RECENT ADVANCES IN BIOTECHNOLOGY AND INFORMATION TECHNOLOGY IN THE POTATO INDUSTRY

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Summary
Recent advances in potato production particularly concern those in biotechnology and information technology. This paper discusses two examples of these advances from the Netherlands. Developments in biotechnology are demonstrated using the methodology and results of the DuRPh project. In this 10 year project, a proof of principle was developed to make potato resistant to late blight (*Phytophthora infestans*) by the introduction of resistance genes from wild Latin American potato species without the use of selectable markers: cisgenesis. Advances in information technology are exemplified by the national Netherlands effort of Smart Potato Farming in which a potato ontology is developed, decision support systems are upgraded with geographical data details and a national data exchange platform (Akkerweb) is successfully exploited.

INTRODUCTION

Since 1995 most resistance genes against diseases and pest have been located in the potato genome and cloned. Through genetic modification many of these resistance genes have already been deployed in new potato varieties resulting in resistance against viruses which drastically reduces the degeneration rate of seed, reduced losses and sprays related to late blight and control of cyst nematodes allowing potato more frequently in the crop rotation. A gene from a bacterium made one variety resistant against Colorado beetle and another against tuber moth potentially reducing losses and use of insecticides. Quality characteristics are also being altered through these techniques, for example, reduction in bruising damage and browning when frying. Precision farming translates as giving potato crops and tuber lots the right treatment at the right time at smallest scale possible. Introduction of GPS systems, sensors and e-Science allows treatment of plants at grid and eventually individual plant level, and offers new opportunities for chain management with real-time data. Decision support systems are being developed for many parts of the production and processing chain. The expectation is that innovative genetic, sensor and big data techniques will benefit potato more than other crops due to the many solvable issues from which this clonally multiplied crop currently suffers. .

Biotech

The first genetically modified potato products were plants with resistance to Colorado beetle (*Leptinotarsa decemlineata*), *potato leafroll virus* (PLRV) and *Potato virus Y* (PVY). These were registered in the United States of America and allowed to grow there. Monsanto brought

NewLeaf to the market, the variety Russet Burbank incorporated with a Bt-gene from *Baccillus thuringiensis* making it resistant to Colorado beetle. Risks of non-acceptance coupled with the high costs of regulatory compliance resulted in the withdrawal of these products from the market at the turn of the century.

BASF created varieties Amflora, Modena and Amadea by modifying the GBSS gene with RNAi resulting in solely amylopectin production in the tubers. BASF also modified the variety Fontane into Fortuna with two late blight resistance genes from *Solanum bulbocastanum* with the aid of *Agrobacterium tumefaciens*. Meanwhile the potato company Simplot in the USA developed a GM line of so called 'innate' potatoes through RNAi, RNA interference disabling poly phenol oxidase reducing black spot bruising and reducing asparagine formation thereby reducing brown discoloration when frying. Future "innate" potatoes are being supplied with the Rpi-vnt1.1 gene to make the crop resistant to late blight.

Information technology

More and more data in agriculture are being generated and captured in databases for further use. The developments in information technology, speed of data transfer, data processing and storage capacity are such that they started to assist growers in managing their crops and customers to make use of data generated in the process of production. To structure data, an ontology of the domain needs to be established first. Subsequently, the database needs to be populated with information regarding the genotype, environment and management. Lastly, use should be made of the database in subsequent strategic, tactical and operational decisions, both of growers and customers. In this contribution, a recent advance in information technology in potato production will be discussed as an example: Smart Potato Farming consisting of a consortium of related projects aimed at collecting, organizing and exploiting data in potato production.

