NUE-CROPS: Improving nutrient efficiency in major European food, feed and biofuel crops to reduce the negative environmental impact of crop production

Final Report

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1. Executive summary

The NUE-CROPS project was a five year integrating project funded by the European Commission between 2009 and 2014. The goal of the project was 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 project addressed this challenge by generating new knowledge about nutrient use efficient (NUE) crop varieties and cropping systems. The project focussed on four of the most important arable crops grown in Europe: wheat, maize, oilseed rape and potatoes. Academic and industrial partners were included from six European countries as well as China, Turkey and the United States. Advanced methods in molecular biology and genomics were used to identify “molecular markers” that can speed up the breeding process; these markers were linked with plant traits that improve NUE. NUE traits included those associated with the rate and depth of root development, biomass partitioning during crop development, nutrient uptake, nutrient utilization, harvest components and maturity type. The role of NUE varieties within cropping systems was investigated in partners’ field trials where different management practices (fertilizer type, tillage, rotation) were studied at a range of locations within Europe and China. Field trial work was complemented with modelling studies where a broader range of management strategies and crop characteristics could be investigated.

The studies of four different major crops highlighted the different pathways to improved NUE for each species. Studies with maize demonstrated that root architecture may be crucial for acquisition of nutrients. Quantitative trait loci (QTL) identified for grain yield were linked with those for root number, lateral root length and density. Root characteristics, especially early root vigour, were also essential for phosphorous use efficiency (PUE) of potatoes, while maturity type in potatoes was more important for nitrogen use efficiency (NitUE). Studies on wheat used an “association genetics” approach where alleles controlling NitUE and associated markers in winter barley were identified and used to inform studies on the much more complex wheat genome. Partners found little interaction with fertiliser level suggesting that that there is little prospect in the European adapted winter wheat gene pool for successfully breeding new genotypes that can produce more yield specifically at low fertilizer levels. The exception was for organically bred varieties, which in some cases out-yielded conventionally bred varieties under organic conditions. Studies on oilseed rape resulted in some high-profile results and one publication in the prestigious journal Nature Genetics. The use of semi-dwarf oilseed rape hybrids (a cross between a normal and dwarf variety) showed promise as a strategy to maintain current yields with reduced fertilizer inputs.

The integration of NUE varieties into cropping systems using modelling approaches showed that the value of different traits related to crop Nit-UE depends on the conditions under which the crops are grown. Design of the rotation with attention to timing of fertilizer inputs, sequence of crops, and if possible, the inclusion of catch crops to “mop up” excess nitrogen, are all crucial to ensure maximum system-scale NUE.

Project outputs have already been published in a number of academic journals and disseminated to the scientific community and the wider public via public presentations. Commercially exploitable results have also been generated which are expected to have a direct economic impact on the breeding sector, as well as indirect benefits to wider society through the more rapid development of NUE varieties and cropping systems.

2. Project summary

The global population is projected to increase by 75 million people per year reaching 9 billion by 2050 (Buttriss and Riley 2013), increasing demands on food production. In order to meet these demands it is predicted that rates of artificial fertilizer application may have to increase threefold if past practices are used to achieve the required 50% increase in food production (Tilman et al. 2001). Due to the ever increasing dependence on non-renewable mineral fertilizers which are associated with significant negative environmental impacts, the sustainability of arable crop production in the future faces increasing uncertainty (Tilman et al. 2002). Although past increases in yields have resulted from higher applications of synthetic fertilizer (there was a ten-fold increase in fertilizer use between 1950 and 2000 (Ghorbani et al. 2010)) and pesticides, further increases are unlikely to produce such effective yield gains as a result of diminishing returns (Tilman et al. 2002). Currently, only 30-50% of applied nitrogen (N) fertilizer is taken up by the crop (Smil 1999; Cassman et al. 2002). The loss of N from the system and its detrimental impact on the environment is of major concern; inorganic N (in particular nitrate) can be dispersed into surface and groundwater, leading to eutrophication (Vitousek et al. 1997). Furthermore, the manufacture of N fertilizers relies on the use of fossil fuels in an energy intensive production system that produces greenhouse gases (in particular nitrous oxide) as a by-product (IPCC 2006). P fertilizers are produced from finite mineral reserves and there is increasing pressure to improve efficiency of their use. Plant-available phosphate is easily “fixed” by soil minerals, making it unavailable to the growing crop. This problem is particularly an issue for crops that have poor rooting systems like potatoes. Soil erosion can result in the movement of this P to waterbodies where it becomes a pollutant, contributing to eutrophication.

The need for sustaining continuous arable crop production for future generations while protecting the environment from further degradation is driving the demand for reductions in synthetic fertilizer input or a complete replacement with alternative fertilizers. Predictions about future population growth necessitate that this reduction in fertilizer use must be achieved while maintaining or improving the current crop yield and quality levels. This goal can only be achieved if innovative systems of farming are developed that dramatically improve efficiency of nutrient use in crop production. The most common definition of nutrient use efficiency (NUE) is the yield of a given crop per unit available nutrient (N and P). This can be used to assess the efficiency of nutrient use of a given cropping system or farm, on an annual or multi-year basis. There are three main factors that influence the NUE of a crop or cropping system:

- Plant genotype can influence NUE due to differences in nutrient uptake (e.g. root characteristics) or nutrient utilization (e.g. maturation type; translocation efficiency). Reducing fertilizer input and breeding plants with increased NUE is currently one of the key goals of research on plant nutrition (Hirel et al. 2007).
- There is also potential to improve NUE through agronomic innovation and best practice. 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; Raun et al. 2002). NUE of the whole crop rotation can be improved through optimizing the sequence of deep- and shallow-rooted crops, with deep rooting crops following those with shallow roots so that they can “mop up” excess nutrients that may have moved down through the soil profile below the rooting zone. Similarly, “catch crops” following the main crop in a rotation can capture excess nutrients and retain them

in an organic form for the subsequent crop. Including legumes in crop rotations can improve the fertilizer NUE since inputs of fertilizer can be replaced with biologically fixed N.

- The environment is the third factor which influences NUE. Environment (including soil type and local weather conditions) impacts on the potential of the crop for optimal growth, and is particularly relevant in environments where water can be limiting (Semenov et al. 2007). The local environment can also impact on the expression of genes, so that genotypes that display particular characteristics in one location may not perform similarly in another place. This is often demonstrated by differences in the ranking of crop varieties when they are arranged in yield order, depending on where they are grown.

The interactions between genotype, agronomic innovation (sometimes referred to as management) and the environment make it very difficult to make broad recommendations about best practice across a range of locations and cropping systems. Understanding the genotype by management by environment (or GxExM) interactions that affect NUE is essential if we are to develop the higher yielding, more nutrient efficient cropping systems of the future.

Most previous R&D to reduce fertilizer inputs has focused on improving agronomic methods, while research to develop more NUE crop varieties has been limited. In this project the main focus was on the identification of crop genotypes with higher NUE, the phenotyping (or characterization) of these genotypes, and the use of this information to identify molecular markers that can be used to speed up the crop breeding process. A molecular marker is a string or sequence of nucleic acid which makes up a segment of DNA located near the DNA sequence of a desired gene. Molecular markers are used by plant breeders to speed up varietal development through marker-assisted selection (MAS). This allows breeders to analyze only a tiny bit of plant tissue from a seedling to find out if that seedling contains the desired gene. If it doesn't, the breeders can quickly move on and concentrate on analysis of another seedling, eventually working only with the plants which contain a specific trait (see www.isaaa.org for more information on these approaches). In the NUE-CROPS project various approaches were used to identify molecular markers including classical quantitative trait loci (QTL) methods, association genetics, genetic bridging (between species) and gene expression profiling (reverse genetics). Studies were conducted in a range of management systems and environments to capture the effects of GxExM on NUE.

The overall aim of the NUE-CROPS project was 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 project focussed on four of the most important arable crops grown in Europe: wheat, maize, oilseed rape and potatoes. Recognizing the importance of GxExM interactions, the project used a mult-site, multi-year experimental approach for all crops. Modelling of whole cropping systems was also included to explore impacts of agronomic innovations and NUE genotypes in a still broader range of environmental and management conditions. Models were also used to look at rotational-scale impacts of innovations and NUE genotypes.

In order to achieve the overall aim of the project, activities were conducted to meet the following objectives:

- **Production of phenotyping data sets** that **(a)** quantified levels of nitrogen use efficiency (**Nit-UE)** in different genotypes of barley, wheat, *Brassica rapa,* oil seed rape, maize and potato, **P-UE** in different potato genotypes, and **WUE** (in maize genotypes) and **(b)** link Nit and P-UE to specific morphological and physiological characteristics of crop plants

- **Production of genotyping data sets** that **(a)** improved genetic maps for all crop species targeted under NUE-CROPS, **(b)** identified for the first time alleles, QTL and/or markers that were linked to Nit-UE for the crops barley, wheat, *Brassica rapa* and/or oilseed rape), **(c)** identified additional alleles, QTL and/or markers for Nit-UE, P-UE and/or WUE for the crops maize and potato and **(d)** allowed alleles, QTL and/or markers to be linked to specific morphological and physiological characteristics of crop plants
- **Production of data sets of gene/protein expression profiles of crops with contrasting Nit-UE, P-UE and/or WUE** under different agronomic (e.g. fertilisation, rotation, tillage, water availability) conditions.
- **Compilation of data sets on the integrated use of genetic and agronomic innovations** that identify strategies to improve NUE in different crop production systems and macro-climatic regions.
- **Provision of training courses** that successfully transferred **state-of-the-art breeding and agronomic knowledge, methodologies and skills** developed as part of NUE-CROPS and already available in the NUE-CROPS consortium to **Early Stage Researchers and** technologists/agronomists.
- **Conducting of dissemination activities** that successfully transferred **state-of-the-art breeding and agronomic knowledge and methodologies** developed under NUE-CROPS (and other projects focused on improving NUE and WUE) to a wide range of user/stakeholder groups.

3. Main project results

The main project results presented here are confidential and not for public release. In many cases potentially commercially exploitable knowledge has been shown which may compromise future competitiveness of the partner organisations. Please contact the Project Coordinator before releasing any of the information included in this results section.

3.1 Improving nitrogen use efficiency in wheat

Background and overall approach

Wheat has a hexaploid genome (with three pairs of the basic seven chromosomes) which makes the identification of (a) QTL and genes associated with nitrogen use efficiency (Nit-UE) and (b) molecular markers suitable for marker assisted breeding for Nit-UE, more difficult than in other crops. Within the NUE-CROPS project the main approach to understand the molecular mechanisms of NUE in wheat was therefore to transfer/apply genetic information from association genetics (AG) studies in barley which is a close relative of wheat, but with simpler diploid genetics to similar studies in hexaploid bread wheat.

The work involved three work-packages:

- WP1.1 - an AG approach was used to identify alleles controlling Nit-UE and associated markers in winter barley.
- WP1.2 - AG data from barley was used to complement the resolution of the mapping of potential Nit-UE QTL in subsequent AG-studies in wheat**.**
- WP1.3 - QTL for Nit-UE in wheat were further validated by studying the effect of, and interactions between, agronomic practices (including fertilisation regimes, rotational designs, crop protection systems) on gene and/or protein expression profiles in wheat genotypes with contrasting NUE.

This strategy was based on the assumptions that elite barley and wheat cultivars showed significant differences in Nit-UE and that the genetic control of Nit-UE is comparable in the two crops. These assumptions were supported by some reports of differences in Nit-UE between barley varieties bred for malting and feed production and heterogeneity for Nit-UE in elite winter wheat varieties and the limited available data on the similarity of QTL mapped for NUE and its components in barley and wheat.

Identification of alleles controlling Nit-UE and associated markers in winter barley

In order to identify the genes and alleles controlling Nit-UE via an association genetics approach, 166 (rather than the originally envisaged 100) elite European winter barley cultivars were grown in five site/season trial combinations at three contrasting sites in Northern Europe (South East England, North East Scotland and central Germany) covering maritime and continental conditions with known available soil mineral nitrogen content. Each trial was grown with three fertiliser regimes in a split plot design with partial replication; no nitrogen application, an intermediate malting regime nitrogen application and a full rate nitrogen application appropriate for feed production. The lines included two and six rowed varieties and those bred for malting quality and feed markets. Unbiased estimates of the genotype and genotype x nitrogen level phenotypes were used to conduct marker association scans to identify candidate QTL. Traits measured included yield, harvest components of yield, phenology and nitrogen content and the genotype efficiencies for uptake and utilisation of nitrogen derived (NitUpE & NitUtE). Analysis of the data revealed that there was considerable genetic variation for all the traits measured and also considerable genotype x environment interaction.

However, importantly, the interaction of genotype with nitrogen levels per se was small in comparison to the genotype interactions with site and season. The NitUtEg (grain N utilisation efficiency) trait was strongly correlated to the realised yield in these trials which could account for the strength of the GxE effects. Moreover the portion of the variation observed for NitUpE that could be assigned to genotypic differences was small and again showed large GxE interactions. Overall these results would indicate the necessity to calculate NUE over multiple sites and seasons and provide some caution to interpretations based on single environment studies in particular if based on unusual growing conditions. It also suggests that breeding specifically for low nitrogen inputs over a wide range of environments is unlikely to be effective. It was also apparent that breeding progress in two- and six-rowed barley for nitrogen utilisation efficiency clearly differed with grain utilisation efficiency of the two-rowed barleys increasing faster over time, a trend that is mirrored in grain yield.

