to 3,000 genotypes from up to several hundred accessions and up to 80 different tuber-bearing Solanum species.

#### Results

In all instances, positive/improved traits could be observed within the three GLKS collections. Thus, e.g. more than hundred accessions resistant to *Globodera pallida*, appr. 20 accessions very resistant to *Phytophthora infestans*, and 50 genotypes resistant to race 18 of *Synchytium endobioticum* could be identified. Detailed results will be presented at EAPR 2014.

### **Conclusions and perspectives**

Phenotypic evaluations lead to better insights into the hidden potential of the different genepools for potato breeding, making them better accessible to breeders and researchers. In the four instances described, positive/improved traits were observed within all three GLKS collections, making IPK's potato genetic resources a comprehensive source of new and improved traits for breeding and research. Here, long-term, permanent evaluation projects have shown to be optimal to reach the aims described.

### BREEDING FOR LATE BLIGHT RESISTANCE UNDER ORGANIC FARMING CONDITIONS

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A network of research institutes, breeding companies and organic farmers aim at breeding potatoes which perform well under organic farming conditions. Especially, late blight caused by *Phytophthora infestans* leads to heavy yield losses. Also, after strong infection varieties differ in growth performance and tuber quality. A total of 159 varieties are grown at three locations in Germany under organic farming conditions for examination of yield, quality and late blight resistance. Additionally, the set is grown under fungicide application to determine maturity. As late blight resistance and late maturity correlate assessments have to be statistically corrected. SSR analyses of P. infestans samples of the different fields are used to explain the Genotype x Environment interaction for resistance scores. Field trials, trait assessment and DNA marker analyses will illustrate the phenotypic and genotypic diversity within the currently available breeding material. Early results show that pre-breeding clones from the Julius Kühn Institut unite high resistance to late blight with high yield stability. Many of them combine yellow flesh colour and early maturity. The clones with the best combinations of traits serve as parents in a breeding program. At two locations the general combining ability of several resistance donors are tested using the progeny of 82 crossings. When possible, molecular marker systems will be used for selection. We will focus on using, evaluating, and developing DNA markers which are connected with *Phytophthora* resistance. Information gained in the process will be used for concerted potato breeding.

## Session 20 (OP) - BACTERIAL DISEASES AND PESTS

## 109

A PECTOBACTERIUM CAROTOVORUM SPECIES COMPLEX IN SEED POTATOES: DIAGNOSIS, PHYLOGENY, MOLECULAR DETECTION AND VIRULENCE.

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The cultivation of seed potatoes is a small but indispensable niche in the potato sector in Belgium. Although multiplication from minitubers is mainstream for the production of pre-basic seed, a significant volume of high grade basic seed is habitually imported every year from several EU countries to be multiplied for several field generations. This remarkable multiplicity of origins elicits the introduction of a diversity of pectinolytic enterobacteria which may be present in or on the seed tubers. After the emergence of a more virulent Dickeya variant, i.e. D. solani, in the first decade of the century, more aggressive Pectobacterium variants are now being increasingly diagnosed in seed potatoes. The disorders are commonly displayed as tuber maceration, blackleg and stem rot. Pectobacterium isolates obtained from diagnostic samples from seed potato cultivations in the past few years were analysed in taxon-specific PCR, displaying P. atrosepticum as major blackleg pathogen and confirming the common presence of P. wasabiae. A substantial number of isolates was assigned to the Pectobacterium carotovorum taxon. The majority of these isolates was identified as P.c. ssp. brasiliensis (Pcb) and their identity was confirmed in MLSA of core gene sequences. The P.c. ssp. carotovorum isolates were classified in several clusters in the MLSA analysis. A phylogeny was also constructed with sequences of the periplasmic pectate lyase (pelY) gene and the dspE effector gene. Sequences of these genes were exploited to develop a TaqMan real-time PCR assay for Pcb. The virulence of the P. carotovorum isolates was further determined on potato, several vegetables and ornamentals to assess the observed highly aggressive nature of Pcb.

## CHARACTERIZATION OF THE POTATO BLACKLEG BACTERIAL SPECIES COMPLEX IN FRANCE

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#### Introduction

Pectinolytic bacteria are economically important potato pathogens in France and in Europe. The FN3PT leads, in collaboration with INRA, a research program on the two genera involved, *Pectobacterium* (P.) and *Dickeya* (D.) [1], including characterization, detection and disease epidemiology. Strains for characterization are isolated from blackleg potato plants [2] collected during annual surveys. Strain typing confirms that symptoms are associated to a bacterial species complex including both *Pectobacterium* and *Dickeya*, and that over 2003-2013, *Pectobacterium* remained prevalent over *Dickeya*. This raised two major issues, for which suitable biological and genetic data were lacking:

- 1) Pectobacterium carotovorum subsp. carotovorum (Pcc) identified on potato represent a very diverse group. The aim of the study was to evaluate the genetic diversity within these subspecies to improve taxonomic knowledge of the Pcc group.
- 2) The virulence to potato of *Dickeya* spp., especially the two present species *D. solani* (D. sol) [3] and *D. dianthicola* (D. diant), was advocated as a factor explaining their recent emergence, but this was not satisfactorily demonstrated using a large collection of strains.

## **Materials and Methods**

Molecular diversity in Pectobacterium

The pectate lyase genes were sequenced in 38 Pa and 99 Pcc field strains and strains representative of *Pectobacterium* species. Sequences were subsequently compared to evaluate genetic diversity present in Pectobacterium potato strains and their taxonomic relatedness.

Virulence typing in *Dickeya* strains

Virulence was tested in tuber maceration bioassays involving 109 *Dickeya* strains and representative strains from all known *Dickeya* species. Strains originated mainly from potato but also from other hosts. Symptom severity data were analysed with ANOVA models to test species and strains differences within D. sol, D. diant and *Dickeya* sp. strains.

## **Results**

Pa PEL genes grouped as one cluster, whereas Pcc sequences were split into 3 groups. The sequences of some strains originally classified as Pcc of these groups matched those of the P. wasabiae and *P. carotovorum* subsp. *odoriferum* reference strains.

Significant pathogenicity differences were shown between *Dickeya* species, with D. sol and D. diant not being among the most pathogenic groups isolated from potato. Analyses also revealed strong differences between strains within each species.

## **Conclusions and perspectives**

Pathogenicity differences between D. diant and D. sol proved non significant, and therefore do not explain the recent identification of the latter species on potato.

*P. wasabiae* is probably present in the bacterial complex involved in potato blackleg. Recent studies showed that this species has long been present in Europe (Poland, Scotland, The Netherlands, Ireland) and in America (Canada, USAS, Peru), but went unnoticed due to lack of specific diagnostic methods [4, 5].

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# VISUAL INSPECTION TO MANAGE BACTERIAL WILT IN SUB-SAHARAN AFRICA: IMPROVING SEED QUALITY AND IMPACTING SMALLHOLDER FARMERS

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Potato is a key cash and food security crop for mid to highland farmers in Sub-Saharan Africa (SSA). Bacterial wilt caused by Ralstonia solanacearum, is one of the most devastating diseases affecting potato in SSA. This soil borne disease spreads largely through infected seed tubers, and has come to infect most potato growing regions in SSA. Irrigation and water run-off down the slopes of potato farms and into waterways, and tools carrying infected soil are other important transmission methods. In a survey conducted in 11 potato growing regions in Kenya over two growing seasons, bacterial wilt was observed on 63% of 145 sampled farms, and disease incidence ranged from 1 to 50%, with a mean of 11% per farm. Another survey conducted in nine divisions within Nakuru county of Kenya, found that the prevalence of bacterial wilt ranged from 36 to 100% of the total 147 sampled farms, and on-farm disease incidence ranged from 0 to 42% (Mwaniki, personal communication). Resistance has eluded breeders, hence, management strategies to reduce the spread and incidence of bacterial wilt rely on using clean seed and good agricultural practices (GAP). While, certification regulations exist in several SSA countries to prevent bacterial wilt infected seed from entering the seed system, they are officially practiced only in Kenya. While certified seed is available in Kenya, quantities of certified seed supply approximately 2% of demand and are available at a few locations across the country, making accessibility and disease spread through the informal seed system a concern. Quality declared planting material (QDPM) is an alternative approach to assess the health status of seed that is based on visual inspection to remove infected plants from seed multiplication plots and GAP, particularly crop rotation, alternative host management and field hygiene. QDPM is promoted for practice among decentralised seed producers who further multiply certified seed in close proximity to farmers, particularly smallholder farmers, thereby increasing geographic and economic accessibility to quality seed. Positively selected seed is another seed source for smallholder farmers. Positive selection is a visual form of seed selection based on saving tubers from the healthiest plants in a crop for the following season's seed. Incidence of bacterial wilt in 25 farmers' fields planting positively selected seed averaged 5%, while 25% incidence was observed in plants obtained from randomly selected seed, known as farmer-saved seed. In another study, positive selection reduced incidence of bacterial wilt compared to farmer-saved seed from 41 to 13% (Kakuhenzire et al., 2013). Of the 100% of plants that were infected with R. solanacearum in the highly susceptible cultivar Revolución, 13% were latently infected and would by-pass visual inspection. Despite some drawbacks from latent infection reducing the ability to remove bacterial wilt infected tubers from seed sources, data support that seed originating from visually inspected plants reduces bacterial wilt incidence and improves yields compared to farmer-saved seed. Considering the widespread distribution of bacterial wilt in SSA, integrating visual inspection and GAP is a feasible option to produce quality seed, and support QDPM as a viable option for a quality control system for seed potato to increase smallholder farmer accessibility to clean seed.

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# MONITORING OF PECTINOLYTIC BACTERIA ORIGINATING FROM SEED POTATOES AND WATER ENVIRONMENTAL SAMPLES ON THE TERRITORY OF POLAND.

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### Introduction

Pectinolytic bacteria classified to the genera *Pectobacterium* and *Dickeya* are causative agents of blackleg and soft rot disease in economically important plants such as potatoes, tomatoes or maize. Agricultural losses caused by these pathogens may even reach 30 % of the total yield [1]. Due to the fact that effective control of mentioned diseases has not been achieved yet [2] and taking into consideration the 8<sup>th</sup> position of Poland among the most prominent potato producers worldwide (FAO, 2011) we performed the long-term monitoring of seed potato fields and environmental water-derived samples to get deep insight into the potential sources of bacterial infection.

### **Materials and Methods**

Symptomatic and asymptomatic potato (*Solanum tuberosum* L.) plants and tubers suspected of blackleg or soft rot disease, accompanying weed and water samples were obtained from The State Plant Health and Seed Inspection Service in Poland. Received plant material was homogenised and its serial dilutions were cultivated on selective-differential Crystal Violet Pectate (CVP) medium in 28 °C to isolate pectinolytic bacteria. Identification of species was achieved due to multiplex PCR reaction with primers specific to *Pectobacterium carotovorum*/*Pectobacterium wasabiae* (Pcc/Pwa), *Pectobacterium atrosepticum* (Pba) and *Dickeya* sp (Dsp). Multiplex PCR was also used to examine water samples. Further analysis for the presence of highly virulent *Dickeya solani* [3] and *Pectobacterium wasabiae* [4] was performed.

## Results

In 2013, out of 248 plant samples (including 124 stems, 74 tubers and 50 accompanying weed) 73 homogenates were assessed as positive for Pcc/Pwa (53), Pba (23) or Dsp (7). In addition, from 1866 water samples 19 contained investigated pectinolytic bacteria: 7 Pcc, 6 Pba and 6 Dsp, respectively. We also confirmed the presence of *D. solani* in 1 potato, 1 weed and 2 water samples. Interestingly, 24 potato and 3 weed samples turned out to be positive for *P. wasabiae*. The geographical distribution of identified species pointed out that *Pectobacterium* sp are widespread on the territory of Poland in comparison to *Dickeya* sp limited only to certain provinces.

## **Conclusions and perspectives**

Taking into consideration the results from similar studies performed in 2005, 2009, 2010 and 2011 we state that the distribution of prevailing bacteria changes over time. Our research provides knowledge on the pathogen sources and possible contamination pathways that could be used in the future to justify undertaking control strategies in certain provinces of Poland.

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### TWENTY YEARS OF APHID SURVEY IN WARE POTATOES IN THE SOUTH OF BELGIUM

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Aphids are an important pest in ware potato in Belgium. Since severe attacks occurring in 1994 and 1996, advisory systems have been implemented and alerts are send to the farmers when insecticides application are required. The development of the aphid advisory system, fed by 20 years of observation, has drastically reduced the need of insecticide in ware potato, with a treatment required every 4-5 years in a mean, instead of 2 or 3 insecticide applications per year. The two main reasons of this reduction was the determination of the role of aphid natural enemies and the identification of two groups of aphid species, each requiring specific adjustments in terms of insecticide treatments.

The advisory system is based on weekly field monitoring between June and August, with 10 to 15 fields followed each year. Aphids as well as their natural enemies (parasitic wasp and entomopathogenous fungi, eggs and larvae of hoverflies, lacewings and ladybirds) are counted and the risk is assessed on basis of both population dynamics. According to the important role of natural enemies in the aphid control, the selectivity of plant protection product potentially applied during the season, including fungicides and insecticides, has also been assessed and information used for the advices given to the farmers.

During the monitoring, eight different aphid species were identified but only four were found to be potentially dangerous: *Aphis nasturtii*, *Aphis frangulae*, *Macrosiphum euphorbiae* and *Myzus persicae*. The behavior of these species and their sensitivity to insecticides was different and they can be separated in two groups, the first with the two Aphis species and the second with the two last one, with adapted advisory to the farmers in term of insecticide use (timing of application, spray volume and choice of product).

# AN ATTRACT AND KILL STRATEGY USING CARBON DIOXIDE COMBINED WITH CONTROL AGENTS FOR KILLING WIREWORMS IN POTATO FIELDS

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In recent years wireworms became the most important herbivorous pest species in potato production systems. The larvae feed on the tubers before harvest, causing qualitative damage and paving the way for secondary bacterial or fungal infections, both resulting in reduced income for farmers. These yield losses are of concern for both organic and conventional growers in many parts of Europe. Control options targeting wireworms are limited, due to the phasing out of effective insecticides; new control options are therefore desperately needed.

The larvae of soil dwelling insects, and wireworms as well, use carbon dioxide gradients, established by growing roots, to find their host plants. This long distance orientation cue is complemented by additional specific volatile cues emitted by the plants, resulting in a final acceptance of a host plant for feeding. Control strategies using biocontrol agents, such as entomopathogenic fungi, depend on high concentrations of spores per m², set against competing microorganisms in the rhizosphere, targeting the pest species. However, control efficacies are generally limited, apart from high costs.

The "attract and kill" strategy (A&K) turns this strategy upside down: instead of bringing the control agent to the larvae, they are attracted to the control agent by combining it with capsules emitting CO<sub>2</sub>. When near to these capsules, mortality of wireworms significantly increases because of contact with the control agent. To make this strategy work under field conditions, the capsules need to fulfill some prerequisites, such as building up a CO<sub>2</sub> gradient significantly higher than the background CO<sub>2</sub> concentration in the soil, maintained for at least several weeks, and the larvae need to be attracted to the capsules to feed on them.

Lab experiments demonstrated that wireworms were clearly attracted to these artificial CO<sub>2</sub>-capsules, but moved away when realizing the fraud. Additional compounds incorporated into these capsules increased their attractiveness towards wireworms. We used these capsules combined with either an isolate of an entomopathogenic fungus, combined with Spinosad or with NeemAzal in several field experiments in Germany 2013. Treatments were applied either into the potato dams, below the tubers, or between the potato rows at two different times during the growing season. We used fields with a high incidence of wireworm damage in previous years. Application of the A&K capsules resulted in significantly lower tuber damage (control: 10% damaged tubers; A&K treatment: 2%) in most, but not all fields, depending on the treatment schedule. Late season application of the A&K capsules did not prevent damage by wireworms because mortality did not immediately occur following contact with the killing agent, thus feeding damage was still observed. Wireworms are known to be extremely resistant even to high doses of insecticides and are able to recover after several days when being exposed to the insecticidal compounds.

Necessary improvements of the A&K strategy for a standardized application routine in the field are tested in field experiments this year at several locations. The implementation roadmap for this strategy will be discussed.

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## Session 21 (OP) - AGRONOMY (2)

## 115

# EFFECTS OF BIOLOGICAL AND INTER-ROW SUBSOILING ON SOIL STRUCTURE, ROOT DISTRIBUTION, N UPTAKE AND POTATO YIELD

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Subsoil compaction is a persistent and increasing problem in agricultural fields. Among other things it is caused by heavy wheel loads and high intensity of wheel traffic on fields [1,2]. It is important, from an economically and environmentally sustainable point of view, to study possibilities of soil loosening techniques. Negative effects due to subsoil compaction are for example reduced root growth, nutrient transport and uptake. In potato production these problems seem to be more accentuated since potato roots are sensitive to soil resistance. Potato root growth restriction has already been observed at 1 MPa whereas roots of other crops can penetrate soils with a soil resistance between 2 and 3 MPa [3]. The objective of this study is to determine the effects of inter-row subsoiling, biological subsoiling and the combination of these two loosening methods on root morphological parameters in a potato crop. We will also evaluate N accumulation and dry matter in potato tubers and establish at what extent changes in soil structure are due to different loosening techniques separately and in interaction.

Potatoes (cv. King Edward), were grown in 2013 at an experimental farm in southern Sweden. The experiment was arranged as a randomized block design with two factors; biological subsoiling and interrow subsoling. The two loosening methods consisted of (1) biological subsoiling by using pre-crops with a strong root system able to grow through compacted soil structures and (2) inter-row subsoiling to 45 cm depth (Agrisem Cultiplow subsoiler) between the rows one week after planting. The preceding crops were spring barley (control), tillage radish (Raphanus sativus cv. Structurator), tillage radish (Raphanus sativus cv. Terranova) and red clover (Trifolium pretense).

The sampling of roots was carried out with soil corers (7.3 cm inside diameter and 5 cm hight) taken 58 days after emergence at three spatial positions; (1) top of the hill, (2) between the hill top and the bottom of the furrow and (3) at the bottom of the furrow. At each position samplings were carried out at 4 different depth layers measured from the soil surface after removal of the hill; 15-20, 30-35, 40-45 and 50-55 cm. The content in each soil core was placed in labeled plastic bags and stored at -20°C. The recovery of roots was done by washing the soil cores under running tap water on a sieve with a mesh of 1 mm. The clean roots were scanned and image analyzed using WIN-RHIZO software system (version 2007a, Regent Instruments). Thereafter, root samples were dried at 60°C for 48 hours for measuring root biomass. Total N concentrations in potato tubers were measured with a CHN-600 analyzer (Leco Co.) on dried material at 65°C for 12 hours. Dry mass in tubers were measured by drying the material at 110°C for 12 hours. For measuring soil mechanical resistance in the field a penetrometer was used: shortly after planting and a few days before harvest.

Analyses of the material are under preparation and the results will be presented during the conference.

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### **IMPROVING UNIFORMITY OF POTATO CROPS**

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### Introduction

Tuber size is an important quality attribute of any potato crop. To maximise marketable yield, growers should aim to minimise variation in tuber size so as to avoid producing tubers that are either too small or too large. Variation in tuber size can be quantified by grading tubers, fitting a normal curve to the data and calculating the coefficient of variation from the mean and standard deviation of the curve [1]. Erratic water supply to the crop and *Rhizoctonia solani* are known to increase variation in tuber size, but the underlying mechanisms responsible for causing variation in tuber size within a crop are relatively poorly understood [2]. Svensson (1966) [3] described the general variability between plants in potato crops, for example in stem number, date of emergence and yield. The main causes of this variation between plants are unclear, as is whether it has any effect on variation in tuber size.

## **Materials and Methods**

The effects of variation in seed weight, emergence and within-row spacing on variation in tuber size were examined in the variety Maris Piper through field experiments and by detailed sampling of commercial crops. In the experiments, the date of emergence of each plant was recorded and each plant was photographed from above several times at the start of the season. Above ground stems were harvested and weighed individually and the tubers from each plant harvested separately and weighed individually. In a separate survey, stems were harvested individually at emergence and at eight later dates during the growing season.

### Results

The data show that the yield of individual stems and plants within a crop can vary widely and that there is a correlation between the yield of each stem and the average weight of the tubers that it produces. Tuber number increased as yield increased, but this was not sufficient to counter the effect of higher yield on average tuber weight. When variation in the yield of stems was smaller, the variation in tuber size was also smaller. None of the treatments examined decreased variation in tuber size however and nor did they alter the weight distribution of above ground stems. No relationship was found between seed weight and stem number per plant, which is surprising considering the relationship between seed weight and stem number per hectare. The yield of individual plants was affected by seed weight and date of emergence but was also influenced by neighbouring plants. The space between plants did not correlate with the yield of individual plants.

## **Conclusions and perspectives**

While there is a relationship between the yield of stems and the average size of the tubers they produce, it is difficult to manipulate the crop to decrease the variation in the yield of stems. This study only examined one variety (Maris Piper) and sampling a commercial crop of the variety Desiree revealed differences in the way the crop grew. Further work will examine how seed weight, emergence and spacing interact with each other in Maris Piper and relationships between seed weight and stem number in several other varieties.

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### MALEIC HYDRAZIDE: SPROUT SUPPRESSION OF POTATOES IN THE FIELD

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### Introduction

In 2005, the active substance maleic hydrazide was released on the Belgian market. Maleic hydrazide is authorized in potatoes as foliar treatment for instore sprout suppression and control of volunteers. The mode of action is based on blocking cell division whilst cell elongation is not affected. The product must be applied at once during the growing season, only after at least 80% of the tubers have reached 25 mm diameter and not later than 3 weeks before haulm killing. The first 24 h after application, no meaningful precipitation should occur to insure sufficiently uptake of the product by the crop.

### **Materials and Methods**

The same field trials were set up for 4 years (2005-2008) and 4 locations per year with application of maleic hydrazide in four different cultivars (Bintje, Fontane, Asterix and Cilena). Maleic hydrazide was applied at different crop stages. The results were compared with un untreated control (reference). The following parameters were assessed: yield, quality, sprouting of the tubers during storage and volunteer control in the next crop. In years with secondary growth the effect of maleic hydrazide on this phenomenon was checked as well. In 2010 and 2011 there were additional trials on multiple locations and with different varieties. The same parameters were assessed. For the cultivar Innovator we also looked at the effect on internal germination during storage.

### Results

When maleic hydrazide was applied too early (80% tubers > 25mm diameter) yield was negatively affected (3 years to 4) except for the cultivar Cilena (fresh market). Internal quality (dry matter, cooking and fry quality) was not influenced by the application of maleic hydrazide. Only Fontane had a slightly lower dry matter content. Maleic hydrazide also influenced appearance of secondary growth. However, the results were very variable depending on cultivar, location and time of application. After harvest, the tubers were kept in storage and assessed monthly on sprouting. Potatoes treated late in the growing season, showed a shorter dormancy period. A part of the tubers was replanted the following spring to verify volunteer control. We can speak of a volunteer reduction of approximately 80% for Asterix, Bintje and Fontane and of 50% at Cilena when applied in the early crop stages. The influence of maleic hydrazide on internal germination during storage was examined on the cultivar Innovator. The tests clearly showed a positive effect for this parameter. After application, the cultivar Asterix showed almost every year a temporarily phytotoxicity (bronze discoloration).

## **Conclusion and perspectives**

During 6 years various trials were done with maleic hydrazide. On multiple locations and in different varieties the effect of this active substance was examined. Treatments in early crop stage showed yield loss, except for the variety Cilena. Treatment with maleic hydrazide allows a delay in sprouting during storage and less volunteer potatoes. There was little difference between the different times of spraying. Only the latest treatment, three weeks before haulm killing, had less impact because at that time too little of the maleic hydrazide could reach the tubers to have a sufficient impact. So the overall message is that maleic hydrazide may not be sprayed when tubers are smaller than 35 mm or after three weeks before haulm killing.

# PLURENNIAL SYNTHESIS OF HAULM KILLING METHODS COMBINING MECANICAL AND CHEMICAL

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### Introduction

For potato cultivation, the obligation is often made to carry out the destruction of the vegetation before natural maturity to set the quality of tubers growing in the ridges to correspond as good as possible to the specifications of buyers (size, dry matter content, ...). The necessity is now to respond at this agronomical demand by reducing the quantities of applied pesticide as objective of "Ecophyto 2018 Plan" launched in France.

### **Materials and Methods**

The study took place during four years (2008-2012) at the ARVALIS-Institut du vegetal Experimental Center of Villers Saint Christophe, in North France. At the same date in mid-Summer two kinds of canopy, corresponding to more or less vigorous potato cultivars (Bintje, Nicola, Kardal, Markies), have been destroyed using combined mechanical and chemical techniques. The main products registered for foliage destruction were used at normal or half dose: diquat [Reglone 2], glufosinate ammonium [Basta F1], carfentrazone ethyl [Spotlight Plus]. They were applied after haulm topping with full soil covering as classical spraying at 200 l/ha (experimental sprayer Pulvelec) or with centrifugal spraying at low volume 35 l/ha (Loof-does equipment) but also with localized spraying on the row at 75 to 150 l/ha (Chafer equipment). Each year a field trial is conducted in a as randomized of possible disposal regarding the use of agricultural machines.

## Results

The results show that flailing prior to chemical application can instantly remove more than three quarters of vegetation but chemicals are necessary for limiting or avoiding regrowth of foliage, especially for vigorous crops.

For localized treatment, the final efficacy of haulm destruction is poorly influenced by the volume applied (75 l/ha vs. 150 l/ha). The beneficial effect of localization is more important for diquat within the 8 to 14 days after application but is not sufficient to escape regrowth of foliage in difficult situation. On this point localization is more efficient with glufosinate ammonium.

The two years in which the centrifugal spray 35 l/ha was introduced in the study, it was observed better results in all situations for this application technique, including comparison with the localized treatment on the row, except for diguat and carfentrazone ethyl applied at half dose.

In most cases, the efficiency of the half dose is reduced in the first few days following application with much larger gaps for the destruction of the stems. Regarding the destruction of the leaves, just a few differences exist after 8 days but no more after 14 days.

A stronger effect of the applied product and spraying technology applied is observed for stems destruction, especially for vigorous crop. Half dose seems sufficient in easy situation but is too insecure in the difficult cases, particularly with diquat.

## Conclusions and perspectives

Most of time, the combination of mechanical and chemical techniques is an interesting technique to achieve in a single pass to a rapid destruction of the foliage with a possible reduction in dose of pesticides applied. The development of specific equipments allows today to optimize this kind of intervention: haulm toppers with large width, integrated sprayer for full or localized spray covering, low volume sprayers, integrated ridge rolling. The main limits are the cost of these specific machines, a work rate reduced compared to a classical sprayer, difficulties to drive in the fields after heavy rainfalls.

## IRRIGATION NEED AND EXPECTED FUTURE WATER AVAILABILITY FOR POTATO PRODUCTION IN BELGIUM

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Potato production in Belgium covers approximately 5 % of the total arable land. Although Belgium is situated in the temperate climate zone, dry periods can occur during summer. Trials on Belgian potato fields indicate a tuber yield decline of 10 to 40 % in dry summers due to water stress [1]. Due to the high water stress sensitivity potato is one of the crops likely to suffer from global climate change. Objective of this study is to calculate the climate impact on water availability and future irrigation need for Belgian potato production.

A soil water balance is used to calculate the climate impact and the future irrigation need of potato. The model is used for irrigation scheduling in yearly approximately 30 Belgian potato fields since 1989. During these years crop parameters, such as transpiration coefficients were calibrated with moisture measurements taken every three weeks during each growing season. This results in a well calibrated soil water balance for Belgian climatological and agronomical conditions (Fig 1).

Three climate change scenarios, derived for Belgium [2], were used as input for the simulations: a high scenario (HI), a mean scenario (MI) and a low scenario (LO) (Fig 2a, b). Scenarios for 2066 to 2095 were calculated with the CCI-HYDR [3] perturbation tool based on ETo and rainfall series recorded in Uccle Belgium between 1961 and 1990. Calculations were made for a silt soil and sand soil. A 15 year reference period between 1998 and 2012 was compared to a forecast period between 2073 and 2087.

In the reference period (1998-2012) average yield reduction due to water stress in non-irrigated potatoes was 21% for sand and 15% for silt soils. In the most extreme HI scenario yield reduction for non-irrigated potatoes increased for the forecast period (2073-2087) to 61% for sand and 51% for silt. In the milder MI scenario yield reduction was 33% for sand and 24% for silt.

Average irrigation need (I) in the reference period (1998-2012) for an optimal production was 121 mm for sand and 80 mm for silt. I in the forecast period (2073-2087) in the HI scenario was 250 mm for sand and 213 mm for silt. In the MI scenario I evolved to 156 mm for sand and 112 mm for silt.

However the calculation neglects beneficial effects on crop yield due to altered CO<sub>2</sub> availability for the plant in the atmosphere [4]. The dramatic yield reductions in this study are consistent with previous general figures for Belgium [3].

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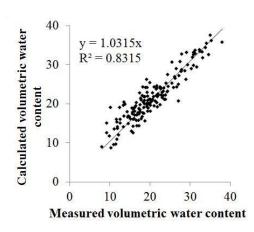


Fig 1: Correlation between calculated and measured volumetric water content (%) in 2012 over 30 potato fields in Belgium.

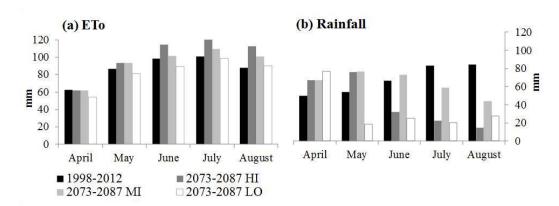


Fig 2: Current ETo and rainfall compared with the forecasted High (HI), Mean (MI) and Low (LO) climate change scencario derrived from climate series in Uccle (Belgium).

# THE EFFECT OF SOIL IMPROVING PRACTICES ON POTATO YIELD, QUALITY AND DISEASES IN SLURRY FERTILIZED LAND

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Potatoes (Solanum tuberosum L.) managed for maximum production show a high nutrient demand and due to the high economic value of potato and the vulnerability to many diseases and pests, pesticides are frequently applied to protect this crop. However, recent legislative restrictions on the use of fertilizers and pesticides and an increasing public concern regarding food safety and the environment call for drastic reduction of the chemical inputs in agriculture. This implies a greater reliance on the self-regulating processes of the soil such as supply of nutrients, water regulation and disease suppression. With the new emphasis on sustainable agriculture comes the awareness of the importance of soil quality to the productivity of the soil. Possible tools for the maintenance and the improvement of soil quality are noninversion tillage systems, crop rotations and cover crops, the use of compost, etc. In order to evaluate the effects of soil improving practices on soil processes and crop production, a long-term field experiment (BOPACT) was initiated at ILVO in 2010. The experiment has a strip split plot design with three factors and four replications. The factors are 1) slurry application (cattle vs pig slurry), 2) tillage practices (ploughing (CT) vs non-inversion tillage (NIT)), and 3) farm compost application (0 (FC0) vs 2 ton C.ha year (FC1)). The trial has a 4-year rotation with maize, potato, summer barley and leek, with cover crops during winter periods. Crop yields and quality parameters (dry matter, N and P content) are determined annually. For potatoes, which were grown in 2011, the harvested tubers were sorted into different size grades after which the underwater weight (UWW) and the incidence of common scab (Streptomyces scabies) and black scurf (Rhizoctonia solani) were determined. Seed tubers infiltrated with Dickeya solani were also planted establishing a random 5% contamination level in each of the objects to evaluate the natural disease suppressiveness of the soil in the plots. Wilting, blackleg and stem rot were scored during the cultivation and composite samples of 200 tubers from the plots were analysed after harvest. After two years, no significant effect of the trial treatments on total potato yield (both fresh and dry matter yield) and the fresh weight yield of marketable-size tubers (>35 mm diameter) has been observed. Amongst the tuber quality parameters, a significantly higher N-content was recorded in the cattle slurry amended plots. The application of cattle slurry also resulted in a significantly higher incidence of black scurf and in a higher occurrence of Dickeya symptoms. Plots with application of farm compost displayed reduced Dickeya infection. Latent infection of Dickeya was also more detected in the tuber samples from the cattle slurry plots. Furthermore, a significant interaction (tillage x compost) effect on UWW was noticed. The application of compost resulted in a significantly higher UWW in the CT plots compared to the NIT plots. As this experiment is still ongoing, we will continue to test the influence of the different soil improvement practices on potato yield, quality and diseases in 2015 during the next crop rotation.

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# FACTORS INFLUENCING PERFORMANCE OF SEED PLOT TECHNIQUE IN SEED POTATO QUALITY IMPROVEMENT AMONG SMALL SCALE FARMERS

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Potato seed-borne diseases are the major causes of continuous low yields in potato production in East and Central Africa (ECA). A small seed-plot technology (SSPT) has been developed as a step towards effective management of the seed borne diseases. This has been successfully promoted and adapted to varying farming conditions, as a self-sustaining technology for on-farm seed potato production, where disease-free seed is planted at high-density in plots that are apparently free from bacterial wilt. The objectives of the study were; to establish the influence of spacing and fertilizer nutrient composition on the performance of small seed plot; and to determine the influence of fertilizer levels on the performance of small seed plot.

The trials were conducted in seven locations for three seasons. Different fertilizer types (DAP and Mavuno NPK) and fertilizer rates (0, 45, 90 kg N/ha) were used at a spacing's of 20\*20 cm and 30\*30 cm, to optimize management for SSPT. Observations were made on the following parameters, number of tubers, tuber yield (Kg), tuber weight (g/tuber), number of tubers per unit area and the number of tubers per plant.All the experiments were designed in a Randomized Complete Block Design. Data was analyzed using Microsoft Excel and Statistical Analysis System (SAS) software version 9.1. ANOVA was used to determine the difference among treatment means, while the significance difference between each treatment means was done further using DMRT at 5% significance level. The results indicated that in most locations tubers were higher with Mavuno fertilizer application compared to DAP fertilizer application, this could be attributed to an increase in stolon number through its effect on Gibberellins biosynthesis in the potato plant, the involvement of gibberellins in regulating stolon number through stolon initiation is reported by [1]. According to [2], NPK affect tuber formation in potato by influencing the activity and phytohormone balance in the plant, especially, on the levels of gibberellic and abscissic acids and cytokinins. Planting in common spacing of 75\*30cm the varieties used in the trials produce about 25 tubers per unit area, whereas the SSPT spacing of 20\*20 cm and 30\*30 cm produced 67 and 54 tuber per unit area on average respectively, this results were in relation to findings by [3,4,5] indicating that tuber production per plant are directly correlated with spacing per plant. In general if clean land is extremely limited the spacing of 20\*20 cm should be chosen to make best use of this part, whereas land is relatively sufficient a spacing of 30\*30 cm seems to be more practicable and economically viable.

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# HOW THE AGE OF TRANSPLANTS FROM IN VITRO-DERIVED POTATO PLANTLETS AFFECTS CROP DEVELOPMENT AND SEED YIELD AFTER FIELD PLANTING

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## Introduction

In vitro produced potato plantlets are commonly used in potato seed production for direct production of minitubers in glass- or screenhouses or for production of transplants followed by field planting. When transplanting to the field, proper haulm development is crucial for achieving high yields. Haulm development may be limited by too early tuber initiation and growth, competing with leaf development. This problem is most pronounced in early cultivars and could be influenced by the conditions during transplant production or the duration of that phase. During this phase, the plants gradually proceed in the degree to which they are induced to tuberise.

This research studies the effects of varying the duration of the transplant production phase, to understand how the development stage of the transplants at the moment of field planting affects their growth and seed tuber production in the field.

## **Materials and Methods**

Transplants from in vitro-derived potato plantlets of cvs Gloria (very early) and Bintje (mid early) were produced during 2, 3 or 4 weeks under controlled conditions, but hardened outside during the last 3 days. All transplants were transplanted to the field on the same date. The experiment was a split-plot experiment with five replicated blocks and harvest date (0, 14, 28, 56, 70 and 84 days after transplanting) as split factor. Ground cover was assessed weekly; other variates needed for a yield formation study were assessed on the harvest dates.

#### Results

At transplanting, haulm weight, tuber weight, and harvest index [tuber/total dry matter (DM)] were higher in older transplants. Two-week old transplants had no tubers at transplanting.

Two weeks after field planting, tuber formation was observed in all treatments. In crops from older transplants, the percentage of the total DM increase in these 2 weeks that was allocated to tuber growth was higher than in crops from younger transplants; crops from 4-week old transplants allocated >85% of the DM to tubers, crops from 2-week old transplants allocated >79% of the DM to the haulm. This resulted in harvest indices of 64% in cv. Gloria and 59% in cv. Bintje after 2 weeks of field growth in crops from 4-week old transplants, and in limited early haulm growth in these treatment.

During the later growing period, the unfavourable DM distribution in crops from older transplants was gradually reverted to that in crops from 2-week old transplants. This normalisation occurred earlier in cv. Bintje than in cv. Gloria and earlier in crops from 3-week old transplants than from 4-week old transplants. Maximum ground cover was achieved later in crops from older transplants and in cv. Gloria it was also lower than in crops from younger transplants.

Due to the differences in ground cover during the growing period, the amount of radiation intercepted at the final harvest and the final tuber yield were higher in crops from younger transplants. No effects of transplant age on harvest index were apparent anymore at that moment.

## **Conclusions**

Seed tuber yield in crops from 2 to 4 week old transplants was highest when crops were grown from younger transplants. This was due to their higher light interception during the growing period. Although younger transplants were smaller at the time of transplanting, they showed a faster increase in ground cover in the field than older transplants, due to a more favourable dry matter allocation pattern across tuber and haulm growth.

# PERFORMANCES OF POTATO CULTIVARS FROM IN VITRO AND CONVENTIONAL PRODUCTION SYSTEMS

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### Introduction

The biological value of potato (*Solanum tuberosum* L.) seed mainly depends on the physiological age and the level of disease infection of tubers. Most of the world potato producers use in vitromicropropagation techniques to acquire healthy tuber seed. By in vitromicropropagation technology, it is possible to produce plantlets and microtubers (McT). Minitubers (MnT) are produced for in vitro derived plantlets or McT. The purpose of this study was to compare the field performance of in vitro derived MnT to conventional tubers (CT).

#### **Materials and Methods**

This study was carried out at EAARI, Erzurum, Turkey.Pasinler, Granola and Caspar were used in the present study. Field trial was carried out for MnT and CT. Observations were recorded as shoot length (SL, cm), the number of shoots (NS), days to tuber maturation (DTM), number of tubers per plantlet (NTP), weight of tubers per plantlet (WTP, g), marketable yield (MY, kg ha-1) and % tuber dry matter (TDM).A completely randomized design (CRD) was used to evaluate three cultivars, two production systems with four replications.

## **Results and Discussion**

It is clear from the results that all plantlet and tuber characteristics studied were higher in MnT derived system compared to CT system. The maximum SL of 60.58 cm, the maximum NS of 11.17 and the maximum NTP of 21.5 were recorded on in vitro originated potato cultivars. Minimum DTM were recorded on cv. Pasinler (90 days) and followed by cv. Granola (102.5 days) on MnT system. The highest WTP was obtained from Pasinler (1986.0 g plant-1) and followed by Caspar (1983.5 g plant-1). Marketable yield showed similar response with WTP. Although MnT system showed higher TDM content (21.66%) than CT system (20.52%), difference was insignificant.Researhers (1) showed that some cultivars are suitable for McT system however some are more suitable microplant based system. Similar results were noted in Ozturk and Yildirim(2) study in which they determined better results from in vitro based potatoes. The presented results are in disagreement with Singh et al. (3) study, in which they noted that all morphological and physiological parameters along with the yield characteristics were better in CT than micropropagated plants.

#### Conclusions

Significant differences were determined between MnT and CT based systems for yield and yield characteristics in the field conditions. Since the presented results showed MnT based system is better on in vivo conditions, and tuber fresh weight was almost two times higher that of CT method suggesting a potential for MnT for field planting. Since genotypes respond differently to examined production systems, it can also be concluded that plant and tuber characteristics studied were variable depending on the genotype. By employing of this system in seed tuber production methodwillprovide economic advantages for seed potato producers.

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## SEED POTATO IDENTIFICATION USING SSR MARKERS IN FRANCE: ORGANIZATION. METHODS AND DATABASE

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French seed potato industry is recognized for its know-how and quality. Single sequence repeat (SSR) markers have long been validated to be an essential tool to fingerprint cultivars, check their identity and manage collections.

In 2001 French potato seed growers' organization (FN3PT) proposed to implement SSR markers in the certification scheme. This led to the set up and publication of a first kit of five markers able to identify 286 cultivars [1]. In the seed potato production scheme (273 varieties multiplied in 2013), fingerprinting is used to check the initial material and the first generations of the multiplication process and also in complement to visual field inspection (in case of doubt). The kit is routinely used in five French labs where around 1500 tests are realized per year. Seven ring tests have been organized for 10 years with the Official Certification Body (SOC) to check the ability of the labs, the markers and the data set [2].

With the increase of the number of cultivars, two additional markers have been implemented into the procedure. Thanks to a grant of the French Ministry of Agriculture, an internet platform has been designed by the FN3PT IT department in order that the molecular profiles obtained could be exchanged interactively between the labs [3]. Login and password are needed to access the secured platform. Collaboration with Inra allowed to include in the database the profiles of 350 worldwide varieties and breeding lines (described in [4]) and the analysis of the transferability of the markers to 3 different gel systems: acrylamide electrophoresis followed by silver staining which was the routine method up to now, LI-COR sequencing system and capillary sequencing system [3].

The database is now containing the profiles of 1193 varieties and 415 hybrids. Information concerning 30 markers is available: 7 of the kit used in the seed certification labs and 23 markers used in the molecular description of the Inra collection. 10 new alleles were added to the previously known panel routinely in

Next step is to obtain the validation of our kit using the ISO 13495:2013 standard. Three markers of our kit are also part of the kit developed by [5], another prospect is to get additional experience on the 6 other markers described in this latter study.

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## UPDATING OF THE MAIN SEED POTATO VIRUSES AND THEIR TRANSMISSION BY APHIDS

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During the last ten years, PVYNTN in seed-potato production was the most abundant virus and a limiting factor for the certified seed. In fact, it is the most important reason for downgrading seed potato lots of Spunta to lower classes and rejection of certification. An updating of other viruses species in four seed production areas (Soliman, Bir Mroua, Batan, Chbika) was realized in spring 2013. Samples were analysed by ELISA using polyclonal antiserum of PVY, PLRV, PVX, PVS and PVA and two monoclonal antiserum of PVY. Results revealed the presence of PVA (99%), PVY (72%) distributed as 65% PVY<sup>N</sup> and 9% PVYO, PVS (68%), PLRV (33%) and PVX (9%). However, this situation becomes more complicated with the detection of two other abundant viruses PVA and PVS infecting seed production in addition to PVY. These three viruses are transmitted by aphids in non persistent manner. Previous works showed that PVY is transmitted by the 15 most abundant aphids captured by yellow water traps and suction trap. These species are Aphis gossypii, Macrosiphum euphorbiae, Myzus persicae, Aphis fabae, Aulacorthum solani, Aploneura lentisci, Brachycaudus helichrysi, B. cardui, Hyalopterus pruni, Hyperomyzus lactucae, Acyrthosiphon pisum, Rhopalosiphum padi, R. maidis, Aphis spiraecola, and Lipaphis erysimi. Among these species M. persicae, A. gossypii and A. spiraecola were the most efficient vectors. For a better understanding of PVA and PVS epidemiology, transmission tests were conducted in the laboratory to identify the main vectors. Five aphid species: A. fabae, A. gossypii, A. spiraecola, M. euphorbiae and M. persicae were used to detect the three viruses PVY, PVA and PVS after the acquisition time on infected plants and their transmission to healthy plants. Detection of viruses on aphids and plants was performed by ELISA test and by molecular method for PVY only to characterise which strain is implicated. The variability of the behaviour of these five aphid species is discussed.

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ADVANCES TOWARD COMMERCIAL IMPORT-EXPORT OF SPROUTS AS SEED-POTATO: BRAZIL-CANADA PIONEERING ON A POTENTIAL NEW ALTERNATIVE (PRE-)BASIC SEED-POTATO STOCK SUPPLY SYSTEM\*.

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Like most potato producing countries laid on tropical and sub-tropical regions, Brazil is also dependent on annual imports of high class seed-potato lots, for (1) genotype; and (2) renewal of (pre)basic seed-potato stocks, caused by fast viral seed-potato degeneration: mainly PLRV, PVY (aphids) and now warning ToCV (whiteflies) [4]. By the years 70-80s, imports of high-grade, basic class tuber/seed-potato lots, were around 13,000 tons, but over the past 10 years have dropped to 5,000 tons. Such drop can be credit to (1) local scientific research on potato virus diagnose, epidemiology and control; and, (2) improvements on lab tissue-culture techniques, leading to increased supply and demand for virus-free (early-generations) propagating material: in vitro plantlets and/or mini-tubers produced inside aphid-proof greenhouses. The alternative sprout/seed-potato technology [1;2], now officially accepted as propagating material [MAPA/IN-32, as of 21-11-2012/Potato], can now certify virus-free, true-to-type, mini-tuber/seed-potato stocks, when originated from sprouts, detached from basic classes (national or imported) tuber/seedpotato lots [3]. The rising challenge of reducing field multiplication of basic seed-potato is linked to increasing offer of basic class seed-potato lots. For 7-year experimental Brazilian import of sprouts (permit issued by MAPA), from the USA and Canada, 2003-09, sprouts detached from basic class tuber/seed-potato lots (G-1), were evaluated just like tissue culture plantlets or micro-tuber (early generations) to produce mini-tubers (inside aphid-poof greenhouse; 10-25°C). Sprouts were efficiently shipped, inside zipped plastic bags, at room temperature, via USP, FEDex, Post Office Express Service (4-5 day for delivering). Average of 1,000 sprouts/year; planted in pots with horticulture substrate, produced mean of 3 mini-tubers/sprout in 75-80 days; confirmed free of major seed-potato virus by ELISA. Comparative performance of sprouts x tissue culture plantlets and micro-tubers (>1<2 cm) did not show significant difference under different mini-tubers/seed-potato producers. Therefore, this innovative export/import of sprouts as seed-potato, revealed a viable, additional seed-potato product to renew (pre)basic seed-potato stocks. The results would strongly support import of sprouts as a new way of increasing supply of certified (G-0) mini-tubers/seed-potato lots. Attempts to commercial import of sprouts, detached from G-1 and G-2 tuber/seed-potato lots from Canada, were made on 2010 and 2013. Proforma invoices were issued to two highly qualified Brazilian seed-potato producers. Unfortunately, we did not achieve the importation due to the impossibility or the delay to MAPA to issue the import permit. In 2010, sprout/seed-potato had not vet officially regulated to produce seed-potato. With the new IN-32, it has been resolved. In 2013, restrictions were due to lack of a risk analysis for sprouts. The export/import of sprouts, detached from basic classes, early tuber/seed-potato generations (G-1-G2), is expected to become soon, an additional seed-potato product to be export-imported, just like the traditional basic tuber/seed-potato lots.

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## Workshop 6 - Nematodes

## 126 bis

HOW MOLECULAR TOOLS CAN SUPPORT AN EARLY AND ACCURATE DETECTION OF PESTS AFFECTING POTATOES: THE EXAMPLE OF NEMATODES FROM *GLOBODERA* AND *MELOIDOGYNE GENERA* 

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Plant parasitic nematodes as soil organisms are complicated pests to manage. Especially some nematodes species, e.g *Meloidogyne* species, are able to parasite many families of plants which makes their management difficult. Additionally some species, e.g *Globodera* species, have specific properties for surviving adverse conditions, which also contribute to their establishment and spread.

Whatever the regulatory status of the nematode, their presence may adversely affect the productivity and the quality of many crops. Specific concerns exist for potato crop for regulated nematodes, *Meloidogyne chitwoodi*, *M. fallax*, *Globodera pallida* and *G. rostochiensis*. Consequently the early detection of plant parasitic nematodes is a strong support for an efficient management of these pests.

New molecular technologies such as polymerase chain reaction (PCR) provide sensitive and high throughput assays in different disciplines including nematology. They allow the detection of pests directly from a complex matrix (e.g. soil, tubers, bulbs, leaves) without morphological separation.

The evaluation of the performance of these assays in routine situation is a key element before their use, in order to establish if they fit for purpose (e.g. level of detection, sensitivity, robustness). The results obtained for the evaluation of real time PCR assays will be presented in the framework of the detection and the quantification of regulated *Meloidogyne* species. The consequences for national surveys will also be documented.

Examples of routine uses of real time PCR assays for *Globodera* detection will also presented. The limits of these approaches, such as the sampling strategies operated, will be addressed.

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# INTEGRATED MANAGEMENT OF POTATO CYST NEMATODE (*GLOBODERA* SPP.) FOR MORE THAN HALF CENTURY IN NORWAY

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Potato cyst nematodes (PCN) *Globodera* spp. are thought to have originated in the Andean region of South America, and have been introduced into Europe after 1850. Subsequently, in Nordic region PCN were detected in Sweden 1922, Denmark 1928, Finland 1946, Faroe Island 1951, Island 1953, and Norway 1955 [1, 2].

Since the first record of PCN in Norway in 1955 there has been more than a half century of managing the nematode. After the first detection of PCN extensive surveys were carried out and regulations were implemented. The first statutory regulation for PCN dates from 1956, and has later been amended several times [3]. The latest amendment was made in 2010 [4]. All regulations prohibit the introduction and spread of PCN with soil and plant materials. Early control strategies included the use of chemical fumigants and resistant potato cultivars in infested fields. The taxonomic separation of the yellow *Globodera rostochiensis* and the white species *G. pallida*, together with emerging information on the existence of pathotypes caused a change in the strategy involving a controlled use of resistant cultivars to avoid the increase of resistant breaking pathotypes. In the preceding decades great emphasis has been placed on documenting freedom from PCN in the production of certified seed potatoes, and on the detection of infested fields and their placement under effective quarantine regulations. In the early 1960s import and movement of all kinds of potato seed was prohibited, as a measure to prevent the introduction of new PCN populations, and to prevent contamination of uninfested land. In addition proper crop rotation and the use of cultivars with resistance have been enhanced [2].

Commercial chemical fumigants, organophosphates or carbamate nematicides have not been used in Norway for more than 40 years. Today, non-virulent *G. rostochiensis* is managed by crop rotation, while infestations by G. pallida or virulent *G. rostochiensis* results in 40-years ban on growing potato [3, 5]. Most Norwegian potato cultivars have the resistance genes, Gro-1 (H1) from Solanum tuberosum ssp. andigena. Crop rotations using non-host crops, alternating susceptible and resistant cultivars are important control measures, but not easy to implement in Norway due to restricted acreage suitable for long rotations. Hence, the use of resistant potato cultivars becomes important, but requires a better knowledge on the species and pathotypes present [3, 5]. An overview of the PCN management strategies and studies done since PCN were detected in Norway will be presented.

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# THE EFFECT OF PYRAMIDING TWO RESISTANCE LOCI TO POTATO CYST NEMATODE GLOBODERA PALLIDA PA2/3 IN POTATO

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### Introduction

Due to heterogeneity for avirulence genes within *Globodera pallida* pathotype Pa2/3 populations, single resistance genes offer only partial resistance. The best strategy to combat PCN infestations is the use of resistant varieties. Two quantitative trait loci (QTL), GpalVsadg derived from the species *Solanum tuberosum* ssp. *andigena* and Gpa5 from *Solanum vernii* individually confer partial resistance. The objective of this research was to determine if pyramiding these QTLs using marker assisted selection provides increased resistance to *G. pallida* Pa2/3.

### **Materials and Methods**

380 F1 genotypes were generated from a cross between ET5848/3 and the variety Innovator which contained the QTLs GpalVsadg and Gpa5 respectively. The genotypes were seperated into four distinct marker classes (no QTLs, both QTLs and either QTL) using a molecular marker for Gpa5 developed previously by Sattarzadeh et al. 2006 and for GpalVsadg by a marker developed in Teagasc. Three tubers per genotype were planted individually in 0.7 litres of soil (kept at 25°C throughout test with continuous watering). Fifteen cysts of *G. pallida* Pa2/3 (Chavornay population) were added to each pot. Cysts counts were taken three months after inoculation. Individual and combined effects of GpalVsadg and Gpa5 were analysed with a general ANOVA. The number of cysts counted per genotype were transformed by taking the natural logarithm of the (mean cyst count + 1), to obtain a more uniform distribution of the variance. Tukey's Pairwise comparison test was used for the multiple comparison of means P<0.05). A suitable contrast was used to test for an additive effect between the QTLs.

