Nitrogen use efficiency in potato: an integrated agronomic, physiological and genetic approach

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Abstract

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Nitrogen (N) fertilizers increased food production over the last 60 years, but also contributed significantly to the use of fossil energy and the total amount of reactive N in the environment. Agriculture needs to reduce N input and increase nitrogen use efficiency (NUE). Legislation like the Nitrate Directive (91/767/EEC) and the Water Framework Directive (2000/60/EC) forces a reduction in N supply in crop production. The effects of this constraint on yield and quality of potato are expected to be significant since N plays an important role in the vegetative development and production of potato. Considerable amounts of N are needed as N recovery is notoriously low due to the small and shallow roots. The overall aim of this thesis is to improve the nitrogen use efficiency of potato under low nitrogen supply. Specific aims are i) to understand the N effects on potato performance, especially under low N input, ii) to quantify the genotypic variation under contrasting N inputs, iii) to identify quantitative trait loci associated with the crop's response to nitrogen. We used ecophysiological models to dissect the canopy development into biological meaningful parameters as phenotyping tools. Two potato populations (a set of tetraploid cultivars and a biparental diploid population) were phenotyped in the field under two contrasting N levels. Additionally, a set of 6 cultivars from three maturity groups (early, middle and late) were phenotyped in more detail under 5 nitrogen conditions combining two input levels and two fertilizers types plus a control without nitrogen fertilisation. The curve-fit parameters were, together with other agronomical traits, used in the agronomic and genetic analysis. Our approach using the ecophysiological models captured the phenotypic response to N, enhancing the interpretation of the nitrogen effects and of the differences among maturity types. The nitrogen effects on canopy development resulted in large differences in light interception, tuber yield, tuber size distribution and nitrogen uptake. There were differences in the response to nitrogen between the diploid biparental population and the set of tetraploid cultivars. In general, in the diploid population, having less vigour and therefore less potential to respond to the extra nitrogen, the time required to complete each phase of the canopy development was longer than in the set of tetraploids. In the set of cultivars the rate of early vegetative growth was higher, the onset of the phase with maximum canopy cover was earlier, and the duration of maximum canopy was longer than for the diploid population. However, in both the diploid and the tetraploid population maturity was the major factor accounting for genetic variation in canopy development and tuber development traits. The genotypic differences were reflected in quantitative trait loci that were either N dependent or N independent, with pleiotropic regions affecting most of the maturity-related traits. Few traits showed quantitative trait loci on common regions that were not maturity

related like those on chromosomes 2 and 6 (association mapping) or linkage groups ma_VI, pa_VIII pa_XI. Maturity obscures other genotype-dependent physiological traits; therefore it is imperative to find traits that are responsible for genotypic variation, but not related to maturity type. Moreover the results showed that nitrogen use efficiency under low nitrogen input is higher than under high nitrogen input, and higher for late cultivars than for early cultivars. Therefore, breeding for nitrogen use efficiency under low input requires direct selection combined with good response to extra nitrogen and should be done within each maturity group. Finally in a broader context we discussed the need of high-throughput phenotyping in breeding for complex traits, like those involving efficiency, to make the most of the large amount of genetic data, all possible based on advances in technology in remote sensing and images analysis.

Keywords: Association mapping, Breeding for low input, Canopy development, Maturity type, Nitrogen use efficiency, Potato, *Solanum tuberosum,* Quantitative trait loci.

To my beloved Family

...to those who are always there

...even from far away

...even after life has left

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Chapter 1

General introduction

César Andrés Ospina Nieto

Potato origin and importance

Potato (mainly *Solanum tuberosum* L.) is a true star in agriculture having a recognized importance in human nutrition and food security throughout history. According to the Food and Agriculture Organization of the United Nations (FAO 2013) potato is the twelfth most important agricultural product produced worldwide, the fourth most important food crop in the world and the number one non-grain food product.

Originated in South America, potato was domesticated as early as 7,000 years ago (Ugent 1970). It was an ancient cultigen in the Andes mountains (Hawkes and Francisco-Ortega 1993) with more evidence of proper cropping from 4500-3000 years ago (Quilter 1991) by pre-Inca natives. Then potato became one of the pillars for the Inca civilization in Peru and for other tribes of pre-Columbian South Americans, all powered by an intrinsic selection during that time. After the invasion of the Spanish in the Conquest period, potatoes reached Europe in the 16th century with the first reported cultivated potato between 1562-1573 in the Canary islands (Hawkes and Francisco-Ortega 1992; Hawkes and Francisco-Ortega 1993; Ríos et al. 2007).

The adoption process in Europe was slow; in some countries, such as Italy and Sweden, potato was still rejected as a food in the late 1700s (Hawkes and Francisco-Ortega 1993) and in many other countries considered as a poor man's crop. Potato was first used to feed animals and only later became part of the human diet. This practical, high-yielding crop and easily processed food finally seduced Europeans. In England potato was established as a field crop in 1800 and then became widely grown in the rest of Europe (Nunn and Qian 2011), encouraging the rapid rise of population enabling the Industrial Revolution (Hobhouse 1985; Salaman et al.). Since its arrival into Europe, potato introductions were selected to adapt the crop to long days, turning it into a major food crop. Later on potato spread all over the world and nowadays its popularity is rapidly growing in developing countries. Haverkort and Struik (Haverkort and Struik 2015) mentioned that potato is a hunger-breaking crop with significant advantages over cereals (like short cycle and high harvest index). It is also suitable to many crop systems as main crop or early crop adaptable to different conditions and different lengths of the crop cycle.

Potato species classification

The first cultivated potato in South America was diploid (Raker and Spooner 2002). Peru has the highest number of wild potato species (Hijmans and Spooner 2001). Landraces are highly diverse with different ploidies (Huamán and Spooner 2002), all having a monophyletic origin from a wild species of the *Solanum brevicaule* complex in Peru (Ames and Spooner 2008; Gavrilenko et al. 2013; Ríos et al. 2007; Spooner and Hetterscheid 2006; Spooner et al. 2007). Spooner (2007) supported the reclassification of the cultivated potatoes into four species: (i) *S. tuberosum* (usually tetraploid 2n = 4x = 48, (ii) *S. ajanhuiri* (diploid 2n = 2x = 24), (iii) *S. juzepczukii* (triploid 2n = 3x = 36),

and (iv) *S. curtilobum* (pentaploid 2n = 5x = 60). Additionally *S. tuberosum* is divided into two Cultivar Groups: The Andigenum Group of upland Andean genotypes containing diploids, triploids, and tetraploids and the Chilotanum Group of lowland tetraploid Chilean landraces. In South America modern cultivars are distributed across two regions (corresponding to the Cultivars Groups): the highlands from Venezuela to Argentina, and the lowlands in Chile. As a result, there was an adaptation to long days (Ames and Spooner 2008; Huamán and Spooner 2002). The tetraploid cytotypes are the highest yielding, and include the Chilean landraces, which have good tuberization at latitudes similar to Europe (Glendinning 1983). Therefore it is mentioned that the potatoes in Europe were introduced originally from the Chilean and Andean germplasm but the Chilean type predominated as a crop (Ríos et al. 2007).

Natural reproduction in potato is variable. Diploid species are often self-incompatible and tetraploid ones self-compatible (Hanneman Jr 1999; Hawkes 1990). Regarding the ploidy level, Hutten et al. (1995) found that diploid progenies had significantly lower yields (due to smaller tubers) and significantly higher underwater weights than tetraploid progenies. From the breeding point of view, Lindhout et al. (2011) proved the principle of F1 hybrid potato breeding using diploid self-compatible potato clones to replace the current method of out-breeding and clonal propagation by an F1 hybrid system with true seeds.

Solanum tuberosum includes thousands of varieties that vary by size, shape, colour, and other sensory characteristics exhibiting perhaps the greatest amount of biodiversity of any major food crop (Hawkes and Hjerting 1989). In addition, there is wide variability in nutrient levels among the tubers of potato varieties as mentioned by Mouillé et al. (2008). This diversity makes the potato crop important for different sectors: human food, animal feed and industry (food and non-food products).

Crop distribution

Worldwide, potato cultivars are cropped under non extreme temperature conditions (ranging from 5 to 25 °C daily average temperatures) at different altitudes, as well as different latitudes, and therefore different photoperiods. The potato crop grows from tropical highlands or lowlands under relatively cold conditions to subtropical areas (high and lowlands), with cold night conditions and also to temperate zones with cold nights and high radiation levels (Haverkort 1990). Haverkort and Struik (2015) defined six potato production systems world wide:

1. Rainy summer crop production; found in, e.g., Northern Europe and the South African High Veld characterized by long growing seasons (180 days),
2. Dryland summer crop production; here rainfall is sparse such as in the North-West of the United States of America.
3. Partly irrigated spring crop production; found in Mediterranean climates such as in North Africa, South America and South Africa.

4. Irrigated autumn crop production; these crops are planted in Mediterranean climates after the summer heat and harvested before winter frosts with a crop cycle of about 100 days.
5. Irrigated winter crops in monsoon climate regions where rice is grown during the rainy summer.
6. Equatorial highlands crop production systems; these are located above 1800 m with two rainy seasons such as in East and Central Africa.

Potato production

World potato production has increased over the past 20 years. However the relative importance of potatoes has shifted from developed to developing countries. Potato production from 1992 to 2007 increased 48% in the developing world, while it decreased by 12% for the developed world (Birch et al. 2012). The overall production worldwide has increased by 24% to 368.1 Mt in 2013 compared with 1992 (FAO 2013). A similar trend is shown in Figure 1, using the Human Development Index (HDI) to classify different categories of countries. Walker (2011) pointed out that the reduction of potato production in developed countries manifests as a progressive decline of the harvested area as a consequence of: i) Modernization of the animal feed sector (especially in Eastern Europe), ii) A rapid diet diversification away from a starchy staple with rising income (especially in Western Europe). The increase in production in developing countries (or formerly considered developing countries) is due to the increase in productivity and area harvested, especially in Africa, China and India (Birch et al. 2012; FAO 2013; Haverkort and Struik 2015). Currently, developing countries account for more than half of the global potato area and production (Haverkort and Struik 2015). The major potato producers during the period 1992 to 2010 have been China, the Russian Federation, India, USA and Poland with India taking over the second place from 2012 onwards as an example of the tendencies between developing and developed countries (Birch et al. 2012; FAO 2013).

Potato as a food

Potato has great nutritional value containing well digestible carbohydrates, important vitamins, as well as one of the best mixes of plant proteins with a high content of lysine (Pęksa et al. 2013), which is an important essential amino acid often lacking in crops like cereals and vegetables (Waglay et al. 2014). The nutritional quality of potato protein is considered high using as reference the amino acid composition and digestibility of casein, which is recognised as the best digestible protein (Eppendorfer et al. 1979; Friedman 1996, 1997). High daily intake of potatoes can supply nutritive compounds, minerals and vitamins essential for humans (Lister and Munro 2000). Additionally, potato contains a good quantity of primary and secondary metabolites that play an important role in a number of human metabolic processes (Friedman 1997). Kolasa (1993)

mentioned that potato nutrition is more than calories since potato provides important quantities of vitamin B6, vitamin C, fibres and minerals like iron, magnesium, zinc and copper (based on data of United States). Srikumar and öckerman (1990) also highlight these aspects of potato based on information from Swedish National Food Administration, Livsmedelskonsumtion 1984. On the negative side, diets including large quantities of potato fries, chips and crisps are related with obesity diabetes and heart disease (Abete et al. 2010; Harvard T.H. Chan School of Public Health, 2014; Mozaffarian et al. 2011).

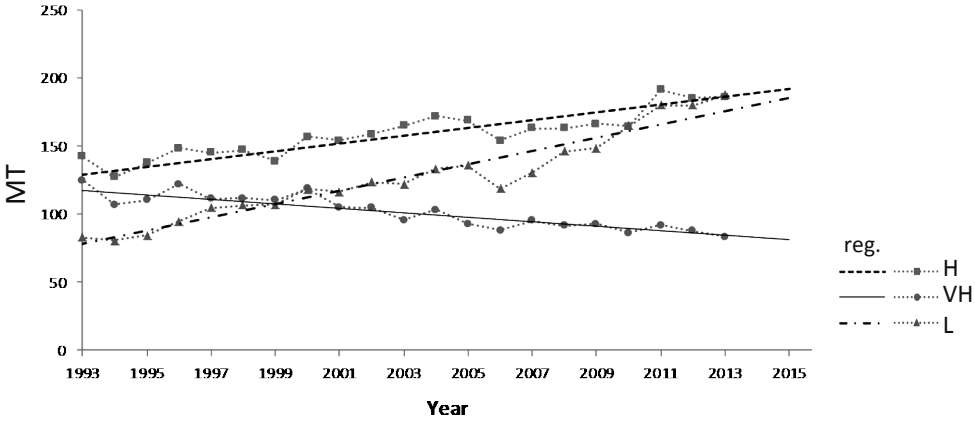

Figure 1 Trends in potato production per group of countries, grouped on the basis of the country classification by the Human Development Index (HDI) as included in the "United Nations Development Programme" (UNDP) http://hdr.undp.org/en/. The categories of HDI considered here were: very high (**VH**), high (**H**), and low (**L**), this last category aggregating countries with medium and low HDI. (see legend). The 'reg.' in the legend is the trend line of each group. Values are in mega tons (MT). Data source: FAO, 2014.

Furthermore, potato is a very efficient food crop in terms of yield and water use, having greater consumable part of its dry matter as well as more protein and minerals per unit area in comparison to cereals (Birch et al. 2012). Therefore, potatoes can play an important role in providing food security for millions of people across the world, mainly in South America, Africa and Central Asia (Thomas and Sansonetti 2009).

The United Nations declared the year 2008 as "The International Year of the Potato", affirming the need to focus the world's attention on the role that potato can play in providing food security and eradicating poverty (Thomas and Sansonetti 2009).

Nitrogen use efficiency (NUE)

Nitrogen (N) is well known as a key factor in plant production because of its important role in the biological process of assimilation and especially in crop growth. With the discovery of the Haber-Bosch process in 1909, synthetic N fertilization was possible allowing an increment in the amount of N forms usable for plants (Smil 2001). Therefore, N fertilization has been associated with the

doubling of agricultural food production worldwide over the past four decades (Hirel et al. 2007), allowing a significant decrease in world hunger, despite a doubling of the population (Godfray et al. 2010; Smil 2001; Tilman et al. 2001). The use of N fertilization is forecasted to double or almost triple by 2050 (Gomiero et al. 2011).

On the other hand, the increase in human population threatens the natural resources (like water) especially in regions of high settlement and intensive agriculture (Vorosmarty et al. 2010). Intensive agriculture has a vast environmental impact since the introduction of chemical fertilization (Gomiero et al. 2011), accounting for 33% of the total annual creation of reactive nitrogen (Dobermann 2005). Moreover, the debate is not only on the use of fertiliser but also on the whole process to produce it, which is energy costly, and has a high environmental impact as well.

Improved nitrogen use efficiency (NUE) is important for a more sustainable agriculture. Several definitions can be found for NUE (Good et al. 2004). In this thesis I follow the definition of Moll et al. (1982) for NUE: the yield produced per unit of available N in the soil. Improving N balance in the production system could be achieved by optimising crop management strategies. Precision farming can be used to improve the timing and rate of N application so that it coincides more closely with crop demand (Dawson et al. 2008). Potential developments in methods of monitoring crop N status in potato could lead to a better "real-time" positioning of N fertiliser supply in space and time based on crop N requirements (Goffart et al. 2008). Additionally, production systems with cover crops and intercropping are alternatives to have a better use of N in the soil, and to reduce N losses (Swain et al. 2014).

Another option to increase the N efficiency is to improve NUE on the basis of a genetic change in physiology of the crop plant itself. Good et al. (2004) mentioned the need of combining traditional breeding, marker assisted selection and even genetic modification designed to improve specific aspects of NUE. Breeding plants with increased NUE at reduced fertiliser input is currently one of the key goals of research on plant nutrition as well as for sustainable agriculture (Hirel et al. 2007; Hirel et al. 2011). Ceccarelli (1996) mentions the importance of breeding under low input, discussing the fact that most of the breeding was done under high input of mineral fertilization due to low heritabilities at low input conditions. Gallais and Coque (2005) showed interaction between the genotype and the level of N for NUE, concluding that the best performing crop cultivars at high N input are not necessarily the best at low N input. For a large number of crops, there is genetic variability for both N uptake efficiency (i.e., the efficiency with which the crop captures available nitrogen resources) and for N utilization efficiency, i.e., the efficiency with which nitrogen taken up is used to produce crop dry matter (Hirel et al. 2007). Variation in NUE at high N input is mainly related to variation in N uptake efficiency (NuptE), whereas at low N input, both components of NUE could play a role, specifically nitrogen utilization efficiency (NUtE) (Gallais and Coque 2005).

Physiological components of NUE

NUE can be considered as resulting from three types of capacities: (i) the capacity of the crop to capture soil N, NuptE, defined as the increment in crop N uptake per unit of increment in soil N supply; (ii) the capacity of the crop to use N for biomass production, N conversion efficiency, NCE; and (given the impact of nitrogen on the phenology of the crop); (iii) the capacity of the crop to allocate carbon to harvestable organs (Sadras and Lemaire 2014). The two main recognized components of NUE are NuptE and NCE (Hirel and Lemaire 2006; Moll et al. 1982; Sadras and Lemaire 2014).

In most crops, during the vegetative stage, young developing leaves and roots behave as sinks for inorganic N uptake, synthesis and storage of amino acids via the nitrate assimilation pathway. These amino acids are further utilized in the synthesis of proteins and enzymes involved in different biochemical pathways and the photosynthetic machinery governing plant growth, architecture, and development. During the reproductive stage the increased supply of nitrogenous compounds is necessary for optimum flowering and grain filling. At this stage, both N assimilation and remobilization become critical and the leaves and shoots act as the source providing amino acids to the reproductive and storage organs (Kant et al. 2011). During tuber bulking in potato there is also intensive reallocation of dry matter to tubers. Vos (1999) mentioned that the balance between the relative sink strengths for nitrogen of canopy and tubers defines the carbon that can be produced in the plant, because it is strongly related to the senescence process: the higher the nitrogen reallocation to tubers, the faster the canopy senescence. Mustonen et al. (2008) mentioned that tuber yield and tuber nitrogen accumulation at plant maturity were related to crop nitrogen supply and that most of the nitrogen is reallocated to tubers; it would imply that tuber nitrogen uptake is representative of the total plant nitrogen uptake.

NUE has been studied in potato, in general using small numbers of genotypes or varieties. Kleinkopf (1981) reported that an increase in N input induced a decrease in the agronomic NUE (i.e., amount of tubers produced per amount of nitrogen supplied), with no difference due to plant growth type. On the other hand, Tiemens-Hulscher et al. (2014) found that early-maturing cultivars have lower NUE; Zebarth (2004b) reported differences in NUtE within maturity groups, suggesting differences in N uptake capacity of the cultivar (N uptake at high N input). Significant variation in NUE characteristics among genotypes and across contrasting environments enhances the importance of screening adapted potato germplasm with respect to N use efficiency characteristics (Zebarth et al. 2008).

Potato canopy development

Potato yield has been described as a function of light interception (Haverkort et al. 1991; Haverkort and Bicamumpaka 1986; Haverkort and Harris 1986; Haverkort and Harris 1987; Struik et

al. 1990). The percentage of ground cover has been reported as a useful trait to assess intercepted solar radiation (Haverkort et al. 1991). Haverkort and Bicamumpaka (1986), studied potato fields under *Phytophthora infestans* infestations and showed how the amount of intercepted radiation, which was linearly related to yield, could be determined by the proportion of ground covered with crop green leaves, emphasizing that radiation use efficiency did not change with changes in ground cover. In addition, Vos (2009) mentioned that potato adapts its foliage development to maintaining productivity per unit of leaf area when N is limited; this means that N affects the canopy dynamics resulting in a small canopy under low nitrogen. Spitters (1990) used this to describe differences among cultivars using crop models.

Crop development in potato is a complex trait, including a series of phenological events from different processes such as canopy growth, sympodial growth, tuber formation, etc. (Struik et al. 2005; Struik 2010). The response of the plant to different environmental factors could be manifested and studied by how developmental processes like canopy cover are affected.

Khan (2012) modelled canopy cover dynamics as a function of thermal time and soil coverage following the Yin et al. (2003) approach, which used a sigmoid curve to determine growth. The thermal time was calculated based on the beta function, describing the response to temperature of several developmental processes (Yin et al. 1995). An application of the canopy develoment model is shown by Khan et al. (2013). In this study, a physiological maturity type criterion based on canopy development parameters was developed as a first step to relate maturity type to phenology, agronomy and physiology. Usually maturity type is assessed using ordinal scales based on visual classification by comparison with a known standard variety (Visker et al. 2003) leading to ambiguous criteria (Haga et al. 2012). This method assesses the foliage senescence which is a stage of canopy development. The physiological maturity type criterion defined by Khan et al. (2013) offered the advantage of improving the understanding of the developmental process. Maturity type of potato plants is a very important factor affecting plant development. It has a strong genetic component, showing a quantitative trait locus (QTL) on chromosome 5 in different genetic studies with possible pleiotropic effects on several traits related to crop development (Celis-Gamboa 2002; D'hoop et al. 2014; Hurtado et al. 2012; Hurtado et al. 2015; Khan 2012; Malosetti et al. 2006). For this maturity type locus, the underlying central regulator has been identified and described as a Cycling DOF transcription factor (CDF) related to plant life cycle and tuberization (Kloosterman et al. 2013). Tiemens-Hulscher et al. (2014) reported maturity type and cultivar effects for several canopy traits under organic and conventional production systems showing that late cultivars tend to have higher yields and larger canopies provided the growth cycle is long enough.

Canopy development based on soil coverage is a powerful and important tool to study factors like N affecting plant development traits (Khan 2012; Tiemens-Hulscher et al. 2014), since nitrogen directly affects important develomental processes as mentioned by Kant (2011).

Relevance of this study

The Nitrate Directive (91/767/EEC) and the Water Framework Directive (2000/60/EC) will force a reduction in N supply to potato crops. However, since the potato crop (compared with many other field crops) has (a) a small and shallow root system and an associated low N-recovery (Hopkins et al. 2008) and (b) poor plasticity in response to variable N bio-availability (Vos 1997), it requires high levels of readily available N throughout the growing season to maintain vegetative growth and high productivity (Vos 1997; Vos and van der Putten 1998). It is therefore assumed that these directives restricting N-fertilisation will have great impact on both yield and quality in potato.

The agronomy and physiology of NUE in potato is extremely complex due to genotype-specific effects of N-supply on crop physiological/morphological characteristics, such as (a) the rate of leaf appearance, individual leaf growth, final leaf size, and the life span of individual leaves, (b) the number of lower and sympodial branches, (c) the overall rate of canopy development (e.g., increasing N supply levels accelerates crop development and the time when maximum canopy cover is reached), (d) light interception by the crop over time, (e) the rate of canopy photosynthesis, (f) the onset of tuberization and (g) final tuber yield and harvest index (Biemond and Vos 1992; Vos 1997, 2009; Vos and Biemond 1992). Moreover, N supply may affect tuber quality in terms of tuber size distribution, tuber dry matter content and protein content and protein quality like digestibility (Biemond and Vos 1992; Ewing and Struik 1992; Joern and Vitosh 1995; Kleinkopf et al. 1981; Vos and Biemond 1992).

However, while there is detailed information available on N-fertilisation regimes required for example to get optimum economic performance based on detailed response curves (Neeteson and Wadman 1987), there is little information on the type of genotypes that can cope with limited N supply and there are no detailed studies on the genetic variation for NUE among the modern cultivars used in Europe and its underlying ecophysiological mechanisms (Lammerts van Bueren et al. 2002; Lammerts van Bueren et al. 2008; Tiemens-Hulscher et al. 2014).

Additionally, as N input is basically limited to a maximum level by legislation to protect the environment, there is a shift in the focus of research from finding the economically or agronomically optimum rate of input to how to make best use of the permitted maximum amount of external supply of N (Vos 2009). This could be addressed by two different approaches:

I. from the fertilization management perspective, improving efficiency of the N supply by precision fertilization, using fractioned limited amounts of fertiliser at particular stages of the crop developmental process.
II. to breed for genotypes that can produce good yield in limited N input instead of under optimum N conditions.

Most of the studies until now included few genotypes or cultivars to understand NUE components and its variation by assessing how available N affects potato crop growth and how to improve the crop fertilization (Zebarth et al. 2008; Zebarth et al. 2004a; Zebarth et al. 2004b). However, research has not yet addressed the question: which genotypes can cope with limited level of N and how do they do it?

There is also limited quantitative and precise information available about genotypic differences in response to contrasting N-fertiliser regimes and types and/or input levels. In addition, there is virtually no information about (a) the genetics of NUE in potato and (b) physiological/morphological characteristics associated with NUE in potato. Such knowledge would however be essential to design new selection criteria and selection methods to select and breed for NUE in potato.

Main objectives:

A. To have a better understanding of how contrasting N inputs affect the performance of potato cultivars with distinct maturity types over the growing period, focusing on low input.
B. To understand the maturity type and N effects by identifying genetic variation in NUE of modern European cultivars using an extensive potato germplasm collection (200 cultivars/genotypes) phenotyped under two different levels of N input.
C. To identify QTL related to crop development traits and N uptake that depend on N input in a diploid biparental population (SH × RH population) previously developed in WUR, by making use of (a) existing genotyping data and (b) existing phenotypic data combined with newly generated data sets.
D. To identify N input dependent QTL/markers using an association mapping approach for crop development and nitrogen uptake (N_upt) using phenotypic data generated from an extensive potato germplasm collection in this study.

Outline of the thesis

This thesis is based on the combination of field experiments and genetic information available from previous projects in which Wageningen University and Agrico Research took part. There are six chapters, including this general introduction (Chapter 1), four experimental chapters (Chapters 2 to 5) and a general discussion (Chapter 6).

This thesis encompasses 3 years of experiments focused on comparing the performance of cultivars or genotypes under contrasting N input levels (i.e. N in the soil plus fertiliser). Phenotyping was based on canopy development (with weekly assessments), final yield, dry matter and N content. We used an ecophysiological model to dissect canopy development, expressing it as

estimated parameters with biological meaning. These estimated parameters can be considered as new traits which are informative and allow the understanding of the developmental process of canopy development, while enhancing the interpretation of the findings.

In Chapter 2 we address objective A, by assessing canopy development and yield components of a few genotypes from different maturity type classes, under different N environments (combination of two types of fertiliser and two contrasting N levels, and a control). The ecophysiological model parameters described the response of the cultivars to N as well as the maturity type differences. Maturity type drives the response to N input. The low N availability had more effect on the late-maturing cultivars. The meaningful model parameters helped to understand the correlation and the chronological relation between important traits like leaf area index (LAI) and tuber bulking.

Chapter 3 describes the phenotypic variation of a large set of potato cultivars for canopy development parameters and NUE under two contrasting N levels. The main effects of N and maturity type were described, as well as the effects of N on the relationship between traits. It is suggested that a general strategy to breed for NUE should focus on low input. The best cultivars for high NUE should combine a high response to N fertilization and high performance under limited input (Objectives B and D).

In Chapter 4 we make use of genotypic information available to identify QTL, and especially those that are depending on the N input in the biparental diploid population SH × RH. The approach combines QTL analysis with the ecophysiological canopy model, where the canopy parameters are the traits used to find QTL. Hotspot regions were found in some chromosomes besides the well reported region on chromosome V. QTL that were N dependent for canopy development, yield and quality traits were shown by comparing the result from separate QTL analyses for each contrasting N level (objective C). There were interactions between the genetic factors associated with agronomic and physiological traits and N input, therefore direct breeding for low N input could offer an advantage.

In Chapter 5 an association analysis is done using the phenotypic data generated in Chapter 3. The ecophysiological model is combined with a Genome-Wide Association mapping (GWAS) analysis to identify N input dependent QTL/markers (Objective D). This approach entails the use of a diverse set of tetraploid cultivars with a much wider genetic background compared with the SH × RH diploid biparental population. Again, the usefulness of the approach using an ecophysiological model to add extra physiological value to the phenotypic data in combination with genetic analysis is shown. N dependent QTL for some canopy development traits were identified within this broad genetic background and the importance of maturity type is discussed as it affects the response of the cultivars and the QTL detection of related maturity traits.

Chapter 6 is the general discussion of the findings of this study. The importance of understanding developmental traits is highlighted as well as their possible use in breeding schemes for NUE. The findings are put in the context of NUE for low input. The importance of maturity type is also

discussed from the perspective of breeders and producers. Finally, the possibilities of using high-throughput technologies to enhance phenotyping or to combine high-throughput phenotyping with approaches like the ones used in this thesis using an ecophysiological model to dissect canopy development, are highlighted.

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Chapter 2

Source and amount of nitrogen affect relations between canopy development and yield traits of diverse potato cultivars

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Abstract

Nitrogen (N) has a huge impact on agronomic, economic and environmental aspects of the potato (*Solanum tuberosum* L.) crop. Policies restricting N use changed the research perspective to how to make the best use of the allowed amount of external N supply, including breeding for the best genotypes under low input. We aim to understand how cultivars differing in maturity type respond to contrasting N inputs and N availability across the growing season. Six (2010) or three (2011) cultivars representing the early, middle and late maturity groups were evaluated under five nitrogen regimes combining two N levels (70 kg N/ha or Low, "L" and 140 kg N/ha or high, "H") and two types of fertiliser (synthetic, "FC", and organic, "FO") plus no application (0 kg N/ha). Assessments included canopy development (CDv) (phenotyped using an ecophysiological model) and agronomic traits. The 2010 experiment showed poorer crop performance than the 2011 experiment because of less conducive field and weather conditions especially affecting the organic fertiliser treatments. The more N available, the better the crop performance, but the lower the N use efficiency expressed as unit of dry matter produced per unit of fertiliser applied. The later the maturity type, the higher the nitrogen use efficiency with higher yield and dry matter percentage but lower N content. Canopy development closely reflected the N effects. With more N available initial CDv was faster, whereas the maximum soil cover achieved was reached earlier and had higher value allowing more light to be intercepted. Late varieties and high N treatments, however, needed more time to reach the maximum LAI. The onset of tuber bulking did not show significant N treatment effects but was earlier for earlier cultivars. For all cultivars onset of tuber bulking was around the middle point between maximum rate of canopy development and the time when maximum soil cover was reached. Maximum tuber bulking progression rate occurred when the LAI was maximum. The cropping period (time until haulm has senesced or is destructed) should allow the cultivar to reach maturity and to relocate as much dry matter to the tubers as possible. In this sense, the cost to maintain the crop for the extra time needed by late cultivars will significantly determine the value, all within the framework of each specific market. In our experiments, low rates of nitrogen tend to affect late varieties more with useful variation within maturity groups that need to be further explored in a wider set of cultivars.

Keywords: Potato, Nitrogen input, Canopy development, Organic and synthetic fertilisers, Haulm and tuber dry matter, Tuber bulking, Radiation use efficiency.

Introduction

Potato (*Solanum tuberosum* L.) crop growth is highly responsive to nitrogen (N) supply. Plants respond to different N inputs levels by adjusting several physiological processes and morphological traits related to canopy development, all having an effect on yield. Vos and Biemond (1992) studied some of these responses. They showed that high N inputs increased the number of basal branches and the length of the main stems, but not the number of main stems. The apical branching and apical leaf proportion were enhanced with more N, the leaves became larger, with higher rate of leaf expansion and longer life spans; all these factors contributed to increasing the total leaf area. N input levels did not affect the rate of appearance of successive organs, but it did affect the total number of leaves (Vos and Biemond 1992).

Nitrogen effects on the canopy result in higher potential to intercept solar radiation over time (because of a denser canopy and a prolonged longevity) and a higher photosynthetic capacity of the plant. However, the intrinsic productivity per unit leaf area does not change (Vos 2009) since only a small effect of N on the photosynthetic capacity of leaves has been reported (Marshall and Vos 1991; Vos and Van der Putten 1998; Vos and Van der Putten 2001). Moreover, the amount of solar radiation intercepted by the crop was shown to be closely related to the dry matter accumulation in the potato crop (Haverkort and Harris 1987; Haverkort and Bicamumpaka 1986).

The changes in canopy due to N and the effects on photosynthesis affect tuber bulking and final yield. A delay in the onset of tuber bulking due to high rates of nitrogen fertilization has been reported (Biemond and Vos 1992; Kleinkopf et al. 1981). Moreover, the N effects depend on whether the N level is above or below optimal values. For instance, an increase in N rate below optimal values increases the yield, whereas above optimal levels (excessive N rate) may delay tuber bulking and maturity (Biemond and Vos 1992). The latter could affect the tuber quality regarding tuber size and dry matter content (Goffart et al. 2008) and even cause a reduction in the final yield if the season is not long enough (Kleinkopf et al. 1981; Vos 2009). On the other hand, Millard et al. (1989) found that increasing N fertiliser raises the N concentration in the tuber dry matter and therefore increases the yield of some nutritionally essential amino acids, hence improving the nutritional quality of tubers.

Vos (2009) mentioned that more yield in response to N could result from:

(a) A larger crop growth rate over the same total crop duration;

(b) Similar average crop growth rate over an extended period of growth; or

(c) Combination of (a) and (b).

The total growth period of plants is prolonged by high rates of N supply mainly because the period of maximum light interception, i.e. where the canopy cover is at the maximum, is extended with higher N nutrition (Vos 2009).

In addition, cultivars can differ in their response to N. Kleinkopf (1981), when comparing potato plants with different growth types under different N inputs, found that cultivars showing indeterminate growth might have higher final tuber yields if other factors are not limiting and if the growing season is long enough. In addition, different cultivars have different requirements for nutrients with different optimal values based on their uptake. Maturity type has been identified as a major factor, with a strong genetic component for complex traits like yield, canopy development (Khan et al. 2012) and senescence (Hurtado Lopez 2012).

The study of the development of the canopy over time has the potential to assess N effects on complex traits such as yield for different cultivars with different plant types and maturity type. The canopy development can be measured as the proportion of ground cover with green leaves (%SC). Haverkort et al. (1991) found that SC% represents the best estimate of the proportion of intercepted radiation and allows the most accurate calculation of the efficiency of conversion into dry matter. Ground cover can be assessed easily across the season since it is a non-destructive and relatively quick method (Burstall and Harris 1983). Khan et al. (2013) developed a model to express the canopy cover as a function of temperature by using cardinal temperatures for canopy growth. The model allows a dissection of canopy growth into assessable parameters that can be treated as new traits in further analysis. All this increases the opportunity to understand how N input affects canopy development, not only as a complex trait but also as a group of individual, physiologically meaningful component traits.

Plant responses to N depend on other factors determining growth such as other nutrients (Vos 2009), water potential in the soil, soil types, and biological and environmental factors in general. In addition, crop management (e.g. organic vs conventional production), crop rotation, cropping practices, type of fertiliser, restrictions on the input levels etc. are important factors determining N availability and N response.

Since agricultural production was largely directed to provide optimal conditions to the cultivars, low input was not a constraint during the last decades in most of the developed countries. This trend is changing since cropping must adapt to environmental changes caused by side effects of agriculture itself. N fertilisers account for 33% of the total annual creation of reactive N or 63% of all anthropogenic sources of reactive N (Dobermann 2005). Potato crops on sandy soil and with high N input (to avoid yield losses due to low N) contribute to N contamination as a result of the naturally shallow and poorly developed root system compared with other main crops such as wheat, maize or sugar beet (Goffart 2008).

In an attempt to mitigate the damage of N pollution, regulations and laws, like the Nitrate Directive (1991) (91/767/EEC) and the Water Framework Directive (2000) (2000/60/EC), have restricted the amount of N input in crop production. This promoted a shift in the focus of research

that goes from finding the financial optimal input rates to how to make the best use of the permitted maximum amount of external N supply (Vos 2009). Consequently, regulation has been pushing for improvements in fertiliser N use efficiency (NUE) in agricultural systems: less N fertiliser per unit food produced (Dobermann 2005). Following this trend, breeding should also change its aims from looking for cultivars that perform well under optimal management conditions to breeding for the best genotypes under low input (Kerbiriou et al. 2014; Tiemens et al. 2014).

The motivation for this study was to provide an insight into how different levels and types of N input (i.e. organic and inorganic) affect the performance of different potato cultivars across the growing season under field conditions in the Netherlands. For this purpose, we focused on agronomic traits such as canopy cover, tuber bulking, biomass production, and N uptake. We aimed to assess and analyse relationships between these traits as well as how the traits develop over time, focusing on low input. In addition, by including representative cultivars from different maturity classes, we aim to have a more realistic view of possible ranges of responses to N in the potato crop development.

Materials and Methods

Field design

Two experiments were carried out for this study, one in 2010 and one in 2011. Table 1 summarizes the details of the design and the agronomy. High quality commercial seed tubers were pre-sprouted and used for planting. All cultural practices such as ploughing, sowing, weeding, and chemical spraying were done uniformly and according to the best practices for the site. Contrasting cultivars for: i) N uptake in tubers at final harvest and ii) canopy development traits under low N input were included (based on data from Agrico Research in 2009). Two cultivars were selected from each of three maturity groups (early, intermediate, and late) for the 2010 experiment. For the 2011 experiment, three cultivars out of the six in 2010 were selected as the most representative ones of each maturity group (Table 1). The maturity scores for the cultivars included in these experiments are: 'Berber' (coded: Bb): 8; 'Innovator' (In): 7; 'Bintje' (Bj): 6.5; 'Fontane' (Fn): 6; 'Kuras' (Kr): 4, and 'Festien' (Fs) 3. These scores are based on the National Dutch Cultivar List of Potato (www.potato.nl and http://www.europotato.org/).

Plots consisted of four rows and only the two middle rows were used for assessments. The distance between rows was 75 cm and between plants within the row 32 cm. The plots included plants to be measured for intermediate and final harvests. At each intermediate harvest 3 plants/row were harvested, leaving 2 plants/row as border between intermediate harvests. At final harvest 10 plants/row in 2010 and 7 plants/row in 2011 were harvested. The five N treatments considered in these experiments were the combinations of two factors:

- two fertiliser types, synthetic fertiliser (FC) with normal release and organic fertiliser (FO) with slow release of nitrogen;
- two N levels, 70 kg N/ha or Low (L) and 140 kg N/ha or high (H);
- the control application consisted of 0 kg N/ha (C).

Table 1 Summary of setup of experiments in 2010 and 2011.

Experiment	Achterberg 2010	Wageningen Born 2011
Planting date	29-04	27-04
Soil type	Non structured [4] Sandy soil	Structured Sandy soil
Cultivars:	n=6	n=3
Early (E)	Berber (Bb), Innovator (In)	Berber (Bb)
Intermediate (M)	Fontane (Fn), Bintje (Bj)	Bintje (Bj)
Late (L)	Kuras (Kr), Festien (Fs)	Kuras (Kr)
Treatments[1]	5 Env[2]	5 Env
FCH	FCH 1	FCH 2
FCL	FCL 1	FCL 2
FOH	FOH 1	FOH 2
FOL	FOL 1	FOL 2
CCC	CCC 1	CCC 2
Blocks	3	3
Plot number:	90=6*5*3	45=3*5*3
Intermediate harvests: (IH)	5 IH	4 IH
DAP[3] for the IH	at 46, 67, 88, 108 and 130	at 49, 68, 82 and 99
Final harvest (in DAP)	180 (haulm destruction168)	131, 139, 154
Harvesting method	Machine	By hand

[1] FCH= fertiliser synthetic high, FCL= fertiliser synthetic low, FOH= fertiliser organic high, FOL= fertiliser organic low; CCC=no application.
[2] Envs: Nitrogen environments are all possible combinations of treatment and year.
[3] Days after planting.
[4] refers to the arrangement of soil separates or primary soil particles (sand silt and clay into units called soil aggregates. An aggregate possesses solids and pore space.

Experimental units were arranged in a "split-plot design" with three blocks, where fertiliser treatments were the main plots and cultivars were the sub plots.

The fertilization was done before planting. For the FO, dry pellets of cattle manure were mixed in the upper 15 cm soil layer. The application was done 1 day before planting in 2010 and 1 month before planting in 2011. The synthetic NPK fertiliser was applied just before planting and mixed homogeneously with the soil. The composition of the synthetic fertiliser was: Calcium ammonium nitrate (N 27%), Triple super phosphate (P_2O_5 45%), and sulphate of potash (K_2O 30%). The cattle manure had a composition of 2% N, 2% K_2O, and 3% P_2O_5. The working coefficients for these nutrients were 0.3, 0.9 and 1% for N, P and K, respectively.

For the synthetic fertiliser treatments and the zero application, phosphorus and potassium (P and K) fertiliser were applied to reach non-limiting levels based on soil analysis. This correction was not done at the organic fertiliser treatments because cattle manure also provided P and K abundantly.

The N in the 0-25 cm soil layer was estimated at 20 kg N ha^{-1}. This, added to the applied synthetic N, resulted in 160, 90, and 20 kg available N per hectare, for the high, low and no application, respectively.

Assessments and data processing

Haulms from sampled plants were cut just above the soil surface and tubers were dug out immediately. The vegetative parts of the plant below ground (stems, roots, and stolons) were not considered in this research, mainly because they only represent 1-2% of the total dry weight and N (Vos, 1997) and because of the technical difficulties to collect them. Plant tissues were washed with water to remove soil materials and then the excess water was removed. Subsequently, the total biomass fresh weight was recorded. Dry matter percentage (DM%) for different fractions was measured as dry weight of a subsample divided by its fresh weight expressed in percentage; drying was done for 48 hours at 70 °C in force-ventilated ovens. The fractions were: A) Tuber including all size classes, B) Green stem and leaf (separating green from yellow or dead tissue). Additionally, N content ([N]) was assessed using the Kjeldahl protocol (1883). Using the leaf subsamples, Leaf Area Index (LAI) was measured with a LiCor leaf area meter (LiCor, Lincoln, USA). Acronyms for all variables considered are listed in Table 3.

Measurements for plant tissues, dry weight, N uptake by plant tissues (tissue dry weight x N content in the tissue) and N application were expressed per square meter (m^{-2}).

Thermal time estimation

Thermal time was calculated for each assessment date and each plot starting at the emergence day and using the Beta function described by Yin et al. (1995). For this purpose, the cardinal temperatures determined for potato growth (Khan, 2012) and hourly temperature recordings were used. Thermal day (td) is the unit for beta thermal time. For 1 calendar day, the thermal time has a value between 0 and 1.

The non linear temperature effect function *g(T)* was use to convert days after emergence (DAE) in beta thermal days (BTT) (see Annex 1A). Where *T* is the air temperature in degrees celcius (°C). The three main temperatures: *Tb* (base), *To* (optimum) and *Tc* (ceiling) for the phenological development of potato were used (T_b= 5.5 T_o= 23.4 and T_c= 34.6; all in °C (Khan 2012, Khan et al. 2013); c_t is the temperature response curvature coefficient also estimated by Khan (2012) to be 1.7.

Canopy development

The percentage of soil cover by the canopy (%SC) was assessed weekly using digital photos taken with a digital camera Canon SXI200 (Canon Inc., Japan). Pictures were taken by setting the camera always at the same place, in order to cover the same three plants in one row. The height of the camera was 80 cm above the top of the canopy. The percentage of green pixels on the photos was estimated using MATLAB® version 7.8.0347 (R2009a), the MathWorks TM program.

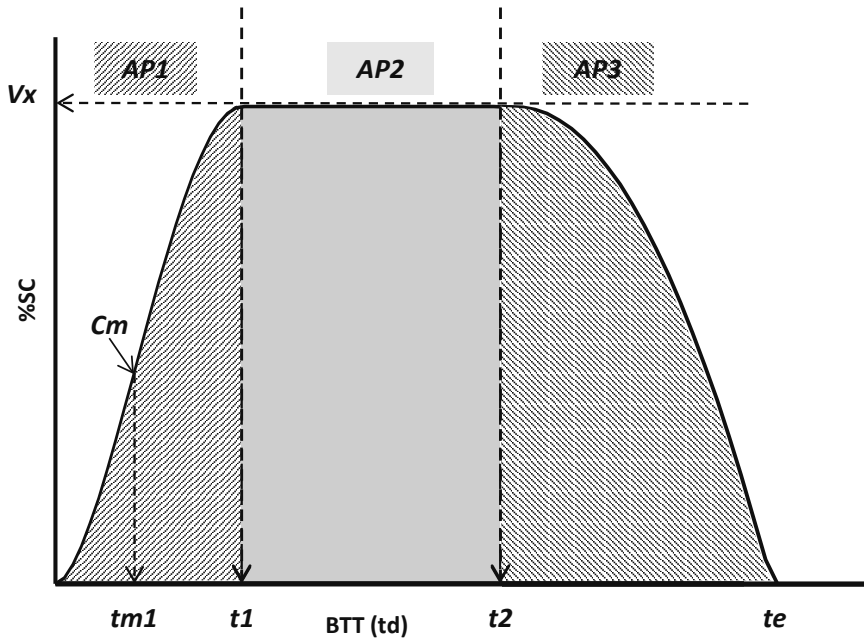

Figure 1 Canopy development curve showing the percentage of soil coverage (%SC) progress across the season in beta thermal time (BTT), as thermal days (td), calculated using the beta function describing potato canopy growth as a function of temperature. The canopy development parameters are indicated as well as the areas under the curve for each of the three phases of canopy development (see main text).

The curve fitting for canopy development was performed using %SC as a function of beta thermal time following the three equations describing each phase of the canopy growth curve (Khan et al. 2013). The procedure was carried out using the nonlinear procedure (PROC NLIN) of SAS. The equations were specified in the procedure (Eqs 1 to 3) and the parameters for these functions were estimated with the GAUSS method as fitting algorithm. The curve fitting was done for each plot separately. Therefore, the estimated parameters were used as a new traits for statistical analysis according to the experimental design to estimate treatment, cultivar and interaction effects.

$$V = V_x\left(1+\frac{t_1-t}{t_1-t_{m1}}\right)\left(\frac{t}{t_1}\right)^{\frac{t_1}{t_1-t_{m1}}} \text{ with } 0 \leq t \leq t_1$$

Eq. 1

$$V = V_x \text{ with } t_1 \leq t \leq t_2$$

Eq. 2

$$V = V_x\left(\frac{t_e-t}{t_e-t_2}\right)\left(\frac{t+t_1-t_2}{t_1}\right)^{\frac{t_1}{t_e-t_2}} \text{ with } t_2 \leq t \leq t_e$$

Eq. 3

Five parameters were estimated in Equations 1 to 3, where *tm1* is the time at which the maximum progression rate of Phase I occurs (the inflexion point in the canopy build up phase); *t1* is the time at which the maximum soil coverage *Vx* is reached. *t2* is the moment at which soil coverage starts to decline and *te* is the moment at which the %SC is zero and the crop is fully senesced. The term *V* is then the predicted %SC using a set of parameters. Furthermore, the maximum progression rate *Cm* was calculated as is shown in Eq. 4 (Khan, 2012; Khan et al. 2013).

$$Cm = \left(\frac{2t_1-t_{m1}}{t_1(t_1-t_{m1})}\right)\left(\frac{t_{m1}}{t_1}\right)^{\frac{t_{m1}}{t_1-t_{m1}}} Vx$$

Eq. 4

In Figure 1 the three main phases of the canopy development are shown and related to the parameters in Eq. 1 to Eq. 4. The areas under the curve of soil coverage are graphical representations of these phases. *AP1* is the area under the curve for Phase I or build-up phase. It covers the period from crop emergence until maximum soil coverage is reached. In this period leaves, stems and branches appear. *AP2* is the area under the curve for Phase II during which the maximum soil coverage is constant and light interception is maximum. Finally, *AP3* is the area under the curve for Phase III or the senescence phase, the period of decline in light interception and soil cover, reaching zero soil coverage at plant death. The total area under the curve *AUC* was also calculated as the summation of the areas of the sub phases. The equation to calculate the areas under the curve are also shown in Khan et al. (2013) (see Annex 1B).

Curve fitting for other traits using the beta function

Due to the flexibility of the beta function to fit curves from quadratic to sigmoid shape (Yin et al. 2003), the procedure described above was used to fit curves for other traits. Using data from the intermediate harvest curve parameters were estimated for dry matter partitioning of leaves, stems, tubers, leaves and stems together in haulm, and also leaf area index. Only Eq. 1 for sigmoid shapes, as well as a restricted version Eq. 5, (Eq. 1 for *tm1* = 0 that is quadratic equation without the constant term as shown by Yin et al. (2003)) and Eq. 4 (to estimate the maximum progression if applicable) were used here.

$$V = V_x(2t_1 - t)\frac{t}{{t_1}^2}$$

Eq. 5

To simplify and to avoid further confusion parameters are identified by adding a prefix as follows: *SC_* for soil coverage, *Tb_* for tuber bulking, *LAI_* for leaf area index, *St_* for stem dry matter, *St_c_* for stem dry matter cumulative, *Lf_* for leaf dry matter and *Lf_c_* for leaf dry matter cumulative. The meanings of the parameters are shown in Table 3.

Onset of tuber bulking (*tb*)

Onset of tuber bulking, *tb*, was estimated as the time in thermal days (td) at which a linear extrapolation of tuber growth predicted zero weight (W_0), using the maximum progression rate (*Cm*) as the slope of the linear equation (Eq. 6). *b* was estimated by using the predicted yield (tuber dry matter, Tb_DM) at *tm1* that was calculated from Eq. 1.

$$W_0 = Cm\ tb + b$$

Eq. 6

Radiation use efficiency (RUE)

Radiation use efficiency, the amount of biomass produced per amount of light energy captured, is especially important to compare the performance of a crop under different environmental conditions. From the incoming radiation, not the entire spectrum is used for photosynthesis. Therefore, PAR (that is the photosynthetically active radiation) was used for the calculation of RUE. PAR is known to be a fraction of the global light radiation that reaches the ground and more precisely a fraction of the shortwave radiation (SI).

Using data from a weather station nearby the experimental field, SI was used for RUE calculations. Accumulative SI for every two-day-interval was calculated and expressed in MJ m^{-2} based on hourly data (average of each hour). The fraction of SI that is incident PAR (PARinc) was calculated

using a factor of 0.45 (PAR/SI) (Howell et al. 1983). The percentage of PAR intercepted (%PARint) by the canopy of the plants was calculated as a function of the percentage of canopy cover estimated every two days (%PARint = 0.895 x %SC +0.043 (r^2 =0.988) (Haverkort et al. 1991)). Subsequently, the PAR intercepted (PARint) was calculated as the product of PARinc and the %PARint. Finally, the seasonal PARint was calculated as the summation of its partial values for each interval from emergence until senescence. All calculations were done on a per plot basis.

Tuber size and weight distribution

At final harvest, tubers per plot were graded by size using a standard procedure for tuber grading in potato. The size categories were 28-30, 30-35, 35-40, 40-45, 45-50, 50-55, 55-60, and 60-65 mm; for each size category the total tuber weight (Tbw) and tuber number (Tbn) were recorded and the data were expressed per m^2. A bell-shaped curve was fitted to each of the data sets, Tbwand Tbn, on a per plot basis as in Eq. 7,

$$Tb = MX * \exp\left(-\frac{(mcl - B)^2}{A}\right)$$

Eq. 7

where *Tb* is either Tbw or Tbn. "*A*" is a dispersion parameter expressing how the weights/numbers are distributed across classes, "*mcl*" is the average size of each size class, "*B*" is the average size at which the "*MX*" or maximum weight/number occurs. The curve-fit parameters were named for each variable as follows: for tuber weight data: TbwA, TbwB, TbwMX and for tuber number data: TbnA, TbnB, TbnMX.

Statistical data analysis

The data sets were analysed using GENSTAT (VSN International Ltd., Version 15). In this paper we present results in two ways: A) Combined data from the two experiments 2010-2011 in order to have an overview of cultivar performance under different N conditions, using a mixed model. B) Analysis of 2010 data, for in depth understanding; in this case the ANOVA approach in Genstat offered the same result as a mixed model as the data set was balanced. Means were separated using a Bonferroni test ($P < 0.05$).

In most of the tables and figures of the results and appendixes, abbreviation for cultivars, treatments and/or N environments (Env) will be used as indicated in Table 1.

Results

Nitrogen environments

To assess the performance of cultivars under different soil N conditions, we defined the N environment as the combination of treatment and year (and site). The year and site were confounded including differences in weather conditions, soil characteristics and their interactions. The result was a set of different scenarios of N availability. Canopy development traits, yield, N content in tubers (Tb_[N]), N uptake, and RUE were measured and analysed. The environment (Env) and cultivar (Gen) main effects were significant for all traits except for *AP3* and *te-t2*. These two traits showed a high variability with no explanation of the sources of variation included in the experiments. On the other hand, the interaction cultivar × environment (Gen × Env) was not significant for *tm1*, *te*, *te-t2*, *AP3*, *AUC*, Tb_[N], and RUE_Tb. This means that the differences between cultivars were similar when they were exposed to different N Env (Table 2).

Table 2 Wald test summary (P values) per trait, using the data of the 2010-2011 experiments. To avoid further confusion the prefix *SC_* denotes parameters derived from soil coverage. 'Gen' indicates the cultivar term in the analysis, 'Env' indicates the nitrogen environment term and 'Gen × Env' indicates the interaction term. For traits acronyms, see Table 3.

Traits	Env	Gen	Gen × Env
SC_tm1	<0.001	<0.001	0.997
SC_t1	<0.001	<0.001	<0.001
SC_t2	<0.001	<0.001	0.005
SC_te	0.003	<0.001	0.898
SC_Vx	<0.001	<0.001	<0.001
SC_t2-t1	<0.001	<0.001	<0.001
SC_te-t2	0.467	0.506	0.323
AP1	<0.001	<0.001	<0.001
AP2	<0.001	<0.001	<0.001
AP3	0.278	0.302	0.221
AUC	<0.001	<0.001	0.267
Tb_DM%	<0.001	<0.001	0.023
Tb_DM	<0.001	<0.001	0.004
Tb_[N]	<0.001	<0.001	0.721
Tb_NUpt	<0.001	<0.001	0.039
TbNUE	<0.001	<0.001	<0.001
RUE_Tb	<0.001	<0.001	0.150

In order to have an overall description of the environments (Envs), a ranking was done using information from all traits. Standardized values based on general averages and standard deviation (including all the plots) for each trait were calculated to avoid problems because of the units. Then, averages per environment for each trait were calculated and combined into an index with no weights. The general ranking of the Envs, from the lowest to the highest index value was: CCC 1, CCC 2, FOH 1, FOL 1, FOL 2, FCL 1, FCH 2, FCL 2, FOH 2, and FCH 1 Annex 2. In this ranking, Tb_DM, *AUC* and *AP2* strongly increased from the low index to the high index Envs. The increment was less strong for N uptake and *t1-t2*, and very small for *Vx*. The other traits were not well represented in this general ranking.

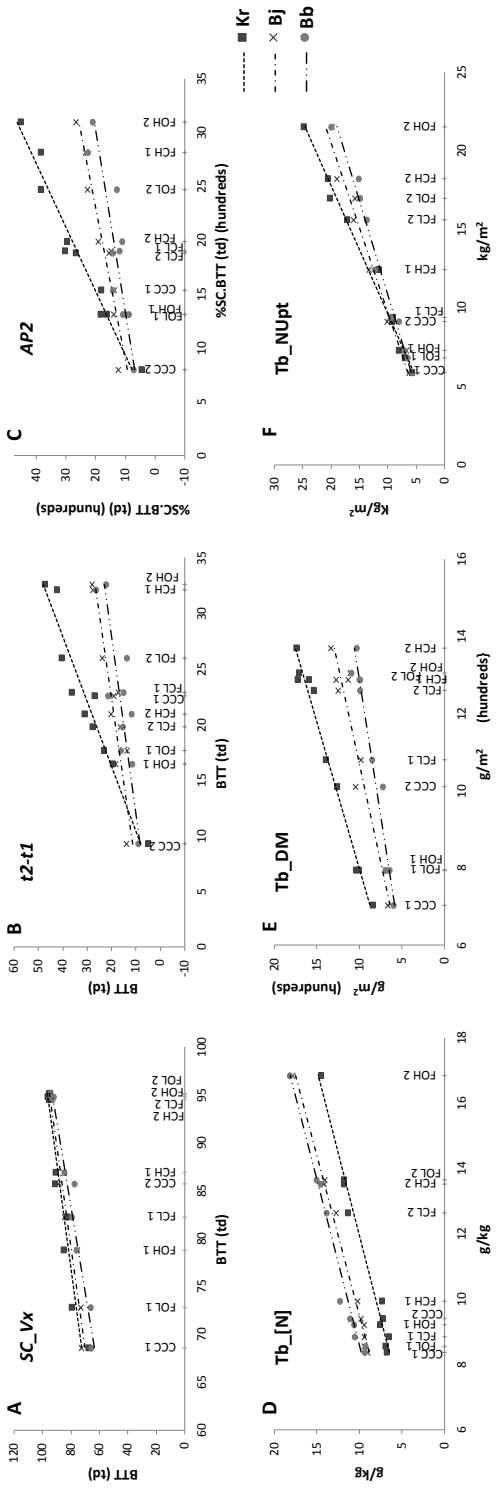

Figure 2 Finlay Wilkinson regression lines of the treatment means for each genotype on the average treatment means. Only the three cultivars common in the two experiments were included. On the X axes treatment means are ranked in ascending order. The units for each plot are the same in both axes. For acronyms of the treatments, see Table 1. A) *SC_Vx* , B) *t2-t1*, C) *AP2*, D) Tb_[N], E) Tb_DM and F) Tb_NUpt. ■ = Kuras, × = Bintje and ● = Berber.

Using yield as indicator of performance, *AP1* and *t1* showed low values in good performance Envs (High index): the plants quickly reached *Vx* and therefore *AP1* was small in comparison with poor performance Envs.

Correlations between tuber dry matter yield (Tb_DM) and some canopy traits were strongly positive: area *AP2* (0.80), *AUC* (0.78) and *Vx* (0.72)(Annex 3). Therefore, yield was highly dependent on how canopy developed, especially in Phase II when the maximum soil coverage was stable and light interception was maximum.

A visualisation of the cultivar × Env interaction is shown in the Finlay Wilkinson regression (Figure 2). This regression gives an idea of varietal adaptability or phenotypic stability. The average for the cultivars in each environment is plotted against the overall average of each environment. It also gives a ranking from the worst to the best environment for each trait. Tuber N content (Tb_[N], Figure 2 D) is an example of a trait with no interaction, with all lines parallel. Kuras stood out as the cultivar with a higher slope for all traits with significant interaction, except for *SC_Vx* (A). For tuber dry matter or yield dry weigth (Tb_DM), and Tuber N uptake (Tb_NUpt) the differences in slope were small and therefore the cultivar × environment interaction was less strong.

Parameters *t2-t1* and *AP2*, which refer to Phase II of the canopy development, showed a cross-over interaction of the performance of the cultivars at the low quality Envs. These two parameters were highly correlated (0.95) and had similar ranking for the Envs, since *AP2* is the product of *t2-t1* and *Vx*. The late cultivar Kuras showed a stronger response for these two variables, with a higher slope of the line (Figure 2B,C). This means that duration of maximum canopy cover is highly responsive to N allowing longer light interception (Phase II) in better Envs. Additionally, since the *SC_Vx* was similar for all cultivars the main response to a better Env was mainly due to an increase in the duration of Phase II.

The Tuber N uptake (Tb_NUpt) showed a cross-over interaction, with 'Kuras' in particular having a high slope (Figure 2F). The two variables that are combined in the N uptake, i.e. Tb_[N] and Tb_DM, exhibited an inverse order of cultivars. It means that the more dry matter produced by a cultivar, the lower the N content in the DM.

In the poorest Env for Tb_DM, the performance of cultivars showed small differences converging in a range between 6 to 9 kg/m^2, whereas in the best Env the differences were larger with a range of 11 to 19 kg/m^2. Moreover, considering that these cultivars are representative of three maturity types there is a trend: 'Kuras' the late cultivar, had a higher yield DM (or Tb_DM) under better Envs, with a higher slope, followed by 'Bintje' (intermediate cultivar) and then 'Berber' (early cultivar). The Tb_N content did not show differences among cultivars, all of them having similar slopes.

Three quadrant approach

This type of graph displays three important relations at once (Vos 2009), based on cultivar and fertiliser type combination per year (Figure 3): Quadrant I relates tuber yield with N applied for each cultivar and type of fertiliser, which is called the "agronomic response". Quadrant II shows

the "physiological response", which describes tuber yield per unit of N uptake. Finally, Quadrant III represents the relation between N applied and N uptake, which gives an indication of the "soil and root related domain". We used this analysis to show the results at final harvest of the two experiments (2010 and 2011). The fertiliser rates were the N levels applied: 0 (0 rate), 7 (low rate) and 14 (high rate) kg/m^2 while the fertiliser types were FC synthetic and FO organic fertiliser. The lines representing each cultivar-fertiliser type combination were compared by evaluating the slopes among the fertiliser levels.

The slope of the segment from 0 application to low rate is referred to as the response to the low input or 0-7. The slope for the segment 0 to the high rate is referred to as the response to the high input or 0-14; finally the slope for the segment from low rate to high rate is referred to as the response from low to high or 7-14 (values of the slopes are in the Annex 4).