Using the genotypic information derived from the 9K barley SNP platform (rather than the 3K SNPs originally envisaged), QTL were found for all traits including nitrogen utilisation and uptake efficiency. The genetic variation observed can be dissected into significant QTL through the use of an association panel with many of these QTL showing interaction with the environment.

Some of these QTL could be associated with known major genes effecting plant architecture (*int-c*) or flowering time (*VrnH-1, ppd-H1, eam6*). The allele effects shown at *eam6* and potentially at other loci do show a correlation of positive NitUE effects with spring alleles potentially selected in malting quality winter barleys to maintain low grain nitrogen under lower nitrogen inputs in malting regimes. There was less evidence for the involvement of candidate genes chosen *a priori* for involvement in nitrogen metabolism and utilisation, such as the cytosolic glutamine synthetases or NAM Gpc-H1.

Some QTL in particular for NitUtE collocated with the known position of various peptide transporters but the clear association of particular genes and alleles with the observed effects has proven difficult. This is in part due to the evident expansion of peptide transporter gene families in the evolution of Triticeae (wheat, barley, rye) genomes relative to the model species rice and Brachypodium. Such expansion may underpin the species' plasticity in response to different forms of nitrogen stress.

Although the NitUE QTL found did exhibit interaction with the environment, some of the underpinning components of in particular NitUtEg could be found in other studies to validate the findings of the NitUE-Crops analysis. This included a reanalysis of AGOUEB association analysis, QTL mapping in a KWS bi-parental population and in particular though the development of NIL lines that will allow further delineation of the effects. Although full validation will require longer term and more detailed experiments the correspondence found for a number of effects is encouraging and forms the basis of the choice of SNPs for future marker assisted selection and genomic selection for improved NitUE in this crop

Identification of alleles controlling Nit-UE and associated markers in winter wheat

High quality yield trials grown in Dundee, Scotland, Cambridge, UK and Seligenstadt, Germany successfully tested 170 wheat varieties (rather than the100 originally envisaged) in conventional farming situations under three controlled levels of artificial nitrogen input. Analysis showed that the interaction of genotype with fertiliser treatment was small in comparison to the genotype interactions with site and season (Figure 1), a picture very similar to that found in winter barley in WP1.1. The amount of interaction with fertiliser level was consistently small and always less than the main genetic effect suggesting that there is little prospect in the European adapted winter wheat gene pool for successfully breeding new genotypes capable of delivering substantially higher yield specifically at low fertiliser level. Our findings showed that the lines that deliver a high yield at the high input level are generally the most successful at lower nitrogen input levels although there is some interaction of genotypes with fertiliser (about 10%) and it is also clear that much of this variation is attributable to the poor responsiveness of older cultivars to the modern levels of high nitrogen. These observations confirm other studies which have shown that there is no 'magic bullet' in the current gene pool of European wheat that will enable breeders to produce high yields at low levels of available nitrogen.

We were however left with the strong indication that high yielding cultivars bred in modern high nitrogen environments are not necessarily at a disadvantage in lower nutrient status situations and that therefore continuing progress with NitUE may be made by continuing to concentrate upon breeding for high output genotypes. It must be stressed that this is not a global conclusion but rather a parochial one confined to the limits of the NW European regions where soil fertility, climatic conditions and the high standard of general agronomy favour the production of yields in the 5-12 t/ha range.

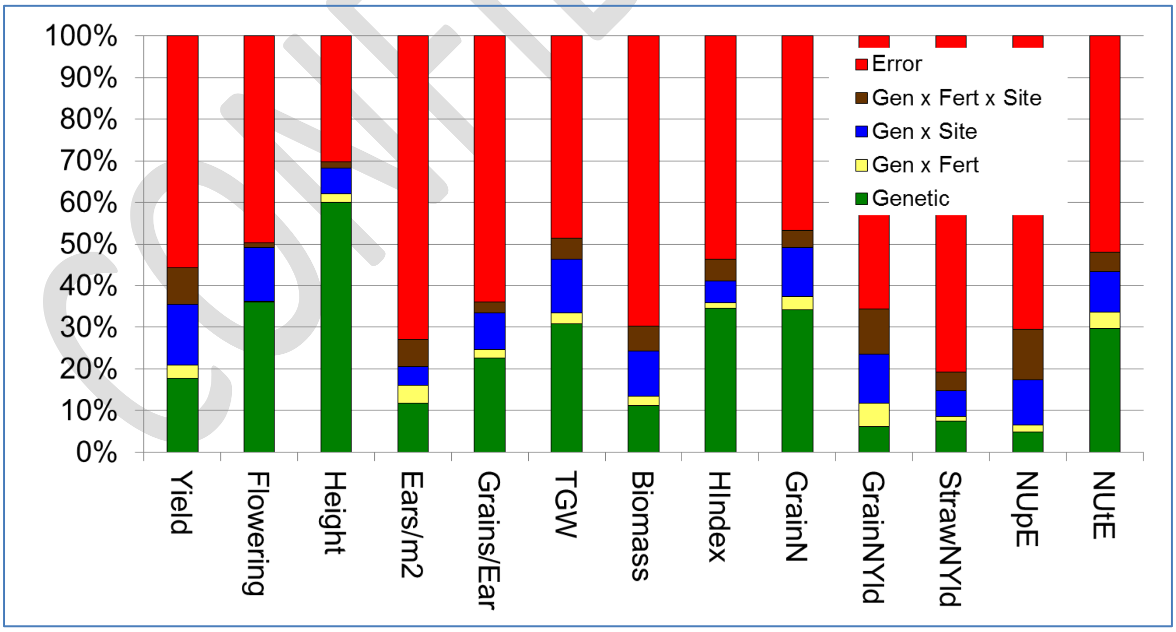

Figure 1 *Bar chart display of the partitioning of the variance components for some of the agronomic variates studied*

A total of 15,542 SNP markers assayed on the 90K chip were polymorphic (i.e. showed variation at between different cultivars p5891 and paul at the assayed sites in the genome) in this European material. These were fairly well distributed but as expected there was a deficiency of markers on the D genome. The level of genotyping is very considerably more than was originally envisaged (~200 SSRs) and reflects the rapidly changing face of wheat genetics. The association mapping analysis was implemented in TASSEL (a software package for investigating relationships between phenotypes and genotypes) and 285 potential QTL ‘clusters’ were identified. Each of these was identified as a significant association of ordered markers with one or more traits. Of these, 18 targets of very high fidelity have been prioritised for follow-up work including potential gene candidate identification.

The candidate gene that was most significantly associated with traits was the *Ppd-D1* marker, which was associated not only with flowering time but also with total biomass, yield, and harvest index. Other significant associations detected by KASPar marker for *a priori* candidate genes were for yield with *vrn-A1* and Straw nitrogen yield with *Pin-D1*.

The close homology between wheat and barley facilitated the interlinking of results from the two species. For example the effect for NitUtEg found on the long arm of barley chromosome 4H was linked to the genomic position of PTR3a like peptide transporter that is known to have a strong expression in senescing leaves. Close homologues of this gene can be found in wheat on the long arm of wheat chromosomes 4B and 4D. The homologous position of 4BL PT3a-like gene is very close to the SNP marker (BS00031652) mapping at 71.5 cM that sits under a cluster of co-segregating QTL for grain N and NitUtEg.

The hot spot on 6A in the region of Tdurum_contig30082_197 at 79cM corresponds to the region at 50-52 cM on the long arm of 6H that shows significant associations with grainN, NitUtEg and yield in barley but is somewhat distant from the position of the potential candidate gene NAM-A1 grain protein gene which is also close to the centromeric region on the genetic map but on the short arm. Although the involvement of homologous candidate genes is yet to be proved, these examples do demonstrate a clear relationship between the positions of genomic regions involved in the control of NitUE in the two crops.

In summary it is clear that elite wheat genotypes show a lot of genetic variation for yield and hence NUE but that the interaction of genotypes with the fertiliser regime is only about 10% of the genotype variation. Moreover much of this observed variation may be attributed to the relatively poor response of older cultivars to nitrogen inputs. It seems therefore that there is no ‘magic bullet’ in the current gene pool of European wheat that will enable breeders to produce high yields at low levels of available nitrogen. However the study of the nitrogen economy of the wheat as a component of yield will greatly facilitate the development of higher yielding varieties with improved Nitrogen Use Efficiency. This work will take advantage of the rapidly improving genotyping capabilities for bread wheat and the close syntenic relationship with barley. The assumptions that underpinned the work in WP1.1 and WP1.2 have been borne out and form the basis for the ongoing more detailed dissection of the genes and alleles controlling Nit-UE in EU winter bread wheat.

Investigating the effect of different fertilisation regimes, agronomic practices and environments on gene and/or protein expression in wheat varieties/genotypes with contrasting Nit-UE

The GxExM interaction that has been reported in the studies on barley and wheat was further explored in studies on three continents using a range of management practices and genotypes. The trials were used to assess agronomic performance and in the case of trials in Newcastle, to also investigate gene expression of selected genotypes under different levels of N supply. Trials were conducted in Northern Europe (maritime climate; UNEW), Central Europe (continental/alpine climate; FiBL), Northern China (semi-arid climate; CAAS) and Pacific North-West (Mediterranean climate; WSU).

Trials at Newcastle University were conducted in two years (2010 and 2012) and compared the performance of four modern, high-yielding UK wheat varieties (Cordiale, Grafton, Gallant and Solstice) with four long-strawed, organically bred wheat varieties from Switzerland (Aszita, Wiwa, Scaro and Laurin). As reported in WP1.1 and 1.2, the season of growth had a large effect on crop performance. The dry conditions that prevailed in 2010 resulted in high grain yields, harvest indexes (HI) and nitrogen harvest indexes (NHI), despite lower total biomass, N uptake and N uptake efficiencies. The wet growing season in 2012 resulted in poor yields despite high N uptake. The results highlight the importance of the translocation of N from the vegetative parts into the grain after N uptake in order to produce high yields. This is reflected in high post-anthesis N uptake in 2010 coupled with a high N translocation. The source of N fertilizer affected NUE parameters more so than application rates, however yields increased with increasing available N. In 2010 the modern, short-strawed varieties significantly out-yielded the organically bred varieties when ammonium nitrate fertilizer was used (see Figure 2); however, when growing conditions were less favourable in 2012, differences between the two groups of varieties at each level of input were less pronounced (see Figure 3).

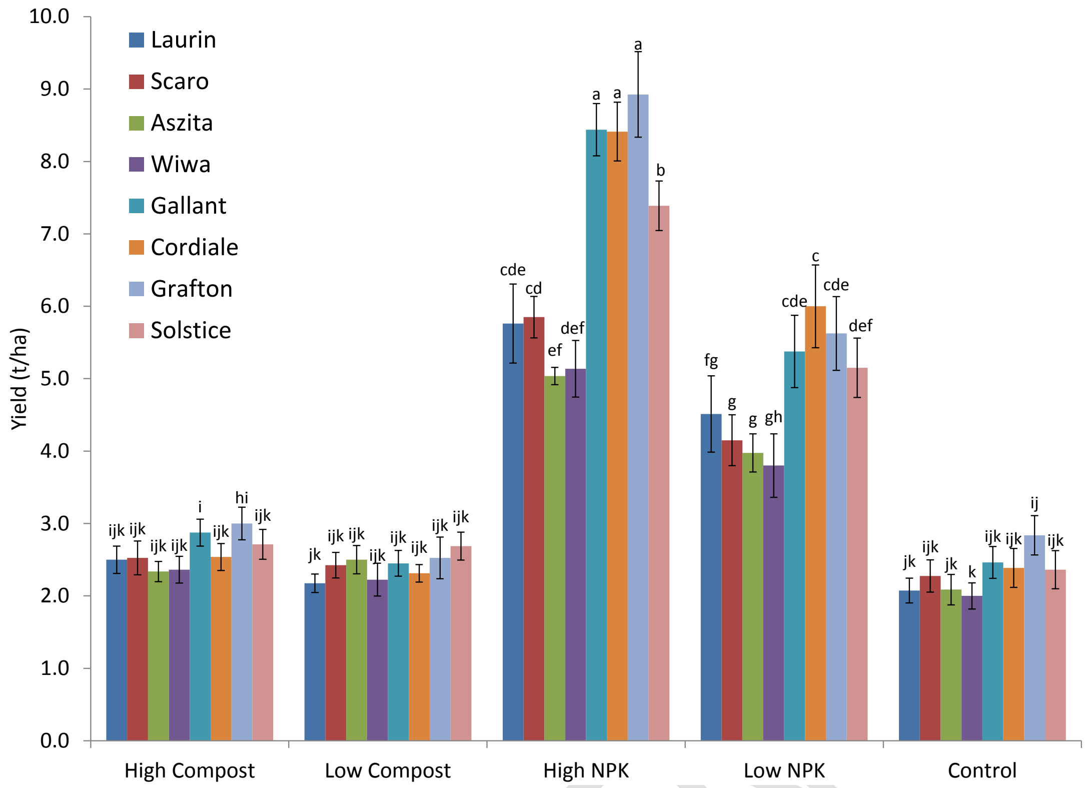

Figure 2. Effects of fertility management (ft) and variety (vr) on wheat grain yield in the 2010 harvest year. Standard errors of the mean (error bars) were calculated from all four field plot replicates.