## **Results and Discussion**

The class lacking either QTL developed the highest number of cysts (mean = 285.6). The class of genotypes containing GpalVsadg, only, formed an average of 76.2 cysts, a significant decrease of 74% (P<0.0001). The class containing Gpa5, but not GpalVsadg, developed, on average, 41.3 cysts, and offerd a reduction of 86% of the number of cysts (P<0.0001). The means of the two single QTL classes were not significantly different (P=0.3546). When both QTLs are present, the average number of cysts is 18.5 cysts, a decline of 94% relative to the no QTL class. According to the analysis of variance (ANOVA) followed by contrast analysis, the joint effect of both loci has a significant effect on reducing the number of cysts (P<0.0001) relative to either of the individual QTL-containing classes, indicating that the two QTLs act in a generally additive manner in response to the population of *G. pallida* Pa2/3 used in this experiment.

### **Conclusions**

This study proves the potential of pyramiding different resistance genes using MAS to increase levels of quantitative resistance levels to *G. pallida* Pa2/3. Pyramiding resistance genes should also protect the durability of these sources.

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### Workshop 2 - Precision agriculture

### 129

#### PRECISION AGRICULTURE IN GEORGIA AND POTATO HIGH YIELDS

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Precision agriculture in Georgia is a new and developing discipline that incorporates advanced technologies to enhance the efficiency of farm inputs in a profitable and environmentally sensible manner. Potato yield monitoring and variable rate application are the most widely used precision technologies. Versatile guidance systems utilizing the global positioning system (GPS) and management zone approaches are also being developed to further increase productivity by reducing error, cost, and time. These technologies provide tools to quantify and manage variability existing in fields across an array of cropping systems. A review of precision farming technologies that are currently being used World Wide would be adopted by the Georgian Farmers. Precision farming yields as threefold advantage. First, it provides the farmer useful information, that can influence their use of seed, fertilizer, chemicals, irrigation. and other farm inputs. Second, economics are optimized by enhanced efficiency of farm inputs. Finally, by varying the amount of farm inputs (fertilizers, pesticides, and irrigation) used for crop production (in vitro and in vivo virus free seeds) and applying those inputs exactly where they are needed, the environment is sustained. Effective soil and water management are essential in order to optimize crop yields and maintain soil functionality for sustainable, profitable in Georgia safe and bio and organic food production. Modern technologies incorporating Geographic Information Systems (GIS), Global Positioning Systems (GPS), remote sensing, and geostatistics provide unique opportunities to advance ecological understanding of pests across a landscape. Increased knowledge of the population dynamics of plant pathogens will promote management strategies, such as site-specific management, and cultural practices minimizing the introduction and impact of plant pathogens.

#### SOME EXAMPLES OF PRECISION AGRICULTURE IN POTATO

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#### Introduction

Site-specific management is the process of adjusting agricultural practices within fields according to the measured spatial variation. It is an alternative to the traditional uniform management of agricultural fields, because it is supposed to increase the profitability of crop production and reduces undesirable environmental impacts by regulating production inputs according to local needs.

This abstract presents the first results ongoing the characterization of the spatial variation in soil and development of potato crops. Study sites were chosen within two traditional, high-productive agricultural regions in Flanders, Belgium: the polder area in northwest East-Flanders and the Belgian loess belt in the southern part of Flanders.

#### Materials and methods

The soil of the polder region is generally composed of an upper clay layer on a sandy substrate. The soils of the Belgian loess belt consist of Tertiary marine sandy and clayey deposits covered by a quarternary loess topsoil. The thickness of the loess cover varies with the position in the landscape. In both regions both soil and crop scans were performed as the first attempts to implement site-specific management in potato.

Detailed soil measurements were performed with a multi-receiver EMI instrument: the Dualem-21S. The sensor was mounted on a sled pulled by an all-terrain vehicle. The Dualem-21S measures simultaneously the apparent electrical conductivity of four different soil volumes up to a depth of 3 m.

The GreenSeeker crop sensing system uses optical sensors to measure and quantify crop development. A sensor located on the underside of the GreenSeeker system emits brief bursts of red and near infrared light, and then measures the amount of each type of light that is reflected back at the sensor. Four sensors were used, mounted on a boom in the front of a tractor, wherefrom the NDVI and WDVI were calculated.

Sampling of tare soil was compared to soil sampling in the field for detection of potato cyst nematodes by using the AMI100 method developed in the Netherlands for monitoring seed potato fields.

#### Results

EMI soil survey in the polder region allowed to map within-field textural variations. In two study sites in the polder regions, ECa differences could be correlated to the textural differences in the subsoil which proved to effect either crop yield or the desiccation of the foliage. Within the third study site, increases in ECa with depth could be correlated to the presence of salt groundwater.

In the loess region, differences in ECa could be attributed to the depth of the Tertiary layer below the loess cover. Further research is needed to correlate these variations to the within-field crop performance.

An interesting and simple application is based on machine tracking during harvest. It was found that soil tare sampling at harvest time was very accurate for early detection of Potato cyst nematodes. First indicate similar accuracy to the AMI100 method. By GPS tracking, each trolley is connected to a specific area in the field.

### **Conclusions and perspectives**

Further research is required to correlate the within-field soil variations to the crop performance of potato plants. These preliminary results were able to correlate soil textural variability to potato development and the desiccation of the foliage. The combination of tracking harvest machinery and soil tare sampling is a promising technique for cheaper and more detailed soil sampling.

# EVALUATION OF POTATO CROP NITROGEN STATUS BASED ON LEAF CHLOROPHYLL AND LEAF FLAVONOIDS CONTENTS ASSESSED BY HAND-HELD FLUORIMETERS DUALEX AND MULTIPLEX

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#### Introduction

In-season indicators of potato Crop Nitrogen Status (CNS) can help for the adjustment of crop N fertilization at specific field scale. The Nitrogen Nutrition Index (NNI) is suggested as the best indicator of CNS. NNI serves as a reference method for quicker, easier and non destructive optical methods. Portable and hand-held optical sensors, based on light transmittance or reflectance readings, have been developed to measure leaf chlorophyll content which decreases with leaf N content [1]. Increase in leaf polyphenolic (flavonoids) content is highly sensitive to leaf N content decrease and the objective was to investigate whether optical assessment of flavonoids contents could be used as early CNS indicators. Using recently developed fluorimeters, we compared in this study the potentialities of leaf chlorophyll content, leaf flavonoids content and ratios of both as CNS indicator. The absolute readings of the indices provided by optical sensors could be biased by factors other than N. In order to alleviate effects of such factors, we tested the potentialities of relative readings obtained by the use of within field reference (well fertilized reference plot versus zero-N reference plot).

#### **Materials and Methods**

Trials were conducted in 2012 and 2013 at the experimental field of the Walloon Agricultural Research Centre in Gembloux (Belgium). The trial included two potato [Solanum tuberosum (L.)] cultivars: Charlotte and Bintje, and six increasing N rates (0 to 250 kg ha<sup>-1</sup>). Leaf flavonoids content was assessed using the Dualex and Multiplex fluorimeters (Force-A, Paris, France). The leaf chlorophyll content was assessed with a Hydro-N Tester chlorophyll-meter (Yara, Oslo, Norway), the Cropscan radiometer (Cropscan, Rochester, USA) and the Dualex and Multiplex devices. The measurements were made at several growth stages from emergence to the start of senescence. Plant tissue samples were collected and analyzed allowing the assessment of the biomass N concentration and the NNI. In order to assess the potentialities of the different generated indices three criteria were studied: (i) sensitivity to N; (ii) earliness of the CNS diagnosis; and (iii) accuracy of the index.

#### Results

Statistical analysis showed that the combined ratio of leaf chlorophyll content to leaf flavonoids content was able to respond successfully to the three criteria. Our study showed that the normalization procedures alleviate the effect of cultivars for almost the N indices provides by the studied sensors. Comparing to the non-fertilized reference plot, the use of the well-fertilized reference plot improves the statistical correlations obtained between N indices and the NNI.

### **Conclusion and perspectives**

The ratio of leaf chlorophyll content to leaf flavonoids content could be used as more valuable tool for assessing potato CNS, comparatively to the indices based on transmittance or reflectance. The establishment of the well-fertilized reference plot appeared as the most efficient way to relate the indicator diagnosis to the actual N status. Further studies are needed to establish critical values of chlorophyll and flavonoids indicators that provide threshold for decision on supplemental N need.

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### 131 bis

THE USE OF HIGH RESOLUTION SATELLITE IMAGES AND CROP GROWTH MODELS FOR MONITORING POTATO GROWTH AND DEVELOPMENT AT FIELD LEVEL: OPPORTUNITIES AND LIMITATIONS

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Belgian potato processors, traders and packers are increasingly working with potato contracts. The close follow up of contracted parcels on the land as well as from above is becoming an important tool to improve the quantity and quality of the potato crop and reduce risks in order to plan the storage, packaging or processing and as such to strengthen the competitiveness of the Belgian potato chain in a global market. At the same time, precision agriculture continues to gain importance and progress. Farmers are obligated to invest in new technologies.

Today the use of geo-information by the (private) agricultural sector in Belgium is rather limited, notwithstanding the great benefits this type of information may offer, as recognized by the sector. The recently approved "iPot" project, financed by the Belgian Science Policy Office (BELSPO), aims to provide the Belgian potato sector, represented by Belgapom, with near real time information on field condition (weather-soil) and crop development and with early yield estimates, derived from a combination of satellite images and crop growth models. An intuitive web based geo-information platform will be developed to allow both the Belgian potato industry and the research centres focusing on the cultivation of the potato crop, to access, visualize and analyze the data and to use them, combined with their own field observations and in close collaboration with the farmers, for improved decision-making.

This presentation illustrates how time series of optical high resolution satellite images can be used to monitor the development of the potato crop over large areas and to get an idea of intra- and inter-field variability. Crop emergence maps show the time (date) and degree of crop emergence and crop closure (in terms of % cover) whereas crop senescence maps reflect the % cover of non-photosynthetic active vegetation. These maps allow for crucial decisions on haulm killing, harvest time and overall planning to be made. Typically, vegetation indices are derived from satellite images. These indices provide information on the crop's productivity. Visualization of vegetation indices often reveals variability within a field. This is considered to be important information for the farmer and the processing industry as they envisage homogeneous product quality and as less variability as possible. Comparison of actual index values with average value allows to locate fields where potato growth is sub optimal. This information can be used by the industry to organize targeted field visits. To validate the satellite based monitoring products, field observations, close range sensing measurements as well as UAV images are used.

Accurate and timely yield predictions are of great economic importance. Potato yield can be estimated by means of crop growth models such as B-CGMS/Wofost, Lintul,... Part of the iPot research will be dedicated to adapt these models from regional to parcel level and to assimilate satellite derived phenology data into these models in order to increase their performance and to obtain better yield predictions.

It is clear that the above techniques already offer plenty of opportunities for improved potato monitoring. However, today there are still some (practical) limitations to overcome such as the limited availability of optical satellite data under cloudy conditions, the cost price of the satellite data, the fact that the crop growth models only cover a few varieties and that more research is required to enable accurate estimation of quality parameters such as tuber size or under water weight.

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# POSTER PRESENTATIONS

Abstracts 132 to 304





### **GENOMICS** (poster session)

### 132

# THE DRAFT GENOME SEQUENCE OF SOLANUM COMMERSONII DUN., A VALUABLE SOURCE OF RESISTANT GENES FOR POTATO BREEDING

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State-of-the-art sequencing technology combined with the availability of the S. tuberosum reference genome now allows the characterization of genetic variations that affect tuber potato crop. However, recent studies have shown that reliance on a single reference genome may underestimate the variability among different genotypes. The wild relatives of the tetraploid (2n=4x=48) cultivated potato provide a rich, unique and diverse source of genetic variation for potato breeding. In particular, the diploid (2n=2x=24) wild species S. commersonii contains a largely untapped reservoir of agronomically important genes, such as resistance to biotic and abiotic stresses. We de novo sequenced the genome of S. commersonii to 103x coverage using Illumina technology producing a 862 Mb draft genome. We predicted 39,290 proteincoding genes supported by transcript data and annotated them on the basis of sequence homology. More than 8,500 S. commersonii-specific gene families were discovered that were not shared with S. tuberosum. In the S. commersonii assembled genome 2,523 non-redundant NBS-encoding R gene candidates were annotated using a Hidden Markov Model (HMM) and manual curation strategy. The annotation of a set of genes involved in abiotic stress response is ongoing. Results on genome-wide orthology and paralogy predictions based on the analysis of the phylogenetic trees will be presented. The draft genome sequence of S. commersonii will substantially increase our understanding of the limitations of the domesticated germplasm and the potential to use wild relatives for crop breeding and evolutionary studies.

# CHARACTERIZATION OF SNAKIN/GASA GENE FAMILY IN SOLANUM TUBEROSUM AND FUNCTIONAL ANALYSIS OF SNAKIN-1

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#### Introduction

Snakin/GASA proteins are widely distributed among plant species. They have been implicated in diverse biological activities: cell elongation/division, root formation, stem growth, flowering time, fruit ripening, and stress tolerance [1-4]. Interestingly, all of them maintain 12 cysteines of the C-terminus in highly conserved positions that are probably responsible for their protein structure and were shown to be essential for their biochemical activity as antioxidants [5, 6].

Snakin-1 is a member of the Snakin/GASA family isolated from Solanum tuberosum that was found to be active against pathogens in vitro and we demonstrated that its overexpression in potato enhances resistance to Rhizoctonia solani and Erwinia carotovora [7, 8]. We recently showed that Snakin-1 silencing resulted in an abnormal phenotype affecting cell division, leaf primary metabolism and cell wall composition demonstrating that it has additional roles in growth and development [9].

#### **Materials and Methods**

Snakin/GASA genes were *in silico* screened in the Potato Genome Sequencing Consortium database using the InterPro GASA domain annotation (IPR003854) as query. For functional analysis, *Snakin-1* transgenic potato lines and conventional protocols were used [9].

#### Results

In this work, we carried out a genome-wide search and 14 novel Snakin/GASA genes were identified in the potato genome in addition to the previously reported genes (*Snakin-1*, *Snakin-2* and *Snakin-3*). Chromosome localization studies of Snakin/GASA genes indicated that they correspond to different loci and are distributed on nine of the 12 potato chromosomes. *In silico* analyses revealed that Snakin/GASA gene upstream sequences carry a variety of potential hormone and stress responsive *cis*-regulatory elements. Expression analyses showed a tissue specific expression pattern for each family member and also some differences from the previously reported data.

Further analyses of transgenic potato lines suggested that the mechanism of action possibly involves the participation of *Snakin-1* in redox homeostasis.

#### **Conclusion and perspectives**

Snakin/GASA family in potato consist of 17 members and phylogenetic analysis based on sequence alignments of the full-length proteins resulted in three major groups. Expression studies indicated that the spatial regulation of these novel Snakin/GASA genes is highly specific suggesting distinct functions. And as it was described for others Snakin/GASA genes, *Snakin-1* may play its role by modulating reactive oxygen species.

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# MAJOR-EFFECTS QTLS FOR CHIP COLOR ARE MAPPED ON CHROMOSOMES I AND VI IN DIPLOID POTATO

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#### Introduction

The low level of reducing sugars in potato (*Solanum tuberosum* L.) tubers is one of the most important requirements in processing worldwide. When reducing sugars concentration is high, the non-enzymatic browning occurs during the frying process. Dark-pigmented chips and French fries are unacceptable for consumers. Low temperature (4°C) storage leads to the partial conversion of starch to glucose and fructose in potato tubers. This phenomenon is known as cold-induced sweetening. In the present study, we report map positions of quantitative trait loci (QTL) for potato chip colour at harvest, after storage at 12 and 4°C and after reconditioning.

#### **Materials and Methods**

A diploid potato segregating F1 population consisting of 92 individuals was used in this study. Chip colour was scored for tubers collected from seedlings (2011), first (2012) and second tuber generations (2013) at harvest (H), after cold storage (CS) and after reconditioning (RC). In addition, chip colour stability for tubers stored at 12°C was also determined. Four slices form each of two tubers per a plant were scored on a scale of 1 to 9, where 9 is very light colour. For genetic analyses Diversity Array Technology (DArT), the JoinMap ® 4 and MapQTL ® 6 software were applied [1].

#### Results

Using 1405 DArT segregating markers, the linkage map of all 12 potato chromosomes was constructed. Total map reached 988 cM. Number of markers on particular chromosomes varied from 29 to 226, while their length ranged from 55 to 108 cM. Chip colour segregated in the mapping population and score ranged from 1 to 9. The main QTLs were detected on linkage group I and VI and depending on the year and time of assessment and explained in year 2012 up to 16.2% (interval mapping LOD 3.53) and 22% (interval mapping LOD 4.95) of variance observed in chip colour, respectively.

#### Conclusion and perspectives

Obtained genetic map will be helpful in searching for specific genes responsible for chip colour. On the base of DNA markers that refer to specific genes, we are planning to find transcript sequences with expression levels highly correlated with phenotypic effects.

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# ALLELE MINING AND EXPRESSION ANALYSIS OF MAJOR CAROTENOID BIOSYNTHETIC GENES IN A COLLECTION OF DIPLOID AND TETRAPLOID POTATO VARIETIES

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Carotenoids are essential components of the human diet due to their beneficial effects, especially in the prevention of eye diseases. Potato germplasm shows strong differences in tuber carotenoid content both in natural and cultivated genotypes, as reflected by the wide variation of tuber flesh color in the yellow-orange range.

In an effort to disclose new allelic variants of the main carotenoid biosynthetic genes, influencing carotenoid accumulation in tubers, a collection of ten potato genotypes with contrasting tuber flesh color was analyzed at the genetic, transcriptional and metabolic level. The focus was on three structural genes: beta-carotene hydroxylase 2 (chy2); zeaxanthin epoxidase (zep); carotenoid cleavage dioxygenase 4 (CCD4), whose relevance in carotenoid synthesis and catabolism had already been highlighted in potato and other species. The varieties under study were profiled as to their carotenoid level and composition by means of HPLC. CAPS markers were employed to verify the occurrence in the selected genotypes of two previously identified alleles, known to have a major effect on the accumulation of carotenoids in tubers (chy2 allele 3, zep allele 1) [1, 2]. To discover new allelic variants, partial genomic sequences including non-coding regions of the genes of interest were cloned and sequenced and SNP polymorphisms were used to define the diagnostic haplotype of each allele. Relative expression level of each gene was determined by qRT-PCR.

High variability was found in the allelic composition of the genes surveyed. The CCD4 polymorphism of potato was analyzed here for the first time. In addition, two new alleles were discovered at the zep locus which had already been thoroughly investigated by other groups. Compared to other variants at the same loci, some of the new alleles identified displayed significant variations in transcript levels, correlated with differences in the amount and profile of tuber carotenoids, suggesting their relevance in carotenoid accumulation.

Populations segregating for these new allelic variants are being produced to verify their actual importance in conditioning high levels of tuber carotenoids with the aim to identify the most suitable haplotypes for the genetic improvement of this trait.

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# APPLICATION OF MOLECULAR MARKERS TO STUDY INTROGRESSION OF ALIEN GENETIC MATERIAL IN INTERSPECIFIC SOMATIC HYBRIDS OF POTATO AND THEIR BACKCROSS PROGENIES

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#### Introduction

World-wide potato is attacked by fungal, viral, bacterial diseases as well as by a lot of pests, like aphids and other insects. The introduction of 'exotic' germplasm from wild species of *Solanum* genus may provide a source of possibly durable resistance to pathogens and pests. Wild diploid tuber-bearing Mexican species *S. bulbocastanum*, *S. cardiophyllum*, *S. pinnatisectum* and *S. tarnii* (2n=2x=24, BB genome) are resistant to foliage-blight, have extreme resistance to potato virus Y (PVY) as well as resistance to Colorado potato beetle. The wild diploid non-tuber-bearing Chilean species *S. etuberosum* (2n=2x=24, EE genome) has extreme resistance to PVY and is an unsuitable host for potato aphids.

#### **Materials and Methods**

Interspecific somatic hybrids between common potato *Solanum tuberosum* (tbr, 2n=4x=48, AAAA genome) and the gene bank accessions of the wild species: *S. tarnii* (trn, GLKS32870), *S. cardiophyllum* (cph, GLKS30108), *S. bulbocastanum* (blb, GLKS31741) and *S. etuberosum* (etb, VIRk-9141) were produced by protoplast fusion [1, 2, 3, 4]. Following backcrossing and embryo or ovule rescue, fertile progenies derived from crosses of wide somatic hybrids with common potato have been produced. For the fusion combination tbr (+) etb BC3 lines were obtained [1]. From combinations between *S. tuberosum* and wild Mexican species BC1–BC4 progenies were produced, some of these showed high levels of resistance to diseases [2–4]. The standard methods of DNA analysis were used.

#### Results

More of 100 nSSR markers [5, 6] and 70 STS and CAPS-markers with known chromosome locations were studied to select the markers which are able to distinguish the genetic material of wild species with B and E genomes into inter-specific hybrids. In each combination from 34 to 48 markers were developed and used for studying of backcross progenies of somatic hybrids. The most BC1 lines retained all the chromosome specific trn-, blb- and etb-fragments. In BC3 and BC4 of combinations tbr (+) trn 3–5 alien chromosomes were detected by markers. Self pollination lines of the BC3 progenies possessed 0–2 trn fragments. Using SCAR, CAPS and SSR markers specific to organeller DNA different types of cytoplasm have been identified in parental lines and in hybrid material.

#### **Conclusion and Perspectives**

Perspectives for involvement into pre-breeding programs the selected BC lines with a certain genetic material from wild species are discussed.

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# CHARACTERISATION OF THE GENETIC BASIS OF FOLIAGE AND TUBER BLIGHT RESISTANCE IN THE POTATO VARIETY SETANTA USING THE INFINIUM 8303 POTATO ARRAY

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#### Introduction

The potato variety Setanta has a useful combination of traits including suitability for crisp production, suitability for long term storage and very high levels of tuber blight resistance. When initially released the variety also had high foliage blight resistance but the aggressive 13\_A2 strains of *Phytophthora infestans* have defeated this foliage resistance. Preliminary experiments suggested that tuber blight resistance to these aggressive strains remains intact, suggesting it is mechanistically and genetically distinct from the foliar resistance. Identifying quantitative trait loci (QTLs) underlying tuber blight in the variety Setanta would prove useful for deploying marker-based strategies for selection of tuber blight resistance using Setanta as a donor. Additionally investigating the QTLs which control foliage and tuber blight will give new perspectives on the interaction between these traits.

#### **Materials and Methods**

185 F1 genotypes were generated from a cross between Setanta and the blight-susceptible breeding clone C1992/42. Leaf material of the parents and 185 progeny genotypes from glasshouse grown plants was used in a detached leaf assay to determine foliage blight resistance using the 5\_A1 genotype of late blight, to which Setanta is resistant. In order to determine variation and inheritance patterns of tuber blight in the population, field grown tubers of the population were used in a tuber blight assay inoculated with a mixture of aggressive 13\_A2 strains. As a basis for QTL mapping we are in the process of analysing the population using the Illumina Infinium 8303 Potato Array, which was developed through the SolCAP project. DNA was extracted from the population and applied to the array. Data analysis for genetic map production is underway.

#### Results

The replicated leaf and tuber blight resistance experiments were analysed by one way ANOVA and significant differences were detected between the genotypes, with the parents performing as predicted. Significantly, Setanta's high level of tuber blight resistance to 13\_A2 strains was confirmed. Both traits exhibit a quantitative inheritance pattern with transgressive segregation, where progeny genotypes with greater resistance and susceptibility scores than the parents were observed. Preliminary analysis of the Infinium array data indicate that from a total of 8303 SNP elements, 1670 SNPs fell below the quality thresholds for genotype calling, and a further 2232 SNPs exhibited a monomorphic pattern in the population, leaving a total of 3902 SNPs for linkage map construction.

#### **Discussions and Conclusions**

The tuber blight assays confirm that tuber-specific resistance in Setanta is still effective against the 13\_A2 strains of blight that have been shown to overcome foliar blight resistance in this cultivar. Genetic map construction is underway in the parents using the Infinium array data, and the map will be used in conjunction with the phenotypic data to identify the genetic components of both tuber and blight resistance/susceptibility in the parents of the cross. In the longer term we hope to develop tuber-blight resistance specific genetic markers for use in breeding.

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#### FINE MAPPING OF THE RO-LOCUS INVOLVED IN TUBER SHAPE ON POTATO CHROMOSOME 10.

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#### Introduction

Tuber shape is an intriguing morphological trait, which displays continuous trait variation ranging from flat, round to oval and long. Initially a single locus model was proposed to explain tuber shape, where multiple alleles rather than multiple loci were proposed to explain quantitative variation [1]. Besides this majoreffect QTL on chromosome 10, other minor effect QTL have been published on chromosome 2 and 11, explaining 8.0 and 5.6 % of the variance, respectively [2].

To obtain a better overview on the loci contributing to variation in tuber shape a genome wide association study (GWAS) was performed. This confirmed that the Ro-locus is the major-effect QTL. Furthermore a recombinant screening was performed to identify the gene underlying the Ro locus.

#### **Materials and Methods**

The panel of tetraploid potato varieties [3] was analysed with a SNP array as described by Vos et al [4]. Recombinants were selected from 1472 seedlings descending from the parental clones CxE [1] with markers flanking the Ro-locus and used to fine-map the physical interval on the potato genome sequence [5]. The parental clones were sequenced and reads were mapped back against the reference genome sequence. This allow SNP calling in parental genomes for marker development of fine mapping. Furthermore regions with homozygosity in one or both parents was used to exclude potential regions of the Ro-locus.

#### Results

Genome wide association analysis identified significant marker-by-trait associations on chromosome 1, 2, 7, 9, 10 and 12. The most significant associations were found to localize on PGSC superscaffold DMB385 on chromosome 10.

Recombinant analysis resulted in the identification of 104 recombinants originating from the female meiosis and 27 recombinants from the male meiosis. Recombinant analysis with additional SNPs within the selected region allowed to confine the Ro locus to a less than 1 Mb interval, comprising superscaffolds DMB385 (568kb), DMB773 (134kb), DMB546 (323kb), and DMB 446 (474kb).

The success rate of marker development for fine mapping was severely reduced in certain areas of DMB385. Analysis of genome sequences displayed a high level of DNA polymorphisms between the parents, but also prolonged tracts of homozygosity was observed in parent C. Homozygous tracts are surprising, because pedigree analysis of this *S. phureja* x *S. tuberosum* hybrid largely excludes identity-by-descend of alleles. The homozygous tracts were instrumental to reject the remaining interval on DMB385. On the remaining scaffolds a few dozens of candidate genes remain including the gene families of non-specific lipid-transfer protein and peroxidases, each comprising many copies.

#### **Conclusions**

The Ro-locus is the major-effect QTL involved in tuber shape across cultivated germplasm. Recombinant analysis and exclusion of homozygous genomic intervals allowed us to refine the region to DMB773 and DMB546, but still no obvious candidate gene can be indicated with a biological function that could explain variation in tuber shape.

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# GENETIC ANALYSIS OF BLACKSPOT BRUISE SUSCEPTIBILITY IN MAPPING POPULATION OF DIPLOID POTATO

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Blackspot bruise of potato tubers is an enzymatic discoloration caused by the formation of dark pigments through the oxidation of phenolic compounds by the enzyme polyphenol oxidase (PPO). It is a complex trait resulted from the interaction of genetic and environmental factors. Despite of the importance of blackspot bruise of potato tubers, there is a little understanding of molecular basis that controls the variation of this trait.

The main goal of our work is to identify specific chromosomal regions responsible for variation of blackspot bruise susceptibility through QTL analysis in mapping population.

The mapping population of diploid potato was developed in cross between parental forms with extremely diverse level of blackspot bruise. These parents are interspecific Solanum hybrids and were selected in Plant Breeding and Acclimatization Institute- National Research Center in Mlochów. Phenotypic evaluation of blackspot bruise susceptibility, enzymatic discoloration of tuber flesh, starch content, tuber weight and shape were conducted in two consecutive years of tuber propagation, in two or three replications. Blackspots were provoked by impact method using hexagonal drum. Blackspot bruising was visually scored according scale 0-100 based on percentage of tuber surface covered by bruises (0-resistant to blackspot bruise, 100- susceptible to blackspot bruise). Evaluations of blackspot bruising were performed in two replications, in each 10 tubers were tested. Distribution of blackspot bruise in mapping population was studied. Repeatability of blackspot bruise between testing years and replications and relationships between this trait and remaining analyzed traits were evaluated by linear Pearson's correlation coefficients. Analysis of variance (ANOVA) was performed to assess the effects of genotype, year and their interaction on the results of blackspot bruise expression. Broad- sense heritability of blackspot bruising for mapping population was estimated. All calculations were performed using STATISTICA for Windows (Soft, Inc., Tulsa, OK,U.S.A.).

On the basis of phenotypic analysis and segregation of DArT markers in mapping population, parental linkage map for blackspot bruise susceptibility will be constructed and QTL analysis will be performed. Studies are in progress.

The work is financed by The National Research Center in Poland, grant: UMO-2012/N/NZ9/01295.

# GENETIC DIVERSITY AND ORIGIN OF CULTIVATED POTATOES BASED ON PLASTID MICROSATELLITE POLYMORPHISM

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#### Introduction

Potato is the most important crop species and one of the oldest crops in South America. At the same time the origin of domesticated potatoes, their genetic structure and classification are still in dispute. In this study we analyzed chloroplast microsatellite (cpSSR) diversity in a wide subset of cultivated and related wild species accessions.

#### **Materials and Methods**

We analyzed 237 accessions of living plants of all cultivated species and 155 accessions of related wild species mainly from subset of VIR potato collection [1] and additionally 150 herbarium accessions of cultivated species (including type material) from WIR and LE herbaria. For living accessions of the subset we used a set of 15 cpSSR markers [2, 3]. Additionally the 241 bp plastid deletion marker [4] was applied both for living and herbarium accessions.

#### Results

All 15 cpSSR loci were polymorphic and identified 127 haplotypes. Dramatic decrease in level of genetic diversity was revealed in cultivated species (88% landraces shared 4 most frequent haplotypes: I, II, III, IV) in comparison with wild ancestors (110 haplotypes, small or individual). Phylogenetic analysis revealed 2 distinct groups.

Group A contained all landraces having haplotypes I and II, included all diploid and triploid members of the *Solanum tuberosum Andigenum* group (*S.chaucha*, *S.phureja*, *S.stenotomum* by a former taxonomic system), most of tetraploid accessions of *S.tuberosum Andigenum* group (*S.tuberosum subsp.andigenum*), and all accessions of *S. curtilobum*. This group contained the majority accessions of wild ancestors from the northern members of the *S.brevicaule* complex.

Another group B comprised most wild species accessions of southern members of the S.brevicaule complex and two subgroups of hybrid cultivated species and their wild ancestors. Group Ba contained almost exclusively accessions of *S.juzepczukii* and *S.ajanhuiri* with haplotype IV and accessions of *S.acaule* and *S.boliviense*. Group Bb contained almost all accessions *S.tuberosum Chilotanum* Group (S.tuberosum subsp. tuberosum) with haplotype III and majority accessions of *S.berthaultii*. All 36 accessions with haplotype III had 241 bp deletion in plastid region ndhC/trnV. The frequency and taxonomic distribution of 241 bp deletion in herbarium material were similar to detected in living plants.

#### **Conclusions and Perspectives**

According to our data genetic differentiation of cultivated species supports recently proposed system [5] of cultivated potatoes. Our future plans are to apply cpSSR markers for herbarium material.

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# HIGH RESOLUTION MAPPING OF THE GPA IV LOCUS AND DEVELOPMENT OF DIAGNOSTIC MARKERS FOR POTATO CYST NEMATODE RESISTANCE.

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#### Introduction

Quantitative resistance to *Globodera pallida* pathotype Pa2/3, originally derived from *Solanum tuberosum* ssp. *andigena*, Commonwealth Potato Collection (CPC) accession 2802, is present in several potato cultivars and advanced breeding lines at Teagasc Oak Park. One genetic component of this resistance, a large effect quantitative trait locus (QTL) on linkage group IV, GpaIVsadg, has previously been mapped in the tetraploid breeding line C1992/31. The GpaIVsadg locus was mapped in an F1 population of 100 individuals produced by crossing C1992/31 with the *G. pallida* susceptible cultivar Record.

We have also physically characterized the locus by sequencing a region spanning approximately 1 million nucleotides around the peak of the QTL effect in three potato genotypes (RH, DM and HB) susceptible to *G. pallida*. This has revealed the presence of an extensive R-gene cluster containing homologues of the blight resistance gene R2. The locus comprises a number of sub-clusters, with a great deal of conservation across different genotypes. We hypothesise that the gene or genes underlying the effect of the GpalVsadg QTL are present in a homologous cluster in C1992/31.

#### **Methods and Results**

In order to progress towards map-based cloning of the genes underlying the QTL, we generated a high resolution F1 population by crossing C1992/31 with the *G. pallida* susceptible cultivar Rooster, comprising of 1600 genotypes. We used our sequence data for the region to develop 4 SNP-based and one SSR based marker interspersed throughout the R-gene cluster in our reference sequences. The flanking markers Primer 44 and Bac 9\_3 have been used to screen the 1,600 genotypes for recombination events. A total of 170 genotypes were found to have undergone a recombination event between the flanking markers, and recombination events were further defined by the application of the remaining three markers.

#### **Discussion and conclusions**

This high resolution recombination-based map will be used in conjunction with a phenotypic data on resistance to *G. pallida* Pa2/3 currently being developed in the population, and a BAC library being constructed in a GpalVsadg triplex genotype derived from C1992/31 to identify candidate genes for the QTL effect, with the goals of developing gene specific markers and for map-based cloning of the underlying gene/s.

### INTERACTIONS BETWEEN PHYTOPHTHORA INFESTANS RXLR EFFECTORS AND POTATO HOST PROTEINS

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Potato (Solanum tuberosum) is the third most important food crop just after wheat and rice. As each crop, potato suffers from different diseases caused by various pathogens. Lacking an adaptive immune system, plants including potato have evolved two-tier monitoring system to detect and deflect pathogen invasion. These two levels of inducible plant defense provide hurdles to infection. Pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) follows the perception of conserved microbial molecules at the surface of plant cells. Plant pathogens secrete effector proteins that suppress PTI. Effectors may be recognized by plant disease resistance (R) proteins, resulting in effector-triggered immunity (ETI) often involving hypersensitive response (a form of programmed cell death) coinciding with restriction of the invading pathogen. Thus, many pathogen effectors have evolved to suppress the programmed cell death as a component of either PTI or ETI. One of the most devastating potato diseases is caused by the oomycete Phytophthora infestans. The pathogen secrets RxLR effectors that are translocated inside the host cells [1,2]. The conserved motif RxLR found in these effectors is needed for their translocation. The number of RxLR effector genes in *Phytophthora* genome is remarkably high, with about 563 predicted in P. infestans [3]. Although sequence information, the little that is known about the manipulation of plant targets by oomycete effectors is focused on those RxLR which are recognized by plant resistance proteins.

The goal of our recently started project is to gain a better insight into the function of RxLR-effectors using the interaction of potato with *Phytophthora infestans*. To identify candidate host targets of *P. infestans* RxLR effectors, a yeast-two-hybrid library composed of cDNA from potato infected with the pathogen is under construction and screening with candidate RxLR effector molecules. Our experiments aim to learn more about the regulation of expression and the delivery system of RxLR-effectors and to identify their molecular targets in the host plant.

In , a more in depth knowledge of RxLR-effector biology is central for understanding and ultimately controlling diseases caused by *Phytophthora*.

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### MAP-BASED CLONING AND ANALYSIS OF THE GENE RESPONSIBLE FOR POTATO PLANT MATURITY

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Potato (Solanum tuberosum L.) originates from the Andes and most wild genotypes are short-day dependent for tuber-formation. Potato reproductive development is regulated by the sensing of environmental cues resulting in the activation of a signalling cascade. Signal molecules synthesised in the perceiving organs are transported to the cells and tissues that go on to develop into flowers or tubers. Although soil nutrients and factors such as water availability and ambient temperature are important signals for reproductive development, the major environmental component for tuberisation is day-length. In diploid research material as well as in tetraploid cultivars a major-effect QTL for Plant Maturity and Earliness of tuber formation has been mapped on Chromosome 5 close to the R1 resistance gene cluster. Using map based cloning together with deep bi-allelic sequencing of chromosome 5, we have now identified a gene coding for a central regulator underlying this major-effect QTL. We show that this gene named StCDF1 belongs to the family of DOF transcription factors that regulates tuberisation and plant lifecycle length, by acting as a mediator between the circadian clock and the mobile tuberisation signal StSP6A. We also show that naturally occurring allelic variants of this protein, evade post-translational light regulation thereby leading to early tuber formation and shortened lifecycle. We propose that this adaption has allowed the cultivation of potato outside the centre of origin, under long-day conditions of spring and summer that prevail in northern temperate latitudes.

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#### MAPPING IN A TETRAPLOID POTATO CROSS USING SNP MARKERS

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Genetic analysis using molecular markers is well established for diploid crops but until recently the tools for mapping and QTL analysis in tetraploids were not readily available. However, this has changed: next-generation sequencing facilitates the identification of large numbers of SNPs, SNP arrays enable the generation of large SNP marker data sets, and software for dosage scoring (fitTetra, Voorrips et al. 2011) efficiently assigns the tetraploid SNP genotypes of individuals. Simulation software (Voorrips and Maliepaard, 2012) allows studying the consequences of different meiotic configurations and inheritance modes for genetic analysis. Methodology for map construction and QTL analysis in tetraploid crops has been presented (Hackett et al. 2013).

We genotyped 240 progeny of a tetraploid potato cross, including parents and three grandparents, using a 20K SNP Infinium array. SNP dosages and recombination frequencies were estimated for different possible tetrasomic marker configurations. We constructed a tetraploid linkage map, with marker positions of SNPs on individual homologous chromosomes, and validated the marker positions against the reference genome of potato and confirmed the division over homologs using grandparental genotyping information. Based on this we compared the estimated genetic (cM) distances with physical (bp) distances on individual chromosome arms. In addition, we quantified the occurrence of double reduction in this potato cross.

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# MAPPING OF QUANTITATIVE TRAIT LOCI FOR STARCH CONTENT IN DIPLOID POTATO ENRICHED WITH THE WILD SOLANUM SPECIES GERMPLASM

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#### Introduction

Starch plays a central role in the regulation of plant metabolism and growth. In potato, starch of tubers is also of a great economic importance. A large number of quantitative trait loci (QTL) for tuber starch content that have been mapped in potato so far, show the high genetic complexity of this trait.

The aim of our study was to localize QTL for starch content in diploid potato enriched with the wild Solanum species germplasm.

#### **Materials and Methods**

The parental clones DG00-683 (PH) and DG08-28/13 (PL) were interspecific diploid hybrids having in their pedigrees: *S. tuberosum*, *S. acaule*, *S. chacoense*, *S. demissum*, *S. gourlayi*, *S. microdontum*, *S. phureja*, *S. stenotomum*, *S. verrucosum* and *S. yungasense*. DG00-683 had over 20% of starch content in potato tubers. The clone DG08-28/13 used as a male parent had low tuber-starch content ranging from 10 to 11%. Crossing PH × PL resulted in 184 F1 individuals that were used as a mapping population. Progeny was assessed for starch content at the seedling stage in 2012 and first tuber generation in 2013 using tuber specific gravity as a guide. K.For genetic analyses Diversity Array Technology (DArT), the JoinMap ® 4 and MapQTL ® 6 software were used as described in [1].

#### Results

The tuber starch content segregated in the mapping population ranging from 9.9 to24.3% and its distribution in 2012 was normal and in 2013 close to normal. DArT analysis resulted in scoring 1595 polymorphic and segregating markers that were placed on the genetic map of total length 1117 cM. Number of markers on particular chromosomes varied from 70 to 228, while their length ranged from 66 to 143 cM. The constructed map was oriented by comparisons to existing DArT maps, e.g. [1]. QTL analysis identified three most important QTL for tuber starch content in this material: on chromosome I (depending on the year of assessment explaining up to 19.2% of variance observed in tuber starch content, interval mapping LOD 8.5), chromosome VIII (respectively, up to 14.2% of variance, LOD: 6.1) and X (respectively, up to 15.0% of variance, LOD: 6.3).

### **Conclusion and perspectives**

The results obtained will be validated in the third year of phenotypic evaluation of the tuber starch content in the mapping population that is planned in 2014. The identified QTL will become a starting point for research aimed at finding the genes and alleles underlying starch content variation in potato tubers.

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The research was supported by The National Science Centre (NCN) in Poland, grant UMO-2011/01/B/NZ2/00181.

# MARKER SNPS ASSOCIATED WITH TEXTURE IN TUBERS OF SOLANUM TUBEROSUM GROUP PHUREJA FROM THE CENTRAL COLOMBIAN CORE COLLECTION

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Solanum tuberosum, is a specie of the Solanaceae family that grown in different regions of the world. In the Andean region is a staple food in fresh consumption or process (chips and French fries). However mostly of the current potato cultivars does not have the require characteristics in texture quality for the industry, so there is a significant need to improve the quality of potatoes. Also classical breeding programs are slow, expensive and difficult to assess in the field due to the complexity of the features that determine the high quality. So the use of molecular approaches, such as in this research (candidate genes, SNPs) and an accurate phenotyping are a feasible alternative to optimize breeding programs supporting the classical breeding.

In this research 110 accessions from of the Colombian Core Collection (CCC) of *phureja*. We harvested fresh tubers and standardized a cooking method, using data of tubers midpoint temperature after cooking. Phenotyping was performed in two different ways, first we calculated the content of total starch (%) in tubers using an enzymatic methodology and we found in the CCC clones percentages ranging from 7.05% to 8.95% of. Second we performed a texture assay profile (TPA) using a texturometer and TA-XTPlus texture analyzer® software. Finally, we used the phenotypic information and genomic results of SNPs markers from a previous work and we performed ANOVA analysis (p =0.05) for each clone, each marker and each parameter using Genstat® software. We found 12 markers with a statistical p-value that are possibly associated with texture, all of those markers were found in genes that are important in photosynthetic pathways. This was a first approach to find genes associated to a complex agronomic trait as texture that also will help breeding programs in the future researches.

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# ORGANELLE DNA ANALYSIS TO STUDY CYTOPLASM EVOLUTION IN MEXICAN POTATO SPECIES

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#### Introduction

Of the approximately 200 potato species, 36% are polyploids and 64% diploids. Origin of polyploids in section Petota was based on analysis of chromosome pairing, nuclear DNA sequencing [1, 2] and GISH data [3]. Based on results of these studies putative progenitors of Mexican polyploid species of series Longipedicellata and lopetala Group was revealed. However there is a restricted data about organelle DNA contributors in the origin of these species. We investigated the cytoplasmic diversity and relationships of Mexican polyploids and their ancestor species through the use of organelle DNA specific markers.

#### **Materials and Methods**

The choice of species and accessions was based on studies mentioned above and included: all Mexican polyploid species; diploid species of series *Pinnatisecta*, *Bulbocastana*, *Piurana*, accessions of *S.verrucosum*.

PCR-analysis was performed using a set of 15 cpSSR markers [4]; PCR products were separated by electrophoresis on a LiCor4300S system. The amplicons of different species were cloned to check the probable changes in the cpSSR loci and flanking regions. Additional the sequences of the same SSRs loci from the GenBank were used. Alignment was conducted using the program MEGA version 5.2. Besides, the set of CAPS and STS organelle DNA specific markers [5] was used to determine the cytoplasm types.

#### Results

The alignment of sequences of 15 analyzed cpSSR loci showed that interspecific polymorphism in some cases arises not only from differences in the numbers of cpSSR repeats but also from point mutations, insertions/deletions. Two of them were species specific. To minimize homoplasy 12 cpSSRs were selected for further analysis.

A high level of intraspecific variability was detected. Phylogenetic analysis revealed 5 distinct genetic groups of accessions of diploid species: *S.verrucosum*, *S.bulbocastanum*, *S.cardiophillum*, species of series *Pinnatisecta* and species of series *Piurana*. Accessions of Mexican polyploid species and diploid *S.verrucosum* were clustered together suggesting *S.verrucosum* as the maternal parent. The obtained cpSSR data were examined for concordance with results of cpRFLP [6], nuclear DNA sequencing [1, 2] and GISH [3]. cpSSR-haplotypes were compared with cytoplasm types to select accessions perspective for introgressive hybridization.

#### **Conclusions and perspectives**

Our study demonstrates that the set of cpSSRs provides a useful tool to study the diversity and the cytoplasmic evolution of potato species.

This study was supported by grants: ISTC 3329 and RFBR 14-04-32300.

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# QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO COMMON SCAB AND COLD-INDUCED SWEETENING FROM THE DIPLOID POTATO SOLANUM CHACOENSE

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#### Introduction

Wild diploid potato has historically been an important source of genetic variation for variety development in *S. tuberosum*. In previous research, the diploid line *S. chacoense* 524-8 had consistently displayed resistance to common scab, a widespread disease caused by the soil-borne bacterial pathogen Streptomyces scabies (Thaxt.) Waksman and Henrici. Line 524-8 had also demonstrated resistance to cold-induced sweetening, which is an important trait for the development of varieties with light fry color after long-term cold storage. The objective of this research was to localize the genetic basis for these resistance traits by mapping quantitative trait loci (QTL) in a segregating diploid population.

#### Methods

Line 524-8 was crossed with the *S. tuberosum* dihaploid US-W4 (2n=2x), which is susceptible to cold-induced sweetening and common scab. Two full-sibs from the US-W4/524-8 family were intermated to create an F1 outbred mapping population with 104 progeny. The population was genotyped using the SolCAP 8303 SNP array, and the linkage map was created with JoinMap 4.1. Field trials were conducted near Antigo, Wisconsin, in a field managed to promote high common scab pressure. Two replicates were used in 2011 and six replicates were planted in 2012. Tubers were kept at room temperature (22°C) for 14 to 21 days after harvest while they were scored for scab lesion type and percent surface area. Then, after 78 days of cold storage at 4°C, a 4 mm slice from each of two tubers was fried at 191°C and visually scored for chip color (light to dark). QTL were identified using MapQTL 6.

#### Results

In the initial phase of linkage mapping, 2605 SNPs clustered into 12 linkage groups. The grouping of all but 15 markers was consistent with version 4.1 of the DM pseudomolecules; the 15 markers were removed. The final linkage map contained 1018 bins, with a total map length of 798 cM, and marker order was consistent with the DM reference genome for all twelve chromosomes. Coincident QTL were identified for scab lesion type and disease severity on chromosome 11, explaining 17% and 24% of the phenotypic variance, respectively, in 2012. QTL for cold-induced sweetening were detected on chromosome 6 in both years (R2 = 0.34 in 2011, 0.19 in 2012) and on chromosome 4 in 2012 (R2 = 0.15).

#### Conclusion

Although QTL for scab resistance have been mapped in other studies, the one identified on chromosome 11 appears to be novel, having originated in the wild species *S. chacoense*. Future variety development based on this research will involve using scab-resistant progeny from the mapping population as 2n gamete donors in crosses with elite tetraploid cultivars.

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#### SSR- AND SCAR-ANALYSIS IN POTATO GERMPLASM COLLECTION MANAGEMENT

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#### Introduction

Over 400 accessions of wild potato species, somatic hybrids, S. tuberosum dihaploids and their progenies, S. tuberosum (4x) hybrids and potato cultivars are preserved currently in the in vitro potato germplasm collection of the Laboratory of Biotechnology (RUE Research and Practical center of NAS of Belarus for Potato, Fruit and Vegetable Growing). Potato genetic resources are an important gene reservoir of valuable agronomic traits like resistance to diseases or pests.

#### **Materials and Methods**

Plant material: 41 accessions of 21 wild tuber-bearing potato species, belonging to 10 series according to J.G. Hawkes; 36 plants - products of 3 interspecific protoplast fusion combinations, including *S. tuberosum*, *S. cardiophyllum*, *S. caripense*, *S. bulbocastanum*, *S. pinnatisectum*; 90 accessions of 23 wild species of different origin.

Markers: 12 SSR-markers, locating on all 12 potato chromosomes; 8 SCAR-markers (TG689, Gro-1-4, Gpa2, SPUD1636, HC, RB629, NL27, UBC864) linked to sequences that control resistance to golden and pale potato cyst nematodes, late blight, PLRV; two set of *Solanum* genomes SCAR-markers, developed in Institute of Agricultural Biotechnology (Moscow, Russia): LEAFY intron2-based marker set and COSII-based set [1, 2].

PCR and visualization of SSRs and SCARs were performed with standard techniques. SSR products were separated with Li-Cor 4300S DNA analyzer.

#### Results

Microsatellite analysis was carried out to fingerprint 41 accessions of 21 wild potato tuber-bearing species. 117 alleles in total were visualized, including 24 rare and 18 unique. The most polymorphic marker was STM1106 (19 alleles), the least - STM1052 (4 alleles). The quantity of samples, that can be distinguished with the only marker, ranged from 29 (for STI005) to 2 (STM1052). The minimal marker set to fingerprint unambiguously all samples of the group was STI005, STM1106, STI046. The only STI005 marker was sufficient to distinguish samples, which didn't belong to the progeny from one crossing.

SSR and Solanum genomes SCAR-markers were employed for detection of hybridity of plants regenerated after protoplasts fusion procedure. It was shown that genomes of 36 plants from 3 fusion combinations were quite unstable but none of them had hybrid nature. All plants were proved to be protoclones of one of parent forms and were excluded from collection.

90 samples of 23 wild species were screened with 8 SCAR-markers of resistance to diseases or pests. All markers, but TG689 and Gpa2, revealed corresponding sequences in wild accessions. Their functionality is under consideration and to be studied further.

#### Conclusion and perspectives

SSR and SCAR markers can be useful tools to manage potato germplasm collection, allowing assessing the genetic diversity, variability, relationships between accessions, excluding double and inappropriate samples. SCAR-markers can be used to identify the presence (or potential presence) of valuable for breeding genes, and trait germplasm collection can be organized.

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### SSR GENOTYPING OF OLD POTATO VARIETIES TO IDENTIFY MISLABELLING AND SYNONYMS FOR AEGIS

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The identity (cultivar name) of potato clones in different collections is not always clear or correct. This is hampering the selection of the Most Appropriate Accessions (MAA's) for the AEGIS\* collection. In particular for old cultivars the clone can be mislabelled (H. Campbell in [1]). This was also observed during work for CPVO on the EU Variety Catalogue [3]. Some variety names have been used more than once (e.g. Gloria 1921, 1937, 1972) and it is not always known to the curator what the true identity of a clone is. The classical differentiation of cultivars based on morphological characteristics is a highly skilled and time-consuming task. To assist in granting Plant Breeders' Rights for potato varieties, a standard fingerprinting method has been developed [2, 3]. The method uses nine microsatellite (SSR) markers. The set was expanded to twelve to increase the level of discrimination. Obviously, somaclonal variants and mutants cannot be separated from the original clone.

For the AEGIS project "The identification of 500 old potato clones having unreliable variety name by means of fingerprinting using 12 microsatellite (SSR) markers to assist in setting up the AEGIS collection for potato cultivars", SASA genotyped 379 clones from eight European potato collections. Additionally, the UK and Ireland donated 24 and 30 fingerprints of heritage varieties. The 433 accessions show 398 different profiles. The 26 duplication groups contain two till six accessions. The largest group consists of blue coloured varieties. In the case of the duplicate group Fortuna / Morgane\_1985, it is unclear which one has been mislabelled. If available in SASA's (not public) database, profiles of crossing parents and/or offspring might resolve this. However, the Potato pedigree database [6] indicates for the name Fortuna four different varieties. In general, a preferably public database containing as much profiles as possible would be helpful. However, the results from different labs have to be harmonized to allow the data to be compatible, which can be a drawn out and potentially expensive process. The harmonization by CPVO.

The results of the genotyping project have been made public at its website [5]. The curators can use them to improve their selection for AEGIS.

\* AEGIS [4] is a European Genebank Integrated System, to establish a European plant genetic resources collection, which would be a virtual European Genebank, maintained with agreed quality standards, and freely available in accordance with the International Treaty on Plant Genetic Resources for Food and Agriculture.