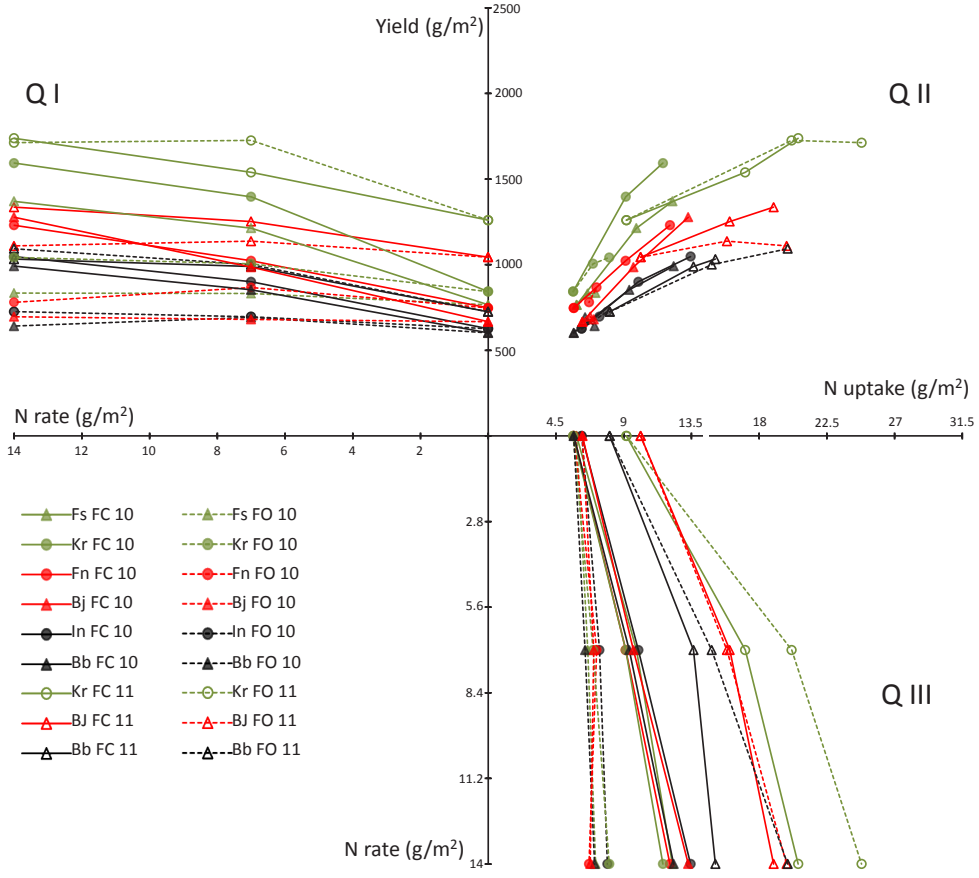

Figure 3 Three-quadrant diagram including data from 2010 and 2011 experiments (close and open symbols in the legend, respectively). Quadrants are referred to as: I, II, III). Colour denotes maturity type; green = late, red = intermediate and black = early. The line type represents the type of fertiliser; Dotted line: FO, Continuous line: FC. Yield is considered as tuber dry matter. For the acronyms in the legend, see Table 1.

In Quadrant I, maturity appeared as a main factor explaining the observed differences in yield for both years when looking at general averages. Typically, late cultivars had higher yield than intermediate and early cultivars (Table 4 and Table 5). Most of the lines (cultivar-fertiliser type combinations) levelled off at high N input. Generally, the response of each cultivar was lower for the segment low to high, than for the 0-7 range, except for 'Bintje' under FO in 2010. Moreover, the reduction in the response was in general lower with FC than with FO.

Furthermore, comparing within the same segment, FC generally showed a stronger response than FO, but not in 2011 for the segment 0 to low input. Here, the response of the cultivar Kuras with FC was smaller than in FO, while cultivar Berber had a slightly lower response with FC. Finally, 'Berber' with FO had also a stronger response in the other two segments 0 to high input and low to high input.

There were very specific interactions between type of fertiliser and year/field conditions. The year 2011 showed smaller differences in response between fertiliser types while in 2010 FC clearly induced a stronger response. In 2010 with FO, 'Fontane' and 'Berber' had a negative response from low to high, i.e. lower yield at higher N rate, but 'Bintje' had higher yields at high N.

In general, 2010 showed lower dry matter yields than 2011 (885.4 and 1206.8 g/m^2, respectively). At the 0 rate, the overall average was 708.2 g/m^2 in 2010, with small differences among cultivars, compared with 1009.6 g/m^2 in 2011 with larger differences among cultivars. These data suggest a higher N availability from the soil in 2011 than in 2010 as a result of soil and weather conditions. Therefore, 2010 could be considered a very low input situation compared with 2011.

In Quadrant II (Figure 3), the maturity type differences were obvious in both years, with the late cultivars accumulating more yield per unit of N taken up. However, as shown in Figure 2D, the later the maturity type, the lower the content of N in the tuber dry matter. Comparing the 0 application treatment from the two years, the N uptake was greater in 2011 than in 2010. Additionally, FO showed large differences in response between years. In 2010 the yield and N uptake hardly improved with application of N (for both rates) using manure, whereas in 2011 it clearly increased. Nitrogen utilization efficiency, NUtE, was considered as the yield relative to the N uptake on tubers at final harvest. NUtE is represented by the slope of the lines in QII, for segments from no application to low or high. FC generally showed higher NUtE in 2010 than in 2011.

In 2010, the late cultivars showed higher NUtE than intermediate and early ones (Figure 3QII). For both fertilisers types, FO and FC, the segment from no application to low had stronger response than the low to high, but for FO some cultivars took up less N at high rate ('Fontane' and 'Berber'). In addition, FO had a larger difference in NUtE among N levels than FC.

In 2011, early cultivars showed higher NUtE than late and intermediate ones, with both fertiliser types. Moreover, the NUtE was higher at low N level than at high N level and the difference between levels was larger for FO than for FC.

Finally, in Quadrant III, cultivars with FO in 2010 did not show a clear response in the N uptake to both levels of N input (high and low rates). On the contrary, FC did show a response. Additionally,

the reduction in the response for the segment low to high was lower with FC than with FO. In this quadrant N uptake efficiency (NUptE) is the slope of the lines in QIII. Here, the late cultivar Kuras showed the lowest NUptE, with both fertiliser types from the "0 rate" to the "low rate". In 2011, the N uptake at "0 rate" was much higher than in 2010. Additionally, in 2011 the increment from the "0 rate" to the "low rate" was higher for all cultivars having higher N uptake efficiency than in 2010 under both fertiliser types. At the "high rate", the NUptE decreased (for both years and both fertiliser types) except for 'Bintje' under FC. Moreover, FO allowed more N uptake than FC for all cultivars in 2011 while in 2010 it was completely the opposite. As mentioned before, there was a great difference in the soil N inherently available between the two years, reflected in the NUptE at the "0 rate". The lack of response of cultivars under FO in 2010 indicated that N was not delivered due to the combination of soil and weather conditions that did not allow a good mineralization. Under these conditions, organic matter in the soil, Carbon:Nitrogen ratio and soil moisture could be important factors influencing the results. In 2011, the situation was totally different with the cultivars showing much better performance under treatments with FO (Table 5).

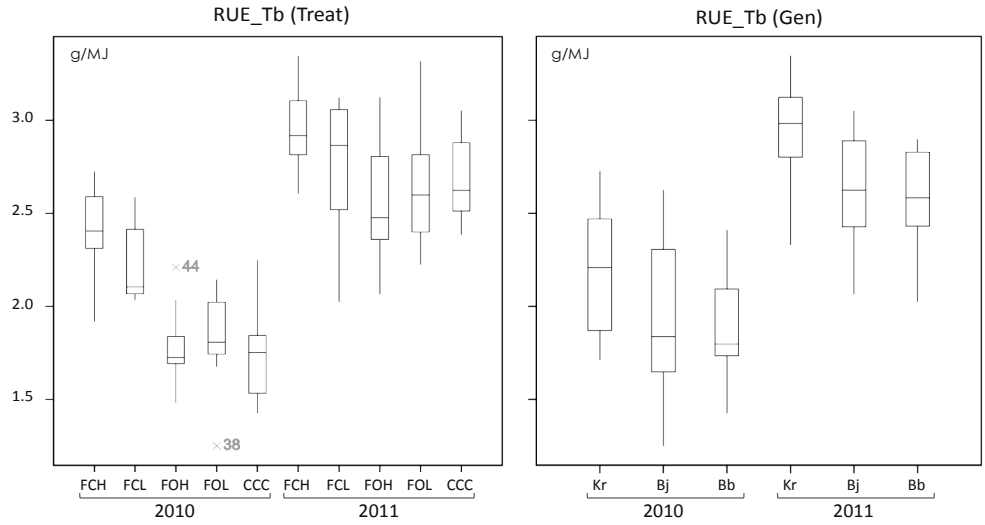

Figure 4 Boxplots for radiation use efficiency (RUE_Tb) using tuber dry matter as biomass. Comparison of treatments (Treat) and cultivars (Gen) across the two-year study. For Treat, X axes show treatments (FCH, FCL, FOH, FOL and CCC) per year 2010 and 2011 (these are also referred to as nitrogen environments). Only cultivars common to both years are included. The green crosses show outliers. For the acronyms of treatment and cultivars, see Table 1.

Light interception and Radiation use efficiency

Considering cultivars at one location and in the same year, the estimation of RUE depends directly on the %SC. However, between years it depends also on the incoming radiation, which can differ

from year to year as well as on other factors like environmental variables and soil conditions. There were significant differences in the RUE for environment and genotypes (Figure 4) but the interaction was not significant (Table 2). In 2011, the treatment with no application had better radiation use efficiency than the treatments with organic fertiliser, indicating luxurious growth above ground that was not translated into tuber weight. For the cultivars common in both years, the maturity type marked the differences between them: the later the maturity the longer the growing period and the more intercepted radiation, translating into higher yield (Figure 4). The average RUE in 2010 was lower than in 2011 (1.95 and 2.72 g DM MJ^{-1} respectively). Additionally, comparing the temperature and incoming radiation of the two years (Annex 5, and Figures 5A and B) 2010 had lower temperatures before planting, less cumulative thermal time (BTT) and lower cumulative incoming radiation at the beginning of the season until about 70 DAP. This period covered the time to reach the maximum soil coverage for most cultivars (Phase I of canopy development). Thus, more thermal time accumulated and more incoming radiation led to a higher potential growth in 2011. In addition, 2011 showed better performance than 2010 when comparing the treatment without application as mentioned above, which means, in general, better conditions on 2011.

Trends of some traits during the growing season

In order to evaluate how traits developed across the growing season, the following section of results is mainly focused on 2010 since phenotyping was more detailed in this season. The 2011 experiment is used for validation. The 2010 data showed a lower base line of N than 2011, when comparing the cultivars performing under the treatment with zero application in both years (Env 5 and 10). This will help to understand the impact of low N input conditions on crop development.

Soil coverage

In 2010, the soil coverage showed differences in response between FO and FC. FO showed values for some traits very similar to those of the no application treatment.

The analysis of the five parameters describing soil coverage in 2010 (Table 4) did not show a significant cultivar effect for *SC_tm1*. *SC_te* did not show treatment effect and for the interaction term, only *SC_Vx* and *SC_t2* were significant (Annex 7). The overall trend showed that high N availability led to higher *SC_tm1*, shorter SC_*t1*, and higher *SC_Vx*, whereas *SC_t2* did not show a clear trend. Therefore, with more N available Phase II of canopy development was reached earlier and had higher %SC that allowed more light to be intercepted (Figure 6A). Moreover, early cultivars had on average lower values for *SC_t1*, *SC_t2* and SC_*te* than late ones. *SC_Vx* was slightly lower (Figure 6B) when maturity was defined as done by breeders and growers, which is based on the initiation of canopy decay.

Table 3 Variable description, units and significance level in the 2010 experiment. 'Gen' indicates the cultivar term in the analysis, 'Treat' indicates the treatmet term and 'Gen × Treat' indicates the interaction term.

Traits	Gen	Treat	Gen × Tret	Description	Units
SC_tm1	0.149	0.004	0.995	Inflexion point in the Phase I: build-up of canopy development	*td*
SC_t1	<0.001	<0.001	0.085	Period from plant emergence to maximum soil coverage	*td*
SC_t2	<0.001	0.049	0.022	Initiation of senescence or Phase III	*td*
SC_te	<0.001	0.362	0.988	Total growing period till canopy is dead	*td*
SC_Vx	<0.001	0.001	<0.001	Maximum %SC reached	*%*
SC_Cm	<0.001	<0.001	0.152	Maximum progression rate of soil coverage during Phase I	*%SC/td*
AP1	<0.001	<0.001	0.037	Area under canopy cover curve for Phase I	*%SC.td*
AP2	<0.001	0.001	0.058	Area under canopy cover curve for Phase II	*%SC.td*
AP3	0.707	0.250	0.718	Area under canopy cover curve for Phase III	*%SC.td*
AUC	<0.001	0.024	0.462	Total area under the canopy curve	*%SC.td*
t1-t2	<0.001	0.005	0.074	Duration of Phase II	*td*
te-t2	0.279	0.421	0.789	Duration of Phase III	*td*
Tb_DM	<0.001	<0.001	0.003	Tuber dry matter	g/m^2
Tb_tm1	<0.001	0.347	0.009	Maximum rate of tuber bulking	*g/td*
Tb_t1	<0.001	0.828	0.723	Period to reach the maximum tuber weight	*td*
Tb_Vx	<0.001	<0.001	0.066	Maximum tuber weight	g/m^2
Tb_Cm	0.160	<0.001	0.843	Maximum progression rate of tuber bulking	$g/(m^2.td)$
tb	<0.001	0.193	0.018	Onset of tuber bulking linear interpolation of Tb_Cm	*td*
Tb_DM%	<0.001	<0.001	0.087	Tuber dry matter percentage	*%*
Tb_[N]	<0.001	<0.001	0.163	Tuber nitrogen content	*g/kg*
Tb_NUpt	0.208	<0.001	0.956	Tuber nitrogen uptake	g/m^2
RUE_Tb	<0.001	0.002	0.011	Radiation use efficiency	*g/MJ*
TbNUE	<0.001	<0.001	<0.001	Tuber nitrogen use efficiency	*g/g*
TbnMX	<0.001	0.004	0.155	Maximum tuber number on the size TbnB	$Tb\ \#/m^2$
TbnB	<0.001	0.076	0.085	Tuber size having the maximum tuber number	*mm*
Tbnd_A	<0.001	0.739	0.551	Tuber number dispersion parameter	
TbwMX	<0.001	<0.001	0.714	Maximum tuber weight on the size TbwB	g/m^2
TbwB	<0.001	0.116	<0.001	Tuber size having the maximum tuber weight	*mm*
TbwA	<0.001	0.568	0.106	Tuber weight dispersion parameter	
LAI_t1	<0.001	0.533	0.034	Period to reach the maximum leaf area index (LAI) in the season	*td*
LAI_Vx	<0.001	<0.001	0.541	Maximum leaf area index (LAI)	
St_t1	<0.001	0.618	0.323	Period to reach the maximum stem dry matter	*td*
St_Vx	<0.001	<0.001	<0.001	Maximum stem dry matter	g/m^2
Lf_t1	<0.001	0.336	0.027	Period to reach the maximum leaves dry matter	*td*
Lf_Vx	<0.001	<0.001	0.008	Maximum leaf dry matter	g/m^2
Hm_t1	<0.001	0.396	0.120	Period to reach the maximum haulm dry matter	*td*
Hm_Vx	<0.001	<0.001	0.005	Maximum haulm dry matter	g/m^2
Lf_c_tm1	<0.001	0.595	0.062	Period to reach the maximum progression rate of cumulative leaf dry matter	*td*
Lf_c_t1	<0.001	0.014	0.198	Period needed to reach total absence of green leaves	*td*
Lf_c_Vx	<0.001	<0.001	<0.001	Maximum cumulative leaf dry matter	g/m^2
Lf_c_Cm	<0.001	<0.001	0.803	Maximum progression rate of cumulative leaf dry matter	$g/(m^2.td)$
St_c_tm1	<0.001	0.583	0.142	Period to reach the maximum progression rate of cumulative stem dry matter	*td*
St_c_t1	<0.001	0.020	0.455	Period needed to reach total absence of green stems	*td*
St_c_Vx	<0.001	<0.001	<0.001	Maximum cumulative stem dry matter	g/m^2
St_c_Cm	<0.001	<0.001	<0.001	Maximum progression rate of cumulative stem dry matter	$g/(m^2.td)$
Hm_c_t1	<0.001	0.013	0.339	Period needed to reach total absence of green haulm	*td*
Hm_c_tm1	<0.001	0.565	0.059	Period to reach the maximum progression rate of cumulative haulm dry matter	*td*
Hm_c_Vx	<0.001	<0.001	<0.001	Maximum cumulative haulm dry matter	g/m^2
Hm_c_Cm	<0.001	<0.001	0.058	Maximum progression rate of cumulative haulm dry matter	$g/(m^2.td)$

Regarding areas under the soil coverage progress curve and the duration of the growing phases (*t1*, *t2-t1* and *te-t2*), there were significant effects of cultivar and treatment for all these traits except *AP3* and *te-t2* (only for treatment), which are parameters related to Phase III of canopy development (Table 3). There was no significant interaction between cultivar and treatment,

excluding *AP1*. In general, the differences between cultivars in the canopy development phases (areas and duration) remain relatively the same across the different treatments. *AP1* had lower values at high N level for both types of fertiliser (FO and FC) although the differences were not significant between levels for the same fertiliser type. Furthermore, the zero applications had higher value than both synthetic fertiliser inputs but lower than both inputs with organic fertiliser. 'Kuras' showed strong response to the treatment with synthetic fertiliser (having small *AP1,* Table 4, Annex 7). For *AP2* and *AUC* higher N input resulted in higher values, although only *AP2* at FCH was significantly different. Comparing cultivars, *AP2* and *AUC* increased from early to late cultivars contrary to *AP1*.

Comparing the two years, the zero application showed better performance in 2011 than in 2010 in terms of dry matter yield (Figure 7A). Moreover, in 2011 *SC_Vx* did not discriminate treatments and the differences between cultivars were also small (Figure 7D). FO showed a different response than FC in both years. For instance, in 2011 values for *SC_Cm* were higher in treatments with FO than in treatments with FC leading to a lower *SC_t1* (Figures 7B and C), whereas in 2010 it was the opposite. The N levels for the synthetic fertiliser showed also the same inversion in trend when comparing both years. For example, *SC_Vx* and *SC_Cm* were higher in FCH than in FCL in 2010 while in 2011 it was the opposite. The year by fertiliser type interaction could be attributed to the differences in weather and site characteristics. Additionally, application of FO was one month earlier in 2011 than in 2010. All these factors may have affected the N mineralization process resulting in different amounts and timing of available N with respect to the crop development compared with 2010.

LAI

The beta function fitted a curve with a quadratic shape for LAI, with *LAI_tm1* parameter very close to 0. Therefore, *LAI_tm1* was not included in the analysis. *LAI_Vx* showed significant differences for both cultivar and treatment, whereas the interaction term was not significant (Table 3). The cultivars had higher LAI under higher FC, 3.5 and 2.6 for high and low levels, respectively. FO followed with 2.2 for high level and 2.0 for low and the control or no fertiliser at last place with 1.6. Cultivars were ranked from 'Festien' 2.6 and 'Kuras' 2.5, then 'Innovator', 'Fontane' and 'Bintje' with 2.4 and 'Berber' with 2.1 (Table 4).

LAI_t1 showed a cultivar effect. Late cultivars needed more time to reach the maximum LAI; 'Kuras' and 'Festien' had 41.4 and 38. 2 td, then the two intermediate cultivars 36.3 and 34.8 td and at last place the early cultivars 33.4 and 29.2 td for 'Innovator' and 'Berber'. The interaction term was just significant; the differences in *LAI_t1* between cultivars were slightly smaller at the treatments with a higher average of *LAI_t1* (Figure 8).

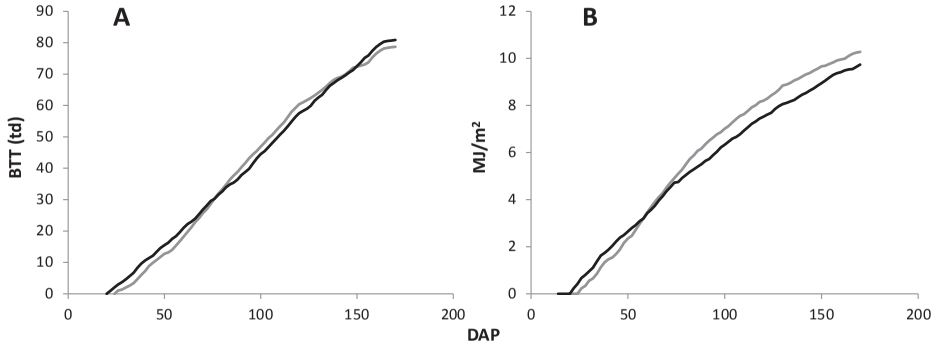

Figure 5 A) Cumulative Beta thermal time, B) incoming radiation, for the growing period in the 2010 and 2011 experiments, The unit along the X axes is days after planting, DAP. The intersection of the lines with the X axes represent average emergence day in each year.

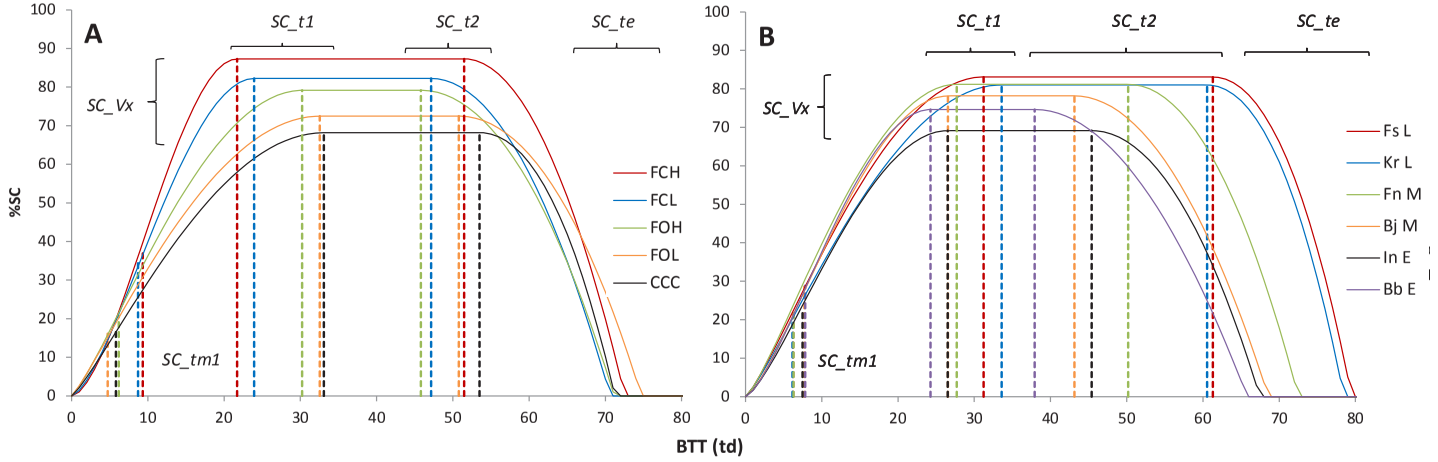

Figure 6 Canopy development curves and parameters for A) Treatments FCH, FCL, (synthetic fertiliser high and low) FOH, FOL (organic fertiliser high and low) and CCC (no application); B) Cultivars Fs (Festien), Kr (Kuras), Fn (Fontane), Bj (Bintje), Bb (Berber) and In (Innovator). Dotted lines represent projections to the curve for the "t" parameters, for acronyms of treatments and cultivars see Table 1 and Table 3 for parameters. The unit along the X axes is thermal days (td) calculated from the beta thermal time (BTT).

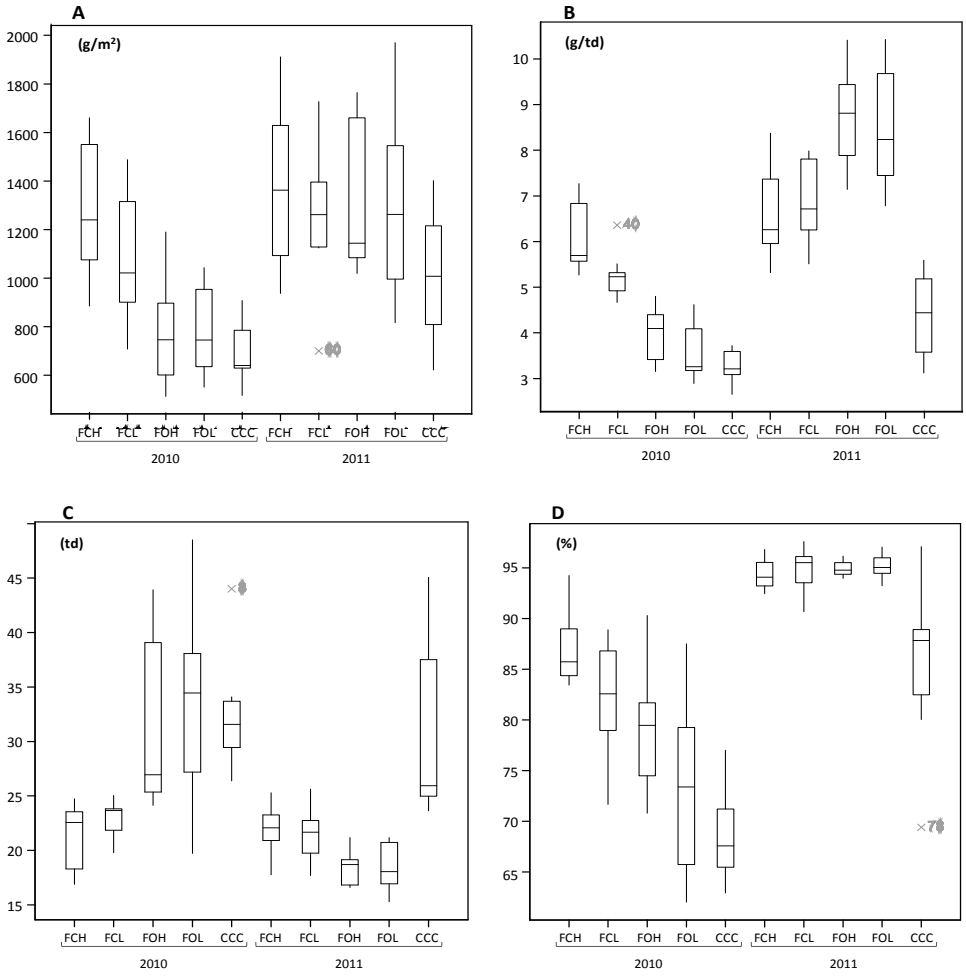

Figure 7 Box plots comparing treatments between the two years (2010 and 2011). A) Tuber dry matter (Tb_DM), B, C, D) canopy development parameters for maximum progression rate (B: *SC_Cm)*, time to reach the maximum soil coverage (C: *SC_t1)* and maximum soil coverage (D: *SC_Vx)*. On the X axes, the treatments (FCH, FCL, FOH, FOL, and CCC) are shown per year. The green crosses represent outliers. For the acronyms, see Table 1.

Dry matter haulm

Curve fitting using the beta function was done using data of stem (*St_*), leaf (*Lf_*), and haulm (*Hm_*) dry matter, that is the combination of both stem and leaf dry matter fraction. The trend showed a quadratic shape as in the case of LAI. For the three data sets, the *tm1* was not significantly different from zero, therefore only *Vx* and *t1* parameters were used as new traits to assess main effects and the interaction between cultivar and treatment. These two parameters were related to the maximum value and to the time to reach that maximum, respectively.

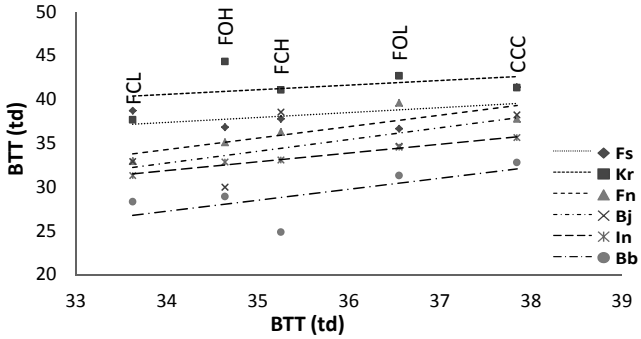

Figure 8 Interaction of cultivars by treatments for *LAI_t1,* Finlay Wilkinson regression lines of the treatment means for each cultivar on the average treatment means. Both axes are in beta thermal time (BTT) as thermal days (td). See Table 1 for the acronyms in the Figure legend.

Additionally, the cumulative variables for stem (*St*_c_), leaf (*Lf*_c_) and haulm dry matter were calculated and a curve fitting was also done, again with the beta function. The trend showed a sigmoid shape, and the three parameters (*tm1, t1 and Vx*) for the three variables were considered as new traits (Table 3).

All parameters had significant differences between cultivars (Table 3); the general trend showed an increase from early to late maturity. However, some exceptions were found. For example, the leaf dry matter cumulative (*Lf*_c_) 'Bintje' had lower *Lf*_c_*tm1* and *Lf*_c_*Vx* than 'Innovator'. For stem dry matter cumulative (*St*_c_), 'Innovator' had lower *St*_c_*Vx* values than 'Berber'.

Additionally the cultivars Innovator and Bintje, early and intermediate respectively, were inverted in the order for traits related with leaf measurements, i.e. 'Innovator' had higher leaf area index and more maximum leaf dry matter than 'Bintje'. Then, 'Innovator' had more leaf development while 'Bintje' showed more stem development.

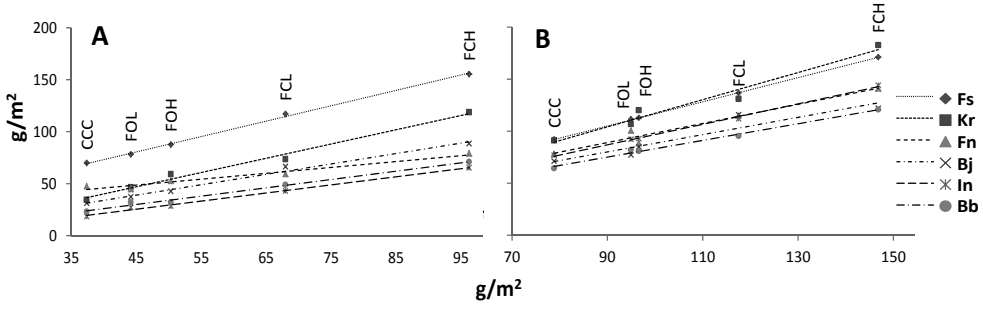

Figure 9 Interaction of cultivars by treatments for A) maximum stem dry matter (*St_Vx*), B) maximum leaf dry matter (*Lf _Vx*). The order of the treatments on the horizontal axes is given by the average of all cultivars for each treatment, and both axes are in g/m^2. See Table 1 for the acronyms in the Figure legend.

Treatment effects were significant for all *_Vx* parameters*, (St_ Lf_, Hm_)* using the cumulative and normal variables (Table 3). The rank of the treatments based on these traits decreased from synthetic fertiliser high (FCH), having the higher values of haulm dry matter for all the cultivars, to FCL, FOH, FOL and to the no application treatment (CCC) with the lowest values (Table 4). The maximum progression rates of the cumulative variables and the period needed to reach total absence of dry matter accumulation was also significant with the treatments following the same rank as mentioned before. The *_Vx* for both stems and leaves, with the cumulative and normal variables, showed interaction. For *St_Vx* late cultivars had a stronger response to the environmental differences, i.e. higher slope in the regression line (following the ranking of the treatment for these trait) (Figure 9A). 'Festien' had considerably higher values for *St_Vx* than all other cultivars in all treatments, while 'Kuras' had lower values than 'Fontane' (intermediate cultivar) in the lowest ranked treatment. The early cultivars had lower values of the trait in all treatments. For *Lf_Vx* (Figure 9B), the late cultivars had the highest response in this ranking of treatments followed by intermediate and early, with no cross-over in the lowest ranked treatment. The differences between late cultivars are likely due to the architecture of the plant as well as whether the cultivar has an indeterminate growth type, as it is the case for 'Festien'.

Then, the haulm dry matter development was highly dependent on maturity type with evident variation between cultivars with the same maturity type. This could be more apparent if a large group of cultivars is compared, taking into account that the maturity classification does not have a distinct border between groups.

Haulm Dry Matter%

The DM% in the haulm increased during Phase I of canopy development (Figures 12A and B). After that, there was a small reduction of the haulm dry matter percentage during Phase II, depending on the maturity type of the cultivar, with the lower point almost at the moment at which Tuber DM% reached its maximum. Overall, the later the maturity type the more constant was the haulm DM% during Phase II of canopy development. 'Berber' which was the earliest cultivar, had the greatest reduction in DM during this period (slope of -0.15 %/td), whereas 'Kuras' had the lowest reduction (slope -0.025 %/td). For the last part of the canopy development there was an increase in DM% as a consequence of the senescence process.

Nitrogen content ([N])

In the haulm, [N] decreased over time (Figure 10). At the beginning of the growing season, the treatment with FC showed higher [N] than FO and control treatments. However, the situation switched at the end of the season, probably due to a late N release from the organic fertiliser. In this sense, treatments with FC should have relatively higher availability of N at the beginning of the growing season, whereas treatments with FO should have relatively more N available later in the season. Additionally, the general trend showed that with more N input, more N was taken up and the content of N was higher in the dry matter. As a comparison, 2011 results showed that N

content was higher for FO than for FC, which may indicate that the available N was higher for FO in 2011 due to early application and better mineralization.

Regarding N content of the cultivars, the differences between the maturity types were clear at the end of the growing season. This could be a consequence of the senescence process, with early cultivars having less N in the canopy due to the relocation to the tubers.

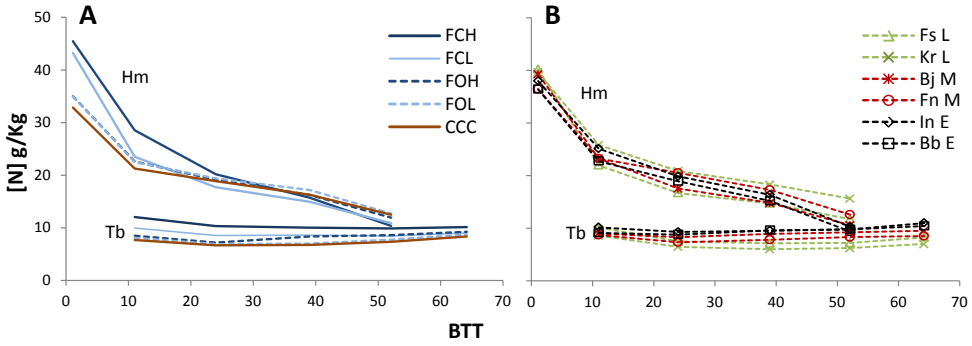

Figure 10 Nitrogen content [N] in haulm (Hm) and tubers (Tb) for the experiment in 2010; including A) Treatment. B) Cultivars. The unit along the X axes is thermal days (td) calculated as beta thermal time(BTT). See Table 1 for the acronyms in the Figure legend.

The [N] in tubers did not vary much during the season; however, for treatments FO and CCC, the [N] increased slightly at the end of the growing season, whereas for FC there was not a clear increase. On the other hand the ranking of treatments at the third intermediate harvests was FCH to FCL, then FOH, FOL and CCC, whereas at the end of the season [N] showed higher values for both types of fertiliser with high N level followed by both types with low level and the control. As stated before, a late release of N could cause the change in ranking and trend.

Tuber yield Dry Matter (Tb_DM)

Data for tuber DM in the 2010 experiment showed a sigmoidal trend; the curve fitting included five intermediate and one final harvest across the growing season (Figure 11). There was a significant cultivar effect on the three parameters (*Tb_tm1*, *Tb_t1* and *Tb_Vx*) whereas treatment and interaction effects were only found for *Tb_Vx* (Table 3). Overall, the later the cultivar, the longer the period with maximum light interception (Phase II of canopy development), the longer the tuber-bulking phase, and the higher the final yield. 'Kuras' had the highest *Tb_Vx* (1186 g/m^2) clearly apart from 'Festien', After followed by 'Fontane', 'Bintje' and finally 'Innovator' and 'Berber' (762 g/ m^2). High availability of N led to higher yield (Table 4). Only the FC showed differences between levels, whereas the two levels of FO and the unfertilized control did not differ significantly.

The maximum progression rate for Tb_DM *(Tb_Cm)* did not change significantly between cultivars but it did change depending on the treatments (with values from 33.7 to 18.4 g/td). On the other hand, the moment at which the maximum rate occurred (*Tb_tm1*) only showed a cultivar effect,

with the late cultivars requiring more time to reach their maximum. In addition, *Tb_Cm* was placed around the middle of Phase II of canopy development where the crops probably had the maximum light interception with the fully developed canopy that supported this maximum accumulation rate of DM into tubers (Figure 13A, B and C).

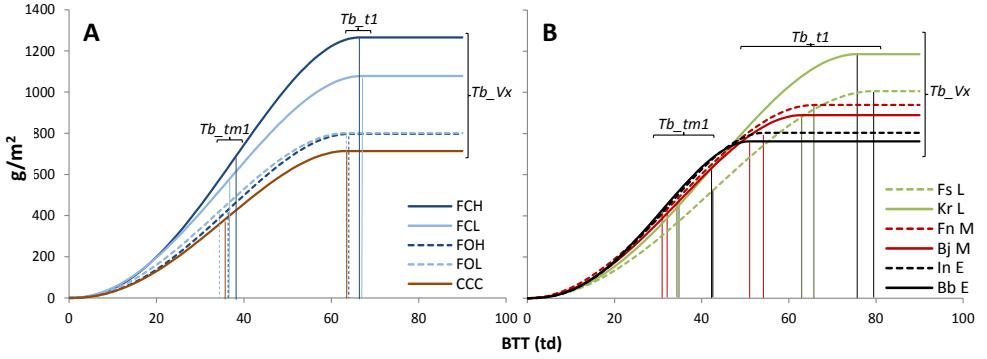

Figure 11 Predicted tuber dry matter yield (Tb_DM) development in g/m^2 using the estimated parameters for: A) treatments, B) cultivars based on data from 2010. The unit along the X axes is thermal days (td) calculated as beta thermal time (BTT). See Table 1 for the acronyms in the Figure legend and Table 3 for acronyms of the parameters.

The onset of tuber bulking (*tb*) showed differences among cultivars with the means ranging from 17.1 to 12.7 thermal days (td). The late cultivars had a delay in the onset of tuber bulking, with 'Kuras' having the largest *tb*, followed by 'Festien', followed by the two early cultivars ('Berber' and 'Innovator') and finally the two intermediate ones ('Bintje' and 'Fontane'). There were no significant treatment differences, and the interaction was significant although without any clear trend. Here, the cultivar Innovator showed different behaviour compared to the other cultivars. For all cultivars *tb* occurred around the middle point between the moment of maximum rate of canopy development (*SC_Cm1*) and the moment at which the maximum SC (*SC_Vx*) was reached (Figures 13A, B and C).

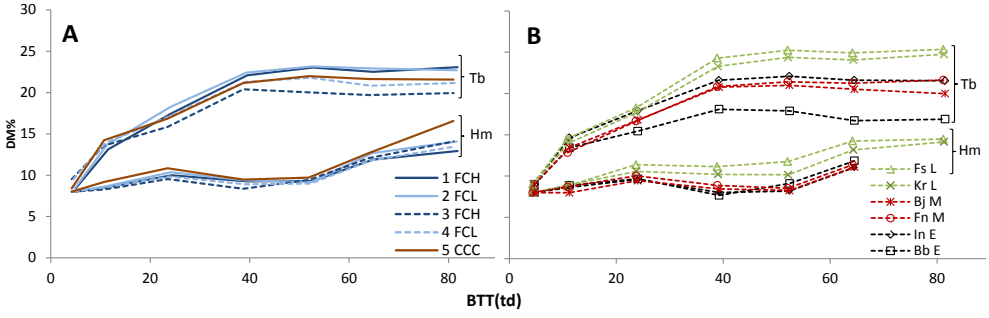

Figure 12 Dry matter percentage (DM%) development for tubers (Tb) and haulm (Hm) biomass; A) treatments; B) cultivars. The unit along the X axes is thermal days (td) calculated as beta thermal time (BTT). See Table 1 for the acronyms in the Figure legend.

Tuber dry matter percentage (Tb_DM%)

The interaction effect between cultivar and treatment was not significant in 2010 (Table 3). Late cultivars had on average higher DM% followed by the intermediate cultivars and then the early cultivars. Additionally, there was also significant variation among cultivars within the same maturity class.

The treatments with organic fertiliser were expected to release N later in the growing season than those with the synthetic fertiliser. FO had lower values for Tb_DM%, even lower than the control in 2010. The ascendant rank of the treatments was: FOH with the lowest values of dry matter percentage followed by FOL, CCC FCL and FCH with the higher values (Table 4). These low values for FO were probably a result of a very low N released due to slow mineralization process in this particular experiment (2010) that conditioned the plant development and its capacity to accumulate biomass especially at the beginning of the growing season and then affecting the final yield. In 2011, the plant development for the treatments with organic fertiliser was as good as for the synthetic fertiliser and this was also reflected in the yield (Tb_DM).

In 2010, FO had a low rate of DM% increment early in the season (before 24 td) with slopes of 0.17 and 0.24 %/td for High and Low N level, respectively (Figure 12A, B) compared with 0.51 and 0.35 %/td for FC and 0.20 for no application. The trend during the season showed that DM% stabilized approximately at 40 td. For treatments with FO, this point varied for cultivars and depended mainly on the maturity type, with a slightly longer period for the late cultivars. The rate of increment in DM% (for the period 24 to 40 td) depended also on maturity type with high values for late cultivars (0.38 %/td) followed by intermediate (0.27 %/td) and early (0.21 %/td) ones (Annex 6).

Tuber size-number (Tbn) and size-weight (Tbw) distribution

Assuming that there is an underlying discrete bell shaped trend for tuber number and weight, data from the grading of tubers into six size classes were used to fit a bell-shaped curve. For tuber number the three parameters differed among cultivars, but not among treatments (Tables 3 and 4); there were also no cultivar x treatment interactions. For the size-weight distribution, the cultivar effects were significant for the three parameters. The treatment effect was significant only for the "MX" tuber weight, showing higher values for higher N. The interaction was significant only for the size with higher weight.

For both weight and number distribution, tubers grew in a continuous way, from small sizes with a high number at the beginning of the growing season to large tubers with a low number at the end the season. This development process means that tuber production could take place permanently, most probably with higher rates at the beginning of the tuber production and very low rates at the end, if there are no limiting factors that stop the process (resources or environmental variables).

Table 4 Overview of means of main factors treatment and cultivar in the 2010 experiment. Maturity score of the cultivars is included, Mt. Letters next to the means values show groups from Bonferroni test with an $\alpha = 0.05$. S.E.D. are included. For treatments and cultivars acronyms see Table 1. For Traits acronyms see Table 3.

		Canopy development parameters												Tuber bulking							N uptake and Radiation use efficiency			
Trait		*SC_tm1*	*SC_t1*	*SC_t2*	*SC_te*	*SC_Vx*	*SC_Cm*	*AP1*	*AP2*	*AP3*	*AUC*	*t1-t2*	*te-t2*	*Tb_DM*	*Tb_tm1*	*Tb_t1*	*Tb_Vx*	*Tb_Cm*	*tb*	*Tb_DM%*	*Tb_[N]*	*Tb_Nupt*	*RUE_Tb*	*TbNUE*
Units		*td*	*td*	*td*	*td*	*%SC*	*%SC/td*	*%SC.td*	*%SC.td*	*%SC.td*	*%SC.td*	*td*	*td*	*g/m²*	*td*	*td*	*g/m²*	*g/(m².td)*	*td*	*%*	*g/kg*	*g/m²*	*g/MJ*	*g/g*
Treatment																								
FCH	1	9.3 c	21.6 a	54.8 a	72.1 a	87.2 c	6.01 c	1003 a	2905 b	1014 a	4922 b	33.2 b	17.3 a	1251.8 c	38.2 a	66.4 a	1265.3 c	33.73 c	15.9 a	23.1 c	10.16 c	12.50 d	2.26 c	78 ab
FCL	2	8.6 bc	24.3 ab	49.0 a	69.5 a	82.3 bc	4.99 bc	1128 ab	2059 ab	1121 a	4308 ab	24.7 ab	20.5 a	1062.0 b	36.2 a	67.1 a	1078.3 b	25.64 b	13.8 a	22.7 c	9.05 ab	9.52 c	2.16 bc	118 c
FOH	3	6.3 abc	30.1 bc	50.4 a	68.5 a	78.8 abc	4.10 ab	1463 c	1625 a	972 a	4060 ab	20.3 a	18.1 a	786.8 a	36.6 a	64.1 a	796.8 a	21.42 ab	15.5 a	19.9 a	9.25 b	7.27 b	1.73 a	49 a
FOL	4	5.2 a	32.7 c	53.5 a	73.5 a	72.7 ab	3.58 a	1504 c	1552 a	978 a	4033 ab	20.8 a	20.0 a	795.5 a	34.4 a	63.4 a	801.0 a	20.87 ab	13.4 a	21.2 b	8.74 ab	6.94 b	1.80 ab	88 bc
CCC	5	5.8 ab	33.3 c	54.6 a	71.2 a	68.4 a	3.21 a	1429 bc	1449 a	786 a	3664 a	21.3 a	16.7 a	708.2 a	35.8 a	63.5 a	713.7 a	18.44 a	14.3 a	21.6 b	8.38 a	5.89 a	1.77 a	354 d
Cultivar	**Mt**																							
Fs	3	6.6 a	31.6 bc	62.5 d	78.7 c	83.3 d	4.25 ab	1582 cd	2613 b	925 a	5120 c	30.8 c	16.3 a	1002.9 d	42.7 c	79.5 d	1006.2 d	20.24 a	16.0 bc	25.4 d	8.26 b	8.34 a	1.79 a	150 c
Kr	4	6.4 a	33.5 c	63.1 d	79.9 c	81.1 cd	4.02 a	1648 d	2429 b	937 a	5014 c	29.6 c	16.7 a	1176.1 e	42.4 c	75.8 cd	1186.0 e	25.43 a	17.1 c	24.8 d	7.01 a	8.28 a	2.19 c	171 d
Fn	6	6.4 a	27.7 ab	53.6 c	71.0 b	81.0 cd	4.66 b	1334 bc	2123 b	958 a	4415 b	26.0 bc	17.4 a	929.7 cd	34.4 b	65.8 bc	938.8 cd	22.85 a	12.7 a	21.6 c	8.51 b	8.16 a	1.85 a	142 bc
Bj	6.5	7.5 a	26.7 a	46.0 ab	66.9 ab	78.2 bc	4.55 ab	1201 ab	1531 a	1100 a	3832 a	19.3 a	20.9 a	861.2 bc	34.8 b	63.1 b	889.6 bc	22.61 a	13.7 ab	20.0 b	9.52 c	8.60 a	1.92 ab	128 ab
In	7	7.5 a	26.7 a	47.2 b	66.3 ab	69.3 a	4.03 a	1055 ab	1451 a	897 a	3404 a	20.5 ab	19.1 a	799.1 ab	32.2 ab	54.2 ab	803.3 ab	24.78 a	13.9 ab	21.5 c	10.98 d	8.99 a	2.04 bc	120 a
Bb	8	7.7 a	24.2 a	42.4 a	63.0 a	74.3 b	4.76 b	1012 a	1361 a	1026 a	3399 a	18.1 a	20.6 a	756.2 a	30.9 a	51.1 a	762.2 a	28.22 a	14.1 abc	16.9 a	10.41 d	8.18 a	1.88 ab	115 a
S.E.D.																								
Treatment		0.83	1.53	1.87	2.51	2.88	0.28	81.52	224.17	132.52	290.95	2.60	2.24	46.36	1.68	4.01	41.29	1.72	1.10	0.22	0.22	0.27	0.10	8.33
Cultivar		0.67	1.49	1.52	1.94	1.34	0.17	98.94	167.50	138.69	151.98	2.11	2.48	26.62	1.10	3.80	32.85	3.02	0.98	0.28	0.25	0.37	0.06	5.17
Interaction		1.51	3.33	3.40	4.35	3.00	0.39	221.24	374.55	310.13	339.84	4.73	5.54	59.52	2.46	8.49	73.46	6.74	2.20	0.62	0.56	0.82	0.13	11.6

Continuation...

		Tuber size, number and weight distribution						LAI and Haulm partitioning (leaf, stem)																			
Trait		*TbnMX*	*TbnB*	*TbnA*	*TbwMX*	*TbwB*	*TbwA*	*LAI_t1*	*LAI_Vx*	*St_t1*	*St_Vx*	*Lf_t1*	*Lf_Vx*	*Hm_t1*	*Hm_Vx*	*Lf_c_tm1*	*Lf_c_t1*	*Lf_c_Vx*	*Lf_c_Cm*	*St_c_tm1*	*St_c_t1*	*St_c_Vx*	*St_c_Cm*	*Hm_c_t1*	*Hm_c_tm1*	*Hm_c_Vx*	*Hm_c_Cm*
Units		*#/m²*	*mm*		*g/m²*	*mm*		*td*		*td*	*g/m²*	*td*	*g/m²*	*td*	*g/m²*	*td*	*td*	*g/m²*	*g/(m².td)*	*td*	*td*	*g/m²*	*g/(m².td)*	*td*	*td*	*g/m²*	*g/(m².td)*
Treatment																											
FCH	1	28.8 b	43.4 a	285.8 a	2444 d	51.0 a	184.8 a	35.3 a	3.51 d	42.0 a	96.29 c	32.5 a	146.8 c	37.1 a	246.90 d	22.38 a	62.0 a	507.0 c	11.92 c	32.3 a	68.3 a	326 c	7.11 d	67.97 a	26.31 a	873.80 c	18.51 d
FCL	2	28.7 b	41.9 a	268.5 a	2183 c	49.3 a	169.7 a	33.6 a	2.63 c	38.8 a	67.99 b	30.0 a	117.5 b	34.8 a	190.00 c	19.26 a	61.2 a	403.5 b	9.54 b	28.3 a	68.3 a	237.8 b	5.02 c	67.56 a	23.26 a	676.20 b	14.31 c
FOH	3	28.9 b	40.1 a	269.9 a	1794 b	48.4 a	198.7 a	34.6 a	2.25 bc	40.1 a	50.34 a	32.0 a	96.6 a	36.9 a	152.60 b	20.82 a	62.4 a	333.9 ab	7.72 ab	28.6 a	68.6 a	172.3 a	3.69 b	68.22 a	24.73 a	538.40 a	11.35 b
FOL	4	26.2 ab	40.9 a	265.9 a	1782 b	48.2 a	157.3 a	36.6 a	2.09 b	40.5 a	44.18 a	33.6 a	95.0 a	37.4 a	143.40 ab	21.26 a	68.7 a	344.7 ab	7.28 a	29.4 a	72.6 a	161.9 a	3.25 ab	72.65 a	25.27 a	533.20 a	10.60 ab
CCC	5	20.6 a	41.4 a	299.5 a	1532 a	49.7 a	177.8 a	37.9 a	1.61 a	41.7 a	37.37 a	35.2 a	78.8 a	39.1 a	120.70 a	23.03 a	69.8 a	284.8 a	5.88 a	30.7 a	73.7 a	132.1 a	2.67 a	73.57 a	27.56 a	444.50 a	8.73 a
Cultivar	**Mt**																										
Fs	3	24.7 b	42.7 b	233.2 ab	1666 a	49.4 bc	190.3 ab	38.3 cd	2.63 b	50.0 d	101.55 d	37.6 d	124.7 c	44.0 d	229.00 e	25.89 d	74.5 d	484.1 d	9.43 b	40.8 d	77.3 c	370.7 d	7.42 e	77.42 c	33.51 c	886.30 e	16.78 d
Kr	4	26.4 bc	42.2 ab	326.5 cd	1915 ab	51.0 cd	230.3 b	41.4 d	2.57 b	49.3 d	66.40 c	37.9 d	126.2 c	43.4 d	198.30 d	26.85 d	72.4 cd	479.2 d	9.64 b	40.1 d	75.9 c	236 c	4.84 d	75.90 c	32.77 c	753.30 d	14.59 c
Fn	6	25.8 b	42.9 b	269.0 abc	1959 b	49.8 bc	169.3 ab	36.3 bc	2.39 ab	42.3 c	56.62 b	32.7 c	104.5 b	37.5 c	164.40 c	20.24 bc	68.1 c	374.2 c	8.14 a	31.3 c	73.5 c	201.1 b	4.06 c	72.99 c	25.31 b	605.70 c	12.12 b
Bj	6.5	36.3 d	39.9 a	191.0 a	2352 c	45.6 a	112.2 a	34.9 b	2.37 ab	36.9 b	53.14 b	32.7 c	94.1 a	35.8 c	154.30 bc	21.97 c	60.7 b	304.2 ab	7.48 a	25.9 b	67.5 b	177.2 b	3.86 bc	67.40 b	24.37 b	521.40 b	11.32 ab
In	7	17.0 a	42.0 ab	364.9 d	1678 a	52.2 d	210.8 b	33.5 b	2.40 ab	34.3 ab	36.41 a	29.6 b	103.5 b	32.7 b	145.00 ab	17.46 ab	61.0 b	336.6 b	8.14 a	21.8 ab	68.6 b	122.8 a	2.66 a	67.32 b	19.70 a	491.70 b	10.74 a
Bb	8	29.7 c	39.6 a	283.1 bcd	2111 bc	47.8 b	153.1 ab	29.2 a	2.15 a	30.9 a	41.27 a	25.6 a	88.7 a	29.0 a	133.30 a	15.69 a	52.0 a	270.3 a	7.99 a	19.4 a	59.1 a	128.2 a	3.25 ab	58.95 a	16.89 a	421.10 a	10.65 a
S.E.D.																											
Treatment		1.62	0.98	28.79	59.70	1.01	24.90	2.54	0.11	2.15	3.58	2.34	4.78	2.02	7.90	2.41	2.33	19.43	0.52	2.66	1.61	14.15	0.25	1.60	2.59	32.80	0.58
Cultivar		1.24	0.86	28.84	90.04	0.68	25.86	1.03	0.09	1.38	3.10	0.91	3.03	0.92	5.44	1.15	1.73	11.62	0.35	1.50	1.58	10.21	0.22	1.46	1.08	21.33	0.41
Intraction		2.78	1.93	64.48	201.34	1.52	57.81	2.30	0.21	3.07	6.94	2.04	6.78	2.05	12.17	2.57	3.87	25.99	0.78	3.36	3.53	22.84	0.50	3.27	2.42	47.70	0.92

Table 5 Overview of means of main factors: treatment and cultivar in the 2011 experiment. Maturity score of the cultivars is included, Mt. Letters next to the means values show groups from Bonferroni test with an $\alpha = 0.05$. S.E.D. are included. For treatments and cultivars acronyms see Table 1. For Traits acronyms see Table 3.

		Canopy development parameters												Yield, Nuptake and radiation use efficiency					
Trait		*SC_tm1*	*SC_t1*	*SC_t2*	*SC_te*	*SC_Vx*	*SC_Cm*	*AP1*	*AP2*	*AP3*	*AUC*	*t2-t1*	*te-t2*	Tb_DM	Tb_DM%	Tb_[N]	Tb_NUpt	RUE_Tb	TbNUE
Units		*td*	*td*	*td*	*td*	*%SC*	*%SC/td*	*%SC.td*	*%SC.td*	*%SC.td*	*%SC.td*	*td*	*td*	*g/m²*	*%*	*g/kg*	*g/m²*	*g/MJ*	*g/g*
Treatment																			
FCH	1	10.8 a	22.0 a	42.9 a	60.5 a	94.5 b	6.59 b	1042 c	1988 b	1116 a	4145 b	21.0 b	17.6 a	1369 b	22.3 bc	13.5 bc	18.22 bc	2.955 a	86 a
FCL	2	11.1 a	21.4 a	41.2 a	61.3 a	94.8 b	6.89 b	990 bc	1881 b	1274 a	4146 b	19.8 b	20.1 a	1260 ab	22.2 bc	12.6 ab	15.59 b	2.750 a	140 b
FOH	3	11.2 a	18.3 a	50.8 b	65.6 a	95.0 b	8.79 c	751 a	3095 c	939 a	4786 c	32.6 c	14.8 a	1305 b	19.4 a	16.8 c	21.51 c	2.555 a	82 a
FOL	4	10.7 a	18.5 a	44.5 a	62.1 a	95.2 b	8.41 bc	803 ab	2470 b	1118 a	4390 bc	26.0 bc	17.6 a	1288 ab	21.0 ab	13.7 bc	16.96 bc	2.649 a	143 b
CCC	5	9.8 a	30.4 b	39.7 a	57.8 a	85.7 a	4.38 a	1503 d	795 a	1076 a	3374 a	9.3 a	18.1 a	1010 a	23.4 c	9.4 a	9.13 a	2.690 a	505 c
Cultivar	**Mt**																		
Kr	4	9.9 a	24.3 b	54.5 c	72.4 c	94.6 b	6.77 a	1206 b	2882 c	1135 a	5222 c	30.3 c	17.9 a	1595 c	25.7 c	11.3 a	18.37 b	2.938 b	242 c
Bj	7	11.1 a	20.8 a	41.2 b	60.0 b	94.1 b	7.34 a	930 a	1932 b	1182 a	4044 b	20.5 b	18.8 a	1176 b	20.7 b	13.8 b	16.17 a	2.645 a	188 b
Bb	8	11.2 a	21.4 a	35.8 a	52.0 a	90.4 a	6.93 a	919 a	1323 a	996 a	3238 a	14.4 a	16.3 a	968 a	18.6 a	14.6 b	14.31 a	2.577 a	143 a
S.E.D.																			
Treatment		0.64	1.10	1.66	2.29	1.21	0.49	53.20	161.30	185.80	122.90	1.76	3.14	76.84	0.45	0.92	1.25	0.12	12.03
Cultivar		0.62	0.65	1.52	1.27	0.92	0.22	50.40	141.90	130.60	85.90	1.53	2.17	53.87	0.23	0.41	0.82	0.10	14.17
Interaction		1.30	1.61	3.22	3.26	2.07	0.63	106.30	305.20	302.30	199.30	3.30	5.06	124.81	0.61	1.18	1.94	0.22	28.54

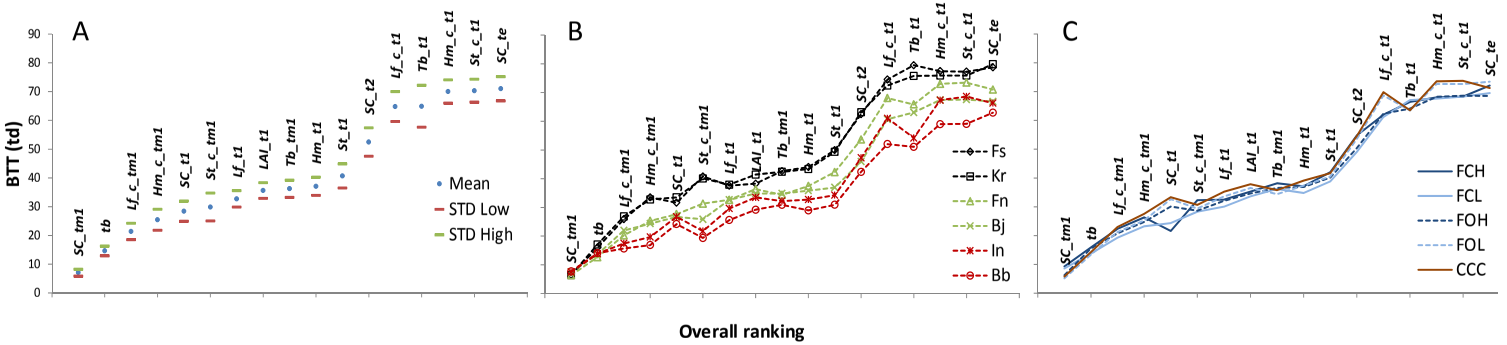

Figure 13 Occurrence of time parameters (Y-axis, in thermal days, td) in relation to the overall ranking of these parameters (X-axis) in the 2010 experiment. A) Overall ranking of these parameters, showing the mean of each parameter and the standard deviation (around the mean) for the experiment, calculated base on means over all enviroments for all cultivar ; B) and C) are cultivar and treatment means respectively, plotted against the overall order. See Table 1 for the acronyms in the Figure legend and Table 3 for the acronyms of the parameters.

Discussion

The performance of cultivars under contrasting N levels could be measured as how much yield was produced in relation to the N available. As expected, our results showed that the more N is available the higher the yield. At low available N, N use efficiency was consistently higher, which is in line with previous reports (e.g., Zebarth et al. 2008).

In this study, we considered as input the available N in the soil before planting and the amount of fertiliser applied to reach input levels. Since FO was included, a working coefficient was also considered. However, the amount of N coming from mineralization processes during the growing season was not predictable. In addition, there was a lack of robust information to accurately estimate the extra N from the net mineralization in the soil. Therefore, our experimental setup that included two years with different experimental sites (due to a mandatory crop rotation) contributed to a variation in the N available and more specifically to the soil N supply.