DuRPh PROJECT

The DuRPh (Durable Resistance against Phytophtora through cisgenesis) project at Wageningen UR was carried out from 2006 through 2015.The project aimed at proving the principle that sustainable resistance can be achieved through cisgenesis. This involves the use of multiple resistance genes from crossable (with *Solanum tuberosum*) wild potato species from North and South America through genetic modification (cloning genes with *E. coli* and transform them with *A. tumefaciens*). The use of existing varieties is essential as these have proven to be of market value whereas the introduction of new varieties is costly and the success rate usually is low. Moreover the introgression of a single resistance gene takes decennia. In 1959, *S. bulbocastanum* (2x) that cannot be crossed directly with *S. tuberosum* (4x) was crossed with *S. acaule* to create the AB-material (3x) that after polyploidisation (6x) six years later was crossed with *S. phureja* (2x) yielding ABP material (4x) that was crossable with *S. tuberosum* (4x) leading to the widely used breeding material ABPT to introduce the *S. bulbocastanum* into modern varieties. The varieties Bionica and Toluca only contain one R-gene which in the past, when only single genes of *S. demissum* were used proved risky, and indeed currently the resistance of these new varieties has not kept up. The nomenclature of the Resistance genes is explained with the aid of the following example: Rpi-vnt1, meaning R= Resistance gene, pi = *Phytophthora infestans*, vnt = *S. venturi*, 1 = the first Rpi gene found in this wild species. Globally costs of control in developed countries but mainly losses due to late blight in developing countries are estimated to be close to € 9 billion (Haverkort et al., 2015). The DuRPh project concluded in 2015 and consisted of five subprojects that are detailed below.

Cloning

The main interest regarding cloning new R genes is to find and clone genes with a broad spectrum of recognition, meaning that the gene allows the potato plant to recognize and offer resistance to many late blight pathotypes, each pathotype differing in a typical protein (an effector) they contain. Late blight resistance is based on the host potato plant being able to detect that it is being attacked by *P. infestans* and effect a hypersensitivity reaction, killing a great number of cells around the invaded spot on the leaf (apoptosis) and resulting in the death of the pathogen. To be able to clone R-genes, first they have to be detected by crossing a wild species with a susceptible variety. If Mendelian segregation in a 50% to 50 % ratio the presence of an R-gene is assumed. If it is more complicated in case of the presence of a stack of R-genes the result may be backcrossed with the susceptible genotype, so-called de-stacking. Through successive mapping, fine-mapping and landing in a BAC-library (bacterial artificial chromosome), some 20 R genes that segregated in 50-50 had been cloned by late 2015.

Transformation

The DuRPh project used three potato varieties to transform into late blight resistant ones, the early table variety Premiére, the mid late variety Désirée and the late maturing starch variety Aveka. Single R genes or stacks up to three were cloned into a binary *A. tumefaciens* vector. At the start of the project, a selectable marker gene, kanamycin resistance, was used to quickly explore which R genes were effective but as this is not a Solanum gene this could not be classed as a cisgenic plant. The following approach was taken to overcome this issue. First, most of the R genes were transferred with *A. tumefaciens* to the variety Désirée to create a set of R genes in an iso-genic background but with varying spectrum of resistance. Broad spectrum resistance are resistant to over 80 % of all isolates occurring in an area, narrow spectrum to less than 10 %. This set was then used in the subproject 'resistance management'. Selected R genes with known resistance spectrum were transferred to study the influence of the genetic background on transformation efficiency and resistance expression, both showing considerable variation. Next combinations of two R genes were made, not all of these were stable in the vector and they varied in transformation efficiency associated with slow growth of the vector. Also it was discovered that if two genes were transferred in one stack, they were not always both effective. Next, marker-free events were created by mass transformation and each transformed plantlet was screened for the presence of the R gene using PCR (de Vetten *et al*., 2003). Transformed plantlets were also checked for freedom of vector backbone. Within the time frame of the project it was not possible to obtain a cisgenic event containing 3 R-genes so an event with 2 R genes was re-transformed with another single different R gene to obtain an event with 3 R genes.