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# VALIDATION FOR ASSOCIATION OF NOVEL CANDIDATE GENES WITH QUANTITATIVE RESISTANCE TO PHYTOPHTHORA INFESTANS IN POTATO

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Late blight of potato caused by the oomycete Phytophthora infestans is world-wide one of the most important bottlenecks of potato (Solanum tuberosum) production. The disease could be better managed by using cultivars with high levels of field resistance, which is more durable and mostly race non-specific. Breeding for quantitative resistance is however, complex and challenging and requires multiple year and location trials. In addition, it is hampered by a correlation between resistance and late maturity. This problem can be tackled by using diagnostic DNA markers, which enhance efficiency and precision of resistance breeding [2]. The aim of this project was the identification of genes for quantitative resistance to P. infestans not compromised by late maturity and the discovery of diagnostic SNP markers. Twenty-three novel candidate genes, which were differentially expressed in quantitative resistant versus susceptible potato genotype pools, were selected from transcriptome data generated through SuperSAGE analysis of nine samples comprising three genotype groups with different resistance levels and three infection time points [1]. The selected candidates were subjected to validation of differential expression by qRT-PCR and allele specific pyrosequencing. Candidates showing reproducible transcriptional regulation in three independent infection experiments as well as with reference to their SuperSAGE expression were tested for association with maturity corrected resistance (MCR) to late blight in a population of tetraploid breeding clones [3]. Novel associations between SNPs and resistance to late blight were identified. In conclusion, comparative transcript profiling combined with association mapping can be used to enlarge breeders toolbox for marker-assisted resistance breeding.

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# 'MELROSE': A NEW ITALIAN POTATO VARIETY WITH IMPROVED TOTAL CAROTENOIDS CONTENT

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Potato, after wheat and rice, is the third most important staple food worldwide, largely due to its environmental adaptability, yield potential, and nutritional value. White- and yellow-fleshed potatoes provide an affordable source for essential nutrients like fiber, vitamins (A, B complex, C), phytochemicals (polyphenols, carotenoids and anthocyanins), minerals (K, P, Mg, Zn, Cu) and they are the most cost-effective source of potassium [1]. In the latest years at CRA-CIN in Bologna, potato breeding programs were active, as part of two research projects focused on "biofortified tubers", with an improved accumulation of secondary compounds valuable for human health and nutrition. Among these metabolites, carotenoids have raised a high interest, especially for the existence in the cultivated and wild germplasm of a strong variation, allowing introgression of the trait in elite cultivars.

Tuber carotenoids mostly belong to the group of pigments known as xanthophylls. These include lutein degeneration and cataracts. The potato tuber is virtually devoid of pro-vitamin A total carotenoids, that play a key role in human nutrition.

By several cross combinations 'high yielding\*high carotenoid content' at tetraploid level, deep yellow-fleshed new potato clones were developed at CRA-CIN during breeding programs started 10 years ago [2]. These new genotypes were checked for the amount of total carotenoids accumulated under field conditions by a preliminary selection based on spectrophotometrical determination, and were tested for yield performance by comparing them with commercial high-carotenoid diploid cultivars (e.g. 'Andean Sunside', 'Mayan Gold' and 'Papapura').

Screening of CRA-CIN's new clones for carotenoid content led to the identification of clone 'ISCI 5/03-1', endowed with an interesting level of total and pro-vitamin A carotenoids. The carotenoid content and type of the mature tubers of this clone was analysed by HPLC-DAD in two seasons, and compared to the content of other yellow- and orange-fleshed potatoes.

At end of 7-years field evaluations and of 2-years official VCU trials, 'ISCI 5/03-1' was released as a new variety named 'Melrose'.

'Melrose' is an early maincrop variety, has oval-shaped tubers with deep pink skin and deep yellow flesh; it carries the resistance to potato cyst nematode Ro1 by H1 gene. Cooking type is CB (rather firm to rather floury). This variety is suitable for long storage (tubers with long dormancy), and its tubers are resistant to enzymatic discoloration after cooking (ACB). 'Melrose' in many storage trials showed good resistance to cold-induced sweetening (ICS) and physiological aging, with low reducing sugars accumulation. The tubers have shallow eyes and moderate washability.

'Melrose', due to its mid-high tuber dry matter (21-22%) is able to retail fresh market (home fries and baking potatoes) and processing (crisps and French fries).

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#### BREEDING LATE-BLIGHT RESISTANT POTATOES FOR ORGANIC FARMING

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#### Introduction

A programme focussed on the breeding of potato varieties for organic farming in Germany was initiated by a network of organic farmers, potato breeders, and research institutes. The project aims at combining low susceptibility to late blight, resistance to other diseases, and quality traits in pre-breeding materials. Late blight in potato, caused by fungus-like oomycete *Phytophthora infestans*, is one of the most disastrous diseases worldwide. Economic losses due to decreased yields and expenses for fungicide application were estimated to amount to €10 billion/yr. [1, 2]. Compared to conventional farming, organic farming is faced with an even higher challenge, due to constraints in the use of fertilizers and pesticides.

Pre-breeding for quantitative, race non-specific late-blight resistance may serve an option for enabling sustainable potato growing in organic farming

#### **Materials and Methods**

A total of 158 varieties and breeding clones were evaluated for their susceptibility to late blight of the foliage and tubers. Four experiments with different test methods, i.e. a field trial, a detached-leaf assay, a tuber-slice assay, and a whole-tuber test were carried out. Infestation of potato tops with late blight in the field was assessed via Delta-rAUDPC values which had been corrected for maturity [3].

#### Results

Maturity scores ranged between 1.0 (very early) and 7.2 (late), with the highest frequency of maturity group 4 (medium). Most of the tested JKI breeding clones showed very low Delta-rAUDPC values, which illustrates the enhancement achieved in breeding for late-blight resistance within this material. A large part of these clones are early maturing, thus exemplifying that the correlation of late-blight resistance and late maturity can be broken up.

Correlations of Delta-rAUDPC values and results of the detached-leaf assay were in a medium range, which illustrates that a detached-leaf assay is of limited reliability in predicting quantitative late-blight resistance among breeding clones.

As expected, infestation of the tubers was only loosely correlated with the infestation of the foliage as assessed in the detached-leaf assay or in the field. Some pre-breeding clones exhibited low infestation of the tubers in tuber-slice and whole-tuber tests. The data obtained so far will have to be confirmed in repeated experiments during the project time.

#### **Conclusion and Perspectives**

The project is expected to provide information on the potential of late-blight resistant potato breeding clones in contributing to sustainable potato growing via reduced use of copper-based fungicides in organic farming systems.

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#### CELL SUSPENSIONS AND SOMATIC EMBRYOGENESIS IN POTATO.

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Liquid medium have been used as excellent alternative to increase productivity in many process such as somatic embryogenesis. It offers many advantages such as the supply and disposal of nutrients to cells surrounding the liquid medium and commercial production, however, liquid medium may negatively affect productivity, taking into account the fact that there are many factors which must be controlled for increasing it. Somatic embryogenesis is a useful "in vitro" process in plant propagation and developmental studies where plant totipotent cells under controlled hormonal conditions induces the formation of somatic embryos though different stages. Somatic embryogenesis in potato has been studied in different varieties and cultivars to evaluate productivity mainly in solid medium. However, few information is available regarding liquid medium. As part of our research, we studied the formation of somatic embryos in Granola cultivar from cell suspensions in relation with factors such as plant growth regulators, cell suspensions age and callus age under "in vitro" conditions. Based on a modified protocol of Vargas et al (2005) we set up an experimental design which evaluated cytokinin: zeatin (Z), kinetin (K) and benzyl adenine (BAP) at 0,5, 1 and 2 mg/l, from 70, 160, 200 and 260 day old embryogenic cell suspensions. Under this conditions, we observed a positive response in MS medium (Murashige and Skoog, 1962) mainly supplemented with 0,5 mg/l - 1 mg/l of zeatin and 1 mg/l de benzyl adenine (BAP) from 260 day old embryogenic cell suspensions of culture Furthermore, callus (2, 4, 6, 8 and 11 months old) previously obtained in MS medium with 4 mg/l of 2,4-dichlorophenoxyacetic acid (2,4-D) were used to set up thick and cell suspensions in MS medium supplemented with 0,5 mg/l of 2,4-D, 0,5 mg/l of K and 1 g/l of yeast extract. After 15 days, thick cell suspensions were filtered to set up fine cell suspensions adding directly MS medium supplemented with 0,5 mg/l of Z for two months. After two months, zeatin was replaced with MS medium supplemented with 1 mg/l od benzyl adenin (BAP) for two month. Finally, benzyl adenine was replaced with 0,1 mg/l of gibberellic acid (GA3). We observed cell clumps from cell suspensions mainly from two month old callus and 6 month old callus after 60 days of culture. When zeatin was replaced with benzyl adenine, we observed a favorable development of embryogenic clumps using 1 mg/l of BAP. "In vitro" plants from somatic embryos were observed in MS medium with gibberelic acid after 135 days of culture. A higher number of plants (44) were observed after 195 days of culture. Our results indicate the effect of cytokinins such as benciladenine and zeatin on the somatic embryogenesis from cell suspensions in Granola cultivar as well as the genotype effect in this process.

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# CREATION OF POTATO SOURCES OF RESISTANCE TO PVY AND PLRV BY MEANS SOMATIC HYBRIDISATION

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#### Introduction

Potato virus Y (PVY) and potato leafroll virus (PLRV) are two of the most important damaging viral pathogens of potato. Using somatic hybridization would be useful to potato breeders for the development of PVY and PLRV resistant potato cultivars. Crucial moment of somatic hybridization is the problem with fertility of somatic hybrids in the crosses with cultivated potato (tbr, 4x). As sources of resistance to viruses is denoted the genotypes with the resistance to PVY and (or) PLVR and the ability for generation of sexual progenies.

#### **Materials and Methods**

Potato interspecific somatic hybrids were obtained by chemical fusion of mesophyll leaf protoplasts. Two SCAR markers were used for recognizing the germplasm of non-tuberous *S. etuberosum* (COS4 [1]) and *S. bulbocastanum* (Sblb-509 [2]) in sexual generations of somatic hybrids. Spontaneous berries were gathered from plants of analyzed genotype grown in greenhouse and (or) in the field. Crosses of analyzed genotype with the tbr, 4x as the male parents were performed in the greenhouse on grown "a brick" mother parents. Resistance to PVY and PLVR was estimated in the test with grafting of analyzed genotype on the tomato plants contaminated by PVY or the potato plants contaminated by PLVR. The alive graft and wilding in 30 days were tested by ELISA.

#### **Results**

### **Conclusions and perspectives**

The introgression of desirable resistance to viruses PLVR and PVY from wild to cultivated potato is realized by means somatic hybridization between cultivated potato (78563-76, 86-6, LDH) and selected accessions *Solanum*. Obtained forms of potato would be useful to potato breeders for the creation of PVY and PLRV resistant potato cultivars.

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#### DEVELOPMENT OF LATE BLIGHT RESISTANT BREEDING MATERIAL

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One of the most devastating pathogens for potato is the oomycete *Phytophthora infestans*. New cultivars with improved host plant resistance needs to be created e.g. through germplasm enhancement. Transfer and introgression of different resistance genes is one way to achieve this.

Using genotypes formerly evaluated by us regarding late blight resistance, hybridizations were performed in 2009 with the goal to combine different genetic sources of late blight resistance. Reciprocal crosses were made using 1) 4 breeding clones, 2) the cultivars Sarpo Mira, Kiva and Superb, 3) a genotype from *S. tuberosum* group *Andigena* (adg) and 4) a hybrid between *S. tuberosum* group *Tuberosum* and *S. tuberosum* group Phureja (tbr × phu). All breeding clones, Sarpo Mira and the tbr × phu hybrid showed high foliar resistance. One of the breeding clones was SW93-1015 which is the female parent to the other three breeding lines included. The resistance mechanisms of SW93-1015 has been studied in a separate project [1] and we have reasons to believe that its resistance is different from the one of Sarpo Mira. The adg-selection had sensitive foliage but was partially tuber resistant and had good tuber characters. Superb and Kiva had moderate leaf resistance but good tuber qualities and tuber resistance respectively. In 2010 seeds from 11 crossing combinations were sown and tubers were harvested. The hybrid populations were grown in field trials (2011–2013) and evaluated for field resistance to late blight. Here we focus on the results for the hybrid populations derived from crosses inlucing Sarpo Mira as one parent.

Sarpo Mira was most successful when used as pollinator. As female parent seeds were produced only when it was pollinated with the breeding clone SW04-3262 or Superb. When crossed with adg or tbr × phu it was without success however, no matter if it was used as female or male parent. Highest level of resistance was found in the hybrid material from SW93-1015 × Sarpo Mira. Good resistance was also found in the offspring from the reciprocal crosses between Sarpo Mira and SW04-3262. Slight infection was observed for the hybrids originating from the cross SW04-2662 × Sarpo Mira. Hybrids derived from different combinations where Superb was one of the parents showed lower resistance compared to their resistant parent. Only offspring derived from Superb × Sarpo Mira were assessed as highly resistant.

As this material is included as a part of the Swedish breeding program for table potatoes it has also been assessed for tuber shape and yield. Selections have been performed yearly and at present a number of hybrid clones with resistance to late blight combined with good agronomic traits are available for use in the breeding program. Recently developed molecular markers for *Phytophthora* resistance in SW93-1015 and Sarpo Mira will be used to examine the existence of these different resistance genes in the selected hybrid clones.

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# DNA MARKER-ASSISTED INTROGRESSION OF RESISTANCE TO *GLOBODERA PALLIDA* (STONE) FROM *SOLANUM SPARSIPILUM*: PRELIMINARY VALIDATION FOR ITS USE IN A CONVENTIONAL BREEDING PROGRAM

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Conventional introgression of pest & disease resistance traits into the cultivated potato is a lengthy, very tedious procedure because they have to be integrated in combination with excellent agronomical and processing performances to match the requirements for modern potato activities. This is the current situation European potato breeders have been facing for the past 60 years for introducing durable resistance to the potato cyst nematode, *Globodera pallida* (Stone) Pa3 into cultivated varieties. As one of the promising tools, the use of DNA- markers has been recently implemented in order to select desirable clones based on genotype rather than on phenotype in a more efficient and fast procedure [1,2].

For many years, Germicopa as a private potato breeder has established pest & disease resistance as one of its top breeding priorities [3]. Through its long lasting partnership with Inra, diverse sources of resistance to potato cyst nematode *G.pallida* Pa3 have been used in conventional breeding programs. Recently, Inra scientific teams have implemented specific DNA markers to trace the major QTL GpaVspl and the minor QTL GpaXIspl for the resistance originating from *Solanum sparsipilum* [4,5].

The purpose of this poster is to report on an assessment of the feasibility and the reliability of using this technique in a running conventional potato breeding program at the tetraploid level. Advanced clones and over 400 progeny seedlings (generated by 17 different bi-parental crosses with at least one resistant parent to *G. pallida* Pa3, from *S. sparsipilum*) have been genotyped with the specific markers and phenotypically assessed for resistance to *G. pallida* Pa3. The consistency between genotypic and phenotypic data has been established for all plant materials. Relevance of this approach will be put into different perspectives: scientific, practicality and economic. Future development for an optimized use of DNA-tools for pre-breeding or advanced selection of resistant cultivars will be discussed.

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GENETIC ANALYSIS OF LATE BLIGHT RESISTANCE GENES AND PLANT ARCHITECTURAL GENES IN TWO SEGREGATING POPULATIONS ORIGINATING FROM TWO WILD POTATO RELATED SPECIES

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In potato, several studies showed that plant or canopy architecture had strong effects on the development of foliar late blight disease. In particular, levels of partial resistance are significantly and positively correlated with plant maturity. This correlation can be explained by the co-localization of resistance and lateness QTLs [1]. The identification of the relative part of architectural traits and growth characteristics in the late blight partial resistance is therefore an important issue to orientate the construction of resistant ideotype.

Two diploid segregating populations, obtained by crossing a dihaploid *S. tuberosum* clone (Caspar H3 and Rosa H1 respectively) with a clone belonging to a wild related species (*S. sparsipilum* and *S. spegazzinii* respectively) were studied. These populations were evaluated for partial late blight resistance using a stem assay and a whole plant assay, for maturity type, and for several architectural traits (plant height, leaf and stem number, leaf size). Quantitative trait loci for architectural traits or maturity type were detected on almost all the chromosomes, depending on the parent. The variance explained ranged from 6 to 26%. Several QTLs for late blight resistance were also identified on several chromosomes. The QTLs presenting the most significant effects (up to 29%) mapped to chromosome 10 in both wild species [2]. A few genomic regions, including the ones identified on chromosome 10, seemed to be involved in both types of traits. Denser genetic maps of these regions on chromosome 10 were constructed in order to investigate further if these QTLs co-localized or not. All these results will be presented in this poster.

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### INCREASING THE EFFICIENCY OF SELECTING NEMATODE RESISTANT POTATO FORMS WITH A SET OF ECONOMICALLY VALUABLE FEATURES IN HYBRID POTATO POPULATIONS

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The Golden potato cyst nematode (*Globodera rostochiensis*) is a quarantine pest causing considerable damage to potato production in Russia. Its distribution area virtually matches the area of the crop production. At present, one of the most efficient control measures is growing potato varieties resistant to the pathogen. Thereupon, research has been conducted on the selection of initial parental plants with dominant genes for resistance to Ro 1 pathotype, as well as on optimization of hybridization types in order to increase the frequency of resistant forms in a hybrid generation in the course of breeding.

Having analyzed the experimental data on the average frequency of nematode resistant hybrids with a set of economically valuable features, we have found that the most considerable results in selecting the most valuable breeding forms were obtained in the following breeding types: resistant × resistant (3.7%) and nonresistant × resistant (3.6%). However, taking into consideration a relatively low frequency of resistant hybrids with a set of economically valuable features, it is practical to use a wide genetic variety of parental plants in breeding which enables to combine major economically valuable features in a hybrid generation, for example, such features as crop capacity, consumption and cooking qualities of tubers, and resistance to biotic and abiotic environmental factors.

# MAPPING COMMON SCAB RESISTANCE, CHIP-PROCESSING AND ASPARAGINE IN TETRAPLOID POTATO USING THE SOLCAP INFINIUM SNP ARRAY

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The USDA-funded SolCAP project has reduced the gap between genomics and breeding by providing the infrastructure to link allelic variation of SNPs in genes to valuable traits. Genome-wide, SNP-based markers for potato have improved genetic mapping and QTL analysis for diploid and tetraploid potato mapping populations. The Potato Infinium SNP Array of 8,303 SNPs was used to genotype a tetraploid mapping population of 207 individuals. The MSV507 population (Tundra x Kalkaska) was phenotyped for 11 traits, including chip-processing quality, specific gravity, and common scab (Streptomyces scabies) resistance. TetraploidMap software was used to generate a genetic map and QTL analysis. 1.276 segregating simplex, duplex, and triplex SNPs were used for mapping. Significant QTL were identified for common scab resistance on chromosomes one, four, and ten. Chip-processing quality QTL were identified on multiple chromosomes including three and four. Significant QTL were also identified for specific gravity, stem-end discoloration, and average tuber weight. These QTL can be used to investigate candidate genes for specific traits, such as scab resistance and chip-processing quality. Acrylamide is a suspected human carcinogen that is found in processed products including potato chips and French fries. Acrylamide is formed from the reaction of the amino acid asparagine and reducing sugars glucose and fructose at high temperatures involved in processing. Two low asparagine clones from the MSV507 population were crossed to create an additional tetraploid mapping population (MSB699) to further investigate low asparagine content and chip-processing quality. The 186 MSB699 progeny have been genotyped using the Potato Infinium SNP Array and tubers will be analyzed for asparagine content. Current results from this on-going research will also be presented. The SolCAP SNP markers have improved genetic mapping and QTL analysis in tetraploid potato.

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# MISMATCH REPAIR DEFICIENCY INCREASES THE RESISTANCE TO COLORADO POTATO BEETLE OF THE SOMATIC HYBRIDS SOLANUM CHACOENSE + POTATO

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#### Introduction

Colorado potato beetle (CPB) is the most voracious insect pest of potato. This insect is notorious for developing very fast insecticide resistance or even resistance to Bt toxin. The best way to control CPB is the use of resistant potato varieties, but very few results have been obtained up till now. *Solanum chacoense* (chc) a wild relative of potato is known to biosynthesize specific glycoalkaloids, i.e. leptines in the leaves, which are repellents for CPB. The aim of our study was to compare somatic hybrids between chc and potato with or without mismatch repair deficiency, for their resistance to CPB.

#### **Materials and Methods**

By genetic transformation, mismatch repair (MMR) deficient lines of high leptine accession of chc were produced [1] and somatic hybrids with cvs. 'Delikat' and 'Desiree' have been obtained via protoplast electrofusion. Progenies were produced by back crossings (BC) of somatic hybrids with cultivated potato (Solanum tuberosum). A laboratory bioassay was used to analyze the effect of potato plants on the feeding of CPB. Larvae of CPB were reared at 25°C under a 16:8 h light:dark photoperiod. Within 24 h after hatching 25 larvae were put on leaves of the two varieties, chc, somatic hybrids or BC lines involving MMR deficient or wild type chc. On every other day the survival of the juvenile stages /larvae and pupae and their weights were recorded. Developmental time, mean relative growth rate (MRGR), mean weight of pupae and adult beetles was calculated. Female and male beetles were identified and transferred pair wise in a Petri dish to estimate the fecundity.

#### **Results**

When the MRGR of CPB larvae were compared, the ones fed on three different BC1 progenies derived from a resistant wild type somatic hybrid were similar to the cultivars, indicating susceptibility to the beetle. Three hybrids involving MMR deficiency were inducing MRGR values comparable with those of the wild species chc, accession with high leptines. To the same group belongs one hybrid with another accession of chc that proved to be resistant to CPB in laboratory bioassays. Another group of genotypes showed values in between the resistant and susceptible ones and included one hybrid with MMR deficient chc, and two other BC1 lines derived from the same somatic hybrid without MMR. The results on larval development, pupae formation, adult beetle development and fecundity supported this data. These results are also sustained by choice tests, hybrid's morphology, data on molecular markers linked to leptine biosynthesis and Fourier Transformed Infrared Spectroscopy analysis.

#### **Conclusions and perspectives**

MMR deficiency increases the resistance of somatic hybrids to CPB, more such hybrids being as resistant as the wild species. The influence of MMR deficiency in increasing the recombination in interspecific somatic hybridization will open a new way to the higher introgression of wild resistance genes into crops gene pool.

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#### PEDIGREESIM: SIMULATION OF DIPLOID AND POLYPLOID MEIOSES AND PEDIGREES

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While the genetics of diploid inheritance are well studied and software for linkage mapping, haplotyping and QTL analysis are available, for polyploids the available tools are limited. In order to develop such tools it would be helpful if simulated populations based on a variety of models of the polyploid meiosis would be available. Also the simulation of breeding schemes would be a great help in developing effective strategies in the breeding of polyploid species, including a.o. potato, rose, alfalfa and leek.

Here we present PedigreeSim, a software package that simulates meiosis in both diploid and polyploid species and uses this to simulate pedigrees and cross populations. For polyploids a variety of models can be used, including both bivalent and quadrivalent formation, varying degrees of preferential pairing of hom(oe)ologous chromosomes, different quadrivalent configurations and more. We show that simulation of quadrivalent meiosis results in double reduction and recombination between more than two hom(oe)ologous chromosomes in frequencies corresponding to theoretical expectations.

This is the first public software that implements a true simulation of quadrivalent formation and polysomic inheritance. It allows to generate data for polyploid and diploid populations, and to investigate different models of polyploid meiosis. The software and manual are available for free from http://www.plantbreeding.wur.nl/uk/software/PedigreeSim.html

# PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF 3 BREEDERS' COLLECTIONS FOR LATE BLIGHT RESISTANCE

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Breeding for late blight resistant varieties is a way to help growers decreasing fungicides impact. Association mapping is a promising way to detect helpful markers to implement markers assisted selection in breeding schemes. Our aim is to construct genotypes stacking R genes and partial resistant factors that may increase the durability of R genes. To do so, it is necessary to get reliable markers. Here we present some preliminary results obtained on 287 hybrids used in or coming from the selection processes of three breeding companies: Bretagne Plants, Comité Nord and Grocep. These hybrids have been carefully chosen by breeders to represent the phenotypic diversity of the genetic pools being in use. The hybrids were experimented for late blight in natural conditions of contamination in 2012 using an augmented design according to [1]. The experiment took place in Ploudaniel, France, oceanic climatic conditions and included Black's differentials. Bintje, Sirtema, Kerpondy, Desiree and Robijn were used as controls in each of the 5 blocks of the experiment. Plots of 5 plants were evaluated 9 times between mid June and late July. rAUDPC, Δa and Δt were computed as described in [2]. These parameters were used to characterize the type of resistance present in the hybrids as explained in [3]. As a preliminary step, 25 markers were used to genotype the collection from which 13 had previously been detected to explain a significant part of late blight resistance in segregating mapping populations [3, 4]. Additional SSR markers will be used by the time of the congress to test for a possible structure in the collection. Marker per marker variance analysis were performed to search for significant association with rAUDPC, Δa and Δt.

43% of the hybrids exhibited resistance: half of them were extremely resistant and the remaining half was partially resistant. Markers mapped on chromosome IV and IX were detected to be significantly associated with late blight resistance (p<0.0001 or p<0.001), presence of the marker being most of the time associated with a decrease of rAUDPC value. Results will be checked taking into account a possible structure of the collection. A second experiment will be settled in 2014.

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# REINFORCING RESEARCH IN POTATO BREEDING – A COLLABORATIVE VIETNAMESE-GERMAN EFFORT

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#### Introduction

In Vietnam potato growing increased rapidly to acreages of 40,000 ha in 2012 [1]. Potato is used as staple food or for processing and is cultivated as a second crop after rice between October and March, mainly in the Red River Delta and the northern highlands. A few non-adapted foreign varieties ('Atlantic', 'Solara') are cultivated, but their tuber yields are low and unstable. Progress is made in terms of technology development for healthy seed-potato production using a hydroponic system. National potato breeding programmes are missing, but own potato varieties are needed, which are well adapted to the specific climatic conditions of the country and combine high yield potential and resistance to important pests and diseases.

In Germany, potatoes have been grown for food, processing and industrial purposes in a crop area of 241,000 ha in 2013 [2]. Chemical plant protection requires high amounts of costly pesticides each year. In a long-term pre-breeding programme at JKI approaches and methods are developed and applied to improve resistance to late blight, viruses and pests in newly established pre-breeding materials of potato. In 2010 a Vietnamese-German research project in the frame of the German Federal Ministry of Food, and Agriculture's Bilateral Co-operation Programme in agricultural research was initiated, with scientists from the Institute of Agro-Biology, Hanoi, and the Institute for Breeding Research on Agricultural Crops, Groß Lüsewitz as project partners.

### Materials, Methods and Results

The activities accomplished so far in this cooperative effort are as follows:

- Know-how on methods of protoplast fusion, in vitro regeneration, as well as methods for disease-resistance testing have been exchanged between the partners.
- Gene bank accessions of wild *Solanum* species resistant to diseases and pests, such as potato virus Y (PVY), late blight and aphids, as well as diploid or tetraploid breeding lines and cultivars which exhibit favourable properties with regard to starch contents, processing, French fries, or crisp quality, were selected for their use in potato breeding programmes for 2010 2013 in Vietnam and Germany.
- Sexual hybrids as well as interspecific somatic hybrids were developed and assessed for resistance to PVY, late blight by detached-leaf assay, foliage-blight attack in a field and estimated for plant and tuber properties in the greenhouse and field in both countries.
- Selected breeding lines assessed to late blight using German and Vietnamese inoculum of *Phytophthora infestans* (P.i.) via a leaf-assay proved to exhibit advanced resistance to foliage-blight in the field. *Solanum bulbocastanum* + cv. 'Delikat' hybrids and BC progenies generated no or slight symptoms after infection with zoospores of P.i. and were among the medium to late maturing entries in a field trial.
- Evaluation of BC offspring for morphology, yield and quality in greenhouse, and for agronomic traits, like plant growth type in the field have been used for selection of genotypes as pre-breeding material.
- In 2013, the first Vietnamese crossing programmes started. Backcrossing progenies of 14 interspecific somatic hybrids and 20 combinations of potato pre-breeding lines and cultivars resulted in 8,400 and 13,000 seeds, respectively. This seed can serve as a start-up for Vietnamese potato breeding programme.

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# RESPONSE OF POTATO GENOTYPES DERIVED FROM WILD SPECIES SOLANUM COMMERSONII TO NEMATODE INVASION BY GLOBODERA ROSTOCHIENSIS

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### Introduction

Potato is the world's most important vegetable crop. Under Nordic conditions potato is often exposed to a combination of low temperature and pest invasion. The aim of this study was to investigate the response of potato genotypes derived from wild potato species *Solanum commersonii*, which are freezing tolerant and originate form Uruguay, to nematode invasion by *Globodera rostochiensis* Woll., a widespread and dangerous potato parasite, originating from the Andes mountains.

#### **Materials and Methods**

The objects under study were wild species *S. commersonii* (highly freezing-tolerant) and 2 potato genotypes (freezing-tolerant and freezing-sensitive) on the one hand and potato cyst-forming nematode (PCN) *G. rostochiensis*, pathotype Ro1 (laboratory population), on the other. Experiments were conducted with plant seedlings in growth chambers in 2012-2013. The plants were proliferated from stem cuttings, and grown for about 7 weeks in growth chambers with a photoperiod of 16 h at a PPFD of 122 µmol•s<sup>-1</sup>•m<sup>-2</sup> and a temperature of 23°C. Part of the plants were then infested by PCN at infestation level of 20 cysts per plant. The ability of PCN to reproduce on the plant roots was estimated.

### **Results and Conclusion**

It was established that all genotypes irrespective of the freezing tolerance were susceptible to *G. rostochiensis*. PCN populations reproduced freely on the plant roots: the reproduction coefficient (Pf/Pi) of the nematode was 2.5-6.0 in *S. commersonii*, 2.5-4.0 – in the freezing-tolerant genotype and 3.0-4.0 – in the freezing-sensitive genotype. Plant infestation parameters varied in the two years of the experiments. The most obvious differences were observed for the wild species *S. commersonii*. However, analysis of the content of newly-formed cysts (total number of eggs, viability, and degree of maturation) showed that a majority were blastomeric or dead eggs: 91.1% in *S. commersonii*, 85.3% – in the freezing-sensitive genotype, and no matured and viable eggs were observed in new cysts in the freezing-tolerant genotype. The results showed that the invasion capacity of the next generation of PCN propagating on the plants decreased considerably. Thus, the relationships between *G. rostochiensis* and the wild species *S. commersonii* and its derived genotypes can be assessed as incompatible host-parasite interactions. Study was supported by joint project of the Academy of Finland and the Russian Academy of Sciences (project N 5).

# SCREENING OF SWEDISH WILD SOLANUM SPECIES FOR RESISTANCE TO PHYTOPHTHORA INFESTANS

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### Introduction

The oomycete *Phytophthora infestans* is the causal agent of potato late blight, a disease with devastative economic impact. Many resistance genes against this pathogen (Rpi-genes) have been successfully transferred from wild *Solanum* species to potato cultivars [1]. However, the pathogen contains fast-evolving effector proteins [2] that can overcome Rpi-gene based resistance in potato. In this project, we want to know if there is variation of resistance against *P. infestans* in *Solanum* species collected from southern Sweden, as a mirror of the unusually high variability in *P. infestans* strains found within fields in Sweden. The only natural hosts for *P. infestans* in Sweden are *Solanum nigrum*, *S. physalipholium* and *S. dulcamara*. The overall goal of this study is to understand the cause of the variation and identify new genes or pathways conferring durable resistance against the pathogen.

#### **Materials and Methods**

Natural populations of the two selfing annuals (*S. nigrum* and *S. physalipholium*) and the outcrossing perennial (*S. dulcamara*) species are under screening for resistance in laboratory and field conditions using detached leaf assays with the Swedish *Phytophthora* isolate SE-030558. A total of 164 individuals of *S. dulcamara* (12 populations), 74 individuals of *S. nigrum* (21 populations), and 10 individuals of *S. physalifolium* (6 populations) are under the resistance screening. We have also generated RNA-seq of *P. infestans* infected leaves from an individual of each species using Illumina HiSeq 2000 Sequencing. Moreover, leaf apoplastic proteins were extracted to quantify targeted peptides using Selected Reaction Monitoring (SRM). The 164 individuals of *S. dulcamara* are in a multi-year common garden experiment.

### **Results**

S. nigrum was resistant and S. physalifolium was susceptible to P. infestans inoculation. Interestingly, there was unprecedented variation of P. infestans resistance in S. dulcamara at the scale of local populations. Generally, all S. dulcamara showed some resistance and we will investigate the mechanisms beyond classic R-genes. Currently, we are analyzing the assembled RNA-seq transcriptomics data of each species, and optimizing and validating our SRM setup.

### **Conclusion and perspectives**

The range of resistance variation and differential symptoms among individuals of *S. dulcamara* may suggest existence of more than one resistance mechanism. The plan is to investigate genetic basis of the resistance variation and *S. dulcamra-P. infestans* interactions using Next Generation Sequencing (NGS) technologies. The cause of this so far unique local variation will be addressed by testing several different local isolates of *P. infestans* as a first approach in order to get an indication of possible local adaptation of pathogen and/or host.

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# SELECTING HYBRID POTATO POPULATIONS BASED ON THEIR FIELD RESISTANCE TO THE PHYTOPHTHORA DISEASE

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One of the major lines of work in selective breeding of potato is developing varieties resistant to the Phytophthora disease. Use of the resistant potato varieties significantly reduces the damage caused by the pathogen. To effectively select and develop new basic material, annual testing of the hybrid populations derived from parents with high (R) and medium (M) field resistance are performed as part of the study. The test results show that nonspecific field resistance (FR) is regulated by the polygenes with additive effect that contributes to transgressive segregation. Transgressive segregation allows for emergence of progeny hybrids exhibiting higher resistance than their parents. Selection of such positive transgressions substantially increases FR in progeny as compared to that of the parents.

In 2008-2013, studies were performed using interspecific backcrosses with the breeding record including genes of several wild species (adg, acl, dem, chc, sto, vrn). FR was estimated during the epiphytoties in 2008 and 2013 as well as by artificial inoculation. Testing of the populations throughout the study period was conducted during short growing season (100-102 days) using a common 9-grade resistance scale where 9 is high resistant, while1 is most susceptible. During 2008 epiphytoty, 31 hybrid populations were tested. Transgressive recombinants (TR hybrids) were selected from sixteen of these populations. Occurrence of transgressive recombinants varied from 0.5 to 6.7 %. TR hybrids with 7-7.9 resistance were selected from 22 populations. Occurrence of these was 0.5-6.7 %.

It has been determined that the occurrence of TR hybrids depends on the type of breeding. When backcross breeding (RxR), in 9 populations, TR hybrids with 8-9 resistance amounted to 1.7 % (varied from 0.6 to 3.6 %), while when RxM was used in 14 combinations, the proportion of TR hybrids was 1.1 % (varied from 0 to 6.3 %).

Resistance of the selected TR hybrids was compared to that of 'Sarpo Mira' (SM) variety. During the tests performed on August 1 and August 8 (prior to haulm removal), FR of SM was 8.5 and 7.5, respectively; yield capacity amounted to 497 g/plant. Seven TR hybrids demonstrated higher FR and yield capacity than SM; five of these had same FR resistance as SM but had higher yield capacity (1000-1432 g/plant). During the second test, two TR hybrids showed higher FR (by 0.5 grade) and yield capacity (1066-1100 g/plant).

The obtained data confirm efficacy of selecting TR hybrids for increased FR in progeny as compared to that of parents and further progress in this respect.

# SNP MARKERS FROM A 20K INFINIUM SNP ARRAY ASSOCIATED WITH POTATO TUBER QUALITY TRAITS

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A non-redundant subset (15138 SNPs) of the ~129000 SNPs identified before (Uitdewilligen et al. 2013) and 4454 SolCap markers (Hamilton et al, 2011) were combined on a 20k Infinium SNP array for genotyping a total of ~2400 tetraploid genotypes. Clear segregation was obtained for 14413 SNP markers using FitTetra software (Voorrips et al. 2011). Along with phenotypes of 538 tetraploids a statistical power for association mapping was obtained with -10log(p)-values ranging up to 44 and 85 for the of the major QTL for maturity (Kloosterman et al, 2013) and flesh colour respectively. For a more complex traits such as UWW (Under Water Weight, a measure for dry matter content in potato tubers) multiple SNPs supported QTLs on almost all chromosomes. To validate the QTL from this discovery panel, an independent validation panel of another 351 advanced clones was analysed. Many QTLs were validated, but others failed. Validation of SNPs from discovery panels in breeders germplasm may fail due to various reasons. False positives can be circumvented by high significance thresholds and proper methods to correct for population structure. False positives may arise from incongruent gene pools, where alleles in the discovery panel are hardly exploited in breeders germplasm. Low allele frequencies have a strong negative impact on the statistical power to validate marker-trait associations. SNP markers may tag more than one allele and the lack of haplotype specificity may blur the identification of alleles that contribute to a trait value. Therefore, haplotype information is required to improve association mapping and cultivar breeding. In addition new mapping populations may be required to validate allele-effects.

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# SV-LINES – AN EFFECTIVE TOOL FOR INTROGRESSION OF 1 EBN DIPLOID POTATO SPECIES INTO BREEDING

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High efficiency has been shown of using self compatible 2 EBN wild diploid potato species *S. verrucosum* (ver) as a bridge for introgression of 1 EBN diploid species into breeding [Polyukhovich et al. 2011]. However, it has some deficiencies. F1 hybrids between ver and 1 EBN species practically were not able to form tubers in moderate climate conditions. A significant proportion of such hybrids were male sterile, those ones which were male fertile were difficult to cross with cultivated potato because of unilateral interspecific incompatibility.

We proposed to use the substituted Sv-lines of *S. tuberosum* (tbr) rather than ver as a bridge in crosses with 1 EBN species. Sv-lines were produced by crossing between ver as the male and acceptor genotypes of tbr diploids (which were able to cross to ver despite unilateral incompatibility). Then F2 were obtained and gomozygotes SvSv (genotypes carrying S-genes of ver) were selected among the F2s, that were further backcrossed by tbr diploids [Polyukhovich *et al.* 2010]. Thus, Sv-lines are the tbr diploids whose St-genes were substituted for Sv.-genes of ver. Like ver, Sv-lines do not have pistillate S-RNAase and due to this they have the same opportunities as ver for using as a bridge to introduce 1 EBN species into breeding. However, interspecific hybrids obtained with them have better tuberization capasity, fewer backcrosses to cultivated potato are needed to produce advanced breeding material. These hybrids have tbr cytoplasm what solves the problems associated with nuclear-cytoplasmic male sterility which is typical for interspecific hybrids on ver cytoplasm.

Comparison of hybridization results (crosses were performed in 2009-2010) of 1 EBN species *S. bulbocastanum* (blb), *S. pinnatisectum* (pnt) and *S. polyadenium* (pld) with SvSv-lines (F2 2x tbr x ver) and ver confirmed superiority of Sv-lines. Hybridization efficiency between Sv-lines and blb comprised 39.4 seeds/pollination, pnt - 58.2, pld – 33.1. In crosses of these species with ver it was 20.8, 4.4 and 18.4 seeds/pollination, respectively. Sv-lines had an advantage in germinating capacity of hybrid seeds (77 and 50%, 67 and 88%, 68 and 46% respectively), ability of hybrids to form tubers (75 and 54%, 74 and 62%, 82 and 83% respectively), weight of tubers from plant (14.7 and 2.9 g, 22.9 and 3.3 g, 20.8 and 2.9 g), male fertility (portion of sterile forms was 26 and 56%, 38 and 35%, 14 and 64% respectively). Interspecific hybrids with SvSv-lines as well as similar ver hybrids crossed to tbr diploids with rather high efficiency (10 and 90, 35 and 19, 20 and 24 seeds/pollination respectively). The majority of the obtained hybrids had DNA-markers that were not available in none of used Sv-lines or ver accessions. In particular, marker RB-629 [Pankin et al. 2011] presented in 6 of 8 blb and all 5 used pnt accessions, was available in 8 of 16 SvSv-linexblb and 15 of 16 Sv-linexpnt hybrids, marker CosII 5B [Drobiazina et al, unpublished] (specific for B genome) — in all blb and pnt, as well as in 15 of 16 Sv-linexpnt and all 16 Sv-linexblb hybrids.

The set of Sv-linexblb and Sv-linexpnt hybrids was selected having high LB-resistance (of score 8.0–9.0) according to the results of three year (2011-2013) field trials.

# TOWARDS SUSTAINABILITY IN POTATO PRODUCTION VIA PRE-BREEDING FOR QUANTITATIVE LATE-BLIGHT RESISTANCE

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### Introduction

Breeding research provides a pre-requisite for establishing resources of plant-based raw materials in the frame of bio-economy. Yet, production of raw materials from plants has to obey the principles of sustainability. Late blight, caused by *Phytophthora infestans*, is a major threat to sustainable potato production worldwide [1]. R-genes derived from *Solanum demissum* have not proven sufficient to provide protection against this pathogen.

The challenge is to breed for a quantitative expression of late-blight resistance, which has the potential of enabling an environmentally sound potato production with less fungicide efforts and, thus, less production costs [2]. As a completive approach towards a sustainable potato production, our pre-breeding activities encompass evaluation of plant genetic resources and selection of pre-breeding potato clones in respect to drought tolerance [3].

#### **Materials and Methods**

A total of 54 breeding clones and cultivars were compared for their foliage-blight resistance using a detached-leaf assay and an infected field trial. When assessing foliage-blight resistance, time of maturity has to be considered, because late maturation is mimicking the effects of disease resistance. For this reason, Delta-rAUDPC values were corrected for maturation time to separate the two characters. Tuber-blight resistance was evaluated by a tuber-slice assay as well as a whole-tuber assay [4].

Yield and starch content were assessed under controlled irrigation in a fungicide treated field trial.

### Results

A number of JKI pre-breeding clones belonging to the early to medium maturity group exhibited low Delta-rAUDPC values, thereby demonstrating the progress achieved in breeding for late-blight resistance, as well as the success in breaking the correlation of resistance and late maturity. Most of the cultivars and genebank accessions showed relatively high susceptibility to late blight in the field. In the detached-leaf assay similar results were obtained.

In addition a few plant genetic resources, as old potato cultivars, were identified as a possible source for quantitative resistance too.

Especially processing potatoes produced high yields combined with high starch content even under drought conditions. Interestingly some clones of the pre-breeding material also showed notable qualities as elevated starch content. However it is necessary to adapt their yield performance to the capacity of current cultivars.

### **Conclusion and perspectives**

The analysis of pre-breeding-material, plant genetic resources as well as several varieties originated from the processing and food sector, showed promising approaches for future breeding activities. The results indicate the potential of pre-breeding clones not only as resistance donors but also for improved starch content. Varieties and genebank accessions provide very high starch contents and yield capacity under dry conditions too. The material tested is a base for future breeding of sustainable produced potatoes as renewable resources.

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### USE OF DNA-MARKERS FOR DETERMINATION OF ALLELIC DOSAGE OF RESISTANCE GENES IN BREEDING MATERIAL

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Using DNA-markers for determination of allelic dosage of resistance genes may help to reveal multiplex parental lines (duplex, triplex, quadruplex). Their application in crosses makes it possible to enlarge greatly the proportion of seedlings having desirable characters. To determine the allelic dosage of the gene, the parental line is crossed to the tester and then segregation is studied on corresponding character in F1 [1]. Detection of DNA-markers of appointed genes in F1 provides with the possibility of getting the most precise segregation figures. The effective testers have to be nulliplex (recessive homozygotes) for the maximum number of studied genes, and to have good male fertility what makes it possible to apply them for a wide range of parental lines.

The aim of the present work was revealing the testers that meet these requirements and their using for determination of allelic dosage of disease resistance genes in a set of perspective parental lines. We used as the initial material for revealing the testers the old American potato varieties Katahdin, Superior, Red Pontiac, Kennebec, Norchip, and Russet Burbank that probably do not carry markers of most resistance genes (according their descriptions in international data bases). In addition, we tested several *S. tuberosum* diploid lines bred in our laboratory for high male fertility and production of 2n pollen what provides their good crossability to tetraploid potato varieties.

The variety Red Pontiac as well as diploid lines IGC17-8, IGC27-11 were selected following the detection of 12 DNA-markers for genes of resistance to *Globodera rostochiensis*, PVX, PVY, PLRV, potato wart disease and late blight in above material. They only had few markers and appeared to have good crossability to tetraploid parental lines. Information on the availability of the markers of resistance genes in parental lines to be studied and in the selected testers helps to choose optimal combination parental line – tester for determination of allelic dosage of resistance genes with minimal inputs.

We have obtained hybrids between the testers and twelve perspective parental lines from the breeding program of the Institute of potato growing of Belarusian NAS. The first results of their study have shown that the parental lines were the simplex on most resistance genes. Nevertheless, some duplex or even triplex lines have been revealed on nematode resistance gene H1 (marker TG 869) and PVY resistance gene Ryadg (marker RYSC3).

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#### GENETIC ENGINEERING OF POTATO TO IMPROVE THE QUALITY TRAITS

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As one of the most versatile food crops, the potato (Solanum tuberosum L.) is used worldwide for human and animal consumption, and as raw material for starch and alcohol production. One of the most important aspects of potato production is tuber quality, that includes biological traits such as proteins. carbohydrates, and minerals; sensorial traits for example flavor and texture); and industrial traits e.g. tuber shape, cold sweetening, starch quality. A recent target of genetic engineering has been the production of potato plants with a higher nutritional value with a good quality. The production of starches with modified amylose to amilopectin ratio represents a good example of the possibilities offered by genetic engineering in improving potato quality traits. Great attention has been attached to improve the essential amino acid composition of tubers and especially their lysine, tyrosine, methionine and cysteine content. It has been also used to improve carotenoid content of tubers. Potato plants carrying a gene for low-weight glutenin have been produced with the aim to improve functional qualities of flour. A gene encoding phosphofructokinase enzyme from bacterium Lactobacillus bulgaricus caused degradation of simple sugars via glycolytic pathway in potato tubers. This transgenic potato tubers not only have lower sugar content, but also, chips prepared from such potatoes are lighter in color than those prepared from nonmodified ones. Recently researchers have studied to improve transgenic potato varieties for the production of proteins and vaccines against several bacterial and viral diseases in human. Since the potato is hampered by its tetrasomic inheritance, high level of heterozigosity, and incompatibility barriers, recent advances in plant biotechnology have significantly improved the possibilities of producing novel genetic variability and efficiently perform selection, especially when biotechnologists pool resources with breeders. The purpose of this study to review genetic engineering studies in potato carried out worldwide and potential of DNA markers for quality breeding based on marker-assisted selection.

# HETEROSIS EFFECT OF POTATO HYBRIDS (SOLANUM TUBEROSUM L.) IN MOUNTAIN CONDITIONS OF TAJIKISTAN

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Intraspecific hybridization of (*Solanum tuberosum* L.) with different crossing schemes was conducted at high elevation of Tajikistan (Jirgatal district, 2700m asl. on 2009). At the selection of parental forms that have been used at crossing, the priority was given to such characteristics as productivity, early maturation, resistance to diseases and tuber storage. Produced potato hybrids are appreciated in terms of morphological traits (growth and development, degree of formation of generative organs and by its productivity). Heterosis effect by the quantitative criteria was different and depended on the crossing scheme. True heterosis (Htru), hypothetical heterosis (Hhyp) and dominance coefficient (H) were estimated by the criteria of weight 1000pcs of the produced hybrid seeds (F1C0), number of tubers, weight of one tuber and productivity of hybrids (F1C1). On the average frequency of selection in F1 has made 11.76 %. More advantageous hybrid combinations were determined for further production of new perspective lines, forms and clones. In this article we was brining results of hybridization and studying of heterosis effect of potato hybrids seeds (F1C0), hybrids (F1C1-2) in duration of 2009-2011 in mountain conditions of Tajikistan.

# SCREENING SOME POTATO CULTIVARS AND BREEDING LINES FOR RESISTANCE TO POTATO WART DISEASE UNDER FIELD CONDITIONS

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#### Introduction

The potato wart (*Synchytrium endobioticum*) became an important disease threaten the potato production in Turkey since 2004. After detection of wart disease around 900 ha area closed to potato production in the Nigde and Nevsehir provinces where main potato production areas in Turkey. It was reported that the causal agent of potato wart disease in Turkey is a new patotype [1]. Hence screening of existing cultivars for resistance/tolerance to this new patotype is very important to cultivar recommendation for the infected regions. A new breeding program for development of wart resistant/tolerant potato lines is also needed for Turkey. In last year we started the crossing some adapted cultivars with resistant cultivars which was determined at earlier preliminary studies. The objective of this study was to evaluate the resistance/tolerance of some potato cultivars and crossing progenies to wart disease under infected field conditions.

#### **Materials and Methods**

This study was conducted in an potato wart infested field (6 sporangium per g soil) in Nevsehir province in 2013. In total, 34 potato cultivars and 500 progenies from 10 families were evaluated in the study. The seed tubers of potato cultivars were planted in a randomized complete block design with four replications using 70 x 30 cm planting distance. Fifty progenies from each of ten families were also planted into same field with 70 x 90 cm distance. Each plant was harvested separately and clean and infected tubers determined, and then rate of infected tubers were calculated. The resistance of cultivars and progenies to wart were scored using 1-9 scale according to EPPO [2].

### Results

The mean values for infection rate of cultivars and families were presented in Table 1 and 2, respectively. The infection rates of cultivars ranged from 0.0% to 8.4% (Lady Olympia). The cultivars Andante, Bettina, Megusta and Pomqueen gave no infection of wart under the field condition.

The families contain resistant parents (Megusta, Bettina and Lindita) gave different mean infection rates. The all progenies from Megusta x Lindita family were found free of wart infection while the progenies of Megusta x Granola family showed 11.8%infection. Granola x Lindita progenies have the highest infection rate (12.0%).

### Conclusions and perspectives

Our studies indicated that some of existing cultivars have field resistance to Turkish patotype of wart diseases. With using these cultivars as parent in a breeding program, it is possible to develop resistant potato lines.

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Table 1. Wart infection rate (%) on tubers of evaluated cultivars.

	Infection rat	te	Infection rate		Infection rate
Cultivars	(%)	Cultivars	(%)	Cultivars	(%)
Andante	0	Marfona	0,8	Surya	4
Bettina	0	Agata	1,1	Alegria	4,2
Megusta	0	Taurus	1,3	Lady Claire	4,4
Pomqueen	0	Galata	1,6	Sagitta	4,6
Lindita	0,1	Agria	2,1	Marabel	5
Savanna	0,2	Krone	2,2	Soprano	5,9
Hermes	0,3	Granola	2,3	Musica	6,1
Van Gogh	0,4	Innovator	2,3	Juwel	6,1
Melody	0,4	Fakse	2,6	Atlantic	6,3
Royal	0,4	Sissi	3,4	Folva	6,3
Brooke	0,5	Spunta	3,5	Lady Olympia	8,4
Lanorma	0,5	130000000000000000000000000000000000000	\$400m		105,552

Table 2. The mean wart infection rate (%) on tubers of families

<u>Families</u>	Infection rate (%)		
Agria x Granola	8,8		
Agria x Megusta	2,9		
Krone x Megusta	3,0		
Bettina x Megusta	5,3		
Van Gogh x Megusta	2,2		
Megusta x Granola	11,8		
Megusta x Krone	9,8		
Megusta x Lindita	0,0		
Granola x Lindita	12,0		
Lindita x Granola	7,1		
Mean	6,3		

#### VARIABILITY AND GENETIC CONTROL OF PROTEINS IN POTATO HYBRID POPULATIONS

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#### Introduction

Potatoes contain significant levels of: carbohydrates, potassium and vitamins  $B_1$  and  $B_6$ . Even the proteins represent 1-3% from tubers (g/100g) (1) through their digestibility, they are important polymers of amino-acid useful in healthy diet and to increase the proteins content should be considered. Selection for a higher level of proteins has been analyzed (2).

#### Materials and methods

Progenies (182-167 genotypes/location) of 4 combinations:  $C_1$  (Bv.X.72-601-6 x Arka),  $C_2$  (Fanal x Bv.X.72-602-14),  $C_3$  (Bv.403/M x Arka) and  $C_4$  (Isna x Alka), 7 parents and 3 standards (Ostara, Desirée, Eba) cropped 4 years in 2 locations (Braşov and M.Ciuc) were studied for the protein contend variability and for its genetic control. Biuret method was used to determine the proteins and analyses of variance (ANOVA) was performed to asses the effects of genotype, year, location and their interaction.