Nevertheless, a qualitative estimation of N available was possible. A comparison of yield at zero application between both years showed how different the soils, sites and weather conditions were, with the 2011 experiment having more fertile and favourable conditions for crop development offering a better soil N supply condition. Zebarth et at. (2005) showed the usefulness of plant bioassay under no N application as an indication of soil N supply. Regarding treatments with FC, the N available was higher with higher fertiliser rate, as it did not depend on mineralization. On the other hand, the FO (dry cattle manure in this case) interacted strongly with the field and year conditions with respect to mineralization. Mineralization processes likely took longer in 2010 due to adverse temperature and soil conditions as well as the very unbalanced carbon: nitrogen ratio. In addition, the time from application to planting was only one day in 2010 compared with a month in 2011 resulting in a late N release in the first year. Therefore, the FO treatments differed greatly between years. In 2010, the yield of the cultivars under both rates of FO was similar, and only slightly better than zero application. At the high N rate, the extra N was evident from the [N] average per treatment as well as from the *SC_Vx* where the high rate showed higher values. Hence, the N available was higher at high rates but late in the season.

Radiation use efficiency (RUE), i.e. the biomass production per amount of light energy captured depends directly on the %SC and LAI considering cultivars at one location in the same season. From season to season, RUE depends on the incoming radiation and is especially important to compare the performance of a crop in a more equivalent way under different conditions. There were differences between years; a lower RUE in 2010 compared with 2011 (1.95 and 2.72 g/MJ). The year 2010 had lower incoming radiation and the temperature was also lower for the vegetative development, Phase I, and for the period with maximum soil coverage (Phase II) of canopy development; these two environmental variables conditioned the plants to a lower potential for dry matter production compare with 2011.

In general, the poor conditions in 2010 led to a very poor performance of the cultivars in that year (low yield and poor canopy development) with low N available at the zero application, representing a low input environment and thus a scenario with low potential dry matter production. The combinations year and treatment resulted in 10 different conditions of available N that we call N Envs. Across these 10 N Envs, higher N input resulted in higher yield and [N] in the dry matter (averages per Envs) with no significant differences for the moment at which the maximum yield was reached. That extra N in the tuber dry matter could be important from a nutritional point of view, as was reported by Millard et al. (1989) who mentioned that more N supply also increased the yield of some nutritionally essential amino acids. Comparing the cultivars, from early to late maturity type, increases in dry matter were accompanied by lower N content in that dry matter as reported in other studies (Ospina et al. 2014, Tiemens et al. 2014). With longer growing periods late cultivars have more time for photosynthesis and to relocate those assimilates into tubers, especially carbohydrates, than early cultivars. Then late cultivars have lower proportion of assimilates containing N than carbohydrates in the tubers, and N appears to be "diluted".

The dry matter yield was strongly related to maturity type, with late cultivars having more time to accumulate assimilates, showing higher DM% and higher yields in a long growing season. However, if the harvest had taken place earlier, regardless of maturity type and with no extra time to reach maturity, some cultivars like 'Festien' (late) would have appeared as very inefficient with less yield than intermediate and early cultivars. Our results suggest that N affects mainly the maximum tuber bulking rate and not the onset of tuber bulking, which depends on the cultivar but is not related with maturity type. The absence of N effect in the tuber bulking initiation is probably due to the lack of data points at the beginning of the season. Kleinkopf et al. (1981) found a delay in the tuber bulking initiation at a very high rate while Dyson and Watson (1971) did not find this effect of N but reported that N slowed the early growth of tubers. However, our experimental conditions (2010) did not involve an excessive N input. Additionally, our experiments lasted long enough to allow the late cultivar to go into senescence, which implies no major restrictions to relocate assimilates into tubers. Our results are in line with Tiemens et al. (2014) who reported higher yield with an increase in N supply when maturity type of the cultivars was later, highlighting the need for a long crop cycle for late maturity cultivars to profit from the additional nitrogen.

Analysing parameters that denote timing of events for the canopy and tuber bulking development (Figure 13), a general chronological sequence of events was defined as follows: Canopy coverage started to develop at emergence, and then maximum progression rate of soil coverage occurred. Later, onset of tuber bulking was followed by the time at which maximum %SC was reached. Then the maximum green leaf DM was reached, followed by the maximum LAI. Next, the maximum progression rate for tuber bulking took place, and after, the maximum dry matter in stems was reached. Subsequently the initiation of canopy decay started, followed by the complete loss of all leaves. Afterwards, the maximum tuber DM was achieved and finally green stems were completely dead as well as the whole canopy. Overall, treatments with low N available had an early end of tuber bulking even before green leaves were completely dead,

and the maximum progression rate of tuber bulking also occurred earlier than the moment at which the maximum LAI was reached. At high N, the plant has enough N to keep investing in the haulm rather than to restrict its strategy to tubers. On the other hand, cultivars were distinctive, and especially maturity type influenced the required time to reach the events, especially *SC_t,1 LAI_t1* and *Hm_t1*.

Vos (2009) discussed the effects of N on the duration of the phases of canopy development and the yield response to N. Our results pointed to an important role of N in Phase I; under high available N (e.g. in the FCH environment) *SC_t1* was shortened, which is the moment at which the *SC_Vx* was reached, and *AP1* also became smaller, even though *SC_Vx* itself was increased. The data indicate that FO in 2010 had very low N availability at the beginning of the season. In contrast, in 2011 *AP1* was smaller for treatments with FO, maybe even more so than in treatments with FC. This would imply that with less N available, *AP1* increased. For *AP2* and *t2-t1* the opposite applies: with less N available, the area or length was decreased. Consequently, the total duration of the crop (*te*) did not change significantly but those of the sub phases did.

Maturity type drives the response of the cultivars to nitrogen, as has been shown by Tiemens et al. (2014) and Ospina et al. (2014); we showed a relationship between maturity type, yield, canopy development as well as LAI. In general, the total area under the curve *AUC* and *AP2* distinguished maturity types and both were strongly related to yield. *AP2* has high relevance since it is the product of the length of the period of Phase II (*te-t1*) and maximum %SC. As mentioned before, during this period plants have maximum light interception, and any variation will be reflected directly in the dry matter yield. If N and other factors are not limiting, *SC_Vx* would reach very high values and the differences in *AP2* would be mainly due to the duration of the phase (*t2-t1*) and, as might be expected, to LAI. Finally, under such conditions, smaller effects of N on canopy parameters would be a good indication of good and stable performance.

Although these experiments did not aim to answer questions about optimal inputs in relation to the productivity or economic return, it is clear that this will depend on the maturity type of each cultivar. As mentioned before, the cropping period (time until haulm is dead) should allow the cultivar to reach maturity and to relocate as much dry matter as possible. In this sense, the cost of maintenance or management of this extra time needed by late cultivars will significantly determine the value, all within the framework of each specific market.

Perspective

With this in-depth crop-physiological study on how low N input affects the canopy development and yield traits of diverse potato cultivars, one should be able to better understand and detect genetic variation for these parameters in a larger set of genotypes. This could be an important step in designing a breeding programme for varieties better adapted to low-input agriculture.

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Supplementary material

Annex 1

A) The nonlinear beta temperature response function ($g(T)$) is used to convert days after emergence (DAE) into beta thermal days (BTT) whose unit is thermal days, td. The equation of function of T, i.e. the air temperature in degrees celcius (°C) and the three cardinal temperatures: T_b (base), T_o (optimum) and T_c (ceiling) for the phenological development of potato were use (T_b = 5.5; T_o = 23.4 and T_c= 34.6; all in °C; Khan 2012, Khan et al. 2013). c_t is the temperature response curvature coefficient also estimated by Khan (2012) as 1.7.

$$g(T) = \left[\left(\frac{T_c - T}{T_c - T_o} \right) \left(\frac{T - T_b}{T_o - T_b} \right)^{\left(\frac{T_o - T_b}{T_c - T_o} \right)} \right]^{c_t}$$

B) Equations to calculate the areas under the curve for each phase of the canopy development using the parameters describing the curve (*t1*, *tm1*, *t2*, *te* and *Vx*, see description in the main text and in Khan et al. (2013)). *AP1* area under de curve for Phase 1, or build-up phase. *AP2* is the area under the curve for Phase II during which the maximum soil coverage is constant. Finally, *AP3* is the area under the curve for Phase III or the senescence phase. The total area under the curve *AUC* is the summation of the areas of the sub phases. (Khan et al. 2013).

$$AP1 = V_x \left[\frac{2t_1(t_1 - t_{m1})}{3t_1 - 2t_{m1}} \right]$$

$$AP2 = V_x(t_2 - t_1)$$

$$AP3 = \frac{Vx(t_e - t_2)}{2t_e - 2t_2 + t_1} \left[(t_e - t_2 + t_1) \left(\frac{(t_e - t_2 + t_1)}{t_1} \right)^{\frac{t_1}{t_e - t_2}} - 2t_1 \right]$$

Annex 2 Standardized values of the traits means across the nitrogen environments. The environments are ordered by the average, which represents an environment index with no weight. Correlations of each value with the index are included in column "correlation" with its respective P value in the next column. For treatments and cultivars acronyms see Table 1. For Traits acronyms see Table 3.

Env / Trait	CCC 2	CCC 1	FOH 1	FOL 1	FCL 2	FCL 1	FOL 2	FCH 2	FOH 2	FCH 1	corr	Pvalue
SC_tm1	0.40	-1.32	-1.12	-1.60	0.98	-0.12	0.77	0.81	1.02	0.17	0.58	0.076
SC_t1	0.89	1.39	0.83	1.27	-0.66	-0.16	-1.16	-0.57	-1.20	-0.63	-0.84	0.002
SC_t2	-1.49	1.14	0.39	0.96	-1.23	0.16	-0.65	-0.92	0.48	1.17	0.20	0.583
SC_te	-1.52	0.91	0.41	1.32	-0.89	0.60	-0.75	-1.04	-0.11	1.07	0.08	0.829
SC_Vx	0.03	-1.74	-0.67	-1.30	0.95	-0.33	0.99	0.92	0.97	0.18	0.62	0.054
t2-t1	-1.98	-0.24	-0.38	-0.30	-0.45	0.27	0.45	-0.28	1.41	1.50	0.88	0.001
SC_AP1	1.17	0.92	1.04	1.18	-0.59	-0.12	-1.23	-0.41	-1.41	-0.54	-0.86	0.001
SC_AP2	-1.71	-0.77	-0.51	-0.62	-0.15	0.11	0.70	0.01	1.60	1.33	0.96	0.000
SC_AUC	-1.75	-1.12	-0.27	-0.32	-0.08	0.27	0.45	-0.08	1.30	1.60	0.94	0.000
Tb_DM%	1.32	-0.09	-1.33	-0.40	0.38	0.77	-0.49	0.49	-1.71	1.06	-0.11	0.763
Tb_[N]	-0.62	-0.98	-0.68	-0.86	0.52	-0.75	0.88	0.83	2.01	-0.35	0.67	0.032
Tb_NUpt	-0.59	-1.19	-0.94	-1.00	0.60	-0.52	0.85	1.08	1.69	0.03	0.75	0.012
Tb_DM	-0.30	-1.52	-1.20	-1.17	0.71	-0.09	0.83	1.15	0.90	0.68	0.77	0.009
Average	**-0.47**	**-0.35**	**-0.34**	**-0.22**	**0.01**	**0.01**	**0.12**	**0.15**	**0.53**	**0.56**	**1.00**	**0.00**

Annex 3 Correlation matrix for 2010 data set. See Table 3 for the acronyms in the Figure legend.

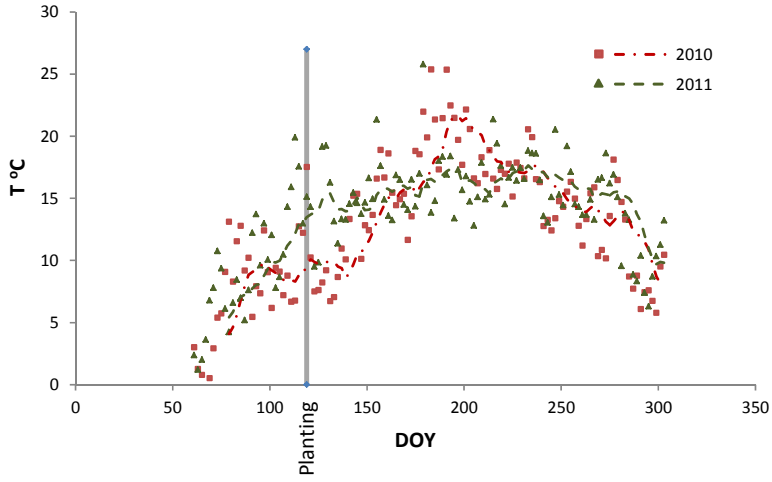
	SC_tm1	SC_t1	SC_Vx	SC_t2	SC_te	SC_Cm	AP1	AP2	AP3	AUC	t1-t2	te-t2	Tb_DM%	Tb_DM	TbnMX	TbnB	TbnA	TbwMX	TbwB	TbwA	Tb_[N]
SC_tm1																					
SC_t1	-0.69																				
SC_Vx	0.35	-0.22																			
SC_t2	-0.16	0.37	0.32																		
SC_te	-0.07	0.37	0.38	0.75																	
SC_Cm	0.59	-0.82	0.68	-0.11	-0.09																
AP1	-0.64	0.93	0.13	0.47	0.49	-0.58															
AP2	0.38	-0.36	0.65	0.71	0.52	0.58	-0.14														
AP3	0.25	-0.11	0.32	-0.38	0.30	0.23	-0.01	-0.17													
AUC	0.18	0.02	0.80	0.74	0.82	0.39	0.29	0.82	0.22												
t1-t2	0.33	-0.34	0.48	0.74	0.49	0.48	-0.19	0.97	-0.30	0.73											
te-t2	0.16	-0.09	0.00	-0.55	0.15	0.04	-0.09	-0.41	0.94	-0.06	-0.49										
Tb_DM%	0.03	0.18	0.38	0.70	0.67	0.05	0.29	0.59	-0.05	0.67	0.58	-0.20									
Tb_DM	0.41	-0.32	0.72	0.49	0.49	0.61	-0.09	0.80	0.12	0.78	0.72	-0.11	0.65								
TbnMX	0.19	-0.25	0.42	-0.26	-0.15	0.41	-0.08	0.03	0.30	0.11	-0.08	0.20	-0.27	0.16							
TbnB	0.15	-0.04	0.30	0.42	0.36	0.17	0.04	0.48	-0.06	0.45	0.45	-0.17	0.48	0.49	-0.39						
TbnA	0.01	-0.03	-0.21	0.14	0.08	-0.05	-0.12	0.08	-0.16	-0.04	0.16	-0.11	0.06	0.10	-0.53	-0.08					
TbwMX	0.53	-0.55	0.57	-0.17	-0.10	0.72	-0.37	0.33	0.28	0.26	0.22	0.12	-0.13	0.53	0.63	0.14	-0.22				
TbwB	0.14	-0.06	0.06	0.40	0.31	0.08	-0.07	0.41	-0.17	0.30	0.45	-0.21	0.39	0.43	-0.66	0.56	0.69	-0.14			
TbwA	-0.03	0.07	0.00	0.31	0.26	-0.06	0.07	0.24	-0.13	0.21	0.27	-0.14	0.28	0.28	-0.37	0.06	0.55	-0.38	0.72		
Tb_[N]	0.35	-0.46	-0.11	-0.67	-0.61	0.32	-0.52	-0.31	0.15	-0.47	-0.35	0.23	-0.53	-0.25	0.07	-0.19	0.01	0.27	-0.11	-0.20	
Tb_NUpt	0.61	-0.58	0.62	0.03	0.07	0.79	-0.39	0.55	0.21	0.43	0.45	0.03	0.25	0.75	0.23	0.31	0.10	0.71	0.30	0.09	0.42
RUE_Tb	0.42	-0.40	0.19	0.02	0.01	0.43	-0.38	0.33	0.02	0.16	0.31	-0.02	0.38	0.72	0.03	0.31	0.23	0.45	0.42	0.26	0.05
Tb_tm1	0.01	0.29	0.45	0.73	0.70	0.00	0.43	0.56	-0.04	0.71	0.53	-0.21	0.69	0.60	-0.02	0.37	-0.03	0.03	0.27	0.27	-0.53
Tb_t1	-0.09	0.21	0.44	0.68	0.65	0.06	0.37	0.56	-0.03	0.68	0.54	-0.20	0.63	0.52	0.02	0.28	-0.06	0.00	0.16	0.17	-0.56
Tb_Vx	0.41	-0.30	0.72	0.48	0.49	0.60	-0.07	0.79	0.12	0.77	0.70	-0.11	0.62	0.99	0.19	0.47	0.09	0.56	0.41	0.25	-0.24
Tb_Cm	0.35	-0.39	0.29	-0.17	-0.13	0.51	-0.31	0.18	0.17	0.10	0.11	0.10	-0.02	0.37	0.21	0.15	0.07	0.49	0.17	0.06	0.33
tb	0.09	0.21	0.18	0.28	0.29	-0.06	0.24	0.18	0.01	0.28	0.14	-0.05	0.25	0.29	-0.01	0.20	0.04	0.05	0.22	0.23	-0.12
LAI_t1	-0.26	0.51	0.12	0.69	0.66	-0.32	0.55	0.31	-0.12	0.49	0.33	-0.20	0.55	0.32	-0.19	0.28	0.05	-0.18	0.27	0.31	-0.60
LAI_Vx	0.50	-0.47	0.70	0.25	0.20	0.72	-0.25	0.69	0.09	0.58	0.59	-0.12	0.39	0.81	0.21	0.37	0.02	0.58	0.33	0.20	0.15
St_t1	-0.17	0.42	0.39	0.78	0.75	-0.11	0.56	0.51	-0.06	0.71	0.49	-0.22	0.74	0.50	-0.10	0.42	-0.06	-0.11	0.25	0.23	-0.62
St_Vx	0.31	-0.23	0.71	0.50	0.46	0.53	0.00	0.76	0.07	0.76	0.67	-0.15	0.63	0.78	0.20	0.41	-0.15	0.34	0.22	0.16	-0.18
Lf_t1	-0.29	0.52	0.16	0.74	0.69	-0.29	0.57	0.36	-0.14	0.54	0.37	-0.23	0.61	0.32	-0.17	0.34	-0.04	-0.19	0.23	0.24	-0.63
Lf_Vx	0.44	-0.32	0.71	0.43	0.42	0.61	-0.10	0.76	0.11	0.73	0.66	-0.11	0.63	0.87	0.09	0.45	0.06	0.41	0.40	0.26	-0.08
Hm_t1	-0.24	0.50	0.27	0.79	0.74	-0.24	0.59	0.44	-0.12	0.63	0.43	-0.24	0.71	0.40	-0.14	0.36	-0.05	-0.19	0.23	0.24	-0.66
Hm_Vx	0.39	-0.29	0.74	0.47	0.45	0.59	-0.05	0.78	0.10	0.76	0.68	-0.13	0.66	0.86	0.17	0.43	-0.05	0.39	0.30	0.21	-0.14
Hm_c_tm1	-0.17	0.46	0.31	0.77	0.71	-0.18	0.55	0.45	-0.12	0.63	0.44	-0.25	0.69	0.43	-0.10	0.36	-0.06	-0.13	0.22	0.23	-0.63
Hm_c_t1	-0.31	0.49	0.18	0.77	0.77	-0.30	0.56	0.40	-0.07	0.60	0.43	-0.17	0.65	0.29	-0.26	0.37	0.03	-0.26	0.30	0.25	-0.65
Hm_c_Vx	0.29	-0.16	0.72	0.60	0.59	0.48	0.07	0.81	0.08	0.84	0.72	-0.16	0.74	0.85	0.10	0.46	-0.03	0.29	0.34	0.25	-0.28
Hm_c_Cm	0.42	-0.33	0.75	0.42	0.40	0.64	-0.10	0.77	0.11	0.74	0.67	-0.12	0.61	0.85	0.19	0.40	-0.03	0.42	0.29	0.19	-0.09
Lf_c_tm1	-0.17	0.43	0.23	0.65	0.60	-0.17	0.50	0.35	-0.11	0.51	0.35	-0.21	0.56	0.37	-0.04	0.28	-0.06	-0.08	0.18	0.21	-0.53
Lf_c_t1	-0.32	0.51	0.17	0.80	0.79	-0.32	0.57	0.41	-0.09	0.60	0.44	-0.20	0.62	0.28	-0.30	0.37	0.07	-0.27	0.32	0.24	-0.66
Lf_c_Vx	0.28	-0.12	0.69	0.64	0.64	0.44	0.10	0.81	0.08	0.85	0.73	-0.15	0.75	0.87	0.01	0.50	0.07	0.27	0.43	0.31	-0.29
Lf_c_Cm	0.49	-0.45	0.68	0.21	0.22	0.72	-0.23	0.64	0.15	0.57	0.54	-0.04	0.45	0.79	0.20	0.34	0.03	0.48	0.29	0.17	0.09
St_c_tm1	-0.11	0.39	0.42	0.77	0.73	-0.07	0.53	0.53	-0.05	0.71	0.50	-0.22	0.75	0.53	-0.07	0.41	-0.07	-0.07	0.24	0.22	-0.61
St_c_t1	-0.29	0.46	0.15	0.75	0.73	-0.30	0.51	0.39	-0.10	0.56	0.43	-0.20	0.65	0.28	-0.29	0.35	0.09	-0.28	0.35	0.31	-0.63
St_c_Vx	0.27	-0.18	0.70	0.56	0.53	0.48	0.05	0.79	0.06	0.80	0.69	-0.16	0.68	0.77	0.16	0.42	-0.13	0.28	0.24	0.19	-0.25
St_c_Cm	0.33	-0.25	0.72	0.47	0.45	0.55	-0.02	0.76	0.08	0.75	0.66	-0.15	0.61	0.77	0.20	0.40	-0.13	0.35	0.22	0.16	-0.16
TbNUE	-0.23	0.35	-0.39	0.23	0.16	-0.44	0.18	-0.13	-0.23	-0.13	-0.02	-0.14	0.16	-0.22	-0.40	0.04	0.14	-0.40	0.12	0.04	-0.38

	Tb_NUpt	RUE_Tb	Tb_tm1	Tb_t1	Tb_Vx	Tb_Cm	tb	LAI_t1	LAI_Vx	St_t1	St_Vx	Lf_t1	Lf_Vx	Hm_t1	Hm_Vx	Hm_c_tm1	Hm_c_t1	Hm_c_Vx	Hm_c_Cm	Lf_c_tm1	Lf_c_t1	Lf_c_Vx	Lf_c_Cm	St_c_tm1	St_c_t1	St_c_Vx	St_c_Cm	TbNUE
RUE_Tb	0.66																											
Tb_tm1	0.22	0.22																										
Tb_t1	0.13	0.12	0.77																									
Tb_Vx	0.77	0.70	0.61	0.54																								
Tb_Cm	0.54	0.43	-0.11	-0.41	0.36																							
tb	0.18	0.16	0.58	-0.05	0.28	0.43																						
LAI_t1	-0.07	0.07	0.69	0.53	0.31	-0.17	0.43																					
LAI_Vx	0.86	0.58	0.29	0.20	0.81	0.53	0.22	0.07																				
St_t1	0.07	0.09	0.83	0.72	0.50	-0.10	0.41	0.79	0.21																			
St_Vx	0.61	0.36	0.57	0.49	0.77	0.31	0.30	0.26	0.77	0.58																		
Lf_t1	-0.09	0.02	0.75	0.60	0.31	-0.20	0.43	0.93	0.07	0.85	0.35																	
Lf_Vx	0.75	0.58	0.45	0.34	0.87	0.47	0.28	0.21	0.91	0.42	0.85	0.23																
Hm_t1	-0.04	0.03	0.82	0.67	0.40	-0.16	0.46	0.87	0.12	0.96	0.49	0.95	0.31															
Hm_Vx	0.70	0.50	0.53	0.43	0.85	0.41	0.31	0.24	0.87	0.52	0.96	0.30	0.96	0.42														
Hm_c_tm1	0.01	0.07	0.84	0.68	0.43	-0.12	0.48	0.85	0.15	0.95	0.52	0.93	0.34	0.98	0.45													
Hm_c_t1	-0.12	-0.10	0.60	0.61	0.31	-0.26	0.18	0.71	0.04	0.77	0.33	0.75	0.21	0.80	0.27	0.72												
Hm_c_Vx	0.61	0.42	0.62	0.53	0.85	0.31	0.32	0.36	0.80	0.63	0.96	0.42	0.92	0.54	0.98	0.56	0.46											
Hm_c_Cm	0.72	0.50	0.49	0.39	0.84	0.45	0.29	0.17	0.88	0.45	0.95	0.23	0.96	0.34	0.99	0.39	0.18	0.95										
Lf_c_tm1	0.01	0.09	0.76	0.57	0.36	-0.08	0.49	0.86	0.13	0.81	0.41	0.93	0.29	0.89	0.37	0.92	0.54	0.43	0.31									
Lf_c_t1	-0.13	-0.12	0.62	0.61	0.29	-0.31	0.21	0.75	0.04	0.77	0.31	0.79	0.19	0.81	0.25	0.74	0.96	0.44	0.17	0.56								
Lf_c_Vx	0.61	0.46	0.61	0.51	0.86	0.32	0.33	0.40	0.81	0.63	0.87	0.43	0.95	0.54	0.94	0.54	0.48	0.97	0.92	0.43	0.47							
Lf_c_Cm	0.77	0.58	0.26	0.18	0.78	0.63	0.21	-0.03	0.88	0.22	0.78	-0.01	0.94	0.09	0.89	0.14	-0.04	0.81	0.92	0.13	-0.09	0.82						
St_c_tm1	0.11	0.13	0.84	0.72	0.53	-0.07	0.43	0.77	0.24	0.99	0.61	0.84	0.45	0.95	0.55	0.96	0.72	0.65	0.49	0.83	0.73	0.64	0.27					
St_c_t1	-0.12	-0.08	0.56	0.57	0.29	-0.25	0.15	0.68	0.05	0.71	0.28	0.71	0.21	0.75	0.25	0.67	0.97	0.44	0.17	0.52	0.91	0.47	-0.03	0.66				
St_c_Vx	0.56	0.32	0.60	0.53	0.77	0.25	0.29	0.31	0.74	0.62	0.99	0.40	0.84	0.53	0.95	0.55	0.43	0.97	0.93	0.42	0.40	0.89	0.74	0.64	0.40			
St_c_Cm	0.62	0.36	0.55	0.47	0.76	0.33	0.30	0.23	0.77	0.55	1.00	0.32	0.85	0.45	0.96	0.49	0.28	0.95	0.96	0.39	0.27	0.86	0.79	0.58	0.24	0.98		
TbNUE	-0.43	-0.14	0.09	0.10	-0.23	-0.29	-0.04	0.30	-0.48	0.20	-0.23	0.32	-0.36	0.28	-0.31	0.26	0.34	-0.22	-0.34	0.24	0.35	-0.22	-0.44	0.18	0.33	-0.20	-0.25	

Annex 4 Slopes for the lines in the three-quadrant analysis and % of change from 0 rate to Low rate and from Low to High rate.

							Q I		Q II		Q III	
Rate	Lable	Type	Treat	Year	Cultivar	Segment (seg)	Slope	% of change in slope from 0-7 seg	Slope	% of change in slope from 0-7 seg	Slope	% of change in slope from 0-7 seg
0	H	CC	CCC	11	Kr	0-7	39.92		35.39		1.13	
7	L	FC	FCL	11	Kr	7-14	28.47	-28.68	56.64	60.07	0.50	-55.44
14	H	FC	FCH	11	Kr	0-14	34.19	-14.34	41.94	18.52	0.82	-27.72
0	L	CC	CCC	11	Kr	0-7	66.54		42.41		1.57	
7	L	FO	FOL	11	Kr	7-14	-1.89	-102.84	-2.85	-106.72	0.66	-57.71
14	H	FO	FOH	11	Kr	0-14	32.32	-51.42	28.96	-31.72	1.12	-28.86
0	H	CC	CCC	11	Bj	0-7	29.75		35.17		0.85	
7	L	FC	FCL	11	Bj	7-14	11.91	-59.99	28.57	-18.77	0.42	-50.74
14	H	FC	FCH	11	Bj	0-14	20.83	-29.99	32.99	-6.19	0.63	-25.37
0	L	CC	CCC	11	Bj	0-7	13.34		16.27		0.82	
7	L	FO	FOL	11	Bj	7-14	-3.98	-129.81	-7.02	-143.13	0.57	-30.89
14	H	FO	FOH	11	Bj	0-14	4.68	-64.90	6.75	-58.49	0.69	-15.44
0	H	CC	CCC	11	Bb	0-7	37.69		47.36		0.80	
7	L	FC	FCL	11	Bb	7-14	6.21	-83.53	29.94	-36.79	0.21	-73.95
14	H	FC	FCH	11	Bb	0-14	21.95	-41.77	43.76	-7.60	0.50	-36.97
0	L	CC	CCC	11	Bb	0-7	39.38		40.68		0.97	
7	L	FO	FOL	11	Bb	7-14	13.21	-66.46	18.31	-54.99	0.72	-25.48
14	H	FO	FOH	11	Bb	0-14	26.29	-33.23	31.13	-23.48	0.84	-12.74
0	C	CC	CCC	10	Fs	0-7	64.02		112.73		0.57	
7	L	FC	FCL	10	Fs	7-14	22.40	-65.00	65.68	-41.73	0.34	-39.93
14	H	FC	FCH	10	Fs	0-14	43.21	-32.50	95.07	-15.66	0.45	-19.97
0	C	CC	CCC	10	Fs	0-7	9.43		89.58		0.11	
7	L	FO	FOL	10	Fs	7-14	0.43	-95.40	5.88	-93.44	0.07	-29.89
14	H	FO	FOH	10	Fs	0-14	4.93	-47.70	55.08	-38.51	0.09	-14.94
0	C	CC	CCC	10	Kr	0-7	79.13		158.57		0.50	
7	L	FC	FCL	10	Kr	7-14	28.06	-64.54	79.42	-49.92	0.35	-29.20
14	H	FC	FCH	10	Kr	0-14	53.60	-32.27	125.76	-20.69	0.43	-14.60
0	C	CC	CCC	10	Kr	0-7	23.26		122.94		0.19	
7	L	FO	FOL	10	Kr	7-14	5.17	-77.76	33.83	-72.48	0.15	-19.18
14	H	FO	FOH	10	Kr	0-14	14.22	-38.88	83.11	-32.40	0.17	-9.59
0	C	CC	CCC	10	Fn	0-7	39.35		79.89		0.49	
7	L	FC	FCL	10	Fn	7-14	29.71	-24.48	70.76	-11.42	0.42	-14.75
14	H	FC	FCH	10	Fn	0-14	34.53	-12.24	75.69	-5.25	0.46	-7.37
0	C	CC	CCC	10	Fn	0-7	17.16		79.38		0.22	
7	L	FO	FOL	10	Fn	7-14	-12.39	-172.22	-172.60	-317.44	-0.07	-133.21
14	H	FO	FOH	10	Fn	0-14	2.38	-86.11	33.02	-58.40	0.07	-66.61
0	C	CC	CCC	10	Bj	0-7	45.61		94.32		0.48	
7	L	FC	FCL	10	Bj	7-14	41.68	-8.61	80.03	-15.15	0.52	7.71
14	H	FC	FCH	10	Bj	0-14	43.65	-4.31	86.91	-7.86	0.50	3.85
0	C	CC	CCC	10	Bj	0-7	1.98		18.44		0.11	
7	L	FO	FOL	10	Bj	7-14	2.30	16.12	-69.59	-477.38	-0.03	-130.77
14	H	FO	FOH	10	Bj	0-14	2.14	8.06	57.57	212.18	0.04	-65.39
0	C	CC	CCC	10	In	0-7	39.12		72.65		0.54	
7	L	FC	FCL	10	In	7-14	21.06	-46.16	42.52	-41.47	0.50	-8.01
14	H	FC	FCH	10	In	0-14	30.09	-23.08	58.21	-19.87	0.52	-4.00
0	C	CC	CCC	10	In	0-7	9.96		59.16		0.17	
7	L	FO	FOL	10	In	7-14	4.26	-57.29	56.22	-4.97	0.08	-55.06
14	H	FO	FOH	10	In	0-14	7.11	-28.65	58.25	-1.54	0.12	-27.53
0	C	CC	CCC	10	Bb	0-7	36.01		68.35		0.53	
7	L	FC	FCL	10	Bb	7-14	19.75	-45.15	46.80	-31.52	0.42	-19.91
14	H	FC	FCH	10	Bb	0-14	27.88	-22.58	58.76	-14.02	0.47	-9.95
0	C	CC	CCC	10	Bb	0-7	13.02		120.83		0.11	
7	L	FO	FOL	10	Bb	7-14	-7.26	-155.80	-80.26	-166.43	0.09	-16.00
14	H	FO	FOH	10	Bb	0-14	2.88	-77.90	29.03	-75.98	0.10	-8.00

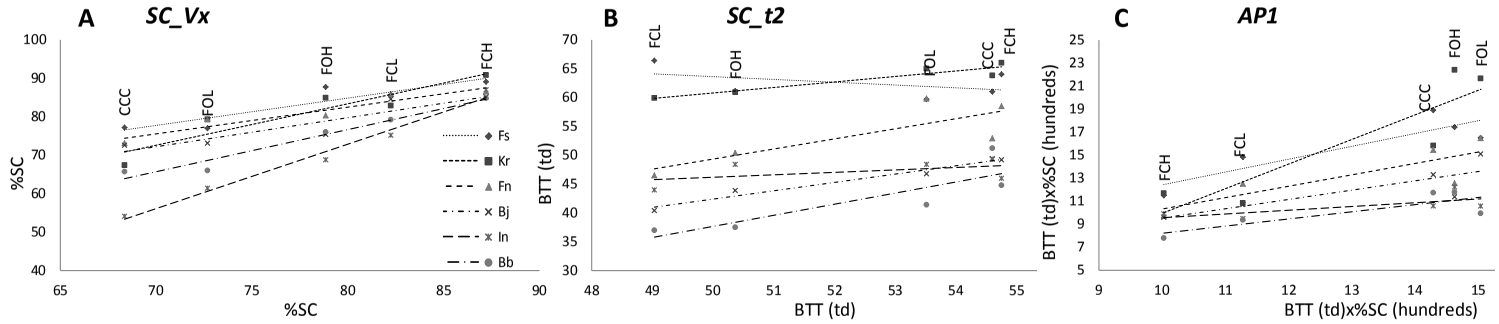
Annex 5 Temperature comparison 2010 and 2011 from March until October. DOY is day of year; T = temperature.



Annex 6 Slopes for the change in dry mater percentage (tuber and haulm) between harvests (segments of the lines in Figure 12). Only some segments are included. For treatments and cultivars acronyms see Table 1.. DAP = days after planting.

	Tubers			Haulm			
Harvest (H)	**H2-H3**	**H3-H4**	**H4-H5**	**H1-H2**	**H2-H3**	**H3-H4**	**H4-H5**
Period DAP	46-67	67-88	88-108	0-46	46-67	67-88	88-108
Thermal days (td)	10.9-23.7	23.7-38.9	38.9-52.1	1.1-10.9	10.9-23.7	23.7-38.9	38.9-52.1
Cultivars							
Significance	***	***			***		
E	**0.21**	**0.21**	**0.01**	**0.23**	**0.13**	**-0.14**	**-0.14**
E In	0.26	0.24	0.04	0.27	0.12	-0.16	-0.16
E Bb	0.16	0.17	-0.02	0.19	0.15	-0.12	-0.12
L	**0.40**	**0.38**	**0.08**	**0.26**	**0.32**	**-0.02**	**-0.02**
L Fs	0.51	0.40	0.07	0.24	0.39	-0.02	-0.02
L Kr	0.29	0.37	0.08	0.28	0.25	-0.03	-0.03
M	**0.28**	**0.27**	**0.03**	**0.10**	**0.21**	**-0.08**	**-0.08**
M Bj	0.26	0.26	0.02	-0.01	0.22	-0.08	-0.08
M Fn	0.30	0.27	0.04	0.21	0.20	-0.09	-0.09
Grand Average	**0.30**	**0.29**	**0.04**	**0.20**	**0.22**	**-0.08**	**-0.08**
Treatments	***	***			***		
1 FCH	0.51	0.30	0.07	0.18	0.22	-0.06	0.00
2 FCL	0.35	0.27	0.06	0.21	0.26	-0.08	-0.01
3 FCH	0.17	0.30	-0.03	0.09	0.19	-0.09	0.07
4 FCL	0.25	0.27	0.04	0.17	0.20	-0.07	0.01
5 CCC	0.20	0.28	0.06	0.36	0.25	-0.11	0.02
Grand Total	**0.30**	**0.29**	**0.04**	**0.20**	**0.22**	**-0.08**	**0.02**

Annex 7 Finlay Wilkinson regression lines of the treatment means for each genotype on the average treatment means. Only the three cultivars common in the two experiments were included. On the X axes the treatment means are ranked in ascending order. The units for each plot are the same in both axes. For acronyms of the treatments see Table 1. A) SC_Vx , B) SC_t2, C) AP1.



Chapter 3

Diversity of crop development traits and nitrogen use efficiency among potato cultivars grown under contrasting nitrogen regimes

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Abstract

Potato (*Solanum tuberosum* L.) requires abundant nitrogen (N) to perform well and has low nitrogen use efficiency (NUE). We assessed phenotypic variation among 189 potato cultivars for NUE and the association between NUE and ecophysiological variables describing canopy development (CDv), under high and low N input. In 2009 and 2010, 189 cultivars were grown with N supply (soil N + fertiliser N) of 75 or 180 kg N/ha at Bant, the Netherlands. CDv was assessed weekly as the percentage of soil covered by green potato leaves (%SC). Data were analysed using a model that described CDv as a function of thermal time, based on the beta function and estimates of cardinal temperatures. Nitrogen significantly affected model-derived, biologically relevant, curve-fit parameters for each cultivar. The *t1* (i.e. thermal time required to reach maximum soil cover (*Vx*)) was higher at low than at high N. Other parameters were higher at high than at low N, especially *Vx* and the period over which it was maintained. Nitrogen also affected tuber dry matter yield, tuber size and weight distributions, N content and N uptake but not tuber dry matter percentage. The total area under the %SC curve was highly correlated with yield in both years. Cultivars performing well under high N also performed well under low N. There was large variation in NUE component traits among cultivars; maturity type partially explained this variation. Variables of the CDv model captured this variation, N effects on light interception and its correlation with yield.

Keywords: Breeding for low input; Canopy development; Maturity type; Nitrogen use efficiency; Potato; Selection criteria.

Introduction

Potato (*Solanum tuberosum* L.) is an important crop for food security, hunger alleviation and cash. Farmers usually apply nitrogen (N) fertiliser to ensure profitable potato production as most N in the soil is present in soil organic matter and crop residues and not readily available for plant uptake (Zebarth and Rosen 2007). High N inputs, combined with the potato's shallow root system (Yamaguchi and Tanaka 1990; Iwama 2008) and irrigated cultivation on sandy soils, increase the chances of nitrate leaching and subsequent contamination of groundwater (Milburn et al. 1990; Errebhi et al. 1998a; Sharifi and Zebarth 2006).

Mitigation strategies to reduce N emissions take a long time to become effective and are closely linked to policy decisions (Dobermann 2005). The Nitrate Directive (1991) (91/767/EEC) and the Water Framework Directive (2000) (2000/60/EC) are forcing a reduction in N supply to crops in Europe. The focus of agronomic research has therefore shifted from finding the optimum rate of input to how to best make use of the permitted maximum amount of external supply of N (Vos 2009).

The potato crop is very responsive to N fertiliser levels (Harris 1992). With more N there is an increase in the number of photosynthetically active leaves as well as in the rate of leaf appearance per plant due to more branching, particularly at the top of the plant (Oliveira 2000). Moreover, plants have larger leaves, with a longer life span, whereas the rate of leaf appearance on a branch is not affected (Vos and Biemond 1992; Biemond and Vos 1992). Consequently, there is sustained leaf production affecting the duration of the growing period mainly by increasing the period of full soil cover (SC) when the daily rate of production is maximal (Vos 1995; Haverkort and MacKerron 2001). In this way, N effects on the canopy characteristics influence the light interception during the growing season. Vos (2009) proposed that potato adapts its foliage development to limited N supply in such a manner that the plant maintains productivity per unit of leaf area while adjusting total leaf area (through changing individual leaf size and branching). Dry matter accumulation of the potato crop is closely related to the amount of solar radiation intercepted by foliage and to the efficiency of dry matter production (Haverkort et al. 1991). Kleinkopf et al. (1981) reported that more available N increased the leaf area index (LAI) and tuber yield. Van Oijen (1991) showed that tuber yield in potato is linearly related to green leaf area averaged over the season. LAI and green leaf cover are closely related before 100% SC is obtained; %SC can easily be assessed and proves to represent the best estimate of the proportion of intercepted radiation (Haverkort et al. 1991).

Nitrogen also affects dry matter partitioning between haulm and tubers. High levels of N delay the onset of tuberisation (Biemond and Vos 1992). N affects yield, tuber size and quality parameters increasing the proportion of large size tubers (Zebarth and Rosen 2007), but only if the duration of the growing season permits the full use of the growth potential. Vos (1997) showed that the N regime drastically affects the N concentration in the total dry matter, although it hardly affects the

proportion of total plant N allocated to the tubers; or the proportion of dry matter allocated to tubers of mature plants. Vos and Biemond (1992) and Mustonen et al. (2010) showed that different N treatments resulted in plants that differed considerably in final dry weight, in N uptake and in foliar development; whereas the final distributions of dry matter and N between haulm and tubers were not affected, but the time sequences of processes leading to these end results were different (Biemond and Vos 1992). In addition, Biemond and Vos (1992) suggested that the pattern of N allocation is conserved.

Zebarth and Rosen (2007) observed that specific gravity is variably affected by N fertilisation, based on contrasting results from other authors (Bélanger et al. 2002; Zebarth et al. 2004a; Joern and Vitosh 1995, in Zebarth and Rosen 2007).

Not much effort has been put into improving performance of potato under low N fertilisation levels. The effects of low availability of N on physiological and morphological characteristics are severe because of the small and shallow root system of the potato crop (Wolfe et al. 1983), especially in early cultivars (Iwama 2008), associated with low N uptake and N use efficiency (NUE). The higher the N input the lower the NUE and N utilisation efficiency (NUtE) (Mustonen et al. 2010; Zebarth et al. 2004b; Gholipouri and Kandi 2012), even though the total N uptake increases with more N applied (Biemond and Vos 1992; Vos 1997). NUE can be considered as the ability of a crop to convert input into output. Most of the potato studies define NUE as total plant dry matter (TDM) per unit of available N in the soil (NS), including the residual N present in the soil and the fertiliser (Errebhi et al. 1998b; Zebarth et al. 2004b). NUE can be broken down into two components: uptake efficiency (NUptE; the ability of the plant to capture N from the soil) and utilisation efficiency (NUtE; the ability to use N taken up to produce yield) (Hirel et al. 2007). Once the crop growth cycle is completed, only a small fraction of N remains in the dead haulm. Then NUE could be considered as tuber dry matter (TbDM) over NS. Mustonen et al. (2010) did not find differences among cultivars, N inputs and seasons for the nitrogen and dry matter relocation from haulm to tubers, whereas Vos (1997) showed that N in the tuber very closely reflects total N uptake.

Most studies on NUE in potato have used only few genotypes or cultivars to analyse NUE components (Zebarth et al. 2004b), N effects on potato crop growth (Kleinkopf et al. 1981; Biemond and Vos 1992; Vos 1997) and how to improve crop fertilisation (Zebarth et al. 2004a; Battilani et al. 2008). Van Delden (2001) reported differences in sensitivity to N shortages between the cultivars Junior (early) and Agria (late). There is also a report of genetic variation on a "mini" core collection of wild potato germplasm (Errebhi et al. 1998b). N uptake capacity (plant N accumulation when N is abundant) was more variable than NUptE among 20 commercial potato cultivars (Zebarth et al. 2004b). Furthermore, studies with high levels of input and contrasting fertilisation regimes showed differences in NUE that were mainly associated with differences in maturity type (Tiemens-Hulscher et al. 2012).

NUE improvement is relevant for several reasons: farmers seek the highest yield with lowest cost of inputs, breeders look for good performance under low input with good response to extra N, researchers and breeders want to understand the genetics, physiology and agronomic mechanisms of this complex trait by converting it or breaking it down into (component) traits amenable for selection and interpretation.

In this paper we aim to quantify genotypic variation in N response and in canopy development (CDv) traits under high and low N input in an extensive collection of potato germplasm selected by D'hoop et al. (2008). We also identify the main factors explaining phenotypic variation for these important traits and their relationships.

Materials and methods

Location and planting material

Experiments were carried out at the Agrico research and breeding station (Bant, Flevoland, The Netherlands), during 2009 and 2010. Planting date was 15 April (2009) or 23 April (2010), whereas the experiments were harvested on 15 September (2009) or 12 October (2010). The soil was typical of the Noordoostpolder: 60 cm of Young-light-clay (35% clay) positioned on a thick layer of sand.

We used a set of 189 cultivars representing the commercial potato gene pool in Europe (Supplementary material: Annex 1). The set has been extensively used for association studies of quality traits and has been described by D'hoop et al. (2008, 2010). In order to reduce the phenotypic variation due to differences in quality of seed tubers, tubers of size class 40/50 mm from each cultivar were obtained from a single propagation at Agrico following standard procedures for potato seed production while ensuring excellent phytosanitary quality.

Experimental design and treatments

In 2009, plots were arranged over two rows, with 16 plants for final harvest. The plant arrangement was 0.33 m × 0.75 m. Cultivars were split into three groups according to their maturity type (early, medium and late) to avoid unequal competition. Based on experience from the 2009 experiment, some changes were made in the layout of the 2010 experiment, mainly to facilitate the weekly canopy cover assessment. Each plot was arranged over four rows having 16 plants for final harvest. The number of control plots (cultivar Fontane) was increased from 10 in 2009 to 18 plots/block in 2010.

In both experiments, two N levels were implemented: i) High N, with 180 kg available N/ha (soil N and fertiliser N combined) as a standard conventional N input level, and ii) Low N, with 75 kg available N/ha as the low input variant. The amount of fertiliser required was calculated based on the soil analysis done at the end of the winter. Fertiliser application was split into two: a basic fertiliser treatment was applied just after planting (N-P-K) on the whole experimental field to reach the amount for low N. A second amount was applied to the high N plots only, before the final

ridging, using KAS (27-0-0). P and K were abundantly available for potato crop growth in both N treatments.

The experimental design was an unbalanced split-plot design with treatment (N) confounded with blocks (with no replicates), maturity groups as sub-blocks at random within the block and cultivar at random but nested within maturity block. The cultivar Agria (yellow skin) was used as a border plant in plots with red skinned cultivars, and cultivar Amorosa (red skin) in plots with yellow skinned cultivars. Control plots were planted at random across the field to estimate the plot-to-plot variation.

Data collection

Emergence date was estimated per plot, as the first date when more than 50% of the plants in the plot had emerged (i.e. first leaf visible).

In the 2009 experiment, SC was assessed using a grid of 0.99 m × 0.75 m divided into 100 squares. The grid was always put in the same place to assess the same plants in each plot. The height of the grid was adjusted to canopy height. A square was counted when it was filled with green leaves for at least 50%. The total number of green squares was used as the estimate for percentage SC. This trait was assessed weekly throughout the growing season from emergence until complete crop senescence. In the 2010 experiment, SC was assessed using digital photos taken with a digital camera Canon SXI200. The camera was mounted 80 cm above a frame and positioned in the middle. The dimensions of the frame were the same as those for the grid. Pictures were always taken on the same spot, with the frame at the top of the canopy in order to cover the same three plants over a row. The percentage of green pixels on the pictures was estimated using a specific script made for this purpose developed by Gerie van der Heijden in MATLAB® version 7.8.0347 (R2009a), the MathWorks™ programme. The two methodologies to assess SC (grid and digital photos) were highly correlated (r = 0.93 for several hundreds of data pairs, data not included).

Maturity was scored using a scale to assess the progress of senescence (Celis Gamboa 2002; modified) in which 1 = green canopy with the first flower buds, 2 = green haulm with abundant flowers, 3 = first signs of yellowness in the upper leaves, 4 = up to 25% of the plant with yellow leaves, 5 = up to 50% of the plant with yellow leaves or lost leaves, 6 = up to 75% as in 5, 7 = up to 90% of the plant yellow or without leaves, 8= entire haulm brown or dead plant. The assessment was done three times within two weeks, scoring three plants per plot, when a typical intermediate maturity cultivar was showing a score of 5 under low N. This assessment will be referred to as maturity assessment (mt_as) avoiding confusion with maturity groups used as blocking factor.

Final harvest

The final harvest took place as late as possible to allow late cultivars completing their cycle. The whole experiment was harvested at once. Sixteen plants were harvested per plot and the following tuber traits were assessed: A) Total tuber fresh weight. B) Tuber size and weight

distribution; for this, six size classes were included: 0-30 mm, 30-40 mm, 40-50 mm, 50-60 mm, 60-70 mm and > 70 mm. For each class the tuber number and tuber weight were recorded. C) Tuber number per meter; obtained for the class 50-60 mm. D) Dry matter percentage (DM%), as dry weight of a sample divided by its fresh weight expressed in percentage. Tubers from all size classes were cut with a French-fries cutting machine before drying at 70 °C for 48 hours. E) N content ([N]) was assessed using the Kjeldahl protocol.

Data processing

The Beta thermal time for each canopy assessment date was calculated from the emergence date for each plot using the Beta function (Yin et al. 2003), cardinal temperatures determined for potato haulm growth (Khan 2012; Khan et al. 2013), and hourly temperatures for each season from the Marknesse weather station (12 km from the site).

A canopy development model was fitted using SC data, the Beta thermal time for each assessment date and the NOLIN procedure of SAS/STAT®. The equations describing each phase of the curve were specified along with starting values for each parameter. After that SAS performed an optimisation process to get estimated parameters and their standard errors. Five parameters were estimated for each individual plot (Khan 2012; Khan et al. 2013). Four *t*-parameters were expressed in thermal days (td): *tm1* (inflection point in the growing phase of the curve), *t1* (when SC stabilized), *t2* (start of senescence), and *te* (when canopy had completely senesced). The fifth parameter, *Vx*, was the maximum SC reached with percentage soil coverage (%SC) as unit.

A bell-shaped curve was fitted per plot and for each of the two data sets to describe the tuber weight and tuber number distribution (Tbw and Tbn respectively). Three parameters were estimated for each data set following the equation.

$$\mathrm{Tb} = \mathrm{MX} * \exp\left(-\frac{(\mathrm{mcl} - \mathrm{B})^2}{\mathrm{A}}\right)$$

Eq. 1

where Tb is either Tbw or Tbn, A is a dispersion parameter expressing how the weights/numbers were distributed across classes, mcl is the middle size of each size class, B is the average size at which the maximum (MX) weight/number occurs. The curve-fit parameters were named for each variable as follows: for Tbw data: TbwA, TbwB, TbwMX and for Tbn data: TbnA, TbnB, TbnMX.

Calculated variables

Based on the parameters estimated with the CDv model, the following variables were calculated (Khan 2012; Khan et al. 2013): *t2-t1* (duration of maximum SC in td), *te-t2* (duration of senescence in td), *Cm* (maximum progression rate of %SC in %/td), *AP1* (area under the curve for canopy build-up phase in %.td), *AP2* (area under the curve for phase of maximum SC in %.td), *AP3* (area under the curve for senescence phase in %.td), and *AUC* (area under the curve for the entire crop

cycle in %.td). In order to express the agronomic variables in a standard way, subsequent calculations and conversions were done as: Yield (Y) in kg/m^2, N content ([N]) in g/kg (determined only in tubers), DM% in percentage. Dry matter yield (Y_DM) in kg/m^2, that is Y×DM%/100. N uptake in tuber (NUpt) in g/m^2, that is Y_DM × [N]. N use efficiency (NUE) as Y_DM/(N input) in kg/g. N utilisation efficiency (NUtE) that is Y_DM/NUpt, in kg/g. N Uptake efficiency (NUptE; NUpt/N input in g/g). Soil coverage yield index (SCYi=*AUC*/ Y_DM in %.td/(kg/m^2)). The variables were analysed without transformation since there were no severe violations to the assumptions required for mixed models analysis.

Statistical analysis

Data were analysed with the 16th edition of the Genstat package. Because of the structure of the data and the objectives of this research, a mixed model was used to study the effects of the main factors year, N levels, and maturity groups, as well as their interactions. Variance components were used to quantify the ratio between the genotypic variance and total variance (genotypic + environmental variance) as a measure of heritability. General trends for the traits were described as well as correlations between them. To answer the main questions in this analysis, the following model was used:

$$Y = yr * N\_lv * Mt + \underline{N\_lv.row} + \underline{N\_lv.col} + \underline{Mt.G} + \underline{E} \qquad \text{Eq. 2}$$

Where terms joined by "*" represent individual effects plus the interactions (*yr*N_lv= yr+N_lv+yr.N_lv*), whereas terms joined by "." represent interaction only. The term *yr* represents year, clarifying that year effects are confounded with possible variation because of the field experiment or changes in the plot layout, the last one assumed to be small. The term *N_lv* is the N treatment that is confounded with field block effects. The term *Mt* is the maturity group excluding control plot information. Corrections for rows and columns are the random terms *N_lv.row* and *N_lv.col*. The term *Mt.G* represents the cultivars nested within maturity groups (random term) and finally the *E* represents the error. All random terms are underlined.

We also used a second model combining information of both years for each N treatment separately:

$$Y = yr * Mt + \underline{yr.row} + \underline{yr.col} + \underline{Mt.G} + \underline{E} \qquad \text{Eq. 3}$$

The variance component for genotype nested within a maturity group (*Mt.G*) was considered the genotypic variance whereas the residual was considered the environmental variance. The plot-to-plot variation was assessed through the control plots and its variance was also included as environmental variance. Calculation by maturity group was possible by setting options in the

mixed model related procedures in Genstat VCOMPONENTS and VSTRUCTURE to get residuals and variance components per *Mt* group.

Additionally, biplots were generated to visualize relationships between traits per N level. The GGE-biplot option in Genstat was used because it allows plotting only the trait loadings or vectors since our interest was on the relationships between traits. This analysis was complemented with correlation matrices. Data were standardized by trait. We excluded traits in which calculation included N input level, i.e. NUE, NutE and NUptE. To define groups of traits, a cluster analysis was performed using the vector loadings from a principal components analysis (PCA). The principal components (PC) 1 to 3 were selected for the cluster analysis based on their variance explained. The cluster number was selected based on the within group sum of squares for different numbers of groups defined (see additional data in Supplementary material: Annex 2).

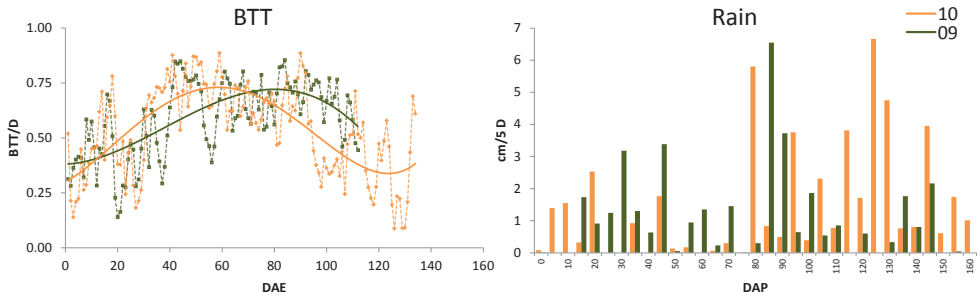

Figure 1 Beta thermal time (BTT) and rain profile over the two growing seasons. The trend in BTT is presented as moving average per day calculated from emergence onwards (DAE: days after emergence) and rainfall is given in cumulative cm per period of 5 days calculated from planting (DAP: days after planting).

Results

Thermal time and rainfall during the two experimental seasons

There were differences in the profiles of thermal time and rainfall during the growing periods of the two experimental years (Figure 1). The daily thermal time had slightly higher values in 2009 from April, around the planting date until emergence. This trend was inversed a few days later until around 70 days after emergence (DAE), when the average thermal time per day considerably dropped in 2010. At this moment the cumulative thermal time was already higher in 2010 than in 2009 (41.2 and 37.6 thermal days, respectively). Furthermore 2009 gave an advantage especially to all early and intermediate cultivars in the canopy build-up phase, since these maturity groups tended to have a quick first phase due to high temperatures. In 2009, late cultivars were disadvantaged in most of the phase with maximum canopy cover (Phase *t2-t1* of CDv) with lower

thermal time values in 2009 compared to 2010. On the other hand the growing period in 2010 had a higher cumulative thermal time than that in 2009 as the former lasted longer (Figure 1).

Table 1 P values for main factors included in the overall model. yr: year, N_lv: N level, Mt: maturity groups. Interaction terms are represented by joining the main terms by a "." For acronyms of traits, see main text.

Trait	*yr*	*N_lv*	*Mt*	*N_lv.Mt*	*yr.Mt*	*yr.N_lv*	*yr.N_lv.Mt*
tm1	<0.001	<0.001	0.822	0.006	<0.001	0.309	0.212
t1	<0.001	0.001	<0.001	0.002	<0.001	0.881	0.057
Vx	0.001	<0.001	<0.001	0.124	<0.001	0.215	<0.001
t2	<0.001	<0.001	<0.001	0.099	<0.001	0.064	<0.001
te	<0.001	<0.001	<0.001	0.320	<0.001	<0.001	0.448
Cm	<0.001	<0.001	<0.001	0.269	<0.001	<0.001	0.338
AP1	<0.001	0.224	<0.001	0.006	<0.001	0.784	0.022
AP2	<0.001	<0.001	<0.001	<0.001	<0.001	0.211	0.117
AP3	<0.001	<0.001	0.553	0.150	<0.001	<0.001	0.140
AUC	0.009	<0.001	<0.001	0.076	0.061	0.034	0.027
t2-t1	<0.001	<0.001	<0.001	<0.001	0.001	0.122	0.229
te-t2	<0.001	0.793	0.008	0.065	<0.001	0.013	0.003
DM%	<0.001	0.915	<0.001	0.023	0.097	0.366	0.153
Y_DM	0.023	<0.001	<0.001	<0.001	<0.001	0.334	0.149
[N]	0.004	<0.001	<0.001	0.467	<0.001	0.207	0.210
NUpt	0.735	<0.001	<0.001	0.002	<0.001	0.233	0.735
NUptE	0.777	<0.001	<0.001	0.074	<0.001	0.313	0.032
NUtE	0.009	<0.001	<0.001	0.001	<0.001	0.124	0.785
NUE	0.082	<0.001	<0.001	<0.001	<0.001	0.999	0.056
SCYi	<0.001	0.242	0.424	0.627	<0.001	0.02	0.162
TbwMX	0.045	<0.001	<0.001	0.432	<0.001	0.351	0.689
TbwB	<0.001	<0.001	0.028	0.329	<0.001	0.012	0.118
TbwA	0.987	<0.001	0.192	0.119	<0.001	0.102	0.015
TbnMX	<0.001	0.437	0.021	0.638	<0.001	0.003	0.550
TbnB	<0.001	<0.001	0.022	0.436	0.026	0.004	0.600
TbnA	<0.001	<0.001	0.015	0.08	<0.001	0.015	0.066
mt_as	0.585	<0.001	<0.001	0.021	0.606	0.993	0.648

In both years planting was done in moist soil. The year 2009 had better rainfall conditions during most of the growing period (while no excessive rain occurred) with the rainfall being more evenly distributed during the entire growing period (Figure 1). The year 2010 had periods with low precipitation between 45 and 75 days after planting (DAP) (Figure 1). Thereafter, both years had 10 days with high precipitation, at around 80 DAP, with one week delay in 2009 compared to 2010. Year 2010 got more precipitation than 2009 until harvest. These differences in rainfall distributions probably affected N availability during the season but also tuber set. Its effects are reflected in the term year and in its interaction with other main factors.

Table 2 Three-way table including means per maturity group (E: early, M: intermediate, and L: late), N levels (N_lv; 1: 180 kg available N/ha; 2: 75 kg available N/ha) and year (2009 or 2010) combination for all traits assessed. W Avg 09 and W Avg 10 represent an average weighted by the number of cultivars in each maturity group for 2009 and 2010, respectively. For acronyms and units of traits, see main text.