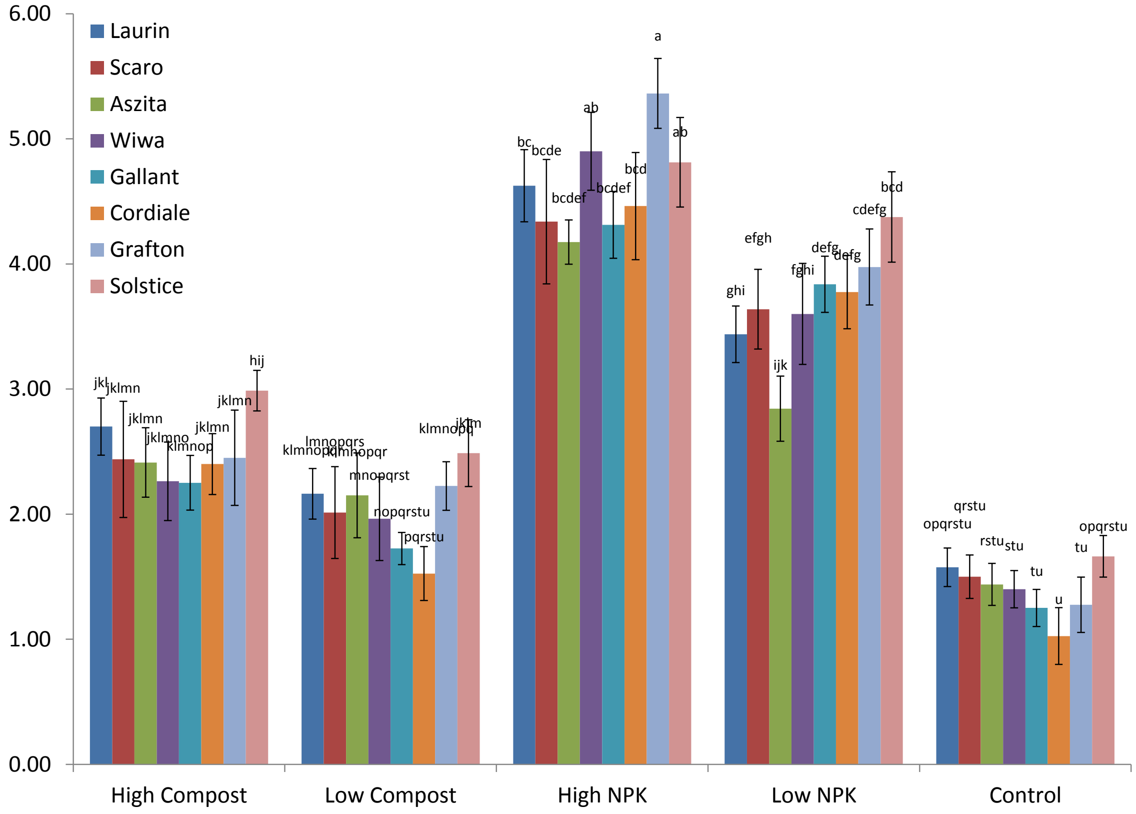

Figure 3. Effects of fertility management (ft) and variety (vr) on wheat grain yield in the 2012 harvest year. Standard errors of the mean (error bars) were calculated from all four field plot replicates.

To gain insights into the molecular effects of low nitrogen inputs used in organic practices compared to conventional systems on plant growth, we used plant material from the Newcastle trial described above and a combined proteomic and transcriptomic approach. The aim was to investigate the effects of contrasting fertility regimes on the wheat flag leaf gene expression and the association between the differentially expressed genes and physiological traits. Flag leaf tissue from the high yielding varieties Grafton and Cordiale were selected for this work.

The abundance of identifiable protein spots and transcripts varied significantly between fertilisation regimes. Differences between photosynthetic status, stress response, nitrogen remobilisation and regulation of RNA processing via transcription factors indicate that the balance between crop development, fertility and resistance to stress are controlled though molecular-based mechanisms.

In agreement with previous studies multiple protein spots were identified as RuBisCo either as whole subunits or as predicted products of degradation (Tétard-Jones et al 2013, 2014). The functional role of RuBisCO is in the Calvin cycle of photosynthesis; however, it is also known to act as a major nitrogen storage compound. Changes in the regulation of RuBisCO synthesis and degradation are initiated upon leaf senescence (after full leaf expansion) (Irving and Robinson 2006). RuBisCO degradation products are transported out of chloroplast cells and may undergo further degradation before being remobilised in nitrogen sink tissues such as developing wheat grains (Mae et al. 1983; Mae 2004; Chiba et al. 2003). However, it has been previously postulated that the visual presence of RuBisCO degradation products during electrophorese indicates an accumulation of these products within chloroplasts due to a bottleneck in the transport process (Irving and Robinson 2006). Field grown plants are frequently exposed to adverse environmental conditions and will unavoidably encounter varying degrees of environmental abiotic and biotic stress during a growth season. Estimates for reductions in productivity in eight economically important crops range from 65 % to 85 % (Bray et al. 2000). It is well known that stress conditions might induce premature senescence of leaves resulting in a shortage of assimilates, ultimately causing an acceleration in the whole-plant maturation process (Gan 2003) and in the end strongly affecting crop productivity. The significant changes in gene expression noted in this study demonstrate the immediate responses of plants to stresses leading to the initiation of senescence.

The resulting insights into the genetic strategies available to mitigate adverse environmental conditions and reduced nitrogen availability may be used to enable the potential development of functional molecular markers in future breeding programs to select for higher nutrient use efficiency.

Trials in Switzerland investigated winter wheat production in organic farming systems. Field experiments with ten winter wheat varieties were performed in the DOK-system comparison trial in 2009 and 2010. This long-term trial consists of a comparison between different management systems. These systems differ in terms of fertilization and plant protection strategies. The level of fertilization increases from the unfertilized control NOFERT to the organic systems with composted manure BIODYN 1 (0.7 livestock units ha_{-1}), BIODYN 2 (1.4 livestock units ha_{-1}) and to the conventional-high-input system CONMIN. This makes 4 farming systems as fixed factors in the field trial design.

The hypothesis tested in these trials was that varieties bred within a specific system will perform best in that system compared to varieties bred for other systems. In the experiments genotypes from three sources: old, organically and conventionally bred varieties developed between 1840 and 2006 were tested including widely grown varieties with (Tommi and Caphorn) and without semi-dwarfing genes (Titlis, Antonius) and contrasting nutrient use efficiency.

If the hypothesis was valid, it was expected that there would be a variety x farming system interaction with significantly higher parameter values for varieties when they were grown within their selection system. These interactions were detected for grain yield, grain N and P but not for the N and P use efficiency. When combined for the 2 years, "variety x system" and "breeding category x system" were significant for the yield parameter. However, on average, it was only under the two "extreme" systems (BIODYN1 versus CONMIN) that varieties from different breeding categories responding differently to farming systems (Figure 3b). Under BIODYN 1, organically bred varieties performed better than old varieties with respect to grain yield, P grain concentration and N use efficiency and were superior to conventionally bred varieties for PUte. Old cultivars were superior for yield only under very poor conditions of the unfertilized control.

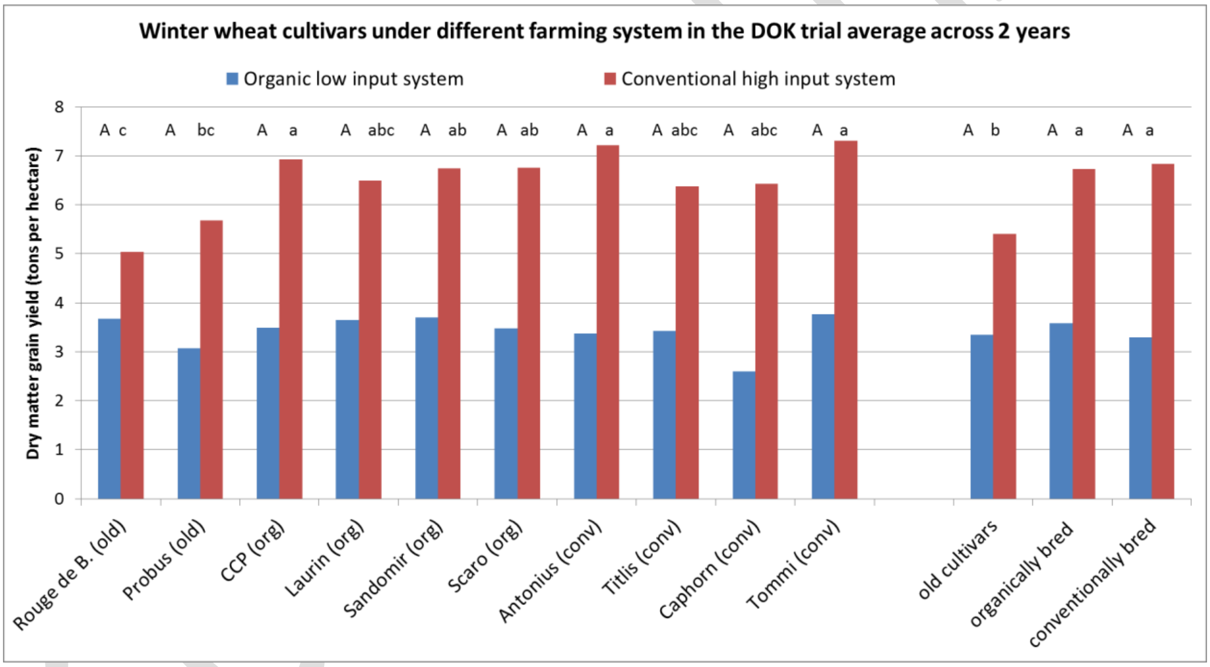

Figure 3b. Effects of farming system and variety (breeding category indicated in brackets) on wheat grain yield across 2009 and 2010 in the long term DOK system comparison trial in Therwil, Switzerland. Different letters indicate significant differences between winter wheat genotypes.

For Nitrogen use efficiency, when calculated across the four systems, no breeding category performed better than the others, and no "variety x farming system" or "breeding category x farming system" interaction was observed. In contrast, more variations among varieties and within the different farming systems were observed for PUtE. The conventionally bred varieties achieved significantly higher values when calculated across all four systems. While the number of varieties from the three breeding categories was very limited and do not allow a general conclusion, the significant genotype x farming system interactions are of greater relevance. It clearly demonstrates that certain genotypes are more or less adapted to a given farming system, e.g., Caphorn with a dwarfing gene performed poorly under organic farming system due to high weed competition, whereas Tommi with similar plant height was among the highest yielding ones. Thus the choice of an adapted variety is decisive for the farmer and needs to be tested in variety trials representing the target environment.

Trials at Washington State University were conducted as part of a larger PhD programme looking at yield and quality of hard, red winter wheat grown in organic and conventional farming systems. This study looked at GxExM effects on the agronomic performance, breadmaking quality, and phenolic acid content of hard red winter wheat produced in western Washington State. Three rates of N (0, 85, and 170 kg N/ha) were surface-applied either as poultry feather meal (PFM) or as sulfur-coated urea (SCU) to field experiments conducted at a total of four sites during the 2009-10 and 2010-11 crop years. The four wheat cultivars showed a large variation in yield (0.3 to 10.1 Mg/ha), hectoliter weight (52.8 to 79.2 kg/hL), grain protein content (7.0 to 14.4%), constant weight micro-SDS sedimentation volume (5.3 to 19.5 cm3/g), constant protein micro-SDS volume (4.6 to 16.7 cm3/g), and Hagberg falling number (HFN) (119.0 to 632.5 s). Although inputs of N had negligible effects on yield, yield was 0.5 to 2.0 Mg/ha higher in the second year compared with the first, and the two cultivars developed since 2005, Bauermeister and WA8022, produced 2- to 3-fold greater yields (4.6 and 6.3 Mg/ha, respectively) than the two cultivars developed prior to 1965, Relief and McCall (2.2 and 2.3 Mg/ha). There were no interactions between N fertilizer rate and variety indicating that the varieties that yielded highest at low levels of N supply, also yielded the best under high inputs. The higher yields in the second year corresponded with a 1% reduction in grain protein content compared with the first year, and additions of both the PFM and SCU fertilizers, primarily at the 170 kg-N/ha level, increased protein content on average 1%. The two “modern” cultivars averaged 9.7 and 10.2% protein, while the two “historic” cultivars averaged 11.5 and 12.4% protein, thus genetic gains in yield have partially offset gains in protein content.

In summary, this study confirmed finding of other studies within the workpackage, supporting the evidence that varieties selected under high-input conditions (e.g. the post-2005 varieties in this trial) were still the most efficient at lower levels of N supply.