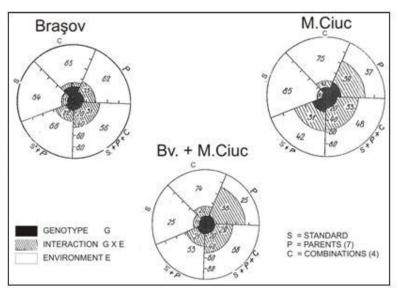
#### **Results and discussions**

chi<sup>2</sup>: distribution average values protein content evaluated bν of was  $\chi^2 = 1/n_1 n_2 \sum_{i=1}^{\infty} (f_1 n_2 + f_2 n_1)^2 / f_1 + f_2$  and all the combinations were significant different ( $\alpha < 0,1\%$ ) for location and year. The limits of variation (mg/ml) were for  $C_4$  (14, 2) and  $C_2$  (19, 2) in Braşov comparing with  $C_4$ (18, 2) and C<sub>2</sub> & C<sub>3</sub> (20, 1) in M.Ciuc. To examine whether the observed differences were caused by segregation, the percent of heterotic transgresive genotypes was assessed. For each of 4 combinations this average percentage ranged from 21% in C<sub>3</sub> to 50% in C<sub>2</sub> (4 years, 2 locations). Values in this study are consistent with other studies (3). Effects were considered random for obtaining estimates of the variance and genetic parameters (Tab.1). The heritability coefficient was small, the parents scored the higher values while the chance of phenotype to represent genotype was reduced. Environment and interaction ExG were very strong. To estimate G, E and GxE contribution into phenotypic content, synthetic groups were made (Fig.1). It has been determined the dominant contribution of E and GxE in protein content expression. Identification of superior genotypes allows selection for this character in Solanum t. populations and potato breeders could decide for a large number of traits in the same time.

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Genotypes	(S <sup>2</sup> G)	(S'E)	(S'GE)	(S'F)	(GCV%)	h.
Parents (P)	3.81***	12.33***	4.25	20.39	9.53	18.
Combinations (C)	0.76*	7.18***	1.72	9.66	4.35	7.8
Standard (Std)	0.46*	7.43***	0.86	8.75	3.61	5.2
Std & P	2.46*	17.84***	4.13	24.42	8.25	10
C, P, Std	2.25***	9.27***	4.1	15.59	7.45	14 4

Test F: \* P = 5 %; \*\*\* P = 1 %; \*\*\*\* P = 0,1 %



USING OF PROTOPLAST FUSION PRODUCTS OF 1EBN SOLANUM X MICHOACANUM FOR INTROGRESSION OF RESISTANCE TO PHYTOPHTHORA INFESTANS INTO S. TUBEROSUM GENEPOOL

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The objective of this work was to introgress resistance to *Phytophthora infestans* both from resistant somatic hybrids S. x michoacanum (+) S. tuberosum and 4x autofused S. x michoacanum into cultivated potato by sexual crosses in both directions. For crossing program we selected resistant to *P. infestans*: one somatic hybrid and three 4x autofused S. x michoacanum and four susceptible potato cultivars: Eugenia, Felka, Flaming and Legenda. All cultivars were resistant to PVY. After about 10 000 pollinations, about 700 berries were obtained when cultivars were used as pollinators and only 2 berries in opposite direction of crosses. In 2013 we received 388 seedlings of eight cross combinations. The ploidy level of pedigree was evaluated by counting chloroplasts in the guard cells. Pollen fertility was estimated using an indirect lactofuschin method based on percentage of regularly shaped and stained pollen grains. The resistance to foliage blight of obtained progenies were assessed in a laboratory test using detached leaf test. Respective parental forms and standard cultivars were tested together. Foliage tests were performed at two different dates in two replicates each. The single replicate was represented by one leaf in witch 1-6 leaflets were evaluated based on 1-9 scale (9 = the most resistant). A genotype with mean infection score between 6 and 9 was assumed to be resistant to P. infestans. All 388 seedlings were tetraploid. Majority of them flowered intensively and produced properly stained pollen grains. Foliage tests of obtained progenies confirmed the successful transfer of resistance to late blight in 64 individuals. Tubers of resistant genotypes have been preserved. Research is currently in progress.

The work is supported by The National Science Centre in Poland, grant: UMO-2012/07/B/NZ9/01901

# STUDY ON BREEDING OF POTATO CLONES ADAPTABLE TO CENTRAL-NORTH INTERSECTIONAL REGION OF TURKEY

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Potato breeding studies are new, although potato has been produced for years in Turkey. But in recent years, studies on developing new cultivars were gained acceleration in Turkey. Potato cultivars grown in Turkey were mainly imported from overseas. This study was aimed at development of new potato cultivar and has been performed since 2007. Beginning materyals of the study were real seeds of 13 hybrid families (Serrana x 104.12LB, MF-1 x TS-4, Serrana x TS-9, Granola x TS-2, Serrana x DTO-33, Serrana x LT-7, Serrana x TS-4, Serrana x TPS-113, Serrana x TPS-67, MF-1 x LT-7, Pentland Crown x TS-2, Granola x Huincul, and Achrina x LT-7) originating from CIP. In the first year of the study about 20.000 seedlings belonging to 13 hybrid families were produced. Then seedlings were planted in the field and seed tubers were produced. Clone selection has been continued by use of clonal selection method. The following yield and tuber parameters were used in clonal selection studies; tuber yield/per hill, nubref of tuber/per hill, average tuber weight, tuber shape, peel smoothness, tuber dry matter content, peel and internal colour of tuber. Detailed quality characteristics of the clones have been evaluated since 2010. In this study, Marfona, Marabel, Agata, Agria, Hermes, Slaney, Layd Claire and Granola varieties were used as standart.

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### **PHENOTYPING** (poster session)

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### FIELD SCREENING FOR POTATO LATE BLIGHT RESISTANCE

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Late blight caused by a fungus *Phytophyhora infestans* has endangered areas of the world where potato is produced and is increasing. Therefore, ten genetically diverse elite potato genotypes including a susceptible check (Bertita) were evaluated under natural infestations. The experiment was a randomized complete block design replicated three times. There was genetic variation for resistant to late blight. Resistance was expressed as adult plant resistance. Potato genotypes wc721-1 and Ruslin Ruaka possessed plant partial resistance with low average diseases progress curve of 27.25% and 32.25% and reduced final stand count of 19.33 and 17.67 and tuber yield 11.24t/ha and 7.46t/ha and 7.46 t/ha respectively. Genotype 392278.4 was tolerant to late blight, genotype wc731-1 and Ruslin Ruaka are potential sources of late blight resistance.

### AGRONOMIC AND PHYSIOLOGICAL RESPONSES OF STARCH POTATO CULTIVARS UNDER DROUGHT AND NITROGEN DEFICIENCY STRESS

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#### Introduction

A nitrogen surplus from agriculture poses a serious hazard to the environment by nutrient contamination of ground, fresh and marine water as well as by emission of ammonia and nitrous oxide. Nitrogen availability, uptake efficiency and leaching are directly related to the soil water content. Increasing periods of drought are expected in Central Europe due to the predicted climate change especially in late spring and early summer. This period coincides with the phase of maximal nitrogen uptake and vegetative development in potato which provide the basis for the subsequent yield potential. This investigation aims at the characterization of starch potato genotypes with respect to nitrogen use efficiency under water limiting conditions.

### **Materials and Methods**

14 starch and 3 reference table potato cultivars were investigated in a pot experiment grown in a rain-out shelter. For the control individual plants were supplied with 1040 mg N and the water capacity of the substrate was maintained at 60% during the entire season. The stress variants included nitrogen deficiency (1/4 N of the control), drought stress (13 days without water supply during the tuber initiation stage) and a combination of both stress treatments. The determined agronomic traits comprised tuber yield, starch and nitrogen content. Water and nitrogen use efficiency were calculated. Physiological responses were monitored by repeated determination of the chlorophyll content (SPAD) of young leaves and by analysis of cell osmolality and contents of total soluble sugars, proline, crude and pure protein in leaves at early and late stage of drought stress.

### **Results**

Genotype dependent differences in response to stress conditions were found for all considered parameters. The agronomic nitrogen use efficiency was generally reduced upon drought stress treatments while the water use efficiency declined under nitrogen deficiency conditions. Cultivars which produced relatively high yields in terms of both tubers and starch at all stress conditions were identified for the given experimental system. On the other hand, some genotypes were persistently negatively affected.

General trends in physiological disturbances were determined while individual genotypes were differentially affected. The chlorophyll content decreased with increasing culture period and was reduced in nitrogen deficient plants. Drought variants showed a distinct decline right after the stress phase but recovered after rewatering with a delayed senescence. Cell osmolality increased under drought stress depending on the cultivar. Some genotypes, including the drought tolerant cv 'Desiree', reached their individual maximum already at an early time point. In contrast, other cultivars further increased the osmolality between day 5 and 13 reaching a level above that of 'Desiree'. The crude protein content in leaves generally increased under drought stress, while the percentage share of pure protein decreased with increasing duration of water deficiency reflecting the progressive disturbance of metabolic activity.

### **Conclusions and perspectives**

Individual responses of cultivars to drought and nitrogen deficiency stress were identified at the agronomic and physiological level. For verification of results the experiment is repeated in a second vegetation period. Differentially responding genotypes will be used for investigations on proteomic alterations during the early stress phase.

Project funded by FNR (Fördernummer 22023311)

# EVALUATION OF GROWTH AND YIELD CHARACTERISTICS OF KOREAN POTATO VARIETIES UNDER HIGH TEMPERATURE

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Over the last 100 years, Korea has been warming faster than the global average. This study was conducted to determine the growth and yield characteristics of potatoes widely cultivated in Korea. Fourteen varieties and Desiree were grown in high temperature area (Gangneung, average sea level 5m) and in cool highland area (Daegwallyeong, asl 800m) in Korea's summer. And then, the growth and yield characteristics of varieties between two areas were compared. In stem length, Desiree and Jayoung and Chuyoung did not show any difference between two areas, while Superior and Atlantic and Jowon showed longer stems under high temperature. Leaves in most varieties, except Desiree and Harveong and Atlantic and Jayoung and Goun and Chuback and Chuyoung, were smaller under high temperature than under cool highland. Chlorophyll contents (SPAD) between two areas were not different in Desiree and Jayoung and Jowon and Dejima, while the one in Chuyoung was much higher under high temperature than under cool highland. There was much bigger yield decrease in fourteen varieties than in Desiree under high temperature. Yield decrease at high temperature area was caused by the increase of small tubers (under 80 g per a tuber) and it was because of the insufficient tuber enlargement. Meanwhile, in physiological disorders, secondary growth tuber's rate was much higher than malformed or cracked tuber's rate at high temperature area. This might be because the main target of potato breeding in Korea was early maturity with a short dormancy. Long time is needed to develop new varieties. Therefore, from these results, it was suggested that potato breeding for heat tolerance be urgently needed in Korea.

# MULTI-ENVIRONMENT TRIALS OF POTATO CLONES AS POTENTIAL VARIETIES FOR CEREAL BASED SYSTEM OF INDO-GANGETIC PLAINS OF INDIA.

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Under changing agro-climatic conditions, new potato varieties will be required for planting early and in main crop season to fit potato into diversified cropping systems and agro-ecologies of India. New, early/medium maturing varieties with heat tolerance, high tuber dry matter and good storability are needed. Short maturing potato will give way for timely planting of succeeding crops such as rice and wheat. The Central Potato Research Institute of India has included the development of early and medium maturing potato varieties among its 2013-2020 research priorities (2). Out of 34.4 million tons of potato grown in India, about 31 million tons are produced in sub-tropical lowlands (1). The present study was undertaken to select early maturing, high dry matter, heat tolerant and good storage quality variety/varieties to introduce potato in new and non-traditional potato growing areas to improve food security and enhance farmer's income.

Four elite clones bred at the International Potato Center were evaluated at three locations: Burdwan-West Bengal, Ladol- Gujarat, and Karnal- Haryana under the All India Co-ordinated Research Project during 2011-12 & 2012-13 to identify candidate clones for release as varieties for cereal based systems of the Indo-Gangetric Plains (IGP) and for the Plateau region of India. The evaluation sites represent hot-humid, semi-arid and sub-humid agro-ecologies of West Bengal, Gujarat and Haryana, respectively. Region-specific popular varieties were planted for comparison. The field trials were conducted during winter under short days. The trial was planted one month earlier than the normal planting time in each region, in order to observe tuberization during relatively high temperatures. Sprouted tubers were planted at 60 cm X 20 cm spacing in a randomized block design with 3 replications each of 2.0 x 2.4 meter size. The clones and control varieties were dehaulmed at 90 days after planting. Total and marketable tuber yield (>20 gram), tuber dry matter content and tuber characteristics were recorded.

In two successive seasons, out of four clones and five control varieties, CIP clone 397065.28, gave the highest total tuber yield of 41.15 t/ha, 56.40 t/ha, and 45.26 t/ha at Karnal, Burdwan, and Ladol, respectively and was 51.5 %, 21.3%, and 14% higher than the best control varieties of regions. Marketable tuber yield of this clone ranged between 38.85 to 50.15 t/ha at three locations compared. The two year results revealed that the selected clone CIP-397065.28 was superior to both clones and control varieties in production. The tuber dry matter content of clone 397065.28 ranged from 17.1 to 21.7% at locations and was 11% higher than Kufri Badshah at Karnal, 4.1% higher than Kufri Jyoti in Burdwan and 16.8% greater than Kufri Badshah at Ladol ,suggesting a promising future for storing in country stores. The selected clone has white skin and flesh color oval tubers. Based on two year performance, CIP 397065.28 has been recommended for on farm trials to be released as medium maturing variety for the cereal based system.

The release of CIP clone 397065.28 resistant to lowland tropical viruses as a new variety will improve food security and enhance income of potato growers by introducing potato into new areas of cereal systems

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# PARTICIPATORY EVALUATION OF NEW DISEASE RESISTANT AND ANDEAN LANDRACE POTATOES IN ETHIOPIA

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Advanced, disease resistant tetraploid clones with highest Fe and Zn concentrations and selected diploid group Phureja accessions were evaluated by farmers and researchers in Ethiopia in 2013 to gain their appreciation of tuber yield and consumption traits using a participatory approach. Nineteen farmers (10 female and 9 male) and five research technicians participated in the evaluation. Participants were asked to list traits for describing and discussing potato varieties at harvest of tubers and after cooking for consumption and rank the best clones from all the clones harvested from the field trial for the traits they identified. Farmers identified five attributes: tuber color, tuber size, tuber number, tuber shape and freeness from any tuber disease and insect pest attack as important criteria for evaluating new varieties at harvest. For both men and women, the most important criterion was freeness from damage of any pest and disease, followed by tuber number and tuber size. However, in ranking of potato tuber characteristics, women placed a higher value on tuber shape, and men did so on tuber color. Farmers also assessed the clones for appearance of boiled potatoes on plates. In this assessment, the recently-released local variety 'Belete' (CIP393371.58) and the new introductions CIP397067.2 and CIP703295 were the top three clones, rated excellent for appearance after boiling by men whereas for women, clones CIP703295, CIP706828 and CIP397067.2 were ranked as excellent. Clone CIP704205 was rated excellent for both taste and texture. This clone produced 27.31 tons ha<sup>-1</sup> total tuber yield and 22.96 tons ha<sup>-1</sup> marketable tuber yield. Other clones most preferred were CIP397067.2 and CIP706828 for taste and CIP704227 for texture. The Andean landrace cultivar group Phureja accession, CIP706828 was favored for its good taste by majority of the panelists and also produced good tuber yield (33.44 tons ha-1 total tuber). Phureja cultivars are characterized by short dormancy which could enable year-round harvests in Ethiopia as a contribution to food security. From this preliminary participatory exercise with farmers, we learn that farmers are open to test new types of potatoes in their farming system. The conventional approach of developing varieties that have the same appearance as those farmers are accustomed to growing may actually restrict the introduction and exposure of farmers to novel, attractive and adapted germplasm.

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# PERFORMANCE OF ADVANCED POTATO CLONES IN MOISTURE STRESS CONDITIONS IN ARID ZONE OF INDIA

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Potato is a drought-susceptible crop and when subjected to water stress results in low yield and poor tuber quality. The development of varieties which can maintain their yield under moisture-stress could improve the productivity of areas where irrigation is a limiting factor. In arid zones, drought in combination with high temperature increases the stress to a new level and significant reduction in tuber yield and quality have been observed [2]. Eight advanced clones (392745.7, 392780.1, 397006.18, 399101.1, 301029.18, 380583.8, 388972.22 and 391580.30) received from the International Potato Center, Peru were evaluated along with two varieties Kufri Pukhraj (early fast maturing) and Kufri Surya (heat tolerant) in an arid sub-tropical region of Western India at Jodhpur during 2012-13 crop season. Five of these clones (392745.7, 392780.1, 397006.18, 399101.1 and 388972.22) were found virus resistant when evaluated in lowland tropics in Peru. The field experiment was laid out in strip plot design with two irrigation regimes: I1= normal Irrigation (eight irrigations) and I2= deficit irrigation (five irrigations) as horizontal factor and ten clones/varieties as vertical factor. Data were recorded on growth, yield and tuber quality attributes at 30, 45, 60, 75 and 90 days. The crop was dehaulmed at 90 days of planting and harvested 15 days later to allow tuber skin set. Drought tolerance index (DTI) was calculated using formulae referred by Hassanpanah [1]. Clone 397006.18 had the highest mean tuber weight per plant (513.7q), which was significantly superior to control Kufri Surya (360.8q) and other two advanced clones 392780.1 (352.3g) and 391580.30 (333.8g). This was also comparable to the control Kufri Pukhraj (483.0q). Severity of moisture stress reduced tuber weight notably and interactions between genotypes and irrigation regime were also distinct. Maximum mean tuber dry weight per plant was also observed in same clone 397006.18 (104.8g) which was significantly higher than both the controls Kufri Pukhraj (76.1g) and Kufri Surya (72.3g). Like other yield attributes, tuber dry weight declined consistently in moderate drought treatment (36.2%) and interactions of irrigation and genotypes were significant. Clone 397006.18 also presented the highest and significantly superior total biomass yield (142.0g). The harvest index (HI) of clone 397006.18 was 0.74 which was at par with other clones and varieties, except for variety Kufri Surya which was higher (0.81). Water stress decreased HI markedly, however, no interactions between irrigation and genotypes were observed. Among all the clones 397006.18 recorded the highest DTI of 1.067. Overall, clone 397006.18 performed best among all the evaluated clones. This clone can further be tested in abjotic stress conditions in other regions to confirm its potential and release as a variety if found promising.

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# SCREENING OF POTATO (SOLANUM TUBEROSUM) GENOTYPES FOR YIELD AND QUALITY TRAITS UNDER LOW NUTRIENT STRESS CONDITION IN SIERRA LEONE

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The trial was conducted at one of the Sierra Leone Agricultural Research Experimental station, located at Madina in Koinadugu District, Northern part of the country, on the 1st July to September 2012.

The main aim of the trial was to identify genotypes for high yields and improve quality characters or traits under low input conditions for sustainable crop production and enchance profits for poor resource farmers involved in its cultivation.

To achieve this aim, the three current potato genotypes under cultivation in the country were used. All three are already improved genotypes from other countries, namely: Resy, Ostira and Nicolas.

The experimental design was a complete block with three replications (collapsed) in which only one factor was used which is genotype.

The morphological data were measure and recorded and they include; stem height, tuber number (large and small), tuber weight, to fresh weight, leaf area at one, two and three months after planting (MAP). Other parameters determined were tuber to top weight ratio (R/T ratio), dry matter content, protein, starch and harvest index at maturity. The soil and weather information were measured before and during the growing period of crop.

Data were analyzed through the general linear model (GLM) analysis of variance (ANOVA), testing of the significant of means and separation of means least significant differences (LSD) by student's Turkey test performed by SASV 8-1.

Results shows significant genotype variations among the three potato genotypes screened in terms of yield production, the component that contribute to yield and quality characters like dry matter percentage, starch and protein contents.

Genotypes with highest and lowest contents of these parameters were identified and recommended either for production or to be used in further crop improvement work to enhanced yield and quality of potato that could ensure food availability, added nutrient and processing values all gearing towards food security, income generation and poverty reduction.

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# TUBER YIELD AND PHOSPHORUS USE EFFICIENCY IN DIFFERENT GENOTYPES OF POTATOES CULTIVATED IN SOUTHERN CHILE

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#### Introduction

World demand for phosphorus (P) fertilizer and increasing environmental regulations will continue to motivate the search for ways to improve P use efficiency (PUE) in potato production [4]. PUE can be defined as the product between P uptake efficiency (PUPE, the ability of crops to absorb P from the soil) and P utilization efficiency (PUTE, the ability of crops to convert the absorbed P into yield). There are several studies assessing PUE in grain crops [1, 2 and 3] and much less is known in potato. Till now, potato crops exhibits considerable genetic variation in uptake efficiency of nutrients like nitrogen, and it is likely that genetic variation also exists in P use efficiency [4]. The aim of the present work was to assess yield responses, PUE and its components in different potato genotypes under field conditions.

### **Material and Methods**

During de 2012-13 growing season, a field experiment was carried out at INIA Osorno in Southern Chile.. Treatments were the factorial combination of 22 genotypes of potatoes and two P rates (P0: 0 and P300:  $300 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ ). Five native potatoes, eight advanced lines and nine cultivars were among the set of genotypes tested. Initial soil P level was 7.2 mg/kg P-Olsen. Above-ground biomass, yield and total P uptake was determined at physiological maturity. Treatments were arranged in a split-plot design where P rates were assigned to main plots and genotypes to the sub-plots randomized in three blocks.

#### Results

Yield, P uptake and P utilization efficiency were affected (P < 0.01) by genotype, P rate and genotype x P rate interaction. Across treatments, yield ranged between 28 and 73 t/ha. Genotypes showed different sensitivities to P deficiency (genotype x P rate interaction) since tuber yield reductions ranged between 40 and 5%. As expected, PUPE was decreased by P fertilization, with 1.90 and 0.20 kg of P uptaked/kg P available under P0 and P300, respectively. Most importantly, great differences were found among genotypes in their abilities to absorb P from the soil, since PUPE under P0 ranged between 2.7 and 1.3 kg P uptaked/kg P available. Regarding PUTE, it was less affected by P supply than PUPE. On average, PUTE measured 2378 and 2048 kg fresh tuber/kg P uptaked under P0 and P300, respectively. PUTE under P0 treatment, showed differences among genotypes, ranging between 2821 and 1886 kg fresh tuber/kg P uptaked. PUE, the product between PUPE and PUTE, averaged 4475 and 412 kg fresh tuber/kg P available under P0 and P300, respectively. Measured under P0, PUE showed important differences among genotypes varying between 5871 and 2814 kg fresh tuber/kg P available. Interestingly, these extreme PUE values were produced by a native genotype and a European cultivar, respectively.

### **Conclusion and perspectives**

This results might be useful for breeding and crop management programes aimed to improve the phosphorus use efficiency of agricultural systems through generation of more efficient cultivars, a better choice of cultivars according to soil P availability and/or adjust P fertilization according to cultivar sensitivities (tolerant vs sensible genotypes). Future work will focus on understanding the ecophysiological determinants of phosphorus use efficiency which might be related to root system traits.

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# LATE BLIGHT (PHYTOPHTHORA INFESTANS) RESISTANCE OF THE ESTONIAN POTATO CULTIVAR ANDO AND ANDO'S HYBRIDS

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### Introduction

The Estonian potato cultivar Ando has demonstrated high isolate-specific late blight resistance to most of the local *Phytophthora infestans* isolates for over two decades. Despite its deployment in potato breeding, the molecular basis of Ando's resistance has not been studied. The putative candidates of Ando's resistance include monogenic resistance genes (R genes) which mostly belong to the NB-LRR class that contain a nucleotide binding domain (NB-ARC) and a leucine rich repeat domain (LRR). The NB-ARC domain is highly conserved and acts as a molecular switch of R protein activity. Based on the conserved NB-ARC domain motifs of the published potato genome (DM1-3 516R44) R gene sequences, the research aims to identify late blight R genes in cultivar Ando by NGS sequencing and develop functional marker(s) for the disease resistance.

### **Materials and Methods**

The late blight resistance phenotype of the resistant cultivar Ando, susceptible cultivars Agra and Frila and F1 hybrids of Ando x Agra and Ando x Frila (100 plants in both populations) was assessed by in vitro detached leaflet inoculation assay and late blight field test. In the field trial, the progression of the late blight infection was estimated as a percentage of total foliage infected. For the molecular analysis of resistance, RNA was extracted from 100 mg of fresh leaf material of Ando, Agra and Frila and 3' RACE was conducted to amplify the 3' regions of expressed R genes using potato R gene NB-ARC domain specific primers.

### Results

Both in vitro and field tests confirmed the late blight resistance of Ando and susceptibility of Agra and Frila. The F1 progeny of Ando x Agra (AA) and Ando x Frila (AF) segregated for late blight resistance. The in vitro inoculation assay identified 48 resistant plants among AA hybrids and 75 resistant plants among AF hybrids. The late blight field assay identified 12 resistant plants among AA hybrids and 26 resistant plants among AF hybrids. 7 AA and 21 AF hybrids showed similar resistance phenotype both in in vitro and field tests, whereas the majority had significant differences in resistance levels according to the two tests. The 3' RACE yielded R gene amplicons in a size range of 400 bp to 2500 bp and preliminary analysis of some of the sequences confirmed their identity as NB-LRR type R genes.

### **Conclusions and perspectives**

The in vitro and field assays corroborated the resistance of Ando and susceptibility of Agra and Frila and asserted the segregation of late blight resistance in Ando x Agra and Ando x Frila F1 populations. The different numbers of resistant and susceptible plants determined in the two late blight assays possibly account for the different nature and timeframe of the tests: in the inoculation test, the extracted leaflets were drop inoculated with *P. infestans* zoospores solution and incubated under artificial light and moisture conditions for 7 days, whereas the field test assessed the natural progression of late blight disease under field conditions during 24 days.

The 3' RACE of R genes using NB-ARC domain specific primers yielded PCR products of 400 to 2500 bp. These R gene 3' sequences of Ando, Agra and Frila will be submitted to Illumina sequencing to determine the R gene sequence regions which correspond to Ando's late blight resistance. The two F1 populations of Ando x Agra and Ando x Frila will be analysed further for co-segregation of the resistance phenotype with respective R genes.

# EVALUATION OF POTATO CLONES FOR RESISTANCE TO BLACK SCURF CAUSED BY RHIZOCTONIA SOLANI AG-3 IN FIELD STUDIES IN TOKAT-TURKEY

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Black scurf and stem cancer of Potato is a serious disease commonly observed in most potato-producing areas of the world. Caused by *Rhizoctonia solani* AG-3 (teleomorph *Thanatephorus cucumeris* [Frank] Donk), this disease is favoured by the capacity of fungus to survive in soil as sclerotia and mycelium in plant debris for long periods, and environmental conditions of low soil temperature and high soil moisture. Management of the disease requires an integrated approach since no single tactic is totally effective. An effective control program combines cultural practices, fungicides, and resistance. The objective of the present study was to evaluate the reaction of potato clones (262 clones derived from 13 hybrid families) for resistance to *Rhizoctonia* black scurf in field experiments during the 2008 and 2009 growing seasons. Seed tubers of each clone were planted in a field with a history of black scurf. Experimental design was randomized block design with three replications. At harvest, the incidence of black scurf on tuber was determined. The incidence of black scurf differed significantly among clones in both years. Based on the results of present study, black scurf incidences of 54 clones out of 262 clones were changing between 10% and 74%. On the other hand 153 clones were found highly resistant to the pathogen (0% black scurf incidence).

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### SUSCEPTIBILITY OF NEWLY BRED POTATO CULTIVARS TO BLACK DOT AND SILVER SCURF

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#### Introduction

In Switzerland, the fresh consumption market washes a large part of the potatoes for better display in the shops. Newly bred cultivars with beautiful presentation have a delicate skin, susceptible to many skin diseases. Among those, black dot (*Colletotrichum coccodes*) and silver scurf (*Helminthosporium solani*) have an important impact on tubers quality. They contribute to visual deterioration and weight loss during storage [1, 2]. The distribution of these two diseases in seed tubers and in the soil is very large in Europe [3]. The pathogens symptoms increase first in the soil, after harvest, during the conservation and after washing and packing process. The symptoms can quickly evolve when the conditions are favorable to diseases development [3, 4].

### **Material and Methods**

Each year in Switzerland, 20 to 25 newly bred potato cultivars, mainly from European breeders, are tested in four locations under field conditions. Each variety is tested for at least two consecutive years. For each field trial, 100 tubers are sampled for visual observation of blemish diseases after 3 months of storage. Tubers are first washed and dried for 24 hours at room temperature. Then the general incidence of both diseases is visually estimated using a 9 grades scale. There is no differentiation between black dot and silver scurf for this routine visual approach of cultivars preliminary screening. Between 2006 and 2012, 128 cultivars were tested.

### Results

Our visual approach showed that most of the tested cultivars are highly susceptible to black dot and silver scurf symptoms development. For the great majority of the cultivars assessed, 35 to 100% of the individual tubers were affected. The higher the number of affected tubers, the higher the severity of the symptoms on each tuber. The average annual variation of symptoms was about 30%. Symptom level of black dot and silver scurf infections also varied with the location, depending on the soil infection with *Colletotrichum coccodes* and the influence of environmental factors on diseases' expression.

### **Conclusions and perspectives**

This study showed that each of the 128 cultivars tested is susceptible to black dot and silver scurf. It would be of interest to determine whether the susceptibility of the cultivars varies for the two pathogens, *Colletotrichum coccodes* and *Helminthosporium solani*).

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### SEED POTATO PRODUCTION (posters session)

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### THE PRODUCTION OF THE POTATO CONSERVATION VARIETY FROM MERISTEM PLANTS

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#### Introduction

We have been researching how to use the meristem method for eradication of viruses of potatoes, multiplication of disease free plants and growing seed potato. The methods of multiplication enable much more opportunities than it has been used at present. The new viruses are spreading rapidly and the quality of seed potato falls down during the years of propagation. Today beside the new varieties the old and ancient varieties, preserved in gene banks, are propagated and taken into production as conservation varieties. They have a lot of interesting properties. But often the conservation varieties are suggestible to viruses and after the long- term seed production are infected widely. Therefore the shorter production cycle is needed.

#### **Materials and Methods**

The plants of the conservation variety 'Väike verev', previously eradicated from viruses, were propagated in vitro. On 15 of May the plants were planted into peat substance in plastic rolls and after 2 weeks were transported to a farm. The plants were planted into a field by hand in first week in June. The planting was carried out according the technology created in EVIKA. The same agrotechnical procedures and fertilising, used in common seed potato production field were carried out. The field was spayed tree times against the late blight infection and harvested by the harvester in 2 of October. In next year, pre-germinated tubers were planted into the field on 28 of April. As the vegetation period was dry, the field was irrigated twice. The tubers were harvested in 25 of September; the green haulms were killed previously.

### **Results and Conclusions**

In 2012 4580 potato plantlets were planted into a farmer's field and 1.5-t of first generation seed tubers were harvested. In 2013 tubers were planted and 45-t of potato with high quality was harvested. It is marketed as exclusive product for consumption. There are several methods for propagation of disease-free initial seed potato material. The propagation of meristem plants by stem-cuttings in vitro and growing in greenhouses, plastic or net houses is more widely used. According to our technology, the plants are grown or multiplied in plastic rolls, and planted into the field by hand or by machine. It is the cheapest, most simple and effective way to get larger quantities of virus-free seed potato tubers and to multiply new cultivars and conservation varieties. In our plant productivity was affected by multiplication and growing methods.

The usage of cheap and non-complicated propagation methods enables to shorten the seed production cycle, which is vital importance in the case of disease susceptible varieties and especially in growing the conservation varieties.

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# POTATO (SOLANUM TUBEROSUM L.) IN VITRO GERMPLASM CONSERVATION IN ACTIVATED CHARCOAL MEDIUM

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An efficient protocol for in vitro long-term storage of a potato (*Solanum tuberosum*) germplasm has been devised. In vitro method of germplasm conservation employing minimal growth strategy involves frequent transfer of cultures to fresh media, constant vigilance and control of storage conditions. Activated charcoal was investigated as an alternative medium for slow-growth in vitro storage of potato microplants. Four different germplasm storage medium in 5 replicate culture tubes (Medium 1 % AC: MS + 10 gl<sup>-1</sup> activated charcoal + 30 gl<sup>-1</sup> sucrose + 7 gl<sup>-1</sup> agar; Medium 2 % AC: MS + 20 gl<sup>-1</sup> activated charcoal + 30 gl<sup>-1</sup> sucrose + 7 gl<sup>-1</sup> agar; Medium 3 % AC: MS + 30 gl<sup>-1</sup> activated charcoal + 30 gl<sup>-1</sup> sucrose + 7 gl<sup>-1</sup> agar and Medium 3 % S: MS + 30 gl<sup>-1</sup> sucrose + 7 gl<sup>-1</sup> agar) were used. The cultures were conserved under continuous illumination at 10 oC for a year and following microplant traits were measured at 1st, 3rd, 6th, 9th and 12th month of storage: microplant height, leaf, node and branch numbers. Potato microplants can be conserved successfully in medium containing MS + 10 gl<sup>-1</sup> or 20 gl<sup>-1</sup> activated charcoal + 30 gl<sup>-1</sup> sucrose + 7 gl<sup>-1</sup> agar under continuous illumination at 10 oC storage temperature.

# A CASE STUDY ON CURRENT POTATO (*SOLANUM TUBEROSUM* L.) MINITUBERS PRODUCTION IN LATVIA AND ITS FURTHER PROSPECTS

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As a vegetatively propagated crop, potato (*Solanum tuberosum* L.) is prone to accumulation and further spread of several diseases influencing its yield and quality. Potato seed production in Latvia is considerably affected by potato viruses infections caused by severe pressure of viruses transmitting vectors. In the second part of the 20th century some popular local varieties were totally infected by viruses and were excluded from potato seed production system.

The studies on virus elimination methods were started in Latvia in the 60s of the 20th century, followed by establishing of in vitro collection of potato varieties and breeding material.

In Latvia, potato seed production system based on virus free in vitro plant propagation material was developed at State Priekuli Plant Breeding institute (SPPBI) in the 80s of the 20th century. Propagation included elimination of potato viruses, aseptic tissue culture establishment, plant micropropagation and minitubers growing in greenhouses and plastic tunnels.

Nowadays potato minitubers production methodology in Latvia is based mainly on trials conducted from 2001 to 2003 by A.Gabere. Potato plants are planted in fertilized peat with pH adjusted to 5.3 in two container types – plastic beds and plastic boxes. Planting density is 45 plants per m2. Destructive harvesting is performed in 80-100 days after planting. Previous tests have shown that minituber number per plant did not significantly differ between two types of planting containers.

From 2009 to 2012 seven varieties were assessed in respect of tuber number per plant and tuber weight distribution. Significant influence of genotype on tuber number per plant was observed (p<0.05). Mean values ranged from 1.79-6.85 tubers per plant. On average more than 50% of harvested tubers weighted more than 20 g, 40% were in range between 5-20 g and 10% - less than 5 g.

Potato viruses transmitting vectors' (aphids) pressure in field is considerable and by reason of viruses infection potato seed material categories drop. It is one of reasons limiting production of certified seed material in sufficient amount. Decreasing of field generations' number by increasing number of potato minitubers obtained in greenhouse can serve as one of the most effective solutions. The area in greenhouses suitable for potato minitubers production at SPPBI is limited. Planting density should be increased with aim to obtain as many minitubers with good field performance as possible.

Minitubers of three potato varieties 'Monta' (early), 'Prelma' (medium early), 'Mandaga' (medium late) were grown in greenhouse in 2013. Tubers were graded in 4 classes by weight (3-5 g, 5-10 g, 10-20 g, >20 g) and in the spring of 2014 these tubers will be planted in field with aim to compare their performance depending on the weight class. At the same time greenhouse trial with various planting densities for the same varieties will be conducted. The aim of the study is to find the most efficient planting density for obtaining largest number of acceptable size minitubers with perfect field performance (high seed tubers yield). This approach will increase the efficiency of minituber (potato initial seed material) production and will improve the effectiveness of potato seed production system.

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# EVALUATION OF THE TRUE POTATO SEED PROGENIES IN FIELD CONDITIONS AND ITS ADAPTABILITY IN CENTRAL ROMANIA

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#### Introduction

One of the pressing problems of the nowadays world is to provide food for a continuously growing population. Also, the world economic crisis leads to necessity of finding some abundant food sources with minimal costs. The True Potato Seed (TPS) technology is dedicated for less expensive potato production, mostly in warm regions where the seed potato cannot be produced and for those who have limited financial resources, but not only.

#### **Materials and Methods**

The goal of this study is to evaluate the vegetative progenies of four potato isogenic lines: Mindy, Zolushka, Gilroy and Catalina (creations of Bejo Zaden company, Netherlands), compared with two control varieties (Santé and Rustic) under field conditions and assessment of phenotypic, quality and yielding uniformity compared with performance of conventional potato varieties. The research was set at the experimental fields of the National Research and Development Institute for Potato and Sugar Beet Brasov in 2010. During the growing season there was made several observations on the potato plant development, from emergence to maturity and how the phenological issues have influenced the yield and number of tubers. Also, were made laboratory determinations on the culinary and technological quality of studied potato genotypes.

### Results

Regarding the percentage of emergence, the best result was recorded for Santé (95.15%) and Zolushka (93.53%), the sprouting was distributed between middle and uniform and there were no significant differences among the six studied potato genotypes. Regarding the overall assessment of the plant, Gilroy followed by Zolushka, was similar with control varieties, which have received the highest scores. In terms of flower and fruit richness, the isogenic lines were obtained higher marks than the control varieties. Under the climatic conditions that characterized 2010, the studied variants were expressed different resistance to the late blight attack. Thus, Rustic showed a very good resistance, Santé was medium resistant and the isogenic lines were strongly affected. The highest number of tubers/plant was registered on Rustic (22.2) followed by Santé (12.2). Among the studied lines, Gilroy had the best results (8.5), followed by Mindy (7.9), Catalina and Zolushka (7.8). Regarding the average weight of tubers/plant, the lowest value was recorded on Rustic (43.5 g). Isogenic lines had intermediate values (from 55.4 to 59.3 g) and the highest value was obtained by Santé (73.3 g). Regarding the total tuber yield, the best results were obtained in control varieties (over 40 t/ha), the isogenic lines have done lower yield (about 23 t/ha). Following the determinations made in the laboratory on the culinary and technological quality, the results showed that Mindy got the lowest percentage of starch content, making it suitable for fresh consumption, while others, due to higher starch content, suitable for processing.

### **Conclusion and perspectives**

Of the four studied potato isogenic lines Catalina was noted having good production capacity, low and compact appearance of the haulm, which made it suitable for mechanized tillage. As well Catalina has the most uniform tubers. Gilroy made the highest number of tubers/plant and Mindy has the highest average weight of tubers/plant. Compared with the control varieties, the four isogenic potato lines showed competitive results in terms of uniformity, productivity, cooking and technological quality.

### MINI-TUBERS: A NEW FRONTIER TO ENHANCED BASIC SEED POTATO PRODUCTION IN KENYA

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Seed potato is seen as a vital input in any potato production system, as it's the physiological basis for vigorous crop development, genetic basis for the adaptation to varied ecological and product preferences. At the Agricultural Development Corporation (ADC) Molo Complex, a formal seed potato production system exists, however over the years, it has been unable to produce sufficient seed to farmers due to shortage of basic seed from the national potato program, lengthy field multiplications hence low yields. The seed shortage has led to farmers obtaining planting material either from the local market, neighbours or own farm saved seed. This has promoted build-up of devastating bacterial wilt and viral disease, low yields and poor quality of tubers. An improved certified basic seed potato production system capable of ensuring sustainability in seed multiplication using minitubers is now underway at ADC through collaboration with CIP. With the new var. K. Mpya and K. Sherehekea the technology has worked well in getting healthy, true to type basic potato seed, rapidly, in a timely manner, under reduced field generations hence lowering costs and raising the plant health quality of the field production generation. With reduced field generations and precise practice of recommended agronomic practices and inspection services by KEPHIS the seed potato produced has improved in trueness, quality and quantity. Potato being a promising food security crop in Kenyan households, production is expected to significantly increase, contributing to poverty alleviation through income generation, provision of employment opportunities through value addition enterprises in production, processing and marketing. Mini-tubers production system is thus seen as a new frontier that will open a window for production of own breeder's seed at ADC, resulting in enhanced sustainability of the formal seed potato production system, to the local small scale farmer in Kenya and Africa as a whole.

### POSITIVE SELECTION TO IMPROVE QUALITY OF FARMERS SEED POTATO IN KENYA

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In Kenya, seed potato quality is often a major yield constraint in potato production as smallholder farmers use farm-saved seed without proper management of seed-borne pests and diseases. Farm-saved seed is therefore often highly degenerated. The efficiency of positive selection techniques in improving quality of farmers saved seed potato and the performance of subsequent potato crops was investigated from 2011 to 2012 in farmers' fields in in Meru County. Positive selection in farmers seed potato contributed to a 30% reduction in for both bacterial wilt and virus disease incidences. The analysis of mean tuber yield/ha and number of tubers/plant revealed that the seeds obtained through positive selection significantly out-yielded by 58.0% and 34.6% respectively, compared to the farmers saved seed. Therefore, positive selection can contribute to improve quality of farmers saved planting material and potato yields hence an important alternative and complementary technology to regular seed replacement, especially in the context of imperfect rural economies characterized by high risks of production and insecure markets. It does not require cash investments and is thus accessible to all potato producers. It can also be applied where access to high quality seed is not guaranteed. The technology is also suitable for land races and not recognized cultivars that cannot be multiplied formally. Finally, the technology fits seamlessly within the seed systems of Sub-Saharan Africa, which are dominated by self-supply and neighbor's supply of seed potatoes.

# POTATO SEED CERTIFICATION AND INTERVENTIONS ALLEVIATING CONSTRAINTS IN POTATO PRODUCTION IN KENYA

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Potato (Solanum tuberosum L.) is a crop of major economic importance worldwide and is considered the second most important food crop after maize in Kenya. National potato production ranges from 4.4t ha-1 to 15t ha-1 with an average of about 6.7t ha-1 although yields of 40t ha-1 are attainable under research conditions. Scarcity of good quality seed is a major constraint in potato production hence improving the quality and availability of certified, disease free potato seed of high varietal purity is of great importance in ensuring optimum potato production. KEPHIS has supported the implementations of various interventions aimed at alleviating constraints in the potato seed supply system. These include seed certification, quality assurance of seed emanating from new technologies such as aeroponics, enhancing capacity in dealing with phytosanitary challenges, providing a platform for exchange of potato germplasm in the region and adoption of modern techniques such as polymerase chain reaction (PCR) and Immuno-Flourescence Microscopy for seed health testing. Other intervention have been undertaking pest risk analysis (PRA) with a view of establishing pest of quarantine concern within the region in order to facilitate importation of superior potato germplasm and seed potato. This is aimed at boosting the existing pool of potato varieties providing a wider choice to farmers. Potato seed production has further improved with entry of more seed companies into potato seed production hence the need for certification to maintain quality and an effective variety release program.

# PRODUCTION OF MINITUBERS USING STEM CUTTINGS IN LOW AND HIGH LIGHT INTENSITY GREENHOUSE

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Stem cutting propagation is among the most productive and highly efficient low cost potato rapid multiplication technique. An experiment was conducted at Kenya Agricultural Research Institute, Tigoni to determine the effect of light intensity on productivity of potato stem cuttings. The mother plants were originally tissue cultured and then raised in trays containing coco peat as the medium. The trays were placed in a greenhouse. When they were three weeks old, stem cuttings were made from them. The cuttings were planted in sand beds. The sandbeds were placed in both 50% shaded and unshaded glasshouses. The 50% shading was achieved by placing a net over the greenhouse so that only 50% of the incident radiation passed through into the glasshouse. The experimental design was a split plot in which the five potato cultivars (Tigoni, Sherekea, K. Mpya, Dutch Robyjn and Purple Gold) were the main plots and the lighting (50% shaded and unshaded) were the subplots. The experiment was replicated three times while the subplot consisted of ten stem cuttings. After three months, mini tubers were harvested from the stem cuttings. Data collected was the number of mini tubers from the ten stem cuttings in each subplot. The data was analysed using Genstat version 12 and mean separation was done using Tukey's test at 5% level of significance. There were significant differences among the potato cultivars. between the lighting intensity and in the interaction between potato cultivars and lighting intensity. Kenya Mpya had the highest mean number of mini tubers per plant (6) and (4) from the 50% shaded glasshouse and un-shaded glasshouse respectively. The shaded glasshouse gave the highest mean number of mini tubers for all the cultivars. This suggests that low light intensity favors mini-tubers production in potato stem cuttings. This could be due to a longer stay-green period that enhanced tuber initiation.

#### PRODUCTION OF POTATO MINITUBERS USING SAND HYDROPONICS IN KENYA

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Potato (Solanum tuberosum L.) is one of the most important food crop in Kenya. However, the yield have been fluctuating over the years at about 6-7 t/ha as compared to more than 45 t/ha in Europe. Availability of seed potato is one of the major constrain in potato production. In order to improve the situation, an economically feasible technique is required to feed the system of commercial seed production with healthy material at a rate that is in accordance with the existing rate of seed degeneration. In this respect, hydroponics technology was recently introduced as an efficient method to produce and propagate minituber, which are healthy seeds without any contamination to pathogens. A study was therefore conducted at Kenya Agricultural Research Institute (KARI), Tigoni hydroponic unit. 7 different genotypes were evaluated the experiment was laid in randomized complete block design with three replication. Comparison of genotypes was performed using Fischer's protected Least Significant Difference (LSD) mean separation procedure at P<0.05. The results indicated that there was significant difference (P<0.05) on number and the weight of minitubers of the different genotypes used. However, there was poor performance in all the potato genotypes used. The results showed the highest mean tuber number were obtained from clone 381381.13 (16.11 tubers/plant). Whereas clone 390478.9 had the highest mean weight of 77.2 gms per tuber. However, the experiment needs to be repeated two more season to come up with conclusive recommendations.

#### **EVALUATION OF SOME CIP GERMPLASM UNDER AEROPONICS IN KENYA**

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Aeroponic technology is useful for rapid multiplication of seed stock for basic seed potato (*Solanum tuberosum L.*) production within a short period of time. The technique has been successfully applied by seed potato producers in Kenya. In addition, aeroponic method has been used by the breeders for production of minitubers of new genotypes. In this regard, a study was undertaken using 10 CIP potato genotypes and their yield was evaluated in the aeroponic unit. The trial was carried out by CIP and Kenya Agricultural Research Institute- Tigoni. These genotypes were obtained from Lima, Peru. The experiment was laid in randomized complete block design with three replications. Yield comparison of genotypes was performed using Fischer's protected Least Significant Difference (LSD) mean separation procedure at P<0.05. The results indicated that there was significant difference (P= 0.001) on number of minitubers of the different genotypes used. The highest performing genotypes were: 392797.22, 398190.735 and 381381.20 with mean value of 68.39 tubers/plant, 67.28 tubers/plant and 66.39 tubers/plant respectively. CIP393371.58 was the least performing genotype with mean tuber numbers of10 tubers/plant. Nonetheless, the experiment needs to be repeated one more season to come up with useful recommendations.

# IS A FUNCTIONAL POTATO SEED SYSTEM POSSIBLE IN A DEVELOPING COUNTRY? EXPERIENCES FROM MALAWI

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Potato production in developing countries like Malawi is constrained by lack of quality seed tubers. Through the collaboration of International Potato Centre (CIP) and the Department of Agricultural Research Services, a potato seed system has been put in place. The seed system has two approaches: informal and formal sub-sectors. Through the informal sub-sector a technique called "Positive and Negative Selection" is used where farmers select the best plants from their fields to be used as seed tuber for subsequent crop. Through this technique smallholder farmers have significantly increased potato yields as high as 50%. Furthermore, there has been drastic reduction in pests and diseases like bacterial wilt and viral diseases. The formal sub-sector starts with pathogen free plantlet from a tissue culture laboratory, through screen houses (aeroponics and sandponics) and finally open field multiplication in disease free fields. The whole process of seed production within the formal sub-sector is regulated by an independent seed certification unit. Positive experiences from the novel Malawi potato seed systems have created demand for quality seed tubers which has also increased the competitiveness of the Malawian potato within the confectionary industries like French flies and crisp making.

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# SUSTAINABILITY OF THE AEROPONIC'S PRODUCTION SYSTEMS OF PRE-BASIC SEED OF POTATO IN PERU

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To implement a sustainable system of production of pre-basic potato seed, it is important to be familiar with every factor involved. This work was, therefore, carried out in two phases. The first was a production phase in a greenhouse, at the International Potato Center (CIP), in Lima, Peru, where two production methods were evaluated (Aeroponics, using rooted cuttings; and Conventional: beds with substrate and rooted cuttings), using two recently released varieties (Chucmarina and Serranita) in a Complete Randomized Design, with four repetitions. The second phase, to determine the sustainability of the production systems, was based on interviews conducted with producers of pre-basic seed who used either the aeroponics system or the conventional one. With the information gathered, the sustainability indicators were constructed in accordance with the methodology and the conceptual framework proposed by Sarandón and Flores (2009), making small modifications according to the methods evaluated.

The lowest total production cost per square meter (US \$37.98/m²), was obtained in the conventional system with both varieties. The highest total income and profitability were obtained by the Aeroponics-Serranita treatment, with US\$125.16/m² and 66.97%, respectively. The largest number of tubers per m² > 5g was obtained with the Aeroponics-Serranita system with 324 tubers/m²; and the lowest was obtained with the Conventional-Serranita system with 54 tubers/m². The largest number of tubers per m² < 5g was obtained with the Aeroponics-Chucmarina system (198 tubers/m²); and the lowest (16 tubers/m²) with the Conventional-Serranita system. The average values of sustainability according to the methodology proposed by Sarandón and Flores (2009) were 2.74 and 2.56, respectively, for the Aeroponics and Conventional systems of production of pre-basic potato seed, which place them in the medium sustainability range.

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# VALIDATION AND DISSEMINATION OF SMALL SEED PLOT TECHNOLOGY FOR SEED POTATO QUALITY AVAILABILITY AND MAINTENANCE FOR SMALL SCALE FARMERS IN BURUNDI

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Lack of quality seed potato is the major constraint to potato production in Burundi. Prevalence of diseases such as Late Blight (Phytophthora infestans), viruses and Bacterial Wilt (Ralstonia solanacearum) contribute to seed degeneration leading to low yields in potato farming systems. Improving the availability of quality seed potato at smallholder farmer level was studied though testing, adapting and promoting a small seed plot technology that would reduce accumulation of degenerative diseases in seed. This technology was evaluated in three provinces (Bururi, Kayanza and Bujumbura Rural) respectively in Buyengero, Muruta and Mugongomanga communes during the long-rain season of 2007. The new technology was tested again by 32 associations of farmers during the second and first rainy seasons of 2010 and 2011, respectively. Small size of pre basic seed potato - about 30-35 mm in diameter - were planted in holes with a spacing of 20 cm X 20 cm (2007 season) and 30 cm X 30 cm (other seasons) for a plot size of 9 m long and 1.8 m wide. This improved technology was compared to normal planting as recommended by the National Potato Research Program: spacing of 80 cm X 30 cm with a fertilization of 200 kg of DAP, 100 kg of KCl and 50 kg of Urea per hectare. Incidence of Bacterial Wilt during plant growth and development, tuber yield, and perception of farmers in respect of the new technology were recorded. Results showed that there were significant differences between the small plot technique and conventional planting with regard to total tuber yield (t/ha), number of tubers per plot and per plant and average tuber weight. Productivity in terms of tubers harvested per unit area was statistically higher for new technology (76.5 tubers /m²) than conventional technique (32.2 tubers /m<sup>2</sup>). There was a significant difference between treatments (P <0.001) for average tuber weight. Assessment of the new technology made by farmers indicated a higher advantage for small plot technique compared to conventional planting as they could notice that 1 m<sup>2</sup> of small plot was sufficient to generate enough seeds for planting 30 m<sup>2</sup> in the conventional system. With knowledge and skills acquired through various trainings conducted through workshops and farmer field schools, majority of trained group members and other farmers living in their neighborhoods established their own small seed plots. A couple of NGOs and CBOs are interested in the new popular technology in the country and some of them like CAPAD (Confédération des Associations des Producteurs Agricoles), ACVE (Action Ceinture Verte pour l'Environnement) and ELVIA have already started its dissemination in Mwaro province.

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## **AGRONOMY** (Poster session)

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## COMPARATIVE ACTION CHLORINE - AND MAGNESIUM - SULPHUROUS FERTILIZERS ON PRODUCTIVITY OF THE POTATO

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For stable high yields of potatoes (for food purposes) with good quality of product in the conditions of the Central Region of Russia in addition to making a sufficient amount of nitrogen, phosphorus and potassium, it is necessary to develop a supply system with magnesium and sulfur, because there is a shortage of these elements over large areas of arable land. Relevant and practically important issue of potato growing is the development of ways to obtain the maximum yield of modern varieties of potatoes; also we need to know the reaction of new potato varieties on elements such as chlorine and sulfur.