Year		2009				2010			
Traits	**N_lv**	**E**	**M**	**L**	**W Avg 09**	**E**	**M**	**L**	**W Avg 10**
tm1	1	9.99	10.66	11.31	10.61	8.53	8.50	8.16	8.41
tm1	2	9.02	9.42	9.84	9.40	7.45	6.63	5.42	6.57
t1	1	17.70	18.10	20.94	18.73	20.49	27.24	28.97	25.51
t1	2	19.86	21.64	24.00	21.70	20.76	30.90	36.85	29.23
Vx	1	90.85	95.47	94.89	93.82	80.83	88.60	90.67	86.63
Vx	2	75.53	83.09	75.95	78.73	67.40	76.57	82.01	75.07
t2	1	35.40	40.03	49.12	40.95	42.52	56.35	63.13	53.68
t2	2	31.96	34.39	37.85	34.54	37.19	53.90	61.39	50.52
te	1	58.37	66.44	77.42	66.74	63.49	68.60	71.96	67.85
te	2	50.24	58.25	73.39	59.74	62.48	67.47	70.97	66.80
Cm	1	8.39	8.90	7.66	8.41	6.41	5.17	4.89	5.50
Cm	2	6.03	5.89	4.91	5.67	5.19	3.96	3.69	4.28
AP1	1	743.7	772.1	938.0	807.0	893.3	1392.0	1539.0	1269.3
AP1	2	778.6	947.7	982.7	902.6	799.4	1452.0	1915.0	1365.9
AP2	1	1618.0	2096.0	2694.0	2099.9	1796.0	2591.0	3107.0	2472.0
AP2	2	920.7	1058.0	1064.0	1015.3	1112.0	1766.0	2020.0	1623.3
AP3	1	1360.0	1634.0	1754.0	1577.0	1100.0	731.1	553.0	803.0
AP3	2	921.1	1306.0	1734.0	1296.9	1099.0	698.7	532.4	783.1
AUC	1	3722.0	4502.0	5351.0	4474.7	3790.0	4715.0	5199.0	4544.9
AUC	2	2620.0	3311.0	3781.0	3214.4	3010.0	3917.0	4468.0	3772.5
t2-t1	1	17.69	21.94	28.18	22.22	22.04	29.11	34.15	28.17
t2-t1	2	12.10	12.76	13.85	12.84	16.43	23.01	24.54	21.30
te-t2	1	22.97	26.41	28.80	25.93	20.97	12.25	8.83	14.16
te-t2	2	18.28	23.86	35.54	25.20	25.29	13.57	9.58	16.28
DM%	1	23.40	24.89	27.93	25.22	22.34	23.37	26.09	23.77
DM%	2	23.38	25.00	27.51	25.15	22.02	23.96	26.04	23.89
Y_DM	1	1.33	1.64	1.81	1.58	1.16	1.53	1.75	1.47
Y_DM	2	1.01	1.25	1.30	1.19	0.86	1.20	1.38	1.14
[N]	1	12.80	11.64	10.25	11.65	12.59	12.13	11.18	12.02
[N]	2	10.78	9.43	8.56	9.63	11.24	10.49	9.45	10.45
Nupt	1	16.74	18.83	18.27	18.00	14.45	18.30	19.32	17.32
NUpt	2	10.70	11.57	10.83	11.09	9.53	12.46	12.80	11.61
NUptE	1	0.93	1.05	1.02	1.00	0.80	1.02	1.07	0.96
NUptE	2	1.43	1.54	1.44	1.48	1.27	1.66	1.71	1.55
NUtE	1	0.08	0.09	0.10	0.09	0.08	0.08	0.09	0.08
NUtE	2	0.09	0.11	0.12	0.11	0.09	0.10	0.11	0.10
NUE	1	0.07	0.09	0.10	0.09	0.06	0.08	0.10	0.08
NUE	2	0.14	0.17	0.17	0.16	0.11	0.16	0.18	0.15
SCYi	1	2800.0	2782.0	2984.0	2841.6	3359.0	3191.0	3123.0	3227.3
SCYi	2	2592.0	2681.0	2928.0	2718.7	3597.0	3339.0	3385.0	3434.6
TbwMX	1	2.72	3.24	2.90	2.98	2.36	2.99	3.09	2.81
TbwMX	2	2.15	2.46	2.20	2.29	1.84	2.37	2.58	2.26
TbwB	1	57.10	62.51	60.92	60.33	54.20	55.87	54.10	54.84
TbwB	2	52.44	54.97	55.09	54.19	50.57	52.65	51.10	51.56
TbwA	1	152.30	206.00	213.60	190.60	173.00	167.80	160.40	167.48
TbwA	2	136.80	133.00	162.70	142.21	151.70	156.20	140.10	150.42
TbnMX	1	20.04	18.55	19.48	19.28	23.64	26.42	30.56	26.64
TbnMX	2	20.63	19.93	20.00	20.17	21.69	24.85	30.61	25.38
TbnB	1	52.49	56.18	54.63	54.57	48.37	50.69	49.33	49.56
TbnB	2	48.10	50.42	50.18	49.61	45.67	47.94	46.80	46.90
TbnA	1	217.80	329.70	305.90	287.06	208.30	215.10	196.60	207.86
TbnA	2	179.10	212.50	218.00	203.20	177.00	185.00	169.50	178.25
mt_as	1	6.24	4.76	3.87	5.00	6.23	4.72	3.79	4.96
mt_as	2	6.86	5.75	4.87	5.87	6.93	5.66	4.76	5.83

Main effects and interactions

An overall statistical model combining the two years, the two N levels and all cultivars (Eq. 2) showed year having a significant effect on almost all crop traits, excluding NUpt, NUptE, NUE, NUtE, TbwA and mt_as (Table 1). The term *yr* included differences in environmental condition, weather

conditions being the most relevant factors. Therefore rainfall and temperature could partially explain the differences between seasons as discussed below. In an overall comparison across years (Table 2 and Figure 2A), in the 2009 trial the plants had faster growth through the first phase of CDv, showing on average a shorter *t1* with a longer *tm1* and reaching a higher maximum %SC (*Vx*) than in 2010. Phase *t2-t1* was shorter, with higher soil coverage (*Vx*) while Phase *te-t2* was considerably longer than in 2010. Therefore senescence started sooner and progressed slowly in 2009, while in 2010 it started abruptly and was quicker. In general, *AUC* was slightly lower in 2009 than in 2010 with a marginally higher dry matter yield and dry matter content than observed in 2010. The N content in the tubers dry matter was almost identical in both years and so were NUpt, NUE and NutE.

Only *AP1*, *te-t2*, DM%, SCYi and TbnMX did not show significant differences between N levels (Table 1 and Figure 2B). High N resulted in a low *t1* with a higher *Vx*, the vegetative development was quicker and more extensive. Phase *t2-t1* was longer with more N input with also higher *AP2* allowing better light interception. *AP3* was lower with more N; that means N affected the senescence process, not only delaying its initiation but making the process to progress quicker. With more N, *te* and *AUC* were higher. The increase in *AUC*, however, was mainly due to an increase in *AP2*. Y_DM and DM% were higher with more N, with no clear differences in DM% (Table 2). On the other hand, the year effect was larger than the N effect for DM%, *Cm*, *tm1*, TbnB, TbnMX, *t1*, *te-t2*, *t2*, *AP1*, SCYi and *AP3*. For the other variables the N effect was larger than the year effect.

Maturity class did not show significant effects on *tm1*, *AP3*, SCYi and TbwA (Table 1 and Figure 2 C). Typically, late cultivars had lower values for *Cm*, *AP3*, *te-t2* and [N]. Generally, the other traits increased in their values, from early to late with the intermediate group showing differences in its relative increase between years. A maturity assessment (mt_as) showed lower scores for the cultivars under high N than under low N. This means a delay in the onset of senescence due to extra N prolonging *t2-t1*, with a green canopy sustained for a longer period.

The interaction term between year and N level (*yr.N_lv*), was not significant for most traits (Table 1), i.e. the N effect was consistent in both years whereas *Cm*, *AUC*, SCYi, *te*, *AP3*, *te-t2*, and tuber number traits did show significant interactions. The interaction between N level and maturity (*N_lv.Mt*) was significant for *tm1*, *t1*, *AP1*, *AP2* which are variables from the first and second phase of CDv, and also DM%, Yield, N and therefore N uptake as well as NUE. On the other hand, the interaction term between year and maturity (*yr.Mt*) was significant for most of the traits excluding *AUC*, DM%, mt_as, TbnB and *t2-t1*.

Additionally, parameters *tm1*, *te-t2*, *AP3*, SCYi and TbwA showed a completely different trend in the second year compared to the first year with values increasing from the early to the late maturity group in 2009, while in 2010 values decreased from the early to the late maturity group. These differences reflected the year-specific CDv trend with a low *t1* and a slow senescence in the year 2009.

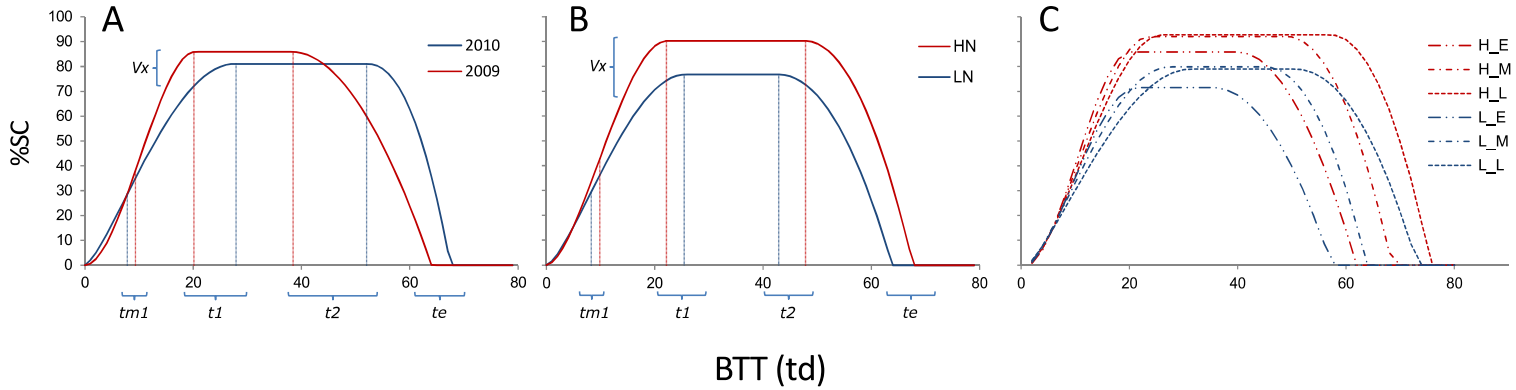

Figure 2 Average fitted curves of canopy development for: A) Years (2009, 2010), B) Nitrogen levels (High N (HN) and low N (LN)), and C) Maturity group × nitrogen level combinations. (H stands for high N level, L stands for low N level; E: early, M: intermediate; L: late). BTT represents beta thermal time in thermal days (td). %SC: soil coverage in percentage.

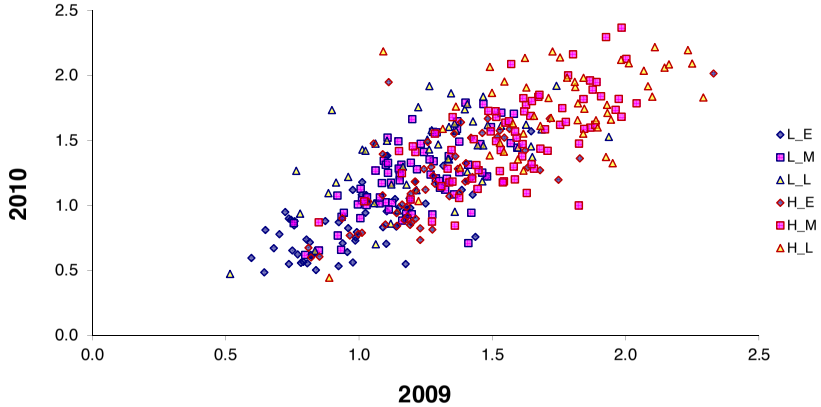

Figure 3 Potato dry matter yield (in kg/m²) comparison: 2009 (x-axis) and 2010 (y-axis), at two N levels. Markers with red border line (N1) 180 kg N/ha and markers with blue border line (N2) 75 kg N/ha). Maturity groups based on breeders' information are: E) early (diamonds), M) intermediate (squares) and L) late (triangles) cultivars.

Yield

Y_DMs were similar in both years (1.356 kg/m^2 in 2009 and 1.321 kg/m^2 in 2010) with a correlation across cultivars of 0.786. (Figure 3). Although the field experiment moved from 2009 to 2010 due to compulsory crop rotation we assumed that sites were similar from year to year, with results supporting this assumption.

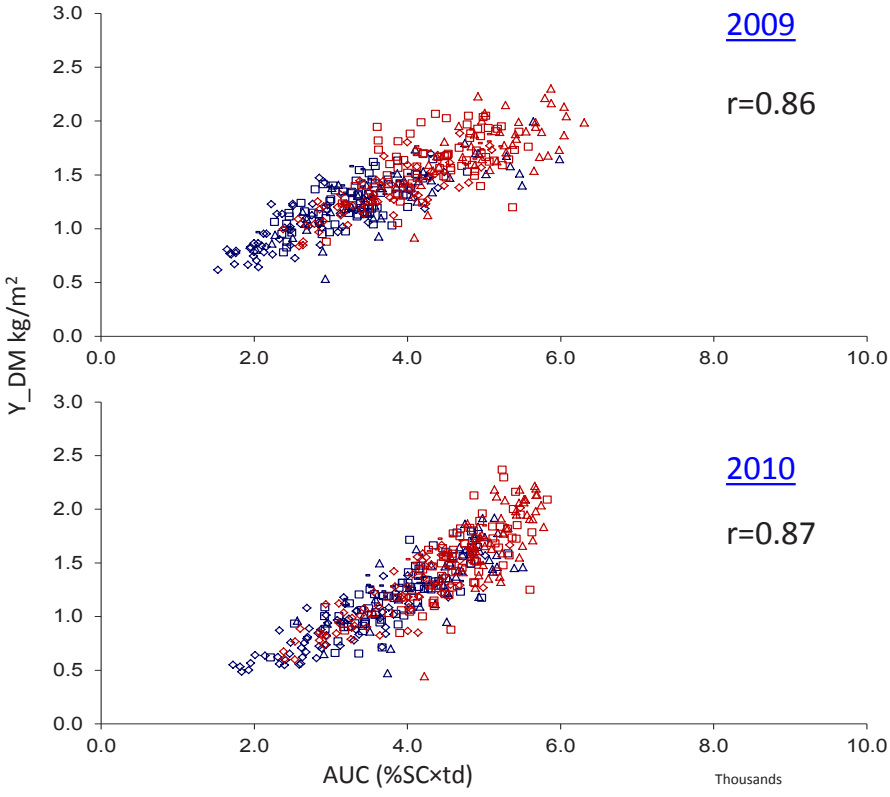

Figure 4 Relation between *AUC* (area under the curve for canopy development) and potato dry matter yield (Y_DM) in both years and under two N input levels. Red symbols are N1 (180 kg/ha), blue symbols are N2 (75 kg/ha). Maturity groups based on breeders' information are: ◇: early, □ : intermediate and △: late cultivars

Overall, high N resulted in higher Y_DM than low N (1.516 kg/m^2 versus 1.160 kg/m^2), with similar correlations across cultivars between years (0.748 and 0.711 for high and low N, respectively). The performance of the maturity groups showed the same trends in both years: late cultivars tended to have higher Y_DM than intermediate and early cultivars. Additionally, the correlations between year, per maturity group at high N level were 0.76, 0.67 and 0.52 (for early, middle and late, respectively) whereas at low N level the correlations were 0.66, 0.64 and 0.59. At low N the performance of genotypes may depend more on other factors affecting the amount of N available to the plant. The soil type and its characteristics were the same, with no evidence of

spatial variation supported by the performance of the control plots as well as information from the experimental station. On the other hand, environmental conditions differed between seasons affecting the performance of the cultivars. For example, radiation affected directly the potential yield whereas rainfall may have affected N processes in the plant and in the soil.

The correlation between Y_DM and *AUC* was 0.86 (2009) - 0.87 (2010) (Figure 4). High N led to higher yield due to an increase in *AUC*, mainly by earlier *t1* and increased *Vx* (Table 2). Therefore the period with maximal light interception was prolonged. Late cultivars had highest Y_DM and *AUC*, followed by intermediate and early cultivars. This trend was similar for both N levels and also for both years. However, under low N, late and medium maturity groups were more scattered. In addition in 2010 the variation within maturity groups was large, especially in the intermediate maturity group (Supplementary material: Annex 3).

NUE decreased with an increase in N input, as did NUptE and NUtE (Table 2). In most cases, these indices increased from early to late maturity with the intermediate group having values close to the values from the late group. Taking the extremes, late cultivars had higher dry matter yield, higher dry matter percentage, more N uptake, but lower N content in tuber dry matter than early cultivars. The late maturity group was therefore more efficient in using the available resources than the early maturity group, although the former needed a longer period of growth than the latter. If efficiency is also defined as a function of growing time, the perspective will probably be changed.

Relationship among traits

Out of the 276 combinations between traits (the lower triangular matrix of 24×24 traits in Table 3) and excluding NUE, NutE and NUptE, 91 correlations had absolute values greater than 0.4 at one of the N levels; 43 of them had a higher correlation at high N whereas 48 had a higher correlation at low N. The trait with most correlations above the absolute value of 0.4 was maturity assessment (14) followed by yield (13), *AUC* (13), *t2* (13), *AP1* (12), N uptake (10), *te* (10), and *AP2* (9). Additionally, mt_as had a negative correlation with 12 of the traits with which it was correlated, whereas Y_DM and *AUC* had most often positive correlations.

Since maturity has been mentioned as one of the factors driving the phenotype of the cultivars we examined how the Y_DM, N content and canopy cover traits were related to maturity per N level. Maturity assessed during the experiment, mt_as, had its highest correlation with *AUC* at both N levels (-0.83 at high and -0.78 at low N) and therefore this trait was the best indicator of maturity together with *te* (-0.71 at high N and -0.75 at low N). Y_DM was also highly negatively correlated with mt_as at both N inputs (-0.69 for high and -0.73 for low N). On the other hand, the correlation with *t2-t1* (-0.60 for high and -0.30 for low N) and with *AP2* (-0.64 at high and -0.30 at low N) considerably changed with N input. These results showed that *AUC* and Y_DM were higher for late cultivars. N content correlated with mt_as (0.56 and 0.57 at high and low N input, respectively); early cultivars had higher N contents than late ones.

Table 3 Correlation matrix illustrated as a heat map for all traits at both N levels, combining the two years per N level. A) High N; B) Low N. For acronyms of traits, see main text.

A

Traits	tm1	t1	Vx	t2	te	Cm	AP1	AP2	AP3	AUC	t2-t1	te-t2	DM%	Y_DM	[N]	NUpt	SCYi	TbwMX	TbwB	TbwA	TbnMX	TbnB	TbnA	mt_as
tm1																								
t1	-0.25																							
Vx	0.26	-0.05																						
t2	-0.23	0.61	0.09																					
te	0.11	0.27	0.19	0.45																				
Cm	0.35	-0.85	0.36	-0.57	-0.20																			
AP1	-0.36	0.98	0.05	0.64	0.27	-0.83																		
AP2	-0.06	0.04	0.32	0.80	0.41	-0.04	0.11																	
AP3	0.36	-0.40	0.22	-0.63	0.39	0.45	-0.41	-0.44																
AUC	0.03	0.25	0.52	0.68	0.85	-0.13	0.32	0.75	0.09															
t2-t1	-0.11	0.06	0.16	0.82	0.38	-0.11	0.11	0.98	-0.51	0.68														
te-t2	0.33	-0.41	0.06	-0.66	0.37	0.42	-0.44	-0.50	0.98	0.01	-0.54													
DM%	0.13	0.10	0.36	0.32	0.45	0.01	0.12	0.39	0.11	0.54	0.33	0.05												
Y_DM	-0.03	0.09	0.58	0.50	0.54	0.05	0.17	0.65	0.02	0.78	0.58	-0.07	0.56											
[N]	-0.03	-0.14	-0.30	-0.36	-0.52	0.04	-0.17	-0.39	-0.11	-0.58	-0.35	-0.07	-0.57	-0.61										
NUpt	-0.09	0.01	0.50	0.38	0.28	0.07	0.10	0.52	-0.08	0.53	0.47	-0.16	0.27	0.79	-0.02									
SCYi	0.16	0.25	-0.24	0.10	0.19	-0.25	0.18	-0.08	0.02	0.03	-0.05	0.06	-0.17	-0.54	0.20	-0.55								
TbwMX	-0.07	0.01	0.25	0.18	0.18	0.04	0.06	0.26	0.00	0.29	0.23	-0.04	-0.06	0.42	-0.12	0.44	-0.29							
TbwB	0.18	-0.02	0.21	0.03	0.21	0.07	-0.02	0.09	0.18	0.21	0.05	0.15	0.02	0.28	-0.10	0.27	-0.19	0.42						
TbwA	0.09	0.02	0.09	0.05	0.19	-0.03	0.02	0.06	0.13	0.17	0.05	0.12	-0.07	0.15	-0.07	0.12	-0.02	0.33	0.73					
TbnMX	-0.27	0.14	-0.01	0.26	0.02	-0.18	0.19	0.22	-0.26	0.13	0.23	-0.26	-0.01	0.08	-0.04	0.08	0.05	0.17	-0.70	-0.36				
TbnB	0.18	-0.03	0.22	0.01	0.17	0.10	-0.03	0.07	0.17	0.19	0.03	0.14	0.04	0.28	-0.08	0.28	-0.22	0.42	0.93	0.49	-0.67			
TbnA	0.13	-0.03	0.13	0.02	0.18	0.04	-0.03	0.06	0.16	0.17	0.04	0.14	-0.04	0.17	-0.08	0.16	-0.09	0.29	0.79	0.81	-0.49	0.64		
mt_as	-0.09	-0.42	-0.38	-0.71	-0.71	0.27	-0.43	-0.64	0.08	-0.83	-0.60	0.14	-0.52	-0.69	0.56	-0.46	0.00	-0.25	-0.27	-0.18	-0.04	-0.26	-0.19	

Colour key

-1 0 1

B

Traits	tm1	t1	Vx	t2	te	Cm	AP1	AP2	AP3	AUC	t2-t1	te-t2	DM%	Y_DM	[N]	NUpt	SCYi	TbwMX	TbwB	TbwA	TbnMX	TbnB	TbnA	mt_as
tm1																								
t1	-0.48																							
Vx	0.04	0.27																						
t2	-0.45	0.74	0.21																					
te	-0.22	0.47	0.09	0.55																				
Cm	0.44	-0.81	0.18	-0.65	-0.48																			
AP1	-0.53	0.96	0.46	0.73	0.45	-0.69																		
AP2	-0.19	0.20	0.29	0.79	0.37	-0.14	0.27																	
AP3	0.34	-0.36	0.09	-0.61	0.29	0.35	-0.33	-0.52																
AUC	-0.28	0.56	0.60	0.75	0.78	-0.34	0.66	0.69	-0.01															
t2-t1	-0.21	0.14	0.05	0.77	0.36	-0.19	0.16	0.96	-0.55	0.57														
te-t2	0.32	-0.42	-0.16	-0.65	0.28	0.30	-0.44	-0.57	0.96	-0.15	-0.56													
DM%	-0.04	0.27	0.20	0.24	0.43	-0.23	0.27	0.13	0.18	0.39	0.10	0.12												
Y_DM	-0.16	0.34	0.60	0.47	0.51	-0.15	0.46	0.49	0.08	0.76	0.36	-0.07	0.53											
[N]	0.02	-0.14	-0.34	-0.19	-0.42	0.05	-0.19	-0.21	-0.25	-0.46	-0.15	-0.16	-0.62	-0.65										
NUpt	-0.21	0.34	0.53	0.46	0.31	-0.15	0.45	0.46	-0.11	0.60	0.35	-0.24	0.19	0.77	-0.05									
SCYi	-0.08	0.25	-0.08	0.30	0.32	-0.25	0.21	0.18	-0.09	0.23	0.20	-0.05	-0.22	-0.42	0.30	-0.33								
TbwMX	-0.16	0.22	0.56	0.32	0.23	-0.01	0.35	0.38	-0.02	0.53	0.26	-0.16	0.10	0.72	-0.36	0.68	-0.35							
TbwB	0.09	0.01	0.16	0.00	0.15	0.04	0.02	0.03	0.18	0.15	0.00	0.13	0.08	0.31	-0.18	0.25	-0.23	0.01						
TbwA	0.04	0.05	0.00	0.10	0.19	-0.07	0.03	0.11	0.05	0.14	0.10	0.05	-0.05	0.11	-0.01	0.11	0.03	-0.39	0.49					
TbnMX	-0.23	0.26	0.19	0.33	0.14	-0.17	0.31	0.28	-0.22	0.30	0.23	-0.25	0.04	0.19	-0.07	0.20	0.10	0.44	-0.73	-0.36				
TbnB	0.06	0.02	0.18	0.01	0.12	0.04	0.04	0.03	0.16	0.15	-0.01	0.10	0.11	0.33	-0.21	0.27	-0.26	0.16	0.95	0.26	-0.67			
TbnA	0.08	0.00	0.12	-0.01	0.17	0.00	0.01	0.00	0.22	0.15	-0.02	0.17	0.02	0.20	-0.12	0.15	-0.09	-0.17	0.72	0.64	-0.56	0.52		
mt_as	0.13	-0.57	-0.40	-0.57	-0.75	0.43	-0.59	-0.39	-0.11	-0.78	-0.30	-0.02	-0.56	-0.73	0.57	-0.48	-0.01	-0.45	-0.26	-0.10	-0.13	-0.28	-0.17	

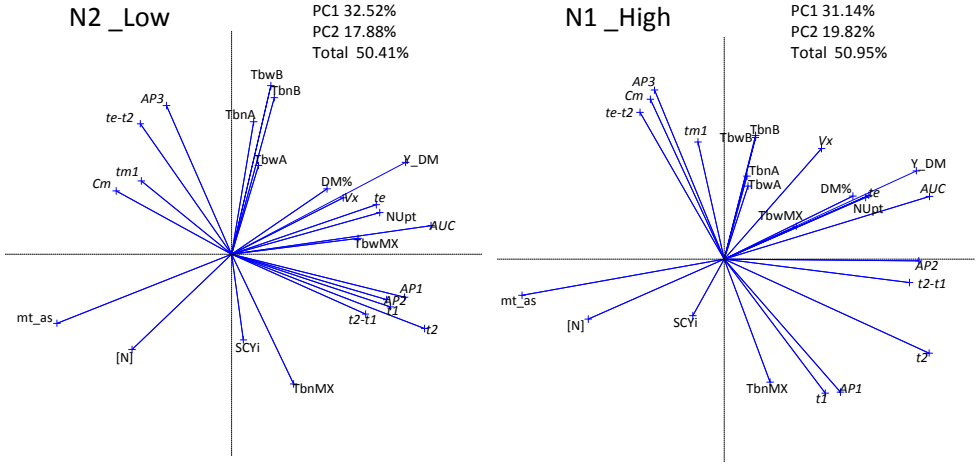

Figure 5 Biplot of trait relationship per N input level. A) Low N input, B) High N input. For trait acronyms, see main text.

At low N level the biplot with traits loading (vectors) showed six groups, two with only two traits and four with more than two traits (Figure 5). The first group included Y_DM, *AUC*, DM%, NUpt, *te*, *Vx* and TbwMX. The tuber [N] and mt_as had a strong negative relationship with all traits in the first group at both N levels. At high N this first group showed smaller angles between the traits excluding *Vx*. The length of the vectors suggests that Y_DM and *AUC* were the most discriminant traits at both N levels.

A second group of traits identified at low N level included *AP1*, *t1*, *t2*, *AP2* and *t2-t1*. At high N level this group was split into three; the *t2* stood alone and there were two groups, (*AP1* and *t1*) and (*AP2* and *t2-t1*), where each area under the curve remained highly correlated with the duration of its respective phase. The third group included TbwB, TbnB, TbnA and TbwA showing very small angles at both N levels. The parameters TbwB and TbnB (size for the maximum tuber weight or number respectively) were highly correlated (0.93 and 0.95) at high and low N levels, respectively. As expected, the highest number of tubers resulted in the highest weight at the same size, with no effect of N on this relation. The correlation between the dispersion parameter for tuber weight (TbwA) and number (TbnA) changed with N level (0.81 at high and 0.64 at low N). The fourth group of traits included *AP3*, *te-t2*, *Cm*, *tm1* was more compact at high N level. The TbnMX grouped with SCYi at low N whereas it aggregated with other traits (*t1* and *AP1*) at high N. The SCYi stood alone at high N.

In general, at high N the areas under the curve (traits depending on *Vx* and duration of each phase (in beta thermal time)) became more correlated or dependent on duration of the phase itself, due to the upper limit that *Vx* values can take. At high N there was more SC (closer to 100%) with more biomass production and with a prolongation of the duration of the total growing period especially by an increase of the second phase of CDv, in which *Vx* was maintained constant.

Table 4 Heat map for heritability per N level (Low and High) and maturity groups (E: early, M: intermediate and L: late) for the traits considered in the N level comparison.

N treatment	Low			High		
Trait	E	M	L	E	M	L
tm1	0.00	0.06	0.09	0.16	0.02	0.17
t1	0.60	0.16	0.24	0.13	0.09	0.30
Vx	0.43	0.29	0.46	0.60	0.25	0.01
t2	0.50	0.24	0.28	0.49	0.37	0.26
te	0.43	0.50	0.26	0.50	0.25	0.07
Cm	0.38	0.24	0.01	0.13	0.05	0.32
AP1	0.55	0.17	0.26	0.06	0.03	0.26
AP2	0.28	0.00	0.30	0.46	0.20	0.26
AP3	0.00	0.00	0.00	0.00	0.00	0.00
AUC	0.67	0.49	0.69	0.79	0.56	0.33
t2-t1	0.09	0.00	0.26	0.33	0.13	0.24
te-t2	0.00	0.00	0.00	0.00	0.00	0.00
DM%	0.84	0.75	0.84	0.92	0.73	0.83
Y DM	0.72	0.65	0.66	0.79	0.65	0.56
[N]	0.36	0.40	0.65	0.33	0.44	0.53
NUpt	0.49	0.23	0.52	0.44	0.27	0.38
SCYi	0.17	0.37	0.56	0.22	0.38	0.32
TbwMX	0.54	0.48	0.49	0.62	0.05	0.43
TbwB	0.70	0.78	0.43	0.71	0.43	0.47
TbwA	0.21	0.18	0.08	0.30	0.15	0.24
TbnMX	0.39	0.72	0.30	0.54	0.53	0.53
TbnB	0.66	0.83	0.60	0.87	0.57	0.62
TbnA	0.32	0.36	0.00	0.48	0.29	0.12
mt_as	0.73	0.66	0.71	0.74	0.63	0.46

Colour key

0 1

Heritability of the traits

Heritability was calculated (Table 4) based on the model combining the two years for each N (Eq. 3). Heritability was 0 or very low for *tm1*, *AP3*, and *te-t2* (for all maturity groups) meaning that observed variation was due to other factors than cultivar. On the whole, the early maturity group had 16 traits with heritability values higher than 0.4, with 12 traits (out of the 16) reporting the highest value at high N level. The intermediate and late groups had only 10 and 12 traits higher than 0.4 and just 2 and 4 traits had the highest value at high N. The heritability changed considerably for each maturity group. This means that the variation for a trait due to cultivar was specific for each maturity group, highlighting the importance of maturity. Moreover, the values also changed because of the N input but there was not a clear trend. For instance, *AUC* had values above 0.4 for all groups at low N whereas at high N the value from the late group dropped considerably (from 0.69 to 0.33). Meanwhile for early and intermediate cultivars the values increased with increasing N (from 0.65 and 0.47 at low N to 0.78 and 0.55 at high N). Additionally *AUC* was the only SC parameter with heritability higher than 0.4 for the three maturity groups.

Performance of cultivars under low nitrogen supply and their response to high nitrogen input

Figure 6 relates the performance of cultivars under low input to the variation in the efficiency of N use when changing the input from low to high. The scatter points were divided into four quadrants using the mean values of both variables as a crossing point for the axes. A dependency between maturity groups and the quadrant in which the cultivars were categorized was found using Pearson's Chi-squared test done by year and combining both data sets (Supplementary material: Annex 4).

Quadrant II shows the best cultivars: these had values above the average for both axes, i.e. higher Y_DM under low N condition and higher response to the change in N input than average. In this quadrant, there were proportionally more late cultivars followed by intermediate and early cultivars. In general, most early cultivars had a lower performance under poor N conditions whereas the response to extra N did not seem to depend on maturity. On the other hand cultivars with good yield under low N tended to have a good yield under high N. The average per maturity group showed the late cultivars with the best performance under both N conditions, intermediate cultivars were more scattered over the general trend and early cultivars tended to have low yield at both inputs. This distinction based on maturity type changed from year to year; it was clearer in 2009 than in 2010; especially intermediate cultivars were more spread in 2010 than in 2009 (Supplementary material: Annex 3).

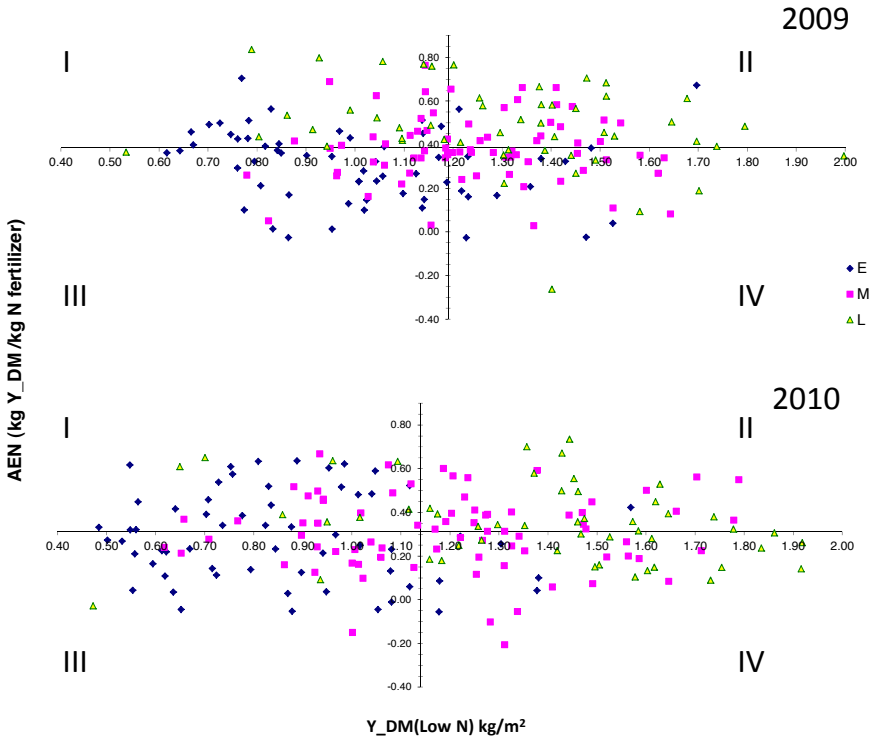

Figure 6 Response of agronomic efficiency of N fertiliser applied (AEN) from low to high N input (vertical axis) in relation to the performance under the low N input (as Y_DM; horizontal axis). I = quadrant (Q)I, high response, low performance under low input. QII = high response and performance. QIII = low response and performance. QIV = low response and high performance. E: early; M: intermediate; L: late cultivars.

Table 5 Percentage of cultivars per maturity group (Mt) consistently in the same quadrant in both years (2009-2010). The totals are from all quadrants per maturity group (right column) or from all maturity groups per quadrant (bottom row). E: early; M: intermediate; L: late.

%	Quadrant				
Mt	**I**	**II**	**III**	**IV**	**Total**
E	13.6	1.7	33.9	5.1	54.2
M	5.6	15.3	9.7	11.1	41.7
L	12.0	18.0	2.0	12.0	44.0
Total	**9.9**	**11.6**	**15.5**	**9.4**	**46.4**

Additionally, Table 5 shows cultivars which fell in the same quadrant in both years for each maturity group. The total percentage per quadrant was calculated for the entire set of cultivars. 46% of all cultivars were consistently in the same quadrant in both years, with the early cultivars showing the highest percentage (54.2%) followed by late and middle cultivars (44.0% and 41.7%,

respectively). In Quadrant II the highest percentage of consistent cultivars corresponded to the late group followed by the intermediate and early groups (18.0%, 15.3%, and 1.7%, respectively). On the other hand, in Quadrant III most consistent cultivars were early (33.9%). This result showed how ranking of cultivars within maturity groups evidently changed, probably due to year effect on the performance and response of cultivars to N but also due to experimental error. Additionally, although the early groups had the highest overall percentage of consistent cultivars in the same quadrant, almost none of them were in the interesting quadrant II. Therefore, performing the analysis per maturity group would be a better approach for selection.

Discussion

Weather conditions had a large influence on N availability and therefore on the response of the cultivars to N input. The two experimental seasons showed different patterns of rainfall. In 2009 season it promoted growth during the first phase of CDv, reaching higher maximum soil coverage *Vx*. In 2010 more rainfall was present later, which positively affected other variables associated with late CDv. These effects of weather conditions differed among maturity classes since the short cycle of early cultivars did not allow them profiting from improved conditions during later phases of CDv. For the build-up phase and the senescence phase, the year effect was larger than the N effect.

On the other hand, extra N enhanced SC, shortening *t1*. As reported by Oliveira (2000) and Biemond and Vos (1992) these effects result from increased rate of leaf appearance, because of more leaves per branch and more branches. The area under the curve for this phase (*AP1*) decreased due to its shorter duration. Subsequently more N resulted in longer maintenance of *Vx* (higher *t2-t1*). Biemond and Vos (1992) indicated that this effect was brought about by increasing the lifespan of leaves and by prolonging the initiation of new leaves. Moreover, under low N the potato plants try to maintain the photosynthetic productivity per unit of area adjusting the foliage development (Vos 2009). On the other hand, this strategy could mean that the plant efficiency would decrease as soon as optimum values for LAI are reached since the intrinsic productivity per unit of leaf area is kept rather constant. During senescence, more N resulted in a slightly shorter *te-t2*, probably associated with the delay of *t2*. Plants at high N had then to face conditions with lower temperatures and lower light intensities making the canopy to collapse rapidly. Additionally, as reported by Biemond and Vos (1992) under low N rates relocation of N from haulm to tubers occurred earlier; we can also speculate, based on observations in the field, that there was also N relocation from lower leaves to upper leaves in the canopy. Consequently, the early senescence at low N could be due to N starvation of lower leaves. As the maximum leaf area was much lower at low N than at high N, this was followed by an earlier decline in canopy cover.

Maturity groups showed a general and characteristic trend for CDv. Our study included many cultivars, each one well characterized. It allows having a more complete picture of the influence of maturity type on NUE related traits. The comparison of the CDv curve for each maturity group

within N treatment showed the parameters *Vx*, *t1*, *t2*, *te*, *t2-t1*, and *AUC* having large differences in both N levels. Additionally these maturity groups had the same relative trend in both N treatments. Moreover, N affected the relationship between the CDv traits and the maturity assessed in the field (mt_as). Some parameters (*AUC* and *te*) were consistently highly correlated with mt_as at both N inputs, whereas for other parameters (*AP2* and *t2-t1*) the correlation varied with N input. Therefore, useful methodologies as the one proposed by Khan et al. (2013), where physiological maturity criteria were used to define maturity in a more assertive way than the traditional assessment, should consider the major effect of some factors such as N.

In addition, there was an evident and consistent delay in maturity due to extra N input. This could result in late senescence and, if the season is not long enough, it could cause a loss at final harvest especially for late cultivars (Kleinkopf et al. 1981). The maturity assessment (mt_as) done in the field showed high heritability, expected for a trait with a strong genetic component. Yield increased from early to late cultivars and so did NUE. This agrees with the findings of Zebarth et al. (2004b), using 20 commercial cultivars. In addition, the maturity grouping (categorical classification) included variation between cultivars for most traits. A change was observed between the variance components and therefore in the heritabilities of most traits when comparing maturity groups. It suggests that selection should be directed within maturity groups. It is important to mention that although late cultivars tend to show higher yield and NUE, there are other reasons to not only have late cultivars such as market and farmers preferences, as well as costs. Finally, NUE has been reported to decrease when N input increases (Zebarth et al. 2004b). We observed the same effect of N on NUE. Low N input is the decisive point in evaluating the potential NUE in potato, even more so if the production system is limited to low input, imposed by laws and regulations (policies) or by management rules (organic production systems). Assuring a good performance at low N means a higher baseline for NUE; this conclusion is supported by findings of Errebhi et al. (1998b) evaluating 39 wild accessions and three varieties for biomass production, N uptake, and NUE. Moreover a good yield under low N could be interpreted as a high ability of a cultivar to use the limited N by probably showing less effect on the canopy traits that strongly respond to N, i.e. *AP2*, *t2-t1* and *Cm*. This could turn into a selection strategy to be adapted and implemented in the early stages of breeding schemes. Offsprings could be classified by CDv and their phenotypic maturity behaviour under a limited N environment that will also allow a high selection pressure for NUE. Parameters such as *Vx*, *t2*, and *t1* could be approximately assessed for these selections by comparing them with typical and well-known cultivars. In combination with other selection criteria like some indicating a good canopy cover, ideotypes could be designed and offsprings could be ranked within the maturity group. In general, breeding for NUE requires combining in a single cultivar a good yield at low N input with a good response to extra fertiliser (Quadrant II in Figure 6). Such cultivars will have a higher basic NUE at low N supply and they will show less decrease in NUE with an increase in N fertiliser supply. Cultivars selected in this way allow farmers either reducing input, and thus costs, or exploring the potential under higher input rates with confidence of a good economic return.

Finally, the present results must be considered within the context of the assumptions mentioned in this paper. This is a first approach to understand the diversity of crop development traits as moderated by contrasting N levels. More experimentation needs to be conducted to confirm our results.

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Supplementary material:

Annex 1 Cultivars used in this experiment; based on D'hoop et al. (2010)

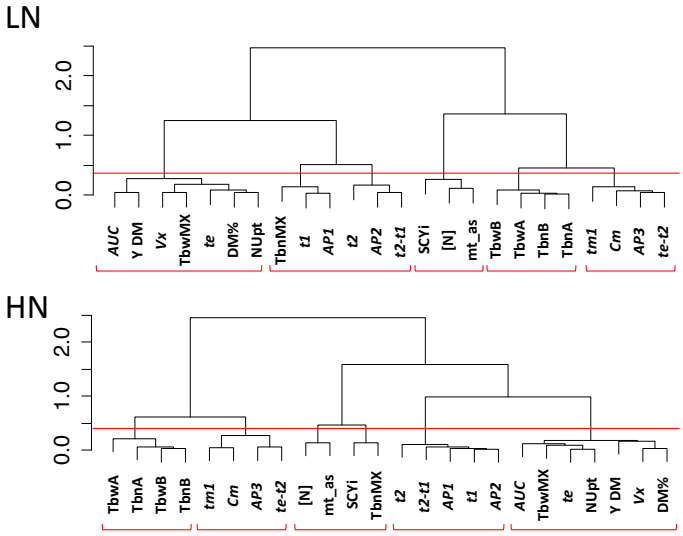
P8 code	Cultivar or breeder's clone	Year of first registration	Country of origin	Parentage	Market niche
P80002	Abundance (Sutton's)	1886	UK	Magnum Bonum x Fox's seedling	ancient cultivar
P80003	Ackersegen	1929	GER	HINDENBURG x ALLERFRUHESTE GELBE	ancient cultivar
P80004	Adirondack	1881	USA	Peachblow x Peachblow	ancient cultivar
P80005	Adora	1990	NETH	Primura x Alcmaria	fresh consumption
P80006	Adretta	1975	GER	LU. 59.884/3 x Axilia	fresh consumption
P80007	Agata	1990	NETH	BM 52-72 x Sirco	fresh consumption
P80008	Agria	1985	GER	Quarta x Semlo	processing industry
P80009	Aljba	1992	NETH	AMINCA x VE 70-9	fresh consumption
P80010	Albion	1895	NETH	REICHSKANZLER x SIMSON	ancient cultivar
P80012	Allure	1990	NETH	Astarte x AM 66-42	starch industry
P80014	Almera	1999	NETH	BM 77-2102 x AR 80-31-20	fresh consumption
P80015	Alpha	1925	NETH	Paul Kruger x Preferent	ancient cultivar
P80016	Am 66-42	/	NETH	VTN 62-33-3 x MPI 19268	progenitor clone
P80018	Am 78-3704	/	NETH	AM 72-3477 x AM 70-2166	progenitor clone
P80019	Amorosa	2000	NETH	Arinda x Impala	fresh consumption
P80020	Ampera	1998	GER	AGUTI x PONTO	fresh consumption
P80021	Amyla	1999	FRA	PROMESSE x ELEMENT	starch industry
P80022	Anosta	1975	NETH	Ostara x Provita	processing industry
P80024	Arcade	1999	NETH	AGRIA x VK 69-491	fresh consumption
P80025	Arinda	1993	NETH	Vulkano x AR 74-78-1	fresh consumption
P80026	Arnova	1999	NETH	Obelix x AR 76-168-1	fresh consumption
P80028	Arran Chief	1911	UK	Paterson's Victoria x Sutton's Flourball	ancient cultivar
P80030	Arran Victory	1918	UK	ABUNDANCE x ABUNDANCE	ancient cultivar
P80031	Arrow	2004	NETH	Solara x Fresco	fresh consumption
P80032	Astarte	1976	NETH	RR 62-5-43 x VTN 62-69-5	starch industry
P80033	Asterix	1991	NETH	Cardinal x VE 70-9	processing industry
P80034	Atlantic	1976	USA	Wauseon x Lenape	processing industry
P80035	Aurora	1972	NETH	Profijt x AM 54-10	starch industry
P80036	Ausonia	1981	NETH	Wilja x KONST 63-665	fresh consumption
P80037	Avenance	/	NETH	Mercury x Florijn	starch industry
P80038	Ballydoon	1931	UK	Herald x British Queen	ancient cultivar
P80039	Bartina	1988	NETH	Saturna x ZPC 62-75	fresh consumption
P80041	Bellini	2001	NETH	Mondial x Felsina	fresh consumption
P80042	Berber	1984	NETH	Alcmaria x Ropta P 365	fresh consumption
P80043	Bildtstar	1984	NETH	Winda x Saturna	fresh consumption
P80044	Bintje	1910	NETH	Munstersen x Jaune d'or (= Fransen)	fresh consumption
P80045	Biogold	2004	NETH	Novita x HZ 87 P 200	processing industry
P80046	British Queen	1894	UK	PATERSON'S VICTORIA x BLUE DON	ancient cultivar
P80047	Caesar	1990	NETH	Monalisa x Ropta B 1178	processing industry
P80049	Charlotte	1981	FRA	Hansa x Danae	fresh consumption
P80050	Cherie	1997	FRA	Roseval x AR 76-199-3	fresh consumption
P80051	Cilena	1981	GER	Gelda x Hria	fresh consumption
P80052	Civa	1960	NETH	(BINTJE x (SASKIA x FRUHMOLLE)) x CIV 49-901	fresh consumption
P80053	Clivia	1962	GER	Seedling 1 x Seedling 17	progenitor clone
P80055	Craigs Bounty	1946	UK	unknown	ancient cultivar
P80056	Craigs Defiance	1938	UK	Epicure x Pepo	ancient cultivar
P80057	Daisy	1998	FRA	GIPSY x CUIPA	fresh consumption
P80058	Deodara	1913	GER	Deutsches Reich x Jubel	ancient cultivar
P80059	Desiree	1962	NETH	Urgenta x Depesche	fresh consumption
P80060	Di Vernon	1922	UK	unknown	ancient cultivar
P80061	Diamant	1982	NETH	Cardinal mutant	fresh consumption
P80062	Ditta	1989	AUT	BINTJE x QUARTA	fresh consumption
P80063	Donald	1996	NETH	Amera x W 72-19-443	fresh consumption
P80064	Doon Star	1926	UK	Templar's seedling x Majestic	ancient cultivar
P80065	Dorado	1995	NETH	Spunta x VK 69-491	processing industry
P80066	Dore	1947	NETH	DUKE OF YORK x BIERMAA 7	ancient cultivar
P80067	Dr McIntosh	1944	UK	(phu x HERALD) x HERALD	ancient cultivar
P80068	Draga (1970)	1970	NETH	SVP 50-2017 x MPI 19268	fresh consumption
P80069	Eersteling	1891	UK	Early Primrose x King Kidney	ancient cultivar
P80070	Early Rose	1867	USA	Garnet Chili seedling	ancestor
P80072	Eden (2000)	2000	FRA	EOLE x PENTLAND DELL	fresh consumption
P80073	Ehud	1965	NETH	Panther x Karna 149	starch industry
P80074	Eigenheimer	1893	NETH	Blaue Riesen x Fransen (Jaune d'or)	ancestor
P80075	Elisabeth	2002	NETH	VE 82-96 x CILENA	fresh consumption
P80076	Eos	2000	NETH	MONDIAL x W 72-22-496	fresh consumption
P80077	Epicure	1897	UK	Magnum Bonum x Early Regent	ancient cultivar
P80078	Escort	1982	NETH	RENTAL x CEB 64-197-16	fresh consumption
P80079	Estima	1973	NETH	NOPOL x G 3014	fresh consumption
P80080	Exquisa	1992	GER	Sigma x Ilse	fresh consumption
P80081	Fabula	1997	NETH	Monalisa x Hudson	fresh consumption
P80082	Felsina	1992	NETH	Morene x Gloria	processing industry
P80083	Festien	2000	NETH	KARTEL x KA 80-1920	starch industry
P80084	Fianna	1987	NETH	KONST 62-660 x AM 64-2	processing industry
P80085	Fichtegold	1945	GER	[(Zwickauer Fruhe x Jubel) x Klara] x Mittelfruhe	ancient cultivar
P80087	Flourball (Sutton's)	1870	UK	unknown	ancestor
P80088	Fontane	1999	NETH	Agria x AR 76-34-3	processing industry
P80089	Fresco	1985	NETH	CEB 60-15-28 x PROVITA	processing industry
P80090	Friesla nder	1990	NETH	Gloria x 74 A 3	fresh consumption
P80092	Furore	1930	NETH	Rode Star x Alpha	ancient cultivar
P80093	Gladstone	1932	UK	ARRAN CHIEF x (MAJESTIC x GREAT SCOT)	ancient cultivar
P80094	Gloria	1972	GER	Ames x Feldes lohn	fresh consumption
P80095	Golden Wonder	1906	UK	Seedling of Early Ros e	ancient cultivar
P80096	Goya (2000)	2000	NETH	AM 78-4102 x KARDAL	starch industry
P80097	Great Scot	1909	UK	Imperator x Champion	ancient cultivar
P80098	Hansa	1957	GER	OBERARNBACHER FRUHE x FLAVA	fresh consumption
P80099	Herald	1928	UK	MAJESTIC x ABUNDANCE	ancient cultivar
P80100	Hermes	1973	AUT	DDR 5158 x SW 163/55	processing industry
P80102	Home Guard	1943	UK	DOON PEARL x CUMNOCK	ancient cultivar
P80104	Impala	1989	NETH	52/72/2206 (BM 52-72) x BIRANCO	fresh consumption
P80106	Innovator	1999	NETH	Shepody x RZ 84-2580	processing industry
P80107	Inova	1999	NETH	NICOLA x IMPALA	fresh consumption
P80110	Jaerla	1969	NETH	Sirtema x MPI 19268	fresh consumption
P80113	Karnico	1987	NETH	Astarte x AM 66-42	starch industry
P80114	Kartel	1994	NETH	KA 77-0133 x AM 78-3736	starch industry
P80115	Katahdin	1932	USA	USDA 40568 x USDA 24642	ancient cultivar
P80116	Kennebec	1948	USA	USDA B 127 x USDA 96-56	ancient cultivar
P80117	Kepplestone Kidney	1900	UK	unknown	ancient cultivar
P80118	Kerpondy	1949	FRA	unknown	ancient cultivar
P80119	Ker's Pink	1907	UK	Fortyfold x (Abundance or Smiths early)	ancient cultivar
P80121	Kondor	1984	NETH	KONST 61-333 x WIJA	fresh consumption

Annex 1 ...continued

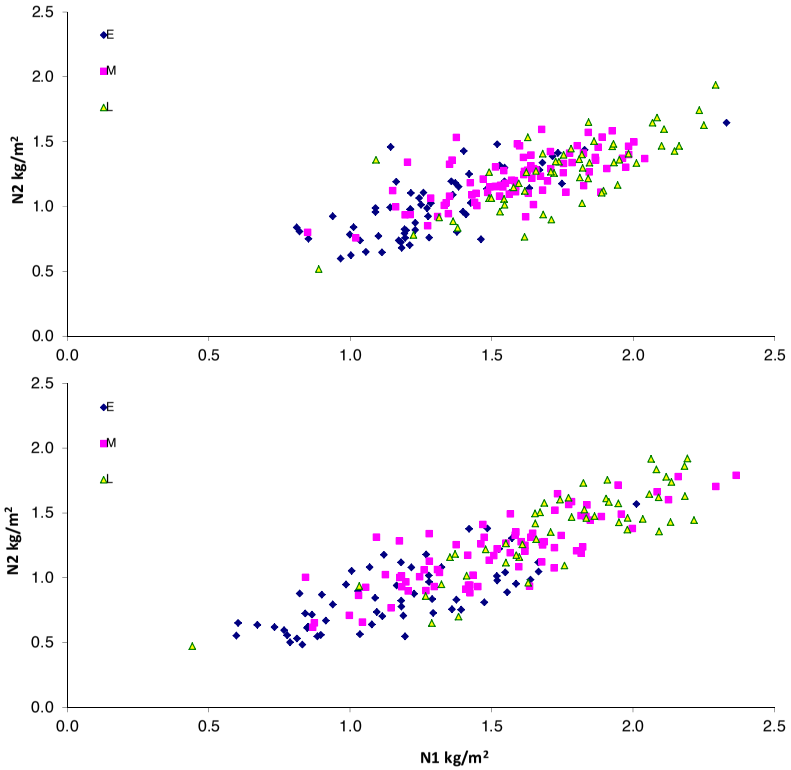
P8 code	Cultivar or breeder's clone	Year of first registration	Country of origin	Parentage	Market niche
P80122	Kuras	1996	NETH	BRDA (=PG 2851) x VK 69-491	starch industry
P80123	Kuroda	1998	NETH	AR 76-1399-3 x KONST 80-1407	fresh consumption
P80124	Lady Christl	1996	NETH	WS 73-3-391 x Mansour	fresh consumption
P80125	Lady Claire	1996	NETH	Agria x KW 78-34-470	processing industry
P80126	Lady Olympia	1996	NETH	AGRIA x KW 78-34-470	processing industry
P80127	Lady Rosetta	1988	NETH	CARDINAL x VTN 62-33-3	processing industry
P80128	Laura	1998	GER	Rosella x L 6140/2	fresh consumption
P80129	Lenape	1967	USA	USDA B 3672-3 x Delta Gold	fresh consumption
P80130	Leyla	1988	GER	7338/812 x CUPA	fresh consumption
P80132	Liseta	1988	NETH	Spunta x VE 66-295	fresh consumption
P80134	Majestic	1911	UK	Unknown breeding line x British Queen	ancestor
P80135	Marfona	1977	NETH	PRIMURA x KONST 51-123	fresh consumption
P80137	Maritiema	1991	NETH	AM 71-125 x VE 70-9	processing industry
P80139	Markies	1997	NETH	FIANNA x AGRIA	fresh consumption
P80140	May Queen	1890	UK	unknown	ancient cultivar
P80141	Mercator	1999	NETH	KARTEL x KA 86-0008	starch industry
P80142	Monalisa	1982	NETH	Bierma A 1-287 x Colmo	fresh consumption
P80143	Mondial	1987	NETH	SPUNTA x VE 66-295	fresh consumption
P80144	Morene	1983	NETH	RENOVA x AM 66-42	processing industry
P80145	Mpi 19268	/	GER	Sdem x Deodara	progenitor clone
P80146	Myatt'S Ashleaf	1804	UK	unknown	ancient cultivar
P80147	Nicola	1973	GER	CUVIA x 6430/101	fresh consumption
P80149	Niska	1990	USA	WISCHIP x LENAPE	processing industry
P80150	Noisette	1993	FRA	AMINCA x PIROSCHKA	fresh consumption
P80151	Nomade	1995	NETH	Elles x AM 78-3704	starch industry
P80152	Noordeling	1928	NETH	BRAVO x AM	ancient cultivar
P80153	Obelix	1988	NETH	Ostara x Renska	fresh consumption
P80155	Pallas	2003	NETH	KW 84-11-280 x VDW 85-72	processing industry
P80158	Peerless	1862	USA	Garnet Chili seedling	ancient cultivar
P80159	Pentland Dell	1961	UK	Roslin Chania x Roslin Sasamua	processing industry
P80160	Pepo (1919)	1919	GER	Deutsches Reich x Jubel	ancient cultivar
P80161	Picasso	1994	NETH	Cara x Ausonia	fresh consumption
P80162	Première	1979	NETH	Civa x Provita	processing industry
P80163	Prevalent	1966	NETH	AMBASSADEUR x LOMAN M 54-106-1	fresh consumption
P80164	Primura	1961	NETH	Sirtema x Majestic	fresh consumption
P80165	Profijt	1949	NETH	Prummel K 264 x Matador	ancient cultivar
P80166	Ramos	2000	NETH	AGRIA x VK 69-491	fresh consumption
P80167	Record	1932	NETH	Trenctria x Energie	ancient cultivar
P80169	Red Scarlett	1999	NETH	ZPC 80-239 x MPAIA	fresh consumption
P80170	Redstar	1997	NETH	Bildstar x VDW 76-30	fresh consumption
P80172	Remarka	1991	NETH	Eidzina x AM 66-42	processing industry
P80173	Romano	1981	NETH	DRAGA x DESIREE	fresh consumption
P80175	Russet Burbank	1908	USA	Mutant of Burbank	processing industry
P80176	Samba	1989	FRA	ROSEVAL x BARAKA	fresh consumption
P80177	Santana	1994	NETH	Spunta x VK 69-491	processing industry
P80178	Sante	1983	NETH	Y 66-13-636 x AM 66-42	fresh consumption
P80179	Saskia	1946	NETH	Rode Eersteling x Herald	fresh consumption
P80180	Saturna	1964	NETH	Maritta x (Record x CPC 1673-1adg)	processing industry
P80181	Seresta	1994	NETH	AM 78-3704 x Sonata	starch industry
P80182	Shamrock	1900	IRL	unknown	ancient cultivar
P80183	Shepody	1980	CAN	Bake King x F58050	processing industry
P80184	Sirtema	1947	NETH	DORSTH 123A x FRUHMOLLE	fresh consumption
P80185	Spunta	1968	NETH	Bea x USDA 96-56	fresh consumption
P80186	Sunrise	1984	USA	Wauseon x USDA B 6563-2	processing industry
P80187	Tahi	1960	NZL	SEBAGO x HARFORD	fresh consumption
P80188	Tasso	1963	GER	seedling x Biene	processing industry
P80189	The Ainess	1934	UK	ABUNDANCE x MAJESTIC	ancient cultivar
P80190	Timate	1984	NETH	Elvira x AM 66-42	fresh consumption
P80191	Triwaldʼs Perfection	1914	UK	unknown	ancient cultivar
P80192	Toyoshiro	1976	JPN	HOKKAI 19 x ENIWA	fresh consumption
P80193	Triplo	2000	NETH	AGRIA x FRESCO	processing industry
P80194	Triumf	1921	NETH	Eigenheimer x Cimbals Neue Imperator	ancient cultivar
P80196	Ulster Glade	1961	UK	ULSTER EMBLEM x adg-hybrid	fresh consumption
P80197	Ulster Knight	1954	UK	CLARKE 736 x CRAIGS DEFIANCE	fresh consumption
P80198	Ulster Sceptre	1962	UK	Pentland Ace x Ulster Prince	fresh consumption
P80199	Ultimus	1935	NETH	Rode star x Pepo	ancient cultivar
P80200	Umatilla Russet	1998	USA	Butte x A 77268-4	fresh consumption
P80201	Up To Date	1894	UK	Patersons Victoria x Blue Don	ancient cultivar
P80202	Urgenta	1951	NETH	FURORE x KATAHDIN	fresh consumption
P80203	Usda 96-56	/	USA	USDA 3895-13 x EARLIANE	progenitor clone
P80204	Ve 66-295	/	NETH	Amelio x HVT 60-8-3	progenitor clone
P80205	Ve 70-9	/	NETH	Atlantis x VTN 62-33-3	progenitor clone
P80206	Ve 71-105	/	NETH	AM 67-136 x AM 67-59	progenitor clone
P80207	Ve 74-45	/	NETH	Sinaeda x AM 66-42	progenitor clone
P80208	Victoria	1997	NETH	AGRIA x ROPTA I 861	processing industry
P80209	Virgo	2002	NETH	NICOLA x AM 78-3704	fresh consumption
P80210	Vivaldi	1998	NETH	TS 77-148 x MONALISA	fresh consumption
P80211	Vk 69-491	/	NETH	VK 64-56 x VTN 62-33-3	progenitor clone
P80212	Voran	1931	GER	Kaiserkrone x Spatgold of Herbstgelbe	ancient cultivar
P80213	Voyager	2003	NETH	RZ 85-238 x OBELIX	processing industry
P80214	Vtn 62-33-3	/	NETH	(V 24/20 x ULSTER KNIGHT)1 x PROFIJT)15 x (VRN I-2	progenitor clone
P80215	W 72-22-496	/	NETH	REDIAD x Y 66-13-636	progenitor clone
P80216	Wauseon	1967	USA	USDA B 4159-8 x KATAHDIN	fresh consumption
P80217	Wilja	1967	NETH	CLIMAX x KONST 51-123	fresh consumption
P80218	Winston	1992	UK	KISMET x DXMP 70	fresh consumption
P80219	Wisent	2005	NETH	Prudentia x Karakter	starch industry
P80220	Y 66-13-636	/	NETH	Y 62-2-221 x AMARYL	progenitor clone
P80221	Yam	1787	UK	unknown	ancient cultivar
P80222	Yukon Gold	1980	CAN	NORGOLD MY x USW 5279-4	processing industry

Annex 2 Correlation between PCA and traits at each N_lv with cluster dendrograms of the trait using the principal components PC from 1 to 5.