Trials in northern China involved a study of strategies to reduce inputs of mineral fertilizers in maize-wheat cropping systems. Prior to 1950 China’s crop production system can be classified as low-input production system with low grain yields, based almost exclusively on organic manure as fertilizer. However, since 1950, use of organic manure continued to increase but was progressively replaced with mineral fertilizers in the pursuit of higher yields following the government’s policy of ‘maximising yield’ to feed its growing population. Today, the national average rate per ha of mineral fertilizer application in China is 2.7 times the average global application rate, however the use efficiency is very low at approximately 35 % (Ellis and Wang.1997; Zhu and Chen., 2002; Jiang et al., 2006; Cui et al.,2010; Zhu and Jin, 2013).This begs the question of what happens to the other 65 % of the applied fertilizer, specifically Nitrogen?

To resolve the question of how to improve NUE through use of organic fertilizers, two related field trials (Ling Xian and Yu Cheng) were set up in Northern China to investigate the responses of 4 wheat and 4 maize varieties with low and high N use efficiencies, to the type and rate of fertilizer application and different rates of irrigation. Ten treatments representing a range of combinations of organic (Org) and inorganic (Inorg) sources of N were selected for intensive monitoring for the

NUE Project. Four winter wheat cultivars with reportedly different NUE (N use efficiencies) were chosen (jimai 19; kenong 9204; shimai 15 and weimai 8) and planted in the sub-plots. The experiment was analysed as a factorial with 4 levels of organic N (0, 45, 120, 240 total N/ha) x 6 levels of inorganic N (0, 45, 90, 120, 180, 240 kg total N/ha) x 4 varieties (although not all possible treatment combinations were included in the design). Organic N, inorganic N and variety all had a significant effect on wheat yield, dry biomass and harvest index. There was an interaction between org and inorg N as well as inorg N and variety; while there was no variety by org N interaction, at least for the first 3 years. The interaction between org and inorg N is shown in Figure 4 which shows that at low rates of total N yields with inorganic N are higher than with organic N; however, at the higher rates of total N application, the source of N has no effect on yield. In fact, when a total of 240 kg N/ha was supplied, 50% from an organic source and 50% from an inorganic source, the yield was equivalent to 240 kg N/ha from an inorganic source only. At the same level of N supply, wheat fertilized with Inorganic N (urea) displayed a yield increase over control that was 3 times that of wheat fertilized with Organic N (cow manure). This indicates that for the first 3 years, manure released 1/3 of its N in an available form. In wheat, maximum yields were achieved with 130 kg available N/ha and may consist of a mixture of urea and/or manure after taking into consideration that only one third of N in the manure becomes available during the wheat season.

The results showed clearly that the current farmers' agronomic practice (240 to 300 kg N/ha for wheat and maize) used too much fertilizer as well as too much irrigation for winter wheat grown on the Huang-Huai-Hai plain of northern China and can be reduced without grain yield losses.

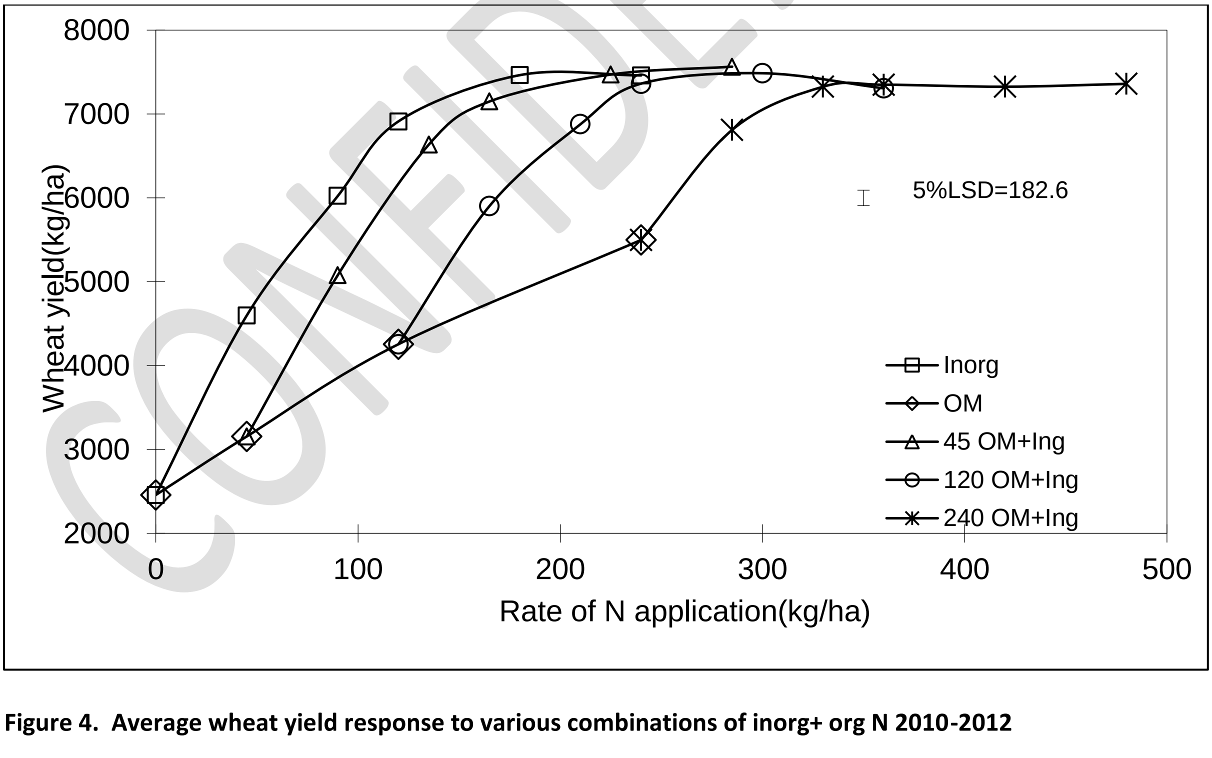

Figure 4. Average wheat yield response to various combinations of inorg+ org N 2010-2012

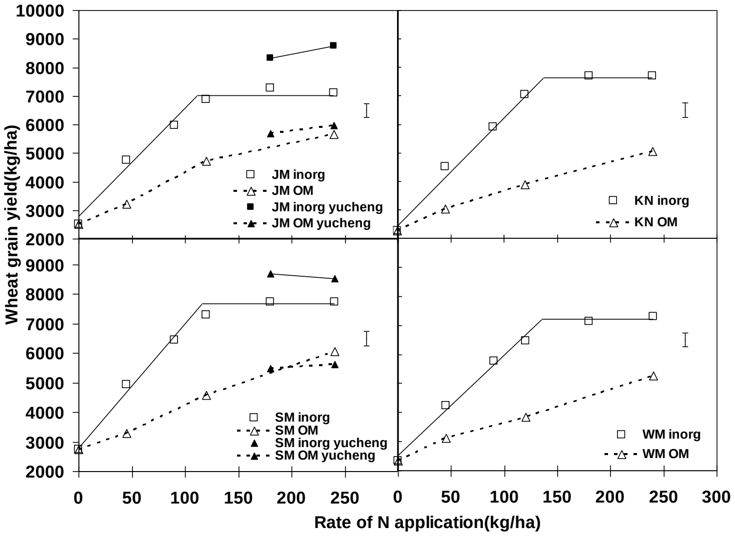

Figure 5. Responses of 4 wheat varieties to inorg N and org N fertilizers; JM=jimai; KN=kenong; SM=Shimai; WM=Weimai

A line+plateau model was fit to the yield data and can be used to judge NUE of the different varieties. It shows that under inorganic N fertilization, the variety Shimai has a higher plateau value than the other four varieties (7666 kg grain/ha for Shimai). It also shows that it reaches this plateau at a lower N rate than the other varieties i.e. the “joint” or inflection point for Shimai is 116.3 kg N/ha compared to an inflection point of 137.6 kg N/ha for Kenong, which also has a relatively high plateau (7645 kg/ha). This means that Shimai not only achieves higher yields than the other three varieties at high levels of N input, but also that it reaches its maximum yield with less N than the other three varieties. Therefore, Shimai shows potential as an NUE variety.

3.2 Improving the nitrogen use efficiency of maize

Background and overall approach

Previous joint R&D by partners 4 ABI, 8 HHUD and 11 KWS (as part of the EUREKA project "CEREQUAL" and several bilateral industry funded projects) using populations based on Central European genotypes clearly demonstrated that selection of maize genotypes under low N-input production backgrounds results in increased yields under such conditions without losing yield potential at high N-input. Similarly, maize breeding projects in China have shown that the average yields of hybrids developed under low N conditions were about 10% higher (higher Nit-UE) both at high and low N supply than those selected under high N conditions. It is therefore considered possible to increase maize yields at reduced N supply.

Since maize is the major feed/food crop in many semi-arid regions of the world and also important in South-East Europe, water use efficiency (WUE) was included as an additional factor in some of the experiments to study the interactions with Nit-UE.

Molecular markers are considered as a promising tool to increase the efficiency of breeding. In maize, the QTL (quantitative trait loci) approach has already been used to identify chromosomal regions involved in the expression of various traits associated with tolerance to abiotic stress, including tolerance to drought. However, the QTLs for NUE identified so far in maize showed inconsistent results across different populations and their suitability for exploitation in MAS programmes is therefore limited.

Genetic resources from cropping systems with traditionally low N inputs are often considered as valuable resources to improve stress tolerance. However, mostly, these exotic populations are not adapted to the environmental conditions in the target environments. Molecular markers could help to overcome the lack of adaptation and to detect and efficiently utilize QTL alleles for Nit-UE.

The main approaches under SP2 were therefore to: (a) identify a wider range of QTL for Nit-UE, (b) establish which QTLs are closely related to specific root morphological/physiological characteristics, N acquisition and grain yield formation at low N supply, and (c) investigate the genetic variability for Nit-UE across a wide range of populations, including genetic resources. In addition, WP 2.3 had the objective to study the effect of Arbuscular mycorrhizae (AM) seed inoculation on stress tolerance, in particular Nit-UE.

Identification of novel QTL for Nit-UE and WUE in SE-European, S-American and Chinese maize mapping populations

WP 2.1 had two major objectives.

1. To evaluate the potential of genetic resources for improving Nit-UE and WUE in maize.
2. To identify QTL for Nit-UE and WUE for more efficient breeding for Nit-UE and WUE.

Main achievement of the SP2 has been the identification of novel quantitative trait loci (QTL) for Nit-UE in four mapping populations of different origin. Two Double Haploid (DH; a genotype formed when haploid cells undergo chromosome doubling; often artificially produced for use in plant breeding.) mapping populations derived from South-American and South-European breeding lines, respectively, were evaluated in field experiments across 7 environments over 2 years with two N levels or two irrigation levels each by Partner KWS. Population 3 developed by the Partner ABI in collaboration with KWS allowed an exploration of the genetic variation for Nit-UE traits generated by chemical mutagenesis in an elite temperate breeding line. The test-cross hybrids of this population were evaluated in field trials across 12 environments including 2 N levels and 2 irrigation regimes at two locations over 2 years. Partner CAAS assessed the population 4 consisting of 200 recombinant inbred lines (RIL) derived from the cross of the large-root maize inbred line with high N-assimilation efficiency and the small-root maize inbred line with a lower N-assimilation efficiency at 2 locations with 2 N levels over 2 years. In parallel two of the populations were analysed under controlled conditions for their root phenotypes (measurable characteristics) at seedling stage. The population 4 was evaluated in hydroponics and in paper roll tests, and, particularly, three stable QTLs were detected for: lateral root density, seminal root number, and root dry weight (WP2.1, CAAS). A sub-population of the population 2 was analysed in paper rolls by the Partner HHUD (WP2.2, see below).

Summarizing the results of the 5 populations in a QTL meta-analysis showed that in total 5262 marker were mapped and 791 QTL were detected for the traits measured (between 2 and 17 traits per population). QTL clusters were found on all chromosomes, often referring to highly heritable maturity related QTL. However, other interesting QTL clusters without correlation to maturity were identified for yield and root traits in the same chromosomal region (e.g. on chromosome 3). The individual population QTL and the QTL cluster are promising candidates for improving stress tolerance and N-use efficiency in European and Chinese breeding materials.

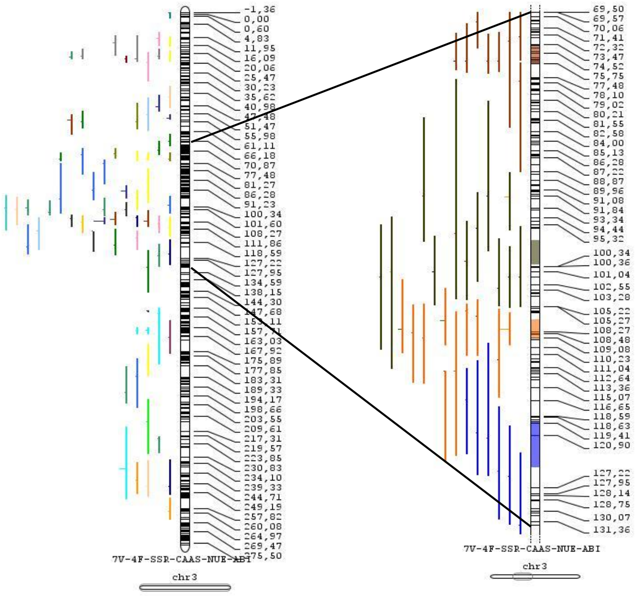

Figure 6. Individual QTL on chromosome 3 and meta QTL in the region between 60 and 135 cM across all experiments. Coloured bars on the left side represent QTL detected in different populations for all traits. Bars with different colours on the right side show 4 meta-QTL for one region on chromosome 3.