Experimental data (2012-2013) were received on growth and development, productivity and quality of three potato varieties of domestic selection depending on entering NPK fertilizers with chlorine in two doses (N90P90K135Cl100 and N120P120K180Cl135) and equivalent doses NPK fertilizers with magnesium and sulphurin the conditions of the sod-podzolic soil of the of the Central Nonchernozem Region of Russia. In the experiment also were compared the action of fractional-local entering of the increased dose of fertilizers of both forms: 2/3 doses - the basic entering before landing + 1/3 - during the hilling rows.

The average and increased doses of mineral fertilizers of both forms (CI and Mg+S) had a positive effect on the growth and development of vegetative weight of studied potato varieties. On varieties Udacha and Golubizna the most significantly increased the mass of foliage under the influence of magnesium, sulfurcontaining fertilizers. Variety Ljubava formed the maximum leaf area and photosynthetic potential for variants with increased dose of both forms of mineral fertilizers. The v. Ljubava was tolerant to chlorine form NPK-fertilizers.

The average and increased doses of mineral fertilizers with chlorine increased productivity depending on the variety - Udacha - Ljubava - Golubizna: on 47-59 %, 46-63 % and 21-42 %, respectively. The average and increased doses of mineral fertilizers with magnesium and sulphur increased productivity of tubers more essentially: Udacha- Ljubava - Golubizna, respectively: on 65-75 %, 50-63 % and 25-60 %. The v. Ljubava was tolerant to chlorine-containing fertilizers. Productivity on variants with fractional application of fertilizers on varieties Udacha, Ljubava reached the level of this indicator on variants with increased dose NPK, and on the v. Golubizna inferior to productivity of tubers on these variants a little. Early v. Udacha and Ljubava rather well tolerated studied forms and doses of fertilizers, however, production received on variants with average doses magnesium, sulfur-containing fertilizers had the advantage. The greatest harm chlorine-containing fertilizers bring to production of middle-ripe v. Golubizna, which notes essential decrease taste under the influence of this form of fertilizers. Fractional entering of the increased dose of mineral fertilizers of both forms put more damage to production, than corresponding equivalent increased doses NPK at single entering.

The increased dose magnesium- sulphur fertilizers, creating positive balance of phosphorus, potassium, magnesium, sulphur and reducing the chlorine content in the soil, allowed to receive a high productivity of a potato (35,7-36,9 t•ha-1) with the increased quality of production.

## EFFECT OF LONG-TERM CULTIVATION OF POTATOES IN CROP ROTATION AND MONOCULTURE ON PLANT NUTRITIONAL STATUS DURING VEGETATIVE SEASON AND TUBER YIELDS

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The study was done on the basis of long-term fertilization experiments, which are conducted continuously at the Experimental Field of Agricultural Chemistry Section SGGW-WULS in Skierniewice since 1923. The study was carried out in 2007-2010 on potatoes var. Hermes cultivated in 3 cropping systems: crop rotation without legumes and without manure, monoculture and 5-course rotation of potatoes (grown in manure-amended soil – 30 t ha<sup>-1</sup>), spring barley, yellow lupines, wheat and rye. In all cropping systems, the following fertilization scheme is used since 1923: CaNPK, NPK, CaPK, CaPN, CaKN, Ca. Potato leaves samples were collected in the initial stage of flowering. These samples were analyzed for basic macro- and micronutrients content.

The highest average yields of potato tubers were obtained in 5-course rotation in the plots fertilized with manure and mineral fertilizers (CaNPK). However, the lowest yields were observed on the plots with Ca (control) and without nitrogen fertilization (CaPK) plots. The average yields of potatoes cultivated in monoculture were almost 60% lower than in crop rotation without legumes and without manure application. The highest decrease in yield of potatoes in relation to the CaNPK treatment was obtained in the plots without nitrogen fertilization among all cropping systems, but the lowest decrease was in monoculture. This situation took place because yields of potato tubers obtained in monoculture were very low and therefore bad site was the only yield-limiting factor (potatoes cultivation in monoculture for 90 years).

Analysis of chemical composition of potato leaves (K, Mg, Ca, Na and P content) indicated that plants grown in monoculture contained about 30-40% (on average) more potassium, magnesium and calcium than these grown in rotation without legumes and manure application. The reason of this situation was poor plant growth and low biomass production. It indicates that assessment of chemical composition of potato leaves should be linked to the mass of the abovegrounds parts. The lowest content of phosphorus and potassium in potato leaves was obtained in the plots not fertilized with these components and the highest in the plots without nitrogen fertilization. Among analyzed micronutrients, predominantly higher content of zinc and copper in leaves was observed in the plots without application of calcium (NPK) and potassium (CaKN), while lower – in objects with CaPK and CaNPK treatments.

### INCREASED WATER USE EFFICIENCY OF POTATOES BY ADEQUATE POTASSIUM SUPPLY

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In the last years, the department of applied research and advisory services of K+S KALI GmbH has focused on improving water use efficiency of plants by optimizing potassium and magnesium supply to crops. Therefore, K+S KALI GmbH cooperated with Sabanci University, University of Kiel and University of Halle. Adequate potassium and magnesium fertilisation improves water uptake, transport and stomata closure of plants, and additionally root growth (Cakmak and Kirkby 2008), transport of assimilate as well as the water holding capacity of the soil (Holthusen et al., 2012, Damm et al., 2011). Much of this work performed by Holthusen et al. (2012) and Damm et al. (2011) based on long term fertilisation trials in Germany in cereals or sugar beets.

In 2013 soil water content measurements were realized in cooperation with the "Deutsche Wetterdienst" on a long term fertilisation trial since 1995 in Cunnersdorf (Saxony) throughout the whole growing period of potatoes. The measurements were done weekly and the plant available soil water content was calculated in percent of the water holding capacity. Fertiliser doses were increased in steps of 120 kg K2O per ha from 0 to 360 kg K2O per ha. Soil water content was measured gravimetrically by soil sampling in the untreated control and the 240 kg K2O per ha treatment in the soil profile from 0 to 60 cm depth. The trial was set up in a randomized block design with four replicates. Every four to five years potatoes are cultivated.

The results show a clear fertilisation effect on potato yield (18%) compared to the untreated control but due to the unsuitable conditions for potatoes in 2013 no further yield increase induced by raising doses occurred. The water content measured by gravity did not differ between the untreated control and the 240 kg K2O per ha treatment. With regard to the relatively consistent development of the soil water content in both treatments it can be concluded, that the same amount of water was used by the crop in both treatments. Thus, the available soil water was used in the fertilized treatment much more efficiently than in the untreated control.

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## INNOVATIVE TECHNOLOGY FOR POTATO GROWING DIRECTED TO INCREASED N FERTILIZATION EFFICIENCY AND WATER CONSERVATION

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Between 2008 and 2013 field trials were performed focused on innovation in potato growing technology in order to reach increased fertilization efficiency and to reduce surface and ground water pollution. Risk of nitrogen losses during potato growing is relatively high [1] and it was found that gaseous nitrogen losses could be also significant in potato growing, since especially compact zones between furrows are prone to nitrogen oxide emission [2]. In the Czech Republic potatoes are mostly grown in light loamy and sand clay soils with percolative water regime. In those soils there is an increased risk of surface and ground water pollution due to higher slope angle and soil percolation. Water flow and nitrate nitrogen leaching is also influenced by rapid preferential flow through the soil [3]. Local application of mineral fertilizers at planting contributes to limitation of nitrate nitrogen formation in soil and risk of water pollution.

Variants with soil separation technology were included into the field trial: a variant with conventional planting and a variant with a soaking groove formed on top of the ridge at planting. A variant with conventional soil preparation with broadcast application of nitrogen fertilizers prior to planting was used for comparison. For stone separation technology local application of nitrification inhibitor-containing fertilizer Alzon and application of urease inhibitor-containing Urea stabil were used. Mineral fertilizers containing these inhibitors were locally applied and locally applied with a soaking groove.

The results show that the highest soil content of nitrate nitrogen after pre-planting fertilizer application was determined in the stage of fourth leaf prior to initiation of intensive N uptake by potato plants. Considering various cultural practices local fertilizer application contributed to the highest reduction of soil nitrate content, which reduced soil nitrate nitrogen compared to broadcast application on average by 40 kg N/ha. After application of evaluated Alzon and Urea stabil expected reduction of soil nitrates did not occur. On contrary, for Urea stabil soil nitrate content was increased. The highest effect of the soaking groove was apparent in drier year 2008, when N use by potato plants was increased from 33 to 44 % for Urea stabil and from 40 to 48 % for Alzon. Nitrogen use from applied fertilizers by potato plants reached 59 % on average of the years. Nitrogen uptake from mineral fertilizers contributed to general potato nutrition on average only in range of 27-38 %. Nevertheless, this percentage also resulted in a significant increase of potato yields compared to non-fertilized control.

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## PLURENNIAL SYNTHESIS OF HAULM KILLING METHODS COMBINING MECANICAL AND CHEMICAL

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## Introduction

For potato cultivation, the obligation is often made to carry out the destruction of the vegetation before natural maturity to set the quality of tubers growing in the ridges to correspond as good as possible to the specifications of buyers (size, dry matter content, ...). The necessity is now to respond at this agronomical demand by reducing the quantities of applied pesticide as objective of "Ecophyto 2018 Plan" launched in France.

#### **Materials and Methods**

The study took place during four years (2008-2012) at the ARVALIS-Institut du vegetal Experimental Center of Villers Saint Christophe, in North France. At the same date in mid-Summer two kinds of canopy, corresponding to more or less vigorous potato cultivars (Bintje, Nicola, Kardal, Markies), have been destroyed using combined mechanical and chemical techniques. The main products registered for foliage destruction were used at normal or half dose: diquat [Reglone 2], glufosinate ammonium [Basta F1], carfentrazone ethyl [Spotlight Plus]. They were applied after haulm topping with full soil covering as classical spraying at 200 l/ha (experimental sprayer Pulvelec) or with centrifugal spraying at low volume 35 l/ha (Loof-does equipment) but also with localized spraying on the row at 75 to 150 l/ha (Chafer equipment). Each year a field trial is conducted in a as randomized of possible disposal regarding the use of agricultural machines.

## Results

The results show that flailing prior to chemical application can instantly remove more than three quarters of vegetation but chemicals are necessary for limiting or avoiding regrowth of foliage, especially for vigorous crops.

For localized treatment, the final efficacy of haulm destruction is poorly influenced by the volume applied (75 l/ha vs. 150 l/ha). The beneficial effect of localization is more important for diquat within the 8 to 14 days after application but is not sufficient to escape regrowth of foliage in difficult situation. On this point localization is more efficient with glufosinate ammonium.

The two years in which the centrifugal spray 35 l/ha was introduced in the study, it was observed better results in all situations for this application technique, including comparison with the localized treatment on the row, except for diguat and carfentrazone ethyl applied at half dose.

In most cases, the efficiency of the half dose is reduced in the first few days following application with much larger gaps for the destruction of the stems. Regarding the destruction of the leaves, just a few differences exist after 8 days but no more after 14 days.

A stronger effect of the applied product and spraying technology applied is observed for stems destruction, especially for vigorous crop. Half dose seems sufficient in easy situation but is too insecure in the difficult cases, particularly with diquat.

## **Conclusions and perspectives**

Most of time, the combination of mechanical and chemical techniques is an interesting technique to achieve in a single pass to a rapid destruction of the foliage with a possible reduction in dose of pesticides applied. The development of specific equipments allows today to optimize this kind of intervention: haulm toppers with large width, integrated sprayer for full or localized spray covering, low volume sprayers, integrated ridge rolling. The main limits are the cost of these specific machines, a work rate reduced compared to a classical sprayer, difficulties to drive in the fields after heavy rainfalls.

## POTENTIAL PRODUCTION OF THE POTATO CROP IN MEXICO

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## Introduction

The potato in Mexico is planted throughout the year in many different environments, it can be found from sea level to altitudes above 3,000 meters. The total area cultivated with this tuber is about 68,928 ha, of which over 60% is under irrigation and the rest under rainfed conditions with a national yield average of 26.8 tons per ha. From total potato production, 56% is consumed fresh, while 29% is used by industry and 15% as seed. In order to identify the better production zones and the technological gaps in the different areas where this crop is produced, the potential yield of potato was calculated at the national level and yield distribution maps were generated representing different production conditions.

#### **Materials and Methods**

Potential production was calculated utilizing a growth simulation model developed by (1). Several cultivars and planting dates were considered to represent production conditions for the whole country. Daily plant biomass and biomass partitioning to the different potato organs were estimated as a function of Solar Radiation and Temperature. Data series from 2710 weather stations were used as model inputs for punctual estimations. Data were interpolated using the inverse distance weighted procedure. The curve of cumulative probability was calculated for every station and the values of the yields corresponding to the 80% of cumulated probability were used to elaborate the yield distribution maps.

### Results

In Figure 1 the map of potato potential production distribution in all the agricultural zones of Mexico is showed. The estimation corresponds to the cultivar Alpha and a planting date of January 20. Potential production varied from 15 tons per hectare in the coastal zones of the Gulf of Mexico and the Pacific Ocean, to more than 100 tons per hectare in the central highlands of the country and the northwest states. Comparing with actual yields at the municipal level, yield gaps were estimated in a rank of 0.67 to 0.1.

## Conclusion and perspectives

Results indicate that there are ample opportunities to improve crop productivity and the present study is being considered as reference in a participative program with potato growers of the major potato producing states in the country.

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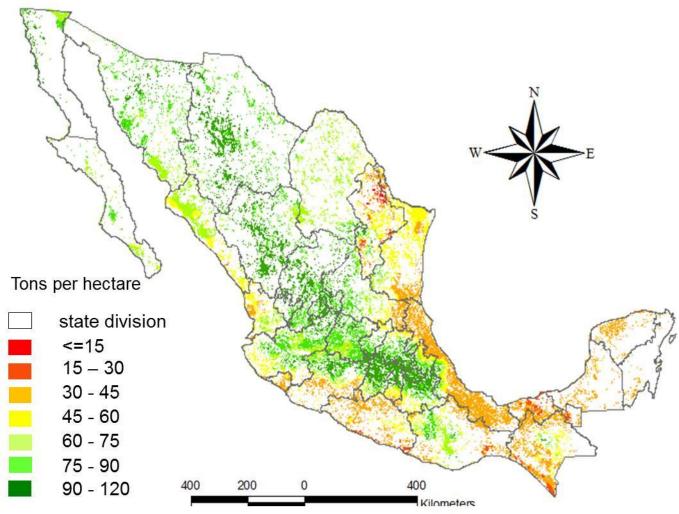


Figure 1. Potential production distribution of potato, cultivar Alpha planted on January 20 in Mexico.

### EFFECT OF IRRIGATION AND POTASH LEVELS ON KEEPING QUALITY OF POTATO

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The investigation comprising four levels of irrigation (25, 30, 35 and 40 mm CPE) and four levels of potash (0, 100, 125 and 150 kg/ha) was carried out at Research Farm of the Department of Vegetable Science, CCS Haryana Agricultural University, Hisar during winter season of 2010-11 and 2011-12 to find out the optimum level of irrigation and potash for obtaining potato tubers with better keeping quality at ambient room temperature. The potato variety used for the investigation was Kufri Bahar. The treatments were laid out in a split plot design with three replications. The irrigation and potash levels both showed significant improvement in keeping quality parameters of potato. Likewise, the values for physiological loss in weight and decay loss of potato tubers (%) at 15, 30, 45 and 60 days after harvest were lowest with irrigation level 40 mm CPE and application of potash @ 150 kg/ha. The two years results suggest that the irrigation level 40 mm CPE along with potash @ 150 kg/ha have shown the best treatment combinations for potato storage at ambient room temperature under semiarid conditions of Hisar (Haryana).

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#### INTEGRATED NUTRIENT MANAGEMENT IN POTATO PRODUCTION

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Due to enhanced population density, pressure on agricultural lands has been increased considerably in Turkey as well as in the world. This intensive land utilization caused deforestation, overgrazing of exposed land and faulty cultivation of steep slopes. A crop production system with high yield targets cannot be sustainable unless balanced nutrient inputs are supplied to soil against nutrient removable crops. Sequential cropping ensures maximization of efficient use of moisture and nutrients from soil. Integrated nutrient management for prevailing cropping systems appears to be one of the effective ways to meet the economical nutrition requirement of crop. Potato is one of the main commercial crops of Turkey and is cultivated on an area of about 172 000 ha, with the 4 800 thousand tons total production and 2,8 ton/ha yield. Current fertilization rates are insufficient to sustain high yields and to replenish nutrient removal by the potato crop. Potato yield could be increased by more than 50% only by improved nutrient management. Great opportunities exist to increase potato yield and quality by improving nutrient management. Potato demands high level of soil nutrients due to relatively poor developed and shallow root system in relation to yield. Compared with cereal crops, potato produces much more dry matter in a shorter cycle. This high rate of dry matter production results in large amounts of nutrients removed per unit time, which generally most of the soils are not able to supply. Hence, nutrient application from external sources as fertilizers becomes essential. The purpose of this review research was to find out the appropriate combination of organic and inorganic sources of nutrients and bio-inoculants for improving yield of potato under different ecological conditions of Turkey.

## RESEARCH REGARDING THE INFLUENCE OF SALINITY ON GROWTH AND DEVELOPMENT OF POTATO PLANTLETS

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### Introduction

Accumulation of salts, in particular those of sodium, is one of the main physiological threats for ecosystems. Salt disturbs plant growth by limiting nutrient assimilation and diminishing quality of water available for plants. The excess of sodium causes destruction of soil structure, that lack of oxygen, cannot support plant growth. For selecting plants with resistance to salinity, can be used *in vitro* culture techniques (thereby, the genotypes can be evaluated in a limited space).

Khenifi and colab. (2011) [2] emitted the idea that the length and weight of microplants root have been influenced by different NaCl (0-120 mM) concentrations used for certain varieties of potato. Farhatullah and colab. (2002) [1] studying the cultivar Cardinal showed that different concentrations of NaCl influenced microplants root length growth *in vitro* and did not respond significantly to the number of roots.

#### Material and methods

The research refers to the salinity influence of different NaCl concentrations (0, 25, 50, 75 and 100 mM) over plantlets height, number and length of internodes and number of leaves. The studied genotypes were: Christian, Roclas, Marfona, Riviera, Tresor.

## **Results**

Concentrations of 100 and 75 mM NaCl had a negative effect less stressed for Riviera and Roclas varieties regarding the average length of internodes. Concentrations experimented did not influence the formation number of leaf for Riviera variety. The lower influence of salinity over plantlets height was observed for varieties Riviera and Roclas that at concentration of 100 mM, reach 7.80 cm and 6.15 cm. Internodes number decrease with increasing NaCl concentration in the culture medium. Riviera variety presented the best resistance to concentrations of 25 and 100 mM (forming between 10.44 and 7.33 internodes / plantlets), was exceeded by the Christian variety at concentrations of 50 and 75 mM NaCl. The average length of internodes was less influenced by NaCl for Riviera and Roclas varieties showing even at concentrations of 100 and 75 mM NaCl quite close values (0.80, 0.82 - 0.65, respectively Roclas variety; 0.82 cm Riviera).

The leaves formation highlights Riviera variety, showing at all four concentrations of NaCl highest number of leaves (even at the maximum concentration of 100 mM presents 10.67 leaves).

Varieties tested behaved like this: Roclas, Riviera and Tresor not differ significantly in the length of plantlets; Riviera and Christian differ significantly in internodes formation from the other varieties; Roclas is distinguished by the higher internodes length (not significantly different from the variety Riviera); Riviera differ significantly from the other varieties by the number of leaves formed.

### **Conclusions**

Graduation of NaCl concentration affects differently the growth and development of plantlets. Concentration of 100 mM NaCl did not affects microplant height for Riviera and Roclas varieties, these recorded the highest values of microplants height. All these considerations lead to the identification of new genotypes with resistance to environmental stress conditions (thermal, hydric and salt conditions).

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## POTENTIAL YIELD OF POTATOES (SOLANUM TUBEROSUM, MILL) GROWN UNDER RAIN FED CONDITION IN TANZANIA

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Potato (Solanum tuberosum, Mill) is one the major food and cash crop in Tanzania grown by small scale farmers without irrigation. In order to estimate yield potential the simulation model described MacKerron and Waister (1985), using various regression relations between accumulated intercepted radiation and tuber dry matter was used. Potential yield in this scenario is defined as the tuber dry matter yield that could be achieved given restricted planting and harvesting dates, defined by genotype and climatic variables. The physiological basis for differences in tuber yield of was studied in terms of intercepted photosynthetic active radiation (IR; MJ m<sup>-2</sup>), tuber dry matter yield (DM) and radiation use efficiency (RUE; g MJ<sup>-1</sup>) in the highlands of Northern Tanzania during the long and short growing season of 2010 and 2011. Treatments were control, fungicide, fertilizer and fertilizer x fungicide, in two local varieties namely "Kenya wani" and Victoria "CIP 381381.20". The crop was not irrigated and rainfall and evapotranspiration determined the length of the growing season. The results showed that variety, fertilizer and fungicide application had large effects on yields without interactions between them. The length of the growing season was 147 days for long rainy and 112 days for short rainy season. Between these limits the amount of radiation intercepted was determined by incident total radiation intercepted by a model crop ranged from 956 to 1192 MJ m<sup>-2</sup>, the maximum RUE was 1.37 g MJ<sup>-1</sup>. The relations between intercepted radiation and tuber dry matter yields were variable, affected by site and season, but not by treatments, and were quadratic during the long rainy season and linear during the short rainy season. Potential yield was 64 tones ha-1, during the long rainy season and 39 tones ha-1 during the short rainy season, and exceeded actual farmer yield by 81 and 71%.

## **PHYSIOLOGY** (Posters session)

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## CHARACTERIZATION OF STARCH POTATOES GROWN UNDER DROUGHT

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Potato (*Solanum tuberosum* L.) is much more sensitive to drought stress than most other crop species. Scarcity of water is a severe problem in many potato growing regions already today and may become even more important in the future due to climate change.

In this respect, increasing drought stress tolerance is an effective approach to secure economic potato production in the future and to reduce water usage for irrigation. Accordingly, drought stress tolerance is an important goal in potato breeding programs, today. But, the complexity of this trait together with the difficulties to induce drought stress under field conditions at defined time points, hampers breeding success for drought stress tolerance. Therefore, this study aims at the identification of physiological traits suited to conduct selection for drought stress tolerance in potato. To achieve this, 34 starch potato cultivars were analyzed in two years pot trials in a rain out-shelter with two levels of irrigation and eight replications per variant. Under control conditions 60 % of water holding capacity was supplied during the whole trial. Drought stress was induced from the date of the three-leaf stage up to ripeness. With the onset of a loss of turgor an amount of water corresponding to three times the daily evapotranspiration was given. To evaluate the effects of this drought stress, samples were collected in two developmental stages (BBCH 17-19 and 60-65). Physiological traits analysed comprised the osmotic adjustment, the amount of free proline, total soluble sugars, crude protein and the chlorophyll content (SPAD). Furthermore, the drought susceptibility index (DSI) [1] was calculated and changes in physiological traits were correlated (Pearson) to the starch yield obtained in these trials.

Drought stress caused a significant relative reduction (53.96 %; P < 0.001) of starch yield. The DSI based on the starch yield ranged from 0.81 to 1.23. The examined physiological traits were also significantly affected by drought stress. Depending on the developmental stages an increase (P < 0.001) of all physiological parameters due to drought stress was observed as well as genotypic differences. Correlating these changes to the yield performance resulted in significant correlations which were strongest in the late sampling for starch yield with soluble sugars (-0.68\*\*\*), osmolality (-0.59\*\*) and crude protein (0.36\*). By applying principle component analysis (PCA) contrasting cultivars with respect to drought stress tolerance were identified based on physiological traits and the drought susceptibility index.

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## CHANGES IN PROTEOME PROFILING OF POTATO TUBERS AND LEAVES UPON SOIL DROUGHT

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Drought is one of the major abiotic stresses affecting plant growth, development and productivity. Potato (Solanum tuberosum L.) is moderately drought sensitive crop (Schafleitner et al., 2007) whose yield is drastically restricted by dehydration. Recent evidence indicates that the reprogramming of gene expression results in the reorganization of plant metabolism under unfavourable environmental conditions. Since variations in drought tolerance have been observed among different potato cultivars in the present experiments the up- and down-regulated proteins in drought-sensitive Cekin cultivar were assayed in order to establish the molecular markers of drought. Therefore, three weeks after tuberisation, potato plants were subjected to soil water shortage for 14 days. Proteins were extracted from middle slices of tubers and from mature non-senescent leaves and separated by two-dimensional polyacrylamide gel electrophoresis. The images were de-noised in the wavelet domain, background was removed using the ALS approach, warping was performed based on the fuzzy warping algorithm and images were standardized using the robust orthogonal least squares method (Zerzucha et al., 2012). The resulting proteins were analyzed by mass spectrometry leading to protein identification and characterization. In potato tubers, three proteins indicate an identity or an extensive homology with the highest probability and belong to the small heat shock proteins (sHSPs). This superfamily is a ubiquitous and evolutionarily conserved group of molecular chaperons that protect proteins from being denatured, misfolded or aggregating under various unfavourable environmental conditions. The expression and accumulation of sHSPs (12-40 kDa) in plants is triggered by extreme temperatures, salinity, drought, osmotic and oxidative stresses. Moreover, in potato tubers new proteins were synthesized de novo upon soil drought, whereas in leaves expression of new proteins has not been observed. Instead of, the up-regulation of proteins was noted. Among them, one of the proteins indicating an identity or an extensive homology with the highest probability is chloroplastic Fe-superoxide dismutase and other chloroplastic enzyme i.e. carbonic anhydrase. The obtained results supported our earlier findings that photosynthetic activity was reduced in susceptible genotypes (Cekin) while tolerant genotypes could continue photosynthesis under soil drought (Boguszewska et al. 2011).

## EFFECT OF DROUGHT STRESS ON PHOTOSYNTHESIS, LEAF WATER POTENTIAL, PLANT GROWTH AND TUBER YIELD IN FOUR POTATO VARIETIES WITH DIFFERENT ROOT MASS

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#### Introduction

In the previous reports, we clarified the effects of improved root system on soil water absorption in deep soil layer (Deguchi et al., 2014; The 19th Triennial Conference EAPR 2014) and hydraulic conductance (HC) from root to leaf (Deguchi et al., 2013; The 17th Joint Meeting of EAPR Section Breeding and EUCARPIA Section Potatoes). In the present report, we will explain the varietal differences in photosynthetic properties, dry matter production and tuber yield performance in different soil water condition among 4 years were analyzed.

#### **Materials and Methods**

Konyu-1, Konyu-2 and Konyu-4, and a check variety Konafubuki (a parent of Konyu varieties) were cultivated in experimental fields of Hokkaido University for 4 years (2008-2011). Planting was conducted in May 6-18. In the middle of June, droughted field under poly-shelters and irrigated field under rain-fed were established. Dry weight (DW) of leaf, stem and tuber, and leaf area index (LAI) were measured at 3 stages: late in June (S1: initial flowering stage, shortly after the set up of poly-shelters), early in August (S2), and late in August (S3). After leaf-yellowing of each variety, tuber dry yield was measured. In addition, on 3-4 dates per year between early June to late August, net photosynthetic rate (Pn) stomatal conductance (gs), and leaf water potential (ψleaf) of the fully expanded top leaf were measured with portable gas-exchange systems (LI-6400, LI-COR) and pressure chamber (PMS 600, Meiwafosis Co. Ltd.).

## **Results and Discussion**

The reductions of Pn, gs and ψleaf in the droughted field relative to irrigated field became clear since middle July before S2 over 4 years. The significant interaction between water treatment and variety was found, and the reductions of Pn and gs were relatively less in Konyu varieties than in Konafubuki. Konyu varieties also showed higher ψleaf than Konafubuki regardless of soil water treatment. For plant growth, although reduction of total DW in droughted field was clear over 4 years, reduction of LAI was significant only in 2008. In addition, although there was no significant interaction between treatment and variety in total DW and LAI, it might be due to moderate evaporative demand in our experimental fields, where the range of monthly mean vapor pressure deficit was much lower (from 0.41 to 0.89 kPa) comparing with other drought prone regions in the world. From S3 to harvesting date, tuber growth rate (TGR) became lower in the droughted field than the irrigated field in 2008 with high radiation and 2010 with high temperature. In these years, the reduction rate of TGR in the droughted field relative to the irrigated field was much higher in Konafubuki (78~85%) than in Konyu varieties (<39%), resulting in less yield reduction in Konyu varieties in the droughted field.

## Conclusion and perspectives

Consistently over 4 years, greater soil water absorption from deeper soil layer and higher HC of Konyu varieties due to their superior root system contributed to smaller reductions in photosynthetic characteristics of leaf from early growth stages. In two years among 4 years, their contribution to dry matter production became clear at the late stage of tuber bulking, and resulted in less reduction of tuber yield due to drought. The presented results partly demonstrated that an improvement of root system would contribute for increasing tuber yield in years and/or regions with high demand of water for plant growth.

### EFFECTS OF COATING CHEMICALS ON SPROUTING OF SEED POTATOES

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## Introduction

In areas with short growing seasons and several months' duration of storage the challenge lies in enabling seed potato (*Solanum tuberosum* L.) sprouting to occur at the preferred time. This challenge is complicated by the effect of the production history and storage conditions [1]. Unwanted seed tuber sprouting during the storage period decreases tuber vigour leading to economic losses [2]. Sprouting mobilizes the starch in the tuber and consumes part of the tuber's biochemical reserves. This results in loss of tuber weight/biomass and withering. The loss in tuber quality is not desirable with respect to the characteristics of seed potatoes, in particular as seed potato quality is measured by its ability to produce sprouts, shoots and daughter tubers [1]. Sprouting should be controlled to occur at the preferred time and in the preferred manner [3]. Numerous methods have been developed for chemical control of sprouting in the storages but less for coating tubers individually. The aim of our study was to minimize loss of minituber weight/biomass and control sprouting by coating tubers with chemicals. Sprouting should be controlled to occur at the preferred time and in the preferred manner.

#### **Materials and Methods**

The effect of the coating chemicals on sprout development was examined by immersing seed potatoes in aqueous solutions containing polymeric and monomeric organic compounds. After these treatments, the seed potatoes were allowed to sprout and the number of sprouts was observed during the storage period. After cultivating seed potatoes in a field, at harvest, the number of stems and tubers were determined.

#### Results

During the storage period, sprouts in treated and untreated potatoes were developed in similar manner. The treatment did not affect on the number of stems, however the treatment significantly increased the number of tubers.

## **Conclusion and perspectives**

Coating seed potatoes by immersing in aqueous solutions containing polymeric and monomeric organic compounds can increase the number of tubers thus positively influencing on production efficiency. In this study, only tubers of cultivar Opera were tested. Further studies are needed to find out whether the impact is similar to potato cultivars generally or just specific to certain cultivar.

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### PROTEIN OXIDATION IN POTATO CULTIVARS DIFFERING IN DEHYDRATION TOLERANCE

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Scavenging of reactive oxygen species (ROS) is a complex event and occurs under strict control of the antioxidant enzymatic and non-enzymatic systems. Ineffective scavenging leads to ROS accumulation resulting in oxidation of protein, lipid and DNA.

Reactive oxygen species (ROS) may cause irreversible carbonylation of proteins, resulting in structural and/or functional modifications. The carbonyl groups of proteins, resulting from the oxidation of proteins, are relatively stable chemically, so that they can be qualitative and quantitative determined, which allows assessing the degree of protein damage. Therefore, carbonylated proteins were determined in leaves, tubers and roots of six potato cultivars differing in decrease of tuber yield due to soil drought. The content of carbonyl groups in proteins was assayed spectrophotometrically with 2,4-dinitrophenylhydrazine (DNPH) by the method of Levine et al. (1994).

The content of carbonylated proteins increased in tubers of all cultivars except the Oberon cv. with the highest yield decrease. The largest increase was observed in tubers of Igor cultivar with the lowest yield decrease.

In leaves of all tested cultivars the content of carbonyl groups decreased upon water deficiency. The largest decrease has been noted in leaves of Ignacy cultivar characterized by the high yield decrease.

The intensity of protein oxidation in roots seems to be independent of the tolerance to drought measured by the yield of tubers.

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## RESPONSE OF DIFFERENT POTATO GENOTYPES TO IN VITRO OSMOTIC STRESS AND FIRST PROTEOMIC COMPARISONS

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### Introduction

For the coming years longer drought periods are predicted for Europe. For the potato as a crop with relatively low drought tolerance, due to its shallow root system [1], this would mean a higher irrigation expense. Because water in many areas is precious and artificial irrigation is expensive, it will become even more important for breeders and farmers to have a range of drought tolerant potato cultivars. At the moment the identification of drought tolerant potato cultivars is expensive and laborious due to field trials and field analysis. Aims of a joint research project (PROKAR) are to screen a set of starch potato cultivars for their reaction upon water deficit and analyze the proteome of selected genotypes in order to identify novel drought responsive proteins, through which biomarkers can be generated. Objectives of this study were to establish an in vitro screening system and to compare differently reacting genotypes in their proteomic response to osmotic stress in vitro.

#### **Materials and Methods**

Potato plants (*Solanum tuberosum* L.) of 9 different cultivars ('Agria', 'Afra', 'Desiree', 'Filea', 'Lambada', 'Milva (DK)', 'Topas', 'Rosella' and 'Saturna') from different breeders were propagated in vitro. Through different sorbitol concentrations (0.1 M, 0.2 M and 0.3 M) within MS medium solidified with Plant Agar, osmotic stress was applied and the reaction of the shoots was documented. Six replicates containing five shoots each were evaluated after 21 days. Proteins were extracted from samples taken after 11 d for two cultivars, separated through 2D-gelelectrophoresis (2D SDS-PAGE) in three biological replicates and will be analyzed with the Delta 2D Software.

## **Results and Conclusion**

In agreement with data obtained by Iwama and Gopal [2], the concentration of 0.2 M sorbitol seemed to be the most suitable one for distinguishing the genotypes in terms of statistic analysis. Genotypes were compared regarding growth parameters on sorbitol containing media and ranked. Genotypes which were considered to be more tolerant to osmotic stress gained positions in this ranking on sorbitol containing media compared to the control. Thereby, genotypes 'Desiree' and 'Filea' were found to be more tolerant, while 'Afra' and 'Topas' were sensitive to osmotic stress. Proteomic analyses are in progress.

## **Perspective**

Currently, 19 starch and table potato cultivars are screened for their reaction to osmotic stress and nitrogen deficiency stress. Samples of leaves and roots will be taken for proteome analysis and differentially abundant spots will be identified by MS/MS-analysis. With this data a GelMap (www.gelmap.de) reference map for a sensitive and a tolerate genotype will be established and will be open for public access at a later time point.

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# DOES PRE-CRYOPRESERVATION LIGHT SPECTRAL QUALITY AFFECT CRYOPRESERVATION SUCCESS OF POTATO (SOLANUM TUBEROSUM L.) SHOOT TIPS IN VITRO?

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Cryopreservation is the most powerful tool for long-term storage of plant genetic resources with minimal space and maintenance requirements. Cryopreservation, the storage of viable material on ultra-low temperature (under -150 oC) is especially suitable for economically important food crops like potato, which varieties can be conserved only vegetatively. Several genebanks have accepted cryopreservation as a reliable storage method and routine cryopreservation has been started [1]. However, the cryopreservation protocol has to be optimized for each species and even variety. Recently, the importance of non-cryogenic factors affecting cryopreservation results has been emphasized. Light is among one of the most important factors affecting plant development and physiology [2, 3]. Therefore, we studied the effect of different light spectral qualities on the growth and morphology of in vitro cultivated potato and whether the photomorphological changes affect cryopreservation success of potato shoot tips. For the experiments in vitro plants of potato varieties 'Varajane kollane', 'Anti' and 'Bintje' were grown in the light provided by cool white fluorescent tubes, warm white HQI lamps, blue LEDs, red LEDs, a mixture of red and blue LEDs (90 % red and 10 % of blue) and white LEDs. After three weeks, the plants were evaluated and cryopreserved by DMSO droplet method. For regeneration, the shoot tips were transferred to conventional growth chamber conditions under cool white fluorescent lamps. The results were evaluated after six weeks of thawing. The preliminary results of the effect of different light spectral quality on growth and cryopreservability will be presented.

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THE EFFECTS OF PRE-PLANTING TEMPERATURE TREATMENT OF POTATO GENOTYPES WITH DIFFERENT FREEZING TOLERANCE ON PLANT MORPHOLOGICAL PARAMETERS, PHOTOSYNTHETIC APPARATUS AND COLD RESISTANCE

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#### Introduction

Plants are routinely subjected to a wide range of abiotic stresses such as cold/heat, drought and salinity. In the North low temperature is one of the most important factors limiting plant growth and crop productivity, in particular, plants exhibit a sensitivity to diurnal temperature fluctuations. At the same time temperature, as a factor, can be used to improve plant resistance to other environmental stresses. For example, temperature drop (DROP) is one of widely used modern horticultural techniques, in which temperature is reduced to suboptimum values that affect plant morphogenesis and biological productivity [1, 2]. The DROP-effects on plant physiological parameters have received much less attention in research. Moreover, the relationship between DROP-effects on the plant and the level of freezing tolerance has not been demonstrated yet. The aim of this study was to analyze the influence of pre-planting temperature treatment on potato genotypes with different freezing tolerance.

#### **Materials and Methods**

Experiments were conducted in growth chambers with wild potato species *Solanum commersonii* (highly freezing-tolerant) and two potato genotypes classified as freezing-tolerant (1020) and freezing-sensitive (2022) genotypes. The plants were proliferated from stem cuttings, and grown for about 7 weeks in growth chambers with a photoperiod of 16 h at a PPFD of 122 µmol•s<sup>-1</sup>•m<sup>-2</sup> and a temperature of 23°C. Part of the plants were then subjected to a temperature drop from 23 to 5°C for 2 h at the end of the night (DROP-treatment). After that all plants were grown under optimal growth conditions (23°C). The morphological and physiological parameters, cold acclimation capacity (CAC) and expression of the cold-related gene (Ci7) of the potato plants were investigated.

### **Results**

DROP-treatment did not affect plant growth and development in freezing-tolerant genotypes, and stimulated these parameters in the freezing-sensitive genotype. Total photosynthetic pigments were accumulated and high Fv/Fm values were observed in *S. commersonii* and 1020-genotype after DROP-treatment. Nothing of that was observed in 2022-genotype. DROP-treated plants of all potato genotypes possessed an enhanced cold resistance as compared with the control. Cold acclimation capacity was related with potato freezing tolerance: CAC gradually increased as freezing tolerance decreased: in *S. commersonii* and the freezing-tolerant genotype CAC-values were 2.1°C and 2.5°C, respectively, in the freezing-sensitive genotype this value was 3.0°C. However, no low temperature-induced gene expression was detected in plants after DROP-treatment.

### **Conclusions**

S. commersonii and the genotypes responded to temperature treatment by different changes in developmental, physiological and molecular parameters. Pre-planting temperature treatment was less effective in freezing tolerant potato genotypes than in the freezing-sensitive genotype. Cold resistance in the freezing-tolerant genotype can probably be improved by other stress factors.

The study was supported by a joint project of the Academy of Finland and the Russian Academy of Sciences (project No 5).

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## PHYSIOLOGICAL AGE AND THE MECHANISMS OF CROP GROWTH AND DEVELOPMENT OF TWO POTATO CULTIVARS

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In New Zealand, seed potatoes are commonly stored inside sheds at ambient temperature, in low temperature coolers or even left un-lifted in the ground between crop defoliation and harvest.

This research describes the response of yield of 'Bondi' and 'Fraser' crops planted with seed potatoes at different physiological ages (PA) generated during the storage phase. The mechanisms of the subsequent crop growth and development are compared amongst treatments.

'Bondi' and 'Fraser' potato cultivars were grown in the field between October 2011 and May 2012 at Canterbury, New Zealand. The seed potatoes planted, had previously been stored and then treated as follows: 'Early' storage phase (comprised between ~5-11 weeks after crop defoliation) of tubers in the 'ground' (un-lifted), 'shed' (ambient temperature) or 'cooler' (storage at 4C°). 'Late' storage phase treatments exposed the tubers to either one or three months further warm-up inside a shed prior to planting. Before the planting date half of the potatoes of each treatment had any sprouts which had developed removed. This added the two further seed potato treatments of 'Sprouts on' and 'Sprouts off'. The experiment was replicated four times using a completely randomized design.

Total yield and number of potatoes differed (P<0.001) between cultivars but were unaffected by any of the treatments. 'Bondi' fresh weight was ~70 t/ha with 30 tubers/m² while 'Fraser' yielded ~53 t/ha with 45 tubers/m². Yield distribution differences (P<0.001) were produced exclusively by 'Sprouts on' versus 'Sprouts off' treatments. The seed potatoes planted with 'Sprouts on' shifted the yield distribution towards larger grades.

'Fraser' 'Sprouts on' crops maintained maximum canopy ground cover for longer (824°Cd, Tb=2°C) and intercepted more total radiation (2202 MJ/m²) than the 'Sprouts off' (718°Cd, 2034 MJ/m²).

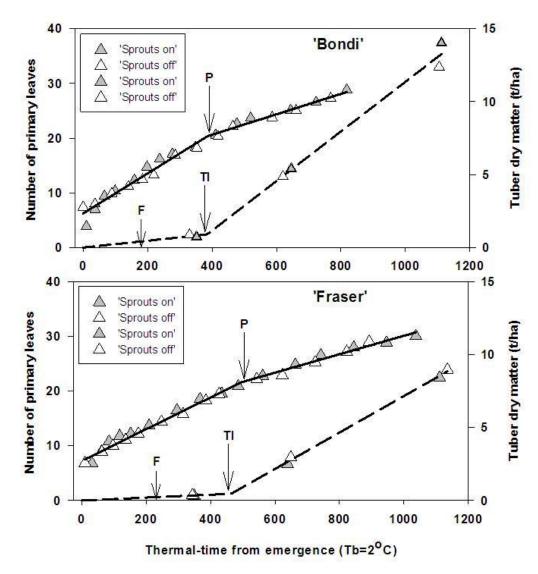
'Bondi' averaged 1.2 g of total DM/MJ total radiation intercepted which was higher (P<0.001) and 'Fraser' 0.9 g of total DM/MJ. The efficiency of conversion of radiation to tubers (g tuber DM/MJ total radiation interception) was 1.1 for 'Bondi' and 0.8 for 'Fraser'.

The 'Sprouts off' treatment produced a lower (P<0.008) maximum LAI of 4.4 compared with 6.8 in the 'Fraser' crop. Maximum LAI (5.5) was unchanged in 'Bondi'.

Phyllochron increased (P<0.001) from 27°Cd to 53°Cd for 'Bondi' at 386°Cd after emergence (Figure 1). 'Fraser' accumulated 495°Cd before the phyllochron increased from 34°Cd to 59°Cd. All crops produced 28 leaves on the main stem (P<0.58); including higher levels of apical lateral branches (sympodium n-2). Time of DM partitioning to tubers was constant within cultivar. 'Bondi' commenced partitioning DM into the tubers (around tuber initiation; TI) earlier (P<0.017) than 'Fraser' at 384°Cd versus 464°Cd, respectively. This time was not different (P<0.87) to the thermal-time values when phyllochron changed for each crop (Figure 1).

PA is carried, at least in part, by the sprout developed in the seed potato. The PA differences generated by the storage treatments were irrelevant from a commercial harvest perspective in the subsequent crop. The link between the time of TI and phyllochron change could contribute to more precise predictions of DM partitioning to tubers in potato crop simulation models.

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**Figure 1** Number of leaves on the main stem and tuber DM against Thermal-time in °Cd from crop emergence. Arrows indicate the time of phyllochron change (P), tuber initiation (TI) and time to first flower (F).

### STUDIES ON OBTAINING ANTHOCYANIN PIGMENTS FROM PURPLE POTATO

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#### Introduction

Potato (*Solanum tuberosum L.*) is one of the principal food crops in the world and the tubers are a good source of carbohydrates (starch), proteins and vitamin C. As a product of plant origin they also contain secondary metabolites (phytochemicals) [1, 2 and 3].

Polyphenolic compounds are a large group of photochemical and depending on their chemical structure they can be divided into the following classes: flavonoids, phenolic acids, tannins, stilbenes and lignans [4]. Anthocyanins (classified as flavonoids) are responsible for the colour found in the pigmented potatoes. The aim of this study was to investigate the effect of temperature  $(30 - 60^{\circ}\text{C})$ , solvent ratio (1:2 and 1:3) and solvent type (ethanol and 1% acidified ethanol) on anthocyanins extraction.

#### Materials and methods

The anthocyanins pigments were extracted from potato (Albastru-Violet de Galanesti variety). For anthocyanin pigments extraction was used the simple extraction in solvent in different conditions in order to find the best condition for obtaining extracts rich in this compounds. Was investigating the effect of temperature, solvent type and solvent ratio.

The total anthocyanins content (TAC) were determined by the pH differential method. This method is based on the property of anthocyanin pigments to change the colour with pH (TAC was expressed as cyanidin 3-glucoside).

#### **Results and conclusions**

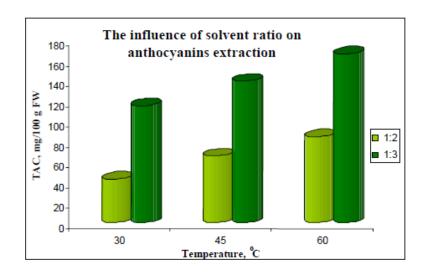
The highest anthocyanin content was found at extraction temperature of 60oC with ethanol (1:3) as solvent (178.25±4.23 mg/100g FW). The lowest anthocyanin content was found at extraction temperature of 30°C with ethanol as solvent (44.41±2.95 mg/100g FW).

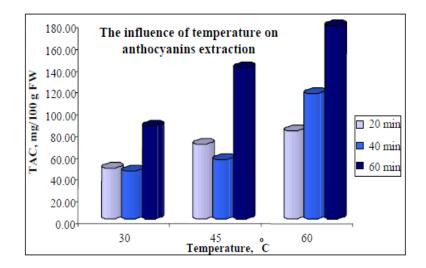
The results are similar with literature reports about purple potato anthocyanin content determined through pH differential method.

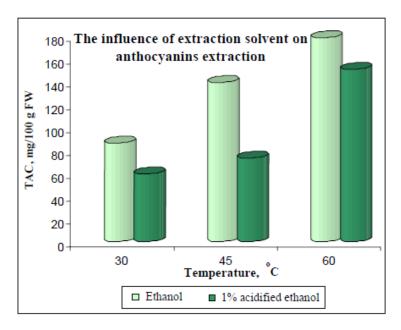
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## COMPARATIVE PROTEOMICS IDENTIFIES CHLORORESPIRATION IN THE METABOLIC SHIFT IN SOLANUM TUBEROSUM DURING SUCCESSFUL PHYTOPHTHORA INFESTANS INFECTION

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#### Introduction

Currently, the fight against *Phytophthora infestans*, the causal agent of potato late blight, is based on the recurrent use of pesticides. However, this has raised environmental issues and resistant strains have emerged, renewing the interest for elucidating the mechanisms of natural resistance and possibly exploiting them to open new ways to manage potato late blight by generating cultivars with durable resistance.

Several experimental strategies have been used to characterize the potato response to *Phytophthora infestans*. Available experimental evidence indicates that the metabolic changes consecutive to infection extend far beyond those supporting the immune responses only.

We present the results of a 2D DIGE study of the potato leaf proteome changes consecutive to *Phytophthora infestans* infection.

#### Results

2D DIGE analysis detected early changes in photosynthetic protein abundance in infected Desire leaves that could adversely affect the chloroplast functional integrity. Surprisingly chlorophyll fluorescence data indicated that the PSII reaction centers remained functional over the biotrophic stage growth. This suggests that an alternative metabolic sink was activated. A clue for identifying this alternative sink was provided by the analysis of the minimal chlorophyll fluorescence, F<sub>0</sub>, which has been demonstrated as the mirror of the plastoquinone pool reduction during chlororespiration. Indeed, as evidenced by Western blot analysis, the thylakoid membranes accumulated higher levels of PTOX, indicating a stimulation of chlororespiration during the compatible relation only.

### Conclusion

This is the first report of chlororespiration activation during successful interaction in the pathosystem *Solanum tuberosum / Phytophthora infestans*, although the actual role of this pathway remains to be clarified.

<u>Acknowledgments</u>. We acknowledge financial support from the National Foundation for Scientific Research and the Foundation for Training in Industrial and Agricultural Research (). We also would like to thank Dr. Viviane Vleeshouwers who provided the transgenic plant Desire/RB, Dr. Marcel Kuntz) for the anti-PTOX antibody and J-L Rolot for the *Phytophthora infestans* strain 99-03/6 (HT).

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## **LATE BLIGHT (Posters session)**

## 223

# EVALUATION OF THE EFFICACY OF REVUS 250 SC FOR THE CONTROL OF POTATO LATE BLIGHT (PHYTOPHTHORA INFESTANS)

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Potato late blight (Phytophthora infestans Mont, De Barvi) is disease of global importance. Total crop failure results when no control measures are taken to manage a blight epidemic on a susceptible cultivar when conditions of high humidity prevail. Control of blight in Kenya is mainly by use of fungicides. Experiments were conducted at Kenya Agricultural Research Centre (KARI) - Tigoni during long rains and short rains of 2009 to evaluate the efficacy of a new fungicide formulation, Revus 250 SC (Mandipropamid 56 g/kg active ingredient), where three application rates of Revus 250 SC (0.5,0.6 and 0.7 l/ha) were compared with Ridomil Gold 68 (WP) (having active ingredient as Metalaxyl-M 40 kg + Mancozeb 640 kg/ha) applied at the rate of 2.5 kg/ha and unsprayed control for the control of potato late blight on potato variety Tigoni. Additionally each product was evaluated at the application frequency of 7, 10 or 14 days. Experimental plots were laid out in a randomized complete block design. Data were collected weekly on blight incidence using a scale of 0-100% at disease onset till the control plot was completely blighted. Blight incidence scores were used to compute area under disease progress curve (AUDPC). Results from these study show both fungicide treatments tested reduced the level of early blight as compared with the untreated check significantly (P= 0.001). Importantly, the data demonstrates the efficacy of Revus 250 SC against blight especially at application rates of 0.6 l/ha and 0.7 l/ha which led to significantly less disease in treated crops compared to control plots. Disease quantity (AUDPC) in plots treated with Revus 250 SC at the rate of 0.5 did not differ significantly from the control plots. Although significant (P=0.001) interactions were observed between the rate of product application and the frequency of application, this trends were similar for both products and higher rates applied at closer intervals gave better disease control. Based on these findings, Revus 250 SC is recommended for use against foliar potato blight at rates of 0.6 or 0.7I/ha. The frequency of application may depend on blight disease pressure and variety tolerance levels.

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DIVERSITY OF *PHYTOPHTHORA INFESTANS* POPULATIONS, THE CAUSATIVE AGENT OF POTATO LATE BLIGHT, IN SELECTED REGIONS OF POLAND IN 2010 – 2012.

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#### Introduction

Phytophthora infestans, belongs to the Oomycota group and is economically the most important potato pathogen worldwide. This organism can reproduce sexually, which causes high genetic diversity. To describe variation within Polish *P. infestans* samples we used genotypic markers: mating type, mitochondrial haplotype, polymorphism at 14 simple sequence repeat (SSR) [1, 2] loci and phenotypic markers: sensitivity to metalaxyl and virulence towards Black's differential set.

#### **Materials and Methods**

Potato leaflets with single late blight lesions were collected in three years 2010, 2011, 2012 in chosen regions of Poland: Mlochow, Boguchwala and Siedlce. Mlochow is the region where protected, commercial fields predominate. Home gardens, experimental fields and allotments dominate in Boguchwala. Early and starch cultivars of potato are commercially cultivated in the area Siedlce. A total number of 365 isolates was obtained and stored in liquid nitrogen. The mating type and mitochondrial haplotype were evaluated using a PCR method. Metalaxyl resistance was tested on rye A agar media with this fungicide. Virulence was tested using 11 potato genotypes, each contains a single R – gene from *Solanum demissum*. 14 SSR markers were amplified with fluorescently labelled primers and separated in a capillary system. Program GeneMapper v. 4.0 was used for genotyping data analyses [1, 2].