Traits	Low N_lv					High N_lv				
	PC1	PC2	PC3	PC4	PC5	PC1	PC2	PC3	PC4	PC5
tm1	-0.42	0.33	0.15	0.15	0.31	-0.09	0.49	-0.12	0.24	0.34
t1	0.73	-0.24	-0.18	-0.40	-0.42	0.40	-0.51	0.60	0.26	-0.30
Vx	0.51	0.26	0.32	0.28	-0.17	0.45	0.37	-0.32	-0.01	-0.22
t2	0.89	-0.33	-0.27	0.00	0.11	0.85	-0.44	0.17	-0.05	0.21
te	0.66	0.23	0.04	-0.53	0.37	0.64	0.32	0.08	0.59	0.05
Cm	-0.54	0.29	0.31	0.52	0.26	-0.28	0.59	-0.62	-0.19	0.17
AP1	0.79	-0.19	-0.09	-0.29	-0.42	0.47	-0.52	0.54	0.20	-0.36
AP2	0.71	-0.20	-0.15	0.42	0.50	0.82	-0.12	-0.26	-0.22	0.42
AP3	-0.30	0.67	0.43	-0.41	0.14	-0.21	0.74	-0.15	0.55	-0.19
AUC	0.91	0.13	0.09	-0.08	0.24	0.90	0.19	-0.09	0.28	0.08
t2-t1	0.61	-0.27	-0.23	0.38	0.56	0.78	-0.19	-0.21	-0.24	0.47
te-t2	-0.42	0.59	0.35	-0.48	0.20	-0.30	0.71	-0.10	0.56	-0.16
DM%	0.44	0.30	0.29	-0.29	0.05	0.56	0.14	-0.30	0.31	-0.06
Y DM	0.80	0.42	0.31	0.21	-0.04	0.84	0.26	-0.30	-0.11	-0.27
[N]	-0.45	-0.43	-0.37	0.10	-0.15	-0.59	-0.16	0.16	-0.31	0.05
NUpt	0.68	0.19	0.12	0.38	-0.21	0.63	0.19	-0.25	-0.39	-0.33
SCYi	0.05	-0.40	-0.32	-0.47	0.41	-0.16	-0.20	0.37	0.54	0.54
TbwMX	0.58	0.07	0.54	0.38	-0.19	0.37	0.32	0.09	-0.36	-0.29
TbwB	0.18	0.78	-0.50	0.17	-0.13	0.21	0.75	0.54	-0.27	0.04
TbwA	0.12	0.40	-0.60	-0.04	0.17	0.14	0.59	0.52	-0.08	0.09
TbnMX	0.31	-0.62	0.57	-0.02	0.07	0.16	-0.58	-0.39	0.12	-0.16
TbnB	0.19	0.73	-0.36	0.20	-0.20	0.18	0.64	0.43	-0.36	0.01
TbnA	0.11	0.66	-0.52	0.01	0.01	0.16	0.63	0.51	-0.23	0.15
mt_as	-0.80	-0.31	-0.14	0.27	-0.06	-0.88	-0.09	-0.07	-0.22	-0.06



Annex 3 Potato yield, under two N levels; N1) 180 and N2) 75 kg N/ha). Maturity groups based on breeders' information are: E) early genotypes, M) intermediate and L) late. Additionally summary tables of regression lines per maturity group and per year.



2009

Maturity	Correlation	Slope	Intercept	r^2
Early	0.742	0.689	0.106	0.551
Medium	0.678	0.526	0.385	0.460
Late	0.762	0.734	-0.023	0.581
Overall	0.778	0.636	0.183	0.606

2010

Maturity	Correlation	Slope	Intercept	r^2
Early	0.725	0.576	0.190	0.526
Medium	0.829	0.687	0.155	0.687
Late	0.842	0.805	-0.026	0.709
Overall	0.870	0.759	0.026	0.758

Annex 4 Contingency tables and Chi-square tests for independence between quadrants and maturity groups in Figure 6. Tests were done using three data sets: 2009, 2010 and the combination of both years.

> #2009 subset**********

Cell Contents

N

Chi-square contribution

N / Row Total

N / Col Total

N / Table Total

Total Observations in Table: 184

2009 Quadrant					
Quadrant	I	II	III	IV	Row Total
E	16	2	30	12	60
	0.19	9.988	13.152	1.136	
	0.267	0.033	0.5	0.2	0.326
	0.364	0.048	0.625	0.24	
	0.087	0.011	0.163	0.065	
L	15	21	2	12	50
	0.775	8.053	9.35	0.185	
	0.3	0.42	0.04	0.24	0.272
	0.341	0.5	0.042	0.24	
	0.082	0.114	0.011	0.065	
M	13	19	16	26	74
	1.246	0.263	0.566	1.726	
	0.176	0.257	0.216	0.351	0.402
	0.295	0.452	0.333	0.52	
	0.071	0.103	0.087	0.141	
Column Total	44	42	48	50	184
	0.239	0.228	0.261	0.272	

Pearson's Chi-squared test

data: 2009

X-squared = 46.6306, df = 6, p-value = 2.217e-08

> #2010 subset**********

Cell Contents

N

Chi-square contribution

N / Row Total

N / Col Total

N / Table Total

Total Observations in Table: 185

2010 Quadrant					
Quadrant	I	II	III	IV	Row Total
E	22	1	31	6	60
	3.758	11.373	13.478	6.157	
	0.367	0.017	0.517	0.1	0.324
	0.489	0.024	0.62	0.122	
	0.119	0.005	0.168	0.032	
L	8	19	2	21	50
	1.424	5.659	9.81	4.543	
	0.16	0.38	0.04	0.42	0.27
	0.178	0.463	0.04	0.429	
	0.043	0.103	0.011	0.114	
M	15	21	17	22	75
	0.577	1.153	0.528	0.229	
	0.2	0.28	0.227	0.293	0.405
	0.333	0.512	0.34	0.449	
	0.081	0.114	0.092	0.119	
Column Total	45	41	50	49	185
	0.243	0.222	0.27	0.265	

Pearson's Chi-squared test

data: 2010

X-squared = 58.6884, df = 6, p-value = 8.309e-11

> #both years**********

Cell Contents

N

Chi-square contribution

N / Row Total

N / Col Total

N / Table Total

Total Observations in Table: 369

2009-2010 Quadrant					
Quadrant	I	II	III	IV	Row Total
E	38	3	61	18	120
	2.834	21.325	26.626	6.259	
	0.317	0.025	0.508	0.15	0.325
	0.427	0.036	0.622	0.182	
	0.103	0.008	0.165	0.049	
L	23	40	4	33	100
	0.052	13.626	19.161	1.419	
	0.23	0.4	0.04	0.33	0.271
	0.258	0.482	0.041	0.333	
	0.062	0.108	0.011	0.089	
M	28	40	33	48	149
	1.753	1.255	1.091	1.611	
	0.188	0.268	0.221	0.322	0.404
	0.315	0.482	0.337	0.485	
	0.076	0.108	0.089	0.13	
Column Total	89	83	98	99	369
	0.241	0.225	0.266	0.268	

Pearson's Chi-squared test

data: 09-10

X-squared = 97.0119, df = 6, p-value < 2.2e-16

Chapter 4

Genetic basis of physiological and morphological characteristics for crop development in a diploid potato population and its response to contrasting nitrogen inputs in a multi-trait analysis

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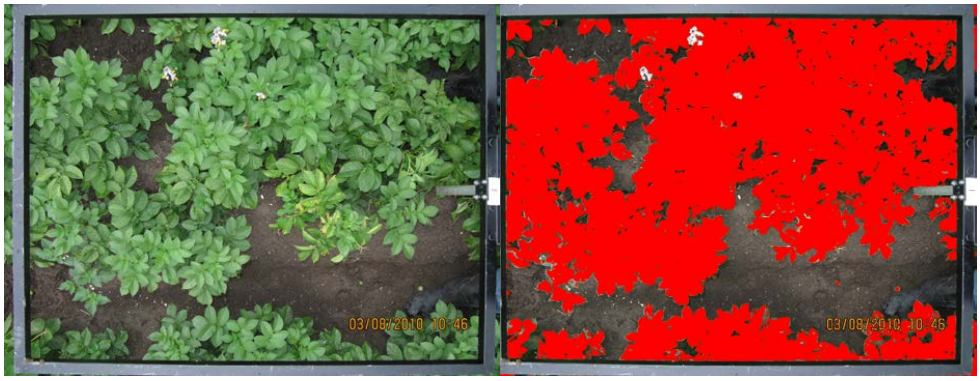

To be submitted

Abstract

Canopy development (CDv) is a complex trait affecting the performance of potato plants. It requires assessments over time to capture the effect of important factors such as nitrogen (N) throughout the growing period. We used an ecophysiological model to dissect the canopy development into biologically meaningful parameters to find genetic factors (quantitative trait loci, QTL) related to canopy development and its dependence on N. We used 92 genotypes of the SH × RH diploid biparental population that is an F1 from a cross between the clone SH83-92-488 as female parent, "SH", and the RH89-039-16 as a male parent, "RH". The genotypes were phenotyped weekly for the percentage of soil cover by green leaves (%SC), agronomic traits and tuber quality traits when grown under 75 and 180 N kg/ha (N soil + fertiliser). The %SC was used to fit a model describing CDv as a function of thermal time and the estimated model parameters were used for QTL analysis. The maternal and parental genetic maps used in the analysis were referred to as ma_ or pa_, followed by the linkage group in Roman numbers. Nitrogen affected CDv traits by influencing the duration of the build-up phase (*t1*), of the phase of maximum soil cover (*t2-t1*), the time to reach onset of senescence (*t2*), the total duration of the growth period (*te*) and the maximum soil coverage. The largest influence of nitrogen was found for the duration of maximum %SC, which greatly impacted the total area under the soil coverage curve (*AUC*) and yield. N also affected the relationships between traits. Maturity groups showed different responses to N. QTL and possible pleiotropic regions identified proved N dependent. In both high N and low N, some QTL were detected on linkage groups ma_III, ma_V, ma_VI and pa_V; these were N independent. QTL that were detected at only one of the nitrogen levels were N dependent: QTL detected only at low N were found in the linkage groups ma_I, ma_III, ma_V, ma_VI, ma_VIII, ma_X, ma_XI, pa_I A, pa_I B, pa_V, pa_VIII and pa_XI, whereas QTL detected only at high N were found in ma_III, ma_V, ma_VI ma_IX and pa_V. Some hotspot regions included QTL only at low nitrogen in linkage groups ma_1, maVIII, ma_X, ma_XI, pa_I A, pa_ I B, pa_VIII pa_XI. The maturity-related QTL on chromosome V was detected for most traits but not for quality traits. A QTL on ma_III was found to be related to the quality traits. We conclude that N affects the expression of genetic factors involved in the regulation of canopy development, yield and quality traits.

Keywords: Diploid potato; Canopy development; Maturity type; multi-trait; QTL; N effects.

Introduction

Crop developmental processes are very important for yielding ability. Such processes are dynamic and involve quantitative and complex traits (Yin and Struik 2008) implying the need for multiple evaluations over the growing period. These traits include not only a snapshot of the processes at a single point in time, but also the development of these processes itself as brought about by the interplay between environmental and biological factors. The development can be described and interpreted by commonly used models like linear models, exponential growth models and S-shaped curves, some with biological meaningful parameters (Schnute 1981). Furthermore, crop physiology models have an extra advantage, adding crop knowledge and giving extra meaning to the parameters estimated. This feature helps in the identification of crucial events, developmental shifts or specific phases in the process. Therefore, those parameters can be used as new traits to assess responses of the crop to particular factors (Yin et al. 2004).

Yin (2003) proposed the beta growth function to describe growth. This is a flexible and suitable function with parameters representing growth traits of interest to characterise growth processes of genotypes and their responses to external factors such as environment and cropping practices. Additionally, ecophysiology-based crop growth models relate elementary crop processes to environmental variables (Yin et al. 2005), allowing physical and environmental factors to be linked to crop physiological knowledge in the understanding of the observed processes. Khan (2012) used the beta function approach in potato, estimating cardinal temperatures for potato canopy growth to quantitatively assess canopy cover as a function of thermal time. The model parameters of canopy cover were found to be related to the ability of genotypes to intercept photosynthetically active radiation and to produce tuber yield, showing high heritabilities. The parameters from the canopy model were used to characterise the performance of potato genotypes under contrasting nitrogen (N) conditions (Ospina et al. 2014), helping to understand the effect of N levels on different stages of the canopy development. The parameters allowed the study of the effects of major factors, such as nitrogen supply and maturity type, their interaction and their relation to yield. Khan et al. (2013) proposed a methodology to categorise genotypes into maturity groups based on these parameters. Furthermore, these parameters also captured the genotypic variation of N-dependent changes in canopy development, showing the potential to detect genetic factors (quantitative trait loci; QTL) that act over time in the development of the canopy cover and probably in the underlying physiological process.

For the combination of growth models and QTL mapping analysis two approaches have been used. The first approach is a one-step procedure combining the characterisation of the development of the trait with the QTL mapping. An example is presented by Ma et al. (2002) who described a statistical infrastructure for mapping quantitative trait loci underlying the development process of constituting a trait where logistic growth curves and QTL mapping are combined in a mixed model approach. Another example of a one-step procedure is proposed by

Malosetti et al. (2006) using nonlinear mixed models. This procedure offers advantages but requires advanced statistical modelling and high computational capacity for inference (Malosetti et al. 2006). The second approach is a simpler two-step procedure. In the first step, observations at multiple time points are used to estimate genotype-specific parameters; in this way the complex trait is dissected into component traits by the use of an ecophysiological model, crop model, growth curve model or mathematical model. Then, in the second step, instead of searching for QTL for the complex trait itself, a conventional QTL analysis is applied to the curve parameter estimates of the first step interpreting these estimates as standard phenotypic traits (Malosetti et al. 2006; Yin et al. 2005). This two-step procedure has been successfully used in several crops: in maize, finding some QTL for leaf expansion as a function of temperature and water deficit (Reymond et al. 2003), in barley, for a model that predicts pre-flowering duration as affected by temperature and photoperiod (Yin et al. 2005) and in rice for the study of flowering responses to photoperiod and temperature (Nakagawa et al. 2005), etc. In potato, the two-step procedure has been used to study the dynamics of senescence and the adaptation under different day lengths (Hurtado 2012; Hurtado et al. 2015) but also to identify QTL related to canopy cover parameters (Khan 2012). With multiple traits describing development processes an integrated QTL analysis combining data from those traits is considered to offer a better understanding of the forces driving plant development (Hurtado et al. 2015; Jiang and Zeng 1995). Moreover, for measurements obtained simultaneously for several traits, it is more appropriate to perform statistical analyses multivariate than univariate. This is even stronger when biological processes are interdependent (Alimi et al. 2013; Jiang and Zeng 1995). Such an approach increases the statistical power by taking into account the correlated structure of multiple traits (Jiang and Zeng 1995; Liu et al. 2007; Malosetti et al. 2008). The multi-trait analysis approach was used in potato with parameters related to senescence, flowering and plant height helping in the understanding of the genetic control by showing pleiotropic regions for these processes (Hurtado et al. 2015).

In this paper, we study the genetic basis of nitrogen effects on the canopy development and other agronomic traits in a diploid population under two levels of nitrogen. We use the multi-trait QTL analysis combining several traits that describe the above-ground development. Canopy development under two contrasting N levels was compared under field conditions. This complex trait was dissected into physiologically meaningful variables using an ecophysiological model for changes in canopy cover, in which soil coverage time series data were described as a function of air temperature (beta thermal time). The estimated variables were used as phenotypic traits to analyse N effects on canopy development and its relation with other agronomic traits and some quality traits. In order to assess how N levels affect the genetic factors related to canopy development and other agronomic traits, a QTL analysis was performed for each N level separately.

Materials and methods

Location

The experiment was carried out at the Agrico research and breeding station at Bant, Noordoostpolder, the Netherlands, in 2011 from April the 20th to September the 22th. The soil type was typical of the Polder created in 1949 and was classified as Young-light-clay (35% clay), composed of a 60-cm layer of clay positioned on a thick layer of sand. The preceding crop was winter wheat, for which straw was removed from the field. A green manure crop (*Raphanus sativus*) was subsequently planted in September and mown in November just before winter ploughing (depth of 25 cm).

Plant material

A total of 94 genotypes from a diploid potato population were included in this experiment: 92 siblings and the two parents. The parents are diploid and heterozygous potato clones: SH83-92-488 was used as the female parent and RH89-039-16 was used as the male parent. They are referred to here as SH and RH, respectively. The population is an F1 diploid (2n=2x=24 chromosomes) progeny called the SH × RH population. The tetraploid cultivar Fontane (intermediate maturity type) was included for reference.

To reduce the phenotypic variation due to differences in quality of seed tubers, tubers of size class 25-30 mm from each genotype were obtained from a single propagation at Agrico following standard procedures for potato seed tuber production ensuring excellent phytosanitary quality.

Field trial and experimental design

The experimental design was an unbalanced split-plot design with two blocks. Within a block (replication), two nitrogen treatments were the main plots and four maturity groups (early, early-medium, medium-late, late) of the genotypes were the sub-plots. Genotypes of similar maturity type were planted at random within maturity subplots. Genotypes were allocated to a maturity group according to their maturity type based on meta information available (Khan 2012). Maturity grouping was done in order to avoid unequal competition.

The N treatments consisted of two input levels: i) High N, with 180 kg N/ha as a standard conventional N input level and ii) Low N, with 75 kg N/ha as the low-input variant. The N input level was considered as the N in the soil estimated in spring plus fertiliser application to reach the required N levels. The fertiliser application was divided into two steps: a basic fertiliser treatment to reach the amount of the low treatment. It was applied just after planting using 23-23-0 (N-P-K) on the whole experimental field. The second step was done to the experimental area used for the high-input treatment before the final ridging, using KAS (27-0-0). P and K were abundantly available for potato crop growth.

Plots consisted of four rows. In each row there were four plants of the assessed genotype flanked by two border plants of the same genotype at each end. A total of 16 plants were available for final harvest. The planting distance was 33 cm between plants and 75 cm between rows.

Traits and assessments

The soil cover was assessed using digital pictures captured with a digital compact camera (PowerShot SXI200.IS, Canon, Japan). The camera was mounted 80 cm above a 99 cm × 75 cm frame and centred in the middle of the frame. The camera was set at the same place each time to cover the same three plant positions in a row. The percentage of green pixels on the pictures was estimated using a specific script made for this purpose developed by Dr. Gerrie van der Heijden in MATLAB® version 7.8.0347 (R2009a), the MathWorks™ programme (Van der Heijden, unpublished).

Emergence date was estimated as the moment when more than 50% of the plants in the plot had emerged. A plant was considered emerged when the first leaf was visible; just after the emerging shoot broke the soil.

Scoring of maturity was done using a senescence scale from 1 to 8 where 8 represents a dead plant and 3 represents the appearance of the first yellow leaves. See Hurtado (2012) and Chapter 3 of this thesis for further details. The assessment was done three times within two weeks, scoring three plants per plot, when a typical intermediate maturity cultivar was showing a score of 5 at the low N treatment. This assessment will be referred to as maturity assessment (mt_as) in order to avoid confusion with maturity groups used as blocking factor (Mt).

Quality traits were assessed by experts scoring potato samples of each plot using ordinal scales for each trait following the standard procedures used by Agrico Research. The traits included were: after cooking darkening (ACD, scale from 3 (dark) to 8 (clear)), smell and taste (GSA, from 4 (disgusting) to 8 (tasteful)), brightness (HLD, from 4 (not bright) to 8 (bright)), cooking distortion (KAW, from 4 (large difference in cooking type between tubers) to 8 (equal sample of cooked tubers)), overall cooking score (KWD, 4 (bad) to 8 (good)), and structure inside the tuber (STR, 4 (soft) - 8 (firm)).

Harvest

The harvest took place as late as possible to allow late cultivars to complete senescence naturally. The whole experiment was harvested at once including the early cultivars that had senesced earlier in the season. Sixteen plants were harvested per plot and the following tuber traits were assessed: A) Total tuber fresh weight. B) Tuber size and weight distribution; for this, six size classes were included: 0-30 mm, 30-40 mm, 40-50 mm, 50-60 mm, 60-70 mm and 70+ mm. For each class the tuber number and tuber weight were recorded. C) Tuber number per meter; obtained for the class 50-60 mm. D) Dry matter percentage (DM%), as dry weight of a sample divided by its fresh weight expressed in percentage; drying was done for 48 hours at 70 °C and a subsample was

taken including tubers from all size classes. E) N content ([N]) determined using the Kjeldahl protocol.

Data processing

The Beta thermal time for each canopy assessment date was calculated from the emergence day for each plot and using the Beta function described by Yin et al. (2003). For this purpose, we used the cardinal temperatures determined for potato haulm growth (Khan et al. 2013; Khan 2012).

Canopy development (see Chapters 2 and 3 for theoretical background) was assessed per individual plot using curve fitting based on the Beta thermal time, for each assessment date and the percentage soil cover. The procedure was carried out using the software SAS/STAT® for statistical analysis. Using the NLIN procedure the equations describing each phase of the curve were specified. This procedure required starting values for each parameter to find the most suitable combination of initial values. After that SAS performed an optimisation process to get estimated parameters and its standard errors. Five parameters were estimated for each individual plot (Khan et al. 2013; Khan 2012): four *t*-parameters expressed in time in thermal days (td), *tm1* (inflection point in the build-up phase of the curve), *t1* (when soil coverage stabilised), *t2* (start of senescence), and *te* (when canopy had completely senesced). The fifth parameter, *Vx,* is the maximum soil coverage reached with percentage soil coverage (%SC) as unit.

A bell-shaped curve was fitted per plot and for each of the two data sets describing the tuber weight and tuber number distribution (Tbw and Tbn respectively). Three parameters were estimated for each data set following the equation.

$$\mathrm{Tb} = \mathrm{MX} * \exp\left(-\frac{(\mathrm{mcl}-\mathrm{B})^2}{\mathrm{A}}\right)$$

Eq. 1

Where Tb is either Tbw or Tbn, "A" is a dispersion parameter expressing how the weights/numbers were distributed across classes, mcl is the middle size of each size class, and "B" is the average size at which the MX or maximum weight/number occurs. The curve-fit parameters were named for each variable as follow: for Tbw data: TbwA, TbwB and TbwMX; for Tbn data: TbnA, TbnB and TbnMX.

Calculated variables

Based on the parameters estimated with the soil coverage model, the following variables were calculated: *t2-t1* (duration of maximum soil coverage in td), *te-t2* (duration of Phase 3 in td), *Cm* (maximum progression rate of %SC in %/td, in Khan et al., 2013), *AP1* (area under the curve for Phase 1 in %.td), *AP2* (area under the curve for Phase 2 in %.td), *AP3* (area under the curve for

Phase 3 in %.td), *AUC* (area under the curve for the entire crop cycle in %.td). In order to express the agronomic variables in a standard way, subsequent calculations and conversions were done as: Yield (i.e. fresh tuber yield; Y) in kg/m^2, N content ([N]) in g/kg (determined only in tubers), DM% in percentage, dry matter yield (Y_DM) in kg/m^2, which is Y×DM%; N uptake in tuber (NUpt) in g/m^2, which is Y_DM × [N]; N use efficiency (NUE) as Y_DM/(N input) in kg/g; N utilisation efficiency (NUtE) that is Y_DM/NUpt, in kg/g; N Uptake efficiency (NUpE) that is NUpt/N input in g/g;, and Soil coverage yield index (SCYi=*AUC*/Y_DM in %.td/(kg/m^2)). The variables were analysed without transformation since there were no severe violations to the assumptions regarding normality and equal variances required for mixed models analysis. Annex 1 provides an overview of the acronyms used and their explanation and units.

Statistical analysis

Main effects and interaction

Data were analysed with the GenStat package (16th edition) using the following mixed model.

$$Y = N * Mt + \underline{bk.N} + \underline{Mt.G} + \underline{bk} + \underline{bk.row} + \underline{bk.col} + \underline{E}$$

Eq. 2

Where terms joined by "*" represent individual effects plus the interactions (N**Mt= N+Mt+N.Mt*), whereas terms joined by "." represent interaction only. The term "*N*" represents nitrogen; the term "*Mt*" is the maturity group excluding control plot information. The term "*bk*" represents the blocks. Corrections for rows and columns are the random terms (*N.row* and *N.col*). The term "*Mt.G*" represents the genotypes nested within maturity groups, since maturity is an intrinsic characteristic of each genotype. Finally *E* represents the error. All random terms are underlined.

Genetic map

The genetic map for the population SH × RH used here was described by Khan (2012). The map consists of AFLP markers heterozygous in one parent and homozygous in the other, segregating in an expected 1:1 ratio in a progeny of 250 genotypes. Two parental maps (SH and RH, respectively) were generated depending on which parent was the heterozygous parent. The maternal and paternal chromosomes are referred to with the prefixes ma_ and pa_ followed by the linkage group number (Roman numbers). Twelve linkage groups were constructed for each of the parental maps; however, for the paternal map, linkage group I was split into two subgroups called "IA" and "IB" due to the lack of sufficient markers between the subgroups. The final number of markers mapped to the maternal and paternal maps was 230 and 177, respectively. Khan (2012) mentioned that these maps were consistent with the ultra-dense map described by Van Os et al. (2006) with small differences probably due to the smaller population size (Annex 5).

Multi-trait QTL analysis

The data was split by N treatment in two subsets to carry out QTL analysis independently for each N treatment. Parameters estimated for canopy development, tuber size weight and number distribution were included as well as agronomic traits and NUE indicators (Annex 1). The best linear unbiased estimators (BLUEs) were obtained for each trait in each subset using a mixed model analysis correcting for random spatial variation, obtaining one value per genotype in each subset. Additionally, each trait was auto-scaled to make traits with different units and scales suitable for multi-trait analysis. It was done by subtracting, for each trait value the trait average and dividing by the standard deviation. The maternal and paternal maps were combined into one map with 25 linkage groups (12 from ma_ and 13 from pa_, because group one is split into two subgroups), allowing the used markers from one parent as a co-factor for possible QTL in the other parent. The QTL analysis was done using the QTL library in GenStat 16, as follows. The best variance covariance matrix among the traits was selected based on the Bayesian information criteria (BIC). First a genome-wide scan using single interval mapping (SIM) was done to identify candidate QTL positions. Thereafter, one round of composite interval mapping (CIM) was done, using the detected QTL candidates on the SIM step as a cofactor (genome wide) to identify QTL effects. After the CIM scan, a backward elimination round was used to remove possibly redundant QTL. The significance of QTL was tested by a Wald test and the p-value of this test was expressed on the –log10 scale. The threshold for QTL detection was calculated using the procedure based on a modified Bonferroni correction, using the effective number of independent test described by Li and Ji (2005) with a genome–wide test level of 0.05, as is implemented in GenStat 16.

Results

Annex 2 provides an overview of the mean values for all traits across maturity types and nitrogen levels investigated.

Main effects of nitrogen and maturity

The experiment showed significant differences between N treatments (Table 1) for most of the traits except for the maximum progression rate of soil coverage (*Cm*), the duration of Phase 3 (*te-t2*), the DM% (almost significant) and tuber quality traits (except for STR). Maturity effects were significant for CDv traits but not for *t1* and *Cm*. Tuber quality traits did not show differences between maturity groups nor did NUtE, DM% [N] SCYi, TbnB, TbwMX, TbwA and Tb_mt. The interaction term was significant for *AP1*, *AP2*, *AUC*, *t2*, *te-t2, tm1*, TbnMX, TbnB, TbwMX NUpt and *t2-t1*.

Nitrogen increased the total area under the curve for CDv (Figure 1), especially by increasing *AP2*. All parameters increased with N except for *Cm*. Yield increased with more weight per tuber, fewer tubers in the small size classes and with an increase in the tuber size parameters in which the

maximum tuber weight and number occurred (TbwB and TbnB respectively). The DM% was slightly lower at high N (but not significantly so). Indices for N use decreased with an increase of N input.

Table 1 p-values (from a Wald test) for fixed main factors and interactions analysed with a mixed model: maturity class (Mt), nitrogen levels (N_lv) and the interaction term (Mt.N_lv) from the model in equation Eq. 1. For explanation of the acronyms in the table, see Annex 1.

Traits	Mt	N_lv	Mt.N_lv
tm1	0.007	<0.001	0.008
t1	0.092	<0.001	0.937
Vx	<0.001	0.007	0.306
t2	<0.001	<0.001	<0.001
te	<0.001	<0.001	0.736
Cm	0.597	0.930	0.603
AP1	<0.001	0.001	0.913
AP2	<0.001	<0.001	<0.001
AP3	<0.001	0.020	0.002
AUC	<0.001	<0.001	<0.001
t2-t1	0.012	<0.001	<0.001
te-t2	0.029	0.113	<0.001
DM%	0.270	0.051	0.690
Y_DM	0.003	<0.001	0.009
[N]	0.259	<0.001	0.122
NUpt	0.006	<0.001	<0.001
NUE	0.006	<0.001	0.030
NUtE	0.075	<0.001	0.055
NUptE	0.015	<0.001	0.496
SCYi	0.066	<0.001	0.664
TbnMX	0.006	<0.001	0.017
TbnB	0.072	<0.001	0.035
TbnA	0.013	<0.001	0.393
TbwMX	0.248	<0.001	<0.001
TbwB	0.005	<0.001	0.306
TbwA	0.052	<0.001	0.521
Tb_mt	0.646	<0.001	0.639
mt_as	<0.001	0.002	0.066
ACD	0.880	0.287	0.509
GSA	0.061	0.948	0.918
HLD	0.776	0.304	0.265
KAW	0.592	0.701	0.660
KWD	0.787	0.503	0.707
STR	0.482	0.013	0.200

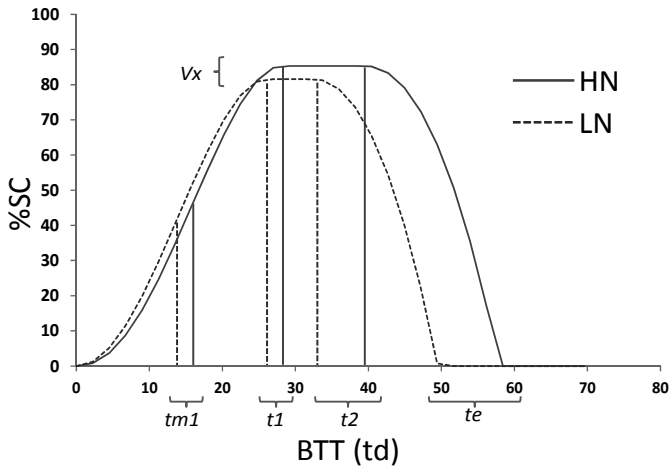

Figure 1 Average fitted curves of canopy development for both nitrogen input levels, high nitrogen (HN) and low nitrogen (LN). Canopy development parameters are shown, for acronyms see main text and Annex 1. On the X axis is the beta thermal time, BTT, in thermal days (td). The Y axis is in percentage of soil coverage, %SC.

Trait	*tm1*	*t1*	*Vx*	*t2*	*te*	*Cm*	*AP1*	*AP2*	*AP3*	*AUC*	*t2-t1*	*te-t2*	DM%	Y_DM	[N]	NUpt	SCYi	TbnMX	TbnB	TbnA	TbwMX	TbwB	TbwA	Tb_mt	mt_as	ACD	GSA	HLD	KAW	KWD	STR
tm1	**0.64**	0.63	0.26	0.55	0.47	-0.17	0.40	0.19	-0.01	0.29	0.17	-0.14	0.10	-0.04	-0.15	-0.07	0.38	-0.04	-0.16	0.08	-0.15	0.01	0.28	0.01	-0.48	0.03	0.10	0.05	-0.24	0.03	0.12
t1	0.64	**0.66**	-0.04	0.41	0.30	-0.73	0.82	-0.18	-0.20	0.04	-0.20	-0.21	0.01	-0.16	-0.09	-0.17	0.30	-0.04	-0.19	0.08	-0.21	-0.02	0.31	0.09	-0.32	-0.02	-0.03	0.03	-0.24	-0.01	0.10
Vx	0.14	0.07	**0.74**	0.42	0.40	0.53	0.40	0.60	0.34	0.80	0.48	-0.09	0.09	0.73	-0.16	0.70	-0.24	0.01	0.35	0.12	0.42	0.34	0.07	-0.15	-0.43	-0.01	0.22	0.02	-0.15	0.01	0.20
t2	0.41	0.56	-0.06	**0.33**	0.72	0.00	0.44	0.81	-0.25	0.74	0.81	-0.47	0.22	0.47	-0.19	0.43	0.15	-0.05	0.18	0.14	0.28	0.29	0.33	-0.09	-0.84	0.01	0.18	0.02	-0.20	0.00	0.32
te	0.47	0.41	0.25	0.33	**0.78**	0.01	0.34	0.61	0.42	0.81	0.58	0.27	0.19	0.46	-0.17	0.41	0.26	-0.25	0.37	0.25	0.34	0.44	0.35	0.07	-0.88	-0.05	0.04	-0.09	-0.10	-0.07	0.18
Cm	-0.20	-0.73	0.53	-0.40	-0.08	**0.66**	-0.46	0.49	0.28	0.38	0.46	0.05	0.06	0.46	-0.03	0.45	-0.28	0.00	0.26	0.05	0.33	0.19	-0.12	-0.18	-0.02	0.05	0.12	0.03	0.01	0.04	0.01
AP1	0.35	0.81	0.55	0.40	0.36	-0.38	**0.56**	0.05	-0.04	0.36	-0.04	-0.23	0.04	0.24	-0.12	0.23	0.02	-0.02	0.05	0.11	0.08	0.15	0.25	-0.01	-0.40	-0.07	0.02	-0.02	-0.23	-0.05	0.18
AP2	-0.07	-0.31	0.10	0.57	0.09	0.41	-0.23	**0.23**	-0.06	0.85	0.98	-0.33	0.21	0.70	-0.16	0.65	-0.09	-0.04	0.38	0.13	0.48	0.38	0.17	-0.15	-0.72	0.01	0.25	-0.01	-0.08	0.00	0.29
AP3	0.22	0.06	0.54	-0.28	0.77	0.30	0.25	-0.22	**0.37**	0.33	-0.15	0.89	0.03	0.19	-0.02	0.16	0.10	-0.23	0.27	0.14	0.19	0.24	0.02	0.13	-0.11	-0.10	-0.12	-0.16	0.09	-0.11	-0.08
AUC	0.25	0.22	0.72	0.31	0.80	0.28	0.51	0.32	0.74	**0.83**	0.77	0.00	0.19	0.79	-0.19	0.74	-0.06	-0.12	0.48	0.21	0.54	0.49	0.23	-0.07	-0.80	-0.04	0.19	-0.05	-0.12	-0.04	0.26
t2-t1	-0.12	-0.33	-0.12	0.58	0.01	0.29	-0.35	0.97	-0.35	0.16	**0.11**	-0.37	0.23	0.62	-0.16	0.58	-0.06	-0.03	0.33	0.10	0.43	0.33	0.15	-0.16	-0.70	0.02	0.22	0.00	-0.05	0.01	0.27
te-t2	0.21	0.04	0.30	-0.32	0.79	0.18	0.11	-0.28	0.96	0.61	-0.36	**0.21**	-0.06	-0.10	0.06	-0.11	0.18	-0.27	0.19	0.14	0.02	0.15	0.01	0.22	0.07	-0.09	-0.22	-0.16	0.15	-0.10	-0.20
DM%	0.08	0.03	0.24	-0.07	0.23	0.10	0.12	-0.04	0.30	0.27	-0.10	0.29	**0.96**	0.22	-0.18	0.16	-0.04	0.33	-0.28	-0.30	0.01	-0.33	-0.20	-0.18	-0.20	-0.13	0.15	-0.37	-0.09	-0.30	0.51
Y_DM	-0.16	-0.10	0.66	0.08	0.34	0.42	0.31	0.34	0.45	0.69	0.19	0.30	0.18	**0.89**	-0.27	0.92	-0.63	0.11	0.61	0.05	0.76	0.47	-0.02	-0.12	-0.52	0.01	0.37	0.01	-0.16	0.03	0.31
[N]	-0.11	-0.33	-0.36	-0.29	-0.33	0.09	-0.50	-0.05	-0.23	-0.45	0.03	-0.15	-0.24	-0.49	**0.54**	0.11	0.18	-0.19	-0.06	0.14	-0.23	-0.01	0.02	0.04	0.21	-0.21	-0.22	-0.19	0.24	-0.22	-0.24
NUpt	-0.23	-0.26	0.56	-0.07	0.18	0.50	0.11	0.32	0.37	0.52	0.20	0.24	0.07	0.86	0.00	**0.68**	-0.58	0.04	0.58	0.12	0.66	0.46	-0.01	-0.12	-0.47	-0.07	0.28	-0.06	-0.06	-0.05	0.23
SCYi	0.48	0.31	-0.14	0.16	0.39	-0.19	0.06	-0.11	0.20	0.10	-0.10	0.28	0.05	-0.60	0.22	-0.61	**0.82**	-0.29	-0.40	0.10	-0.47	-0.18	0.31	0.09	-0.11	-0.03	-0.28	-0.08	0.01	-0.08	-0.09
TbnMX	-0.05	-0.07	0.13	-0.10	-0.27	0.09	0.02	-0.01	-0.16	-0.11	-0.05	-0.21	0.34	0.03	0.04	0.09	-0.19	**0.84**	-0.49	-0.73	0.20	-0.68	-0.54	-0.21	0.18	0.20	0.17	0.17	-0.21	0.20	0.22
TbnB	-0.16	-0.13	0.07	0.10	0.16	0.14	-0.02	0.26	0.13	0.23	0.25	0.10	-0.41	0.40	-0.19	0.34	-0.31	-0.66	**0.84**	0.45	0.63	0.88	0.21	0.23	-0.38	-0.05	0.14	0.03	0.10	0.01	-0.12
TbnA	0.18	0.13	0.09	0.10	0.42	0.00	0.12	-0.01	0.37	0.33	-0.01	0.36	-0.24	0.17	-0.17	0.08	0.11	-0.67	0.58	**0.54**	-0.12	0.75	0.72	0.28	-0.23	-0.18	-0.11	-0.15	0.24	-0.17	-0.23
TbwMX	-0.21	-0.23	0.44	-0.08	-0.06	0.38	0.08	0.23	0.12	0.27	0.14	0.00	0.09	0.71	-0.20	0.69	-0.64	0.50	0.17	-0.19	**0.59**	0.42	-0.09	-0.08	-0.38	0.03	0.24	0.07	-0.14	0.07	0.10
TbwB	0.00	0.02	0.07	0.14	0.33	0.05	0.07	0.16	0.25	0.31	0.15	0.24	-0.40	0.33	-0.19	0.24	-0.11	-0.78	0.91	0.83	-0.03	**0.83**	0.62	0.29	-0.44	-0.13	0.07	-0.03	0.12	-0.06	-0.18
TbwA	0.23	0.22	-0.07	0.17	0.42	-0.15	0.10	-0.04	0.27	0.23	-0.01	0.31	-0.25	0.03	-0.16	-0.07	0.20	-0.62	0.52	0.83	-0.30	0.79	**0.58**	0.21	-0.33	-0.21	-0.08	-0.13	0.09	-0.17	-0.13
Tb_mt	-0.18	-0.04	-0.12	0.06	-0.09	-0.11	-0.04	0.07	-0.14	-0.07	0.11	-0.13	-0.07	-0.04	0.20	0.08	-0.07	-0.01	0.22	0.20	0.00	0.21	0.08	**0.86**	0.01	-0.05	-0.06	0.05	0.09	0.02	-0.33
mt_as	-0.48	-0.44	-0.20	-0.40	-0.90	0.18	-0.36	-0.09	-0.63	-0.71	-0.04	-0.65	-0.26	-0.28	0.33	-0.12	-0.35	0.19	-0.10	-0.34	0.07	-0.25	-0.37	0.08	**0.73**	0.06	-0.12	0.06	0.09	0.06	-0.19
ACD	-0.05	0.08	0.13	0.23	-0.02	0.01	0.17	0.26	-0.11	0.13	0.23	-0.17	-0.18	0.14	-0.05	0.15	-0.08	0.16	-0.01	-0.20	0.17	-0.10	-0.23	0.11	0.02	**0.72**	0.37	0.88	-0.33	0.94	0.18
GSA	0.01	0.09	0.07	0.01	0.03	-0.08	0.12	-0.05	0.02	0.05	-0.06	0.01	0.30	0.02	-0.07	-0.03	0.03	0.16	-0.11	-0.16	0.03	-0.14	-0.08	0.03	-0.07	0.12	**0.36**	0.25	-0.08	0.32	0.50
HLD	-0.06	0.07	0.02	0.31	-0.02	-0.02	0.11	0.33	-0.19	0.08	0.32	-0.23	-0.36	0.10	0.00	0.13	-0.07	-0.02	0.17	-0.05	0.08	0.09	-0.09	0.23	0.03	0.85	0.02	**0.73**	-0.28	0.96	0.00
KAW	-0.04	-0.14	-0.19	-0.25	-0.15	0.02	-0.22	-0.20	-0.04	-0.27	-0.16	0.00	-0.12	-0.18	0.19	-0.09	-0.01	-0.11	0.01	0.13	-0.12	0.04	0.05	0.01	0.23	-0.19	-0.15	-0.14	**0.29**	-0.31	-0.26
KWD	-0.06	0.10	0.07	0.26	-0.03	-0.03	0.16	0.26	-0.15	0.09	0.24	-0.20	-0.27	0.10	-0.02	0.12	-0.06	0.08	0.06	-0.16	0.13	-0.04	-0.20	0.17	0.04	0.93	0.09	0.94	-0.18	**0.69**	0.10
STR	0.01	0.04	0.19	0.12	0.20	0.05	0.14	0.16	0.15	0.28	0.10	0.13	0.57	0.23	-0.23	0.10	-0.03	0.20	-0.11	-0.15	0.12	-0.13	-0.02	-0.08	-0.27	0.09	0.47	-0.13	-0.17	-0.03	**0.56**

Figure 2 Heat map for the correlation matrix between 31 traits excluding NUE, NUtE, and NUptE. The upper triangle corresponds to correlations between traits at high nitrogen level. The lower triangle corresponds to low nitrogen level while the diagonal consists of correlations between the two N levels for each single trait. For explanation of acronyms, see Annex 1.

Genotypes with good yield under low N tended to have a good yield under high N (correlation between yield at both N levels: 0.89) (Figure 2 and Annex 3). Yield partially depended on maturity type (Figure 3) and was increasing from the early to the middle-late maturity group, while the late group showed lower values than the middle-late one. The same was observed for *Cm*, NUpt and NUptE. In this population the distinction between the maturity groups middle-early and early and middle-late and late was not clear probably having an influence on the observed trend of the traits across maturity groups. Tuber nitrogen content increased with a decrease in DM% from late to early (Figure 3). Nitrogen seemed to be diluted with an increase in carbohydrates that are the main components of the tuber dry matter. Additionally, the variation for yield was larger at the higher N level. The efficiency in using N decreased with an increase in N input. The magnitude of this decrease would be equivalent to the change in response to N. High N input increased yield, N content and therefore N uptake, while DM% was slightly reduced (Annex 2).

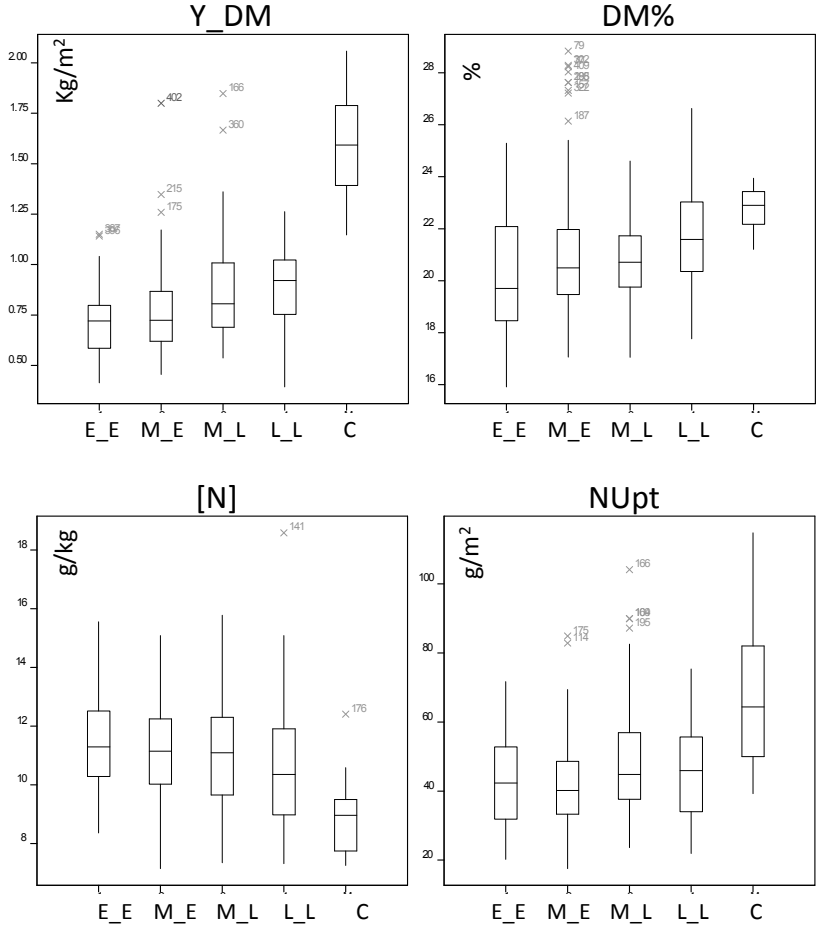

Figure 3 Comparison of maturity groups across nitrogen supply levels for Yield (Y_DM), Dry matter percentage (DM%), Nitrogen content in tuber ([N]) and Nitrogen uptake (NUpt). The maturity groups are: E_E=early, M_E=middle early, M_L=middle late, and L_L=late. C is a tetraploid reference cultivar of intermediate maturity type ('Fontane').

Canopy development traits showed a strong response to N treatment: with high N input the area under the curve for Phase 1 (*AP1*) became larger by a longer *t1* and higher *Vx*. The same N effect was observed for Phase 2: *AP2* became larger (Figure 1 and Annex 2), allowing more light interception by both an increase in the maximum soil covered and by an elongation of the period for which the maximum was maintained.

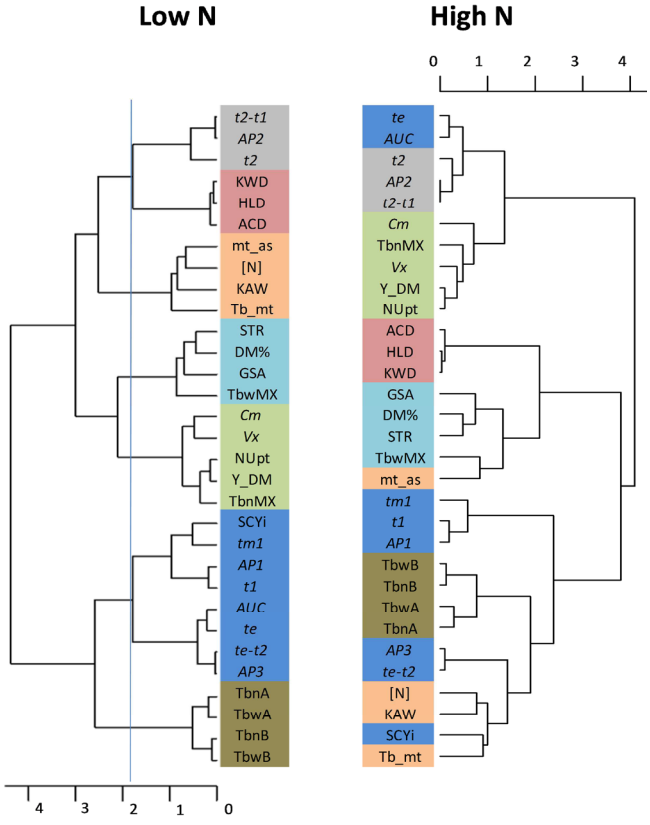

Figure 4 Comparison of the hierarchical cluster analysis (using Ward's method) between traits at high and low N, using (1- absolute value of Pearson correlation coefficient) as a degree of dissimilarity. Low nitrogen grouping is used as a reference with seven groups in different colours.

Relationships among traits

Excluding the traits NUE, NUtE and NUptE, a Mantel test between trait distance matrices (containing 1 - absolute value of Pearson correlations of all pairs of traits) for each N input (high and low) showed a correlation value of 0.81 between both matrices, i.e., traits were similarly correlated at both N levels. However, Figure 4 shows some important changes in the grouping of traits. Taking as a reference the grouping at low N, seven groups were formed. Group 2, which included parameters from Phases 2 and 3 of the canopy development and the total growing period, was split up at high N. Additionally, the parameters from Phase 2 grouped with *AUC* and *te* at high N input.

Comparing the correlations between traits at the two N treatments, it was observed that for seven traits the trait with which they had the highest correlation under the influence of N level was different (*t2*, *AUC*, [N], SCYi TbnMX, Tb_mt and KAW) (Figure 2). Out of these seven traits, four had higher absolute correlation values than 0.4 (i.e. a highly significant correlation) at both N levels. Two of these traits had the highest absolute correlation value at high N: *t2* became more closely correlated to mt_as (-0.84) whereas at low N the highest absolute value was with *t2-t1* (-0.58), while for *AUC* the highest absolute correlation was with *AP2* (0.85) at high N and with *te* (0.80) at low N. Moreover, two traits had highest absolute correlation values at low N: SCYi with TbwMX (-0.63) (whereas at high N the highest absolute value was -0.63 with Y_DM); and TbnMX with TbwB -0.78 (whereas at high N the highest absolute value was with TbnA (-0.73).

Multi-trait QTL analysis

A QTL analysis was performed using 84 genotypes grown under two N input levels. Two separate analyses were done using BLUEs, obtained for each N level including canopy development traits, other agronomic traits and some quality traits. The multi-trait QTL analysis showed regions affecting several traits related to canopy developmental process, helping with the understanding of the genetic control of the process. There were more QTL detected with the multi-trait analysis than with the single QTL analysis (Annex 4). Most of the major QTL found in the single-trait analysis were also recovered in the multi-trait analysis.

Nitrogen input effect on the regions detected is shown (Figure 5 and Figure 6). This effect was observed in the different QTL detected for a trait when comparing high and low N level. In general, QTL in regions accumulating several QTL, which might indicate possible pleiotropic regions, were significant with both high N and low N input. There were more QTL detected at low nitrogen (12 hot spots with at least 3 QTL) than at high nitrogen (6 hot spots). QTL detected at both nitrogen levels were on the linkage groups ma_III, ma_V, ma_VI and pa_V, these QTL are N independent.

QTL that were detected at only one of the nitrogen levels are N dependent. QTL detected only at low N were found in the linkage groups ma_I, ma_III, ma_V, ma_VI, ma_VIII, ma_X, ma_XI, pa_I A, pa_I B, pa_V, pa_VIII and pa_XI, whereas QTL detected only at high N were found in ma_III, ma_V, ma_VI ma_IX and pa_V (Figure 6 and Annex 6). In addition, some hotspot regions included QTL only at low nitrogen in linkage groups ma_1, ma_VIII, ma_X, ma_XI, pa_I A, pa_ I B, pa_VIII pa_XI, whereas there was only one region with QTL only detected at high nitrogen on ma_IX; the other regions on ma_III, ma_V, ma_VI and pa_V included QTL detected at both N inputs.

The major QTL hotspot detected on the paternal linkage group V (pa_V) was linked to most of the canopy development traits and only one quality trait (all with a pick marker at 18.18 cM position; Table 2 and Figure 5). This region on pa_V coincides with the well-known maturity locus (Celis-Gamboa 2002; Collins et al. 1999) that is also reported in studies of the dynamics of development processes (like flowering, senescence, plant height increase) in different populations (Hurtado-Lopez et al. 2015; Khan 2012; Malosetti et al. 2006). Most of the canopy development traits showed

QTL in this region in at least one N level, excluding the maximum progression rate of canopy development (*Cm*). Nine traits out of 12 with common QTL at both N levels, in the pa_V region, showed higher explained variance at high N than at low N while in the other linkage groups with common QTL the variance explained was higher at low N input than at high N input.

Table 2 Quantitative trait loci detected for agronomic and quality traits under two N levels from multi-trait QTL analysis: H=High and L=Low. The values in the table are the explained variance in percentage. For explanation of the acronyms, see Annex 1.

Trait	N level	ma_I	ma_III	ma_V	ma_VI	ma_VIII	ma_IX	ma_X	ma_XI	pa_I A	pa_I B	pa_V	pa_VIII	pa_XI
	position	125.6	*22.5 75.2	57.1	*51.1 87.2	0	0	87.5	9.2	28.5	63	18.2	17.4	46.4
tm1	H		4.5									28.5		
tm1	L		5.0			4.3			7.0	11.5		7.9		
t1	H				12.0							11.5		
t1	L		13.9					6.4	7.7			9.4		
Vx	H			7.2	8.1							34.1		
Vx	L											22.7		
t2	H											51.5		
t2	L	6.5	20.8										6.7	
te	H											63.1		
te	L					5.7						43.2		
Cm	H			4.6	21.2									
Cm	L		8.4						4.5				9.6	5.3
AP1	H											22.0		
AP1	L		7.2	4.7								22.0		
AP2	H				5.2							35.6		
AP2	L	7.0			5.4			8.4						4.9
AP3	H											8.7		
AP3	L					5.5						41.1	5.5	
AUC	H											62.4		
AUC	L											50.1		
t2-t1	H				5.2							28.6		
t2-t1	L	6.6			4.2			8.8						
te-t2	H													
te-t2	L			6.3		6.1						32.0		
DM%	H		19.6											
DM%	L					8.8					9.7			
Y_DM	H			7.7								21.1		
Y_DM	L				7.0				6.5			16.2		
[N]	H			6.3			10.8							
[N]	L					7.2					5.8	4.6		14.4
NUpt	H				4.7							20.1		
NUpt	L				6.8				4.6			10.9	16.9	
SCYi	H			5.2										
SCYi	L				7.0			7.3	7.8			4.5		
TbnMX	H		16.2											
TbnMX	L	4.6									5.3	5.1		11.8
TbnB	H		8.4	6.9								7.9		
TbnB	L										6.8			
TbnA	H		8.8											
TbnA	L		4.4		4.4						7.9	18.0		9.3
TbwMX	H											7.8		
TbwMX	L				5.4			6.3						6.1
TbwB	H		10.3									10.8		
TbwB	L										9.6	10.4		4.9
TbwA	H		5.2									6.8		
TbwA	L										8.1	9.6		10.9
Tb_mt	H			10.5	10.5									
Tb_mt	L			4.9						9.0				
mt_as	H											56.6		
mt_as	L		4.7			4.7						35.8		
ACD	H		29.4											
ACD	L	7.3	39.7					8.1						
GSA	H			6.4										
GSA	L									5.5	5.3			
HLD	H		25.3											
HLD	L	13.3	26.8	5.3				10.2						
KAW	H		8.9				5.0							
KAW	L													
KWD	H		25.7											
KWD	L	11.7	31.2					11.7						
STR	H		12.9				7.8					7.4		
STR	L	5.1									7.0			

For each trait the marker with the highest p-value (i.e. the pick marker) for the QTL detected is shown in both nitrogen levels. QTL present only at one N level are N dependent while those common to both N levels are N independent.

*for some traits in this linkage groups there were two pick markers; however, we cannot ensure that they are two different QTL therefore we combined both in one column. For details see Annex 6.

There were six QTL exclusively detected at high N input in pa_V for the following traits: *t2*, *AP2*, *t1-t2* (parameters related with Phase 2 of canopy development), TbwB, TbnMX and STR. Five QTL were detected only at low N input (for the traits *te-t2*, [N], TbwMX and TbwA). Moreover, the QTL region in pa_V explained a great part of the variance for most of the canopy traits and at high N (*tm1*, *Vx*, *t2*, *te*, *AP2*, *AUC* and *t2-t1* with values above 25%) with values up to 63% and 62% for *te* and *AUC*, respectively (Table 2); these values are even higher than the variance explained for the maturity assessment mt_as (56%). At low N input, values are lower for the traits mentioned before but higher for traits related with the senescence phase (*AP3*, and *te-t2*).

The strong negative correlation between *te* and mt_as is also illustrated by the strong effects showing a very intense colour in Figure 6, with blue and red, respectively, for each trait in pa_V. This is because the longer the period is, the later the variety will be, and the lower the value for maturity, as it was measured on a scale from 3 (late) to 8 (early). As mentioned earlier, a QTL for maturity type at this position has previously been reported; therefore this region affects all canopy developmental traits that are correlated with the maturity assessment (Figure 2).

The development of the canopy as described by the parameters from the curve was affected by N levels as well as the QTL detection, which is a nitrogen by QTL interaction. As an example to show this interaction we describe QTL for *t2*. QTL for this trait on pa_V was exclusively detected at high N input with strong effect as shown in Figure 6. Meanwhile, a QTL with also a large effect showed up, only detected at low nitrogen, on ma_III, as well as other QTL but with smaller effects on ma_I and pa_VII (Figure 6). Moreover, traits that are highly correlated with *t2*, like *t1-t2* and *AP2*, did not show the QTL on ma_III. Typically, the nitrogen input by QTL interaction is specific for each trait.

Regarding tuber size and tuber weight distribution, for the maximum tuber number and weight (TbnMX and TbwMX, respectively) and for the size at which the maximum weight of tubers occurred (TbnB), QTL were all N dependent with small effects, excluding two QTL for TbnMX (in ma_III at high N and pa_XI at low N) which explained more variance than 10%.

TbwB (the size at which the maximum weight of tubers occurred) had a QTL which explained more variance than 10% on ma_III at high N and a QTL N independent on pa_V. Finally, the tuber dispersion parameter "A" for tuber weight (TbwA) showed a QTL at low N in pa_XI; while for tuber number (TbnA) there was QTL on pa_V at low N, both explaining more variance than 10%. There were others QTL but with smaller effects.

The tuber cooking quality traits ACD, HLD, and KWD showed a strong QTL on ma_III that was independent of N input level; QTL on ma_I were present only at low N (including STR with very small effect). For ACD, HLD, and KWD, there were no QTL on pa_V but on ma_V.

The maturity assessment mt_as had a nitrogen independent QTL with a strong effect on pa_V and some other nitrogen independent QTL with small effects on ma_III, ma_VIII.

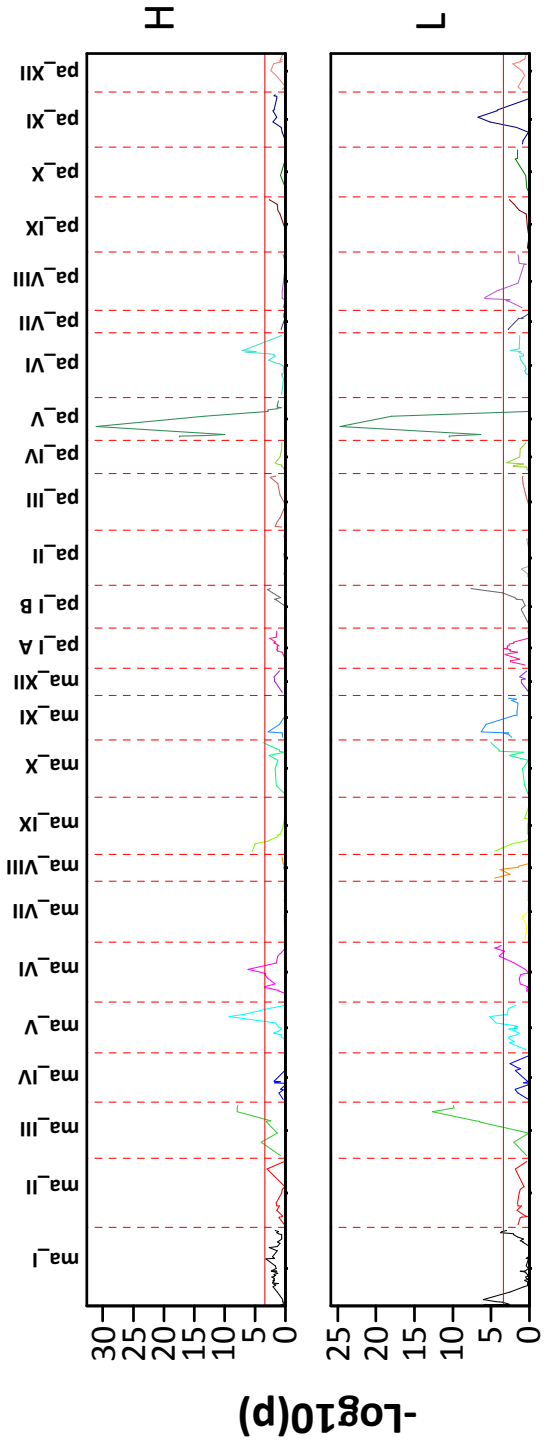

Figure 5 Significance of QTL on a -log10 scale (-log10(p) profile across linkage maps) for the multi trait QTL analysis. Maternal linkage groups are indicated as "**ma_**" (for the SH parent) the paternal linkage groups as "**pa_**" (for RH). The horizontal red line is the Li-Ji threshold for significant QTL (3.4-log10(p)), H is the profile from the high nitrogen level and L is the profile from the low nitrogen level.