Identification of genes and physiological/morphological root characteristics associated with QTL for Nit-UE

Root architecture is crucial for acquisition of nutrients from the soil; and the selection for specific root traits improving Nit-UE is likely to be more productive than conventional breeding. The main goal of this work package was to link Nit-UE QTL to specific genes, root morphological characteristics or physiological functions in maize. We analysed seedlings of seven maize inbred lines, the parents of the population 2 studied under WP2.1, for their root morphological characteristics and response to nitrate. The lines differ strongly in their root architecture however revealed a similar reaction to altered nitrate supply. The two parent lines with contrasting root architecture and the derived sub-population consisting of 60 DH-lines were selected for QTL mapping of root morphological traits. In total, 36 QTL were detected for ten root traits. Clustering of the QTL for different traits were observed, with some of the clusters to be overlapping with the root QTL detected in another population (WP2.1, CAAS) and/or reported in literature. Two loci influencing 3 root traits (crown root number, lateral root length and density) coincide with the QTL for grain yield identified in the same population by the Partner KWS.

In addition we performed a comprehensive time-course analysis of early root architecture of two European maize inbred lines differing in Nit-UE. The line SL adapted to low N was developed in a long-term project on Nit-UE, whereas the line TH was selected under conventional N supply. The most important finding was the detection of genotype x nitrate interaction as regarding to lateral root (LR) growth (Figure 7). SL demonstrated similar LR lengths at three tested nitrate concentrations, thus being less sensitive to different N supply, than the inefficient line TH. The fact that the differences in LR growth characteristics correlated with the agronomic performance of the two lines under field conditions, suggested that the different NUE of TH and SL might be, at least in part, explained by LR parameters. To gain insight into this phenomenon and understand the molecular bases of the genotype-specific differences we initiated an analysis of gene expression in the roots of the two lines.

In the previous project a large segregating DH population derived from the SLxTH cross was evaluated for grain yield performance under high and low N condition (Presterl et al., unpublished data). Two major yield-QTL (NUE1-QTL and NUE2-QTL) were detected at the maize chromosomes 8 and 10 respectively under low N supply. Our earlier data suggested an association of LR growth characteristics with the NUE1-QTL. However evaluation of the available Near Isogenic Lines (NILS) revealed that screening of maize roots at early seedling stage couldn't be used for further restriction of the NUE1-QTL region. Remarkably an assessment of other set of NILs carrying NUE2-QTL revealed a possible link between this QTL and specific root parameters. Further research is needed to validate the detected association and evaluate its possible causal relationships with corn yield and NUE.

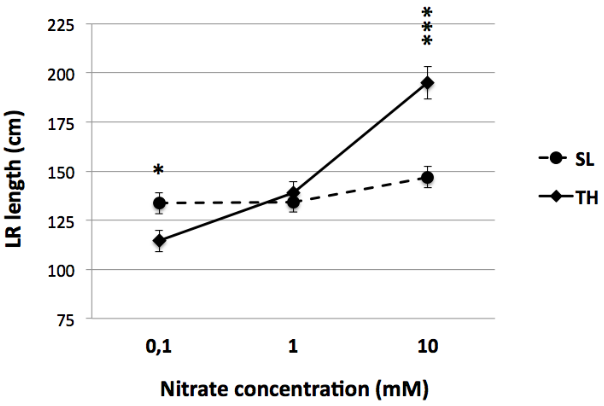

Figure 7. Genotype x Nitrogen Interaction. Lateral root (LR) length of 12 day-old seedlings of two maize lines grown in paper roll system under different nitrate supply. Average values and standard errors are shown. Significant differences are designated as: * - $p<0.05$, ** - $p<0.01$, *** - $p<0.001$.

Identification of genes related to mycorrhizal development associated with QTL for Nit-UE and WUE

Maize is efficiently colonized by Arbuscular mycorrhizae (AM) fungi and studies showed that maize lines differ in their physiological and molecular responses to AM infection and AM development. The objective of this study was to evaluate the genetic variability of the effect of AM seed inoculation on yield and stress tolerance, particular tolerance to low soil N conditions.

To evaluate the genetic variability of the effect of AM seed inoculation, 6 field experiments were conducted between 2009 and 2011. A hundred elite maize lines from North West Europe and 100 lines derived from old flint-maize landraces were evaluated as testcrosses with and without AM seed inoculation to estimate genetic variability at conventional high and low soil N environments. In addition, different sets of commercial hybrids were evaluated across a wide range of environments, including low N, drought, and organic farming conditions. One trial was conducted to optimize the method of seed treatment.

The overall effects of AM seed inoculation were small and in most cases non-significant. Under severe N deficiency stress AM seed inoculation effects were in tendency stronger, resulting in an average yield increase of 3-5%. Interactions between genotypes and treatment were observed indicating genetic variability in the reaction to AM infection. However, the reproducibility of effects of AM seed inoculation of individual genotypes across years was variable and generally low. Finally, it can be concluded that the concept of mycorrhizal seed inoculation as evaluated in the present study will not be economically successful under typical European farming conditions. However, this does not mean that they are not beneficial in other conditions; this has been proven particular under low P in other studies.

Comparison of gene and/or protein/metabolite expression profiles in maize genotypes with contrasting Nit-UE under different fertilisation and irrigation regimes

(Work within WP2.4 was closely linked to WP1.3, which focused on similar studies in wheat; see SP1)

The use of organic-nitrogen sources has been proposed as a way to reduce potential environmental pollution from N losses to water and the atmosphere, while sustaining grain yields. However, the physiological response to organic N application is largely unknown. In the present study, we compared the expression of genes related to N assimilation and remobilization processes during the grain filling stage of maize grown in field conditions. Five N treatments in which N was supplied as organic and/or inorganic forms at different rates (organic/inorganic N): 0/0, 0/120, /0/240, 120/120, and 240/0 kg N/ha were conducted. It was shown that, while yield was increased with increasing N rates, no significant difference was found in the yield, N uptake and physiological N utilization efficiency between the 0/240, 240/0 and 120/120 treatment. Nevertheless, organic N application tended to decrease N accumulation in the crop but increase N utilization efficiency. The genes related to N assimilation activity in the leaves, such as ZmNR1 and ZmFd-GOGAT1, were not affected by the form of N applied. However, in the pure organic N supply treatment (240/0), the genes related to N remobilization activity in the leaves such as ZmGS1.1 and ZmGDH1, were up-regulated, especially in the older leaves. These data suggest that using a purely organic N source might cause insufficient N supply. As a result, physiological N utilization efficiency was increased by up-regulating genes related to N remobilization processes.

3.3 Improving the nitrogen use efficiency of oilseed rape

Background and overall approach

Oilseed rape (*Brassica napus*) is the most important oilcrop in Europe and the second most important oilseed worldwide. It is traditionally produced with high N-fertilizer inputs to obtain maximum grain yields. This results in significant levels of nitrate leaching into the groundwater, because a large proportion of N is left in the field (in the form of crop residues) after harvest. Moreover, new regulatory standards in the European Union demand a reduction in greenhouse gas emissions from growing crops for biofuels, and today a considerable part of the rapeseed oil is used for the production of biodiesel. Oilseed rape production indirectly contributes to greenhouse gas emissions mainly by the energy input to produce mineral nitrogen fertilizer.

Brassica napus is an allotetraploid species (a natural hybrid between *B. oleracea* and *B. rapa*) with often a large number of gene copies of the same gene and a very complex pattern of inheritance. In contrast, genetics in the diploid crop *B. rapa* and the diploid model species *Arabidopsis thaliana* is more straightforward. The main approach to understand N use efficiency in oilseed rape within the NUE-CROPS project was therefore to increase our knowledge on N use efficiency in *Arabidopsis* and *B. rapa* and then to transfer and apply this in *B. napus*.

Summary of Results

Nitrogen use efficiency in Arabidopsis thaliana – the model plant species

Within the world of plant biology, model plants are species that have growth and reproductive properties that provide ease of use in the lab. They are frequently small, hardy organisms with short generation times and small genomes. *Arabidopsis thaliana* is the most frequently studied model plant species and belongs to the Brassicaceae family. Within the NUE-CROPS project *Arabidopsis* was used to identify genes involved in N use efficiency and use them as syntenic (occurring on the same chromosome) markers to target oilseed rape.

The population used consisted of a set of 350 distinct natural accessions of *A. thaliana*, collected world-wide. This population is very densely genotyped using 250,000 single nucleotide polymorphism (SNP) markers. The screening of this population took place in a climate chamber where plants were exposed to two N-supplies: N-deficiency and control. The population was also screened in a high throughput phenotyping platform under N-deficient conditions.

Genome-Wide Association (GWA) mapping was used as a tool to identify Quantitative Trait Loci (QTL) in association with N use efficiency. Candidate genes associated with fresh and dry weight, N use efficiency, N usage index, water content, chlorophyll fluorescence, and N concentration have been identified. The most promising candidate genes co-localized with QTL are MICRORNA 160, A. thaliana SYNTAXIN OF PLANTS 22, Albino 3, Bax inhibitor 1, and RING/U-box superfamily protein.

Nitrogen use efficiency in Brassica rapa - a closely related species

Brassica rapa is one of the diploid ancestors of oilseed rape *B. napus* and it is grown both as an oilseed crop and as a vegetable. The objectives of this research were to quantify the genetic variation for N use efficiency in a wide collection of germplasm, to identify QTL in mapping populations, and to analyse functional relationships between plant morphological characteristics and N use efficiency.

A world-wide collection of 146 accessions was grown in field trials in Southwest China at two N levels (0 and 120 kg N ha^{-1}). The traits measured were biomass production at 82 and 180 days after sowing, leaf N content, plant height, leaf number, leaf weight and seed yield. The same material was tested in pot experiments at two N input levels. For genotyping of this material 206 polymorphic InDel (insertion or the deletion of bases in the DNA of an organism) markers distributed over the *B. rapa* genome were developed and mapped. In addition, 53,587 SNPs (single nucleotide polymorphisms) and 5,501 InDels were identified by re-sequencing. This dataset of SNPs and InDels was used to perform Genome Wide Association Selection (GWAS) for ten investigated traits. This led to the identification of 45 significant markers in the field experiment and 35 significant markers in the pot experiment with only 4 overlapping markers. Natural sequence variation was observed for the two genes *BrFLC1* and *BrFLC2*.

In an alternative approach, QTL were identified in two segregating populations, one with 160 recombinant inbred lines and one with 119 doubled haploid lines. The four parents of these populations were resequenced to design InDel markers. For both populations high density linkage maps were constructed. The two populations were tested in pot experiments at both low and high N levels and biomass, plant height, leaf number, leaf weight, leaf width and length and leaf N content were measured. A large number of QTL could be detected in each of the two populations, but only a few of them were detected in both populations.

The lack of co-localizations for NUE QTLs between populations using either approach demonstrated that NUE is a complicated trait with different parents harbouring very different NUE genes. Successful breeding strategies may require accumulation of genes from different parents. The lack of co-localization between studies also provided an indication that the traits related to NUE were largely affected by environments; although the 4 overlapping loci for markers identified in the GWAS study should be relatively stable in a range of environments.

Nitrogen use efficiency in Brassica napus – oilseed rape

A promising strategy to improve N efficiency in oilseed rape could be the use of semi-dwarf hybrids. Such hybrids are the result of a cross between a dwarf type and a normal type, and they are about 30 cm shorter that normal hybrids (Figure 8). Semi-dwarf hybrids are of special interest, because they might need less N per unit seed yield than normal types, due to their reduced vegetative biomass.Therefore the objectives were to compare the agronomic performance of semi-dwarf and normal oilseed rape hybrids at low and high N supply.

Figure 8: Comparison between semi-dwarf (left) and normal-typ (right) hybrids.

A population of 150 oilseed rape hybrids, segregating into 75 semi-dwarf and 75 normal hybrids was developed from a DH population generated from the cross 'Alesi-*bzh*' x 'H30'. 'Alesi-*bzh*' is a dwarf line carrying the mutated *Bzh* dwarf gene, ‘H30’ is a resynthesized oilseed rape line. The DH population is segregating into dwarf and normal type lines. All DH lines were crossed with a normal type, male sterile tester. The resulting testcross hybrids (TC) segregate into semi-dwarf and normal type hybrids. These hybrids were tested in field experiments at two locations in Central Germany in three years at low (without N fertilization) and high (177 kg N/ha fertilised) N supply. These genotypes were tested in two different experiments with harvest at the end of flowering and at grain maturity, respectively. In total about 3,900 field plots were sown, harvested, and analysed.

On average, semi-dwarf hybrids produced a significantly higher seed yield (2.4 t/ha) at low nitrogen supply compared to normal hybrids. At high nitrogen supply both growth types produced similar seed yields with a non-significant difference of 0.2 t/ha. Semi-dwarf types had significantly less straw than normal types at both nitrogen supply levels. The harvest index of semi-dwarf types was significantly higher at low and high nitrogen supply compared to normal types. The variation for seed yield, straw yield and harvest index at low and high nitrogen supply is shown in Figure 9. In comparison to normal hybrids, semi-dwarf hybrids revealed a significantly higher N use efficiency at both N levels. Variation in N uptake efficiency was the most important parameter for genetic variation in N use efficiency.