## Results

The A1 mating type (69.5%) and Ia mitochondrial haplotype (72.7%) dominated in the tested samples of Polish *P. infestans* population. Most of the isolates were sensitive to metalaxyl (66.3%). All tested isolates were able to overcome gene R4. Almost 100% isolates were virulent against R1, R11, R3 and R7. Fairly often we found virulence against R10, R5, R8, R2 and R6. The virulence against R9 was rare. SSR analyses are in progress.

## Conclusion and perspectives

Getting to know pathogen population characteristics is helpful in plant protection and potato cropping. The analysis of data from this study will result in answering such questions as: i) Did the *P. infestans* population change in tested years? ii) Are there any differences between populations from tested regions depending on dominating kind of potato cultivation? It should also enable comparisons between current *P. infestans* population from Poland and other countries.

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#### EFFECT OF POTATO VIRUS S INFECTION ON LATE BLIGHT RESISTANCE IN POTATO

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Late blight, caused by *Phytophthora infestans*, is a destructive disease of potato. Defender is the only cultivar in the U.S. with foliar and tuber resistance to this disease. However, this cultivar exhibits susceptibility to infection by Potato virus S (PVS) and severe symptoms appeared on leaves after infection with PVS. PVS is widespread in potato fields in the U. S. To investigate potential interactions between *P. infestans* and PVS, detached leaves of Defender and Ranger Russet (susceptible to late blight), that were either PVS-infected or non-infected, were inoculated with *P. infestans* BF05. The amount of sporulation and the extent of lesion expansion on inoculated leaves were measured to estimate late blight severity. When inoculated with *P. infestans* only, as expected, Defender exhibited discrete, relatively small, dark purple to black lesions and on an average had twenty times fewer sporangia compared to Ranger Russet. However, in Defender plants infected with PVS, lesion expansion and sporulation increased significantly compared to PVS-free Defender. The increased severity of late blight in PVS-infected Defender suggests that PVS negatively impacts late blight resistance in this cultivar. This study demonstrates that late blight resistance in cultivars to be released should be screened for PVS susceptibility.

## THE RECEPTOR-LIKE PROTEIN ELR IS PROVIDING A NOVEL LAYER OF RESISTANCE TO POTATO LATE BLIGHT

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The notorious late blight disease in potato is caused by the Irish famine pathogen *Phytophthora infestans*. To fend off pathogens, plants rely on two classes of immune receptors. Some receptors reside on the plant cell surface whereas others are located inside the plant cell. Until now, most intracellular resistance (R) genes to *P. infestans* have been defeated. However, surface immune receptors might provide another layer of immunity. Here we show that the receptor-like protein ELR (elicitin response) of the wild potato *Solanum microdontum* provides a novel layer of resistance to *P. infestans*. We found that transformation of ELR into a susceptible potato cultivar enhanced resistance to *P. infestans* isolates. Besides, ELR could recognize a broad spectrum of elicitin proteins secreted by many other *Phytophthora* pathogens. We hypothesize that combining surface receptors like ELR with specific R genes has a potential to confer durable resistance to potato late blight.

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# VARIATION IN POPULATIONS OF LATE BLIGHT (PHYTOPHTHORA INFESTANS) COLLECTED IN WALLOON REGION (BELGIUM) IN 2010 - 2013

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Late blight, caused by the oomycete *Phytophthora infestans*, is one of the most important diseases in potato production worldwide. Under favorable conditions, the disease can kill unprotected haulm in a couple of weeks. 404 isolates of *Phytophthora infestans* were collected on potato in the main production areas of south of Belgium (Wallonia) and on tomato from 2010 to 2013. They were characterized for mating type, sensitivity to Metalaxyl and virulence. Mating type was tested by growing isolates on rye agar with known reference isolate of the A1 or A2 mating type, virulence patterns were determined using Black's differential set of potato clones and Metalaxyl resistance was assessed with a floating leaf disk method.

The frequency of A2 mating type isolates of *Phytophthora infestans* increased in Belgium since 2003. In 2013, 68% of isolates were found to be A2. All known virulence genes were found in Belgian isolates. Most of the isolates were able to overcome seven R-genes (R1, R3, R4, R7, R10 and R11). 37 different races were found among 201 isolates and the most common races were 1-3-4-7-10-11 (17%) and 1-2-3-4-5-6-7-8-10-11 (16%). Among 227 isolates, 50% were resistant to Metalaxyl and 50% were sensitive. The observation of mating types in relation to their sensitivity to Metalaxyl showed that mating types A2 occurred preferably in conjunction with resistance: 94% of Metalaxyl resistant isolates were A2.

## CHARACTERISICS OF PHYTOPHTHORA INFESTANS THE CAUSE OF POTATO LATE BLIGHT DISEASE

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Eighty six isolates of *Phytophthora infestans* dating back to 2006, recovered from potato tubers were on sale in Alexandria markets, Egypt, were characterized for mating type, colony morphology, and isozymes. Bothe A1 and A2 mating types were detected in the isolate collection, however, the A2 constituted 5.6% of the total isolates made while the A1 mating type isolates constituted 94.1%. The self-fertile phenotype also detected but at a lower percentage of 0.3% of the total isolates. This indicated that Mexico, the probable origin of the disease, is no longer the only place where A2 mating type is ever exists. The lumpy phenotype was the only trait observed linked to the A2 mating type isolates on rye A agar medium. The self-fertile isolates, however, exhibited colonies of a waxy appearance with little aerial hyphae and the culture were backed full with oospores. The A1 mating colonies were of smooth white abundant aerial hyphae. All the isolates investigated were monomorphic at the Gpi-1 locus and were homozygous of genotype Gpi-1 100/100. Greater variation was identified for peptidase at the Pep-1 locus. The homozygous genotype Pep-1 100/100 and the heterozygous genotype Pep-1 92/100 were identified. However, the genotype Pep-1 100/100 was more common and constituted 69% of the total isolates investigated (86) while the Pep-1 92/100 constituted only 31%. Consequently, alleles Gpi-1 100 and Pep-1 100 occurred in 100% of the investigated isolates while allele Pep-1 92 occurred in only 31% of the isolates. The appearance of the A2 mating type outside Mexico and the variation revealed in the population of *Phytophthora infestans* investigated supported the hypothesis of a second worldwide migration of the fungus from its origin which could constitute a threat to potato cultivation around the world.

## OCCURRENCE OF MATING TYPES A1 AND A2 OF *PHYTOPHTHORA INFESTANS* (MONT.) DE BARY IN SPAIN

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The oomycete *Phytophthora infestans* causes the late blight disease, causing death of leaves, stem and tubers of the plant. It can also completely destroy the cultivation in a short time period, and is therefore considered as the most serious problem for production of Solanaceae crops worldwide. Changes in the pathogen virulence originate through sexual reproduction. P. infestans is heterothallic with two known mating types, A1 and A2. The oospores are tolerant to adverse conditions and can survive in the soil. It is becoming increasingly difficult to control the disease in Spain, leading to an ever more intensified use of fungicides. A total of 55 isolates were collected from commercial fields of the major potato-growing areas in Spain during 2011 to 2013. Mating type was determined using the conventional pairing assay and PCR markers. All isolates were characterized by placing a mycelia plug of unknown isolate plug in a rye agar medium [1] 2 cm away from a plug of either an A1 or A2 reference isolate. The Petri dishes were incubated in the dark for 15 days at 18°C. If the culture produced oospores with the A2 reference, the isolate was classified as A1 mating type; an isolate that did not generate oospores was determined as A2 [5]. For identification by PCR, DNA was extracted using the commercial kit DNeasy® Plant Mini Qiagen. Mating type A1 was determined by applying the specific primers S1A and S1B [3], for amplifying a specific DNA fragment corresponding to the S1 gene, which is present in the mating type A1, but not in A2. For the detection of A2 isolates we used the primers PHYB-1 and PHYB-2 [4]. Also, in order to confirm the previous results, a CAPs assay was used with the specific markers W16-1 and W16-2 [2]. PCR products were cleaved with HaelII restriction enzyme and the digestion products were separated by agarose gel electrophoresis. The results revealed the presence of both mating types corresponding to 43.6 % isolates of A1 and 56.4% isolates of A2, respectively. This is the first report of the molecular identification of the A1 and A2 isolates of P. infestans in Spain. The co-existence of the two mating types may enable the pathogen to reproduce sexually, thus enhancing the diversity of its population countrywide.

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## **Acknowledgements**

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### **BACTERIA** (poster session)

### 230

EFFECT OF A NON-PATHOGENIC STREPTOMYCES SP., USED AS AN ANTAGONIST AGAINST POTATO SCAB ON POTATO FIELDS, ON SOIL BACTERIA DYNAMICS AND DIVERSITY.

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A non-pathogenic *Streptomyces* strain has shown antagonistic activity against potato common scab in the field in Northern Finland [1]. The impact of this strain on soil bacterial population dynamics was studied in a field experiment. The experiment included four treatments: an untreated control (0+0), a single antagonist application in the beginning of the growing season (1+0), an untreated control on a plot with an annual antagonist application in the previous three years (0+3), and an antagonist application in the beginning of the growing season on a plot with an annual antagonist application in the previous three years (1+3). Each treatment had four replications. The antagonist was applied at planting as a spray on seed potatoes cv. Bintje placed in a furrow. Five samples were taken from potato rhizosphere from each plot at early tuber formation and combined to one composite sample per plot. A deep-sequencing approach was applied, where the aim is to receive sequences from most, if not all, species in the sample. From each sample 400 000 sequences of the bacterial 16S-rRNA gene variable regions 3 and 4 were analyzed.

Consecutive years of antagonist treatment were observed to have an increasing one-directional effect on the soil bacterial dynamics. While a small but a non-significant effect was observed both between an untreated control (0+0) and a single treatment (1+0), and between three years (0+3) and four years (1+3) of treatments, a clear and significant change in soil bacterial population dynamics was found when an untreated control (0+0) and the four-year treatment (1+3) were compared. Seventeen phyla were significantly altered in percent proportions; the phyla in major decline was *Actinobacteria* (down from 36.8% to 26.0%), while *Proteobacteria* (up from 17.8% to 25.4%) was the most abundant one of the 15 phyla that increased their percent proportion. The family *Streptomycetaceae* declined significantly from 0.68% to 0.39%, but unfortunately, the current Illumina sequencing technology can't produce sufficiently long sequences to yield enough variance from the 16S-rRNA gene to allow species-level identification. The bacterial diversity increased with every additional yearly antagonist treatment, the estimated number of species in the untreated control (0+0) was 73 000 and in the four-year treatment (1+3) 83 700.

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# MONITORING AND FIELD ASSAYS OF *RHODOCOCCUS ERYTHROPOLIS* R138, A BIOCONTROL AGENT AGAINST BLACKLEG AND SOFT-ROT BACTERIA

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Every year in France, blackleg and soft-rot bacteria *Pectobacterium* and *Dickeya* cause a loss of about ten thousand tons of potato seeds. The virulence of *Pectobacterium* is regulating by quorum-sensing, a cell-to-cell communication system based on recognition and concentration of the acyl homoserine lactone family signal. Currently there is no treatment against blackleg and soft-rot diseases. A quorum-quenching strategy developed in the laboratory is based on the introduction of the *Rhodococcus erythropolis* R138 strain as a biocontrol agent (Cirou et al, Appl. Environ. Microbiol. 2012). This bacterium isolated from potato hydroponic culture is able to degrade the quorum-sensing signal and disturb the virulence of the pathogenic bacteria. To monitor R138 and pathogen *Pectobacterium atrosepticum* CFBP 6276 populations, their genomes were sequenced and specific molecular markers of the strains have been developed. Field experiments were conducted by the company SIPRE in Achicourt (62). Both populations of R138 and Pa6276 were monitored in the rhizosphere and in potato plants. Blackleg symptoms were quantified to evaluate the incidence of this biocontrol treatment. Introduction of the biocontrol strain R138 in field associated with a biostimulation treatment allows the growth of R138 population in the rhizosphere. Protection of potato plant depends of the formulation and weather conditions during the application.

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#### PARTICIPATORY FARM-LEVEL INNOVATION IN BACTERIAL WILT CONTROL

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Research on the development of Bacterial wilt (Ralstonia solanacearum) control technology was undertaken using the Farmers Research Extension Group approach supported by sessions (FREG session) harmonized with crop phenology. The study was conducted in Shashemen district (Ethiopia) from 2010 to 2012 seasons with the involvement of 250 farmers of which 64 were women farmers and the rest 186 were men. The purpose was to assist farmers in developing healthy potato farms, which are more productive, profitable, and sustainable. Using this approach, experiments on the role of crop rotation and the use of disease free potato tuber seed on disease incidence and tuber yield were studied with the full involvement of farmers' research group. The modified FREG approach was found to be effective in stimulating farmer participation by considering their goals in the targeting and design of innovations. Before the inception of the project, more than 90 % of the farmers were not aware of the causes of potato diseases and most of the farmers (95 %) believe that any type of disease is caused by rain and mist whereas, others don't even guess the causes. After subsequent sessions and demonstration at field level, more than 65 % of the farmers in the group distinguish the causative agents of late blight, potato wilt and viruses and able to differentiate symptoms damaged caused by diseases and insect pests. Under wear potato production scenario 4% to 32 % correspondingly the loss was 28.6 % to 67.34 % in seed production scenario. Both one and two season's rotation potato with beans and cabbage significantly (P>0.01) reduced the wilt incidence from 81% to 14% and increased tuber yields to 59 % and 84 % respectively.

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#### DEVELOPING A 'FRIENDLY' DIAGNOSTIC METHOD TO IDENTIFY DICKEYA SPECIES

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#### Introduction

Pectinolytic bacteria are economically important potato pathogens in France and in Europe, as they cause field and lots losses as well as downgrading and rejections for the certification of seed potatoes. Developing diagnostic methods is important to identify and discriminate the two genera involved in diseases, *Pectobacterium* (P.) and *Dickeya* (D.) [1], which are able to cause similar symptoms (tuber soft rot and blackleg on stems). Annual field surveys conducted by the French Federation of Seed Potato Growers (FN3PT) on potato plants exhibiting blackleg symptoms, revealed that, besides the prevalent genus *Pectobacterium*, two *Dickeya* species could be detected at variable frequencies. Among them, the new species *D. solani* [2] was identified, as well as *D. dianthicola*, historically the predominant species and still prevalent among *Dickeya* in France. New diagnostic methods for *D. dianthicola* and *D. solani* based on the TaqMan technology have recently been proposed [3, 4] and proved effective on pure bacterial cultures. However, a robust and friendly identification methodology, applicable on both pure cultures and on plant material, is still lacking for routine survey and diagnostic purposes. The present study presents the development of a set of reliable molecular markers allowing to identify and discriminate *Dickeya* species in large scale routine tests.

#### **Materials and Methods**

Pectate lyase sequencing was performed on a set of *Dickeya* spp. strains including 15 CIRM-CFBP *Dickeya* from all species and 53 *D. solani* and *D. dianthicola* potato strains isolated [5] from potato fields in France and Europe. Restriction enzymes sets were selected to evaluate their ability to discriminate between *D. dianthicola* and *D. solani* in a Cleaved Amplified Polymorphism Sequence method.

#### Results

Two enzymes were identified, which allow to specifically detect *D. dianthicola* and *D. solani* respectively. The corresponding CAPS were tested on both pure cultures and macerates from symptomatic and symptomless plants, and provided an accurate identification of *Dickeya* species in all cases.

#### **Conclusions and Perspectives**

The developed CAPS allow the specific detection of *D. solani* and *D. dianthicola*, alone or in mixed infections. The method is robust over a large collection of isolates, is well suited to routine purposes and does not require specific equipment besides standard PCR machines. This new tool was also successfully used on samples stored for several years to confirm or refine earlier identifications.

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# FIRST REPORT OF *PECTOBACTERIUM CAROTOVORUM* SUBSP.*BRASILIENSIS* ON POTATOES IN MOROCCO THROUGH 16S RRNA AND PELY GENE SEQUENCING

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Pectobacterium is one of the most common pathogen affecting potato plant in Morocco, but the phylogenetic position have not been fully elucidated. Twenty strains were isolated from potatoes showing soft rot symptoms. They characterized using biochemical and pathogenicity features and tested positive in a PCR assay using primers Y1 and Y2 / P143 and P145. Sequencing and phylogenetic analysis of the partial PelY gene have shown that the isolates were divided into 3 clusters with high similarity (97-99%). The BLASTn results of PelY sequences comparing with nucleotide sequences from GenBank, 12 strains showed 99% identity with the comparable sequence of periplasmic pectate lyase of Pectobacterium carotovorum subsp. carotovorum (14005.1), 3 strains showed 98-99% similarity with the comparable sequence of periplasmic pectate lyase of Pectobacterium carotovorum subsp. carotovorum WPP14 AN: (ZP 03830273), and 5 strains showed 97%-99% similarity with the comparable sequence of periplasmic pectate lysase of Pectobacterium carotovorum subsp. brasiliensis PBR1692 (ZP 03828738). The nucleotide sequences of the genes encoding 16S rRNA were determined and compared in order to determine and confirm its relative phylogenetic position and taxonomy. The strains were subdivided into 2 clusters Pectobacterium carotovorum subsp. carotovorum and Pectobacterium carotovorum subsp. brasiliensis with high similarity (98 to 99%). This is the first report of isolation of Pectobacterium carotovorum subsp. brasiliensis in Morocco.

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# INVESTIGATING MOLECULAR ASPECTS OF *DICKEYA SOLANI – SOLANUM TUBEROSUM* INTERACTIONS IN BLACKLEG DISEASE

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#### Introduction

Losses in potato production both in field and storage due to blackleg disease have increasingly been reported in Europe. This has been attributed to the spread of *Dickeya solani* that causes a more aggressive disease development compared to other blackleg disease bacteria such as *Pectobacterium atrosepticum* and *Pectobacterium carotovorum* subsp. *carotovorum*. Currently there is no fully resistant potato variety available and identification of resistance sources has been unsuccessful [1]. Additionally, studies of the interactions between *Dickeya solani* and potato at the molecular level have been hindered partly due to complicated disease etiology, a strong environmental influence on disease development and lack of efficient testing systems [2]. We have developed a blackleg disease testing system that enables quick screening of in vitro grown potato clones. Using this system we have tested transgenic potato lines disrupted in hormonal signaling. A crossing population obtained from blackleg resistant and susceptible parents was also tested and their RNA has been sequenced to identify molecular features for resistance/susceptibility.

#### **Materials and Methods**

Dickeya solani strain Ds 0432-1 was used [3] to infect in vitro and greenhouse grown potato cultivars: Desirée, Sarpo Mira, Magnum Bonum and clone SW93-1015.[4] In addition, in vitro grown potato clones in Desiree background with altered plant hormone levels, NahG (salicylic acid deficient), coi1 (jasmonic acid insensitive) and a crossing population obtained from SW93-1015 x Desirée have been analyzed. Further validation of these results was performed on greenhouse grown cultivars and clones using a standard greenhouse based blackleg disease response test. [5].

#### **Results, Conclusion and Perspectives**

With our novel testing method based on in vitro grown potato cultivars and clones we demonstrate an increased throughput screening for blackleg disease response. The response of clones observed in this in vitro system was similar to that obtained from the established green-house assay. We also demonstrate that cultivars Desiree and Sarpo Mira are moderately resistant while Magnum Bonum and clone SW93-1015 are susceptible. Furthermore with this new blackleg disease screening method we have tested an array of clones, cultivars and hormone transgenics which provides molecular insights into disease development in potato stems. Currently we are also investigating alternative approaches such as exogenously applied inducers of resistance against blackleg disease.

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# SCAB DISEASE TOLERANCE AND YIELD POTENTIALS OF NEWLY DEVELOPED POTATO CLONES IN SOUTH AFRICA

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Potato (Solanum tuberosum L.) is the world most important food crop after wheat, rice and maize. It plays a major role in fulfilling the daily nutrition requirements and food security especially for people in the developing world. South Africa is the largest producer and exporter of potatoes in Africa. However, quality and quantity of production of potato in South Africa and elsewhere in the world is often undermined by pests and diseases including scab diseases of potato caused by Streptomyces spp. Although there are wide range of control measure of scab diseases, developing resistant potato cultivars is the most reliable, environmental friendly and long term technique. Potato clones were developed by crossing different parental lines. Fifty-one fourth generation clones were planted in the tunnel on sterile silica sand growing medium for testing their resistance to scab diseases of potato. Common scab (Streptomyces scabies) and fissure scab isolates were plated onto Oat Meal Agar medium for fifteen days and the inoculum were collected from each of the plates and mixed in 20 liters of distilled water. The Growing media of each of these clonal lines were inoculated with these spore suspensions. Every agronomic requirement of these lines was attended throughout the growing season. After harvest, scab symptoms were recorded and analyzed using JMP statistical software. Moreover, field trials were conducted to study yield potentials of some of the clones at Roodeplaat and Cedara locations, South Africa. The result showed that, potato clones 06-677-151, 07-671-44, 07-619-52 and 06-678-8 were consistently found to be scab disease tolerant and better yielder.

#### EFFICIENT INOCULATION METHOD OF POTATO TUBERS WITH DICKEYA SPP.

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#### Introduction

To conduct experimental glasshouse or in field research studies on pectin lytic bacteria of genus *Dickeya*, effective and reproducible inoculation methods are necessary. Tubers are indeed considered as a source of inoculum [1] and have consequently largely been used in epidemiological studies to be artificially inoculated. Different methods have been described so far, such as "wounding" methods, vacuum infiltration and soaking methods [1-3].

Although relative efficiencies of these methods cannot be clearly established, they all present inconveniences, especially regarding their ability to be used for large scale field trials, when many hundreds of potatoes have to be inoculated. The need for validating a reliable method for *Dickeya* is an issue, since all the pre cited methods have been developed for *Pectobacterium*, which used to be the major blackleg agent on potato in Europe. The aim of this protocol is to present a reliable method which is not costly and easy to apply on a large number of tubers.

#### **Materials and Methods**

Procedure takes 3 days and is the following: warm-up of the refrigerated tubers in a dark room at a temperature of  $20 \pm 2^{\circ}$ C (RT°), soaking of tubers in water during 2 hours at RT°, storage of tubers in the dark for 22 hours at RT° in a humid atmosphere (opening of lenticels), soaking of tubers in bacterial suspension for 12 hours the dark at RT°, drying tubers in crate during 12 hours in a dark ventilated room at RT°. The inoculated tubers can be stored at 12°C for up to one month before planting.

Field trials to assess susceptibility of five cultivars to *D. dianthicola* and aggressiveness of 2 *D. dianthicola* and 3 *D. solani* isolates were carried out during 2 years. Experimental assays were set as split plots with 4 repetitions of 100 plants for each cultivar or isolate and for control plants. Inoculations were performed with a 10e5 cfu/ml bacterial density. Blackleg diseased plants were counted repeatedly during the growing season.

#### Results

A 100% emergence rate of the control tubers and a 99% emergence rate of the inoculated tubers indicate that inoculation does not affect survival of tubers and that the density of bacterial suspension is adequate. Analysis on results of both years together reveals a significant difference of susceptibility among cultivars and a significant difference of aggressiveness between isolates. Interaction is noticed but does not affect the reproducibility of the method, as the analysis of both years separately shows that the ranking of cultivars and isolates does not significantly change from one year to the other.

#### **Conclusion and perspectives**

This *Dickeya* inoculation method is reliable and effective as it allows blackleg symptoms to develop in field conditions. It allows performing large scale field trials and can be used by any researcher around the world.

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# DETECTION OF STREPTOMYCES EUROPASCABIEI IN POTATO COMMON SCAB LESIONS IN FINLAND

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Streptomyces scabies, S. turgidiscabies and S. acidiscabies are the most well-known species causing potato common scab worldwide. In addition, S. europascabiei first described in France [1] is currently regarded as the main common scab causing species in Europe [2]. In Finland, S. scabies and S. turgidiscabies have been described as the causal species of common scab based on morphological and physiological characterization [3] and comparison of the 16S rRNA gene sequences [4]. However, S. scabies and S. europascabiei cannot be distinguished by investigating the 16S rRNA gene sequences, because the sequences are almost identical. The aim of this study was to establish, if S. europascabiei, the commonly found species in Europe, is also present in Finland.

Bacteria were isolated from scab lesions of potatoes grown in different parts of Finland. PCR primers formerly believed to be species-specific to S. scabies were used to select isolates for the Hpy99I restriction analysis, where the ITS region of the 16S operon sequence is used to distinguish S. scabies from S. europascabiei. Isolates assigned to S. scabies in the previous studies [3, 4] and the S. scabies type isolate ATCC49173 were included in the tests. For a more precise view of the relationship between the isolates and S. scabies, a part of the BacA1 gene was sequenced from a few isolates. BacA1 can be considered as a 'normal' gene that accumulates substitutions at an average rate, unlike the 16S rRNA.

None of the isolates from the field grown potatoes, which were found positive by PCR using the *S. scabies* specific primers, or the isolates assigned to *S. scabies* in the previous studies could be restricted with Hpy991. All these isolates were assigned to *S. europascabiei* suggesting that *S. europascabiei* is widely present in Finnish field soils. Furthermore, it seems that in the previous studies carried out in the 1990s, *S. europascabiei* may have been mistaken for *S. scabies*, as the restriction analysis of the ribosomal DNA spacer region was not performed. The BacA1 sequences from the isolates shared ca. 95% nucleotide sequence identity with *S. scabies*. It was the closest match out of the 16 *Streptomyces* species sequences found in the database, however, sufficiently different to be considered a different species.

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# EXPRESSION OF POTATO RESISTANCE TO PECTINOLYTIC BACTERIA IN RELATION TO TEMPERATURE, TIME OF INCUBATION, AND THE NUMBER OF BACTERIA USED FOR INOCULATION

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#### Introduction

The goal of presented studies was to investigate the influence of temperature and time of incubation on the expression of resistance to *P. atrosepticum* and *P. carotovorum* in potato cultivars, and to estimate the lowest number of bacteria *D. solani* which can cause the infection of potato tubers of two cultivars, which differ in level of resistance to pectinolytic bacteria.

#### **Materials and Methods**

Exp. 1. Potato cultivars: Irga, Romula, Raja, Medea, Gandawa, and the diploid genotype DG 94-15. Bacterial isolates: *P. carotovorum* strain SCC3193, *P. atrosepticum* 2/06 M. Ten tubers per cultivar were tested with each of bacterial strain, incubation: 48 h at 30°C, and 72 h at 24°C. The weight of rotten tissue was measured. Two way analysis of variance was applied.

Exp. 2. Potato cultivars: Irys and Glada. Bacterial isolate: *Dickeya solani* IFB0099. Serial dilutions of bacteria in Lysogeny Broth liquid medium grown for 16 h at 34°C were plated on LB agar medium. The number of bacteria was estimated after 24 hours of incubation in 34°C. Ten tubers per cultivar were inoculated with 10 µl of bacterial suspension of different dilutions. Tubers were kept at 26°C for 72 h before the rotten tissue was weighted.

#### Results

Exp. 1. Analysis of variance showed significant influence of potato genotype, and the interaction of the genotype by the incubation type for both tested strains of bacteria. Cultivars Irga, Medea and Raja rotted significantly higher when incubated in temperature 24°C for 72 h than in 30°C for 48 h. The cultivar Gandawa showed opposite reaction. The most resistant was the clone DG 94-15.

Exp. 2. 23/60 tubers of susceptible cv. Irys and 16/30 of medium resistant cv. Glada showed symptoms of infection after inoculation with 10  $\mu$ I of bacterial suspension containing from 12-160 CFU.

#### Conclusion and perspectives

The cv. Gandawa expressed higher resistance when tested in lower temperature  $(24^{\circ}\text{C})$ , but not in higher temperature  $(30^{\circ}\text{C})$ . Similar reaction was observed for medium resistant cv. Sleza in other studies with *D. solani* and *P. carotovorum* for two temperatures  $26^{\circ}\text{C}$  and  $34^{\circ}\text{C}$  (not published). Longer time of incubation in lower temperature  $(24^{\circ}\text{C}, 72 \text{ h})$  should be applied for testing the resistance of potato genotypes.

The threshold level for infection with bacteria *D. solani* of susceptible cultivar Irys and medium resistant cultivar Glada was very low, starting from about 16 – 160 bacteria. It was in agreement with previous studies, that *D. solani* rotted tuber tissue at a low density of 103 CFU mL-1 [1]. The hypothesis that for the inoculation of the cultivar expressing higher level of resistance the higher critical level of bacteria is needed was not proved. The high aggressiveness of *D. solani* strain was confirmed.

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#### DICKEYA DIANTHICOLA AND D. SOLANI - EMERGING PATHOGEN OF POTATO IN RUSSIA

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Several regions of Russia were assayed for potato bacterial diseases at 2001-2004, 2009-2011, and at 2013. Bacteria causing black leg and soft rot diseases of potato at 2001-2004 belonged to genera *Pectobacterium* only, but *Dickeya* sp. was found at North Caucasus region on corn and ornamental plants. For the first time, strains of *Dickeya dianthicola* were isolated from potato in 2009 in very few samples (3.5% positive of total number 430) obtained at Lipetsk, Novgorod, Moscow, Voronezh, and Bryansk regions. They were tested for biochemical traits, virulence and genetic properties (MLST with 8 genes). A few strains from Voronezh region were identified as *D. solani*. All the *Dickeya* strains were virulent for potato, tomato, tobacco and Iris plants, and could be identified by real-time PCR with ADE1/ADE2 primers with original ADE3 fluorescent Taqman® probe (Karlov et al, 2011). At 2013 *Dickeya* sp. have been found with frequency near 30% of 386 tested potato samples from 9 regions of Russian Federation. High economic losses are expected from rapid spreading of the Dickeya pathogens.

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# INCIDENCE OF BACTERIAL WILT CAUSED BY *RALSTONIA SOLANACEARUM* E. F. SMITH ON SOLANACIOUS IN NIGER AND DETERMINATION OF PHYLOTYPES INVOLVED

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The brown rot caused by *Ralstonia solanacearum* (syn. *Pseudomonas solanacearum*, syn. *Burkholderia solanacearum*), was first reported in Niger in 1982. Since then, it has regularly been cited across the country. The largest number of households has been observed in 2006, particularly on a variety of potato called in haussa Dan Hadjia or Kouli-kouli and on tomato.

Surveys were conducted in 2006 and 2007 in three major producing regions of potato (Agadez, Dosso and Tillabéri), according to a gradient altitudes ranging from 184 to 1546 meters. The extent of the disease was assessed by calculating the disease incidence.

The region of Agadez, characterized by low night temperatures, sandy clay soil, a surface irrigation with water drawn from the depths, showed averages rates close to 1%. Soil of Dosso, Niamey and Tillabéri regions are predominantly sandy with higher minimal temperatures. Irrigation is by spraying plants foliage using surface water (river) or watertable deep to stream soil, sometimes maintaining a perennial vegetation. The disease is more severe in these regions than the others since the average incidence rates are ten folds higher with more pronounced symptoms.

#### **BROWN ROT IN GEORGIA**

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Brown rot caused by *Ralstonia solanacearum* is a serious and economically important bacterial disease of potato and tomato. The bacterial agent is listed by EPPO and most national plant protection organizations including Georgia as a quarantine organism. Both potato and tomato are valuable crops for Georgian farmers, and the disease can cause losses of over 50%.

During 2010-2013 tomato plantations were observed in field and greenhouse conditions in different geographic regions of Georgia and several of them were infected by *Ralstonia solanacearum*. During potato field survey and harvest diseased tubers were collected from commercial varieties: Julia, Marfona, Picasso, Finca, Agria, Alliance, Marabeli. Diagnostic tests indicated the presence of *R.solanacearum* in potatoes. Bacterial wilt symptoms were also detected on ornamental plants in Batumi botanical garden.

Isolated pathogen streaking on TZC (2,3,5-Triphenylte tetrazolium chloride) agar medium, wear colonies like typical *R. Solanacearum* - large, milky white, flat, irregular and fluidal and after three days incubation develop pink to blood-red coloration in the centre. Identification of the pathogen was done by PCR using two primers pairs: OLR1; Y2 (Fegan & Prior). Pathogenicity test was done in Greenhouse for tomato, potato and pepper seedlings.

Results of research show that quarantine pathogen R. solanacearum is spread in Georgia.

Georgian strains are characterized by growth temperature 20-25 <sup>O</sup>C and they were more pathogenic to potato and tomato plants, then to pepper. It has various host plants in Georgia and that indicates its biodiversity.

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### HATCHING OF GLOBODERA PALLIDA CYSTS IS INHIBITED BY ALLYL ISOTHIOCYANATE BUT NOT BY BRASSICA JUNCEA BIOFUMIGATION

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Potato cyst nematodes (*Globodera pallida* and *G. rostochiensis*) infestation can reduce potato yield up to 80% [1]. Juveniles of *Globodera* spp. attack potato roots and when developed into females they produce eggs, which remain inside their body. The female body swells and toughens, thereby forming a protective cyst. Even after many years in soil, in the absence of host plants, cysts can contain viable eggs. Potato cyst nematodes can be controlled by crop rotation, application of nematicides, growth of resistant potato cultivars or biofumigation. Biofumigation with *Brassicaceae* is the incorporation of glucosinolate-rich plant material into the soil [2]. Glucosinolates are sulfur-containing secondary metabolites of *Brassicaceae* that are involved in the defense of the plant. The release of these toxic compounds is the basis of biofumigation [3].

Toxicity of allyl isothiocyanate was tested in vitro on encysted potato cyst nematode G. pallida. Fifty percent reduction in hatching was found within 2 h of exposure to 0.002% allyl isothiocyanate. Sinigrin (2-propenyl glucosinolate) is the precursor of allyl isothiocyanate and is the main glucosinolate of *Brassica juncea* [4]. Based on the in vitro results we hypothesized that biofumigation with *B. juncea* would reduce hatching of *G. pallida* cysts in vivo and higher sinigrin levels would have a stronger effect. Plant genotype, sulfur fertilization and insect herbivory increased sinigrin concentration of *B. juncea*. Increasing sinigrin concentration of *B. juncea*, however, did not affect *G. pallida* hatching after biofumigation. The absent effect of biofumigation was most likely due to lower concentrations of allyl isothiocyanate in vivo compared to in vitro conditions.

The sinigrin concentration in the plant material could be further increased, or the ratio of plant material to soil could be increased. The ratio we used in our pot experiment, realistic for field conditions, corresponds to the incorporation of a total aboveground biomass of 25 Mg ha-1 to a 10 - 12.5 cm depth in the field. This ratio could be increased by shallower incorporation, thereby concentrating the plant material, but this would likely leave cysts in deeper layers unexposed. These results show that it is difficult with realistic circumstances to reach sufficiently high levels of toxicity via *B. juncea* biofumigation to reduce hatching of *G. pallida*.

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# ASSESSMENT OF A REAL TIME PCR ASSAY FOR THE DETECTION OF *MELOIDOGYNE FALLAX* IN ROOT STOCK MATERIAL

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Meloidogyne chitwood and M. fallax are major pests for important crops such as potato, carrot, tomato or salsify by inducing galls and distortions of rootstocks. They are EU regulated nematodes, according to Directive 200/29/CE, for which National Plant Protection Organizations (NPPOs) have to organize surveys and when necessary to establish a national regulatory control system.

Because the extent of damage on rootstock is cultivar dependent and additionally affected by several other factors, visual inspection for symptoms like galling or swelling is not sufficient to evaluate the status of a suspect lot of rootstock. The NPPO needs to reliably detect the pests in order to define the status of a lot.

In the framework of national official survey and since 2011, French NPPO uses a real time PCR assay (Clear Detection®) that was validated by Anses for the detection of both root-knot nematodes in soil (<a href="http://www.anses.fr/fr/documents/LSV\_nemato.pdf">http://www.anses.fr/fr/documents/LSV\_nemato.pdf</a>). The PCR assay was sensitive (5 juveniles) and specific (no cross reaction) (1). Since 2008 several outbreaks of *M. chitwoodi* and *M. fallax* were detected in France. This highlighted the need to extend the use of this PCR assay on any matrix, including soil, tubers, roots or bulbs.

In this study, the real time-PCR test was combined with a specific preparation sample in order to reduce the loss of nematodes during the extraction process avoiding sieving and/or centrifugation steps (2). The roots and/or peels of rootstock material are freeze-dried and grinded prior to DNA extraction. DNA extraction is performed by using the Wizard Promega Food kit on a KingFisher magnetic particle processor.

The whole process was evaluated according to EPPO recommendations (PM7/98), and its performance criteria such as sensitivity, specificity, repeatability or reproducibility were evaluated for the M. fallax.

The sensitivity of the adapted procedure combined with real time PCR for rootsctock was defined as ten juveniles and one female stage in a subsample of 50 potato tuber peels. This process was repeatable and reproducible.

No cross reaction was observed on various rootstock materials (*Solanum tuberosum*, *Daucus carota*, *Beta vulgaris*, *Allium cepa...*). The possible inhibition of PCR amplification due to specific components of the various rootstock material was also evaluated.

As already shown and confirmed here, a molecular detection of *Meloidogyne* species within soil, tubers or any rootstock can be easily performed in any routine laboratory, experienced with the technology. This allows undertaking more nematological analysis, whereas morphological technics require highly skilled staff.

Furthermore, this molecular approach allows the early detection of the pests in asymptomatic material, including surveys performed in the framework of international trade.

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# DISPERSAL OF POTATO CYST NEMATODE IN NORTHWEST RUSSIA (REPUBLIC OF KARELIA AS AN EXAMPLE)

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The rates and modes of dispersal of potato cyst nematode (PCN) through the territory of Northwest Russia, including former USSR republics where PCN was detected for the first time (Lithuania, 1948, and Estonia, 1953), Leningrad Region and the Republic of Karelia (1973-2013) were investigated. Analysis of the records showed that PCN expansion followed two pathways: gradual northwards spread (an average 1-2° over 2-5 years), and intensive expansion around the sites of first findings, where being an invasive species with growing population PCN established rapidly in the soil nematode communities in local potato fields. In the Republic of Karelia PCN was first found in 1976 in a private field in south-western Karelia, the infection level being 4 cysts/100 g of soil. This fact indicated that the nematode had spread 2° northwards within the 3 years since previous detection (Leningrad Region, 1973). However, already in 1976-1977 thirty private fields infected with PCN (2.8 ha<sup>-1</sup> with an average of 18 cysts/100 g soil) were revealed in south-western and central Karelia. Some of them (13%) had a high level of PCN infection (40-52 cysts per 100 g of soil). It was conjectured that PCN had been introduced to the Republic of Karelia at least 3-5 years earlier.

During 10 years (1985-1987) the nematode was observed in 60 ha<sup>-1</sup> of private fields and farms in southern, south-western and central Karelia with 2-400 cysts/100 g of soil. PCN infection rates remained low in 40% of area, and reached high values in 30% of the potato fields. Such aggressive spreading allowed the nematode to establishing in the soil nematode communities in potato fields up to 63° N. PCN turned into an issue for it grows rapidly in new areas, jeopardizing potato crops, decreasing their productivity, disturbing the soil ecosystem by changing the nematode community structure [1-3].

In the northern part of Karelia gradual PCN dispersal lagged behind (2° northwards over 12-15 years). The nematode was detected there in 2004-2009. The latest pest finding was registered from 65° N. Intensive PCN expansion is limited by severe climatic conditions for potato growing and small crop areas, mostly private fields.

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# INCREASED INTERNATIONAL EXCHANGE: WHAT HAPPENS WHEN THE YAM NEMATODE IS INTRODUCED TO POTATO?

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There is an increasing interest for potato production in Africa, where the crop offers an alternative to traditional food crops and provides extra income as a fresh-market commodity. Yam is a tuber crop that can be stored for several weeks and a traditional source of starch in many tropical parts of the world. A major problem in vam is the vam nematode, Scutellonema bradys, which keeps developing during storage of yam and can result in considerable production loss. With increasing exchange of both yam and potato between continents, we wanted to investigate the possible threats of S. bradys on potato production in the EU, and confirm reports of potato infection by S. bradys in Africa [1]. The host suitability and damage symptoms of the nematode were evaluated in a pot test on five potato varieties commonly grown in Europe and Africa: Désirée, Nicola, Spunta, Diamant and Draga. The yam nematode was able to reproduce and caused brown root lesions, a scaly surface appearance of the tubers, as well as tuber rot. Reproduction and damage symptoms varied with potato variety, but larger scale experiments are needed to establish more realistic field-related numbers. We also investigated the probability of nematode entry, spread and establishment in Europe by performing a pest risk analysis (PRA). This PRA showed that the likelihood of entry of S. bradys from third countries into the European Union is high due to importation of contaminated yam tubers from infested regions of Africa, although reaching a susceptible host (potato, tomato) is less probable. However, establishment of the nematode is considered possible in the most southern parts of the EU where temperatures are high and tomato is present. The study highlights the need to reduce the risk of nematode entry and spread in the EU region. It also showed that expansion of potato production in Africa should take notice of the presence of S. bradys.

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# POPULATION DYNAMIC OF *MELOIDOGYNE FALLAX* IN POTATO CROPS AND MULTIPLICATION IN STORED TUBERS

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In the European Union, *Meloidogyne fallax* (Karssen) and *M. chitwoodi* (Golden, O'Bannon, Santo & Finley) are registered as quarantine nematodes according to Directive 2000/29/EC.

Within the context of monitoring those quarantine pests, an experimentation was conducted with the aim of studying (i) the level of contamination of potato tubers by *M. fallax* on ultra-early harvested crops and (ii) the capacity of *M. fallax* to develop within stored tubers.

In that respect, a follow-up of the populations dynamic was undertaken on tubers from their initiation to the harvest time i.e. 98 days after planting. The nematological analyses combined both morphobiometrical and molecular techniques.

As a result, the findings proved that even if the potatoes are harvested very early, that does not prevent the tubers from being contaminated by *M. fallax*. Indeed once the tubers start to develop, the penetration of second stage juveniles (J2) is observed. Those juveniles grow inside the tubers to develop into females which can be detected a few weeks before the harvest. Even if the level of infestation with *M. fallax* is limited at harvest time, the production of a new generation inside the stored tubers can be observed.

# A STRATEGY TO ENHANCE POTATO RESISTANCE TO THE PHYTONEMATODE BY USING TEMPERATURE FLUCTUATIONS

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#### Introduction

The potato cyst nematode (PCN) *Globodera rostochiensis* Woll. is one of the most serious potato pests. Pathotype Ro1 of *G. rostochiensis* is widespread in NW Russia. Despite quarantine measures, PCN has tendency to progressive spreading. For control of plant diseases the significant efforts are being made to develop novel PCN management methods alternative to synthetic pesticides that unfriendly to the environment. For this purpose in NW Russia temperature fluctuations can be used for control PCN populations due to particular climatic pattern – Nordic conditions are characterized by quite instability and short-term temperature fluctuations occurred in a daily circle. Taking into account the recent data concerning a modulation with temperature of plant immune responses on fungal and virus infections [1,2] it can be supposed that plant treatment by temperature lied on the base of strategy of potato resistance enhancement to PCN by temperature fluctuations (TEMPFLU-strategy) may provide a commercially viable and alternative to existing pest management methods.

#### **Materials and Methods**

Potato plants of susceptible to *G. rostochiensis* potato cv Nevsky were grown at 20-23°C with 16/8-photoperiod during 14 days in climatic chamber, then part of them were exposed to short temperature drop from 23 to 5°C for 2 h at the end of the night for 6 days (DROP-treatment) or remained at 20-23°C (control). After that plants were infected by PCN (10 cysts per plant) and grown under optimal growth conditions (23°C) for 2 months. Plant height, number of leaves, plant biomass, photosynthetic rate, photosynthetic pigment content, and tuber yield were assessed. Nematode development and population density were investigated.

#### Results

It was established that PCN infestation prohibited the most important physiological processes of potato plants. A daily short-term temperature drop promoted active physiological state of infested plants: primed (DROP-treated) nematode infested potato plants did not differ in growth rate and productivity from non-infested control plants. In addition level of plant nematode infestation was decreased by 75%. Under using TEMPFLU-strategy the potato plants became less suitable hosts for PCN – juvenile development inside roots was delayed, final nematode population was reduced. Moreover diapause of new-formed cysts wasn't broken by potato root diffusate and subsequent invasion of plants was difficult. Thus, nematode life cycle was disturbed.

#### **Conclusion and perspectives**

TEMPFLU-strategy is important step in the formation of incompatible host-parasite interactions that will result in successful PCN management.

The study was supported financially by Program of Biology Department RAS "Biological resources of Russia" (N 01201262103).

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# TOWARDS AN INTEGRATED CONTROL AND PREDICTION OF *ALTERNARIA* SPP. IN THE FLEMISH POTATO CULTIVATION

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Alternaria species, including A. solani and A. alternata are a serious threat for potato cultivation and heavy infections can lead to significant yield losses. Both species cause necrotic symptoms, which cannot visually be distinguished. Last years, both pathogens became more and more important in NW Europe. This can be attributed to the combined effect of climate change, a reduced use of mancozeb in combination with specific fungicides against Phytophthora infestans and the increased production of high-yield susceptible cultivars. Successful control of the disease depends on accurate identification and a tailored approach. It can be accomplished through various means: use of resistant cultivars, appropriate farming techniques e.g. careful tillage and crop rotation, as well as fungicide applications that affect the growth of fungi.

The main goal of this research is to unravel the interaction between abiotic and biotic factors influencing the pathogen complex in order to apply IPM to fight early blight in the Flemish potato cultivation.

To reach this goal, we can distinguish five steps. The first consist of data collection. During the summer of 2013, 124 fields in Flanders were monitored. This season the disease incidence was low and no significant differences between regions were found. In a second step, we try to identify the population structure at different time points during the season. Therefore, we collected 186 leaf samples and it seemed that *A. alternata* was the predominant species during 2013. Additionally, we assess the aggressiveness and genetic diversity of the isolates. A similar procedure will be repeated during the next three years, to obtain insight in the *Alternaria* complex under different conditions.

Furthermore, the sensitivity of *A. alternata* and *A. solani* isolates to fungicides is determined. Therefore, both the spore germination capacity and mycelium growth rate is assessed. The first results indicate that all isolates were able to grow on field dose, 1/10 and 1/100 field dose of Amistar and none of the isolates was able to grow on field dose or 1/10 field dose of mancozeb or difenoconazool. Additionally, there was a great diversity in resistance between the isolates, even between isolates from the same field. In the light of fungicide resistance, isolates will be screened for the presence of F129L or G143A mutations, which result in a reduced susceptibility towards QoI fungicides. Also temperature requirements for mycelium growth and sporulation are investigated.

Concerning the impact of nitrogen, it was concluded that 200 kg N/ha significantly reduced the *Alternaria* incidence compared to 160 kg N/ha. To study the impact of other abiotic stress factors, such as drought and temperature pot and field experiments will be set up. Preliminary tests concerning the pathogenicity of the isolates revealed that all isolates were able to infect potatoes, as well as other host plants such as radish, tomato and cauliflower. Furthermore, there is a great diversity in pathogenicity between the isolates. In a next step the toxin producing capacities of the isolates will be unraveled.

Finally, we will combine the gathered information to build a model to predict *Alternaria* outbreaks. To deliver our model to the farmers and industry, it will be incorporated in a decision support system. This DSS will be useful to obtain a field-specific prediction of the *Alternaria* risk and take appropriate measures to limit yield and quality losses.

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# THE NEW PROBLEMS OF POTATO QUALITY DISEASES IN TUNISIA WITH REFERENCE TO THE BLACK SCURF (RHIZOCTONIA SOLANI)

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Quality diseases are considered as a new threat to the potato crop. During the last years, quality depreciation due to these diseases has become a serious problem in Tunisia, both for local and international markets. Black Scurf *Rhizoctonia solani* is the main quality disease and affects particularly the emerging export market of the fall crop. Exclusion rates on potato plots due to this fungus rose suddenly during 2005-2007 and 2010-2012. Average seed quality, frost incidentals and low farmer skills are the main factors for *Rhizoctonia solani* spread.

Seed treatment against the black scurf has been recently introduced for the fall crop in Tunisia. In this presentation, a new pesticide based on Fludioxonil was evaluated as an alternative to classic fungicides based on the Pencycuron. Fludioxonil efficiency was studied according to the applied dose and the number of treatments. All seeds were treated by dipping. Dipping mixture was about 300 I / ton of potato seeds. Results showed a better efficiency of the Fludioxonil than the Pencycuron. Harvested tubers from Fludioxonil-treated seeds were up to 50% less infested with black scurf than those from Pencycuron-treated seeds. With a second treatment during the crop cycle trough drip irrigation, black scurf infestation rate was still above 50% for Pencycuron treatment while it was about 10% for Fludioxonil treatment. No statistical difference was found between the 200 ml and 250 ml doses of Fludioxonil used in the experiments.

Based on these results, Fludioxonil treatment against the black scurf seemed to be more efficient than the Pencycuron. Alternating these two fungicides may be a good option to decrease treatment costs and limit fungicide resistance risks.

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# FIELD SIMULATION OF THE FOLIAGE DAMAGES CAUSED BY THE COLORADO BEETLE IN POTATO CROP IN ORDER TO ESTIMATE AN ECONOMIC THRESHOLD VALUE OF DEFOLIATION

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The Colorado beetle *Leptinotarsa decimlineata* (Col.; Chrysomelidae) is a historically important pest for the potato crop in Belgium. However, the foliage damages caused these last years by this insect are limited, with in most fields only few spots of defoliated plants on the border sides. Despite of this, insecticides are still intensively applied in commercial and private fields to control this pest.

In order to clarify the economic importance of the Colorado beetle damages on potato yield and tuber quality, a set of trials were carried out in Belgium during four years. Potato plants were mechanically defoliated at different dates with different degrees of defoliation to simulate Colorado beetle damages. Tuber yield and quality (size and dry matter content) were assessed at harvest. The trials were performed two years with the variety Charlotte, mainly used for the fresh market, and two other years with the variety Bintje, mainly used in the processing industry for French fries production. Charlotte is a variety harvested quite early in the season (September) with a determinate foliage development, while Bintje has an indeterminate foliage development and is harvested later in the season (October). These two varieties could thus be differently impacted by defoliation during the season and can be considered as indicator of a set of varieties with an intermediate behavior in term of foliage and tubers development.

The results obtained with Charlotte indicate that an economic threshold value of about 5 to 10 percent of the foliage damaged at the field level can be established. Defoliation levels higher than 50% has also reduced the mean tuber size and the dry matter content at harvest. No differences between early or late defoliation were observed. With Bintje, the economic threshold defoliation level was established between 10 and 20 percent. As for Charlotte, only high defoliation levels had a significant impact on tuber size and dry matter content. With Bintje, defoliations that were performed late in the season (mid-august) had no significant impact on tuber yield.

These results suggest that, on an economically aspect, only severe attacks of Colorado beetle justify the use of insecticides. However, there was some variability between the different field sites and years: plants that were stressed or not fully developed (due to drought periods, N fertilizer defaults, slow growth or period with low temperature, etc..) seems to be more sensitive to the loss of part of the foliage than those cropped in normal conditions.

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# WILD SPECIES AS GENETIC RECOURCES OF RESISTANCE TO COLORADO POTATO BEETLE (LEPTINOTARSA DECEMLINEATA)

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#### Introduction

The Colorado potato beetle (CPB) is the most important insect pest in many potato-growing areas of the world. Control of this pest has become more difficult as it quickly becomes resistant to new insecticides. An alternative approach of control is to breed resistant potato varieties.

#### Materials and methods

Wild diploid tuber-bearing Mexican potato species were screened for resistance to CPB. Somatic hybridization by protoplast fusion and sexual back crosses were applied to combine the genomes of these wild species and with that of cultivated potato to produce pre-breeding lines. A bioassay was used to analyze the effect that feeding on potato plants has on the development of CPB. Larvae of CPB were reared at 25°C under a photoperiod of 16:8h. Twenty-four hours after hatching 25 larvae were put on the leaves of varieties, wild species of potato, somatic hybrids and different back cross progenies. On every other day the survival of the juvenile stages /larvae and pupae and their weights were recorded. Developmental time, mean relative growth rate (MRGR), mean weights of pupae and adult beetles were calculated. Female and male beetles were identified and pairs transferred to Petri dishes to determine their fecundity.

#### **Results**

Larvae, fed on the leaves of potato varieties took 25–32 days to complete their development, passing through four larval instars and a pupa before emerging as adults. 17 days after hatching 84% (21) of the larvae fed on leaves of cv. Rasant were still alive and had a mean weight of 127mg. 27 days after egg hatch 10 female and 3 male adults had emerged from the pupae.

Adult females reared on the cvs. Agave, Delikat, Rasant or different BC lines from trn (+) cv. Delikat hybrids laid eggs, from which larvae hatched. That the beetle is able to complete its life circle on these cvs. confirms that the bioassay successfully simulated the situation in nature.