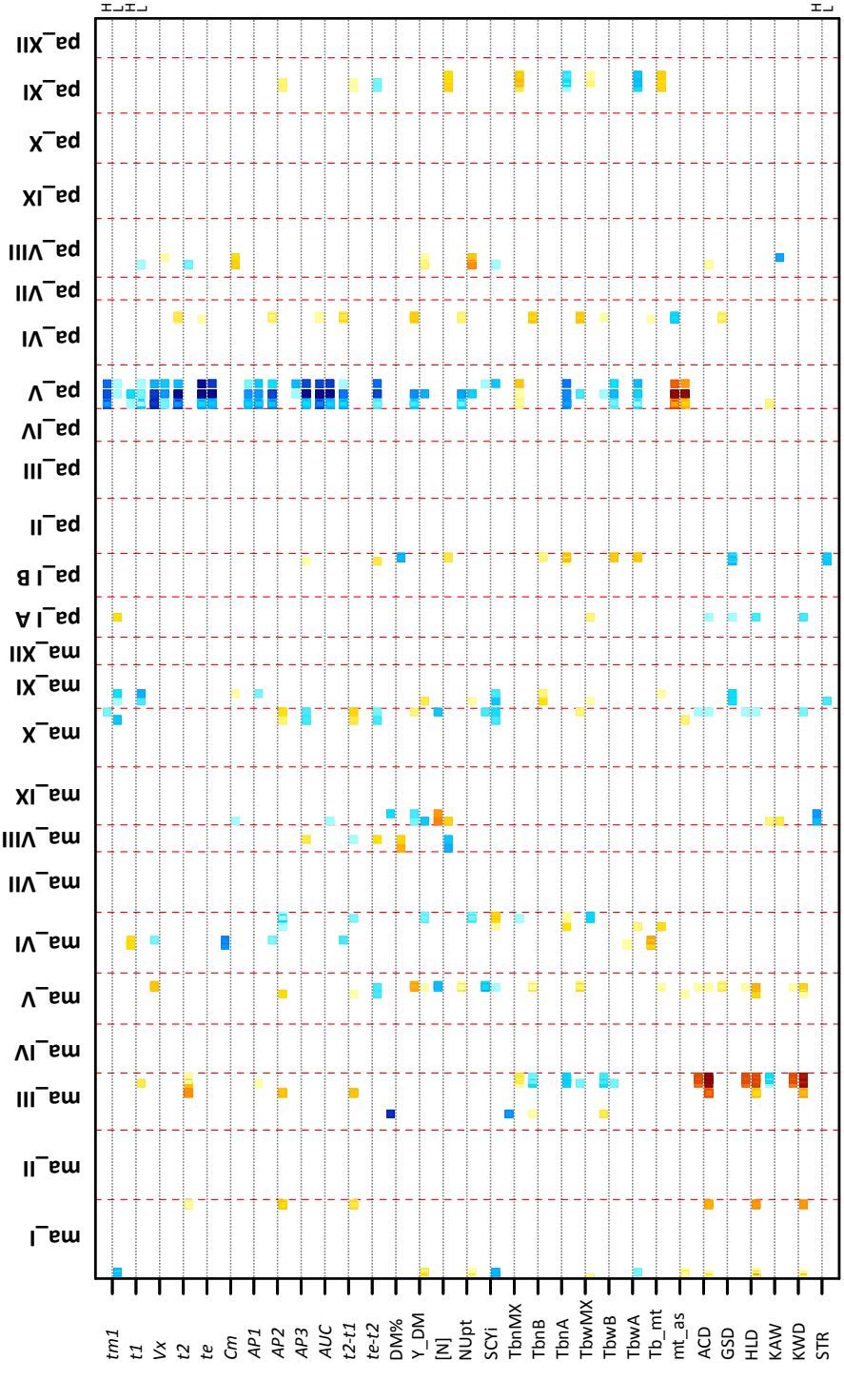

Figure 6 Multi-trait QTL linkage analysis. The intensity of the colours represents the variance accounted for (the darker the colour the more variance accounted for). Effects are shown simultaneously for high and low N (above and below the horizontal dotted line respectively in front of the trait label). Linkage groups are named "**ma_**" for the maternal (SH) linkage map and "**pa_**" from the paternal (RH) linkage map.

Discussion

We studied the genetic factors involved in canopy development by dissecting this complex trait into biologically meaningful parameters to help us understand how the crop and the different maturity type groups respond to contrasting N inputs. This study used a diploid biparental F1 population of potato, but this population showed similar response to N levels for most traits measured as compared to a set of tetraploid cultivars described in Chapter 3 (Ospina et al. 2014). The main difference was in Phase I of CDv, where for the SH × RH population the time to reach the maximum canopy cover was longer at high N than at low N and therefore *AP1* became larger. Moreover, there was not a significant change in the growth rate due to extra N, perhaps because diploid genotypes in general have less vigour than tetraploids and hence they may have been less capable to profit rapidly from the high N input conditions during the vegetative building up phase. However, by having more resources, as is the case at high N, the genotypes continued to increase in leaf area for longer and therefore other phases of CDv did show the same response as observed in the mentioned reference study. In addition to the differences in crop development mentioned above, we observed differences in vigour when comparing in the field the reference tetraploid variety (that is the result a breeding programme) and the genotypes; the genotypes looked weaker and at final harvest the tuber size, for any of the maturity classes, was evidently smaller than for the reference cultivar.

At high N input, Phase 2 of CDv was more important for the general performance of the genotypes, becoming longer and finishing later as reported previously with cultivars (Ospina et al. 2014; Vos 2009). As a result, the plants had a longer period in which the canopy had its maximum coverage, which led to a higher proportion of light intercepted as mentioned by Haverkort et al. (1991). Therefore, the accumulation of dry matter was higher with more N input. Moreover, NUE was higher with less N (Annex 2) as reported previously in tetraploid potatoes (Ospina et al. 2014; Vos 2009; Zebarth et al. 2004), as well as for a selection of *S. andigena* diploids (Zebarth et al. 2008). The increase in NUE at low N was mainly due to an increase in uptake efficiency (the ratio of tuber nitrogen uptake over the N input), even if N content and dry matter yield were lower. The nitrogen efficiency indexes were not included in the QTL analysis (but values of the traits are listed in Annex 2) because the traits are ratios of the yield or N uptake and the input (respectively NUE and NUptE at final harvest) and then the correlation with the original trait within a nitrogen level is "1", which means that the QTL for the indexes will be the same as those for the original trait. The indexes are only meaningful when comparing the two N input levels. For cultivars, as shown by Ospina et al. (2014), the later the cultivar is the higher is the nitrogen use efficiency. Here, with this SH × RH population the relationship is not very clear since early and middle early groups were very close in terms of yield and nitrogen use efficiency and late and middle late were also very close. However, the middle late group was the most efficient at both N levels having a better balance between canopy development and yield. These middle late genotypes had slightly higher yield than late genotypes with a lower area under the curve of canopy development (*AUC*). Then the

relationship: Higher *AUC* equal to higher yield across maturity groups is disturbed for the late group, in this population. It is important to mention that Tiemens-Hulscher et al. (2014) found the trait *AUC* to be the best predictor of performance both in terms of fresh tuber yield and in terms of nitrogen efficiency for cultivars since the trait *AUC* integrates all aspects of canopy development. Additionally the ratio of *AUC*/yield dry matter, SCYi, could be an interesting trait that relates these two important major traits that although highly correlated are not the same (as just shown when comparing the middle late and late groups). For SCYi a QTL was found on ma_X that was not present for the component traits and at low nitrogen level, indicating that other regions rather than the maturity locus on pa_V could be important.

Maturity (mt_as evaluated as the initiation of senescence) summarized the development of the canopy because the evaluation considers the stage of the canopy at a specific moment that is the result of all the phases and physiological events that happened before. However, the study of the factors, events and phases previous to that point improves the understanding of maturity type. Early genotypes not only showed yellow leaves early but also required little time to reach the maximum coverage and had a short Phase 2. Consequently, the moment at which canopy cover started to decay was reached earlier. Phase 3 was considerably shorter under low N input as described in Ospina et al. (2014) and Vos (2009). Additionally, yield tended to increase from early to middle late genotypes at low N where the very late group had lower yield than the middle late group (the same occurred at high N but less clearly so). An explanation may be that at the low N level very late genotypes did not have the capacity to sustain their longer growing period and senesced earlier than they normally would do. This behaviour is an example of the interaction between maturity type and N input. It shows how the very late group in this population is more susceptible to low N conditions, probably having a higher minimum N requirement.

As mentioned by Hurtado et al. (2015) multi-trait analysis combining several traits related to a developmental process helps in the understanding of genetics driving the plant development. Our results showed some QTL as well as regions affecting several traits, which might be pleiotropic regions, being affected by N (Figure 6). Low N level allowed the detection of more QTL than high N. There was no indication that variance explained by the QTL detected was consistently higher at any of the N levels. For example, among the QTL detected at both N levels and for the same traits, some showed higher variance explained at low N. Then, this aspect has to be considered for the particular trait of interest.

This study is the first attempt in potato to see how N affects the genetic factors related to canopy development and NUE. There are few studies in other crops with similar objectives (Hirel et al. 2007; Hirel et al. 2011) with some examples in maize (Hirel et al. 2001) and rice (Wei et al. 2012). Results in maize showed that QTL for NUE and yield at low N were a sub set of the QTL detected at high N (Bertin and Gallais 2001), another study detected QTL for yield and NUE at low N (Agrama et al. 1999). These two studies show how the nitrogen input affects the detection of QTL for NUE related traits. Similarly, our results showed nitrogen specific effects on genetic regions, i.e. nitrogen dependence of QTL. On pa_XI a QTL was present for nitrogen content ([N]) at low nitrogen; pa_VIII

had a QTL for N uptake (NUpt) and for yield dry matter a QTL was present on ma_V (to mention the ones with higher effects of the QTL on pa_V).

Some authors reported that under growth-limiting conditions there is important variation that cannot be assessed under favourable conditions (Ceccarelli 1996) and this would apply to a highly complex trait involving different processes such as yield in potato. Low N could change the importance of the processes related to the complex traits which is also reflected in the genetic factors involved in these traits. Rice plants for example tended to alter a series of physiological, biochemical processes and gene expression for surviving under nitrogen-deficiency conditions (Hirel et al. 2007; Wei et al. 2012). In potato, Vos and Biemond (1992) reported effects of nitrogen on leaf appearance, branching, etc. However, to further understand the genetic basis of these responses, more extensive experiments including different levels of nitrogen supply should be performed. We show that the response of the genotype depends on the N input level affecting the detection of QTL as shown with the multi-trait analysis (Figure 3), then those extra regions that are particular at low nitrogen level would probably not be selected if breeding is done under high N conditions.

On the other hand, Khan (2012), using the same population, did show a large QTL×E interaction for all the CDv traits which means that most of the QTL for canopy development traits were specific for each environment, that is, QTL were not stable across environments. Our experimental setup did not allow confirmation of those findings, because we did not repeat the experiment over locations or years. However, by having N as the major source of variation, since all the other conditions were uniform in our experimental setup, QTL detected exclusively at one of the two N levels are considered as N dependent, with N affecting the proportion of the variation accounted for by genetic factors and their detection depending on the trait.

The major QTL on chromosome 5 for maturity type as previously reported by different authors (Celis-Gamboa 2002; Malosetti et al. 2006; Anithakumari et al. 2012; Hurtado et al. 2015) was found here as well. The gene was reported by Kloosterman et al. (2013), an allelic variation of the CDF1 gene (Cycling DOF Factor) related to phenology, plant maturity and onset of tuberization. This region is important for all phases of canopy development, especially for the total duration of the growing period represented with, *te,* and total area under the canopy cover progress curve (*AUC*), with 63% (for *te*) and 62% (for *AUC*) of variance explained at high nitrogen. Additionally, this region accumulates QTL for traits correlated with the duration of the growth period, such as yield and N uptake. Moreover, this region seems to be related to different traits under different N levels, suggesting that QTL associated with growth are sensitive to N supply.

Moreover, selection for low input should be done directly under low input conditions, since there are N dependent QTL, ensuring that the genetic factors dependent on low N will be maintained. Additionally, the selection for NUE must include both N conditions to allow the assessment of this trait by combining good performance at low N with a good response to extra N, even more so since different genetic factors are expressed at different N levels.

On the other hand, selection in the early stages of the breeding scheme must focus on defining the maturity of the clones. Then, it would be possible to select within a maturity group in order to break the linkage shown by the high correlation of maturity type with some traits of interest, for example, to be able to have early cultivars that are as good as late cultivars in nitrogen use efficiency or with high dry matter content. For this purpose marker-assisted selection might be developed allowing a larger population with small variation in maturity, so the selection would be concentrated on the traits of interest, accumulating other genomic regions, different from the maturity region, even if the effects are small.

Further work should be done to improve the genotyping on this population, since there are many phenotypic data sets that could lead to a more precise localization of QTL and/or genes for the understanding of important processes for the development of new varieties. It could be done by using modern genotyping platforms developed for potato that could allow extrapolation or integration of the result to breeding schemes.

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Supplementary material

Annex 1 Acronyms for the traits used in figures and text.

Traits	Description
tm1	Inflection point Phase I, build-up phase of canopy development
t1	Period from plant emergence to maximum soil coverage
Vx	Maximum % soil coverage reached
t2	Initiation of senescence or Phase III
te	Total growing period until canopy is dead
Cm	Maximum progression rate of soil coverage during Phase I
AP1	Area under canopy cover curve for Phase I
AP2	Area under canopy cover curve for Phase II
AP3	Area under canopy cover curve for Phase III
AUC	Total area under the canopy curve
t2-t1	Durations of Phase II
te-t2	Duration of Phase III
DM%	Tuber dry matter %
Y_DM	Tuber dry matter or yield dry matter
[N]	Tuber nitrogen concentration
NUpt	Tuber nitrogen uptake
NUE	Nitrogen use efficiency
NUtE	Nitrogen utilization efficiency
NUptE	Nitrogen uptake efficiency
SCYi	Soil coverage yield index
TbnMX	Maximum tuber number
TbnB	Average size with the maximum tuber number
TbnA	Tuber number dispersion parameter
TbwMX	Maximum tuber weight
TbwB	Average size with the maximum tuber weight
TbwA	Tuber weight dispersion parameter
Tb_mt	Tubers per meter
mt_as	Maturity assessment
ACD	After Cooking Darkening
GSA	Smell and Taste
HLD	Brightness
KAW	Cooking distortion
KWD	Overall cooking score
STR	Structure inside tuber

Annex 2 Means per trait, maturity (Mt) group, and N level. For explanation of acronyms, see Annex 1. E_E=early, M_E=middle early, M_L=middle late, and L_L=late.

Means										
N level	**H**				**L**				**H Total**	**L Total**
Mt groups	**E_E**	**M_E**	**M_L**	**L_L**	**E_E**	**M_E**	**M_L**	**L_L**		
Traits										
tm1	14.87	15.42	15.78	18.13	13.49	14.03	13.63	15.06	15.88	14.01
t1	26.99	27.59	27.82	30.48	25.04	25.69	26.06	27.68	28.05	25.99
Vx	80.14	82.96	89.47	92.12	76.66	80.60	85.16	86.10	85.43	81.69
t2	35.60	37.61	42.42	46.58	32.51	31.96	32.42	34.25	39.87	32.63
te	54.34	55.77	59.12	65.55	46.34	47.79	50.41	56.41	58.01	49.65
Cm	4.79	5.04	5.37	4.92	4.94	5.05	5.13	5.05	5.03	5.04
AP1	1011.10	1058.49	1135.27	1244.25	911.40	981.95	1082.13	1128.62	1099.04	1014.56
AP2	700.44	834.99	1323.29	1474.45	567.33	508.48	528.75	571.12	1029.39	538.77
AP3	1003.49	1029.91	1015.17	1196.10	725.60	872.51	1044.42	1281.34	1052.12	952.24
AUC	2721.88	2911.06	3490.86	3923.82	2205.75	2354.91	2659.33	2984.18	3183.17	2504.49
t2-t1	8.80	10.06	14.54	15.90	7.48	6.31	6.31	6.56	11.83	6.64
te-t2	18.54	18.22	16.67	19.17	13.88	15.83	17.96	22.18	18.15	17.03
DM%	20.06	20.91	20.80	21.53	20.34	21.13	21.21	21.80	20.80	21.09
Y_DM	0.74	0.80	0.99	0.96	0.66	0.68	0.81	0.78	0.86	0.72
[N]	12.43	12.40	12.58	11.93	10.56	10.21	9.84	9.50	12.36	10.08
NUpt	9.23	9.80	12.37	11.35	6.93	6.84	7.95	7.27	10.51	7.18
NUE	0.04	0.04	0.05	0.05	0.09	0.09	0.11	0.10	0.05	0.10
NUtE	0.08	0.08	0.08	0.09	0.10	0.10	0.10	0.11	0.08	0.10
NUptE	0.51	0.54	0.69	0.63	0.92	0.91	1.06	0.97	0.58	0.96
SCYi	3741.07	3772.08	3663.39	4236.18	3408.24	3560.10	3334.76	4023.08	3829.81	3563.84
TbnMX	14.30	12.96	12.64	10.68	18.94	17.06	15.52	11.83	12.78	16.18
TbnB	42.11	42.91	46.37	46.51	39.19	39.64	41.09	41.61	44.15	40.22
TbnA	212.18	239.12	250.61	314.91	138.54	137.43	166.69	289.63	249.60	172.96
TbwMX	1.09	1.07	1.58	1.41	1.11	1.02	1.11	0.96	1.25	1.05
TbwB	47.36	49.00	52.24	55.05	43.20	43.57	46.02	49.58	50.46	45.15
TbwA	150.60	170.57	164.76	272.86	107.68	104.78	119.52	195.10	184.05	125.83
Tb_mt	12.38	12.67	12.37	12.84	12.95	12.99	12.90	13.27	12.57	13.02
mt_as	5.71	5.46	4.62	3.74	5.20	4.88	4.36	3.68	5.01	4.62
ACD	6.01	6.11	5.81	5.75	5.92	5.83	5.79	5.72	5.95	5.82
GSA	5.20	5.45	5.69	5.31	5.28	5.42	5.72	5.19	5.42	5.41
HLD	6.03	6.01	5.81	5.63	5.67	5.93	5.86	5.57	5.90	5.78
KAW	7.30	7.33	6.96	7.13	7.20	7.32	7.22	7.22	7.20	7.25
KWD	5.69	5.74	5.57	5.35	5.48	5.68	5.58	5.33	5.62	5.54
STR	5.97	6.28	6.42	6.45	6.57	6.45	6.63	6.66	6.27	6.56

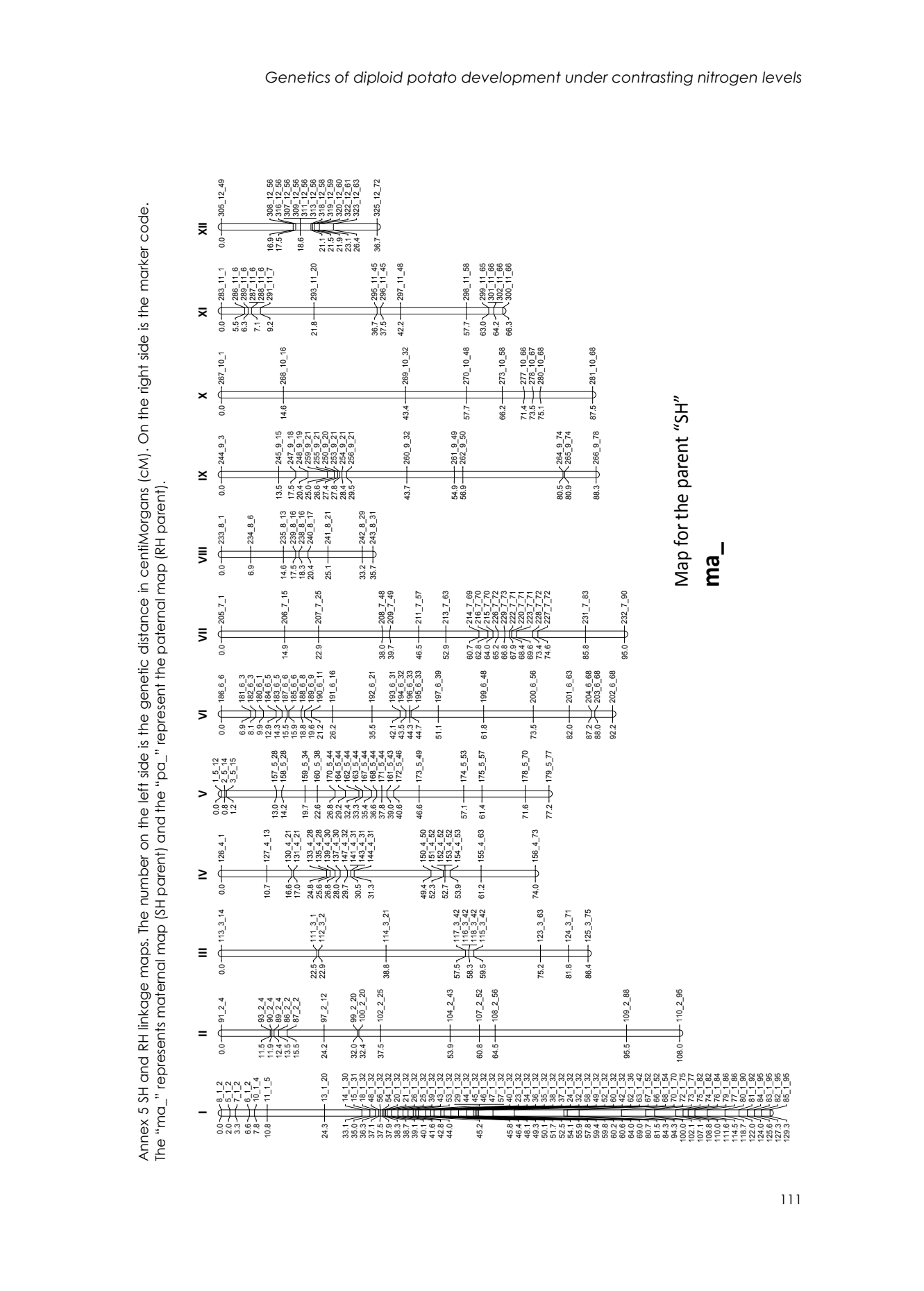
Annex 3 Linear regression parameters for yield values between high and low N inputs per maturity group. E_E=early, M_E=middle early, M_L=middle late, and L_L=late.

Maturity groups	Correlation	Slope	Intercept	R2	Std dev N H	Std dev N L	Mean N 1	Mean N2
E_E	0.888	0.692	0.148	0.788	0.222	0.186	0.723	0.642
M_E	0.896	0.610	0.189	0.803	0.177	0.121	0.799	0.676
M_L	0.816	0.528	0.292	0.665	0.210	0.136	0.926	0.781
L_L	0.783	0.720	0.097	0.614	0.193	0.177	0.971	0.795
Overall	0.887	0.632	0.184	0.788	0.200	0.147	0.853	0.723

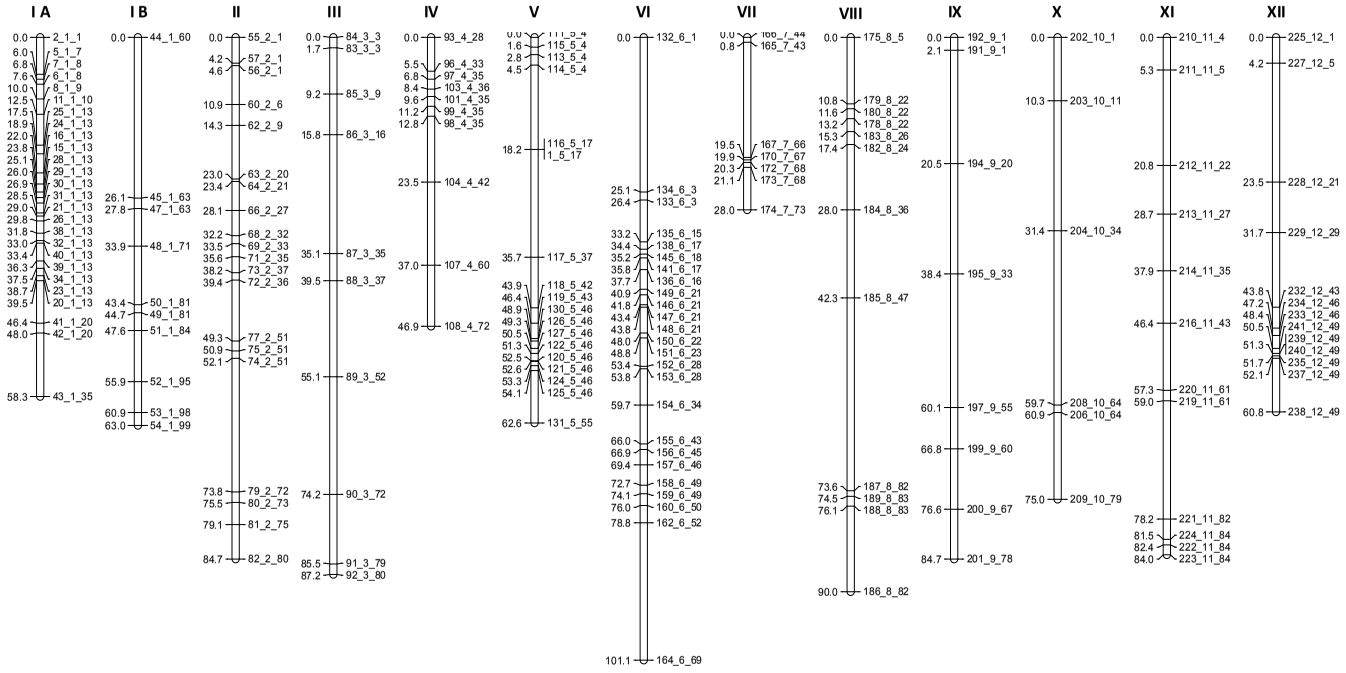
Annex 4 Quantitative trait loci detected with single trait QTL analysis for agronomic and quality traits under two N levels: H=High and L=Low. The explained variance is shown as a percentage (%VarExpl). For explanation of the acronyms, see Annex 1.

Trait	Marker	Linkage Group	QTL position in cM	N level			
				H		L	
				%VarExpl	-log10(p)	%VarExpl	-log10(P)
tm1	**116_5_17**	pa_V	18.18	28.38	5.65		
t1	**196_6_33**	ma_VI	44.31	13.93	3.32		
Vx	**109_2_88**	ma_II	95.54	12.49	3.66		
	114_5_4	pa_V	4.53	21.02	4.84		
	116_5_17	pa_V	18.18			19.33	3.87
t2	**115_3_42**	ma_III	59.52			15.27	3.51
	116_5_17	pa_V	18.18	46.53	9.77		
te	**116_5_17**	pa_V	18.18	58.76	13.96	43.11	8.93
	153_6_28	pa_VI	53.8	7.99	3.50		
Cm	**197_6_39**	ma_VI	51.12	21.28	4.98		
AP1	**116_5_17**	pa_V	18.18	18.70	3.75	24.28	4.83
AP2	**116_5_17**	pa_V	18.18	32.50	6.52		
AP3	**116_5_17**	pa_V	18.18			46.84	10.23
	51_1_84	pa_I_B	47.64			10.74	3.69
AUC	**116_5_17**	pa_V	18.18	54.58	11.93	45.82	9.59
t2-t1	**116_5_17**	pa_V	18.18	26.05	5.18		
te-t2	**116_5_17**	pa_V	18.18			39.04	8.38
	51_1_84	pa_I_B	47.64			12.08	3.78
DM%	**111_3_1**	ma_III	22.51	17.71	4.60	18.24	4.70
	208_10_64	pa_X	59.71	13.55	3.78	12.76	3.59
Y_DM	**116_5_17**	pa_V	18.18	17.50	3.52		
[N]	**247_9_18**	ma_IX	17.51	13.81	3.32		
	248_9_19	ma_IX	20.39	16.43	3.91		
NUpt	**116_5_17**	pa_V	18.18	19.69	3.94		
TbnMX	**107_4_60**	pa_IV	37.04	15.20	3.53	11.77	3.22
	228_12_21	pa_XII	23.54			15.70	3.69
TbnB	**127_4_13**	ma_IV	10.69	13.82	3.25		
	98_4_35	pa_IV	12.84			16.16	3.73
TbnA	**116_5_17**	pa_V	18.18			22.50	4.48
	131_4_21	ma_IV	17.03	13.85	3.30		
TbwB	**109_2_88**	ma_II	95.54	14.25	3.79		
	127_4_13	ma_IV	10.69	12.56	3.38		
Tb_mt	**187_6_6**	ma_VI	15.48	13.57	3.72		
	81_2_75	pa_II	79.14	12.83	3.51		
mt_as	**116_5_17**	pa_V	18.18	54.33	13.23	33.19	6.66
	154_6_34	pa_VI	59.74	8.68	3.75		
ACD	**123_3_63**	ma_III	75.23	31.21	7.09	37.36	8.97
	85_1_95	ma_I	129.34			9.66	3.18
HLD	**123_3_63**	ma_III	75.23	27.66	6.23	27.95	6.72
	83_1_95	ma_I	125.64			16.97	4.72
KWD	**123_3_63**	ma_III	75.23	28.93	6.53	32.45	7.71
	83_1_95	ma_I	125.64			14.65	4.31
STR	**117_3_42**	ma_III	57.5	14.12	3.78		
	54_1_99	pa_I_B	63.03	13.63	3.76		

Annex 5 SH and RH linkage maps. The number on the left side is the genetic distance in centiMorgans (cM). On the right side is the marker code. The "ma_" represents maternal map (SH parent) and the "pa_" represent the paternal map (RH parent).



Annex 5 continuation...



Map for the parent “RH”

pa_

Annex 6 Summary for QTL detected at H and low N per linkage group*: P value, +: percentage of explained variance, □: parent form where the high value comes, 1=SH and 2=RH.

Lk. Groups		ma_I			ma_III				ma_V			ma_VI				ma_VIII			ma_IX			ma_X			ma_XI			pa_I_A			pa_I_B			pa_V			pa_VIII			pa_XI		
(cM)		125.6			22.51			75.23				57.12			51.12			87.21			0			0			87.52			9.16			28.46			63.03			18.18			17.42			46.37		
Trait	Nlv	Pv *	%eV +	h_pt □	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt
tm1	H							0.041	4.5	2																										0.000	29	1						
tm1	L							0.034	5	2										0.040	4.3	2							0.011	7	1	0.002	12	2				0.009	7.9	1						
t1	H													0.000	12	2																			0.003	12	1						
t1	L							0.000	14	2																0.016	6.4	1	0.008	7.7	1							0.005	9.4	1						
Vx	H											0.008	7.2	2	0.004	8.1	1																			0.000	34	1						
Vx	L																																			0.000	23	1						
t2	H																																			0.000	52	1						
t2	L	0.010	6.5	2				0.000	21	2																													0.030	6.7	1			
te	H																																			0.000	63	1						
te	L																			0.018	5.7	2															0.000	43	1						
Cm	H											0.035	4.6	2	0.000	21	1																											
Cm	L							0.006	8.4	1																			0.042	4.5	2								0.009	9.6	2	0.035	5.3	2
AP1	H																																			0.000	22	1						
AP1	L							0.011	7.2	2	0.034	4.7	2																							0.000	22	1						
AP2	H													0.021	5.2	1																				0.000	36	1						
AP2	L	0.008	7	2													0.021	5.4	1							0.006	8.4	2														0.043	4.9	2
AP3	H																																			0.009	8.7	1						
AP3	L																			0.020	5.5	2														0.000	41	1	0.049	5.5	2			
AUC	H																																			0.000	62	1						
AUC	L																																			0.000	50	1						
t2_t1	H													0.020	5.2	1																				0.000	29	1						
t2_t1	L	0.010	6.6	2													0.043	4.2	1							0.005	8.8	2																
te_t2	H																																											
te_t2	L											0.014	6.3	1						0.014	6.1	2														0.000	32	1						
DM%	H				0.000	20	1																																					
DM%	L																			0.003	8.8	2												0.002	9.7	1								
Y_DM	H											0.007	7.7	2																						0.000	21	1						
Y_DM	L																	0.008	7	1									0.014	6.5	2						0.000	16	1						
N	H											0.014	6.3	1									0.001	11	2																			
N	L																			0.008	7.2	1												0.016	5.8	2	0.047	4.6	2			0.001	14	2
Nuptake	H													0.028	4.7	1																				0.000	20	1						
Nuptake	L																0.010	6.8	1										0.039	4.6	2						0.002	11	1	0.001	17	2			

Annex 5 continuation...

Lk. Groups		ma_I			ma_III						ma_V			ma_VI				ma_VIII			ma_IX			ma_X			ma_XI			pa_I_A			pa_I_B			pa_V			pa_VIII			pa_XI		
(cM)		125.6			22.51			75.23			57.12			51.12			87.21	0			0			87.52			9.16			28.46			63.03			18.18			17.42			46.37		
Trait	Nlv	Pv *	%eV +	h_pt □	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt	Pv	%eV	h_pt
SCYid	H										0.025	5.2	1																															
SCYid	L																0.009	7	2				0.010	7.3	1	0.007	7.8	1							0.050	4.5	1						
TbnMX	H				0.000	16	1	0.010	7.2	2																																	
TbnMX	L	0.031	4.6	1																																							
TbnB	H				0.012	6.7	2	0.005	8.4	1	0.010	6.9	2																															
TbnB	L																																	0.009	6.8	2									
TbnA	H				0.025	5.3	2	0.004	8.8	1																																	
TbnA	L							0.044	4.4	1							0.037	4.4	2															0.005	7.9	2	0.000	18	1			0.005	9.3	1
TbwMX	H																																			0.013	7.8	1						
TbwMX	L																0.021	5.4	1				0.017	6.3	2																0.024	6.1	2
TbwB	H				0.003	9.4	2	0.002	10	1																											0.003	11	1					
TbwB	L																																	0.002	9.6	2	0.003	10	1			0.044	4.9	1
TbwA	H				0.027	5.2	2																														0.020	6.8	1					
TbwA	L																																	0.004	8.1	2	0.004	9.6	1			0.003	11	1
Tb_mt	H										0.001	11	2	0.001	11	2																										
Tb_mt	L										0.031	4.9	2																0.007	9	1											
mt_as	H																																				0.000	57	2					
mt_as	L							0.038	4.7	1										0.032	4.7	1														0.000	36	2					
ACD	H							0.000	29	2																																	
ACD	L	0.007	7.3	2				0.000	40	2													0.007	8.1	1																		
GSA	H										0.013	6.4	2																														
GSA	L																												0.034	5.5	1	0.022	5.3	1								
HLD	H							0.000	25	2																																	
HLD	L	0.000	13	2				0.000	27	2	0.025	5.3	2										0.002	10	1																		
KAW	H							0.004	8.9	1											0.030	5	2																				
KAW	L																																											
KWD	H							0.000	26	2																																	
KWD	L	0.001	12	2				0.000	31	2													0.001	12	1																		
STR	H							0.001	13	2											0.007	7.8	1													0.016	7.4	1					
STR	L	0.024	5.1	1																												0.008	7	1									

Chapter 5

Association mapping of physiological and morphological traits related to crop development under contrasting nitrogen inputs in a diverse set of potato cultivars

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To be submitted

Abstract

Nitrogen (N) plays an important role in potato development and production. Abundant N is required for the crop to perform well. However, the more nitrogen is applied to a crop, the lower the nitrogen use efficiency (NUE) is, and the higher the negative impact on the environment. Effects of nitrogen input levels on potato are well documented for above-ground and below–ground traits. A previous study on canopy development with a growth model showed cultivar variation and effects of N and maturity type. In this study we assessed whether the variation of model parameters (physiological traits) and agronomic traits under contrasting N levels allows the detection of quantitative trait loci (QTL). For this, phenotypic data collected under two contrasting N supply levels (75 and 185 kg N/ha including soil N + fertiliser N) were used to estimate those physiological traits and to use them in a genome-wide association study (GWAS) with kinship correction. Twenty-four traits and 10,747 markers based on simple nucleotide polymorphism (SNPs) from a 20K Infinium array for 169 cultivars were combined in the analysis. Nitrogen levels affected most traits and most of the relationships between them. We showed how N levels influenced the detection of marker-trait associations; some were N-dependent while others could be detected at both N levels. Ninety percent of the N-independent associations accumulated on a hotspot on chromosome 5. Other regions with multiple associations were identified on chromosomes 2 and 4. After maturity correction of the phenotypic data, the only N-dependent QTL that remained were for soil coverage yield index and the tuber size class with the maximum tuber number (SCYi and TbnB respectively). Furthermore only the region on chromosome 2 accumulated several QTL. This confirmed the major role that maturity type plays in canopy development. The results indicate the potential to integrate marker assisted selection (MAS) for maturity type in breeding schemes, with the main purpose of improving characteristics within a narrow range of maturity types. Then the objective will be to break the strong links between maturity type and traits like dry-matter content, nitrogen content and nitrogen use efficiency.

Keywords: Potato, N effects, Canopy development, Association mapping, Maturity type.

Introduction

In potato cropping, farmers often abundantly apply nitrogen (N) fertiliser to ensure profits because potato plants are highly responsive to extra N (Harris 1992). This practice reduces the nitrogen use efficiency (NUE) of the crop (Ospina et al. 2014; Zebarth et al. 2004), which is already rather low because of a shallow root system and the common cultivation in sandy soils. Leaching of excess N causes eutrophication of ground and surface water and is therefore a serious threat to the environment in potato production. Governmental regulations limiting the N supply have been installed and these make it necessary to improve N use efficiency at lower levels of input. Moreover, the N fertiliser regulations are specified for maturity types, at least in the Netherlands (CDM, 2012), emphasizing the need to incorporate the effects of maturity type in NUE studies.

Effects of nitrogen availability on above-ground and below-ground crop development have been widely studied. High N input increases individual leaf size and leaf longevity (Biemond and Vos 1992; Vos and Biemond 1992), promotes branching (Oliveira 2000) and therefore supports a sustained leaf production, which enlarges the period of full soil cover (SC) (Haverkort and MacKerron 2000; Vos 1995). Therefore, the crop intercepts more solar radiation and accumulates more dry matter with more N (Haverkort et al. 1991), all resulting in higher yield, but lower NUE (Ospina et al. 2014; Zebarth et al. 2004).

An increase in nitrate available for the plant was reported to lead to a reduction of the proportion of dry matter allocated to roots but also to an increase in the total root surface and root length (Sattelmacher et al. 1990). These authors also attribute differences in N uptake efficiency of two cultivars to the general differences in root morphology and to a particular N response of the cultivars. Moreover, high N availability tends to suppress or delay tuber bulking and to affect dry matter partitioning between haulm and tubers (Biemond and Vos 1992). Additionally, N input also affects tuber size and quality parameters, including tuber dry matter content, tuber starch content, tuber protein content, tuber nitrate content, and processing quality (Tiemens-Hulscher et al. 2014; Zebarth et al. 2004). With more N the proportion of large tubers was shown to increase, the fry colour to become darker, while the effect on tuber dry matter content was ambiguous (Bélanger et al. 2002; Zebarth and Rosen 2007; Zebarth et al. 2004).

Studies on canopy cover have shown a high correlation between the ability of genotypes to intercept photosynthetically active radiation and to create tuber yield (Haverkort et al. 1991; Vos 2009). Khan (2013; 2012) studied the canopy development (CDv) of potato using an ecophysiological model in which canopy growth is a function of thermal time, following the beta function as described by Yin et al. (2003). This methodology allows the dissection of the complex trait of canopy growth into model parameters with biological meaning (Struik 2005; Yin and Struik 2008; Yin et al. 2004). The analysis of the curve parameters as new traits allowed to capture differences in N response among cultivars, maturity types, and among cultivars within the same maturity class, facilitating the understanding of the N effects on different stages of CDv (Khan et

al. 2014; Khan 2012; Ospina et al. 2014). Furthermore, those canopy cover traits had high heritabilities (Khan 2012), and some of them showed high correlations with yield, maturity and N content, allowing an interpretation of how NUE of potato is affected and showing potential as selection criteria for NUE (Khan 2012; Ospina et al. 2014; Tiemens-Hulscher et al. 2014). In addition, these parameters were found to be related to genetic factors (quantitative trait loci; QTL) that act during development of the canopy cover and are probably involved in the underlying physiological processes (Chapter 4). The combination of this ecophysiological growth model and QTL analysis is a two-step approach where the first step is to model the complex trait identifying biologically relevant parameters demonstrating genetic variation and the second step is to use these parameters as new traits to find QTL (Malosetti et al. 2006; Yin et al. 2005). In potato the two-step procedure was used to study the dynamics of senescence and the adaptation in potato under different day lengths (Hurtado 2012), and to identify QTL related to canopy cover parameters (Khan 2012) as well as QTL related to the N effects on the canopy cover parameters in a diploid mapping population (Chapter 4 of this thesis).

In recent years, association mapping approaches have become more and more popular for genetic studies, offering a series of advantages that include higher mapping resolution and results that are applicable to a wider genetic background (Zhu et al. 2008). Association mapping (AM) identifies QTL by examining marker-trait associations resulting from linkage disequilibrium (LD) between markers and trait functional polymorphisms across a set of diverse germplasm (Zhu et al. 2008). AM copes better with tetraploid, non-inbred crops, like potato (Li et al. 2010) than linkage analysis using segregating biparental tetraploid populations for which tetrasomic inheritance is complicated (Luo et al. 2001). AM can detect QTL at the tetraploid level within a genetic background that is more representative of the breeding germplasm of the crop (Malosetti et al. 2007). Moreover, AM procedures can effectively compare a greater portion of the variation within a species while the traditional linkage analysis is limited to the variation in the two parents of the segregating population (Wang et al. 2008). However, in AM it is important to consider the effect of population structure and/or kinship because any association may partially be caused by population admixture, leading to plausible but false marker/trait associations (Wang et al. 2008; Yu et al. 2005; Zhu et al. 2008). The success of association mapping efforts depends on the possibilities of separating LD due to genetic linkage from LD resulting from other causes (Malosetti et al. 2007).

Several papers reported on association mapping studies in tetraploid potato. Gebhardt et al. (2004) and Simko (2004) reported markers associated with resistance to diseases using a form of *t*-test. Malosetti et al. (2007) proposed an AM approach based on mixed models with attention for the incorporation of the relationships between genotypes, whether induced by pedigree, population substructure, or otherwise. D'hoop (2008) applied a simple regression based AM approach for quality traits in potato with promising results for these traits in a large set of tetraploid cultivars. In this chapter we combined the model for canopy development and the association analysis to study the genetic basis of developmental physiological and agronomic traits in relation to N contrasting levels. We performed genome-wide AM for canopy development parameters

and agronomic traits in a set of 169 tetraploid potato cultivars. Our cultivar set was phenotyped and studied for canopy development under contrasting N levels. In addition to effects of environmental factors we observed genetic variation in the canopy development traits and in agronomic traits (Ospina et al. 2014), as required for a genome-wide association analysis. Moreover, we analysed N-dependence of the detected QTL to show the genetic response to such an important factor and to demonstrate the usefulness of the canopy development analysis in combination with genetics studies.

Materials and methods

The experimental design, data collection, and processing to generate the phenotypic information used in this paper were described in detail by Ospina et al. (2014). Therefore a brief description suffices here.

Location and planting material

Experiments were carried out at the Agrico research and breeding station (Bant, Flevoland, The Netherlands), in 2009 and 2010. We used a set of 189 cultivars representing the commercial potato gene pool in Europe (Supplementary material: Annex 1). The set has been extensively used for association studies of quality traits as described by D'hoop et al. (2008; 2010).

Experimental design and treatments

In both experiments, two N levels were applied i.e. i) High N, with 180 kg available N/ha (soil N and fertiliser N combined) as a standard conventional N input level, and ii) Low N, with 75 kg available N/ha as the low input variant. The amount of fertiliser required was calculated based on soil analysis done at the beginning of the growing season. Fertiliser application was split in two: a basic fertiliser treatment (N-P-K) was applied just after planting on the whole experimental field to reach the amount for low N. A second amount was applied to the high N plots only, before the final ridging, using Dolomite-ammonium nitrate DAN (27-0-0). P and K were abundantly available for potato crop growth in both N treatments.

The experimental design was an unbalanced split-plot design, with 16 plants per genotype per field plot, with treatments (N) as whole-plots (with no replicates), maturity groups as sub-plots randomized within whole-plots and cultivars nested and randomized within maturity sub-plots. In addition, sixteen (2009) or twenty (2010) field plots with a reference cultivar were planted at random across the field to estimate the plot-to-plot environmental variation without confounding to cultivar variation.

Data collection

Emergence date was estimated per plot as the first date when more than 50% of the plants in the plot had emerged (i.e., first leaf visible). The percentage of soil cover (SC) was assessed weekly

over three plants per plot all through the growing season from emergence until harvest. Maturity was scored using a scale to assess the progress of senescence (Celis Gamboa 2002, modified) in which 1 = green canopy with the first flower buds, 2 = green haulm with abundant flowers, 3 = first signs of yellowness in the upper leaves, 4 = up to 25% of the plant with yellow leaves, 5 = up to 50% of the plant with yellow leaves or lost leaves, 6 = up to 75% as in 5, 7 = up to 90% of the plant yellowed or without leaves, and 8 = entire haulm brown or dead. This assessment is referred to as maturity assessment (mt_as) to avoid confusion with the maturity index used to form maturity groups as blocking factor (Mt).

Final harvest

The final harvest took place as late as possible to allow late cultivars to complete their cycle. The whole experiment was harvested at once. Sixteen plants were harvested per plot and the following tuber traits were assessed: A) Total tuber fresh weight. B) Tuber size and weight distribution in six different classes i.e. 0-30 mm, 30-40 mm, 40-50 mm, 50-60 mm, 60-70 mm, and > 70 mm. For each size class the tuber number and tuber weight were recorded. C) Tuber number per meter for the class 50-60 mm. D) Dry matter percentage (DM%), as dry weight of a sample divided by its fresh weight expressed in percentage. Tubers from all size classes were cut with a French fries cutting machine before drying at 70 °C for 48 hours. E) N content ([N]) in the tubers was assessed using the Kjeldahl protocol.

Data processing

A canopy development model was fitted using the NOLIN procedure of SAS/STAT®, with percentage soil cover as the dependent variable of Beta thermal time counted from emergence day until each assessment date. Five parameters were estimated for each individual plot (Khan 2012; Khan et al. 2013). Four *t*-parameters were expressed in thermal days (td): *tm1* (inflection point in the growing phase of the curve), *t1* (when SC stabilized), *t2* (start of senescence), and *te* (when canopy had completely senesced). The fifth parameter, *Vx*, was the maximum SC reached with percentage soil coverage (%SC) as unit.

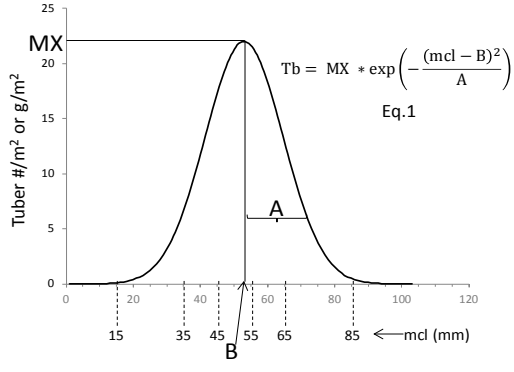

Figure 1 Bell shaped curve and parameter representation of equation 1 (Eq. 1). Parameter names are explained in the text, section Data processing.

A bell-shaped curve was fitted per plot for tuber weight and tuber number data sets separately (Tbw and Tbn respectively) to describe their distribution. Three parameters were estimated for each data set following the equation Eq. 1. (see Figure 1).

Where Tb is either Tbw or Tbn, "A" is a dispersion parameter expressing how the weights/numbers were distributed across tuber size classes, "mcl" is the average size of each tuber size class; "B" is the average size at which the maximum ("MX") weight/number occurs. The curve-fit parameters were named for each variable as follows: for Tbw data: TbwA, TbwB, TbwMX and for Tbn data: TbnA, TbnB, TbnMX.

Calculated variables

Based on the parameters estimated with the CDv model, the following variables were calculated (Khan 2012; Khan et al. 2013): *t2-t1* (duration of maximum SC in td), *te-t2* (duration of senescence in td), *Cm* (maximum progression rate of %SC in %/td), *AP1* (area under the curve for canopy build-up phase in %.td), *AP2* (area under the curve for phase of maximum SC in %.td), *AP3* (area under the curve for senescence phase in %.td), and *AUC* (area under the curve for the entire crop cycle in %.td). In order to express the agronomic variables in a standard way, subsequent calculations and conversions were done as: N content ([N]) in g/kg (determined only in tubers), DM% in percentage. Dry matter yield (Y_DM) in kg/m^2, which is Y×DM%/100. N uptake in tuber (NUpt) in g/m^2, which is Y_DM × [N]. N use efficiency (NUE) as Y_DM/(N input) in kg/g. N utilisation efficiency (NUtE) that is Y_DM/NUpt, in kg/g. N Uptake efficiency (NUptE; NUpt/N input in g/g). Soil coverage yield index (SCYi=*AUC*/ Y_DM in %.td/(kg/m^2)). The variables were analysed without transformation since there were no severe violations to the assumptions required for mixed model analysis. (All trait acronyms are summarised in Annex 5).

Statistical analysis

Data were analysed with the Genstat package (16th edition). The model in Eq. 2 combining information of both years was used for each N level.

$$Y = yr * Mt + Mt.G + \underline{yr.row} + \underline{yr.col} + \underline{E}$$

Eq. 2

Where terms joined by "*" represent individual effects plus the interactions (*yr*Mt= yr+Mt+yr.Mt*), whereas terms joined by "." represent interaction only. The term *yr* represents year, clarifying that year effects include variation due to the experimental field. The term *Mt* is the maturity group excluding control plot information. Corrections for rows and columns are the random terms (*yr.row* and *yr.col*). The term *Mt.G* represents the cultivars nested within maturity groups, since maturity is an intrinsic characteristic of each cultivar. Finally *E* represents the error. All random terms are underlined.

The genetic correlations between traits were estimated as the Pearson correlation based on the estimated genotypic means BLUEs i.e. best linear unbiased estimates (excluding all other terms in

Eq. 2). In addition, in order to understand relationships between traits and to define groups of traits, hierarchical cluster analysis was done using these genetic correlations. We excluded traits for which calculation included N input level, i.e., NUE, NutE and NUptE. Additionally, biplots were generated to visualize relationships between traits per N level, included as Supplementary material (Annex 2).

Association mapping

The analysis included the 169 potato cultivars out of the total set of 189 cultivars for which genotypic data were available. SNP data were generated using a 20K Infinium SNP array (Vos et al. 2015). 14,587 markers were successfully scored in (a maximum of) 5 dosage classes per SNP using fitTetra (Voorrips et al. 2011). The dosage classes are nulliplex, simplex, duplex, triplex and tetraplex depending on the number of copies of the allele being quantified (0 to 4). Only SNPs having allele frequencies greater than 5% in at least two of the dosage classes were considered. Therefore a total of 10,747 SNPs were used to perform the GWAS.

The GWAS was performed using a mixed model including a kinship matrix to account for population structure. The kinship matrix was estimated using 764 SNPs markers randomly distributed over the genome. The mixed model including Kinship was:

$$\underline{Trait\ (y)} = Marker\ (m) + \underline{genotype} + \underline{residual}\ where\ var(\underline{genotype}) = K\sigma_g^2\ and\ K = Kinship\ matrix$$

Eq. 3

Linkage disequilibrium between markers has been extensively studied by Vos et al. (to be submitted) and D'hoop et al. (2010) using the same cultivar set. From that study, the linkage disequilibrium decay was estimated between 2 to 4 Mb. We considered LD as 4 Mb and a window of 8 Mb i.e. the apposition of a marker + & - 4 Mb).

The association analysis was done using fitted values for the observations, (BLUPs i.e. best linear unbiased predictions) using the model of Eq. 2. Four phenotypic datasets corresponding to combinations of two years and two N levels were the input in this analysis. In the results section we only consider associations with a $-\log10(p) > 4$. Then the focus was to find marker-trait associations consistent across the two years, for each N level. Next, we compared results from the two N levels defining associations detected at both N levels as common (cmn), and exclusive either for high N (HN) or for low N (LN). The last two categories are considered N-dependent marker-trait associations.

Since maturity is known to have a strong effect on the traits considered in this study (Khan 2012; Ospina et al. 2014) BLUEs excluding the main effect of maturity group were calculated. From each estimated value the effect of the maturity class was subtracted, and these maturity type-corrected BLUEs were used as input in the association analysis with population structure correction.

Results

Phenotypic data

The phenotypic dataset used for the association analysis was collected and analysed as described by Ospina et al. (2014). Here we investigated the genotypic correlations between all traits at high N, low N, and across N input levels (Figure 2). As the correlations were calculated based on estimated genotypic main effect values, these are effectively genetic correlations (i.e. after excluding the effects due to other terms in the model Eq. 2). A summary of data for all traits per N level is included in the Supplementary material (Annex 3).

Genetic correlation matrices for both high and low N conditions are shown in Figure 2 (corresponding to the right upper and left lower triangles, respectively). A Mantel test to compare the two genetic correlation matrices showed a high and positive association between the Pearson correlations under high and low N (Mantel test r=0.9384, significance=0.001), which was also reflected in similar grouping of the traits in a hierarchical clustering using Pearson correlation as similarity measure (See dendrograms in Figure 3). However, there were slight differences between the clustering results at each N level.

	TbwA	TbnA	TbnMX	TbwB	TbnB	DM%	[N]	*AP2*	*t2_t1*	*t2*	mt_as	*te*	*AUC*	TbwMX	*Vx*	Y_DM	NUpt	*Cm*	*t1*	*AP1*	*AP3*	*te_t2*	*tm1*	SCYi
TbwA	**0.52**	0.84	-0.34	0.65	0.49	-0.10	-0.07	0.18	0.17	0.18	-0.23	0.25	0.24	0.33	0.16	0.20	0.22	-0.11	0.09	0.12	0.11	0.09	0.04	0.02
TbnA	0.71	**0.64**	-0.48	0.77	0.61	-0.10	-0.07	0.17	0.17	0.21	-0.27	0.27	0.25	0.27	0.16	0.17	0.20	-0.14	0.14	0.16	0.12	0.09	0.05	0.07
TbnMX	-0.44	-0.57	**0.87**	-0.67	-0.66	0.16	-0.17	0.22	0.20	0.15	-0.09	0.08	0.18	0.15	0.20	0.17	0.09	0.10	-0.03	0.01	-0.04	-0.07	-0.07	-0.03
TbwB	0.57	0.77	-0.75	**0.83**	0.90	-0.07	-0.04	0.14	0.15	0.18	-0.25	0.27	0.20	0.43	0.04	0.20	0.26	-0.09	0.11	0.10	0.11	0.11	0.06	-0.07
TbnB	0.38	0.60	-0.70	0.96	**0.81**	-0.09	-0.02	0.08	0.10	0.15	-0.20	0.18	0.11	0.38	-0.05	0.16	0.21	-0.13	0.14	0.12	0.00	0.02	0.01	-0.11
DM%	-0.10	-0.06	0.19	-0.01	0.04	**0.94**	-0.64	0.53	0.51	0.62	-0.59	0.53	0.61	-0.04	0.39	0.59	0.32	-0.30	0.38	0.42	-0.03	-0.11	0.05	-0.09
[N]	-0.02	-0.11	-0.26	-0.13	-0.16	-0.67	**0.76**	-0.64	-0.61	-0.70	0.68	-0.61	-0.71	-0.23	-0.46	-0.75	-0.27	0.29	-0.38	-0.46	0.03	0.12	0.01	0.24
AP2	0.03	0.08	0.22	0.15	0.18	0.32	-0.56	**0.66**	0.99	0.89	-0.74	0.57	0.83	0.33	0.61	0.78	0.60	0.03	0.13	0.23	-0.32	-0.42	0.02	-0.14
t2_t1	-0.01	0.04	0.16	0.13	0.16	0.31	-0.48	0.96	**0.58**	0.88	-0.72	0.55	0.79	0.32	0.50	0.75	0.57	0.02	0.09	0.17	-0.35	-0.43	0.02	-0.15
t2	0.04	0.14	0.22	0.22	0.25	0.58	-0.71	0.74	0.70	**0.81**	-0.90	0.70	0.87	0.31	0.56	0.79	0.55	-0.36	0.55	0.60	-0.30	-0.40	0.12	-0.05
mt_as	-0.14	-0.22	-0.17	-0.29	-0.31	-0.61	0.75	-0.55	-0.46	-0.86	**0.89**	-0.83	-0.89	-0.34	-0.57	-0.77	-0.56	0.42	-0.62	-0.65	0.01	0.11	-0.21	-0.03
te	0.12	0.19	0.21	0.21	0.22	0.60	-0.71	0.51	0.45	0.77	-0.91	**0.80**	0.88	0.31	0.41	0.66	0.45	-0.37	0.49	0.50	0.42	0.35	0.26	0.14
AUC	0.11	0.20	0.27	0.23	0.25	0.56	-0.77	0.73	0.60	0.86	-0.88	0.87	**0.88**	0.37	0.71	0.84	0.63	-0.22	0.43	0.53	0.14	0.00	0.09	0.01
TbwMX	-0.34	-0.14	0.43	0.02	0.14	0.13	-0.51	0.56	0.45	0.51	-0.51	0.43	0.61	**0.55**	0.28	0.48	0.52	-0.04	0.09	0.16	0.04	-0.01	-0.14	-0.31
Vx	0.10	0.19	0.23	0.19	0.20	0.27	-0.53	0.52	0.29	0.53	-0.53	0.44	0.77	0.59	**0.65**	0.67	0.61	0.05	0.31	0.48	0.01	-0.20	-0.02	-0.06
Y_DM	0.09	0.19	0.23	0.28	0.31	0.57	-0.82	0.66	0.55	0.75	-0.81	0.73	0.87	0.75	0.69	**0.91**	0.83	-0.15	0.33	0.45	-0.04	-0.17	-0.08	-0.47
NUpt	0.04	0.16	0.14	0.31	0.35	0.31	-0.38	0.49	0.40	0.52	-0.55	0.47	0.64	0.73	0.61	0.82	**0.66**	0.02	0.15	0.27	-0.03	-0.15	-0.13	-0.51
Cm	-0.04	-0.11	-0.09	-0.13	-0.14	-0.45	0.40	-0.02	-0.03	-0.60	0.60	-0.54	-0.38	-0.12	-0.04	-0.35	-0.18	**0.62**	-0.77	-0.73	0.02	0.02	-0.07	-0.10
t1	0.08	0.16	0.15	0.19	0.21	0.50	-0.54	0.12	0.01	0.71	-0.77	0.67	0.64	0.30	0.46	0.54	0.34	-0.81	**0.70**	0.96	-0.02	-0.09	0.22	0.17
AP1	0.10	0.19	0.19	0.22	0.24	0.50	-0.62	0.22	0.07	0.73	-0.78	0.68	0.74	0.41	0.63	0.65	0.45	-0.71	0.97	**0.65**	-0.03	-0.14	0.03	0.08
AP3	0.14	0.15	0.10	0.07	0.05	0.18	-0.24	-0.09	-0.20	-0.04	-0.34	0.55	0.39	0.15	0.31	0.29	0.23	0.00	0.18	0.23	**0.18**	0.97	0.10	0.24
te_t2	0.11	0.06	0.03	-0.02	-0.04	0.10	-0.07	-0.28	-0.32	-0.25	-0.17	0.42	0.10	-0.07	-0.08	0.04	0.00	0.03	0.01	-0.01	0.90	**0.20**	0.12	0.25
tm1	0.01	-0.08	0.01	-0.14	-0.18	-0.24	0.24	0.01	0.00	-0.19	0.17	-0.16	-0.17	-0.14	-0.02	-0.24	-0.20	0.26	-0.24	-0.32	0.01	0.01	**0.47**	0.39
SCYi	0.05	-0.02	0.00	-0.12	-0.15	-0.10	0.21	-0.04	-0.06	0.06	-0.04	0.15	0.07	-0.38	0.03	-0.39	-0.47	-0.07	0.15	0.08	0.14	0.13	0.30	**0.70**

Figure 2 Heat-map of Pearson correlations between traits using genotypic values: correlations at high N are in the upper triangle, correlations at low N are in the lower triangle. The diagonal contains the correlations for each trait between high and low N. The trait order was defined by cluster analysis using the HN correlation matrix. For an explanation of the acronyms of the traits, see paragraphs "Data analysis" and "Calculated traits" in the Material and Methods section.