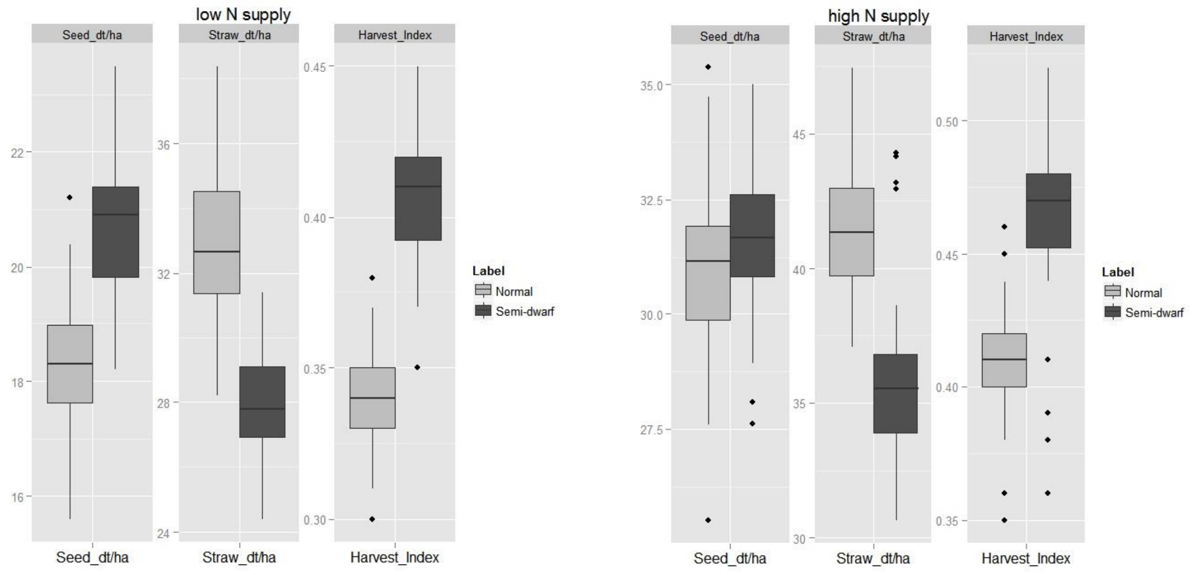

Figure 9: Mean values for seed yield, straw yield and harvest index of 54 semi-dwarf hybrids and 54 normal hybrids across environments with low nitrogen (five environments) and high (four environments) supply.

For QTL mapping a linkage map was developed from 139 DH lines which were genotyped with 541 (432 SNP and 109 SSR) genetic markers. QTL mapping identified a large QTL on linkage group A06 with pleiotropic effects (one gene influences multiple, seemingly unrelated phenotypic traits) on seed yield, straw yield, harvest index and nitrogen use efficiency at low and high N supply. Due to the position of the QTL on linkage group A06 next to the dwarf gene *Bzh* it is most likely a direct effect of the dwarf gene itself. A second QTL with pleiotropic effects on seed yield, harvest index and nitrogen use efficiency was identified on linkage group A07 at low N supply. QTL for seed yield and harvest index on linkage group A07 had been detected in other studies as well. Besides the two QTL on linkage group A06 and A07 several other QTL with minor effects were detected.

In conclusion the semi-dwarf hybrids produced higher seed yields and revealed a higher N use efficiency than normal type hybrids, especially at N deficient conditions. Therefore the use of semi-dwarf hybrids in oilseed rape cultivation is a promising approach to maintain current seed yields with reduced mineral fertilizer inputs.

3.4 Improving the nitrogen use efficiency of potatoes

Introduction

Potatoes require abundant nitrogen to perform well. 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). Moreover, the EU nitrate directive (91/767/EEC) makes nitrogen use efficiency (NUE) a very important trait and will force farmers to reduce inputs of fertilizer N, affecting the physiological and agronomic performance of the crops.

The potato crop is highly responsive to N fertilization (Harris 1992). N effects on potato development have been reported on canopy structure, light interception, photosynthesis, dry matter (DM) accumulation and yield (Oliveira 2000, Vos and Biemond 1992; Biemond and Vos 1992). Most of the studies on NUE in potato have used only a 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 fertilization (Zebarth et al. 2004a; Battilani et al. 2008). Additionally, maturity type is a major factor driving performance and development of the genotypes with high heritability and strong associations with other yield determining factors; maturity type has not been included as a factor in previous studies.

To increase our understanding of the genetic basis of NUE, phenotyping under field conditions was done on: A) a set of tetraploid (having four times the haploid number of chromosomes) potato cultivars (189 cultivars) and B) on a diploid (having double the haploid number of chromosomes) population (100 genotypes) both under contrasting nitrogen inputs and including a wide range of maturity types. Additionally meta-data for the diploid population including 6 environments were included. Canopy development (CDv) was studied using an eco-physiological model, allowing the dissection of this complex trait into biologically meaningful parameters. The model uses a function of thermal time based on the cardinal temperatures for canopy development in potato. The resulting model parameters were used as traits to understand the response of the plant, their relationship with traditional agronomical traits and NUE index. To find genetic factors responsible for the N response both a QTL linkage analysis was performed as well as an association analysis. Complementarily, a small set of cultivars was used to study in more detail the plasticity of the plant response to different N available.

Summary of key findings

Through the intensive phenotyping of the F1 population in the first workpackage (WP 4.1.1) we were able to analyse a large set of physiologically relevant variables. These variables are summarized in Table 1.

Table 1 Description of parameters reproduced from phenotyping the RH x SH population. td stands for thermal day.

Trait	Description	Unit
Canopy cover dynamics		
t_{m1}	Inflexion point during canopy build-up phase	*td*
t_1, D_{P1}	Period from plant emergence to maximum canopy cover (time at which the maximum is reached and duration of period since emergence, respectively)	*td*
t_2	Onset of canopy cover senescence	*td*
t_e	Time of complete senescence of canopy cover or crop maturity	*td*
v_{max}	Maximum level of canopy cover	%
c_{m1}	Maximum canopy growth rate during build-up phase	% td^{-1}
c_1	Average canopy growth rate during build-up phase	% td^{-1}
c_3	Average canopy senescence rate during decline-phase	% td^{-1}
D_{P2}	Total duration of maximum canopy cover phase	*td*
D_{P3}	Total duration of canopy senescence phase	*td*
A_1	Area under canopy curve for D_{P1}	*td %*
A_2	Area under canopy curve for D_{P2}	*td %*
A_3	Area under canopy curve for D_{P3}	*td %*
A_{sum}	Area under whole green canopy curve	*td %*
Tuber growth		
t_B	Onset of linear phase of tuber bulking	*td*
t_E	End of linear phase of tuber bulking	*td*
ED	Total duration of tuber bulking	*td*
c_m	Rate of tuber bulking	g td^{-1}
w_{max}	Maximum tuber dry matter at crop maturity	g m^{-2}

Moreover, based on meteorological data, the parameters indicated in Table 1, and the nitrogen concentration measured by chemical analysis, we were able to calculate the Radiation Use Efficiency (in g per MJ) and the nitrogen use efficiency (in g per g). The variable RUE refers to the slope of the relation between tuber dry matter (g m^{-2}) and cumulative intercepted radiation (MJ m^{-2}). NUE is defined as the ratio between total tuber dry weight and tuber N uptake.

Our results further indicated good correlations across the environments for most physiological traits, e.g. A_{sum}. However, the correlations varied much for some traits like t_1 or V_{max}. It is obvious that traits more sensitive to environment and/or G×E interaction show more heterogeneity among the environments (Figure 10). Ranking of genotypes changed across the environments with maximum variability observed within the F1 population under low N environment.

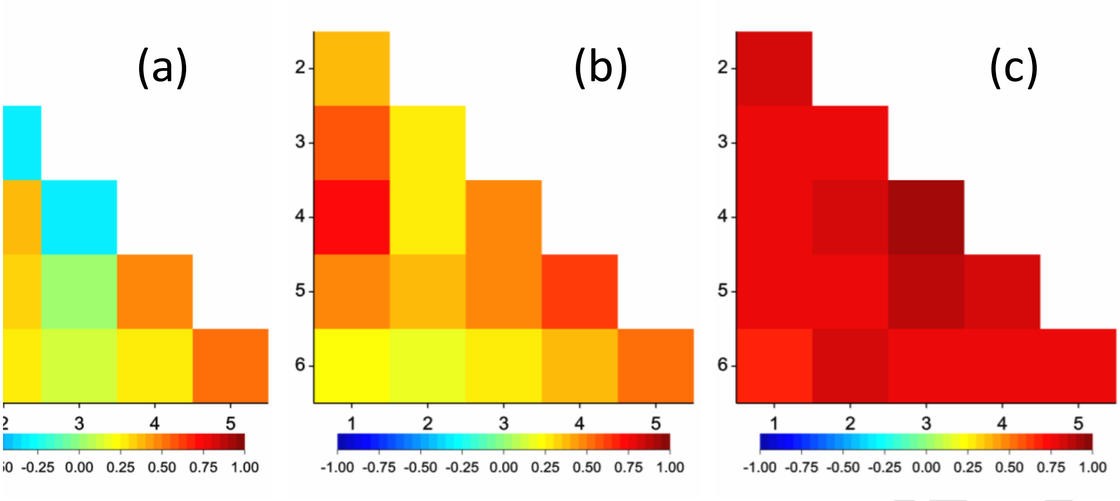

Figure 10 Heat maps revealing correlations between six environments for traits (a) *t1*, (b) V_{max}, and (c) A_{sum} within F1 population of potato. For description of traits see Table 1.

The results indicated that length of the canopy build-up phase (t_1 or D_{P1}) was conserved with few genotypic differences during early growth stages of the potato. In contrast, the duration of maximum canopy cover (D_{P2}) and the canopy decline phase (D_{P3}) varied greatly with maturity type, with late maturing genotypes having longer D_{P2} and D_{P3} and thus accumulated higher area under the whole green canopy curve (A_{sum}). Figure 11 schematically highlights various situations of a potato ideotypes under both optimum and resource poor (low N) conditions.

Our results further indicated significant ($P<0.01$) and strong genetic correlations between tuber yield and most of physiological model traits (Table 2). For instance, traits such as t_2, t_e, V_{max}, c_{m1}, D_{P2}, A_2, A_{sum}, t_E, *ED*, N uptake, and use efficiency showed very high (> 0.80) genetic correlations with w_{max}. The results therefore suggested that a high indirect response could be obtained by selecting genotypes for these traits to improve tuber yield in potato. However, our results also suggested that while using these traits as a criterion for selection, their causal physiological relationships and trade-offs must be considered simultaneously.

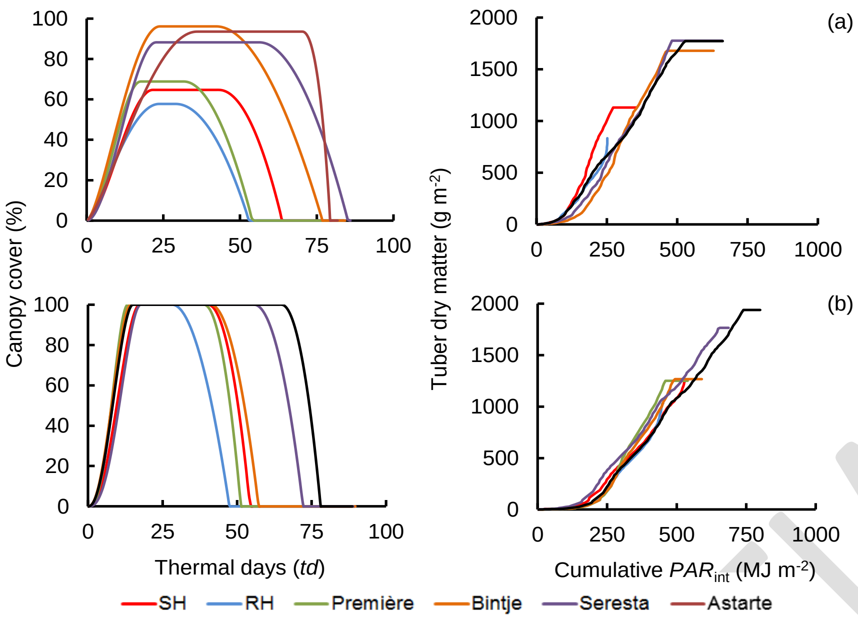

Figure 11 Schematic representation of the course of green canopy cover and tuber dry matter production of 4 standard cultivars varying in maturity type, and 2 parental genotypes (SH and RH) of the F1 population for (a) low N situation and (b) optimum N situation

Table 2 Genetic correlation coefficients (rG) between physiological traits and measured tuber yield (wmax), expected selection response (R) for physiological traits and for wmax, correlated selection response (CR) and efficiency of indirect selection (CR/R) for wmax estimated from physiological traits of potato. Estimations were based on F1 population mean across experiments. td stands for thermal day

Trait	Unit	r_G	*R*	*CR*	*CR/R*
t_{m1}	*td*	0.24**	0.31	0.06	0.29
t_1/D_{P1}	*td*	-0.05^{NS}	0.2	-0	-0.1
t_2	*td*	0.70**	0.22	0.22	0.57
t_e	*td*	0.75**	0.27	0.25	0.57
Vmax	%	0.84**	0.18	0.23	0.94
c_{m1}	% td^{-1}	0.53**	0.48	0.15	0.57
c_1	% td^{-1}	0.46**	0.48	0.14	0.42
c_3	% td^{-1}	0.17**	0.27	0.03	0.4
D_{P2}	*td*	0.85**	0.98	0.27	0.73
D_{P3}	*td*	0.15**	0.39	0.04	0.2
$A_1^{\dagger}$	*td %*	-	-	-	-
A_2	*td %*	0.84**	1.07	0.27	0.69
A_3	*td %*	0.41**	0.44	0.11	0.46
A_{sum}	*td %*	0.78**	0.49	0.26	0.59
c_m	g td^{-1}	-0.20**	0.78	-0.1	-0.2
t_E	*td*	0.66**	0.53	0.22	0.49
ED	*td*	0.64**	0.93	0.21	0.48
RUE	g DM MJ^{-1}	-0.25**	0.41	-0.1	-0.2
N uptake	g m^{-2}	0.85**	0.29	0.24	0.91
NUE	g DM g^{-1} N	0.43**	0.28	0.13	0.39
w_{max}	g m^{-2}		0.29		

** Significant at 1%, ^{NS} Non-significant. † Estimation was not possible due to zero genetic variance (σ^2_G).

The QTL results clearly showed that one particular chromosomal position at 18.2 cM on paternal (RH) linkage group V was controlling nearly all the traits, rendering the population on which this research was done less suitable for detecting QTLs for canopy cover traits that are not associated with maturity type. For example, it was notable that QTL for traits like t_e, t_2, D_{P2}, A_2, A_{sum}, c_m, t_E, ED, and tuber yield *per se* were co-localized in the majority of the environments, making it difficult to select for one of these traits without also selecting for the other ones. This QTL was associated with major additive effects on most of the traits and explained more than 50% of the phenotypic variance.