When fed on the leaves of gene bank accessions of wild species of potato: *Solanum stoloniferum*, S. *bulbocastanum* (blb), S. *pinnatisectum* (pnt), and S. *tarnii* (trn), only 3–4 larvae survived 17 days and had low mean weights of 14–74mg. Only a few infertile adult beetles emerged from the pupae.

More than 80% of the larvae reared on several BC progenies of trn (+) cv. Delikat and pnt (+) cv. Rasant hybrids reached the fourth instar 15 days after hatching and had a mean weight of 100mg. Fed on the leaves of these plants 12–18 larvae were able to complete their development and emerge as adults. Several of the females laid eggs, which subsequently hatched.

Other blb (+) cv. Delikat hybrids and BC lines were intermediate in their suitability as hosts between the cultivars and wild species of potato. The mean weights of the larvae fed on the leaves of the different hybrids were markedly lower than those fed on the leaves of the varieties.

A limited number of interspecific somatic hybrids of the combination blb or trn (+) cv. Delikat were not suitable hosts for CPB, as the larvae quickly died off or exhibited delayed development and had a low MRGR when reared on these plants.

#### **Conclusions and perspectives**

The wild species of potato used in this study possess low attractiveness for CPB. The produced potato breeding lines with improved CPB resistance can be used as genetic resources in pre-breeding programmes.

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# GENETIC CHARACTERISATION OF POTATO VIRUS Y ISOLATES FROM SEED POTATOES IN BELGIUM

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Potato virus Y (PVY), one of the most important and worldwide pathogens in potato production, is transmitted by a variety of aphid species in a non-persistent manner. Several PVY lineages have been differentiated. The two main strain groups O and N are responsible for most of the damage in potato growing. Apart from O and N, PVYC, PVYZ and PVYE are also recognized as PVY strain groups [1]. Over the past years, a lot of progress has been made in understanding the molecular properties of PVY strains. PVYO and PVYN are considered to be the two parent groups from which several subgroups (PVYNTN, PVYN-Wi and PVYN:O) derived as a result of one to three recombinations [2]. The NTN strain (PVYNTN) is the causal agent of the potato tuber necrotic ringspot disease (PTNRD). Wilga and PVYN:O strains mainly produce mild symptoms and can even remain latent in potato. However, symptoms caused by the same PVY strains can vary a lot, also depending on the potato cultivar. Molecular studies revealed that even within the subgroups considerable genomic variations evolved through recombination in different parts of the genome [3]. Available data indicate that the N strain group (including PVYN, PVYNTN and PVYN-Wi strains) is the most common strain group in Europe. In Belgium, strains belonging to the N group have been reported as the most prevalent by means of serological test, but no detailed information on the relative importance of the PVY groups in Belgium has been published to date.

We report here on a survey performed on Belgian seed potatoes harvested in 2010 in which 2700 individual tubers from individual lots, originating from 54 farms, were screened for PVY presence. This study presents the relative distribution of the PVY lineages across seed potato lots in Belgium. We concluded that NTN is the most dominant group in all parts of Belgium and genetically belongs to the NTNa type. Additionally, the presence of the Wilga strain was confirmed and genetically fully characterized for the first time in Belgium. All Wilga strains belong to the N-Wi subgroup and no N:O variants were identified. PVYO and PVYN appeared less important and were also more restricted in their geographical distribution. No PVYC, PVYE or PVYZ was put in evidence. Further discussion on the genetic variability of strains is presented and based on whole genome sequencing of a selection of nine PVY isolates.

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# DETECTION OF POTATO VIRUS Y (PVY) STRAINS IN SEED TUBERS IN AMIK PLAIN-HATAY OF TURKEY

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#### Introduction

Potato production in Turkey is conducted in Mediterranean, Aegean and Black Sea regions using early cultivars. The main potato producing provinces are Nigde, Nevgehir, Izmir, Bolu and Afyon. Potato wart disease, caused by Synchytrium endobioticum fungus, that appeared in 2004 have caused decreases in potato acreage since that year. This pathogen has led to the production of potato in new regions. This study was conducted to investigate the status of Potato virus Y (PVY) strain types in seed tubers grown in Hatay province due to there is lack of information on the presence of PVY strains in our region and PVY is the most important viral agent causing economic losses in our region and Turkey.

#### **Materials and Methods**

Potato seeds obtained from growers and collected new planted tubers from potato fields in Amik plains of Hatay, Turkey. Tuber samples tested for presence of PVY strains by bioassay and ELISA.

#### **Results and Conclusion**

The results of ELISA tests revealed that tubers were infected with Potato virus Y (PVY) and Potato virus X (PVX). PVY infection was found in plants obtained from germinated seed tubers by the rate of 67.9%. Total of 53 leaf samples were not positively tested for presence of PVX. Of the 109 tuber samples tested were found to be infected with PVY and PVX, 48.6% and 1.8%, respectively. It was found that none of the seed tubers were singly infected with Potato leaf roll virus (PLRV). Mix infection rate of PVY+PLRV (2.7%) was higher than PVY+PVX (%0.9). PVX was found a very low rate in tuber samples when compared with PLRV. The result of monoclonal antibody for PVY strains showed that the frequency of PVYN was 64.1%. PVYO, PVYC and PVYNTN were not found in tested tuber samples. PNYN was found as causal PVY strain in both dually infections with PVYN+PLRV and PVYN+PVX. The results of ELISA were supported by exhibiting of vein necrosis symptom on *Solanum nigrum* and tobacco test plants and by observing necrosis symptom in a few cutting tubers (2%). This result showed that the PVYN was the most common strain and it was suggested that there might be other PVY strains by a low rate in potato growing area in the region. The symptoms related to infections of other pathogens such as Synchytrium endobioticum etc. were not inspected on seed tubers in 2013.

Recent reports on reclassification of PVY strains are showing that intensive studies by using advance techniques on population of PVY strains in potatoes and characterization of the new PVY isolates in potato and other host crops in Turkey are necessary to clear the understanding of present status of PVY strains in Turkey.

## NATURAL TRANSMISSION OF POTATO VIRUS Y (PVY) IN EASTERN MEDITERRANEAN REGION OF TURKEY

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#### Introduction

Potato is one of the main crops in Turkey where the production is about 4.8 million ton. In recent years, importance of early potato cultivation has been increasing in Eastern Mediterranean region including Çukurova and Amik plains of Turkey. Potatoes are grown as early crop between January and late May-June in the region. Although, some potato viruses including PVA, PVS, PLRV, PVX, and PVY are widespread in the plains, aphid transmissible viruses are the main pathogens in potato fields.

#### **Materials and Methods**

In this study, PVY was found to be still one of the most common virus cause economic losses in potato production areas in the Eastern Mediterranean region by biological indexing and ELISA.

#### Results and conclusion

Weeds (Amaranthus retroflexus L., Chenopodium album, Convolvulus arvensis L., Portulaca oleracea, Sinapis arvensis L. Solanum nigrum and Sonchus oleraceus L.) were determined to have important role as reservoir both viruses and their vectors in potato fields. Although, PVY is efficiently transmitted by aphid species such as Myzus persicae (Sulzer), Cuscuta sp. was also found to be a vector to transmit the some pathogens including viruses and phytoplasmas (PVY and stolbur phytoplasma etc.) from infected weeds and potato plants to healthy ones due to the population of that parasitic plant has been increasing in potato field in the region in recent years. Although, necrotic strain of the virus (PVYN) was only found to be present in potato fields in Çukurova and Amik plain, tuber necrosis symptom was inspected in PVY infected tubers related to potato tuber necrotic ring spot disease (PTNRD) caused by PVYNTN in the seed tuber samples getting from growers in the region.

Turkey imports seed potato from European countries. Due to lack of of certified seed, transmission of the important viruses by infected tubers is still the main problem in potato cultivation including seed production in Turkey.

# DETECTION AND VISUALIZATION OF POSPIVIROIDS IN POTATO, POTATO LEAFROLL VIRUS AND APHIDS IN SUPPORT OF TRANSMISSION STUDIES

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To date, viroids are known as the smallest plant pathogens on earth. In potatoes (*S. tuberosum*, L.), Potato spindle tuber viroid (PSTVd) causes a severe, spindle-like malformation of the tubers and dwarfing, rolling and twisting of the foliage, leading to important agricultural losses [1]. Because of its potential economic impact, PSTVd is a quarantine pest in Europe. Next to PSTVd, also other members of the family Pospiviroidae infect *Solanaceous* species. Whether these pospiviroids are transmitted in a non-mechanical way through insects is still unclear. In this study, pest species, such as aphids, are tested for viroid presence and transmission by means of techniques such as RT-qPCR and confocal microscopy, and small-scale transmission experiments. Next to direct transmission, there is the possibility that viroid RNA is co-encapsulated within the virion (e.g. Potato leafroll virus, PLRV)[2]. This phenomenon is called trans-encapsidation and might have large epidemiological consequences. Using the virus as a transport vehicle, viroids might ultimately (co-)infect host plants of the virus. Trans-encapsidation can be visualized by means of transmission electron microscopy, or demonstrated via virus purification, micrococcal nuclease treatment and (q)RT-PCR. Here, we give an overview of our results on viroid localization in plants, insects and viruses. Finally, we summarize the epidemiological consequences of this work.

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COMPARATIVE ANALYSIS OF VIRUS-SPECIFIC SMALL RNA PROFILES OF THREE BIOLOGICALLY DISTINCT STRAINS OF POTATO VIRUS Y (PVY) IN PVY-INFECTED POTATO (SOLANUM TUBEROSUM) CV. RUSSET BURBANK

H. Pappu<sup>1</sup>, K Naveed <sup>2</sup>

Deep sequencing technology has enabled the analysis of small RNA profiles of virus-infected plants and could provide insights into virus-host interactions. Potato virus Y (PVY) is an economically important viral pathogen of potato worldwide. While much is known about this virus, little or no information is available regarding host response to PVY infection. In this study, we investigated the nature and relative levels of virus-derived small interfering RNAs (vsiRNAs) in potato cv. Russet Burbank infected with three biologically distinct and economically important strains of PVY, the ordinary strain (PVY-O), tobacco veinal-necrotic strain (PVY-N) and tuber necrotic strain (PVY-NTN). The analysis showed vsiRNAs of 20-24 nt in PVY-infected plants. Considerable differences were present in the distribution of vsiRNAs as well as total small RNAs. The 21 nt class was the most prevalent in PVY-infected plants irrespective of the virus strain, whereas in healthy potato plants, the 24 nt class was the most dominant. vsiRNAs were derived from every position in the PVY genome, though certain hotspots were identified for each of the PVY strains. Among the three strains used, the population of vsiRNAs of different size classes was relatively different with PVY-NTN accumulating the highest level of vsiRNAs, whereas PVY-N infected plants had the least population of vsiRNAs. Unique vsiRNAs mapping to PVY genome in PVY-infected plants amounted to 3.13, 1.93 and 1.70% for NTN, N and O, respectively. There was a bias in the generation of vsiRNAs from the plus strand of the genome in comparison to the negative strand. The highest number of total vsiRNAs was from the cytoplasmic inclusion protein gene (CI) in PVY-O and PVY-NTN strains, whereas from PVY-N, the NIb gene produced maximum total vsiRNAs. In addition to previously reported conserved microRNAs, 258 non-conserved miRNAs as well as 6 novel miRNAs were identified in PVY-infected potato plants. These findings indicate that the three PVY strains interact differently in the same host genetic background and provided insights into virus-host interactions in an important food crop.

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#### OCCURRENCE OF VIRUSES INFECTING POTATOES IN TANZANIA

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Potato (Solanum tuberosum L.) is an important food and cash crop in Tanzania whose production is performed mainly by smallholder farmers in the Southern highlands [3]. Currently, there is no certification system of seed potato and little is known about viral diseases of potato and their associated symptoms in Tanzania. In 2011, a total of 219 potato leaf samples were collected from 16 potato fields in Mbeya region and tested for virus infection by double anti-body sandwich DAS-ELISA [1]. Of these, 20 samples were pressed on FTA cards, and analysed by reverse-transcription (RT)-PCR [3]. Virus-like symptoms were observed in most fields including yellowish-green mosaic, leaf rolling, and veinal necrosis. The most common, globally distributed potato viruses, Potato leaf roll virus (PLRV), Potato virus A (PVA), Potato virus M (PVM), Potato virus S (PVS), Potato virus Y (PVY), and Potato virus X (PVX) [2] were detected by DAS-ELISA. PVS and PLRV were the most prevalent viruses. All ELISA-positive samples tested positive by reverse transcription (RT)-PCR, but PVY-positive samples were not tested by RT-PCR. Four and five samples ELISA-negative for PVX or PVA, respectively, were positive when tested by RT-PCR, suggesting that the actual incidence of these viruses may be higher than detected by DAS-ELISA. Complete coat protein (CP) encoding sequence of five viruses (PLRV, PVX, PVA, PVS and PVM) were sequenced without cloning (GenBank Accession Nos. KC866618 through KC866622, respectively). The CP sequences were closely related with virus isolates from other countries, which suggested that viruses might have been introduced to Tanzania through potato trade or through introducing new cultivars without indexing for viruses. Results of this study suggest the supply of clean potato seeds to farmers as well as incorporating control methods in order to reduce re-infection with viruses. Further studies that involve isolates from all major potato growing areas would generate more information for better understanding of these aspects with respect to potato viruses. To our knowledge, this is the first report of symptomology, serological, molecular and evolutionary analysis of viruses that infect potatoes in Tanzania.

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### VIRUS SYMPTOMS AND INFECTION IN DIFFERENT CLONES OF POTATO VARIETY `AGRIE DZELENIE'

K. Liiv 1,2, V. Rosenberg 2, M. Mänd 1

There are four common potato viruses currently found in Estonia: PVY, PVX, PVM and PVS. In addition, PVA and PLRV have been found earlier, but have not been present recently. However, the presence of the six viruses mentioned above, is routinely tested in laboratory, because the visual identification in the field is usually not possible. The same virus can give different symptoms. The symptoms variability may be due to the potato variety or of the different viruses combination. Moreover, virus infection-like symptoms can be caused by non-viral factors for example by insects.

This makes difficult to identify the virus visually before it has been actually tested in laboratory and compared with visual symptoms.

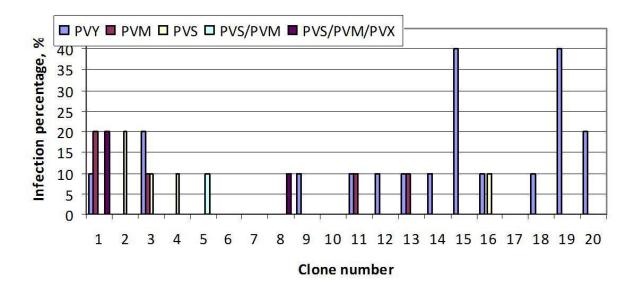
The aim of the research was to compare visual virus symptoms to the laboratory virus tests results in different clones of one potato variety. The study was carried out using the potato variety `Agrie Dzeltenie´, which originates from Latvia. `Agrie Dzeltenie´ is known to be early to middle early variety with a high yield. The variety was selected because it is relatively late blight resistant, does not get the normal scab, but is susceptible to viral disease.

The experiment was carried out in the test field. Ten tubers of 20 clones were planted. All test plants were described for nine visual symptoms: mosaic, rugosity, necrosis, rolling of leaves, growth retardation, discoloration, top necrosis, dead leaves hang, boat-shaped leaves on 3 point scale: 3 - high, 2 - middle and 1- low. The leaf samples of all plants were tested by ELISA for occur of PVX, PVS, PVA, PVM, PVY and PLRV. From 200 tested plants- 49 were visually injured and 151 were evaluated as not injured. Percentage of test results in all clones together were: PVY 10% (20 plants), PVM 2.5% (5 plants), PVS 2.5% (5 plants) and mixed viruses PVM/PVS 1.5% (3 plants), PVM/PVS/PVX 0.5% (1 plant). Viruses PVA and PLRV were not found. Additionally we found that there were differences in virus infection between the clones. Some clones were not infected by any virus and some clones were infected by one virus (PVS or PVY), whereas other clones were infected by the mix of viruses PVM/PVS and PVM/PVS/PVX (Figure 1.).

The most common virus symptoms of the different clones were mosaic, rugocity, necrosis, growing retardation and top necrosis. Visual assessment was not identified four symptoms: rolling of leaves, discoloration, dead leaves hang and boat-shaped leaves. Different clones showed a variety of symptoms of different strengths (Figure 2.). All clones differed from each other in symptoms as well as in the amount of infected plants.

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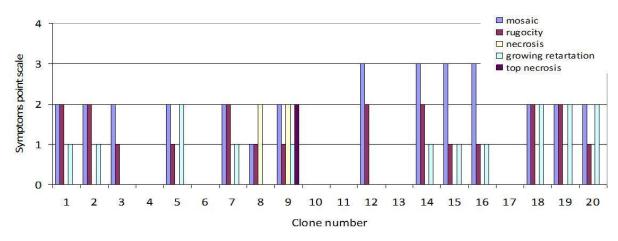


Figure 1: Virus infection percentage in different clones of variety `Agrie Dzeltenie´, analyzed by ELISA test

**Figure 2:** The symptoms varying degree in different clones of variety `Agrie Dzeltenie' (3 point scale: 3 - high, 2 - middle and 1- low)

# OCCURRENCE, DISTRIBUTION AND CHARACTERIZATION OF POTATO MOP-TOP VIRUS IN NORTHWEST OF PAKISTAN

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Potato mop-top virus (PMTV) has fragile rod-shaped particles, transmitted by the soil-borne plasmodiophorid vector, Spongospora subterranea f. sp. subterranea (Wallr.) Lagerth. [1] and is a type species of genus Pomovirus, family Virgaviridae. The Northwest of Pakistan is leading seed potato producing area of the country. Long presence of powdery scab in potato in the region led us to investigate the occurrence of PMTV in potato cultivars commercially grown in the region. We surveyed the hilly region particularly, Malakand and Hazara divisions over three years i.e., 2010-2012. We found that PMTV is prevalent in Northwest of Pakistan and were detected in almost all potato cultivars commercially grown in the region [2]. The highest mean percent incidence (19.0%) of the virus was reported from a field in zone F (Abbottabad) followed by 15.6% in zone G (Manshera) in Hazara division. In Malakand Division (Zone A-E) the incidence was lower (5 to 12%) than Hazara Division. We observed an increase in virus incidence in 2012 as compare to 2010 and 2011 in all major potato growing zones. The identity of the virus was confirmed through bait bioassay, enzyme-linked immunosorbent assay (ELISA) and reverse transcription-polymerase chain reaction (RT-PCR) in leaves and roots of bait plants, tubers of commercially grown cultivars and also in zoospores of its vector, S. subterranea. A products of 566 bp were amplified from coat protein region of PMTV RNA 3 in both root and leaf samples of baited plants. The virus was detected in 10 potato cultivars commercially grown in the region using DAS-ELISA and RT-PCR [3]. Our results indicate that bait plant bioassay, infectivity assay, ELISA and RT- PCR can detect PMTV in roots and leaves of baited plants, field samples, zoospores of S. subterranea and tubers of 10 potato cultivars commercially grown in the region.

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#### DIAGNOSTIC OF THE QUARANTINE AND DANGEROUS POTATO VIRUSES IN RUSSIA

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Potato is an important food crop in Russia. One of the reasons of the loss of quality seed potatoes are viral diseases. To assess the infectiousness and reduce the risk of importation of infected material is necessary to conduct sampling in the field and testing imported seed potatoes. During importing potatoes there is a risk of spreading absent quarantine or dangerous viruses in Russia: Andean potato latent virus (APLV), Andean potato mottle virus (APMoV), Potato yellowing virus (PYV), Potato yellow dwarf virus (PYDV) (all EPPO A1 list, absence in Russia).

In our work were evaluated methods of diagnosis and identification of some quarantine for Russia and the EPPO-region potato viruses. As a positive control were used reference isolates of different strains (APLV-Col PV-0060, APLV-Hu PV-0061, APLV-Col-2 PV-0062, APMoV-B PV-0057, APMoV-C PV-0058, APMoV-H PV-0059, PYV PV-0706, PYDV PV-0719) obtained from the DSMZ collection (Germany).

In the past, the main method for detecting potato viruses was ELISA. In our research, 2009–2013 years, were tested ELISA kits of different manufacturers: Adgen-Neogen (UK), Bioreba and Loewe (both-Switzerland), DSMZ (Germany). Were used the reference isolates of the viruses and potato leaves of different varieties and origins.

For detection and identification of quarantine pest EPPO recommends using the alternative method for confirmation the results. Because of absent information about existing of the PCR tests for studying viruses, primers were designed and molecular diagnostic was developed. For APLV, APMoV and PYV the primers APLV Rep F/R, APMoV Rpol F/R and PYV-rep3-F/R were designed to RNA-dependent RNA polymerase (RdRp) gene. In addition, for the diagnosis of virus non-quarantine for Russia, PYDV, were designed the pair of primers (PYDV F/R) to the phosphoprotein mRNA (P mRNA) gene.

For developing PCR with new primers were used the references isolates of DSMZ (Germany) and VNIIKH (Russia). Were demonstrated high specificity and absence of cross-reactivity between the studied potato viruses, as well as the absence of false-positive reactions with various viruses that infected potatoes (PVX, PVY, PVS, PVM, PVA, PLRV, PVT, PBRSV, TBRV, TRSV, TSWV). Nonspecific reaction with the sap of the healthy potato plants also not observed.

All samples that showed a positive reaction even against the one of the ELISA kit were checked by new primers. During the analyzing of the ELISA results were shown lots of nonspecific reactions of the kits with the samples and false-positive results with non-European potato virus isolates. All results were confirmed by the new designed primers.

According to the results the best specificity are shown the DSMZ ELISA kits: APLV As-0005, APMoV As-0002, PYDV As-0023 [1]. In 2013, were tested the potato plants against ELISA kit DSMZ PYV As-0599 (the singular produced commercial kit) and the positive results were obtained. After the PCR with the primers PYV-rep3-F/R, these results were not confirmed; the plants were free from PYV. By conducting additional experiments were shown that samples infected by PVY and DSMZ kit have nonspecific reaction presumably with it.

Thus, it was shown that for the diagnosis APMoV, APLV and PYDV preferably use the DSMZ kits, as well as a confirmatory test with PCR primers designed in our study. For testing samples against PYV also preferably use new PCR primers that had been mentioned or use DSMZ kit and after test all positive samples against PVY.

### FREQUENCY AND TYPE OF NECROTIC REACTIONS CAUSED BY PVYNTN ON POTATO TUBERS AT DIPLOID LEVEL

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Potato virus Y (PVY) has a world-wide distribution and is one of the most important pathogens affecting potato. A PVYNTN strain was first found in Europe in 1984 (Beczner et al., 1984) and was later reported by numerous authors. It appears that most of the PVY strains and isolates can provoke more or less intense necroses on the tubers of some potato genotypes, if they are exposed to certain conditions. To get more information about the PVY necroses causing factor in potato genome the research on the diploid level was performed. Diploid material was provided by Agriculture Canada Research Station. The first showed that the necroses appear after PVY infection only on certain potato genotypes. Two diploid clones were identified as the donors of virulent genes which are involved in the appearance of the necroses on tubers (Dolničar, 2005). In further research, 14 different, some potentially PVY resistant diploid families and some susceptible ones were tested. 100 seeds of each combination were sown in a seed bed and replanted in 10 x 10 cm pots. All tubers were harvested and multiplied in a screenhouse next year. 10 tubers per genotype were planted in the open field and exposed to the natural infection with PVY during the growing period. They were visually inspected after harvesting in September. Non necrotic tubers were stored at 15°C until March and visually inspected two times during this period. The presence of PVY in non-necrotic tubers was checked by DAS-ELISA using commercially available antisera (Bioreba). Within the tested diploid families, necrotic tubers were counted, the ratio of necrotic tubers was estimated for each individual genotype and type of necrotic reaction was determined.

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### **DISEASES** (poster session)

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# SIMPLE NEW METHOD FOR DNA AND RNA ISOLATION FROM POTATO TUBERS FOR MOLECULAR SCREENING OF DISEASES

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There is need to identify a simple, cheap and efficient protocol that can be used for extraction of high quality DNA and RNA for purposes of molecular work. Potato is an important crop in the sub-Saharan region for food safety and improved livelihoods. There are various local applications of molecular tools in germplasm characterization and disease diagnostics and the available methodologies are expensive for developing Countries. There is need to develop a simple, fast and locally available inexpensive protocol that yields high quality nucleic acids. A new molecular method has been developed. Extraction buffer; 0.2M EDTA, 0.1M NaCl and 0.3M Tris HCl. 15% SDS added, incubated at 65oC for 30 min and spun. Ethanol was added to the supernatant and spun to pellet the nucleic acids which were washed and air dried. The results was analyzed on 1% agarose electrophoresis. The results were compared with the CTAB and Dellaporta and had higher yield than the two. Quantification was done by a nanodrop. The method gave high yield and pure DNA and RNA for use for further analysis. The nucleic acids were used in screening of diseases by PCR and RT PCR.

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# TUBER BLEMISHES: PRESENTATION OF THE NEW COLLABORATIVE WEBSITE FOR UNDERSTANDING AND DIAGNOSING TUBER (SUPERFICIAL) ALTERATIONS

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Potato tubers may be affected by a large range of blemishes leading to rejection or downgrading of potatoes. Among these, a number of frequently occurring blemishes have less clear origins than others. They are cited in the literature under variable denominations, and are sometimes loosely attributed to known pathogens following field experience rather than by scientific demonstration (Kochs postulates). These problems/difficulties linked to the complexity of diagnosing tuber blemishes led French and Scottish scientists to jointly organize a workshop during the past EAPR triennal Meeting in Oulu, Finland, in 2011: it gathered together 80 participants from 20 countries. Following the expressed interest of the audience, we agreed to set up a collaborative tool to share knowledge on known, doubtful and unknown origins of blemishes. This website is the product of this effort. Its objectives are:

- to classify blemishes on the basis of their appearance, in an attempt to reveal their possible causes and prevent possible confusion.
- to describe the causal agents of typical blemishes, and to relate them to the different symptoms they are likely to cause.

This site is therefore organized according to each of these objectives. It so provides the possibility of accessing in a simple way information starting either from the observation and categorization of symptoms, or from a known or putative pathogen.

Live demonstration of the website will be available on-site.

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MANCOZEB, A KEY FUNGICIDE FOR THE INTEGRATED CONTROL OF THE MOST FREQUENT AND DETRIMENTAL POTATO DISEASES: LATE BLIGHT (PHYTOPHTHORA INFESTANS) AND EARLY BLIGHT (ALTERNARIA SOLANI).

S Duvauchelle, JP Pollet

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#### Introduction

A sustainable production has to be profitable (good yield, quality, benefit), respectful to the environment and to human beings (producer and consumer). Crop protection must follow the same targets. Integrated Pest Management (IPM) is the method to control late blight, the more severe and frequent disease on potatoes in Europe but also early blight which has increased since some years.

For an integrated control of *Phytophthora infestans*, the first action is the prevention through prophylaxis measures including the destruction of primary inoculum sources.

It is necessary to have good knowledge of the pathogen in the cropped area, the aggressiveness, the virulence (current or evolution of the varietal resistance), the status of fungicide resistance (actual resistance or prevention for such phenomena). The Decision Support Systems have to integrate these data, to give advices according to epidemiologic risks and grower practices (treatments, irrigation...). These advices should propose a choice of fungicides according to their action on the plant and on the pathogen, the stage of development of the crop and the disease pressure.

Even if mancozeb is registered since 1948 in the United States, and 1962 in Europe, United Phosphorus Ltd (UPL) has conducted new studies to confirm the high performances, and show new ones.

### Methods

To confirm the efficacy of mancozeb in an integrated strategy, field trials are set up with replications. To confirm the interest of mancozeb as a tool of management of the durability of the efficacy of the fungicides (frequent resistance of *Phytophthora* to the phenylamines, high risks to others "unisite" fungicides as the Carboxylic Acid Amines (CAA) more and more frequent, declined efficacity of fluazinam on green 33 strains in Netherlands, or after using too low doses in Denmark; mutation F129L in *Alternaria solani* leading to lower efficiency of strobilurines). Laboratory studies have been conducted by Huub Schepers of Wageningen University on late blight.

Market survey to better understand mancozeb usage in France have been performed

### Results

The field trials confirm the interest of this" multisite" fungicide in alternation with "uni-site"fungicides. The laboratory studies show the interest of associations with mancozeb. The survey demonstrate a high use in all type of production according to the DSS advices, with a higher use frequency in low plus-value production such as starch potatoes driven by the excellent performance/price ratio of this fungicide.

#### Conclusion

In IPM, mancozeb is an efficient fungicide, and it is an essential tool to manage the durability of all the other fungicides.

### INTEGRATED POTATO PLANT PROTECTION MANAGEMENT

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Since January 2014, European producers must implement integrated pest management as imposed by the Directive 2009/128/EC establishing a framework for Community action to achieve the sustainable use of pesticides. Integrated pest management is defined as the combination of all preventing and protection methods against bio-aggressors which can minimize the use of plant protection products. However, the reduction of dependency to pesticides implies the appeal to available non chemical methods, which are more or less scarce in arable crops.

For six years, ARVALIS - Institut du Végétal has been developing studies on potatoes protection to build really effective integrated protection methods for this crop.

These studies have covered a large range of actions:

- Synthesis of different preventive measures to discourage the development of bio-aggressors (prophylaxis) [1],
- The development of an innovative decision support system (Mileos®) to assess the risk of late blight dissemination (*Phytophtora infestans*) for minimizing the requirements to fungicides [2],
- The test of elicitors combined with fungicides allowing a significant reduction of applied pesticides quantities [3],
- The experiment for haulm killing of alternative mechanical methods (included flailing) [4], combined or not with complementary optimized application of desiccant issued from bio-control innovations [5]
- The evaluation of new natural or organic sprout suppressants applied during the storage period [6].

Associated with sustainable protection against insects and weeds, the results issued from these works should allow potatoes crops to be the first French arable crop well conducted in respect with integrated pest management rules, according to the European Directive 2009/128/EC. However, the elicitors have not been yet registered as true plant protection products according to the European pesticides legislation. This is the main difficulty we have to take care before broadcasting this new way of potato's protection.

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### **BIOLOGICAL CONTROL** (poster session)

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# MANAGEMENT OF LATE BLIGHT OF POTATO BY THE USE OF BIO-EFFECTORS OF FUNGAL ORIGIN

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Phytophthora infestans, the causal agent of late blight, is a major threat to potato production in conventional as well as organic productions systems. In organic cultures, this pathogen is mainly controlled by copper fungicides. However, this compound may cause environmental problems by accumulation into soil. The European Commission has set rules to decrease drastically its use in the close future. Therefore, alternative approaches are needed to control *P. infestans* in a context of reduced or no application of Cu.

Among the innovative approaches is the use of bio-control microorganisms. From the literature, it is well known that microorganisms may directly or via the production of metabolites control, at least partly, root and shoot pathogens. Here we aimed to screen a collection of filamentous fungi, yeasts and arbuscular mycorrhizal fungi for their bio-control effects against *P. infestans*.

Based on a literature survey, more than 100 fungal candidates were selected from the Mycothèque de l'Université catholique de Louvain (MUCL), one of the world's largest collections of fungi. Their effects against *P. infestans* were tested in vitro with dual cultures. 24 microorganisms were found to have an activity against the pathogen. These candidates were further tested in the greenhouse on potato plants infested with *P. infestans*. And 5 of them seem to induce a strong decrease of *P. Infestans* development during the first week post infection.

These successive selections led us to a restricted library of microorganisms, representing a promising source of bio-control agents against this major potato pathogen.

# SCREENING OF FUNGAL BIO-EFFECTORS WITH ELISA MICROPLATE FOR CONTROL OF POTATO LATE BLIGHT

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Late blight, caused by the oomycete *Phytophthora infestans*, is the most devastating potato (*Solanum tuberosum*) disease worldwide. Infections can lead to total yield loss, due in particular to the rapid completion of the pathogen life cycle within 3-5 days. In Europe alone, control costs and agricultural losses generated by P. infestans are estimated to 1 billion Euros per annum [1].

Copper-based fungicides have been used for decades against *P. infestans*. Their success is due to multisite activity, which lowers the risk of pathogens developing resistance. However, copper is persistent in the environment and their recurrent application led to its accumulation in soils. Conscious of the environmental harm (e.g. on beneficial soil organisms and animals) caused by copper-based fungicides, the EU decided to replace them with other strategies of control of late blight in organic farming [2].

Numerous studies have demonstrated the efficacy of microorganisms such as *Trichoderma spp.* and others fungal antagonists for the biocontrol of plant pathogens and were considered as a potential alternative to control *P. infestans*. Biocontrol agents may act in various ways against their targets (eg. parasitism, competition, production of antimicrobial metabolites). The aim of our study was to combine these different modes of action by associating different fungal biocontrol agents and antimicrobial compounds in a single treatment to control more effectively *P. infestans*.

The study was initiated by development of a rapid method on 96-well microplate with PDA medium for the screening of 150 extracts and culture supernatants of fungi and oomycetes. Fungi and oomycetes were selected from the literature for their biocontrol properties among a collection of above 30,000 strains of filamentous fungi and yeasts like fungi of the Mycothèque de l'Université Catholique de Louvain (MUCL, Belgium). The most promising fungi and oomycetes selected for their strong antagonist activity toward *P. infestans* were tested on leaf disc and plantlet in vitro for confirmation of their efficacy.

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# ENHANCING RESISTANCE OF POTATO CULTIVARS WITH PHOSPHONATE FUNGICIDES IN MANAGEMENT OF LATE BLIGHT IN KENYA

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Management of Late blight caused by Phytophthora infestans has been found to be among the most expensive endavours in potato production. The appearance of new and more aggressive Phytophthora infestans populations and climatic factors which encourage disease development further complicates the management the disease. Chemical control of the disease is possible with the use of fungicides however many of them pose environmental or health hazards in developing countries. The need for safe and economical management alternative for the resource constrained farmers in developing countries prompted the need for evaluating the efficacy of Phosphonate fungicides, the potential of combination with potato cultivars and economic impact as late blight control alternative. The trials were conducted at the University of Nairobi Kabete campus. Phosphonate formulation Agrifos 400 was compared with the conventional fungicide Ridomil and Dithane. Planting was done on 3m x 3m plots with 4 rows and 10 tubers per row of potato cultivars with different resistance levels. Experimental units were replicated three times in a split plot design. The results showed an effect of Phosphonate formulations on potato late blight when applied early to the crop. There was no significant disease control in highly resistant cultivars as was observed in the susceptible cultivars. It was also more economical using Phosphonate formulation Agrifos 400 compared to conventional fungicides Ridomil and Mancozeb. The study showed that there is significant potential in the use of relatively inexpensive Phosphonate fungicides as alternative to the hazardous conventional fungicides.

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# THE ALARM PHEROMONE E-B-FARNESENE AS BIOCONTROL SEMIOCHEMICAL TO REDUCE POTATO VIRUS Y PROPAGATION IN POTATO FIELDS

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The Potato virus Y (PVY) Potyvirus is transmitted to potatoes in a non-persistent manner by aphids. E-B-Farnesene (EBF) is a largely interspecific aphid alarm pheromone which, depending on multiple factors, might have an impact on aphids feeding, winging, moving or escaping behaviors. In addition, predators and parasitoids may also be stimulated by EBF to find aphids.

In preliminary field trials, EBF had been shown to impact PVY field transmission by aphids. With the view of developing an innovative biocontrol method for PVY infestations on potatoes, we assessed the impact of EBF on the virus propagation under field trials carried out in 2013. To ensure both virus and vector pressure, PVY was inoculated on 11% of the plants. Aphids (*Myzus persicae*) were artificially introduced in the plots. Potato leaves were sampled at four collection dates during the growing season and stored at -20°C before RNA extractions. Quantitative RT-PCR was performed to determine the PVY infection rates in the potato plots depending on whether EBF was released or not.

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# VVH 86 086, THE FIRST BIOCONTROL PRODUCT FOR WEED CONTROL WITH HAULM KILLING AND DESSICANT EFFECT

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Developped since 2008 with 70 trials contracted in France and Europe by the company JADE, VVH 86 086 is a first novel biocontrol product with a haulm killing effect for potatoes.

All trials were carried out according to Good Experimental Practice (GEP), results analysis and reporting of all efficacy were carried out in accordance with EPPO guidelines.

Extracted from a natural process, VVH 86 086 is an emulsifiable concentrate (EC) containing Nonanoïc acid. It is a strict contact activity for weed control, dessicant and haulm killing usage with a fast action. Without toxicity for users, consumers and the environment, VVH 86 086 is the new solution to control grass competition and to reduce chemical weed killers usages.

The product VVH 86 086 is aimed to be used as a solution in the integrated grass management programs. It is therefore developed to be used as a stand-alone after grinding or in programmes for haulm killing on potato.

## **WEEDS** (poster session)

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# POTENTIAL FOR REDUCING INPUTS OF HERBICIDE ACTIVE INGREDIENTS IN POTATO WEED MANAGEMENT

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Systems with reduced pesticide inputs, integrated pest management systems and alternative strategies of ecological raw material and food production have been recently more and more developed and applied. Due to a change in potato growing technology (stone separation technology) weed management has been only focused on herbicide applications in recent years [1]. The aim of our trials was verification of potential for reducing inputs of these substances while maintaining corresponding weed control level in potato growing.

Between 2010 and 2013 field trials were established in the potato production region of the Czech Republic in order to verify efficacy of reduced rates of pre-emergent herbicides in potatoes compared to commonly used generalized rates. The trials were performed under conditions of stone separation done prior to planting. Clomazone, flurenacet, flurochloridone, linuron, metribuzin and prosulfocarb were used for pre-emergent applications.

The highest weed control efficacy on studied weeds (*Capsella bursa-pastoris*, *Galium aparine*, *Chenopodium album*, *Lamium purpureum*, *Polygonum convolvulus*, *Viola arvensis*, *etc.*) was determined in linuron and metribuzin combined with clomazone and in a herbicide containing combination of metribuzin and flufenacet.

The efficacy of reduced herbicide rates was more dependent on course of the year, weather conditions at application and weed pressure, similarly as reported by other authors [2]. Only in one of the four years the weed control effect was significantly decreased in these variants compared to variants of standard active ingredient rates. However, on average of years it was recorded that reducing high (generally used) rates of pre-emergent herbicides to 50 % results in decrease of efficacy on dicotyledonous weeds, usually present in the potato production region, from 91 % to 85 %. This difference was not statistically significant. The best results for reduced rates were also obtained in linuron or metribuzine-based herbicides combined with clomazone and a herbicide containing combination of metribuzin and flufenacet.

Herbicide applications in field trials resulted in 27–117 % increase of potato yield in individual years compared to non-treated control. Potato yield derived from variants treated with reduced rates of herbicide active ingredients was comparable to variants with standard rates in all years. The trials indicated that reducing high generalized rates of herbicides is possible while maintaining weed control efficacy and potato yield and quality level.

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### WEED CONTROL IN POTATO CROP USING DIFFERENT METRIBUZIN DOSES

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The paper present resuts of a two year study regarding the influence of different metribuzin doses in potato crop.

Field experiments were carried out in the years 2011-2012 to the National Institute of Research and Development for Potato and Sugar Beet – Brasov, Romania and in Feldioara area, to a local farmer.

All variants treated with metribuzin influenced significantly the increase in potato yield in comparison with the control variants, in which weeds were not suppressed by chemicals. All metribuzin variants gave a significantly higher yield compared to the control one.

On all variants with metribuzin were obtained a uniform yields, which ranged from 25.42 to 29.73 to ha<sup>-1</sup> in 2011, and from 6.28 to 9.55 to/ha in 2012. The year 2012 was caractherized by a very severe drought. For good results it is important to take account of the potential weed reserve in soil and to see the correlation between meteorological data and the intensity of weed infestation.

## **POST- HARVEST and STORAGE (poster session)**

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### THEORETICAL AND PRACTICAL KNOWLEDGE OF POTATO STORAGE

E. T. van der Ploeg (Omnivent, NL)

Mention and demonstration on theoretical and practical knowledge of potato storage. Omnivent can bring a very interesting combination of new insight theoretically in which we also develop new products through Research and Development within our company (examples are **OmniBreeze**: compact pad Humidification system and **OmniRecup**: for high efficient energy cold recovery, in extreme high temperature environments to reduce running hours of refrigeration and ventilation). The practical solutions of potato storage are also based on scientific knowledge on potato cultivation and storage during the years.

In an upcoming biobased economy and a growing demand of food, biomass is playing an important role. To supply these streams, processes involved in the whole agricultural production chain should be improved. The storage process is one of the processes that should be optimized. From a processing point of view, a continuous stream of biomass is desirable. However, the production of, in particular, agricultural products is often non-continuous. Therefore, a storage facility that operates as buffer and maintains, or even improves, the quality of the product is a crucial link between the production location and processing industries.

To keep the quality of a potato at least at a certain level, most of the indoor storage facilities are climate controlled. However, even in the state of the art potato storages, significant losses of quality are the reality. These losses can be reduced if insight between quality and storage strategies, and in particular ventilation strategies, is obtained. This is feasible by doing experiments in the field, and can be stated more generally by making use of the modern simulation techniques, like CFD [1].

For dynamic simulations of potato storages, the development of an indoor climate model of a potato storage facility was investigated. The spatially distributed storage model includes moisture, temperature and flow balances. This model is the basis for simulation, design and, in a follow-up study, control of potato storage facilities. In this research, the mathematical model was implemented in an software environment that solves the set of equations making use of a finite element method (FEM). After calibration and validation of the model, some scenario studies related to the potato quality and energy consumption and to alternative designs of storage were conducted. These scenario studies support the choice of an optimal flow rate and of the position of the ventilation outlet valves.

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### BE CIPC COMPLIANT: A STEWARDSHIP INITIATIVE FOR GREAT BRITAIN

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### Introduction

There have been only seven MRL exceedances (above 10 mg/kg) for the sprout suppressant Chlorpropham (CIPC) in Great Britain since the establishment of a 5-year potato industry stewardship initiative in 2008. The stewardship programme was reviewed by the independent Advisory Committee on Pesticides (ACP) in September 2013. ACP concluded that progress had been made in implementing best practice for CIPC application and store management. However, continuing exceedances are unacceptable and industry committed to the continuation of the stewardship scheme operating on sound scientific principles.

#### Methods

A new stewardship initiative, Be CIPC Compliant [1] has been developed to by industry to ensure that the key messages are developed and delivered to all the relevant participants in potato supply chains. Particular attention is being given to crops held in box stores held at low temperatures (5oC or below) for fresh market use as these account for the majority of exceedances (5 out of the 7 crops).

In addition to the Stewardship Best Practice messages, the potato industry will continue to monitor CIPC residue levels as part of its due diligence to protect consumers. Over the period of the initial stewardship programme only one sample was found to exceed the MRL from 1998 samples tested. The differences between the outcomes of the two sampling strategies adopted by PRiF and industry are being investigated, modifications to the sampling guidelines have been implemented and additional data will be collected to investigate the associated factors that may affect residue levels.

### Results

The outcomes from research studies undertaken, primarily at Sutton Bridge CSR, and in collaboration with industry have led to specific new recommendations on CIPC use being implemented for the 2013-14 storage season. The emphasis is on store owners to take greater responsibility for their crops, their stores and those applying CIPC and advising on its use.

### a) Early application

- Apply the first the first treatment within 3 weeks after harvest (or at the earliest occasion thereafter) even in the absence of signs of breaking dormancy.
- Use low speed fan assistance in all bulk stores and box stores with positive ventilation to aid distribution. Fit inverters to stores that don't yet have this capability.

### b) One application (cold stores)

• In cold stores with a holding temperature of 5°C or below, use just one application of CIPC ensuring the treatment is applied before the temperature is reduced below 7°C. Recirculate air for at least 6 hours, without cooling, prior to application to ensure temperatures are even at the time of treatment.

### Conclusions and perspectives

The potato industry in Great Britain relies on CIPC as its main sprout suppressant and it is used on about c1.8 million tonnes of stored crop. The compliance with a) stewardship Best Practice guidelines, b) adoption of new CIPC use recommendations and c) the additional information being sourced during industry residue monitoring on e.g. store type and management, date of, and temperature at application, will all contribute to improving and understanding the variables affecting the uniformity of distribution of CIPC in stores and reduce the future risk of MRL exceedances.

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<sup>&</sup>lt;sup>2</sup> Sutton Bridge CSR, AHDB Potato Council, Sutton Bridge, UNITED KINGDOM

# COMPARISON OF D-CARVONE AND ETHYLENE TREATMENTS DURING STORAGE OF SEED POTATOES TO INCREASE THE NUMBER OF PROGENY TUBERS

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### Introduction

Agria and Innovator are both high yielding cultivars. Whereas Agria is the first potato cultivar produced in Switzerland with 22% of the planted surfaces, Innovator is the fourth one with 6% of the surfaces [1]. In spite of their high productivity, both of them produce few progeny tubers with an average of 7-12 tubers for cv. Agria and 7-10 tubers for cv. Innovator [2].

In this work, two treatments were compared for their capacity to improve the progeny tubers number produced by these cultivars. Actually, increasing the seed yield of these two high yielding varieties would make their multiplication easier and improve their market share. Ethylene as D-carvon are known to have an effect on increasing the number of progeny tubers [3, 4]. Their efficiencies on cv. Agria and Innovator were quantified and compared in this study.

### Material and method

Seed tubers of cv. Agria and Innovator were stored 120 days in four different atmospheres and temperatures: (i) 4°C without treatment (ii) 6°C with ethylene (Restrain® system) (iii) 8°C with ethylene (Restrain® system) (iv) 8°C with repeated D-Carvone application (Talenton®). Seeds were then planted in field in plots of 50 plants with 4 replications (split-plot design). The following observations were done: length and number of sprouts before planting, emergence date, number of stems of the plants, yield and number of tubers per tuber size (<25mm; 25-35mm; 35-50mm; >50mm). This trial was repeated two years.

### Results

D-Carvone better controlled germination during the storage compared to ethylene. Seed treated with ethylene (8°C) faster emerged and the total and commercial yield harvested was higher compared to the other tested treatments. The number of harvested tubers was higher for seed treated with D-Carvone and ethylene but this effect was stronger for one year of experiment, particularly for seeds treated with ethylene (8°C).

### Conclusion and perspectives

D-Carvone and ethylene were both efficient to increase the number of progeny tubers of cv. Agria and cv. Innovator. Nevertheless, those products should be tested with other cultivars before a large scale use, especially for cultivars physiologically weak and susceptible to de-sprouting.

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### EARLY DETECTION OF POTATO STORAGE DISEASES BY GAS ANALYSIS

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Blackleg' disease of potato caused by bacteria such as *Pectobacterium atrosepticum* and *P. carotovorum* results in losses of ca. £ 30 Million per year to the UK Potato industry and is a serious problem in store. Other bacterial and fungal diseases can also cause affect tubers, at harvest, or in store. Some of the most common include late blight (*Phytophthora infestans*), dry rot (*Fusarium sp.*), gangrene (*Phoma spp.*) and pink rot (*Phytophthora erythroseptica*).

Current disease diagnostic methods are based on visual symptoms, molecular techniques, such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA). These are often time consuming, labour–intensive and may require destruction of the samples. The difficulties ineffectively sampling from all potato tubers in boxes or bulk hamper enables disease to develop in inaccessible areas. There is an increasing need for diagnostic techniques that can provide rapid, accurate and reliable detection of potato diseases at an early stage to help manage stores. We are developing a technology to smell affected tubers to identify different potato diseases, using gas sensing technology. This should enable entire stores or consignments of tubers to be continuously and remotely sampled for disease and provide rapid, accurate and reliable detection of potato diseases at an early stage to help management decisions. Such technology may also be useful in the detection of quarantine diseases at ports of entry. Our recent results will be reported here.

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### REDUCTION OF POTATO TUBER BLIGHT DEVELOPMENT IN STORAGE

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### Late blight in potatoes:

Caused by the oomycete pathogen *Phytophthora infestans*, late blight is the most important disease worldwide, threatening yield and quality of potatoes.

When not controlled adequately, late blight epidemics have the potential to destroy canopies (leaf blight) resulting in considerable yield losses.

Late blight epidemics in the canopy also infect tubers during the growing season (tuber blight), resulting in soft rot of potatoes either pre- or post-harvest. Tuber blight may unexpectedly result in severe losses during storage when latent infections become apparent and soft rot sets in.

### Infinito®:

Bayer's late blight product Infinito® has become established in recent years as a key product in European late blight control strategies

Infinito combines the properties of the active substances fluopicolide and propamocarb-HCl.

Main features of Infinito® result in effective leaf blight control and outstanding protection of tubers: contact, penetrant, translaminar and systemic activity;

long lasting efficacy;

impact on spore viability;

sporicidal activity.

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## TUBER QUALITY (poster session)

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# COMPARISON OF CHEMICAL COMPOUNDS AMONG TWO VARIETIES OF COLOR POTATOES BY ANTI-INFLAMMATORY ACTIVITY

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Potatoes were first introduced outside the Andes region four centuries ago, and have become an integral part of much of the world's food. Potatoes were first introduced into Europe in the 16th century and Korea in the early 19th century. It is the world's fourth-largest crop, following rice, wheat, and maize. In the nutritional aspects, potatoes contain abundant vitamins and minerals, as well as an assortment of phytochemicals such as carotenoids and natural phenols. Chlorogenic acid constitutes up to 90% of potato natural phenols. Due to the high content of potato functional compounds, it has known that potatoes are effective in the prevention of various human diseases. Recently, color potatoes 'Hong-young' (HY: Red color potato) and 'Ja-young' (JY: Purple color potato) were developed by RDA, and it has reported that they have high effect on bioactivity. So it will be expected that is high content of functional compounds. This study was carried out to obtain a basic information for the improvement of human health and the development of variety through analysis of organic compounds, contents of three CQA(3caffeoylquinic acid, 4-caffeoylquinic acid and 5-caffeoylquinic acid) and five anthocyanin(petunidin-3-pcumaroylrutinoside-5-glucoside, pelargonidin-3-p-cumaroylrutin-oside-5-glucoside,peonidin-3-pcumaroylrutinoside-5-glucoside, pelargonidin-3-p-feruloyl-rutinoside-5-glucoside and peonidin-3feruloylrutinoside-5-glucoside) to color potatoes is HY and JY. The analytical results on organic compounds in color potatoes were shown as follow. The contents of CQA and Anthocyanin of JY variety were shown to be higher than HY, while CQA and Anthocyanin were appeared to be highest in peel of JY. Overall, JY had higher amount of physicochemical properties than HY. The anti-inflammatory activities of CHCl3 fraction of JY's peel was evaluated for inhibitory activities against lipopolysaccharide (LPS) induced nitric oxide (NO) and prostaglandin E2 (PGE2) production as well as inducible nitric oxide synthase (iNOS) and cyclo oxygenase-2 (COX-2) protein expressions in RAW264.7 cell lines. The fraction high inhibitory activity for both tests with IC50 values ranged from 25 to 50 µg/ml. The results of analysis study are in remarkably consistent with the anti-inflammatory study. Therefore, JY is expected to be highly valuable items for the development and applications of a functional food (Anti-inflammatory agent, etc.).

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# DEVELOPMENT AND TESTING OF A PHOTO SCALE TO ASSESS THE EXTERNAL TUBER QUALITY OF POTATOES AFTER WASHING

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The importance of external qualities of potatoes has increased permanently in food retail. In July 2011 the German Handelsklassenverordnung was abolished, which regulated the minimum quality requirement of potatoes. Measures were taken in Bavaria to maintain the high level of quality and ensure that Bavarian potatoes are still compatible. This motivated the Bavarian State Research Center for Agriculture (LfL) together with the Landeskuratorium für pflanzliche Erzeugung in Bayern e.V. (LKP) to develop and test a photo scale for scoring the external quality of washed potato samples. It was titled Waschkarten.

Between 2008 and 2010 Waschkarten were developed on the basis of photos in a three-step approach: 1. step: Collection, processing and evaluation of 203 potato samples from the main growing regions in Bavaria and taking reference photographs. The visual appearance of washed potatoes was assessed from scale 1 (very low deficiencies) to scale 9 (very high deficiencies). Because of large differences between samples, it was finally possible to form four well distinguishable groups. 2. step: Photo documentation and definition of four quality classes Premium (best visual impression), Klassisch, Standard and Natur (weakest visual impression), 3. step: Selection of photos that represent the minimum requirements for each class. These were applied to the Waschkarten to help LKP inspectors to define the quality class of washed potatoes.

Evaluation of the field data of these samples showed a correlation between the classification of external appearance and soil type, humus content and variety.