Selection

After a successful transgenic or cisgenic event, with the vector *A. tumefaciens* transferring one or more R-genes to a cell of recipient variety and the production of plantlets through a callus stage, a number of tests are carried out to finally select genotypes. Genotypes that carry the target genes with as few copies as possible, without vector backbone (parts of the plasmid of *A.*

tumefaciens) of which the R gene stack is a part are selected. The resulting plant and subsequent tubers are resistant to late blight and true to type. The number of transfer DNA, T-DNA insertions or the number times the same R-gene is transferred to the genome could not be ascertained with the Southern blot technique in marker free events so it was done with PCR and R-gene specific primers. Similarly, backbone screening took place to remove undesired events. Subsequently, late blight resistance was assessed with a detached leaf assay in the lab and with field trials. Finally, it is important that the new genotype only distinguishes itself from the original variety, the wild type through its resistance to late blight but that all other characteristics remain the same so growers and processors do not have to take special measures regarding the new genotype. In 2014, 22 events mainly transformed Désirée but also Première and Aveka were tested in the field.

Resistance management

In the past, in conventional breeding programs, a single R gene from *S. demissum* was introgressed that conveyed resistance to all hitherto present late blight pathotypes. As soon as a new pathotype is introducedthrough the atmosphere, through mutation, or because of variation following sexual replication of the two mating types A1 and A2, that is not recognized by the resistant variety, its resistance is overcome. Resistance is overcome more rapidly if it is based on a narrower spectrum of *Phytophthora* isolates and subjected to high spore densities caused by incomplete control and large areas of this variety. Supplying a variety with two, three or four R genes considerably reduces the chance of the variety becoming susceptible to new pathotypes.. Monitoring the single R-gene events by planting them as a differential set in three different areas in the country allows the recording of broken R genes. When two R genes in a stack are left unbroken, seed potatoes with ~ a new stack should be bulked to have a new stack ready for growers within a few years. In the unlikely event that only a single R-gene is present, growers are advised to spray a few times during the season with a low dose fungicide. Monitoring plots with Désirée events in 2013 showed that the weak, narrow R3a gene from *S. demissum* delayed infection by three days. The results showed that there was a clear effect of specificity and of stacking. In the two final years of the DuRPh project a field trial was conducted near Wageningen to demonstrate the effectivity of resistance conveyed by specificity and stacks combined with low doses (25 % of conventional dose) of fungicide sprays. The synergistic effect of stacking was similar to that of adding low dose to a narrow spectre resistance gene.

Communication

The DuRPh project dedicated 10 % of its budget to communication. The objective of this was to inform society, all stakeholders such as consumers, consumer organizations, growers, non-governmental organizations, policymakers, journalists and peer scientists of the motifs, procedures and outcomes of the project. This was in order to allow all involved or interested to judge the advantages and disadvantages of the cisgenic breeding approach. It was not an objective to convince or to achieve societal acceptance of this approach nor to stress its advantage compared to transgenesis. Over the years hundreds of interviews and presentations were given for laymen and scientific peers and many articles in newspapers appeared and films shown on television channels in the Netherlands and abroad (example http://youtu.be/veX6VXAfUoU). The website (www.durph.nl) was updated when related news appeared and contributions to webinars (example http://nas-sites.org/ge-crops/2014/09/22/webinar-november-6/ were recorded.

SMART POTATO FARMING

The national aspiration in the Netherlands to arrive at Smart Potato Farming consists of various research and development efforts. First is the description of the domain or creation of an ontology; second, the collection of crop data, mainly from recordings and decision support systems (DSS); third, data collection from sensing associated with precision farming; and fourth, the development of a platform (Akkerweb) to facilitate user interaction. In this section, each of these aspects are shown under the national Netherlands umbrella activity of **S**mart **P**otato **F**arming (SPF),

Ontology

In structuring data, it is most helpful to create an ontology so those working with the data have a common understanding and to allow for numeric techniques to identify correlations. An ontology is a description of a domain, say 'seed potato production' in terms of instances in classes, super-classes and sub-classes that are described with features and attributes. The ontology completed with instances is a knowledge base.