Looking at one trait at a time, the highest correlation at each N level is between the same traits. Exceptions were *tm1*, *t2*, *te,* TbwMX, TbnA and mt_as. For each of these traits the highest correlation (to whichever other trait) was different for low N and high N. In general, with the diagonal in the correlation matrices excluded, there were 169 out of 552 combinations with absolute correlation coefficients lower than 0.4 at both N levels (using 0.4 as threshold, i.e. equivalent to a significance level smaller than 3.5×10^{-10} to prove correlation is different from 0, to

describe the matrix Figure 2). Additionally, there were more pairwise correlations with absolute values higher than 0.4 at low N than at high N showing the overall effect of N on trait relationships.

The diagonal of the matrix (Figure 2) shows the correlation coefficients between N levels for each trait. *AP3* and *te-t2* were the least consistent traits across N levels with very low values (0.18 and 0.20, respectively). The traits showing the highest positive correlations between N levels were DM%, Y_DM, mt_as, *AUC* and TbnMX. So the expected interaction of these traits with N level across the cultivar set is lowest.

Looking at hierarchical clustering of traits (Figure 3); Yield (Y_DM) was grouped closer (more similar) to the CDv parameters *AUC, t2, te,* and to mt_as (maturity), [N] and DM% at low N than at high N input level. At high N level NUpt, *Vx* and TbwMX were closer to Yield. Furthermore, there were five traits clustering together at both N conditions. This group included all the traits from tuber size-weight and size-number distribution but not TbwMX (left hand side in both HN and LN hierarchical trees in Figure 3), all being highly correlated to each other, as they describe the same phenomenon i.e. tuber size distribution.

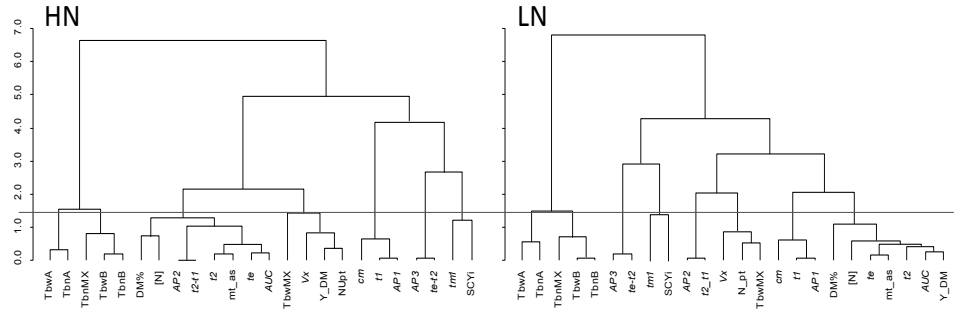

Figure 3 Hierarchical cluster analysis of the traits at both N levels (HN and LN, high and low N, respectively). 1 minus the absolute correlation between each pair of traits was considered as measure of dissimilarity. For an explanation of the acronyms of the traits, see "Data analysis" and "Calculated traits" sections. The blue line is an arbitrary threshold at which clusters of traits resulting from the two dendrograms are compared.

The box plots (Figure 4) illustrate the variation between maturity groups at both N levels for selected traits. The differences between maturity groups (Mt) were not significant for the traits *tm1*, *AP3*, SCYi and TbwA (Annex 4; *AP3* is shown as an example in Figure 3). [N] is an example of a trait for which the differences between Mt were significant, supported by a positive and high correlation with the maturity assessment (mt_as). *AUC* and Yield also were significantly affected by Mt, with a negative correlation with mt_as.

The effect of N level was significant for most traits except *AP1*, *te-t2*, DM%, SCYi, and TbnMX (see also Annex 3). In Figure 4 DM% is shown as an example of a trait that was unaffected by N levels. [N], *AUC* and Y_DM were strongly influenced by N input. Moreover, *AUC*, which is a parameter accumulating temporal and spatial progression of canopy development (that is directly linked to the amount of intercepted light and therefore photosynthetic potential over the whole growth

cycle) was highly positively correlated with yield at both N levels. More N input increased the growing period and promoted vegetative growth, and this may support higher yields, provided the growing season is long enough for the potato tuber yield to benefit from the prolonged canopy development. Finally, the tuber size with maximum weight (TbwB) was significantly affected by N but not by Mt and is a representative of a group of traits that behaved consistently different than other traits levels included in the analysis at both N levels (Figure 2).

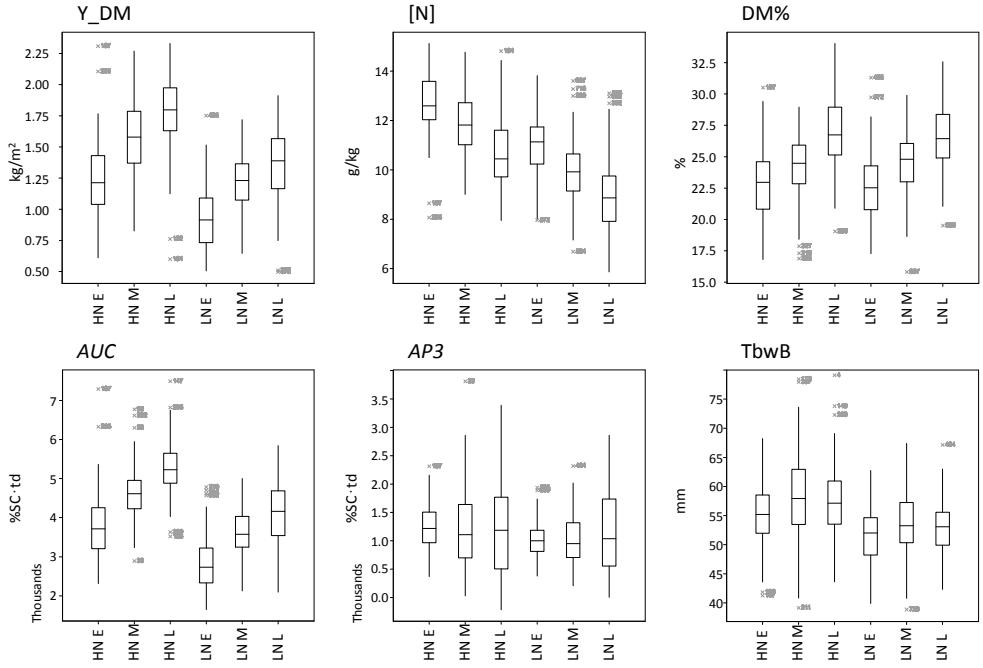

Figure 4 Boxplots of some traits to illustrate the data variation between maturity groups and nitrogen levels. The grouping factor on the X axes is a combination of N level and maturity group as HN (High Nitrogen) in combination with the maturity group HN E (early), HN M (middle), and HN L (late). LN (Low Nitrogen) in combination with the maturity group LN E (early), LN M (middle), and LN L (late). The traits included are Y_DM yield dry matter, [N]: nitrogen content, DM%: dry matter percentage in tubers, *AUC*: area under the curve for canopy development, *AP3*: area under the curve for the phase 3 of CDv (canopy decay) and TbwB: size tuber class where the maximum tuber weight occurs.

Association mapping

The association mapping was performed with kinship correction to minimize false positive associations. The marker-trait associations reported here had –log10(p) values greater than 4 and explained at least 10% of the variance. The results of the association mapping are presented as marker-trait associations to generally describe the output of the analysis, to have an overall impression of the N level effect on the detection of associations in our dataset and to assess the co-localization of association with markers related to maturity. QTL were defined using a linkage disequilibrium window of 8 Mb as mentioned in the Materials and Methods section.

An overall summary is shown in Table 1A. The majority of the marker-trait associations were year dependent, with only 166 associations (out of 950) detected in both years, reflecting a strong influence of environmental conditions. We focused on marker-trait associations detected in both years to compare the results for high and low N levels. In general, more marker-trait associations were detected under high N input than under low N.

Table 1 Number of marker-trait associations detected by genome-wide association analysis using data not corrected (A) and corrected for maturity (B). The correction was done as explained in the Material and Methods section. The associations included fulfilled the criteria of having -log P value > 4 and explained variance > 10%.

A) Data not corrected for maturity

Associations	Detected in at least one data set				Detected in both years			Marker maturity related (%) [5]	
	Mk_T_set[1]	Mk_T[2]	Mk[3]	T[4]	Mk_T	Mk	T	Mk_T	Mk
Total	950	601	282	24	166	74	20	53.0	27.0
Common to High N & Low N					50	19	8	88.0	68.4
N dependent to									
High N					69	42	14	44.9	31.0
Low N					47	33	12	27.7	33.3

B) Data corrected for maturity

Associations	Detected in at least one data set				Detected in both years			Marker maturity related (%)	
	Mk_T_set	Mk_T	Mk	T	Mk_T	Mk	T	Mk_T	Mk
Total	348	233	181	24	86	67	17	2.3	3.0
Common to High N & Low N					8	8	3	0.0	0.0
N dependent to									
High N					48	42	12	4.2	4.8
Low N					30	21	13	0.0	0.0

[1]marker trait set association, this count considers all marker-trait associations from different data sets (there are 4 data sets from the combination of year and N level). [2]marker trait, here the same marker-trait association over different sets is counted as 1. [3]marker is the number of markers involved in a given count of marker-trait associations. [4]trait is the number of traits involved in a given count of marker-trait associations. [5]percentage of markers showing association with the maturity trait (mt_as).

Overall, twenty traits showed associations that were present in both years (irrespective of the N levels). A QTL for maturity assessment (mt_as) in our experiment was detected in the region on chromosome 5 reported as maturity-related in the literature (Celis-Gamboa 2002; Collins et al. 1999; Hurtado et al. 2015; Kloosterman et al. 2013; Visker et al. 2003). This region was an association hotspot with 11 traits associated (*AP1, AP2, AUC*, mt_as, [N], *t1*, *t2*, *t1-t2*, *te*, *te-t2*, and Y_DM). On chromosome 2, there was another region accumulating associations for six traits (SCYi, *AP1*, *t1* TbnA, TbwA, and TbwMX), while there was a region on chromosome 3 with markers associated to mt_as, TbnA, TbnB, TbnMX, and TbwB (Figure 5).

Trait associations detected at both N levels (Table 1A) Common to high N & low N) were considered N-independent associations. Eighty-eight percent of these associations were with markers also associated with mt_as within a window of 8 Mb (see Material and Methods) on chromosomes 5 or 3. Eleven QTL for eight traits were N-independent (Table 2). Six of these QTL (for

AP2, *AUC*, [N], *t2*, *te*, and *te-t2*) were located on chromosome 5. The other two traits were SCYi (with QTL on chromosomes 1, 2, and 11) and TbnB (with a QTL on chromosome 3).

Marker trait associations detected at only one N level were considered N-dependent associations. At high N, 45% of these associations involved mt_as associated markers (on chromosome 5 and 3), all within the LD window of 8 Mb. Eleven traits with 24 QTL were exclusively detected at high N (Table 2), and 9 of these traits (*AP1*, SCYi, *t1*, *t2-t1*, TbnMX, TbwA, TbwMX, *tm1* and Y_DM) did not have QTL co-localizing with maturity assessment (Table 1A, Table 2).

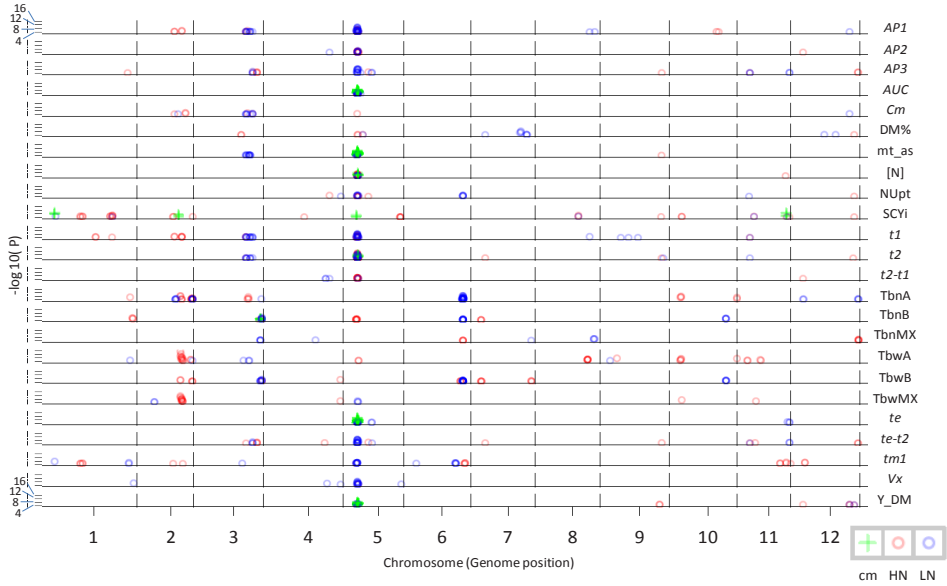

Figure 5 Visualisation of genome-wide association analysis (data not corrected for maturity). cmn= common associations between N levels (green +), HN = associations at high nitrogen (red circle), and LN = association at low nitrogen (blue circle). All traits are included, and only associations detected in both years with –log10(p) value greater than 4 are shown. A higher intensity of the colour corresponds to a higher number of marker-trait associations located in close proximity of each other.

At low N, 27.7% of the N-dependent marker-trait associations co-localized with mt_as (Table 1A). Seven traits (DM%, *t2_t1*, TbnA, TbnB, TbnMX and TbwB, TbwMX) showed a total of 11 QTL with markers not co-localizing with mt_as (Table 2). Moreover there was an N-dependent QTL for mt_as detected at low N on chromosome 3 and QTL for *te-t2* detected at low N but co-localizing with mt_as on chromosome 5. More QTL were detected at high N level than at low N level and even fewer QTL were detected at both N levels (Table 2).

Association after maturity correction

Maturity had a strong effect on the traits measured both at high and low N and the genomic region associated with maturity type is related with most of the traits measured. To gain more insight into the effect of maturity type and to allow detection of maturity-type-independent QTL,

we corrected for the effect of the maturity classes (i.e. the differences in the means of the trait values between the maturity classes), effectively equalizing the trait means per maturity group. The relative differences between genotypes were maintained within the maturity group but corrected when comparing genotypes across maturity groups.

Table 2 Peak markers of QTL consistently detected, using data uncorrected for maturity.

N Level	Trait	Chromosome	Genome Position	Marker	-log10(p)	Explained Variance
cmn Total	*AUC*	5	**316045624**	PotVar0079081	8.16	24.43
	mt_as	5	**316045624**	PotVar0079081	8.46	25.10
	t2	5	**316307819**	PotVar0080570	6.73	16.16
	TbnB	3	**216014512**	solcap_snp_c2_616	5.12	15.72
	te	5	**316045624**	PotVar0079081	7.76	23.18
	Y_DM	5	**316611906**	solcap_snp_c2_50302	6.17	17.20
	SCYi	1	**4041250**	PotVar0045583	8.27	21.18
		2	**131733600**	PotVar0120916	5.96	18.04
		5	**314920671**	PotVar0025024	5.23	15.31
		11	**757973524**	PotVar0112496	7.17	20.40
	[N]	5	**316307819**	PotVar0080570	6.36	19.29
cmn Total	8	11				
HN	*AP1*	2	**127511213**	solcap_snp_c2_15749	4.61	11.28
		2	**135122688**	PotVar0046300	5.33	12.33
		5	**315893706**	PotVar0026425	5.70	14.33
	AP2	5	**316045624**	PotVar0079081	5.33	18.00
	t1	1	**46273159**	PotVar0132293	4.82	11.35
		2	**127511213**	solcap_snp_c2_15749	5.28	13.23
		2	**135122688**	PotVar0046300	5.23	12.00
		5	**315893706**	PotVar0026425	6.95	16.68
	t2-t1	5	**316045624**	PotVar0079081	5.16	17.23
	TbnA	2	**134943142**	PotVar0045853	4.24	10.77
		2	**146198944**	PotVar0002966	5.07	12.61
		3	**203612959**	solcap_snp_c1_3637	5.88	13.38
	TbnMX	12	**832589670**	PotVar0052600	5.08	15.99
	TbwA	2	**134242234**	PotVar0128476	9.59	19.71
	TbwMX	2	**134943142**	PotVar0045853	6.14	15.09
	tm1	1	**32843979**	PotVar0000007	4.62	13.17
		6	**427042067**	PotVar0040538	5.12	13.61
		11	**751753201**	solcap_snp_c2_44269	4.70	13.56
		11	**757973524**	PotVar0112496	5.35	15.86
	Y_DM	9	**627531669**	PotVar0094025	4.56	14.51
		12	**823287226**	PotVar0037640	4.38	12.39
	SCYi	1	**30559567**	PotVar0037260	4.65	13.53
		1	**61310626**	solcap_snp_c2_20888	5.04	15.92
		8	**543834623**	PotVar0060623	5.30	15.34
HN Total	11	24				
LN	DM%	7	**484592357**	PotVar0092426	7.48	11.85
		7	**490792384**	solcap_snp_c2_38787	4.09	20.97
	mt_as	3	**204691153**	solcap_snp_c2_29678	4.37	14.45
	t2-t1	4	**283407138**	PotVar0116182	4.21	10.32
	TbnA	6	**424940350**	solcap_snp_c2_56145	5.44	13.99
	TbnB	6	**425163888**	PotVar0074198	4.20	13.30
		10	**695881376**	solcap_snp_c1_13524	5.57	11.05
	TbnMX	3	**216014512**	solcap_snp_c2_616	4.34	11.33
	TbwB	3	**217632046**	PotVar0021118	5.84	18.26
		6	**424915228**	PotVar0074004	5.17	15.42
		10	**695881376**	solcap_snp_c1_13524	5.27	11.14
	TbwMX	2	**106818648**	solcap_snp_c2_4515	4.29	11.05
	te-t2	5	**316045624**	PotVar0079081	6.53	19.22
LN Total	9	13				

"cmn" represents N-independent QTL i.e. QTL detected for both N levels. "HN" and "LN" represent N-dependent QTL, i.e. QTL exclusively detected at high N level or QTL exclusively detected at low N level, respectively. For the trait acronyms see sections "Data processing" and "Calculated traits". Only QTL detected in both years are included.

The total number of associations detected with the phenotypic data corrected for the maturity main effects (CD) was 348, with 181 markers (Table 1B), much lower than with the non-corrected data (NCD) (Table 1A). The number of associations consistently found in both years at either N

level was almost half compared with the NCD, but the number of markers involved was very similar (74 compared with 66 for the NCD and CD, respectively). This is because most of the trait associations co-localizing with the maturity assessment in the NCD disappeared after correction, as expected. The number of marker–trait associations common to both N levels after the correction was only 8, involving 3 traits (SCYi, TbnB and DM%).

Table 3 Peak markers of QTL consistently detected in both years (corrected and not corrected for maturity). For the trait acronyms see sections "Data processing" and "Calculated traits". N level: whether the associations are detected at high nitrogen (HN), low nitrogen (LN) or common to both nitrogen levels (cmn).

					Not Corrected		Corrected	
Chromosome	**Genome Position**	**Marker**	**Trait**	**N level**	**-log10(p)**	**Explained variance**	**-log10(p)**	**Explained variance**
1	**4041250**	PotVar0045583	SCYi	cmn	8.27	21.18	9.65	22.06
1	**4041250**	PotVar0045583	*tm1*	ln			6.36	14.49
1	**32843979**	PotVar0000007	SCYi	hn	4.74	12.93	4.91	13.58
1	**32843979**	PotVar0000007	*tm1*	hn	4.62	13.17	4.92	13.82
1	**63469625**	solcap_snp_c1_9676	SCYi	hn	5.07	14.44	5.59	15.39
1	**81815164**	PotVar0060997	TbnA	hn			5.60	14.32
2	**127511213**	PotVar0060997	*t1*	hn	5.28	13.23	5.33	11.55
2	**131733600**	PotVar0120916	SCYi	cmn	5.96	18.04	6.93	17.51
2	**134242234**	PotVar0128476	TbwA	hn	9.59	19.71	9.31	20.68
2	**134943142**	PotVar0045853	TbnA	hn	4.24	10.77	4.29	10.80
2	**134943142**	PotVar0045853	TbwMX	hn	6.14	15.09	6.36	15.32
2	**146198944**	PotVar0002966	TbnA	hn	5.07	12.61	5.67	14.50
2	**146303689**	PotVar0003077	SCYi	hn			4.19	10.62
3	**166741184**	solcap_snp_c1_15204	DM%	cmn			5.43	13.98
3	**203612959**	solcap_snp_c1_3637	TbnA	hn	5.88	13.38	5.60	12.92
3	**212481799**	PotVar0030333	[N]	hn			4.33	12.00
3	**212547269**	PotVar0030515	*te-t2*	ln			4.49	10.32
3	**212548683**	PotVar0030515	*te-t2*	hn			5.08	11.57
3	**213525966**	PotVar0121169	[N]	ln			4.61	10.65
3	**216081835**	solcap_snp_c1_151	TbnB	cmn	5.12	15.72	5.38	16.01
3	**216081835**	solcap_snp_c1_151	TbnMX	ln	4.34	11.33	4.73	11.96
3	**217630938**	solcap_snp_c1_151	TbwB	ln	5.85	18.74	5.28	17.75
4	**283533011**	solcap_snp_c1_15513	*AP2*	ln			4.46	10.31
4	**285470025**	PotVar0088487	*Vx*	ln			4.90	11.19
4	**289210701**	solcap_snp_c2_39807	DM%	hn			4.84	11.99
4	**300280572**	PotVar0015935	TbwMX	hn			5.21	10.61
5	**314920671**	PotVar0025024	SCYi	cmn	5.23	15.31	5.83	15.50
5	**360216448**	PotVar0082077	SCYi	hn			4.09	12.72
6	**424406145**	PotVar0082077	TbwB	ln	5.02	14.67	4.46	12.57
6	**424940350**	solcap_snp_c2_56145	TbnA	ln	5.44	13.99	4.40	12.65
6	**427042067**	PotVar0040538	*tm1*	hn	5.12	13.61	5.22	13.84
8	**543834623**	PotVar0060623	SCYi	hn	5.30	15.34	5.52	15.66
9	**624408926**	PotVar0051600	*AP3*	ln			4.54	10.23
10	**695881376**	PotVar0051600	TbwB	ln	5.27	11.14	5.57	11.22
10	**695908422**	solcap_snp_c2_57635	TbnB	ln	5.57	11.15	5.97	11.16
11	**719755658**	solcap_snp_c2_33657	NUpt	ln			5.09	12.32
11	**724842000**	PotVar0058777	SCYi	hn			4.46	12.67
11	**751753201**	solcap_snp_c2_44269	*tm1*	hn	4.70	13.56	5.24	14.68
11	**757973524**	PotVar0112496	SCYi	cmn	7.17	20.40	7.34	21.01
12	**823269906**	PotVar0037718	*t1*	ln			4.21	10.98
12	**827080788**	solcap_snp_c1_11644	mt_as	hn			4.58	11.04
12	**832202879**	PotVar0052761	TbnA	ln			4.13	10.59
12	**832589670**	PotVar0052600	TbnMX	hn	5.08	15.99	5.13	15.96

There were 24 QTL for 11 traits commonly detected with both datasets (CD and NCD) (Table 3), with more QTL detected at high N than at low N (13 and 6 respectively) while 5 QTL were common to both N levels. A QTL for SCYi on chromosome 5 (no QTL detected for mt_as at this position) was consistently detected at both N levels in both analyses (with data CD and NCD). Furthermore, there were 17 QTL for 11 traits detected only after the maturity correction. Three QTL were N-independent (for DM%, *te-t2* and [N]), while 14 QTL were N-dependent with seven QTL at high N (for TbnA, SCYi, DM%, TbwMX, SCYi, and mt_as) and seven QTL at low N (for *tm1, t1 , Vx, AP2, AP3*,

NUpt, and TbnA). There were regions accumulating QTL for three or more traits on chromosome 3, 4, and 12.

Discussion

In this study we combined canopy development modelling with an association mapping analysis to reveal the genetic basis of developmental, physiological, and agronomic traits with varying N availability. We applied established methodologies as used by D'hoop et al. (2008; 2010). The association analysis was done after correction for relatedness, which is the accepted standard because it decreases the probability of false positives (Malosetti et al. 2007; Yu et al. 2006). In potato, D'hoop et al. (2010) showed an increased level of LD within specific cultivar groups demonstrating the importance of correcting for relatedness.

Our results showed effects of N levels on the relationship between traits based on the genetic correlation (Figure 3), similar to the result based on phenotypic correlation for both N levels reported in Chapter 3 (Ospina et al. 2014). We demonstrated the effect of N input on canopy development and yield traits (Figure 4), as well as the strong contribution of maturity type, which is the major factor determining development, to the genetic variation. The genetic variation resulted in QTL consistently detected in both years at both N levels for 20 of the 24 traits included in this study. Many of these QTL accumulated in a single region on chromosome 5 that is known to be linked to maturity type as shown by Kloosterman et al. (2013) who identified an allelic variation of the CDF1 (Cycling DOF Factor) gene at this locus which strongly influences phenology, plant maturity and onset of tuberization, reflecting the importance of this region for quantitative developmental traits.

Effects of nitrogen availability on potato development were reported by many authors (Bélanger et al. 2002; Biemond and Vos 1992; Haverkort et al. 1991; Khan 2012; Oliveira 2000; Ospina et al. 2014; Tiemens-Hulscher et al. 2014; Vos 2009; Vos and Biemond 1992; Zebarth and Rosen 2007; Zebarth et al. 2004). In general, more available nitrogen advances, enhances and prolongs soil coverage as a result of improved haulm growth (Kleinkopf et al. 1981) as well as the initiation of more leaves with a longer life span (Vos and Biemond 1992). The three phases of canopy development (see "Calculated traits" in Materials and Methods section) responded to N with cultivars having a faster build up phase of the canopy, resulting in a shorter time to reach maximum coverage (*t1)*, a higher maximum cover (*Vx*) for a longer period (*t2-t1*) at high N input, all resulting in higher photosynthetic potential (Ospina et al. 2014; Vos 2009).

Most traits included in this study had relatively high genetic correlations between high and low nitrogen conditions, except for *AP3, te-t2* (Phase 3 of CDv) and *t1*. These high correlations reflect the consistency of the genotypic behaviour under varying N availability, at least for canopy development parameters associated with the period of maximum canopy cover. Phase 3 of CDv was difficult to phenotype precisely due to the senescence process itself, which starts initially with

yellow leaves until an uncertain point when the canopy collapses. The yellowness could start early if conditions are not favourable but the crop continues to take up nitrogen. On the other hand, wind and rain can accelerate the collapse and those factors are difficult to predict. Therefore Phase 3 parameters showed the largest random error, explaining the low heritabilities of *AP3* and *te-t2* (Ospina et al. 2014). The relationships between some of the traits based on their genetic correlation coefficients were slightly different between N input levels. For instance, at low N, yield has a higher absolute correlation with *AUC* than at high N, as a result of changes in the relationship with other traits. Under high N input there are no nutritional constraints for canopy development leading to an expansion of the duration of the potato growth phases (Ospina et al. 2014; Vos 2009). However, it is known that with high N input, important traits determining yield like LAI as well as the radiation use efficiency are positively affected (Chapter 2). LAI continues to increase even when the soil coverage is 100% (Haverkort et al. 1991; Haverkort and Bicamumpaka 1986); the maximum coverage is also faster reached and longer sustained at high nitrogen input (Ospina et al. 2014). Therefore, although yield and *AUC* are highly correlated at both N conditions, the contribution of LAI and RUE under high N may therefore not be fully captured by the *AUC*. This could also be reflected in the QTL detected: QTL common to both N levels for yield and *AUC* were found on chromosome 5 (Table 2) and co-localized with QTL for maturity (mt_as), while QTL exclusively detected at high N for yield were located on chromosome 9 and 12. A possible explanation is that the latter two might be associated with the contribution of RUE and/or LAI to yield.

Genomic regions with possible pleiotropic effects were detected on chromosomes 2, 5 and 6 (Figure 5). The QTL hotspot on chromosome 5 is the most noticeable, accumulating QTL for 50% of the traits on this maturity-related region, similarly as shown by Hurtado et al. (2015) with developmental traits related to senescence and flowering and with plant height. Most of the traits with QTL in this region were highly correlated with maturity assessed in our trials (mt_as), emphasizing the importance of maturity and the genomic region on chromosome 5 for crop development. Moreover, as a general remark the co-localization of QTL is mostly determined by the correlation between traits. Furthermore, there was an N dependency of some QTL for several traits. The region on chromosome 2 accumulated QTL for 6 traits (*AP1*, *t1*, TbnA, TbwA, TbwMX and SCYi) at high N input, while the region on chromosome 6 is related to four traits (with QTL for TbnB, TbnA, TbwB and *tm1*) at low N input. This shows the strong effect of available N on the genetic response as well as its complexity.

Regarding the N dependent QTL, at high N input more QTL involving more traits were detected than at low N input, with also a higher percentage of marker-trait associations on chromosome 5. Gallais and Hirel (2004) found in maize more QTL for some traits at high N input than at low N (vegetative development, N uptake and yield components) while for other traits it was the opposite (N utilization efficiency and protein content). This is a reflection of the difference in the expression of the genetic variability between high and low input N that may be trait-dependent.

Trait by nitrogen interaction is translated into QTL by nitrogen interaction, in those studies as well as in our study.

N-independent associations were mostly located at the maturity locus on chromosome 5 (data uncorrected for maturity). Khan (2012) used a similar phenotyping approach to study potato canopy development and reported a major QTL hotspot on chromosome 5 in a diploid biparental population (SH × RH) affecting all parameters of the canopy cover curve in several environments. We reported in Chapter 4 of this thesis (using the same diploid biparental mapping population) the same QTL region on chromosome 5 at both high and low nitrogen levels. In addition, QTL for growth and yield traits in this region were found in drought tolerance QTL mapping in the greenhouse of the C × E diploid mapping population (Anithakumari et al. 2012) and in multiple environments for the same population (Hurtado et al, 2012; 2015). Therefore, the overall and predominant effect of maturity on canopy development and on yield appears to be stable across different environments, nitrogen conditions, and populations.

N-independent QTL different than those of the maturity locus on chromosome 5 were found only for SCYi and TbnB (Figure 5). These two traits were not correlated with the maturity assessment (mt_as) (Figure 2). For SCYi there were N-independent QTL on chromosomes 1, 2, 5 and 11 while in the diploid mapping population SH × RH (Chapter 4) N-independent QTL were found on chromosomes 5 and 10 (referred as linkage groups V and X in the multi-trait QTL analysis, Chapter 4) this might suggest that the genetic background as well as the population type influence the genomic regions related to a trait. For TbnB we found N-independent QTL as well as a QTL at low N level on chromosome 3. Schönhals (2014) also found associations for tuber number on this chromosome (as well as on chromosomes 1, 5 and 6), using markers in candidate genes that were functionally related to tuber yield and starch. The comparison of the result with previous reports is difficult because different markers were used in different populations. For the markers used in the detection of QTL with the SH × RH diploid biparental population in Chapter 4 there are no physical positions available (these markers were not used in this association analysis), while for the SNPs used here in the association mapping, there are no genetic positions known on the SH × RH genetic map.

After the maturity correction, the number of N-independent marker-trait associations was drastically reduced. Since most of the traits were maturity-related, the maturity correction was expected to have a strong impact on the detection of QTL. Only the N-independent QTL for SCYi and TbnB remained after the correction (these were not linked to maturity, and the traits did not correlate with maturity). Similarly, D'hoop (2011) showed the impact of maturity. In their phenotypic analysis, the presence or absence of maturity as a term in the model influenced the genotypic effects for two traits studied: underwater weight and maturity trait (both traits are physiologically correlated) but not for the majority of quality traits, which were not correlated with maturity. Their association analysis using maturity-corrected values in a model with a correction for relatedness showed a reduction of the marker trait associations detected for these two traits

(underwater and maturity) while for other quality traits there was no clear trend (D'hoop et al. 2014).

The maturity correction of our data using predefined information of the cultivars (the maturity grouping factor) was effective in removing the maturity effect although it might have affected the detection of association, most probably reducing the power since the overall variation was reduced. However, it allowed detection of new QTL (Table 3) that did not co-localize in the main region related to maturity on chromosome 5. For instance, a new N-independent QTL for DM% on chromosome 3 was detected. This QTL was expected to be N-independent since the DM% trait did not have a significant nitrogen effect (Annex 4). The results with the diploid population (Chapter 4) also showed a QTL for DM% on chromosome 3. In general, the maturity dependency of some QTL resulted from the physiological relation of canopy development traits and agronomic traits with maturity (these relations are discussed by Ospina et al. (2014) (Chapter 3) and in Chapter 2). Kloosterman et al. (2013) identified the causal gene within the chromosome 5 maturity locus. Allelic variation of the CDF1 (Cycling DOF Factor) gene at this locus strongly influences phenology, plant maturity and onset of tuberization. The CDF1 gene has a great effect on plant life cycle length by acting as a mediator between photoperiod and the tuberization signal. This major effect acts on several processes of the plant resulting in a strong linkage between maturity, traits related to CDv and yield. Khan (2012) also mentioned the dependency of tuber yield with its components (specially tuber bulking parameters and CDv traits) as these are physiologically and genetically related, i.e. genotypes with higher tuber bulking rates show limited haulm growth and canopy duration, leading to an early maturity type (Khan 2012).

We have shown how genetic factors determining canopy development and yield traits in potato cultivars interact with N levels. The different QTL regions detected for a trait under contrasting N conditions may imply that the phenotypes are the result of a trade-off between these QTL regions. The detection of N-dependent QTL emphasizes the importance of direct selection under limiting N conditions only if the QTL contribute to the traits of interest. The contribution of genetic factors to growth and yield is affected by N input, different interactions between the traits under low N than under high N, and therefore different contributions of the traits to the observed phenotype. Ospina et al. (2014) mentioned that to breed for NUE under low input the strategy should be to select for high yield under low N and combine this with a high responsiveness to more N input. This allows for selection of better adapted genotypes in N limiting conditions. In addition, to bypass the strong linkage with maturity that is observed for developmental traits, NUE and yield, the selections should be done within each maturity group. Thus, the phenotyping should be made more discriminative to exploit the variation in a narrow maturity category. Additionally, the strong correlation of most of the traits mentioned with maturity can mask useful genetic variation for these traits, as exemplified in this chapter. An early selection is required to increase the number of individuals in the target maturity class. This might be achieved by developing marker selection for maturity. Small differences in the existing trait will then be detectable allowing selectors and breeders to identify new traits and be more discriminant in their assessments for these other traits.

In general, the reports in other crops of QTL detection under contrasting N conditions have shown great influence of environmental conditions. For example, in barley the detection of QTL have been reported to show extensive G x E/QTL x E interaction, with QTL changing between years irrespective of N levels (Kindu et al. 2014). In maize, contrasting results have been reported when looking for QTL under high and low N input. QTL for grain composition and NUE-related traits detected at low N correspond to QTL detected at high N input (Bertin and Gallais 2001), while other results showed that QTL for NUE are only detected at low N input (Agrama et al. 1999). Hirel et al. (2011) suggested that depending on the RIL population, the response of yield to various levels of N fertilization could be different and thus controlled by a different set of genes. We only report QTL consistently detected in both years for each N level. Therefore, our research is not directly addressing GXE interaction, for which more experiments would be needed. Table 1 shows the influence of the year in the detection of association (only 166 associations detected in both years, out of 950 detected irrespectively of the year). In the context of our experimental setup, nitrogen level was the major control factor driving the differences within this very constant physical environment. Therefore the nitrogen dependency of some QTL could be interpreted as QTL x N interaction.

Our approach focused on contrasting N input levels using a single N application, and it is a first step to understand the genetic factors involved in the response of potato to N. It is important to mention that fertilization practices like split application might have an additional effect on the plant response to nitrogen, especially in relation to the different maturity types. Additionally, soil mineral N supply during the growing season is difficult to control and understand (Haverkort and MacKerron 2000) since it is a dynamic factor. Goffart et al. (2008) mentioned that soil mineral N supply is influenced by several predictable and unpredictable factors, such as weather conditions, chemical and physical soil properties, type and evolution of organic matter previously incorporated in the soil, cultural practices, maturity type of the cultivar, and crop duration. This N dynamic in the soil could result in different levels of N available. This difference will affect the crop development response of the cultivar and thus the variation of the traits, thereby affecting the consistency in the detection of QTL or marker trait associations.

Finally, the understanding of the influence of an intrinsic major genotypic factor such as maturity type is valuable to refine breeding strategies, and to develop cultivars suitable to low N input or otherwise limiting conditions. Furthermore, the results presented here suggest that breeding schemes should be done within maturity groups, with the main idea of improving characteristics that are highly influenced by maturity, like DM%, N content and NUE, within a maturity group.

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Supplementary material

Annex 1 Cultivars used in this experiment; based on D'hoop et al. (2010).

P8 code	Cultivar or breeder's clone	Year of first registration	Country of origin	Parentage	Market niche
P80002	Abundance (Sutton'S)	1886	UK	Magnum Bonum x Fox's seedling	ancient cultivar
P80003	Ackersegen	1929	GER	HINDENBURG x ALLERFRUHESTE GELBE	ancient cultivar
P80004	Adirondack	1881	USA	Peachblow x Peachblow	ancient cultivar
P80005	Adora	1990	NETH	Primura x Alcmaria	fresh consumption
P80006	Adretta	1975	GER	LU. 59.884/3 x Axilia	fresh consumption
P80007	Agata	1990	NETH	BM 52-72 x Sirco	fresh consumption
P80008	Agria	1985	GER	Quarta x Semlo	processing industry
P80009	Ajiba	1992	NETH	AMINCA x VE 70-9	fresh consumption
P80010	Albion	1895	NETH	REICHSKANZLER x SIMSON	ancient cultivar
P80012	Allure	1990	NETH	Astartex AM 66-42	starch industry
P80014	Almera	1999	NETH	BM 77-2102 x AR 80-31-20	fresh consumption
P80015	Alpha	1925	NETH	Paul Kruger x Preferent	ancient cultivar
P80016	Am 66-42	/	NETH	VTN 62-33-3 x MPI 19268	progenitor clone
P80018	Am 78-3704	/	NETH	AM 72-3477 x AM 70-2166	progenitor clone
P80019	Amorosa	2000	NETH	Arinda x Impala	fresh consumption
P80020	Ampera	1998	GER	AGUTI x PONTO	fresh consumption
P80021	Amyla	1999	FRA	PROMESSE x ELEMENT	starch industry
P80022	Anosta	1975	NETH	Ostara x Provita	processing industry
P80024	Arcade	1999	NETH	AGRIA x VK 69-491	fresh consumption
P80025	Arinda	1993	NETH	Vulkano x AR 74-78-1	fresh consumption
P80026	Arnova	1999	NETH	Obelix x AR 76-168-1	fresh consumption
P80028	Arran Chief	1911	UK	Paterson's Victoria x Sutton's Flourball	ancient cultivar
P80030	Arran Victory	1918	UK	ABUNDANCE x ABUNDANCE	ancient cultivar
P80031	Arrow	2004	NETH	Solara x Fresco	fresh consumption
P80032	Astarte	1976	NETH	RR 62-5-43 x VTN 62-69-5	starch industry
P80033	Asterix	1991	NETH	Cardinal x VE 70-9	processing industry
P80034	Atlantic	1976	USA	Wauseon x Lenape	processing industry
P80035	Aurora	1972	NETH	Profijt x AM 54-10	starch industry
P80036	Ausonia	1981	NETH	Wilja x KONST 63-665	fresh consumption
P80037	Avenance	/	NETH	Mercury x Florijn	starch industry
P80038	Ballydoon	1931	UK	Herald x British Queen	ancient cultivar
P80039	Bartina	1988	NETH	Saturna x ZPC 62-75	fresh consumption
P80041	Bellini	2001	NETH	Mondial x Felsina	fresh consumption
P80042	Berber	1984	NETH	Alcmaria x Ropta P 365	fresh consumption
P80043	Bildtstar	1984	NETH	Winda x Saturna	fresh consumption
P80044	Bintje	1910	NETH	Munstersen x Jaune d'or (= Fransen)	fresh consumption
P80045	Biogold	2004	NETH	Novita x HZ 87 P 200	processing industry
P80046	British Queen	1894	UK	PATERSON'S VICTORIA x BLUE DON	ancient cultivar
P80047	Caesar	1990	NETH	Monalisa x Ropta B 1178	processing industry
P80049	Charlotte	1981	FRA	Hansa x Danae	fresh consumption
P80050	Cherie	1997	FRA	Roseval x AR 76-199-3	fresh consumption
P80051	Cilena	1981	GER	Gelda x Hirla	fresh consumption
P80052	Civa	1960	NETH	(BINTJE x (SASKIA x FRUHMOLLE)) x CIV 49-901	fresh consumption
P80053	Clivia	1962	GER	Seedling 1 x Seedling 17	progenitor clone
P80055	Craigs Bounty	1946	UK	unknown	ancient cultivar
P80056	Craigs Defiance	1938	UK	Epicure x Pepo	ancient cultivar
P80057	Daisy	1998	FRA	GIPSY x CULPA	fresh consumption
P80058	Deodara	1913	GER	Deutsches Reich x Jubel	ancient cultivar
P80059	Desiree	1962	NETH	Urgenta x Depesche	fresh consumption
P80060	Di Vernon	1922	UK	unknown	ancient cultivar

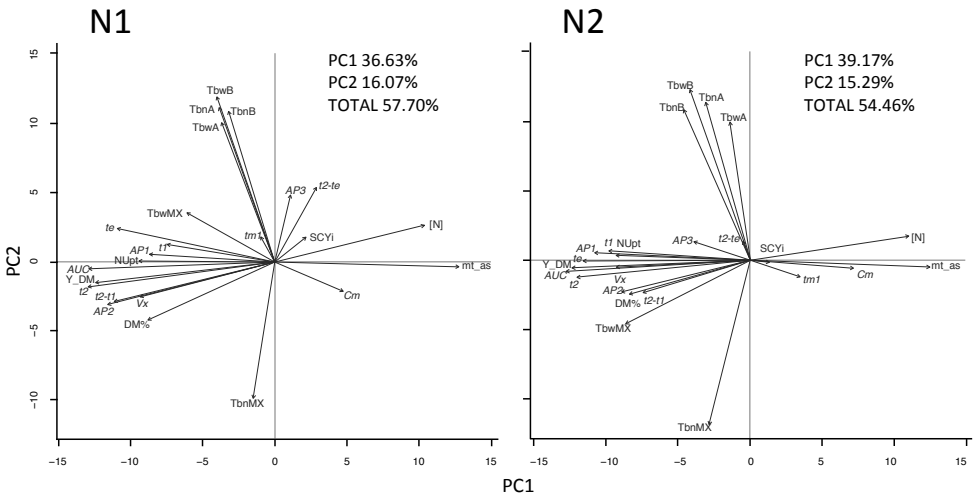
P8 code	Cultivar or breeder's clone	Year of first registration	Country of origin	Parentage	Market niche
P80061	Diamant	1982	NETH	Cardinal mutant	fresh consumption
P80062	Ditta	1989	AUT	BINTJE x QUARTA	fresh consumption
P80063	Donald	1996	NETH	Amera x W 72-19-443	fresh consumption
P80064	Doon Star	1926	UK	Templar seedling x Majestic	ancient cultivar
P80065	Dorado	1995	NETH	Spunta x VK 69-491	processing industry
P80066	Dore	1947	NETH	DUKE OF YORK x BIERMA A 7	ancient cultivar
P80067	Dr Mcintosh	1944	UK	(phu x HERALD) x HERALD	ancient cultivar
P80068	Draga (1970)	1970	NETH	SVP 50-2017 x MPI 19268	fresh consumption
P80069	Eersteling	1891	UK	Early Primrose x King Kidney	ancient cultivar
P80070	Early Rose	1867	USA	Garnet Chili seedling	ancestor
P80072	Eden (2000)	2000	FRA	EOLE x PENTLAND DELL	fresh consumption
P80073	Ehud	1965	NETH	Panther x Karna 149	starch industry
P80074	Eigenheimer	1893	NETH	Blaue Riesen x Fransen (Jaune d'or)	ancestor
P80075	Elisabeth	2002	NETH	VE 82-96 x CILENA	fresh consumption
P80076	Eos	2000	NETH	MONDIAL x W 72-22-496	fresh consumption
P80077	Epicure	1897	UK	Magnum Bonum x Early Regent	ancient cultivar
P80078	Escort	1982	NETH	RENTAL x CEB 64-197-16	fresh consumption
P80079	Estima	1973	NETH	NOPOL x G 3014	fresh consumption
P80080	Exquisa	1992	GER	Sigma x Ilse	fresh consumption
P80081	Fabula	1997	NETH	Monalisa x Hudson	fresh consumption
P80082	Felsina	1992	NETH	Morene x Gloria	processing industry
P80083	Festien	2000	NETH	KARTEL x KA 80-1920	starch industry
P80084	Fianna	1987	NETH	KONST 62-660 x AM 64-2	processing industry
P80085	Fichtelgold	1945	GER	[(Zwickauer Fruhe x Jubel) x Klara] x Mittelfruhe	ancient cultivar
P80087	Flourball (Sutton's)	1870	UK	unknown	ancestor
P80088	Fontane	1999	NETH	Agria x AR 76-34-3	processing industry
P80089	Fresco	1985	NETH	CEB 60-15-28 x PROVITA	processing industry
P80090	Frieslander	1990	NETH	Gloria x 74 A 3	fresh consumption
P80092	Furore	1930	NETH	Rode Star x Alpha	ancient cultivar
P80093	Gladstone	1932	UK	ARRAN CHIEF x (MAJESTIC x GREAT SCOT)	ancient cultivar
P80094	Gloria	1972	GER	Amex x Feldeslohn	fresh consumption
P80095	Golden Wonder	1906	UK	Seedling of Early Rose	ancient cultivar
P80096	Goya (2000)	2000	NETH	AM 78-4102 x KARDAL	starch industry
P80097	Great Scot	1909	UK	Imperator x Champion	ancient cultivar
P80098	Hansa	1957	GER	OBERARNBACHER FRUHE x FIAVA	fresh consumption
P80099	Herald	1928	UK	MAJESTIC x ABUNDANCE	ancient cultivar
P80100	Hermes	1973	AUT	DDR 5158 x SW 163/55	processing industry
P80102	Home Guard	1943	UK	DOON PEARL x CUMNOCK	ancient cultivar
P80104	Impala	1989	NETH	52/72/2206 (BM 52-72) x BIRANCO	fresh consumption
P80106	Innovator	1999	NETH	Shepody x RZ 84-2580	processing industry
P80107	Inova	1999	NETH	NICOLA x IMPALA	fresh consumption
P80110	Jaerla	1969	NETH	Sirtema x MPI 19268	fresh consumption
P80113	Karnico	1987	NETH	Astarte x AM 66-42	starch industry
P80114	Kartel	1994	NETH	KA 77-0133 x AM 78-3736	starch industry
P80115	Katahdin	1932	USA	USDA 40568 x USDA 24642	ancient cultivar
P80116	Kennebec	1948	USA	USDA B 127 x USDA 96-56	ancient cultivar
P80117	Kepplestone Kidney	1900	UK	unknown	ancient cultivar
P80118	Kerpondy	1949	FRA	unknown	ancient cultivar
P80119	Kerr's Pink	1907	UK	Fortyfold x (Abundance or Smiths early)	ancient cultivar
P80121	Kondor	1984	NETH	KONST 61-333 x WILJA	fresh consumption

Annex 1 ...continued

P8 code	Cultivar or breeder's clone	Year of first registration	Country of origin	Parentage	Market niche
P80122	Kuras	1996	NETH	BRDA (=PG 285) x VK 69-491	starch industry
P80123	Kuroda	1998	NETH	AR 76-199-3 x KONST 80-1407	fresh consumption
P80124	Lady Christl	1996	NETH	WS 73-3-391 x Mansour	fresh consumption
P80125	Lady Claire	1996	NETH	Agria x KW 78-34-470	processing industry
P80126	Lady Olympia	1996	NETH	AGRIA x KW 78-34-470	processing industry
P80127	Lady Rosetta	1988	NETH	CARDINAL x VTN 62-33-3	processing industry
P80128	Laura	1998	GER	Rosella x L 6140/2	fresh consumption
P80129	Lenape	1967	USA	USDA B 3672-3 x Delta Gold	fresh consumption
P80130	Leyla	1988	GER	7338/812 x CULPA	fresh consumption
P80132	Liseta	1988	NETH	Spunta x VE 66-295	fresh consumption
P80134	Majestic	1911	UK	Unknown breeding line x British Queen	ancestor
P80135	Marfona	1977	NETH	PRIMURA x KONST 51-123	fresh consumption
P80137	Maritiema	1991	NETH	AM 71-125 x VE 70-9	processing industry
P80139	Markies	1997	NETH	FIANNA x AGRIA	fresh consumption
P80140	May Queen	1890	UK	unknown	ancient cultivar
P80141	Mercator	1999	NETH	KARTEL x KA 86-0008	starch industry
P80142	Monalisa	1982	NETH	Bierma A 1-287 x Colmo	fresh consumption
P80143	Mondial	1987	NETH	SPUNTA x VE 66-295	fresh consumption
P80144	Morene	1983	NETH	RENOVA x AM 66-42	processing industry
P80145	Mpi 19268	/	GER	Sdem x Deodara	progenitor clone
P80146	Myatt'S Ashleaf	1804	UK	unknown	ancient cultivar
P80147	Nicola	1973	GER	CLIVIA x 6430/101	fresh consumption
P80149	Niska	1990	USA	WISCHIP x LENAPE	processing industry
P80150	Noisette	1993	FRA	AMINCA x PIROSCHKA	fresh consumption
P80151	Nomade	1995	NETH	Elles x AM 78-3704	starch industry
P80152	Noordeling	1928	NETH	BRAVO x JAM	ancient cultivar
P80153	Obelix	1988	NETH	Ostara x Renska	fresh consumption
P80155	Pallas	2003	NETH	KW 84-11-220 x VDW 85-72	processing industry
P80158	Peerless	1862	USA	Garnet Chili seedling	ancient cultivar
P80159	Pentland Dell	1961	UK	Roslin Chania x Roslin Sasamua	processing industry
P80160	Pepo (1919)	1919	GER	Deutsches Reich x Jubel	ancient cultivar
P80161	Picasso	1994	NETH	Cara x Ausonia	fresh consumption
P80162	Première	1979	NETH	Civa x Provita	processing industry
P80163	Prevalent	1966	NETH	AMBASSADEUR x LOMAN M 54-106-1	fresh consumption
P80164	Primura	1961	NETH	Sirtema x Majestic	fresh consumption
P80165	Profijt	1949	NETH	Prummel K 264 x Matador	ancient cultivar
P80166	Ramos	2000	NETH	AGRIA x VK 69-491	fresh consumption
P80167	Record	1932	NETH	Trenctria x Energie	ancient cultivar
P80169	Red Scarlett	1999	NETH	ZPC 80-239 x IMPALA	fresh consumption
P80170	Redstar	1997	NETH	Bildstar x VDW 76-30	processing industry
P80172	Remarka	1991	NETH	Edzina x AM 66-42	processing industry
P80173	Romano	1981	NETH	DRAGA x DESIREE	fresh consumption
P80175	Russet Burbank	1908	USA	Mutant of Burbank	processing industry
P80176	Samba	1989	FRA	ROSEVAL x BARAKA	fresh consumption
P80177	Santana	1994	NETH	Spunta x VK 69-491	processing industry
P80178	Sante	1983	NETH	Y 66-13-636 x AM 66-42	fresh consumption
P80179	Saskia	1946	NETH	Rode Eersteling x Herald	fresh consumption
P80180	Saturna	1964	NETH	Maritta x (Record x CPC 1673-1adg)	processing industry
P80181	Seresta	1994	NETH	AM 78-3704 x Sonata	starch industry
P80182	Shamrock	1900	IRL	unknown	ancient cultivar

P8 code	Cultivar or breeder's clone	Year of first registration	Country of origin	Parentage	Market niche
P80183	Shepody	1980	CAN	Bake King x F58050	processing industry
P80184	Sirtema	1947	NETH	DORST H 123A x FRUHMOLLE	fresh consumption
P80185	Spunta	1968	NETH	Bea x USDA 96-56	fresh consumption
P80186	Sunrise	1984	USA	Wauseon x USDA B 6563-2	processing industry
P80187	Tahi	1960	NZL	SEBAGO x HARFORD	fresh consumption
P80188	Tasso	1963	GER	seedling x Biene	processing industry
P80189	The Alness	1934	UK	ABUNDANCE x MAJESTIC	ancient cultivar
P80190	Timate	1984	NETH	Elvira x AM 66-42	fresh consumption
P80191	Tinwald'S Perfection	1914	UK	unknown	ancient cultivar
P80192	Toyoshiro	1976	JPN	HOKKAI 19 x ENIWA	fresh consumption
P80193	Triplo	2000	NETH	AGRIA x FRESCO	processing industry
P80194	Triumf	1921	NETH	Eigenheimer x Cimbals Neue Imperator	ancient cultivar
P80196	Ulster Glade	1961	UK	ULSTER EMBLEM x adg-hybrid	fresh consumption
P80197	Ulster Knight	1954	UK	CLARKE 736 x CRAIGS DEFIANCE	fresh consumption
P80198	Ulster Sceptre	1962	UK	Pentland Ace x Ulster Prince	fresh consumption
P80199	Ultimus	1935	NETH	Rode star x Pepo	ancient cultivar
P80200	Umatilla Russet	1998	USA	Butte x A 77268-4	fresh consumption
P80201	Up To Date	1894	UK	Patersons Victoria x Blue Don	ancient cultivar
P80202	Urgenta	1951	NETH	FURORE x KATAHDIN	fresh consumption
P80203	Usda 96-56	/	USA	USDA 3895-13 x EARLAINE	progenitor clone
P80204	Ve66-295	/	NETH	Amelio x HVT 60-8-3	progenitor clone
P80205	Ve70-9	/	NETH	Alcmaria x VTN 62-33-3	progenitor clone
P80206	Ve71-105	/	NETH	AM 67-136 x AM 67-59	progenitor clone
P80207	Ve74-45	/	NETH	Sinaeda x AM 66-42	progenitor clone
P80208	Victoria	1997	NETH	AGRIA x ROPTA J 861	processing industry
P80209	Virgo	2002	NETH	NICOLA x AM 78-3704	fresh consumption
P80210	Vivaldi	1998	NETH	TS 77-148 x MONALISA	fresh consumption
P80211	Vk69-491	/	NETH	VK 64-56 x VTN 62-33-3	progenitor clone
P80212	Voran	1931	GER	Kaiserkrone x Spatgold of Herbstgelbe	ancient cultivar
P80213	Voyager	2003	NETH	RZ 85-238 x OBELIX	processing industry
P80214	Vtn 62-33-3	/	NETH	((V 24/20 x ULSTER KNIGHT)1 x PROFIJT)15 x (VRN I-3	progenitor clone
P80215	W 72-22-496	/	NETH	REDBAD x Y 66-13-636	progenitor clone
P80216	Wauseon	1967	USA	USDA B 4159-8 x KATAHDIN	fresh consumption
P80217	Wilja	1967	NETH	CLIMAX x KONST 51-123	fresh consumption
P80218	Winston	1992	UK	KISMET x DXMP 70	fresh consumption
P80219	Wisent	2005	NETH	Prudenta x Karakter	starch industry
P80220	Y 66-13-636	/	NETH	Y 62-2-221 x AMARYL	progenitor clone
P80221	Yam	1787	UK	unknown	ancient cultivar
P80222	Yukon Gold	1980	CAN	NORGLEAM x USW 5279-4	processing industry

Annex 2 Biplot of trait relationships per N input level. For acronyms see the sections "Data processing" and "Calculated traits" of Materials and Methods.



Annex 3 Means of traits per maturity group (E: early, M: intermediate, and L: late), and N levels (N_lv; 1: 180 kg available N/ha; 2: 75 kg available N/ha). The data from the two years (2009 or 2010) are combined per nitrogen level. For acronyms and units of traits, see main text or Annex 5.

	High Nitrogen			Low Nitrogen		
Trait	**Early**	**Middle**	**Late**	**Early**	**Middle**	**Late**
[N]	12.70	11.89	10.72	11.01	9.96	9.01
AP1	818.5	1082.1	1238.5	789.0	1199.9	1448.9
AP2	1707.0	2343.5	2900.5	1016.4	1412.0	1542.0
AP3	1230.0	1182.6	1153.5	1010.1	1002.4	1133.2
AUC	3756.0	4608.5	5275.0	2815.0	3614.0	4124.5
Cm	7.40	7.04	6.27	5.61	4.92	4.30
DM%	22.87	24.13	27.01	22.70	24.48	26.78
mt_as	6.23	4.74	3.83	6.90	5.71	4.82
NUE	0.07	0.09	0.10	0.12	0.16	0.18
Nupt	15.60	18.57	18.80	10.12	12.02	11.82
NUptE	0.87	1.03	1.04	1.35	1.60	1.58
NUtE	0.08	0.09	0.10	0.09	0.10	0.11
SCYi	3079.5	2986.5	3053.5	3094.5	3010.0	3156.5
t1	19.10	22.67	24.96	20.31	26.27	30.43
t2	38.96	48.19	56.13	34.58	44.15	49.62
t2-t1	19.87	25.53	31.17	14.27	17.89	19.20
TbnA	213.05	272.40	251.25	178.05	198.75	193.75
TbnB	50.43	53.44	51.98	46.89	49.18	48.49
TbnMX	21.84	22.49	25.02	21.16	22.39	25.31
TbwA	162.65	186.90	187.00	144.25	144.60	151.40
TbwB	55.65	59.19	57.51	51.51	53.81	53.10
TbwMX	2.54	3.11	3.00	2.00	2.42	2.39
te	60.93	67.52	74.69	56.36	62.86	72.18
te-t2	21.97	19.33	18.82	21.79	18.72	22.56
tm1	9.26	9.58	9.73	8.24	8.02	7.63
Vx	85.84	92.04	92.78	71.47	79.83	78.98
Y_DM	1.25	1.58	1.78	0.94	1.22	1.34

Annex 4: p values for main factors included in the analysis using mixed model REMEL yr: year, N_lv: nitrogen level, Mt: maturity groups. Interaction terms are represented by joining the main terms by a "." For acronyms of traits, see main text or Annex 5.

Trait	yr	N_lv	Mt	N_lv.Mt	yr.Mt	yr.N_lv	yr.N_lv.Mt
tm1	<0.001	<0.001	0.822	0.006	<0.001	0.309	0.212
t1	<0.001	0.001	<0.001	0.002	<0.001	0.881	0.057
Vx	0.001	<0.001	<0.001	0.124	<0.001	0.215	<0.001
t2	<0.001	<0.001	<0.001	0.099	<0.001	0.064	<0.001
te	<0.001	<0.001	<0.001	0.320	<0.001	<0.001	0.448
Cm	<0.001	<0.001	<0.001	0.269	<0.001	<0.001	0.338
AP1	<0.001	0.224	<0.001	0.006	<0.001	0.784	0.022
AP2	<0.001	<0.001	<0.001	<0.001	<0.001	0.211	0.117
AP3	<0.001	<0.001	0.553	0.150	<0.001	<0.001	0.140
AUC	0.009	<0.001	<0.001	0.076	0.061	0.034	0.027
t2-t1	<0.001	<0.001	<0.001	<0.001	0.001	0.122	0.229
te-t2	<0.001	0.793	0.008	0.065	<0.001	0.013	0.003
DM%	<0.001	0.915	<0.001	0.023	0.097	0.366	0.153
Y_DM	0.023	<0.001	<0.001	<0.001	<0.001	0.334	0.149
[N]	0.004	<0.001	<0.001	0.467	<0.001	0.207	0.210
NUpt	0.735	<0.001	<0.001	0.002	<0.001	0.233	0.735
NUptE	0.777	<0.001	<0.001	0.074	<0.001	0.313	0.032
NUtE	0.009	<0.001	<0.001	0.001	<0.001	0.124	0.785
NUE	0.082	<0.001	<0.001	<0.001	<0.001	0.999	0.056
SCYi	<0.001	0.242	0.424	0.627	<0.001	0.02	0.162
TbwMX	0.045	<0.001	<0.001	0.432	<0.001	0.351	0.689
TbwB	<0.001	<0.001	0.028	0.329	<0.001	0.012	0.118
TbwA	0.987	<0.001	0.192	0.119	<0.001	0.102	0.015
TbnMX	<0.001	0.437	0.021	0.638	<0.001	0.003	0.550
TbnB	<0.001	<0.001	0.022	0.436	0.026	0.004	0.600
TbnA	<0.001	<0.001	0.015	0.08	<0.001	0.015	0.066
mt_as	0.585	<0.001	<0.001	0.021	0.606	0.993	0.648

Annex 5 acronyms for traits included in the analysis.