The QTL-by-environment (QTL×E) interaction phenomenon was evident mainly for traits determining the canopy build-up and senescence phases, maximum canopy cover (v_{max}), rates of canopy growth and senescence (c_{m1}, c_1, and c_3), duration (D_{P1}) and area under canopy cover (A_1) when canopy cover reaches its maximum, duration (D_{P3}) and area under canopy cover (A_3) of canopy senescence, resource (radiation and N) use efficiencies and tuber yield *per se*. Figure 12 illustrates the QTLxE interaction for tuber yield *per se*.

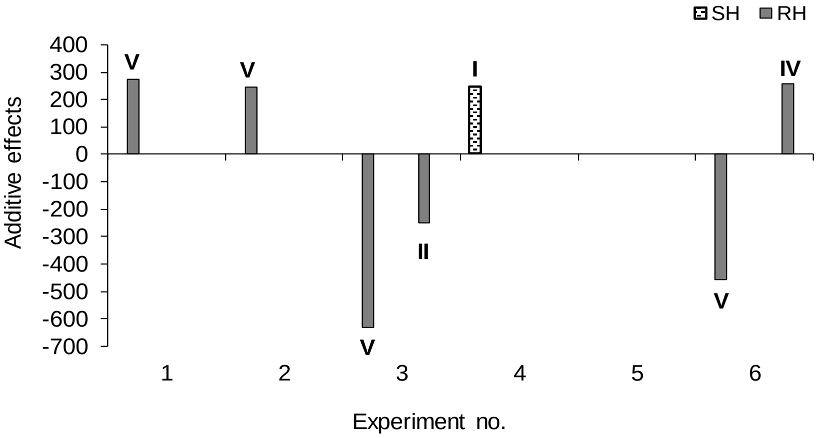

Figure 12 QTL×E additive effects for tuber yield (g m^{-2}) parental (SH and RH) genomes for six experiments. Letters above or below the bars indicate different linkage groups.

Additional detailed phenotyping of the 100 SH × RH genotypes

In 2011 an extra genotyping of a diploid population (SH X RH) with 94 genotypes was carried out to assess its response to two contrasting N input levels (75 and 180 kg/ha, respectively). 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 (

Table 3). Yield partially depended on maturity type, although in this population the distinction of maturity groups was not clear with the genotypes from different maturity types overlapping. In general, the later the genotype, the higher the yield, DM%, as well as the N use efficiency. N content increased with a decrease in DM% (Figure 13). Nitrogen seems to be diluted when carbohydrates (the main components of the tuber DM) are being accumulated. Additionally, the variation for yield was greater at the higher N input. The efficiency in using nitrogen decreased with an increase of N input. The magnitude of this decrease is equivalent to the change in response to nitrogen. On the other hand, higher N input positively affected the yield, N content and therefore the N uptake; DM% was not affected by N input.

Canopy development traits showed a strong response to N treatment; the area under the curve for phase 1 (A_1) became lower with high N input rates while A_2 became larger allowing more light interception by both an increase in the maximum soil covered and by an elongation of period for which the maximum was maintained.

Table 3 Regression parameters for the yield comparison within a given maturity group at each level of N input. N1: High N, N2: Low N. E_E=Early, M_E middle early, M_L=middle late and L_L= late. Std dev and Means in kg/m^2.

Maturity groups	Correlation	Slope	Intercept	R^2	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

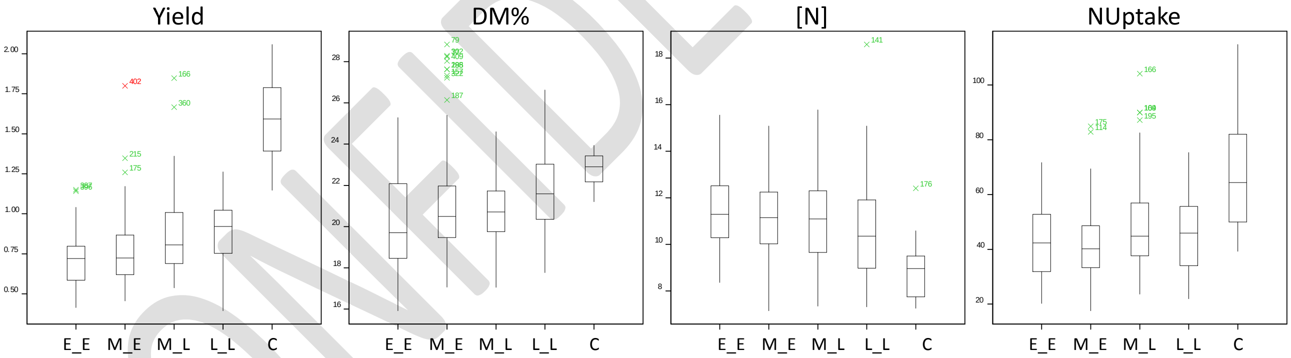

Figure 13 Comparison of maturity groups; E_E=early, M_E=middle early, M_L=middle late, and L_L=late, C= cultivar with middle maturity type (for comparison), for Yield Dry matter percentage DM%, Nitrogen content in tuber and Nitrogen uptake

Identification of potential QTLs for N-dependence of canopy development and Nit-UE

Table 4 QTLs detected for agronomical and quality traits under two N levels: H=High and L=Low. The explained variance is shown as a percentage (%VarExpl)

Trait	Marker	Linkage Group	QTL position	N level				Trait description
				H		L		
				%VarExpl	-log10(P)	%VarExpl	-log10(P)	
t_{m1}	**116_5_17**	pa_V	18.18	28.38	5.65			Inflection point Phase I, build up phase of canopy development
t_1/D_{P1}	**196_6_33**	ma_VI	44.31	13.93	3.32			Period from plant emergence to maximum soil coverage
v_{max}	**109_2_88**	ma_II	95.54	12.49	3.66			Maximum %SC reached
	114_5_4	pa_V	4.53	21.02	4.84			
	116_5_17	pa_V	18.18			19.33	3.87	
t_2	**115_3_42**	ma_III	59.52			15.27	3.51	Initiation of senescence or phase III
	116_5_17	pa_V	18.18	46.53	9.77			
t_e	**116_5_17**	pa_V	18.18	58.76	13.96	43.11	8.93	Total growing period till canopy is dead.
	153_6_28	pa_VI	53.8	7.99	3.50			
c_{m1}	**197_6_39**	ma_VI	51.12	21.28	4.98			Maximum progression rate of soil coverage during Phase I
A_1	**116_5_17**	pa_V	18.18	18.70	3.75	24.28	4.83	Area under canopy cover curve for phase I
A_2	**116_5_17**	pa_V	18.18	32.50	6.52			Area under canopy cover curve for phase II
A_3	**116_5_17**	pa_V	18.18			46.84	10.23	Area under canopy cover curve for phase III
	51_1_84	pa_I_B	47.64			10.74	3.69	
A_{sum}	**116_5_17**	pa_V	18.18	54.58	11.93	45.82	9.59	Total area under the canopy curve
D_{P2}	**116_5_17**	pa_V	18.18	26.05	5.18			Durations of phase II
D_{P3}	**116_5_17**	pa_V	18.18			39.04	8.38	Duration of phase III
	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	Tuber dry matter %
	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			Tuber dry matter (yield)
[N]	**247_9_18**	ma_IX	17.51	13.81	3.32			Tuber nitrogen concentration
NUtE	**248_9_19**	ma_IX	20.39	16.43	3.91			Nitrogen utilization efficiency
UptE	**116_5_17**	pa_V	18.18	19.69	3.94			Nitrogen uptake efficiency
TbnMX	**107_4_60**	pa_IV	37.04	15.20	3.53	11.77	3.22	Maximum tuber number
	228_12_21	pa_XII	23.54			15.70	3.69	
TbnB	**127_4_13**	ma_IV	10.69	13.82	3.25			Tuber number average size with the maximum tuber number
	98_4_35	pa_IV	12.84			16.16	3.73	
TbnA	**116_5_17**	pa_V	18.18			22.50	4.48	Tuber number dispersion parameter
	131_4_21	ma_IV	17.03	13.85	3.30			
TbwB	**109_2_88**	ma_II	95.54	14.25	3.79			Tuber weigth average size with the maximum tuber weight
	127_4_13	ma_IV	10.69	12.56	3.38			
Tb_mt	**187_6_6**	ma_VI	15.48	13.57	3.72			Tubers per meter
	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	Maturity assessment
	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	After Cooking Darkening
	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	Brightness
	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	Overall cooking score
	83_1_95	ma_I	125.64			14.65	4.31	
STR	**117_3_42**	ma_III	57.5	14.12	3.78			Structure inside tuber
	54_1_99	pa_I_B	63.03	13.63	3.76			

A total of 44 QTLs were found for all traits at both N levels, 15 on the linkage group pa_V that has been previously reported for maturity (Table 4). This QTL appeared related to t_{m1}, *te*, areas under the canopy cover progress curve for the three phases of CDv, length of phases 2 and 3, yield (Y DM), NUptE, TbnA and the maturity assessment. On linkage group pa_VI there was another QTL related to maturity only at high N. It also confirmed that CDv and yield depended largely on the maturity type of the genotype.

Only 10 QTLs were common at both N levels. Out of these common QTLs, 4 were on pa_V related with traits A_1, A_{sum}, and mt_as with only A_1 having higher variance explained at the low N level than at the high N level. The other 6 QTLs were found on other linkage groups related with DM%, TbnMx, ACD, HLD, and KWD. Twelve QTLs were detected exclusively at low N for A_{P3}, t_e-t_2, TbnMX, TbnA, TbnB, ACD, HLD, and KWD, whereas 22 QTLs were detected only at high N.

In general, QTLs related with multiple traits (pleiotropic regions) were significant in both High N and Low N on the linkage groups pa_V, ma_V and ma_III . QTLs only detected at low N were found in the linkage groups ma_I, ma_VI, ma_VIII, pa_II, pa_VII and pa_XI, whereas QTLs detected only at high N were found in pa VI (Figure 14).

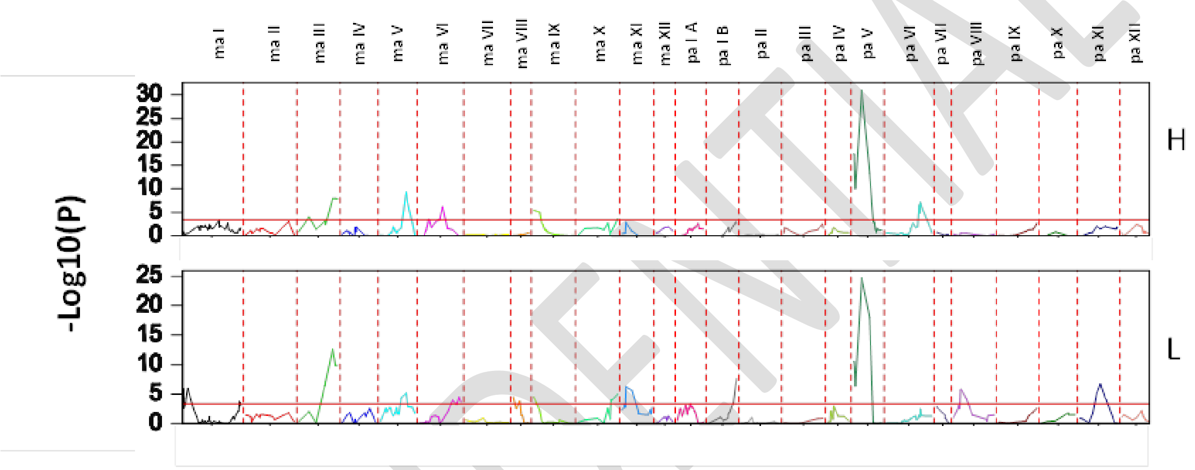

Figure 14 Log profile across each linkage groups for the multi trait QTL analysis. The horizontal red line is the threshold for significant QTLs, H is the profile from the high nitrogen level and L is from the low nitrogen level

The fact that QTLs were found exclusively for one of the N levels for some traits showed how the QTL expression was affected by N and how N affected the proportion of the variation accounted for by genetic factors. Breeding and selection must focus on the early stages of the breeding scheme to define the maturity of the clones and then select within a maturity group. Additionally the selection must include both N conditions since different QTLs are expressed at different N levels.