One approach described by Haverkort *et al*. (2006) and Haverkort and Top (2011) is to organize the ontology in five groups: 1) **S**ociety with e.g. consumer classes and classes of products such as organic and labelled that determine the required performance, 2) **P**erformance of the crop resulting from yield and recovery as determined by seed, environment and management, 3) **G**enotype or propagation material as determined by variety, seed size, health and physiology, 4) **E**nvironment consisting of climate and soil and 5) **M**anagement of the crop. In a squence: :

S » **P** » **G** »**E** » **M.**

The ontology makes use of concepts or classes. For example, biocides are a subclass of agrochemicals and have their own subclasses i.e. fungicides, herbicides, nematicides and insecticides (which are therefore sub-subclasses of agrochemicals). General properties of agrochemicals are their timing of application and dose rate giving them "slots" or "restrictions" such as not allowed to apply 3 weeks before harvest. An instance elsewhere in the database may be e.g. variety Pentland Dell planted on April 15 in field New Acre, seed size 40-55 mm, class E and purchased from SeedCo. A widely used standard language is the Ontology Web Language (OWL) called Protégé that was developed by Stanford University in the USA.

Crop management (DSS)

Well-developed potato farming systems increasingly make use of decision support systems (DSS) for strategic (at the farm level, future oriented), tactical (before planting a particular field) and operational decisions. DSS all have in common that measurements are made, that the resulting data is inserted into a quantitative tool (a table or a model) which produces a quantitative output which the grower or the advisor uses to make a decision. An example of a decision support based strategic decision would be whether to grow potatoes in a given field if a heavy infestation with potato cyst nematodes is present, or to grow starch potato only as there are sufficient starch varieties available with resistance (reducing the population) and tolerance (yields not overly depressed by the current nematode population). The measurement here consists of sampling the soil, determining species and pathotype and the number of cysts and viable eggs. The outcome is benchmarked against response curves of varieties with different tolerance and resistance against the various potato cyst nematode races and the optimal combination is chosen. If needed the DSS may also suggest type and dose of a nematicide.

Tactical decisions are the choice of variety and the use of the variety: a table variety requires a different nitrogen application regime from a crop destined for chipping. Based on the pre-planting residual amount of nitrogen in the soil determined from soil samples and depending on the variety and use, a pre-planting nitrogen rate is advised. After planting, a new supplemental rate of nitrogen is advised based on the amount of nitrogen present in the soil and/or foliage, or on the amount of nitrate in the leaf petioles, or based on crop reflection in infrared (shows biomass) and in green (shows percentage nitrogen in the biomass). The findings in sampling and sensing are compared to previously established calibration lines and appropriate advice is provided.

A wide application is operational decision support for late blight control. This DSS consists of a grower completing a form online detailing which variety is grown, which fungicide was applied previous;y (when and at which dose). The DSS collects past late blight development related weather events such as amount and duration of rain, dew, leaf wetness, and temperature; it collects spore density and presence of foci in the area and from information on variety maturity it assumes how many new unprotected leaves have appeared since the last treatment. Based on the weather forecast the grower is given spray advice including if, when, which fungicide and at what dosage. An irrigation planner also uses past irrigation amount, past and forecast weather, ground cover, and soil water holding capacity to advise on the time and rate of irrigation. A crop growth model such as LINTUL-POTATO-DSS (Haverkort *et al*., 2015b) may be used in strategic decision to calculate expected average yields and deviations due to erratic temperature (frosts and heat waves) and precipitation (floods and drought) events. It may, however, also be used for the operational decision to harvest a particular field based on the predicted yield at any given moment. This model requires information on planting depth time daily maximum and minimum temperatures, solar radiation, precipitation and evapotranspiration; these weather related data are readily available from a weather station. Soil water holding capacity following rooting depth and percentage clay and silt of the soil is also required. There are more DSS, such as planting rate, in MAPP, the management advisory package of potato developed at the James Hutton Institute at Dundee. There are the DSS based on insect trapping such as aphid counts to advise on haulm killing dates for certain seed potato classes in the Netherlands, Colorado beetle trapping for insecticide sprays in America and pheromone trapping of tuber moth for control in stores in North Africa. A most comprehensive DSS is NemaDecide developed in the Netherlands to reduce populations of potato cyst nematodes and free living nematodes based on rotation frequency of potato, type of nematode, choice of variety and support of a granular nematicide. This very successful and effective program is widely applied by seed potato growers to answer questions such as how do I detect the presence of PCN at an early stage? Which measures should I take to avoid infection and thereby losing my licence to grow seed potatoes? And what is the effect of potential control measures? For growers of starch and ware potatoes NemaDecide answers questions like 'how do I control PCN infection and should I apply a granular nematicide at what rate of return?' NemaDecide was extended in 2015 with a geographic interface and the sampling report is now accompanied by geographic coordinates so a grower can apply location specific control or management measures.