For testing and implementation of the Waschkarten quality auditors of the LKP were trained. Between 2010 and 2012 they assessed about 10,000 samples per annum from lots of table and processing potatoes. Additionally, they collected data on the region of origin, variety, soil type, humus content of the soil, irrigating system and the interval between haulm killing and harvest. The relative frequency of the classes differed depending on the variety. On silty soils the optical appearance was better than on peaty soils. On average, potato lots for processing performed surprisingly slightly better than those for the retail market. Overall, the Waschkarten have proven applicable in practical use and thus haven give the processors and traders important information about the external appearance of a lot. Therefore, the photo scale was introduced for evaluation of the variety trials at the LFL as well.

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# DIETARY MINERALS AND BIOACTIVE COMPOUNDS IN A SET OF PURPLE AND RED FLESHED POTATO GENITORS FOR BREEDING

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Potatoes are considered a good source of minerals and phytochemicals which research suggesting may provide a range of health benefits. Several studies have focused particularly on the nutritional profile of intensely pigmented native potato cultivars or wild tuber-bearing Solanum species. However, although some promising accessions have been described, native and wild potato species present often undesirable characteristics which difficult their production in template areas and even their inclusion in breeding programs. Because of that, we have evaluated the contents of some dietary minerals magnesium, potassium, iron, and zinc -, antioxidant activity, total phenolics, total carotenoids, and total anthocyanins in a set of 19 long day adapted red and purple fleshed cultivars of S. tuberosum ssp tuberosum and S. tuberosum ssp. andigena in order to identify suitable genitors with enhanced nutritional quality for the breeding programme. Minerals were analyzed via ICP-OES spectrometry. The analysis of hydrophilic antioxidant activity were performed using two methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,20-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) [1]. Total phenolics were determined using the Fast Blue BB azo-based assay [2]. Total anthocyanin and total carotenoid content were also determined by spectrophotometric methods [3, 4]. We have found high phytochemical content values among the set of 19 purple and red fleshed cultivars. We have detected particularly high levels of phenolics, anthocyanins, and antioxidant activity in intensely colored purple and red samples, such as the HZPC clone HOT-02-7001 and the cultivars Vitelotte, Peru Purple, and Highland Burgundy Red. Also, high magnesium, potassium, zinc and iron values were found within the collection. Compared to reference data, average levels of these minerals were 3 to 8 fold higher depending on cultivar, flesh color, and mineral [5]. The identification of promising material adapted to long day conditions can facilitate the breeding of new cultivars with improved nutritional characteristics.

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# IMPACT OF COOKING METHOD AND TUBER GLUCOSE CONTENT ON ACRYLAMIDE FORMATION IN HOMEMADE FRENCH FRIES

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Acrylamide (a "probable" human carcinogen) forms in foods from a chemical reaction between asparagine and reducing sugars. This reaction is part of the Maillard reaction, which leads to colour, flavour, and aroma changes in cooked foods. Acrylamide formation usually occurs at elevated temperatures when frying or baking (above 120 °C). In potatoes, reducing sugars levels are considered as the main driver of acrylamide formation. Since its discovery in food in 2002, the international research community has explored numerous strategies for reducing acrylamide in food products. In November 2013, the European Commission set an "indicative" level of 600  $\mu$ g/kg in "French fries ready-to-eat". However, if much work has been devoted to the mitigation of acrylamide in potato crisps and French fries, the literature data on acrylamide in home-cooked potato dishes is limited.

This study assesses the impact of three cooking methods on acrylamide in homemade French fries for different varieties and sugar levels (glucose).

To cover as widely as possible the range of glucose content, five varieties, contrasting on this character were used: Agata, Marabel and Bintje, commonly used on the fresh market; Innovator and Lady Claire, used respectively for frozen French fries and crisps, interesting as results for their low sugar content. The potatoes were stored six months at  $4.5^{\circ}$ C,  $6.5^{\circ}$ C and  $8.5^{\circ}$ C (with CIPC) before the tests. 40 tubers from each condition were assessed for glucose content (Gluco-LIS® Blue method) and used in three frying experiments (strips of  $10 \times 10 \text{ mm}$ ):

- FS: Laboratory/restaurant deep fryer Frymaster: 40 strips in 17 I of oil, 5 min at a stable temperature of 180°C (= reference) [assimilated to domestic frying of individual portion (150 g)]
- FM: Domestic deep fryer Kenwood: (according to the recommendations of the manufacturer) 1 kg of strips in 3.5 l of oil, 14 min starting at 180°C. Measurement shows that the frying temperature drops to 115°C after 2 min and slowly reached 160°C at the end
- FA: Low fat/air fryer Seb Actifry: (according to the recommendations of the manufacturer) 1 kg of strips for 1 spoonful of oil, 45 min (~ 150°C)

Fried samples (20 French fries) were judged on colour by means of the CNIPT/ARVALIS colour card (from 0 = pale yellow to 5 = dark brown) and acrylamide content determinated by LC/MS/MS.

A large variability of tuber glucose content (0.01 % to 0.83 % of fresh weight) and French fry colour (0.0 to 5.0) were obtained in accordance with other variety-storage ARVALIS trials. Similarly, the acrylamide content of French fries ranged from < 50  $\mu$ g/kg to > 3500  $\mu$ g/kg. Overall, acrylamide content was well correlated with French fry colour (r2 = 0.83), both depending on frying method. On average, the French fry colour and acrylamide content were respectively 2.5, 2.1, 1.6 and 1511, 1004, 722  $\mu$ g/kg, respectively for FS, FM and FA. For a given cooking method, the level of acrylamide was well correlated with glucose tuber content (r² = 0.68, 0.86 and 0.82, respectively for FS, FM and FA).

Based on these results, to respect a maximum acrylamide level of  $600 \,\mu g/kg$  in homemade French fries, tuber glucose content should not exceed 0.06, 0.14 and  $0.19 \,\%$  fresh weight, respectively for FS, FM and FA frying methods. On the material tested, independently from variety and glucose tuber content, the use of a low fat/air fryer has reduced by approximately  $50 \,\%$  acrylamide in French fries, compared to a deep frying at  $180 \,^{\circ}\text{C}$ .

# POTATO TUBER IMPACT SENSITIVITY IS INFLUENCED BY PERIDERM MAGNESIUM AND CALCIUM CONTENTS

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### Introduction

Mechanical impact leads to visual defects in the periderm of potato tubers (Solanum tuberosum) called "thumbnail cracks". The name comes from the shape of the crack in the tuber skin, which looks similar to a thumbnail. Thumbnails can develop from a slight injury that just breaks the tuber periderm but does not bruise the underlying tissue. After the damage occurs, tubers subjected to low humidity will form the characteristic thumbnail cracks. This tuber defect in fresh-market potatoes is a quality problem because it significantly reduces the consumer acceptance of potatoes [1].

The aim of this study was to evaluate the content of cell wall material and main minerals in the periderm of potato cultivars with different hardiness to thumbnail cracks.

### **Materials and Methods**

After six months of storage, eight potato cultivars were mechanical treated following a standard procedure and the development of thumbnails was recorded. Also, investigations on the composition of the cell wall were performed and compared with the susceptibility to thumbnails.

### Results

The results of the tuber scoring showed significant differences between the eight examined varieties in terms of the expression of the thumbnail symptom.

The content of cell wall material (CWM) of the periderm was significantly negative correlated (r = -0.749\*) with the occurrence of thumbnails, i. e. the higher the CWM content the lower the tuber hardiness against this symptom. In cultivars with low hardiness the CWM content ranged from 29.8 to 38.5 g kg<sup>-1</sup> FM and in cultivars with high hardiness it varied between 18.8 and 29.4 g kg<sup>-1</sup> FM. The content of CWM in the non-periderm was not correlated with the occurrence of thumbnails.

Magnesium and calcium contribute to elastic and gelatinous properties of pectin as main component in the tuber periderm [2]. Both minerals were significantly positive correlated (r = 0.828\* in periderm; r = 0.716\* in non-periderm) with the thumbnail occurrence, i. e. the higher the content of these minerals, the higher the tuber hardiness. In the periderm of cultivars with high hardiness, the sum of both minerals ranged between 3.75 and 4.52 g kg $^{-1}$  CWM, whereas in cultivars with low hardiness it was about 20 to 25 % lower. In the non-periderm similar relationships were observed.

### Conclusion

Potato tubers are characterized by variety-dependent hardiness against thumbnails. The extent of this defect is significantly influenced by tuber properties, as the content of CWM in the periderm and the content of Ca and Mg in the CWM.

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### THE INFLUENCE OF CROPPING SYSTEM ON COOKING QUALITY OF POTATO GENOTYPES

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### Introduction

One of the factors determining potato tuber flesh darkening after peeling or enzymatic darkening is dry matter content [1], as well as the tuber cell turgor. Dry matter content in tubers depends on genotype and the cropping system - especially availability of nitrogen in soil – and intensity of starch accumulation. In tubers with higher dry matter content increases the trend of enzymatic darkening. The tuber flesh darkening after cooking is determined by genotype, but in the potato flesh darkening process the rate of ascorbic acid and chlororganic acids is essential [2]. Ascorbic acid binds iron jones and restrict involvement of compounds which set the darkening. The amount of ascorbic acid in tubers is influenced by the availability of nitrogen in soil.

The aim of the research was to determine darkening of the potato flesh after peeling and cooking depending on genotype and growing technology.

### **Materials and Methods**

Different potato varieties were cultivated in conventional cropping system with two different N rates (60 kg/ha and 120 kg/ha) and in organic cropping system. The field trials were arranged in three fields during 2011-2013. Six varieties with different maturity were tested in all three growing technologies. Additionally four genotypes were tested only in two conventional fields. Dry matter and ascorbic acid content in tubers, hardness of tubers, boiled tuber taste, darkening of tuber flesh after peeling and boiling was determined.

### Results

Dry matter content in tubers was significantly determined by genotype (p<0.05). Neither genotypes nor cropping system significantly influenced boiled tuber taste. The significant influence of cropping system on tuber hardness was detected in year 2011. The influence of cropping system on enzymatic darkening was not significant. The correlation between dry mater content and tuber hardness was significant (p<0.05). Correlation between tuber enzymatic darkening after peeling was not significant neither with dry mater content nor tuber hardness.

Significant influence of genotype and cropping system on ascorbic acid content in tubers was determined in part of assessments, in cropping system with higher available nitrogen content in soil ascorbic acid content in tubers was lower. The influence of genotype and cropping system on tuber flesh darkening after cooking was significant (p<0.05) only in year 2011. Correlation between tuber flesh darkening after cooking and content ascorbic acid was not significant (p>0.05).

### **Conclusion and perspectives**

The significant correlation was found between dry matter in tubers and tuber hardness of analysed potato genotypes and both parameters did not influenced tuber flesh enzymatic darkening. Results present no significant influence of ascorbic acid content in tubers on flesh darkening after cooking.

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### DEVELOPMENT AND SENSORY EVALUATION OF "AKARA" FROM POTATO FLOUR

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### Introduction

Flour from potato were developed in to "Akara" a common Sierra Leonean snack to address post-harvest lost and replace rice flour which are very expensive in making "akara This study evaluated the sensory characteristics of "akara" developed from potato flour."

#### Materials and methods

The potato tubers were obtained from the demonstration farm of Njala Agricultural Research Centre, Njala, Sierra Leone. The flour was obtained by peeling, washing, chipping, sun drying and milling of the potato roots. The "akara" was served to thirty trained panelists to evaluate the taste, flavour, colour, texture, appearance and general acceptability using a nine point hedonic scale. Result indicated likeness of the "akara" with respect to taste, colour, flavour, texture, appearance and general acceptability. Mean scores ranges of attributes evaluated were: taste (2.20 to 3.45), colour (2.50 to 4.10), flavour (2.10 to 4.30), texture (2.05 to 4.05), appearance (2.15 to 4.24) and general acceptability (3.00 to 4.45).

#### Results

Results indicated that taste, flavour, colour and texture of "akara" developed were significant. Potato/rice flour composite "akara" indicated no significant (p>0.05) difference in terms of taste with that of the 100% potato.

### Conclusion

Sensory attributes of the "akara" developed from various sources indicated that rice/ potato composite flour can be used to prepare potato-based snack with no significant variance in consumer acceptability. This recipe is recommended for promotion as a mean of addressing the post harvest losses of potato which is a serious problem in Sierra Leone as reported by a recent study [1].

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### DEVELOPMENT AND SENSORY QUALITY CHARACTERISTICS OF POTATO BASED PRODUCTS

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The aim of this study was to evaluate the sensory characteristics of snacks developed from potatoes. The potatoes were obtained from the local market in the Northern region of Sierra Leone. They were washed, boiled before peeling and pureed using a blender. The mashed potatoes were divided into three portions with one portion each used for developing muffin, fish cake and pancake. The snacks were processed using a composite of 20, 50 and 100 percent of the pureed potatoes. 100 percent wheat flour was also used to develop the different snacks to act as control. The products were served to sixteen trained panelists randomly selected from Ministry of Agriculture Forestry and Food security, Farmers, Traders, Teachers, secondary school pupils and Non Governmental Organizations in Koinadugu districts to evaluate the samples using a nine point hedonic scale in batches of four each. Results generally indicated that addition of potato to wheat flour significantly affected the sensory qualities (taste, flavour, colour and texture) of snacks developed. 50% potato/wheat flour composite snacks indicated no significant (p>0.05) difference in terms of taste and colour with that of the 100% wheat flour. However, significant differences were observed among 20 percent composite potato/wheat flour snacks. The mean scores from the 100 percent potato muffins developed exhibited lower values than all the composite snacks. The results of the sensory attributes of the composite snacks developed from various sources indicated that 50 percent potato/wheat flour composite snacks can be used to develop quality products such as muffins, fish cakes and pancakes with no significant variance in sensory attributes. This Products could be promoted among the Sierra Leone population especially among people involve with food processing, food marketing and value addition. This will also enable sustainability and improvement in the livelihoods of farmers in Sierra Leone that are involved in potato production.

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# EFFECT OF GROWING TECHNOLOGY ON ACRYLAMIDE PRECURSOR CONTENT IN POTATO TUBERS

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#### Introduction

In recent ten years, during potato processing into fried products, an extraordinary attention has been paid to acrylamide (AA) content, which is potentially carcinogenic to human health. Reducing sugars (RS) and free amino acid asparagine (Asn) are acrylamide precursors [1]. Processing contaminant acrylamide is generated by reaction of these compounds. The Confederation of the Food and Drink Industry (CIAA) developed a 'Toolbox' [2]. In addition to the technology of food production the "Toolbox", updated in 2011, also involves not completely clarified effects of cultural practices (N and S fertilization)

#### **Materials and Methods**

In a field trials established in Potato Research Institute Havlickuv Brod, Valecov Research Station between 2007 and 2009 an effect of year, variety, nitrogen and sulphur fertilization was studied on acrylamide precursors (RS and Asn) and acrylamide content. Following varieties were bred for low accumulation of RS during storage: Pirol, Lady Claire, Crispy. Fertilization variants involved nitrogen and sulphur levels, following rates were used: 40 kg N/ha, 160 kg N/ha, 40 kg N/ha + 40 kg S/ha, 160 kg N/ha + 40 kg S/ha.

#### Results

Potato yield was statistically significantly affected only by variety. Year, variety and fertilization variant had a statistical significant effect on starch content. RS are important acrylamide precursors. [3] refers to the importance of variety that acrylamide content in potato products could be substantially reduced especially by selection of cultivar possessing low RS concentration. All studied factors, i.e. year, variety and fertilization variants had a statistical significant effect on Asn content. The results show that year affected AA content similarly as for RS. Within the tested varieties no trend of significant effects of RS and Asn was found, in case of N fertilization effect it was found that for increased N rates tendency of higher AA content is determined. This finding corresponds to the results of [4].

### **Conclusions**

The dependence of RS content in tubers and AA content in fried products, namely chips was confirmed. Year and variety had the highest effect on RS content. In dry and warm year the lowest RS content was found. All studied parameters had a statistical significant effect on Asn content. The relation between Asn and year was opposite than for RS, the content was significantly the highest in the year with highest rainfall deficit (2007). The effect of variety was similar as for RS. Variants with increased nitrogen rate had significantly higher Asn values. Sulphur rate were not significantly expressed.

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# POTATO ANTIOXIDANTS: EFFECT OF ENVIRONMENTAL CONDITIONS AND AGRONOMICAL PRACTICES

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Potato (*Solanum tuberosum* L.) is the most important food crop in the world after wheat, rice and maize with a total planted area of almost 19.25 million hectares and production of 374.4 million tons in 2011. As for the harvested area, potato ranks 7th after wheat, rice, maize, barley, sorghum and rapeseed worldwide. Potato is mainly known to supply dietary fibre, carbohydrates, high-quality proteins, vitamins and minerals. Moreover, in recent studies, it has been shown that potato has antioxidant properties. Many studies suggested health benefits of diets rich in potato phenolic acids as antimutagenic, anticarcinogenic, glucose-lowering, and cholesterol-lowering. Potato tubers contain phenolic compounds including hydroxycinnamic acids, the predominant being chlorogenic

acid and flavonoids, such as catechin, epicatechin and anthocyanins. Potato contains low amounts of carotenoids, such as beta-carotene, indicating that potato is not a good source of pro-vitamin A carotenes; more important are the oxygenated carotenoids, the xantophylls, such as neoxanthin, violaxanthin, antheraxanthin, lutein and zeaxanthin. Potatoes produced in organic cultivation when compared to conventional method contain higher levels of antioxidant capacity polyphenol content and chlorogenic acid. Genetic variability determined from potato tubers will provide an opportunity for future breeding studies directed specifically at enhancing the antioxidative matrix by directed selection for higher concentrations of compounds having these properties. The present paper gives an overview on the potato antioxidants and on parameters impacting their contents in the tuber. Furthermore, it will give an insight into potential health-promoting effects and bioavailability of antioxidants.

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# EXPLOITING THE KNOWLEDGE OF FARMERS ON THE PROCESSING AND UTILIZATION OF POTATOES IN NORTHERN SIERRA LEONE.

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Potatoes (Irish potatoes as it commonly called) are produced by resource poor farmers who see it as a cash crop and called it a rich man's food. In Sierra Leone, its production is low due to it climatic adaption as compared to sweet potatoes which can be grown anywhere in the country. The study aimed at exploiting the knowledge of farmers on the processing and utilization of potatoes. Both key informants and focus group discussions were held in four villages that were randomly selected in the Koinadugu district. Two focus group discussions were held in each village with a maximum of ten participants per group (men and women were separately interviewed). The key informant's interview focuses mainly on the community leaders, farmer group leaders, traders, and household heads. The finding shows that all the participants were aware of the food value of the potatoes but they consider it as a rich man's food. Most of the potatoes they produce are taken to other areas in the country particular in the cities where festivals and ceremonies are often held. When they lack access to markets, due poor road network and high transportation cost the women process the potatoes into different dishes. They boil the potatoes, peel, add palm oil, salt and pepper to it to feed for the day. They also add the boiled potatoes to gravy, stews, and soups. Apart of boiling and eating with gravy, stews or soups, they also use potatoes as complementary foods for their babies. They boil, peel, mash and add powdered fish, some bit of palm oil, and cube magi and serve to their babies. The traders have serious challenge with perishability of the crop which calls for urgent post-harvest handling and storage of the potatoes. Availability of new processing techniques will add more value to the crop. This will increase their income and improve their livelihoods. New products will also be introduced into the country which will provide diverse ways in which the crop can be utilized.

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# MODELING CLIMATE CHANGE IMPACT ON EARLY POTATO CROP AND THE RISK OF FROST DAMAGE IN NORTHERN EUROPE

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Potato (Solanum tuberosum L.) is one of the fourth most important crops after wheat, rice and maize in Europe. The development of the potato is strongly dependent on temperature. In south Europe, high temperature and a declined precipitation amounts is expected to lower the crop productivity in future. At the same time, there is a general tendency to plant potato crops at latitudes above 55°N as the temperature increase in these regions will create a favorable environmental condition for potato to growth. Today, temperature conditions allows to plant potatoes earlier in the season, but this will at the same time increase the risk of frost damage. In this study we aim to assess the impact of climate change on early potato crop and the risk of frost damage in northern Europe. We developed a degree-day model for early potato crop from emergence to maturity and their response to future climate change. The model is based on three different approaches for calculating temperature sums: i) linear growth; ii) upper threshold growth and iii) curve fitted function to estimate the growth of potato. The potential impact of climate change on early potato crop was projected based on a 2°C temperature threshold to estimate the timing of the emergence under two different planting dates: January, 1st (EP1) and April, 10th (EP2) for the time period 1991 until 2100. The climate change signal was assessed using three greenhouse gas emission scenarios (Representative Concentration Pathways: RCP2.6, RCP4.5 and RCP8.5) based on 0.5° latitude x 0.5° longitude gridded data for the period of 2021-2050 (near future) and 2071-2100 (future). For the reference period 1991-2010 we used gridded observed data and compared with two different climate models. The results indicate if the potato planted in the beginning of April (EP2) may not be severely damaged by frost. However, the earlier emergence due to early planting (EP1) increases the probability of frost damage in spring during all years. In general, over the period 1991-2100, all model results indicate a shift in time of the growing season into one month earlier, the average annual change correspond to 3.6 days. Our finding suggest that planting potato in April at latitudes above 55°N may be more favorable due to the reduced risk of frost damage in comparison with an even earlier planting date (EP1). In addition, the early planted potato crops may also prevent the risk of attacks by Colorado potato beetle (Leptinotarsa decemlineata, Say) in future. Today, in many of these regions, the beetles are not yet established, but they could become harmful for late potato in future.

### SEED POTATO DEVELOPMENT PROJECT IN TANZANIA

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### Introduction

Potato is playing an increasingly significant role in food security in sub-Saharan Africa. In highlands of south-western Tanzania potato and maize are the main food crops. However, systematic development of potato production is needed to improve the currently low yields. Experiences in other East African countries indicate that using healthy seed potatoes, yields can be increased by 2-3 fold in the local production systems. The overall objective of the Seed Potato Development Project in Tanzania (SPDP-Tz) is to improve the livelihood of small farmers and rural populations through improving the local capacity to ensure and sustain production, supply, and use of healthy seed potatoes. To achieve the aim, SPDP-Tz strives to establish capacity for pre-basic seed potato production, seed potato certification, seed potato crop management and diagnosis and control of potato diseases and pests.

### **Materials and Methods**

The programme is implemented in the south-western highlands of Tanzania in Mbeya, Njombe and Iringa by the Tanzanian Agricultural Research Institutes (ARI-Uyole and MARI). The Tanzanian Official Seed Certification Institute (TOSCI) will be responsible for quality assurance. These institutes work in a partnership with the International Potato Center (CIP) and the whole seed potato sector of Finland (MTT ProAgria, SPK, Finpom, Perunamestarit, Evira, Oulu Univ. Appl. Sci. and Univ. Helsinki (coordinator) providing trainings. SPDP-Tz is supported financially by the Ministry for Foreign Affairs of Finland.

### Results

The project started in 2012. Fourteen Tanzanian experts have been trained in Finland. After return to Tanzania they have established pre-basic seed production of four potato varieties suitable for the local growth conditions. Facilities and trained personnel are in place for in vitro propagation of potato in ARI-Uyole and ARI-Mikocheni, and also for minituber production using aeroponic and sand-hydroponic systems introduced by CIP. Diffused light storages for seed potatoes are in place. The first field generations of seed potatoes have been produced in the Igeri sub-station (>2000 m above sea level). They will be distributed for further propagation to 59 established farmer groups. Farmers will be trained to "select the best" [1], i.e., harvest the tubers of healthy-looking potato plants for use as seed. Disease surveys are on-going and the first report on viruses occurring in potato crops in the south-western highlands of Tanzania is available [2].

### **Conclusion and perspectives**

The personnel trained in SPDP-Tz has successfully initiated seed potato production in Tanzania. Potato is a well-established crop in the south-western highlands of Tanzania and local small farmers have experience on cultivation of the crop under the prevailing climate and conditions. This is supposed to enhance adoption of improved cultivation practices and proper use of the healthy seed potatoes which will be made available for the local farmers for the first time. It is pivotal to build and strengthen the whole potato value chain in Tanzania, which requires efforts that are beyond the scope of SPDP-Tz.

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## SUSTAINABLE POTATO PRODUCTION (poster session)

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### CHARACTERIZATION OF THE GEOGRAPHICAL ORIGIN OF POTATO TUBERS IN GERMANY

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#### Introduction

The distinctive characteristics of an agricultural product result among others from climate, soil conditions, production systems and local breeds. The European Union developed a policy to protect geographical indications using Protected Geographical Indication and Protected Designation of Origin [1]. The determination of the geographic origin is important for protection of the consumer from deception and overpayment and of the food industry using raw material. In this study, we investigated the suitability of stable isotopes ratio to characterize the geographical origin of potato tubers cultivated in three typical potato-growing areas in Germany.

### **Materials and Methods**

Several cultivars were grown during 2010 and 2011 in three German regions: northern region (about  $52^{\circ}N$ ), western region (about  $50^{\circ}N$ ) and southern region (about  $48^{\circ}N$ ). Tubers of medium size (35 to 65 mm diameter) without visible damage and green spots were used as samples. The determination of the stabile isotopes 2H, 18O and 13C in freeze-dried material was performed using an isotope ratio mass spectrometer coupled with a Conflo III interface and Elemental Analyser. Isotopic ratios were expressed as delta notation ( $\delta$ ).

### Results

Stable isotopes  $\delta^{18}O$  seems to be the parameter with the highest discriminatory power amongst the determined stable isotopes, mainly for differentiating between potato samples obtained from the southern region and the two other regions. The long distance from coasts to a region, along with the progressive effect of the higher altitude allows a greater depletion of the heavy isotopes of water ( $^2H_2^{18}O$ ). Therefore, samples obtained from the southern region, tended to lower  $\delta^{18}O$  values. Furthermore, more complex data analysis, such as discriminant analysis, is required to increase the ability of isotopic data in discriminating samples from the northern and western regions, the two regions with relatively low continental and altitude effects. In the present study, other stable isotopes also contributed to the discrimination of potato samples from the northern, western and southern regions. Nevertheless, because of environmental effects some samples might be misclassified. Therefore, combining stable isotopes with other parameters such as macro- and micro-elements could be more suitable to increase the discriminatory power of the parameters characterizing the geographical origin of potato tubers.

### Conclusion

By means of the stable isotopes  $\delta^{18}O$  and  $\delta^{2}H$  it was possible to discriminate most of the potato samples originating from the southern region from the samples coming from the two other regions. Also, the application of canonical discriminant analysis increased the ability of isotopic data to discriminate between samples from the northern and western regions.

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# EFFECTS OF GM POTATO CULTIVAR MODENA ON SOIL BIOTA REMAIN WITHIN THE RANGE OF EFFECTS INDUCED BY GENOTYPIC VARIATION

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Current techniques allow commercial development of marker-free genetically modified (GM) crops, i.e. cultivars without plant-transformation markers such as kanamycin resistance. One of these crops is a potato cultivar that is modified in starch composition. Normally, starch in potato tubers consists of amylose and amylopectin in a ratio of about 1:5, but tubers of the cultivar Modena produce amylose-free starch. This was accomplished by the inhibition of the transcription of the granule-bound starch synthase gene [1], which is essential for amylose production. The resulting amylose-free starch is an attractive ingredient for a range of industrial applications.

Since this particular trait is a direct inhibition of a pathway in the carbohydrate household and a plant-transformation marker is absent, environmental effects due to this GM crop can be related to its metabolism. Depending on the nature of the modifications, soil systems could be affected by GM plants through a trait-mediated shift in root physiology, root exudates and/or litter quality [2]. We conducted a field experiment at two sites in which microbial activity and nematode communities were assessed at two time points from plots grown with cultivar Modena, its parental isoline and four conventional cultivars. Besides that, we evaluated whether plant litter decomposing under controlled conditions resulted in distinct effects on carbon and nitrogen cycling and if Modena prompted a home field advantage, i.e. plant litter might decompose more rapidly beneath the cultivar it was derived from than beneath a different cultivar [3].

No GM induced changes were observed in soil biota, while significant effects of location and time of sampling were detectable. Analysis with plant traits and soil characteristics revealed that soil moisture and organic matter explained a large part of the variation in both microbial and nematode data. The only observed GM induced effect was a slight home field advantage for Modena in nitrogen mineralization, yet this was not consistent. Location effects were observed on decomposition. In combination with from previous studies on other components of the soil food web [e.g. 4], we conclude that the GM cultivar Modena had no distinct effects on soil biota or soil processes during either its growing season or its litter decomposition as compared to conventional potato cultivars.

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MERINOVA: METEOROLOGICAL RISKS AS DRIVERS OF ENVIRONMENTAL INNOVATION IN AGRO-ECOSYSTEM MANAGEMENT: APPLICATION TO POTATO CULTIVATION IN BELGIUM (HTTPS://MERINOVA.VITO.BE)

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The BELSPO funded project 'MERINOVA' deals with risks associated with extreme weather phenomena. The major objectives of the proposed project are to characterise extreme meteorological events, assess the impact on Belgian agro-ecosystems, characterise their vulnerability and resilience to these events, and explore innovative adaptation options to agricultural risk management. The project comprises of five major parts that reflect the chain of risks:

- (i) Hazard: Assessing the likely frequency and magnitude of extreme meteorological events by means of probability density functions;
- (ii) Impact: Analysing the potential bio-physical and socio-economic impact of extreme weather events on agro-ecosystems in Belgium using process-based modelling techniques commensurate with the regional scale;
- (iii) Vulnerability: Identifying the most vulnerable agro-ecosystems using fuzzy multi-criteria and spatial analysis:
- (iv) Risk Management: Uncovering innovative risk management and adaptation options using actornetwork theory and fuzzy cognitive mapping techniques; and,
- (v) Communication: Communicating to research, policy and practitioner communities using web-based techniques.

The different tasks of the MERINOVA project require expertise in several scientific disciplines: meteorology, statistics, spatial database management, agronomy, bio-physical impact modelling, socio-economic modelling, actor-network theory, and fuzzy cognitive mapping techniques. The MERINOVA project concentrates on promoting a robust and flexible framework by demonstrating its performance across Belgian agro-ecosystems, and by ensuring its relevance to policy makers and practitioners. Impacts developed from physically based models not only provide information on the state of the damage at any given time, but also assist in understanding the links between different factors causing damage and determining bio-physical vulnerability. Socio-economic impacts enlarge the basis for vulnerability mapping, risk management and adaptation options. A strong expert and end-user network is established to help disseminate and exploit project results to meet user needs.

The outlined project methodology will be demonstrated for the case of potato cultivation in Belgium.

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# STUDYING THE INTERACTION BETWEEN ENDOPHYTIC BACILLUS SPECIES AND THE POTATO HOST PLANT

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There is an increasing interest in studying endophytes. Endophytes are microorganisms living inside plants without doing any visible harm. Research has shown that these microorganisms are involved in promoting plant growth. This is done by production of antibiotics capable of eliminating pathogens, direct competition towards pathogens for nutrients, detoxification of toxic compounds and induction of plant defence signalling. Therefore, such organisms are popular to study and possibly use in agriculture as an alternative to chemical control of diseases.

Based on research done on mycorrhiza and rhizobia, it is shown that beneficial microbes initially induce the defence system of plants only to suppress it later in order to be able to colonize.

In many randomly sampled plants, including potato, the bacterium Bacillus mycoides has been found. While it has occasionally been seen inside plants, not much is known about its role. Preliminary studies have shown that it colonizes plants rapidly. Experiments using micro electrode ion flux estimation techniques have demonstrated that B. mycoides can temporarily suppress the immune system of potato plants as well.

We are interested in unravelling the mechanisms by which B. mycoides enters the plant. In order to do this a large-scale survey was made where hundreds of isolates of B. mycoides were obtained from bulk soil, rhizosphere and endosphere. Genetic fingerprinting allows us to classify B. mycoides into various genetic groups. Goal here is to find particular typical groups of endophytes and compare them to typical non-endophytic groups of B. mycoides, suggestion active selection by either plant and/or the microorganism.

Studying differences on both a genetic and phenotypic level between these groups provides insight into what makes a successful endophyte. As B. mycoides is a very common soil- and endosphere organism and is easily cultured, it makes for a good case study.

From the plants point of view we study the interaction with various cultivars and possibly identify key molecules or genes involved in the symbiosis.

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# SUSTAINABLE POTATO PRODUCTION THROUGH USE OF CROP ROTATION AND CROP INTERCROPPING

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Potato (*Solanum tuberosum*) production in Kenya is increasing as a result of people opting for potato crop other than other food crops. However, due to poor fertility management, continuous potato farming among other challenges potato production levels are low than expected [1]. Crop rotation has been used for long and has numerous benefits such as breaking pathogen cycles, using nutrient at different soil depths as well as some crop families fixing nitrogen in to the soil. Crop intercropping has numerous benefits especially due to the crop diversification thus enhancing food security and nutritive foods [2]. A study has been conducted at the University of Nairobi Kabete Campus farm using six crop rotation patterns and a control with potato only for three seasons. The aim of the experiment is to determine the best economically viable potato rotation pattern. Crops used for the rotation patterns included legume (garden peas lupin bean and lima bean), fodder crops (Napier grass, Lucerne and desmodium) brassica (kales and cabbages) and sweet potatoes. Analysis of variance for potato yield in the various rotation and intercropping pattern at the third season were significant at (P <0.05) with p values of 0.007. Crop rotation pattern with potato planted after lupin bean intercropped with sweet potatoes and cabbage had the highest yield. Closely they were followed by those with garden peas. In legume crops planted after cabbages can prove beneficial for East African farmers.

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### BENEFICIAL MICROBES: A ROLE IN FOOD SECURITY?

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The most pressing issues facing the human race today is that of global food security, ever more vulnerable in the wake of major disturbances e.g climate change. Agricultural practices, globally, have to become more sustainable. Legislative changes in relation to the control and use of agrichemicals (especially in Europe) are dictating a more integrated approach for disease management. Although advances in plant science help us to develop and manage crops better, and the use of agrichemicals can provide a short-term solution to some disease problems, a much better approach is to develop and incorporate alternative crop management practices throughout the world. Over the last few years, much research has focussed on the use of beneficial rhizobacteria in developing integrated disease management systems for improving crop quality, whilst reducing the use of agrichemicals. The beneficial microbes naturally colonized the nutrient-rich rhizosphere and benefit plants in a number of ways. Our research project "VALORAM" (http://valoram.ucc.ie), funded under FP7, examined the role of Andean microbial communities in sustainable development of potato cropping systems. The focus of the research is on how certain rhizobacterial inoculants can enhance plant growth and offer disease protection. During the course of our research in VALORAM laboratory and field studies were conducted in order to characterize the beneficial properties of rhizobacteria that had been isolated from the rhizosphere of potato. Also, the volatilome of the selected isolates was characterised using gas chromatography /mass spectrometry (GC/MS). Some of these isolates, trialled in the field in their respective countries of origin i.e Bolivia, Peru and Ecuador, showed significant increase in the yield of potato. This strategy and a number of these bacterial isolates show promise for future incorporation into an integrated (potato) disease management programme(s).

## SOCIO-ECONOMICS (poster session)

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# INFLUENCE OF POTATO GROWING TECHNOLOGY ON YIELD, REALISATION PRICE, REVENUES AND COSTS OF PRODUCTION IN THE CZECH REPUBLIC BETWEEN 2010 – 2013 YEARS

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The technology of soil de-stoning prior to potato planting has been expanded in the Czech Republic during 1990s. At present, this technology is a standard for ware potato growing incl. seed potatoes (conventional technology). In the organic farming system growing measures are identical to conventional system, absence of chemical products for plant protection and mineral fertilizers must be balanced with a choice of appropriate measures for potato plant growth and development (1). Potato growing economy is necessary to evaluate in a longer period (at least for three years). The aim of the study was to compare potato growing technologies as regard as revenues, average yields and cost for production.

Economic results were evaluated from potato growing in conventional and organic farms during 2010 and 2013 years. Four organic and nine conventional farms were included into the set of evaluated potato growers. For evaluation and comparison of both technologies from economic viewpoint gross margin was used. Database containing economic results of PRI Havlickuv Brod and economic data from individual companies were used for economic analyses of potato growing in individual years.

Conventional (CT) and organic technology (OT) of potato growing has many differences resulting from way they are done. Agricultural farms in the Czech Republic mostly use conventional technology (CT) for potato growing on larger plots; only a very low portion of potatoes is grown using organic technology (OT). In the year 2011 were organic potatoes in the Czech Republic grown on 172 ecofarms with small overall area 280.7 ha (2). Economic comparison of both technologies: average potato yield was 14.58 t/ha (50.9 %) reduced on ecofarms compared to conventional technology. For CT a statistically significantly higher potato yield was recorded compared to OT. Quite opposite situation was recorded for average realization price — 0.29 EUR/kg potatoes for organic farms and for conventional farms 0.13 EUR/kg. Average realization price was statistically significantly increased in organic technology compared to conventional technology. Total costs per hectare of potatoes were higher in conventional technology (3 337 EUR), in organic farming they were lower by 15 % (2 896 EUR); total costs per kilo of potatoes were higher in organic farming (0.19 EUR/kg) whereas in conventional technology they were lower by 42 % (0.11 EUR/kg). OT was statistically significantly more costly compared to CT on the significance level of 0;01. Rate of profitability without inclusion of operating supports ranged between 13.1 % (CT) – 47;7 % (OT).

By economic comparison of conventional and organic technology of potato growing substantial differences were found in basic economic factors. Average yield is conclusively higher in CT (by 14.58 t/ha). Average realization price was 0.16 EUR/kg higher, overall revenues in OT were twice higher than in CT (by 162.15 EUR/t of potatoes). Cost of production (total rate of costs per tonne of main product) was higher for OT by 71;5 % (by 80.44 EUR/t of potatoes). We found, that both evaluated technologies reached positive rate of profitability with supports and also without them.

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### ORGANIC POTATO PRODUCTION (poster session)

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## EVALUATION OF COPPER-FREE LEAF TREATMENT PRODUCTS FOR THE CONTROL OF LATE BLIGHT IN ORGANIC POTATO FARMING

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Potato late blight (*Phytophthora infestans*) can cause severe losses in potato yield and quality in organic farming. Still, in organic production *P. infestans* can only be effectively controlled by the application of copper fungicides. Due to their accumulation in the soil and expected detrimental effects on environment and non-target organisms, a reduction in the usage of Cu fungicides is urgently required. Within the course of a project aiming at the reduction and avoidance of Cu in organic farming, trials are being performed investigating the efficacy of different Cu-free products for the control of *P. infestans* leaf infections. The tests will allow the identification of potential alternatives replacing or amending copper based fungicides in the future.

We have tested several commercial and non-commercial organic products in in vitro leaf assays, potted plant assays and field trials. In total, 21 Cu-free alternative products were tested so far. In the in vitro abscised leaf assay, leaves were inoculated approx. 3 hours after spray application of the product. A water agar plug kept the droplet of a sporangial suspension of *P. infestans* in place, ensuring equal infection pressure in all variants, even when leaf surface properties were altered by the product. Leaves were incubated at 15°C for 5 days and disease rated as the number of leaves and % leaf area affected. Each test consisted of 10-15 leaflets per product. Potted plants grown from tubers or eye plugs were infected by a sporangial suspension spray approx. 3 h after application of the product. Plants were incubated at 20°C for 7 days, with plastic bags put over the plants for two days. Disease was rated as the number of leaves and % leaf area affected.

Only few of the substances tested in our laboratory assays proved to be effective against Phytophthora leaf infections. Among the most promising was a commercial garlic product, chitosan, a knotweed product and two more test products. In higher concentrations, some of these products were almost as effective as the Cu control with regard to disease incidence and severity. Sodium phosphonates, although highly effective, are currently not acceptable in organic farming due to residues problems. A citrus product also provided fairly good protection, but has been banned from the market in the meantime. In addition to the lab assays described above, field tests were performed with a selection of Cu-free leaf treatment products in 2012 (Bacillus subtilis, chitosan and citrus). The field trials showed that even products that provided good protection in the lab were not necessarily effective under field conditions. Thus, these products alone are unlikely to be a full substitute for copper in the medium or short term. Therefore, mixed or alternating applications of copper and Cu-free products will be tested during the further course of the project. Some of the more promising products have not yet been tested in the field and will be included in field trials in 2014. Together with other agricultural and technical measures (such as leaf removal or seed treatment) foliar applications can be part of a management strategy to reduce the extent of leaf infections and to minimise the deposition of sporangial inoculum on the soil surface and the potato crop in organic farming. Such tuber infestations are thought to be among the major pathways of Phytophthora inoculum into the field, serving as starting points for subsequent late blight epidemics.

# INFLUENCE OF ORGANIC EXTRACTS ON THE SOIL MICROBIOLOGICAL ACTIVITY IN THE POTATO PLANTATION

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The testing was carried out in State Stende Cereals Breeding Institute from 2011 to 2012. The aim of the study was to identify the biological activity of soil in potato plantation by using extracts from organic products in two cultivation systems: conventional and organic. The following extracts from organic products were used: peat elixir and vermicompost extract obtained at +45° C. The study was carried out in a potato plantation with the variety 'Borodjanskij rozovij'. Extracts from organic products – peat elixir and vermicompost extract application tended to have a positive impact on the number of microorganisms in the soil both in organic and conventional cultivation systems in the potato plantation. However, a tendency was observed that the number of microorganisms in conventional cultivation system was higher than in the organic cultivation system. It was observed in nearly all extract application options that the number of microorganisms was higher at the end of the summer and when the tubers were treated before planting.

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## FROM RESEARCH TO PRACTICE (poster session)

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# POTATO LATE BLIGHT: MANAGING THE RISK WITH UP-TO-DATE AND FIELD SPECIFIC INFORMATION.

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#### Introduction

As in most potato production areas around the world, the potato late blight disease (*P. infestans*) remains a major concern for potato growers in Flanders, Belgium. Despite frequent application of fungicides, widespread attacks of the disease can be observed in farmer's fields during periods of highly favourable weather. The PCA has been running a late blight warning system since 1993, an advisory service that now reaches about 1000 potato growers in Flanders.

### **Materials and Methods**

A network of 48 automatic weather stations collects the necessary meteorological data, which form the core of the disease model and sub models.

A robust database application performs the calculations which allow for a simulation of the development of the disease: germination and infection of spores, latent period in the plant tissue, lesion formation (lesion size, lesion growth, infectious period), spore production (density), spore release and survival. In all these steps of the disease cycle, meteorological conditions play a decisive role.

Differences in cultivar susceptibility can be accounted for in this quantitative approach, by using appropriate values for the several components of resistance: infection efficiency, incubation rate, lesion growth rate, infectious period and sporulation intensity.

### Results

The output gives a view on the spatial and temporal development of the disease, due to linking the application with a GIS. Regional weather forecast data are incorporated daily, allowing for a prediction of the progress of the epidemic and hence risk assessment.

In 2012-'13, an interactive web application has been developed with the aim to give the end-user/potato grower access to all relevant data for his situation at a glance, showing him the actual and forecasted risk for his location and field. A list of potato cultivars was added, as well as a database of late blight fungicides and their characteristics - rainfastness, duration of activity, protection of new growth, curative action. The website also shows a daily refreshed map of the late blight monitoring service, with indication of degree and size of the infection source, and crop type.

### **Conclusions and perspectives**

After input of the field data - field name, location, cultivar, date of emergence- a field- and location specific risk management is possible, based on the evolution of the crop protection, crop growth and disease development. This leads to a better timing of the applications, a better crop protection and less unnecessary sprayings.

# SELECTIVITY LIST OF PESTICIDE USED IN POTATO FOR BENEFICIAL ARTHROPODS, FROM RESEARCH TO FARMERS.

### JP Jansen

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Beneficial arthropods, as aphid predators and parasites, are the key of an effective aphid control in potato. However, these insects are exposed to plant protection products applied during the season, especially fungicides used to control late blight and insecticides used to control aphids and Colorado beetle. The conservation of natural enemies' population by the use of products that are selective for them is required in the context of IPM.

From 1996 to 2002, a research program has been initiated in order to develop tools to establish pesticide selectivity lists. These tools were first used to build pesticide selectivity list in Potato (2004) and open field vegetables (2006). The list used in potato are based on ecotoxicology trials performed on the main aphid natural enemy found in potato in Belgium: the parasitic wasp Aphidius rhopalosiphi (De Stefani-Perez) (Hym.; Aphidiidae) and larvae of the predators Adalia bipunctata L. (Col.; Coccinellidae) and Episyrphus balteatus (Degeer) (Dipt.; Syrphidae). The results are crossed with the phenology of these beneficial organisms to deliver a selectivity list of easy use for farmers, with products rated from green (selective) to red (non-selective) by period of application.

The lists are diffused to the farmers yearly by the organisms in charge of the advisory systems and also used for several guidance document (IPM, specific labels, etc...). They are regularly update to include all changes in the list of product available, as compounds that are newly registered and old one that are removed. The last update (2012) is presented in this poster, with a short presentation of the methodology applied.

### ONGOING NEW RESEARCH PROJECTS

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Genome ENgineering Improvement for Useful plants of a Sustainable agriculture (GENIUS) Rogowsky, P.<sup>1</sup>, Chèvre A.M.<sup>2</sup>, Nogué F.<sup>3</sup>, Déjardin A.<sup>4</sup>, Chevreau E.<sup>5</sup>, Mazier M.<sup>6</sup>, Barret P.<sup>7</sup>, Guiderdoni E.<sup>8</sup>, Matthis L.<sup>9</sup>, Sallaud C.<sup>10</sup>, Matt M.<sup>11</sup>, Pierron J.P.<sup>12</sup>, Bonnel E.<sup>13</sup>, Foucrier S.<sup>14</sup>, Toppan A.<sup>15</sup>, Trannoy L.<sup>16</sup>, Kermarrec, M.P.<sup>17</sup>, Guihard, G.<sup>17</sup>, Chauvin, L.<sup>17</sup>, Chauvin, J.E.<sup>17</sup> <sup>1</sup> INRA UMR0879 RDP Reproduction et Développement des Plantes INRA-RDP, ENS de Lyon, Bât LR5, 46 allée d'Italie, 69364 Lyon Cedex 7, France <sup>2</sup> INRA UMR1349 IGEPP Institut de Génétique Environnement et Protection des Plantes INRA IGEPP, domaine de la motte, BP35327, 35653 Le Rheu, France <sup>3</sup> INRA UMR1318 IJPB Institut Jean-Pierre BOURGIN INRA - Institut Jean-pierre Bourgin, BAT 7, RD10, 78026 Versailles, France <sup>4</sup> INRA UR0588 AGPF Unité de recherche Amélioration, Génétique et Physiologie Forestières INRA AGPF, 2163 avenue de la Pomme de Pin, CS 400001 Ardon, 45075 Orléans, France <sup>5</sup> INRA UMR1345 IRHS Institut de Recherche en Horticulture et Semences INRA - UMR IRHS, 42 rue Georges Morel, BP60057, 49071 Beaucouzé, France <sup>6</sup> INRA UR1052 GAFL Génétique et Amélioration des Fruits et Légumes INRA- GAFL, Domaine Saint-Maurice BP94, 84143 Montfavet Cedex 9, France <sup>7</sup> INRA UMR1095 GDEC Génétique Diversité et Ecophysiologie des Céréales INRA - GDEC, 234 Avenue du Brezet, 63100 Clermont-Ferrand, France <sup>8</sup> CIRAD UMR108 Amélioration Génétique et Adaptation des Plantes méditerranéennes et tropicales CIRAD- AGAP, Boulevard de la Lironde, TA A DIR/PS3, 34398 Montpellier Cedex 5, France CELLECTIS SA, 8 rue de la croix Jarry, 75013 Paris, France 10 Biogemma BIOGEMMA, Site de la Garenne, CS90126, 63720 Chappes, France <sup>11</sup> INRA UMR1215 GAEL Economie Appliquée de Grenoble INRA UMR GAEL, BP47, 38040 Grenoble Cedex 9, France <sup>12</sup> Faculté de Philosophie de l'Université Jean Moulin Lyon 3 Faculté de Philosophie de l'Université Jean Moulin Lyon 3, 15, quai Claude Bernard, 69007 Lyon, France <sup>13</sup> GERMICOPA GERMICOPA, 1 Allée Loeiz Herrieu, 29334 Quimper, France <sup>14</sup> Société Nouvelle des Pépinières & Roseraies Georges Delbard Route de Commentry, 03600 Malicorne, France <sup>15</sup> Vilmorin & Cie Vilmorin & Cie, BP1, 63720, Chappes, France <sup>16</sup> INRA Transfert

World agriculture needs to guarantee food security, replace fossil resources, decrease its environmental impact and adapt to a changing global climate. Whereas France and other European countries presently choose to meet the genetic aspect of these challenges by the sole use of breeding, an increasing number of agriculturally important countries enlarge the available gene pool via transgenesis. Despite a certain

political concerns transgenesis is already an indispensible technology for French seed companies and public scientists to remain competitive at an international level.

Recent scientific advances in the field of transgenesis now provide answers to certain reserves of citizens and blur the border between breeding and transgenesis. In particular the advent of nuclease technology opens the way to extremely precise modifications of plant genomes at pre-determined sites. In this context it is strategic to ascertain top-level know-how in transgenesis in France, to actively participate in the debate of these new technologies and to demonstrate their applicability in a wide range of crop species.

The project **GENIUS** (*Genome Engineering Improvement for Useful plants of a Sustainable agriculture*) will provide French researchers and plant breeders with state of the art know-how, the necessary biological material and connected intellectual property rights for precise genome modifications in a variety of crop species, laying the basis for high throughput functional genomics and efficient plant breeding. The biological material produced in case studies will either be brought directly to the seed market or first undergo trait optimisation. The technical information and ethical framework provided to French citizens and decision makers may ultimately lead to a reduced regulatory burden for risk assessors and applicants. To reach these goals, in an unprecedented effort, GENIUS has assembled a consortium of 15 public and private partners – 10 public research units in biological or social sciences with 5 biotechnology and seed companies. This consortium will create synergy between field- or species-oriented entities into a technology-oriented community.

The project started on September 1st, 2012 and will be developed over a period of 7 years and 4 months. Partnership

### 10 public partners

- INRA (8 units): fundamental biological research/ human and social sciences
- CIRAD : fundamental biological research
- Lyon3 University: human and social sciences

### 5 private partners

- Cellectis: nucleases use and desig
- Biogemma: biotech subsidiary of seed companies (in GENIUS: wheat and maize)
- Germicopa : potato breeding
- Delbard : rose and apple breeding
- Vilmorin & Cie: multi-species breeding (in GENIUS: legal issues)

### Objectives

- Develop and improve methods of cellular engineering for nine crops (wheat, maize, rice, rapeseed, tomato, **potato**, poplar, apple, rose)
- Improve the methods and the throughput of transgenesis for the validation of genes with agronomic interest
- Allow French researchers to maintain high level technical know-how and expertise in the field of genome engineering

### Means

- Optimize existing transgenesis protocols
- Develop new technologies (nucleases) in partner labs
- Explore novel ways of transgenesis
- Conduct case studies up to a pre-breeding level

# <u>IPOT</u>: <u>Industrial POT</u> ato monitoring in Belgium using remote sensing and crop growth modelling

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Belgian potato processors, traders and packers are increasingly working with potato contracts. The close follow up of contracted parcels on the land as well as from above is becoming an important tool to improve the quantity and quality of the potato crop and reduce risks in order to plan the storage, packaging or processing and as such to strengthen the competitiveness of the Belgian potato chain in a global market. At the same time, precision agriculture continues to gain importance and progress. Farmers are obligated to invest in new technologies.

Today the use of geo-information by the (private) agricultural sector in Belgium is rather limited, notwithstanding the great benefits this type of information may offer, as recognized by the sector. The recently approved "iPot" project, financed by the Belgian Science Policy Office (BELSPO), aims to provide the Belgian potato sector, represented by Belgapom, with near real time information on field conditions (weather-soil) and crop development and with early yield estimates, derived from a combination of satellite images and crop growth models. An intuitive web based geo-information platform will be developed to allow both the Belgian potato industry and the research centres focusing on the cultivation of the potato crop, to access, visualize and analyze the data and to use them, combined with their own field observations and in close collaboration with the farmers, for improved decision-making.

This poster briefly presents the iPot project objectives, methodology and expected outcome and shows some preliminary results from the start of the 2014 campaign. Between mid-May and the end of June 2014 three potato fields in Gembloux (were monitored from emergence till canopy closure. UAV images (RGB) and digital (hemispherical) photographs were taken at ten-daily intervals. Vegetation indices (fCover, LAI) derived from high resolution satellite images (22m pixel size) will be cross-referenced with these reference data. As such crop emergence maps will be generated, showing the time (date) and degree of crop emergence and crop closure (in terms of % cover).

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