Traits	Description	Units
tm1	Inflexion point in the Phase I: build-up of canopy development	*td*
t1	Period from plant emergence to maximum soil coverage	*td*
t2	Initiation of senescence or Phase III	*td*
te	Total growing period till canopy is dead	*td*
Vx	Maximum %SC reached	*%*
Cm	Maximum progression rate of soil coverage during Phase I	*%SC/td*
AP1	Area under canopy cover curve for Phase I	*%SC.td*
AP2	Area under canopy cover curve for Phase II	*%SC.td*
AP3	Area under canopy cover curve for Phase III	*%SC.td*
AUC	Total area under the canopy curve	*%SC.td*
t1-t2	Duration of Phase II	*td*
te-t2	Duration of Phase III	*td*
Y_DM	Tuber dry matter	g/m^2
DM%	Tuber dry matter percentage	*%*
[N]	Tuber nitrogen concentration	*g/kg*
NUpt	Tuber nitrogen up take	g/m^2
NUE	Tuber nitrogen use efficiency	*kg/g*
NUptE	Tuber nitrogen up take efficiency	*g/g*
NUtE	Tuber nitrogen utilization efficiency	*kg/g*
SCYi	Soil coverage yield index	$(\%.td)/(kg/m^2)$
TbnMX	Maximum tuber number	$Tb\ \#/m^2$
TbnB	Tuber size having the maximum tuber number	*mm*
TbnA	Tuber number dispersion parameter	
TbwMX	Maximum tuber weight	g/m^2
TbwB	Tuber size having the maximum tuber weight	*mm*
TbwA	Tuber weight dispersion parameter	
mt_as	Maturity assessment	3 to 8

Chapter 6

General discussion

Preamble

This thesis was part of a European Union project aiming to "reduce the environmental impact of crop production", while "maintaining or improving current yield and quality levels" and "increasing sustainability and competitiveness" of European crop production systems. The scientific concept was to develop knowledge, models and tools required to select/breed for nutrient use efficiency in four major crop species (wheat, oilseed rape, potato and maize). Within this framework, this thesis presents results obtained in order to have a better understanding of the phenotypic response of genetically diverse potato germplasm to contrasting nitrogen (N) input treatments using eco-physiological curve-fit models describing canopy development as phenotyping tools. Using available resources including genotypic data to perform genetic analysis for i) a cultivar set (for association mapping) and ii) a diploid biparental population SH × RH (for linkage based QTL mapping), we generated phenotypic data under contrasting N treatments to answer the following questions:

A. How does variation in N input and fertilizer types affect the performance of potato cultivars differing in maturity type during the growing period, with an emphasis on physiological behaviour of potato under low N input?
B. Is there genetic variation in the nitrogen use efficiency (NUE) of modern European cultivars, using an extensive potato germplasm collection (200 cultivars/genotypes) phenotyped at two different levels of N input?
C. Are there N dependent quantitative trait loci (QTL) for physiological/morphological traits related to crop development (canopy development and tuber bulking) and nitrogen uptake (N_Upt) in a diploid mapping population (SH × RH population)?
D. Can we identify N dependent QTL/markers for crop development, tuber bulking and nitrogen uptake (N_Upt) using an association mapping approach?

In this thesis we integrated phenotyping, crop physiology and genetics studies. Our approach consisted of adding value to the phenotypic data by the use of an eco-physiological model, in this specific case, to describe canopy development by estimating meaningful parameters related to the process. We showed that the parameters from the model can be considered new traits to allow the detection of differences in response to N input, which was the major factor considered in the experiments. Moreover, we identified the genetic response to that major factor based on this physiologically enriched data. This approach combines the understanding of the plant response, integrating crop physiology knowledge and genetic analysis towards more conscious breeding processes.

In this discussion, I summarize the main findings identified in the experimental chapters and I reflect on the possibility to integrate them thus deriving general and robust implications for potato breeding under low N input.

Overview of the research approach described in this thesis

In general, there were two main aims in this study as shown in Figure 1:

Understanding of N effects on canopy development, tuber bulking and agronomic traits; and

Assessing whether the QTL identified differed between different levels of nitrogen supply.

Chapters 2 and 3 were directed at the first aim, while Chapters 4 and 5 deal with the second aim.

The phenotypic data generated for this thesis were mainly collected to assess the effects of N supply on agronomic and physiological traits. The use of canopy development was an asset since it is directly related to the capacity to intercept and use radiation and thus to produce yield. Furthermore, it is a simple and robust phenotyping method that allowed us to capture the response of the plant to N input during different stages of crop development. Moreover, by using this data in the canopy development model, the gathered phenotypic data became biologically more meaningful and produced insightful information regarding the phenology and production capacity. The behaviour of the crop response was successfully linked to physiological processes to explain those effects. Therefore, the dissection of a complex trait into meaningful parameters was possible to overcome the difficulty of analysing single points all through the season or scale values that are intrinsically complicated to properly interpret.

Moreover, the tuber bulking process was also quantitatively dissected into several biologically relevant parameters for which genetic variation could be assessed and the impact of nitrogen supply on the expression of that genetic variation could be evaluated.

In Chapter 2, the effects of contrasting types (slow release manure and fast release synthetic fertilizer) and levels of N input on the performance of potato cultivars representative of different maturity types were assessed over the entire growing period. The eco-physiological model parameters reflected the response of the cultivars to nitrogen as well as the maturity differences and their interactions. The results showed the importance of considering the interactions between N supply (type and amount), soil conditions and weather conditions, together resulting in (partly unpredictable) variation in N availability. Overall, low N availability led to an early halt to tuber bulking, occurring even before all the green leaves turned yellow. Moreover, the maximum rate of tuber bulking at low N available occurred before the moment at which the maximum leaf area index (LAI) was reached. With more N, the plant continued to invest in haulm growth for a longer period of the crop cycle than with less N, rather than to invest all available assimilates in tuber growth. Additionally, the more N input, the more yield dry matter (Y_DM) and the higher the nitrogen content ([N]) in that dry matter. Potato plants profit from extra nitrogen by an increased

canopy development, accumulating considerably higher amounts of carbohydrates in their tubers, while the extra nitrogen taken up is only partially used for accumulation of proteins in the tuber. On the other hand, cultivars differing in maturity type were clearly distinctive in the time required to reach some of the physiological events. The early cultivar needed less time to reach maximum progression rate of cumulative leaf dry matter (*Lf_c_tm1*), less time to reach the maximum haulm progression rate (*Hm_c_tm1*) and less time to reach maximum haulm dry biomass (*Hm_t1*). Additionally, the later the maturity type of the cultivar, the longer the time to reach the maximum rate of tuber bulking, and the maximum dry matter yield. Furthermore, late cultivars had higher yield dry matter content with lower nitrogen content in that dry matter, provided the growth season was long enough to complete the crop development. The length of the growing period is a key point to profit from the extra nitrogen as pointed out by Tiemens-Hulscher et al. (2014), otherwise later cultivars would yield less.

Overall, later maturity cultivars are able to yield more, by taking up more nitrogen and developing a larger canopy. Because of the relocation of assimilates at the end of the growing season, the N uptake in the tubers is still higher than for early cultivars but the N content, [N], in the tuber appears to be "diluted". Consequently, a higher yield leads to higher nitrogen use efficiency for late cultivars. The maturity type drives the response of the cultivars to N supply as reflected in the high correlation of the maturity assessment (mt_as) with yield, canopy development as well as LAI. However, within maturity classes there is a possible range of genotypic responses because of the grouping itself, but most importantly because of the physiological variation in genotypic behaviour even at exactly the same physiological maturity type; therefore there is room to exploit existing genetic variation.

In Chapter 3 we used a diverse set of cultivars to obtain a more complete picture of the influence of the maturity type on the canopy development, tuber bulking, N use efficiency and related traits. The main effects of N input and maturity were confirmed. Maturity type is a major factor conditioning the whole performance of the cultivars, not only because it determines the duration of the crop cycle but also because it defines the way in which the resources are used. Generally, later cultivars showed higher dry matter content (DM%), yield and NUE but lower nitrogen content ([N]). There was also an effect of N on the relationships between traits. The *t1* (i.e. thermal time required to reach maximum soil cover (*Vx*)) was longer at low than at high N. Other parameters were higher at high than at low N, especially *Vx* and the period over which it was maintained (*t2-t1*). Moreover, genotypic variation for the traits at each N input level, which is the first requirement for genetic analysis, was confirmed. Additionally, at high N input there was a reduction in the variation among cultivars for most of the soil coverage parameters compared with the low N input, excluding *tm1*, *AP3*, *te-t2* and *t2*. However, for *tm1*, *AP3* and *te-t2* the heritability was 0 or very low (for all maturity groups) meaning that observed variation for these traits was due to other factors than cultivar.

Interaction between N level and maturity was significant for *AP1*, *AP2*, yield and N uptake. For *AP1* and *t1* the N effect was larger for later cultivars than early ones. Additionally, these two traits

showed smaller values at low N input. *AP2* and dry matter yield showed large responses for late cultivars under high N. For N uptake and NUE the intermediate and late cultivars had very similar responses to N. However, at low N input the intermediate cultivars had slightly higher N uptake than the late ones. The environmental differences between the two years included in Chapter 3 influenced canopy cover and probably also N availability. In 2009 the growth for Phase 1 of canopy development was quicker and a higher maximum soil coverage (*Vx*) was reached. In 2010 there were considerable amounts of rainfall later in the growing season, which positively affected Phase 2 of canopy development. The effects of weather conditions differed among maturity classes since the short cycle of early cultivars did not allow them to profit from improved conditions during later phases of canopy development.

In addition, we discussed in Chapter 3 that selection in breeding schemes should be done within maturity groups because of the overriding maturity effect. Finally, the result in this chapter pointed out that the best cultivars for NUE should combine a high response to N fertilization with a high performance under limited input. We proposed to include this strategy in breeding by assessing genotypes under contrasting conditions.

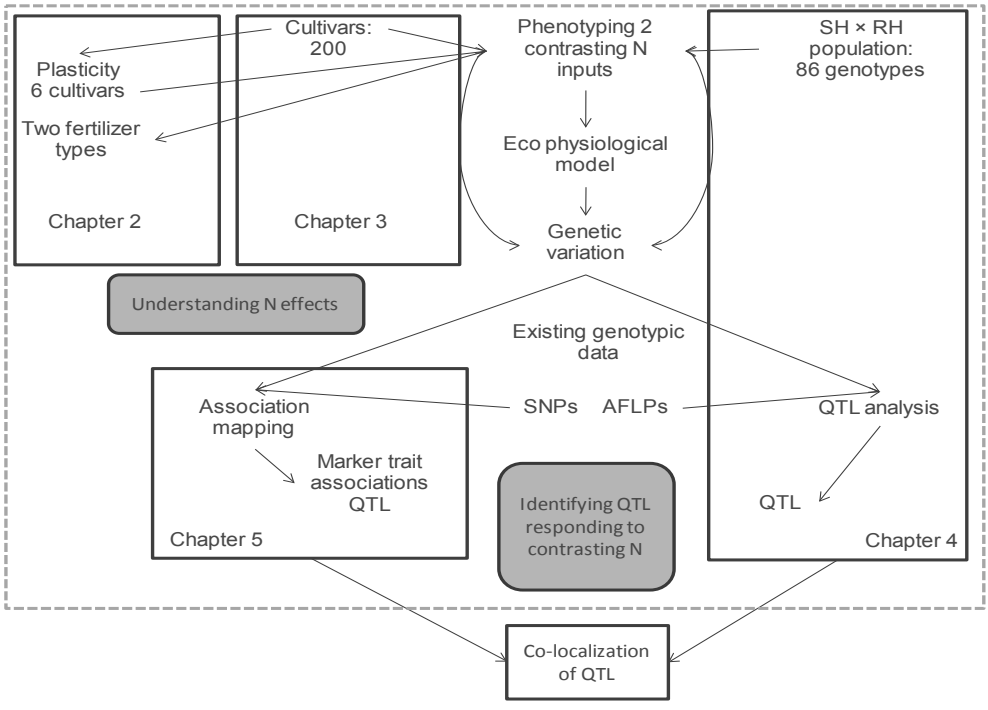


Figure 1 Schematic overview of the research presented in this thesis. The dashed line shows the topics included in this thesis.

The combination of phenotyping soil coverage during the entire growing season and its quantification over time using the eco-physiological model to understand canopy development was an effective approach to show effects of N and maturity in potato as well as the differences among cultivars. In order to further prove the usefulness of this physiological phenotyping tool (phenotypic data analysed with the eco-physiological model), we used two approaches to identify QTL responding to N contrasting input. We used a biparental diploid population in a linkage QTL mapping analysis in Chapter 4 and a large set of cultivars in an association mapping analysis in Chapter 5. These two approaches imply two different genetic backgrounds that lead to different strengths from the analysis. For both the biparental population and the cultivar set, there was genotypic information available although based on different types of molecular markers (Figure 1). For the SH × RH population, on the basis of Amplified Fragment Length Polymorphism (AFLPs) from the high density map study by Van Os et al. (2006), while Single Nucleotide Polymorphism (SNPs) data was available for the cultivar set (Vos et al. 2015). Additionally, both the diploid population and the cultivar set cover a range of maturity types, which is an important factor for the crop physiology of potato and for the canopy cover parameters as show by Tiemens-Hulscher et al. (2014).

In Chapter 4 we studied the genetic basis of nitrogen effects on canopy development, tuber bulking and agronomic traits in a biparental diploid population. The phenotyping was done under two contrasting N treatments and canopy cover was dissected into parameters as it was done in Chapter 2. QTL that were N-dependent or N-independent were identified by comparing the QTL analyses with phenotypic data obtained either at high or at low N input level. Our approach combined a multi-trait QTL analysis with an ecophysiological model of canopy development using the parameters as input traits in the QTL analysis as shown by Khan (2012). The parameters are enhanced phenotypic traits with physiological meaning to understand the canopy dynamics.

Our results showed some QTL for individual traits and for multiple traits (pleiotropic regions) being affected by N input. Low N input allowed the detection of more QTL than High N. Additionally, there was no indication that variance explained by the QTL detected was consistently higher at any of the N levels. The major QTL on Chromosome 5 for maturity type as previously reported by different authors (Celis-Gamboa 2002; Malosetti et al. 2006; Kloosterman,et al. 2013) was also detected. This region on Chromosome 5, in the paternal linkage group V (pa_V), accumulated QTL for most traits but not for quality traits. There were interactions between QTL associated with agronomic and physiological traits and N input. For example: Nitrogen content in tuber ([N]) had a QTL in ma_IX at high N and a QTL in p_XI at low N both explaining more than 10% of the variance of the trait; the duration of Phase 2 (*t2-t1*) had a strong QTL on pa_V only at high N (29% of the variance explained); the tuber DM% had a QTL on ma_III only at high N (19% of the variance explained); *te* had a QTL on ma_III at low nitrogen and a QTL on pa_V at nigh N (explaining 21% and 51% of the variance, respectively). Hotspot regions were found in some chromosomes in addition to the well-reported region on Chromosome 5. Moreover, there were regions like the one in pa_VIII and in pa_XI with QTL for several traits only detected at low nitrogen. This chapter shows

the usefulness of the canopy development curve parameters in genetic studies to detect genetic response due to N input.

In Chapter 5, an association mapping approach was followed to find QTL using the phenotypic data generated in Chapter 3. Therefore, this is a similar approach as the one in Chapter 4 combining the eco-physiological model with genetic analysis (genome wide association analysis, GWAS). Again, QTL dependent on or independent of nitrogen level were identified in this broad genetic background. We effectively showed the influence of N on the detection of marker trait associations, with some of these associations being N dependent while other ones were not. The same region on Chromosome 5, as identified in Chapter 4, linked to maturity was also a hotspot for QTL for most of the traits in the GWAS analysis. Additionally, two other regions were found on Chromosome 2 related to tuber size-weight and size-number distributions. Furthermore, the results showed the importance of maturity and the genomic regions related to this trait, which have been reported in several studies (Celis-Gamboa 2002; D'hoop et al. 2014; Hurtado et al. 2012; Khan 2012; Malosetti et al. 2006); they support the possibility to integrate marker assisted selection (MAS) for maturity in breeding schemes, with the main purpose of improving characteristics that are strongly linked to maturity like dry-matter content, N content and N use efficiency.

In our approach we wanted to compare the positions of QTL of the segregating diploid population and the cultivars set, as it is shown in Figure 1. However, the genotypic information did not overlap between both populations: Neither the physical position of most of the markers used in the SH × RH genetic map nor the genetic position (in the SH × FH population) of the SNPs used in the association analysis were available. Therefore, comparison of the QTL positions of Chapters 4 and 5 was not possible. The co-localization would give a validation of the QTL detected especially because of the different genetic background implied in each analysis. Therefore, it is important to validate new resources, in this case markers in existing populations that have been the base of previous and important studies in order to get more out of the phenotypic data available.

Nitrogen effect on canopy dynamics

The importance of the canopy for yield formation in potato has been shown in several studies (Haverkort and Bicamumpaka 1986; Haverkort et al. 1991; Tiemens-Hulscher et al. 2014; Vos 2009). The intercepted radiation by the canopy is highly correlated with dry matter production, and can be determined by canopy cover traits. Moreover, ground cover or soil coverage is a simple method in comparison to LAI to assess the amount of light intercepted (Haverkort et al. 1991). Furthermore, the effect of N on the leaf size, rate of branching and the partitioning of dry matter are well documented (Biemond and Vos 1992; Vos and Biemond 1992), allowing a good interpretation of N effects on the canopy development dynamics.

Soil coverage is an excellent option as a phenotyping tool to study the dynamics of canopy cover across the growing season as a proxy of canopy development. It requires phenotypic evaluation

over time, in our case weekly, of the percentage of soil coverage (%SC), that is, the area covered by the canopy of the crop plants. The estimation of the coverage was done based on visually counting of grid squares filled with green leaves or based on photographs taken above the canopy, using image analysis as is shown in Chapter 2. The use of pictures was more efficient but involved a photo processing step to estimate the area covered by green leaves based on counting of green pixels. The methodology requires the absence of weeds, but in general normal weed management was enough in our experiments. This methodology allows the evaluation of more genotypes in a more objective approach, while keeping visual track of each plot development. During the onset of senescence the two approaches gave slightly different results especially because the accuracy of the photos better describes the progression of this process.

This approach offered the advantage of a quantitative estimation instead of qualifying the plant using a scale related to a visual impression on a fixed comparative point in the growing season, as it is done with maturity assessments. Moreover, the gathered longitudinal data (assessment over time) was treated as a whole to describe the progress of the trait over time as a function of beta thermal time (Yin et al. 2003). A parametric model for canopy growth developed by Khan et al. (2013) using the beta function was used. It is a quantitative approach to dissect canopy cover dynamics over the season into parameters defining the curve shape. Furthermore, the model divided canopy cover development in three phases (Khan et al. 2013) that are related to the vegetative development (Vos 2009) and to physiological and phenological processes. Phase 1 is the build-up phase during which the maximum progression rate of canopy occurred; it goes from plant emergence to the moment in which canopy cover reach a maximum value (*Vx*). Phase 1 is represented by the area under the curve of canopy development (*AP1*).

Next is Phase 2 (i.e. represented by the area under the curve of Phase 2, *AP2*), that is the phase during which the maximum soil cover is sustained. Although in this phase the canopy cover is maximal and does not change significantly, the leaf area index might still change while this phase progresses. LAI increases with the soil coverage until the moment the coverage reaches a maximum (*t1*). During this period the fraction of the photosynthetically active radiation intercepted is equivalent to the %SC. In Phase 2, starting at *t1*, LAI continues to increase until it reaches a maximum while %SC does not increase (Haverkort et al. 1991). Maximum LAI and *Vx* are greatly affected by N. Subsequently, LAI starts to decline until the end of maximum canopy cover (*t2*). Figure 2 shows the effect of the N levels on the relationship between %SC and LAI for late and early cultivars; with high N input the relationship is as described by Haverkort et al. (1991). However, at low N input or without N input there is no clear increase of LAI after the maximum soil coverage *Vx* was reached, meaning that under limiting conditions the LAI is equivalent to %SC in Phase 1 and Phase 2 of the canopy development; this response is explained by the adjustment on branching and leaf expansion (Vos 1999; Vos and Biemond 1992). In addition, values of LAI during the senescence phase (the blue points in Figure 2) show a distortion of the relationship. The differences due to variation of maturity type are not as clear as the differences due to variation in N input. However, soil coverage is higher for late cultivars and the increase of LAI (after the

maximum soil coverage is reached) is more clearly detectable at high N input than at low N . In Phase 2 of the canopy development, the maximum tuber bulking rate (*Tb_tm1*) occurred and it comes later than the maximum LAI (Figure 13 in Chapter 2). The *Tb_tm1* varied depending on the combination of nitrogen and cultivars with not a clear trend.

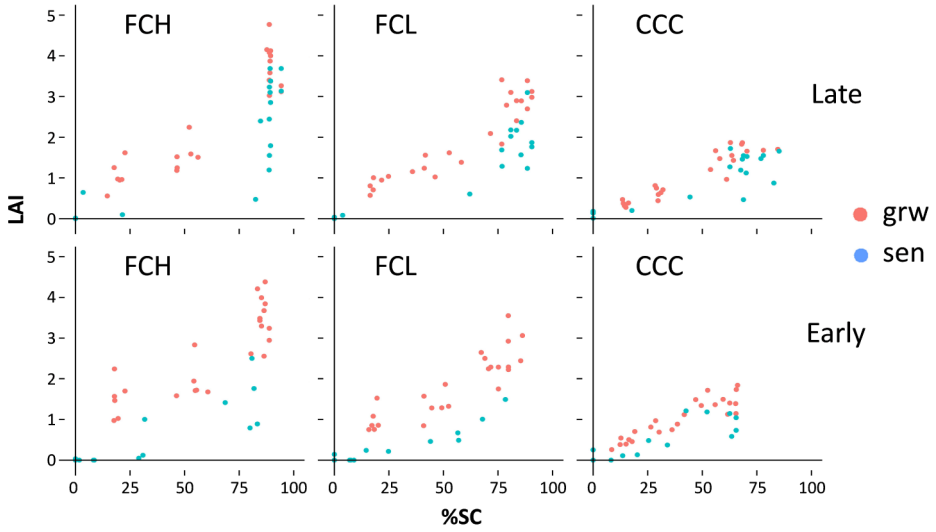


Figure 2 Relationship between Leaf Area Index and percentage of soil coverage for late and early maturity cultivars under different nitrogen inputs: FCH means high input of synthetic fertilizer. FCL, low input of synthetic fertilizer and CCC no fertilizer input. The red dots (grw) are assessments before senescence. Blue dots are assessments after senescence started (sen).

Finally, Phase 3 or the decline phase takes place; in this phase the maximum tuber weight is reached and canopy biomass is declining and assimilates and metabolites are relocated to tubers. This is represented by the area under the curve of canopy senescence (*AP3*). The estimation of *AP3* is difficult, since most of the variation is due to environmental factors strongly affecting both the duration and the area under the curve. As an example Figure 3 shows the response of canopy development for two cultivars in 2009 and 2010. The Phase 3 was very long with the observed values having a strong decrease at high N input and with both early and late cultivars in 2009, where there was more rainfall at the beginning of the season. In 2010 Phase 3 progressed faster while during Phase 1 progress was slow and took longer; here there was a drought period at the beginning of the growing season (Chapter 3).

In Phase 3 when leaves starts to turn yellow and the %SC decreases, the canopy collapses, a process which could be accelerated by wind or rain. Therefore, the distribution of the leaves is no longer uniform, leading to a poorer relation between LAI and canopy cover. Moreover, the leaves are yellowing and it is not always easy to discriminate between yellow and green leaves. After the end of maximum canopy cover, *t2,* the radiation use efficiency is lower, photosynthesis is reduced and the relocation of assimilates is higher so the crop rapidly senesces.

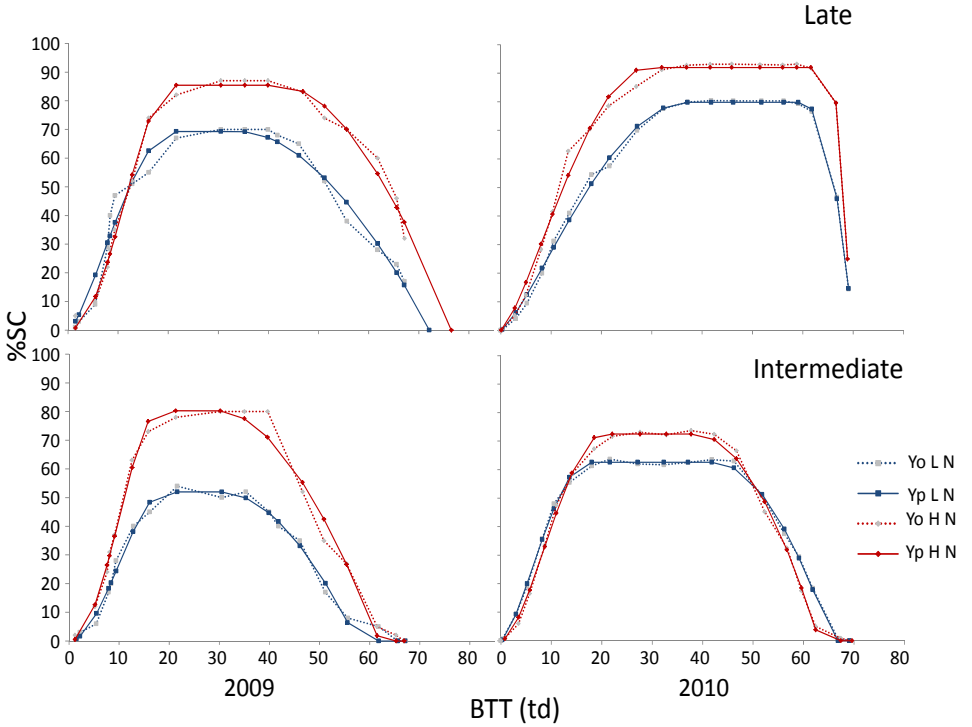


Figure 3 Canopy development for two cultivars (late and intermediate) under two nitrogen inputs (high nitrogen in red and low nitrogen in blue, for the dotted and continues lines); in two years (2009 and 2010): L N, low nitrogen; H N, high nitrogen. The observed values (Yo) and the fitted values (Yp) are included per panel (see legend in the figure). The X axes is beta thermal time (BTT) in thermal days (td) units.

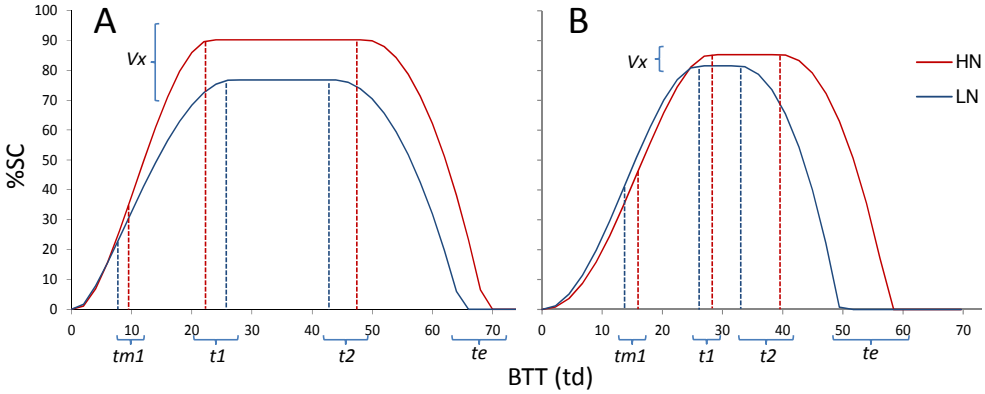


Figure 4 Fitted curves of canopy development for A) the set of cultivars, B) the biparental diploid population. HN represents the high nitrogen input and LN represents the low nitrogen input. Five parameters from the curve are shown: *Vx,* maximum soil coverage; *tm1,* time to reach the maximum progression rate of soil coverage; *t1,*time to reach the maximum soil coverage; *t2*, time for the start of senescence and *te,* time to reach the complete senescence of canopy.

The curve fit parameters are considered physiological and developmental traits with biological meanings as it is explained in Chapters 2 and 3 and also by Khan et al. (2013). The differences in these parameters between genotypes (or cultivars) successfully captured N effects as well as the maturity type effects. These differences are linked to differences in intercepted solar radiation with its potential photosynthetic capacity and with the ability to accumulate biomass and yield.

The summation of the three phases, i.e. the total area under the curve of canopy development (*AUC*), shows the capacity of the crop to intercept light throughout the growing season (Tiemens-Hulscher et al. 2014). With higher values for *AUC* the photosynthetic capacity and the yield are increased. Our results are in line with Khan et al. (2013) reporting *AUC* as highly related to yield, if the season is long enough to allow natural senescence (Tiemens-Hulscher et al. 2014). This parameter reflects the dynamics of the canopy showing significant maturity type and nitrogen effects but not an effect of their interaction (Chapters 2 and 3). More nitrogen input enhances the vegetative development resulting in a higher *AUC*, mainly by an early *t1* and a higher *Vx*. Tiemens-Hulscher et al. (2014) concluded that *AUC* is the best predictor of performance in terms of yield and nitrogen efficiency.

The canopy development analysis was also able to capture the variation in N response in both a biparental diploid population and in a large set of diverse cultivars. These two different groups (having different ploidy levels, different vigours of the plants and different genetic backgrounds) had very similar responses in terms of their canopy dynamics to contrasting N inputs. The differences in the canopy coverage dynamics were mainly in the build-up phase and in the Phase 2 (Figure 4). For the biparental diploid population, the time to reach the maximum canopy cover (*t1*) was longer at high N than at low N. However, the rate of increment in soil coverage did not change but the maximum coverage reached did change. Consequently, the area under the curve (*AP1*) representing the build-up phase became slightly larger. For the diploid population, having less vigour and less potential to respond to the extra N, the time required to complete each phase of the canopy development increased. Hutten et al. (1995) reported that diploid potato progenies had significantly lower yields (due to smaller tubers) and higher under water weights than tetraploid progenies; these can be understood as lower vigour. For the cultivars, which had been bred to benefit from optimal crop system conditions, the duration of the Phase 1 was reduced in response to more N input. This means a shorter *t1 (*period to reach the maximum coverage) showing a higher growth rate, faster growth during Phase 1 and reaching a higher *Vx* (maximum soil coverage) at high N. Phase 2 became longer and accounted for a longer proportion of the whole growing period. Vos and Biemond (1992) mentioned that the canopy of potato plants responds to more N not by changing the rate of appearance of leaves on a branch but by increasing branching, having faster leaf elongation and longer individual leaf lifespan. From our results in Chapter 3 we can summarise that the response to low N vs high N input is like the effect of early maturity vs late maturity for most of the traits. For example *AUC, Vx* and *t2* values increase from early to late cultivar and from low to high N input. However the traits of Phase 1, *t1, AP1*, and *tm1,* showed higher values at low N input than at high N input and smaller values

for early cultivars than for late cultivars. The nitrogen content also showed the same trend: lower values at low N input than at high N input and higher values for early cultivars than for late cultivars.

Canopy cover analysis with the ecophysiological model allows a better use of a more intensive phenotyping to assess the influence of different factors like drought and crop management on the plant and crop development. Additionally, the possibility to use phenotyping platforms in the field makes the combination of the canopy dynamics with other interesting traits based on measurements of canopy reflectance or emissions from visible to near infrared wavelengths, a very interesting approach. Traits such as chlorophyll, photosynthesis activity, nitrogen, leaf area index and plant biomass, expand the possibilities of understanding the plant response applying models to describe seasonal or even daily dynamics of these trait.

Potato breeding for low N input and NUE

Although high input systems may provide large yields, they create a fundamentally unsustainable environment (Fess et al. 2011). Selection under optimal conditions exploits the genetic potential in response to luxurious conditions and management, tuning the conditions in order to get the best response of the genotypes. Under low input the direction changes to finding cultivars able to perform relatively well given low and variable N availability, as mentioned by Tiemens-Hulscher et al. (2014) in organic farming. Moreover, agronomic research has shifted from finding the optimum rate of input to how to make the best use of the permitted maximum amount of external supply of N (Vos 2009).

Generally, when evaluating breeding for low input conditions, the discussion is directed to whether or not varieties developed under optimal conditions will perform well under limited conditions; and whether the selection for low input performance should be done indirectly, under optimal conditions. Typically, varieties that have been bred and tested in nearly optimum conditions across several locations, do not perform well in marginal environments or without the external inputs from which they were selected for (Ceccarelli 1996; Murphy et al. 2005). Hirel et al. (2007) mentioned that under low input conditions maize yield is inevitably lower but direct selection under low N fertilization input would be more effective than an indirect selection under high N fertilization input. Furthermore, modern varieties have been shown to be outperformed by traditional farmer varieties under low-yielding conditions. Lammerts van Bueren and Myers (2011) mentioned that there is substantial evidence that breeding for low N condition is more efficient under severe stress than under high input conditions in several crops. In potato, our results from Chapters 2 and 3 showed that N input affects the relationship between the physiological and agronomical traits and therefore the importance of the measured trait to explain specific processes. An example is the maximum soil coverage (*Vx*). Under abundant N input, all genotypes tend to reach the maximum coverage, then the areas under the curve become more dependent on the duration of the phase that on the *Vx* because the values of soil coverage are very close to

100%. Additionally, as it was observed in the field, the differences in these values of *Vx* detected at high nitrogen are more related to the canopy density, while at low N input these differences in *Vx* are more related to the ability of the cultivar or genotype to invest in expanding its canopy.

Moreover, the significant N by maturity type interaction (Chapter 3) for the traits related to Phase 1 of canopy development, the duration of the Phase 2, the *AUC* and yield, showed the possibilities to improve for better adaptation to low nitrogen supporting the direct selection. In addition, in Chapters 4 and 5 the N dependent QTL suggest that different genomic regions are more or less important for a trait depending on the N input. Thus, the direct selection would lead to higher frequencies of different genes or regions responding to the low N condition. However, this is not conclusive as the high correlation of yield between high and low N input could be interpreted in favour of indirect selection.

In addition, a major complication of studies under limited input conditions, like organic production systems, is the intricate relationship of several factors and their interactions. This relationship is not understood and generates uncertainty in the interpretation of the results. In this sense experiments having few limiting major factors are an ideal framework to understand what is happening with the crop while trying to compensate or adjust its development to complete its biological cycle. As shown in Chapter 3, the variation at low N input tends to be higher for most of the traits. In our experimental conditions, the change in variation was mainly due to N input, which was the main factor driving the differences. Then, the response of the cultivars showed their capacity to adapt to those conditions, ensuring a possibility to select for low N input conditions. Moreover, the adjustments of the crop are not appreciable under high N input selection because they are not happening or because traits advantageous in low-input systems are often overlooked, as mentioned by Ceccarelli (1994) and Fess et al. (2011).

Furthermore, our results showed that at low N input there were some associated markers and QTL that were not detected at high N. This QTL × N interaction indicates that limiting N conditions result in a change in the importance of the associated genomic regions and therefore in differences in genotypic response. We only reported consistent associations over two seasons (Chapter 5) ensuring that QTL were not due to the QTL × Year interaction.

We argued that breeding for low N input should include low input conditions, but more research is needed to find traits that are directly linked to the adjustment that the crop makes under limiting N conditions. Observations in the field experiments discussed in Chapters 2 and 3 suggest that another trait to be considered under low input should be the ability of the crop to retain leaves. Variation was observed, although this trait was not included in this thesis. Moreover, NUE (yield per unit of N input) showed higher values under low N input in comparison with a high N input (Chapters 2 and 3; Zebarth et al. 2004). Therefore, a reduction in input will intrinsically increase the efficiency. The best cultivars adapted to limited conditions would have the highest NUE and yield would be higher in these limited conditions. At high N input, the most important one is yield. To

select a cultivar for NUE, both low and high N should be combined; i.e., good NUE under low input and with good response to the extra N.

The N input affects the vegetative development, light interception, and thus photosynthesis and yield, all reflected in the canopy development throughout the season (Marshall and Vos 1991; Vos 2009; Vos and Van der Putten 2001; Vos and van der Putten 1998). Vos (2009) showed that the potato strategy to cope with limiting N is to adjust light interception per plant, but intrinsic productivity per unit of leaf area does not change, since N concentration in leaves of potato plants is not significantly affected by N nutrition. Additionally, the relocation of assimilated N could be an important factor determining efficiency. Vos (1999) mentioned that relocation accounts for 20 to 40 % of the dry matter yield and this phenomenon is particularly important for N: The balance of sink strengths of N to top and tubers determined the quantity of additional dry matter produced and the starting of senescence.

On the other hand, maturity type also affects NUE: late cultivars showed higher efficiency than early cultivars with considerable variation within each maturity group (Chapter 3); this is valid if the growing season is long enough to allow a profit from the prolonged and bigger canopy. Tiemens-Hulscher et al. (2014) reported that early cultivars had higher NUE in organic production where the growing cycle is restricted as a control measurement to prevent spread of late blight. In our conditions the late varieties had a longer growing period and the response to N had an effect on extending the Phase 2 (*AP2*) of canopy development. Consequently, there is a prolonged period with maximum light interception, more potential photosynthesis and yield as mentioned before.

Measurements like harvest index are useful to find the ideotype of potato that maximizes the productivity of the canopy but for potato this is difficult to use because potato plants are harvested after senescence of the haulm. An alternative trait is the soil coverage yield index (SCYi) that is the ratio of soil coverage (in this case area under the curve of the soil coverage i.e. *AUC*) and yield. Indeed, results showed that this trait had QTL different than those related to yield or *AUC* and were also different for those detected of maturity. This could be an interesting trait to exploit and it also showed the possibility to look for other relationships between traits to help in the improvement of the plant's NUE.

Potato breeding and maturity

Maturity of potato cultivars plays a major role in determining crop management and some of the qualities of the varieties. It is a trait based on the cultivar's timing of tuber formation, or on the timing of leaf senescence combined with sagging of plants and completion of apical canopy growth (Struik et al. 2005). In this thesis we used the definition of breeders that is based on canopy senescence comparison. Maturity type is a complex trait that controls the crop's life span from sprouting of the tuber to flowering, tuber formation, maturation and senescence (Gebhardt et al.

2004), for which genomic regions and mechanisms have been elucidated (Kloosterman et al. 2013; Visker et al. 2003).

Maturity is a stable and important trait and it can be used in potato breeding to separate cultivars in field trials into three maturity classes: early, intermediate and late, to reduce inter-plot competition (D'hoop et al. 2014). The same approach was used in the experiments presented in his thesis, with the set of tetraploid cultivars as well as with the diploid population (Chapters 3 and 4). Maturity was also considered as a factor in the statistical analysis with the varieties nested within maturity groups. The results throughout this thesis, Chapters 3 and 4, showed a strong maturity effect on traits related to canopy development and on yield dry matter, percentage of dry matter, N content, as well as NUE indices. D'hoop et al. (2011) showed that the predicted values for the traits maturity and underwater weight were influenced by the correction for maturity class during phenotypic analysis, but many quality traits were not. In our experiments, cooking quality traits did not show a relation with maturity values (Chapter 4), supporting the results reported by D'hoop et al. (2011; 2014). In addition, in our study the maturity effect was even more pronounced since we showed it had an effect on most of the canopy traits and agronomic traits physiologically related to the duration of the crop cycle.

Maturity groups showed a general trend for canopy development in the cultivar set. The earlier the cultivar, the shorter was Phase 1 as well as the duration of Phase 2 during which the maximum canopy cover is sustained. Additionally, these maturity groups had the same relative trend in both N treatments. Our study included many cultivars, each properly characterized for maturity type gathering information from several experiment in the breeding process. It allows a more complete overview of the influence of maturity type in NUE-related traits. The later the genotype was, the higher the yield and the NUE were. In the diploid SH × RH mapping population, there was not a clear trend of the parameters for the different maturity groups. For Phase 1 only the middle late group had a shorter period to reach maximum canopy cover (*t1*). In Phase 2, the maximum coverage was higher with later-maturing genotypes. However, the distinction between maturity classes was probably not as optimal as for the cultivar set. The maturity grouping in the diploid population was based on limited phenotyping including few years of experiments previous to this study. With the (tetraploid) cultivars, the maturity types had been assessed over several years, in different conditions and management practices, resulting in a better and accurate overall assessment of this trait. Additionally, the cultivar set is indeed more diverse and more spread in the maturity scale than the diploid population.

The maturity type acts as an anchor, determining major plant characteristics and the response to some other factors than N. Maturity assessed in the field was highly correlated with total area under the curve of canopy development (*AUC*), with dry matter yield (Chapters 3 and 4), and is linked to late blight resistance (Bormann et al. 2004; Bradshaw et al. 2004; Colon et al. 1995; Visker et al. 2003). These characteristics determine the market target (e.g. industry or staple food) of cultivars as well as the production system in which a cultivar should be grown.

The strong relationship between several phenotypic characteristics and maturity is a disadvantage when the breeding objective requires a break of this linkage. For example, resistance in potato is often associated with plant maturity type, as most resistant genotypes are also the ones that mature the latest. This is a handicap for breeders and growers who aim to get early maturing crops to shorten the time of tuber production, but also like to include resistance to late blight (Danan et al. 2011). Our results indicated that the same problem exists for agronomic traits like NUE, yield, DM% and [N]. However, within each maturity class there is variation in the parameters that can be exploited (Chapters 3 and 4). *AUC*, for example, showed a strong correlation with yield but there was variation within a maturity group (Chapter 3, Figure 4). This is supported by results of Tiemens-Hulscher et al. (2014) who found significant maturity and cultivar effects on *AUC* across several experiments. Moreover, D'hoop et al. (2011) showed maturity class dependency of the variance components for traits such as under waterweight and maturity assessment leading to variable heritability of these traits among maturity classes.

Therefore, it is crucial for genetic studies and for practical breeding purposes to have a robust maturity characterization to define the class for each genotype or cultivar. Canopy development parameters have been shown to be useful in defining the maturity type with a physiologically based criterion (Khan et al. 2013; Khan 2012) and could help to characterize cultivars and possible parental material in a more appropriate way. However, a global approach by grouping cultivars not only based on one physiological trait at the time but on several of these traits like *t1*, *t2*, *te*, *AUC* and *tm1* could bring advantages considering the relationship between these traits.

It is important to understand and to overcome (if possible) the strong linkage between some characteristics and maturity, for example, to develop varieties with a short growth cycle and relatively high dry matter content, high NUE or high resistance to late blight. However, as postulated by Śliwka et al. (2006) and Collins et al. (1999) the negative correlation between the length of vegetative period and late blight resistance is rather physiologically determined and therefore difficult to break (excluding resistance from R genes). Colon (1994) mentioned that the induction of tuberization by a treatment of short photoperiod in *S. microdontum* has been shown to be associated with a slight, but significant loss of resistance, attributing the linkage to physiological characteristics. Visker et al. (2003) on the other hand found that late blight resistant linkage with maturity is also genetic. They mentioned that it could be due to either a single gene with pleiotropic effects, or to two close linked genes; these conclusions were based on results after a correction for maturity in the QTL analysis. For agronomical traits such as dry matter, yield and N content, as they are the expression of complicated physiological mechanisms depending on the duration of the growing period, the linkage with maturity is most probably a physiological linkage.

Potato populations selected within a determined maturity group could increase the chances of finding possible breaks in the linkage with maturity for these characteristics. Additionally, it will increase the chance of detecting other regions that could be hidden because of the variation due to maturity. In this regard, correction for maturity in genetic analysis, by determining the markers related to maturity and using them as co-factors in the analysis, offers an approximation

to the strategy of populations within a narrow maturity group. It has been done in Chapter 4 using QTL detected in simple interval mapping as cofactor for the composite interval mapping. However, having an extended population within one maturity class truly offers a condition of more accuracy in determining the variation of other traits and the possibilities to select for those traits rather than for maturity.

High-throughput phenotyping

In modern breeding there has been a continous development of techniques showing great potential to be used for genetic improvement programmes. However, the availability of reliable and robust phenotypic data remains a limiting factor. It is good to remark that the core of breeding is the selection based on observation, i.e. based on phenotyping. In order to improve breeding efficiency, especially for quantitative complex traits, the need for high-throughput phenotyping can increase the ability of dissecting complex traits such as yield and NUE.

For genetic studies, it is important to be able to gather phenotypic data rapidly and accurately over a large number of genotypes and, even better, over different environmental conditions as it is needed in breeding. The phenotypic information is used to further dissect the genetics of the traits using powerful quantitative genetic techniques to find QTL and markers explaining the observed variation. Additionally, the phenotypic dissection of complex traits into physiologically meaningful component traits offers a better understanding of the biology and physiology towards a more conscious and intelligent breeding. Cabrera-Bosquet et al. (2012) mentioned that both high-throughput phenotyping and genomic selection have in common that their approach is empirical, enabling breeders to use genomic and phenotypic profiles without understanding the underlying biology, although high-throughput phenotyping has the potential to improve the understanding of crop physiology.

The use of enormous amounts of phenotypic information is becoming more common in breeding. With advances in whole genome sequencing technologies the cost to generate genotypic data is low (Furbank and Tester 2011; Patel et al. 2015). Because genetic information is not a limitation, molecular breeding strategies like genomic selection are possible, using all marker data as predictors of performance and consequently delivering more accurate predictions (Jannink et al. 2010). Such strategies still require phenotypic data to estimate and to update predictions (Cabrera-Bosquet et al. 2012) like those of breeding values from training populations. Field-based phenotyping is increasingly recognized as the only approach capable of delivering the required throughput in terms of numbers of plants or populations required in genetics studies, as well as an accurate description of trait expression in the real-world cropping systems (White et al. 2012). Traits with great potential to increase yield are often complex. Adding strength to the phenotypic data by modelling is an important step to understand the underlying processes while increasing crop physiology knowledge. High-throughput phenotyping may help in the systematic incorporation of modelling phenotypic traits, thus facilitating the phenotyping over time (Cabrera-Bosquet et al.

2012) to understand developmental traits or their dynamics in order to improve not only knowledge but also breeding schemes. Our approach is an example of modelling a complex trait and its dissection into physiologically meaningful traits that deserves to be analysed further.

Therefore, phenotyping for the study of a developmental process with eco-physiological modelling gets more relevant. Our results showed that modelling canopy cover allowed us to study the effect of a major factor, N input, on the plant development. The model parameters captured the response to contrasting N conditions, giving an impression on how the development was affected, improving our understanding of the whole process and its interaction with other factors, like maturity type. The importance of canopy for the plant biomass production is undeniable, and the understanding of its dynamic becomes more relevant since canopy is directly related to other complex traits at the crop scale, like radiation use efficiency, photosynthesis, NUE and yield.

High-throughput phenotyping has been developed as platforms in greenhouse systems (Araus and Cairns 2014; Yang et al. 2013) where great advances have been made to cope with more phenotypic data; therefore, a systematic and efficient data management and data processing are required (e.g. software like PHENOME (Vankadavath et al. 2009)). In this regard, there has been a great development in image analysis processing (Hartmann et al. 2011) and programming; then repetitive tasks can be easily transformed into programme codes to save time. Most of this phenotyping is based on image analysis like visible light imaging, infrared and hyperspectral imaging (Yang et al. 2013). Some of these technologies are becoming useful in field conditions (Araus and Cairns 2014; Walter et al. 2015). Moreover the range of traits that can be evaluated directly and non-destructively using measurements of reflectance is growing with the better understanding of plant biology, sensor and imaging technologies, and data analysis (White et al. 2012).

In general most traits suitable for remote sensing are related to the canopy of the crop since the measurements are not destructive or invasive (Prasanna et al. 2013). Methodologies to perform the measurements are based on proximal remote sensing analysis, including different sensor types using radiation reflected (or transmitted) with wavelengths corresponding, on the electromagnetic spectrum, to visible/near-infrared (VIS-NIR) radiation and far-infrared (or thermal) radiation emitted by the crop (Araus and Cairns 2014; Cabrera-Bosquet et al. 2012). Thus, the understanding of canopy development is important and should be further combined with other possible remote sensing techniques, including the integration of different sensors and the use of appropriated platforms. In this regard, the technology is becoming low cost (Cabrera-Bosquet et al. 2012) and more exploitable. Then, to become operational in the field, platforms have been developed using different setups implemented in different carriers such as tractors or unmanned aerial vehicles (White et al. 2012; Zhang and Kovacs 2012).

Applications of these remote sensing approaches to study nitrogen use efficiency could be possible. Mauromicale et al. (2006) using chlorophyll fluorescence and content found differences

in the response of potato to N supply, discriminating between genotypes, plant age and yield performance under field conditions. In wheat, a study evaluating canopy spectral reflectance was shown to predict grain nitrogen use efficiency in soft red winter wheat (Pavuluri et al. 2015). In both cases these measurements could be done by using proximal sensing methods in high-throughput platforms allowing the investigation of more genotypes or more sites or to study the dynamic of the traits.

In the context of our research study, interested in low N input and N use efficiency, the use of high-throughput platforms could advance the accuracy, increase the amount of data collected, increase the speed of data collection and the usefulness of the findings. For example, the use of aerial platforms such as polycopters could be implemented to do the phenotyping done in this thesis: to take pictures over the crop cycle for the evaluation of canopy cover. A picture taken from a known height could contain several plots positionally referenced by few recognizable points visible in the field and in the picture. Several pictures could overlap in the area covered, facilitating the image processing and increasing the precision of the measurements. Then, several pictures could track the development of the crop at even daily intervals or less, measuring different traits depending on the sensors used. Such a methodology will greatly reduce the time of a single assessment and could open the possibility to increase the number of assessments, the number of experimental fields or to include more related traits. Moreover, the canopy cover model could be applied using such a phenotyping platform and could be combined with other possible measurements, like chlorophyll fluorescence, to improve the interpretation of the results opening a whole range of possibilities.

The state of technology in remote sensing, image analysis, data management and analysis and modelling of plant processes makes us to reflect on how to obtain phenotypic data. It requires multidisciplinary efforts to put together technical aspects in a synergistic effort. As a result, more phenotyping will go beyond the observation of the naked eye and will be available to the eyes of breeders.

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Summary

Potato is an important food security crop that helps to fulfil the diet energy needs of many people worldwide. Potato characteristics like the very high harvest index, adaptability to a variety of environmental conditions as well as to different crop productions system, the excellent quality of food (as source of carbohydrates, minerals, vitamins, etc.), have resulted in an increase in production in developing countries.

Nitrogen (N) plays and important role in the potato vegetative development and production with high N requirements in order to maintain good performance, compensating the low N-recovery and nitrogen use efficiency, due to the small and shallow root system. These latter characteristics, in conjunction with the cultivation on sandy soils (ideal for harvesting) result in high losses of nitrogen and pollution.

Potato exemplifies the impact of nitrogen fertilizers in food production. On the one hand, nitrogen supply has increased the food production over the last 60 years, but on the other hand, it has also contributed significantly to the total amount of reactive N in the troposphere. Agriculture needs to reduce N input and increase nitrogen use efficiency (NUE) of the crops. Therefore, legislation like the Nitrate Directive (91/767/EEC) and the Water Framework Directive (2000/60/EC) forces a reduction in nitrogen supply in crop production. Nitrogen input reduction will have a large impact on yield and quality of potato as potato is highly responsive to N input.

With more N input, potato plants increase the production of leaves, branching, leaf area index (LAI), the duration of the crop cycle, and the dry matter production although N has been reported to delay the onset of tuberization; the positive effects of N input on the canopy development enhances light interception and photosynthetic capacity. Nitrogen also affects some quality traits like tuber size and weight distribution, the tuber dry matter content, starch content, protein content, nitrate content, and processing quality. Moreover, potato is mentioned to adapt its foliage development to limited N supply in such a manner that the plant maintains productivity per unit of leaf area while adjusting total leaf area. Therefore, the study of the variation in nitrogen response is complex with most of the nitrogen effects being related to canopy development.

The nitrogen response also depends on the maturity type of the cultivar. Late cultivars are expected to profit more from the extra nitrogen than early cultivars. Moreover, NUE has shown to be higher for late cultivars. Moreover, the development of soil coverage also reflects the maturity type of the cultivar which is usually assessed by comparing the senescence state of the canopy among cultivars. Therefore, the study of canopy traits became important for assessing the variation in nitrogen response.

Under this context this thesis aimed i) to understand the N effects on potato performance under low N input, ii) to quantify the genotypic variation under contrasting N inputs in order iii) to identify

quantitative trait loci, QTL, involved in the N response, all aiming towards improving NUE under low input.

In this thesis, an ecophysiological model of canopy development was used as a phenotyping approach to study the variation in nitrogen effects on different phases of the crop for different maturity types. Parameters of the curve describing the soil coverage progress (weekly assessed, as a function of beta thermal time) were the traits together with some yield components.

In Chapter 2 the aim was to understand how cultivars differing in maturity type respond to contrasting N inputs (combination of two types of fertilizer and two contrasting N levels, and a control) and N availability across the growing season (2010 and 2011). The results showed the importance of considering the interactions between N supply (type and amount), soil conditions and weather conditions. The meaningful model parameters helped to understand the relations, and the chronological changes in these relations, between important traits like LAI and tuber bulking. Moreover, the results pointed out how maturity type drives the response to N input as shown by the relationship between maturity type, yield, canopy development and LAI. Canopy development parameters were useful to describe the response to different nitrogen conditions. Therefore, the ecophysiological model as used here appeared an excellent approach to study stability in the performance of cultivars. Our results evidenced the importance of studies including more genotypes within maturity classes. There is a possible range of genotypic responses because of the physiological variation in genotypic behaviour even at exactly the same physiological maturity type. Therefore, there is room to exploit existing genetic variation to improve traits related to the response to nitrogen input.

Chapter 3 describes the phenotypic variation of a large and diverse set of potato cultivars for canopy development parameters and NUE under two contrasting N levels. The main effects of N and maturity were described, as well as the effects of N on the relationship between traits. Maturity type is a major factor conditioning the whole performance of the cultivars. Later cultivars showed higher dry matter content (DM%), yield and NUE but lower nitrogen content ([N]). There was also an effect of N on the relationships between traits. Interaction between N level and maturity type was significant for the area of the canopy development progress curve during the build-up phase and the canopy consolidation phase, yield and N uptake. It is suggested that a general strategy to breed for NUE should focus on low input. The best cultivars for high NUE should combine a high response to N fertilization and high performance under limited input. It is discussed that selection in breeding schemes should be done within maturity groups because of the overriding maturity type effect. We propose to assess genotypes under contrasting nitrogen conditions.

In Chapter 4, the aim was to study the genetic basis of nitrogen effects on canopy development, tuber bulking and agronomic traits using a biparental diploid population (SH × RH). The approach combines QTL analysis using existing genetic information with the ecophysiological canopy model by using the canopy parameters as traits to find QTL. The effect of N input on the related QTL was

assessed by comparing the results from separate QTL analyses for each contrasting N level. QTL that were nitrogen dependent or nitrogen independent were found. The major QTL on the parental Chromosome 5 (pa_V) for maturity type was detected. This region accumulated QTL for most traits but not for quality traits. Moreover, there were regions like the one in pa_VIII and in pa_XI with QTL for several traits only detected at low nitrogen. There were interactions between the genetic factors associated with agronomic and physiological traits, and N input, suggest that breeding for low N input done directly under this nitrogen condition could offer an advantage above doing indirect selection. This chapter shows the usefulness of the canopy development curve parameters in genetic studies to detect genetically diverse responses to N input.

In Chapter 5 the model parameters describing canopy development were used to perform a genome-wide association study (GWAS) to assess the genetic basis of developmental, physiological and agronomic traits in relation to contrasting N levels. The approach was similar as in Chapter 4 since in both cases the parameters from the ecophysiological model were used to study the genetic variation in response to nitrogen input. However, a much more diverse and wider genetic background was used in this chapter (a set of tetraploid cultivars) compared with the diploid biparental population (SH × RH) in Chapter 4. The results effectively showed the influence of nitrogen on the detection of marker trait associations, with some of these associations being dependent on the nitrogen input while other ones were not. As in Chapter 4, a region on Chromosome 5 linked to maturity was also a hotspot for QTL for most of the traits in the GWAS. Moreover, two other regions were found on Chromosome 2 related to tuber size/weight and size/number distributions. The importance of maturity type was once more confirmed, especially for developmental traits. It is possible to integrate marker assisted selection (MAS) for maturity type in breeding schemes in order to break the strong linkage between maturity and traits like dry-matter content, N content and N use efficiency. The main purpose should be to pre-select genotypes with a more similar maturity type and then look for variation in those characteristics that are strongly linked to maturity type.

Chapter 6 is the general discussion of the findings of this study. An overview of this thesis is given while the results from the chapters are compared. The importance of understanding developmental traits by using models was highlighted as well as their possible use in breeding schemes for NUE. The canopy cover analysis using the ecophysiological model allowed the study of the nitrogen effects on the canopy dynamics. This approach helped to understand the complexity of these N effects by the articulation using previous knowledge on this matter. Moreover, the phenotyping approach for canopy development was shown to be useful to understand the effects of variation in nitrogen input at different (agronomic, crop physiological and genetic) levels. The importance of the findings was put in the context of NUE for low input, discussing the relevance of direct selection. Maturity type in potato breeding was discussed given its major role in determining the behaviour of the cultivar or genotypes. The canopy development parameters allowed a better understanding of maturity type, since the traditional assessments are a huge simplification of complicated developmental processes. Finally, the possibilities of using

high-throughput technologies to enhance phenotyping or to combine these with approaches like the use of ecophysiological models to dissect canopy development were highlighted.

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César Andrés Ospina Nieto
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About the author

César Andrés Ospina Nieto was born in Palmira, Valle del Cauca Colombia, on the 25th of April 1978. He grew up in Palmira where some of the most important agricultural research centres in Colombia have their headquarters and therefore he was greatly influenced by agricultural activities. With the belief in the fundamental importance of food production to sustain any level of the society inculcated in his family, César enrolled in the bachelor programme for agronomic engineering at the Universidad National de Colombia in the Faculty of Agronomy based in Palmira. He joined CORPOICA (Colombian Corporation for Agricultural Research) as intern student to do his bachelor thesis in phytopathology in citrus. In this project César worked at CIAT (International Centre for Tropical Agriculture) to do the molecular characterization of the pathogen in citrus. After that, he worked as guest researcher at CIAT executing a similar study in the tropical fruit *Annona muricata* L. (Soursop). One day while going home, César had a nice conversation with Martin Fregene, cassava geneticist at CIAT and shortly thereafter César got a position as a technician in the Cassava Genetics lab in 2002. There he worked on marker assisted selection (MAS) for resistance to the Cassava Mosaic Disease (CMD). César was promoted to research assistant being also in charge of the experimental fields, crosses, and phenotyping, supporting all the projects in the cassava genetics group. He continued to be engaged in lab duties related to marker assisted selection and in the Generation Challenge programme and in the phenotyping support service. After gaining massive experience in lab and field, in 2005 César decided to initiate his masters in plant breeding at the Universidad National de Colombia, in Palmira in parallel with his job. The thesis was conducted as part of a project in MAS for CMD in collaboration with a national research centre in Tanzania. In this project César did field and lab work as well as some capacity building for researchers in Tanzania in MAS. In 2008 César took a major step in his life and moved to Europe to improve his English to further develop his professional career, to be with his wife (married one year early), and to build a family. With an eye on these goals César applied for a PhD position in Wageningen University within a project funded by the European Union entitled "Nitrogen use efficiency in potato and integrated physiological morphological an genetic approach", which was designed as a collaboration between Wageningen UR Plant Breeding and Crop Systems Analysis. This thesis presents the outcome of this research experience that helped in the further development and strength of César's skills (team work, development of methodologies, experiment, innovation, and data analysis) and also marks a new step full of new challenges and opportunities to come.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)
- Overview of Nitrogen Use Efficiency (NUE) in potato: physiological and genetic approach (2010)

Writing of project proposal (4.5 ECTS)
- Nitrogen Use Efficiency (NUE) in potato, an integrated agronomical, physiological and genetic approach (2010)

Post-graduate courses (3.6 ECTS)
- Mixed model based QTL mapping; PE&RC (2012)
- Mixed linear models; PE&RC (2012)
- Bayesian statistics; PE&RC (2012)
- The art of crop modelling; PE&RC (2013)

Laboratory training and working visits (4 ECTS)
- Phenotyping of physiological traits; AGRICO (2009, 2010)
- Potato breeding scheme; AGRICO (2011)

Deficiency, refresh, brush-up courses (0.6 ECTS)
- Generalized linear models (2012)

Competence strengthening / skills courses (1.2 ECTS)
- Workshop presentation skills; PE&RC (2014)
- Career assessment; PE&RC (2015)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.8 ECTS)
- PE&RC Weekend (2011)
- PE&RC Day (2012)
- PE&RC Weekend (2015)

Discussion groups / local seminars / other scientific meetings (6.3 ECTS)
- Abiotic stress group meetings (2010-2013)
- CSA and PPS Seminars (2010-2014)
- Soil-plant interaction group (2011)

International symposia, workshops and conferences (7.5 ECTS)
- NUE-Crops annual meetings (2009-2010)
- Potato breeding after completion of the DNA sequence of the potato genome (2010)
- Eucarpia section organic & low input agriculture; poster presentation (2010)
- Eucarpia breeding for nutrient efficiency; oral presentation (2013)

Lecturing / supervision of practicals / tutorials (3 ECTS)
- Organic plant breeding (2010)
- Research methods in crop science (2012, 2013)

Supervision of MSc students (6 ECTS)
- Effects of different sources and levels of nitrogen supply on yield, tuber size and weight distribution on a diverse set of potato cultivars (2010-2011)
- Quantifying plasticity of Nitrogen Use Efficiency (NUE) on selected potato genotypes in response to contrasting nitrogen input types and levels (2011)

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