Precision farming

Precision farming is aimed at not treating the whole field in a similar manner but rather location specific, just in time, and with as little input as possible. This means that measurements and sensing data are needed to detect variation; consequently, variable rates of applications (VRA) inputs such as seed potatoes, topdressing of nitrogen (Van Evert *et al*., 2012), herbicides (Kempenaar *et al*., 2014, Van Evert *et al*., 2012) and fungicides following DSS are applied which requires specific adaptations to machinery. There are a few more challenges: 1) acquiring spatial information from soil and crop on time and at the right level of resolution i.e. as a multiple of the width of the planter, spreader or sprayer; 2) adequate communication between sensor, farm computer and machine - this is not always feasible and despite the wealth of DSS that work on the whole field level with non-machine mounted sensors there is a need to adapt real time application on a tractor and applying machine. More than 65 % of the Dutch arable farmers nowadays use global positioning systems, mainly for navigation. Gradually, more and more growers are purchasing sprayers that allow spatial variation in application. To mimic the real farmer and to assist growers in taking spatial and temporal strategic and operational decisions, a so called "Smart Farm" was created in 2012 at the research and demonstration farm of the Agricultural college CAH Vilentum at Dronten. Gamma radiation based soil scans yielding information on soil organic matter were carried out by the Altic and Dacom companies and crops were scanned with remote and proximal reflection sensors to calculate NDVI values at various resolutions. Electric conductivity (EC) and electromagnetic (EM) soil sensing technologies were used to map spatial variation in soil properties. Combined with yield maps the objective was to generate VRA maps of the fields that machines use as input. WDVI and NDVI indices from remote and nearby sensors can be used to make application maps. An example being the use of VRA Reglone dosing for potato haulm desiccation based on crop NDVI values. .

A user exchange platform

In the Netherlands an information exchange platform exists that standardizes information to facilitate exchange between growers, advisors, contractors and clients, and avoids redundancy of information supply. The umbrella organization is "Agroconnect" for exchange of any agricultural data such as poultry, flowers etc. "Editeelt" was established under this umbrella specifically for field crops, and mainly for registration purposes. . From the examples above in the sections "Crop management" and "Precision farming" it is obvious that the amount of data generated in potato production with the use of DSS increasingly assisted by geo-information and sensing rapidly increases. To facilitate exchange for all users in 2012 an information exchange platform Akkerweb (www.akkerweb.nl) was introduced. It originated from the development of the decision support system for the control of plant parasitic nematodes NemaDecide (GeoNema). Akkerweb offers GIS functionality and a number of generic applications, such as web services, to download satellite data or provide the ability to generate an application map. Third parties can use the Akkerweb platform to rapidly develop new applications. The Akkerweb environment serves as a connection between geographical field data and relevant additional information. Akkerweb is deployed by the largest farm cooperative in the Netherlands, and open for any other user. Wageningen UR uses the Akkerweb and FiSpace platforms/environments to develop new advisory concepts and specific Apps for farmers such as a recent yield predictor based on LINTUL-POTATO-DSS.

Akkerweb offers accounts for users, different background maps, basic apps for crop rotation and field boundary information, soil and soil moisture information, and an increasing number of management Apps. Users (farmers and farm advisors) can share data with otherselected users. Akkerweb has links to farm management information systems and can be used to order soil and crop analyses from agricultural laboratories.

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