Identification of phenotypic variation on a potato cultivar set

Using the set of 200 cultivars phenotyped in 2010 and 2011, our data confirmed the findings for the diploid population i.e. N affects trait relationships. In general, at high N the areas under the curve (traits depending on V_{max} 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 V_{max} values can take. At high N there was more canopy cover (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 V_{max} was maintained constant.

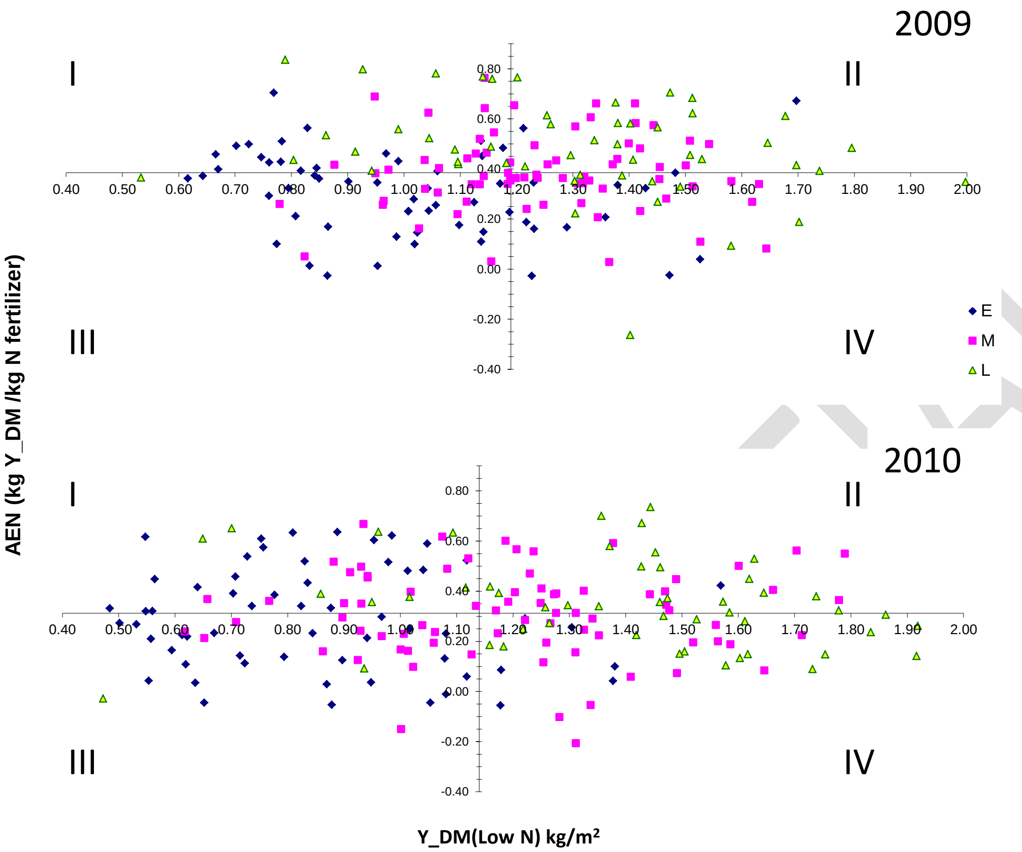

Figure 15 Response of agronomic efficiency of N fertilizer applied (AEN) from low to high N input (vertical axis) in relation to the performance under the low N input (as Y_DM; horizontal axis). I = quadrant (Q)I, high response, low performance under low input. QII = high response and performance. QIII = low response and performance. QIV = low response and high performance. E: early; M: intermediate; L: late cultivars

Figure 15 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. 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 as shown with the diploid population. 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.

Additionally, 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.

Finally, NUE has been reported to decrease when N input increases. Assuring a good performance at low N means a higher baseline for 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. A_2, t_2-t_1 and C_{m1}. This could turn into a selection strategy to be adapted and implemented in the early stages of breeding schemes. Offspring could be classified by canopy development (CDv) and their phenotypic maturity behaviour under a limited N environment that will also allow a high selection pressure for NUE. Parameters such as V_{max}, t_2, and t_1 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 offspring 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 fertilizer (Quadrant II in Figure 15). 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 fertilizer supply. Cultivars selected in this way allow farmers to either reduce inputs, and thus costs, or explore the potential to use higher input rates with confidence of a good economic return.

Association mapping for canopy development and NUE related traits

Association analysis is under way; the initial results showed SNPs related to maturity on chromosome V also affecting most of the traits at both N levels. Some of the associations showed dependency on N rate as happened with A_{sum} on chromosome 3 whereas at low nitrogen the association was not significant. In contrast A_{p1} showed a more intense signal at low N. This result showed that the association approach allowed the identification of genetic factors in a physically localized position on the genome allowing the identification *in silico* of possible candidate genes.

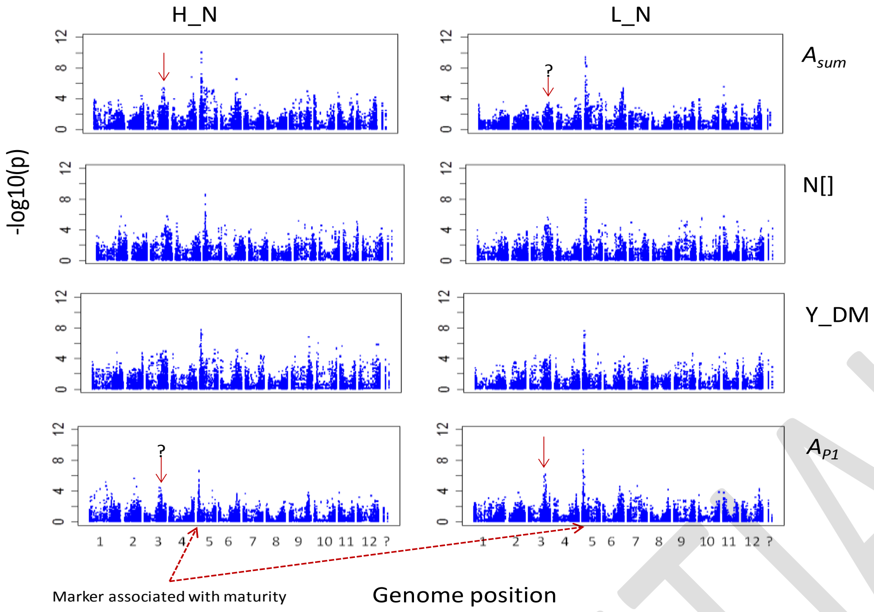

Figure 16 Manhattan plot displaying genome wide association mapping result of regression with kinship analysis. For A_{sum} =total area under the curve for canopy development, N[] =nitrogen content, Y_DM = yield dry matter and A_{p1} area under de curve for phase I. The Y-axis shows the significance level (-log10) and X axis genome position, numbers are the chromosomes.

The present result has shown the importance of the use of ecophysiological models to understand and dissect complex traits such as canopy development and NUE. This approach showed that both linkage QTL and association analysis with the parameter traits allowed detection of N dependent genetic factors. The findings from the current study can further be strengthened with studies using new populations, and integrating novel resources like highly detailed genotyping. In addition, it is important to investigate differences in potato response to organic and mineral sources of N.

3.5 Improving the phosphorus use efficiency of potatoes

Background

A disproportionately large amount of phosphorus (P)-fertilizer is applied to potatoes (*Solanum tuberosum* L.) compared to other field crops (White *et al.*, 2005b, 2007). This can result in large P-losses from fields and, consequently, environmental degradation (Dampney *et al.*, 2002; Davenport *et al.*, 2005). The high P-fertilizer requirement of potatoes is thought to be due to the their inability to acquire P effectively from the soil (Dampney *et al.*, 2002; White *et al.*, 2005b; Syers *et al.*, 2008; Fageria *et al.*, 2011). The potato crop generally recovers <10% of broadcast P fertilizer in the year it is applied (Dampney *et al.*, 2002; Syers *et al.*, 2008) and, although optimizing the timing, quantities and methods of P-fertilizer application helps reduce inputs of P-fertilizer and P-losses to the environment (e.g. Davenport *et al.*, 2005; Marshall, 2007; White *et al.*, 2007; Syers *et al.*, 2008; Burns *et al.*, 2010; Defra, 2010), the impact of these agronomic methods alone to reduce the amount of P-fertilizer applied to the potato crop has been limited (Defra, 2014). To reduce P-fertilizer inputs and environmental pollution further requires the development of potato varieties that use P-fertilizer inputs more efficiently to produce commercial yields. However, there has been little attempt to do this (Trehan and Sharma, 2005; White *et al.*, 2005b).

Agronomic phosphorus use efficiency (PUE) is commonly defined as crop dry matter (DM) yield per unit of P available in the soil (g DM g^{-1} P_{soil}; White *et al.*, 2005a). This is numerically equal to the product of P uptake (acquisition) efficiency (PUpE), which is defined as the P acquired by the crop per unit of available P (g P_{crop} g^{-1} P_{soil}), and crop physiological utilisation efficiency (PUtE), which is defined as the yield per unit P acquired by a crop (g DM g^{-1} P_{crop}). This work, which was largely conducted at The James Hutton Institute (JHI), aimed: (1) to quantify genetic and environmental variation in PUE, PUpE and PUtE, (2) to identify chromosomal loci (QTL) affecting PUE, PUpE and PUtE, (3) to identify physiological mechanisms underpinning PUE, PUpE and PUtE and (4) to determine the effects of N-fertilizer management on PUE, PUpE and PUtE in potatoes.

Genetic and Environmental Variation in PUE, PUpE and PUtE

A NUE_Crops Core Collection, comprising 32 *Solanum tuberosum* genotypes, was grown in the field in 2009 and 2010 both with and without the application of P fertilizer (Table 5). Tuber yields and tuber mineral concentrations were determined at commercial maturity and mineral concentrations in diagnostic leaves were determined at canopy closure. Substantial genetic variation was observed in tuber yield, leaf P concentration ($[P]_{leaf}$) and tuber P concentration ($[P]_{tuber}$) among Tuberosum (standard potato varieties), Phureja (specialised group characterized by the absence of dormant tubers) and Diploid Hybrid genotypes (Table 1). The yield, and therefore the PUE, of Tuberosum genotypes, averaged across both years, was greater than that of Diploid Hybrid genotypes or Phureja genotypes, whether grown with or without P-fertilizer application (Table 1). There was a strong positive relationship between tuber yield when grown without P-fertilizer application and tuber yield when grown with P-fertilizer application among genotypes in both 2009 and 2010, which is consistent with previous reports that tuber yields of potato genotypes grown with low P inputs are correlated with their maximum yield potential (Sattelmacher *et al.,* 1990). However, both $[P]_{leaf}$ and $[P]_{tuber}$ of Tuberosum genotypes, averaged across both years, were similar to those of Diploid Hybrid genotypes and Phureja genotypes, whether grown with or without P-fertilizer application (Table 1), indicating that PUtEs of Tuberosum, Diploid Hybrid and Phureja genotypes were similar, but

Tuberosum genotypes had greater PUpEs than either Diploid Hybrid or Phureja genotypes. These observations are consistent with previous studies indicating that differences in PUE between potato genotypes are correlated with PUpE rather than PUtE (Sattelmacher *et al.*, 1990; Trehan and Sharma, 2005; White *et al.,* 2005a). These observations were reported in the publishable report of White *et al.* (2012).

			Yield (high P) (kg plot-1)		Yield (low P) (kg plot-1)		Yield loss (%)		[P]leaf (high P) (mg g-1 DM)		[P]leaf (low P) (mg g-1 DM)		[P]tuber(high P) (mg g-1 DM)		[P]tuber (low P) (mg g-1 DM)		Yield/[P]leaf (high P)		Yield/[P]leaf (low P)	
Genotype	Group	n	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se
99.FT 1 (5)	Diploid Hybrid	2	10.07	1.96	8.17	0.02	17.4	14.8	4.03	0.10	4.41	1.68	2.00	0.24	1.72	0.05	2.49	0.42	1.96	1.14
HB.171 (13)	Diploid Hybrid	2	5.72	0.27	6.15	0.02	-7.1	20.8	3.83	0.28	3.63	0.52	2.80	0.04	2.31	0.40	1.50	0.04	1.72	0.73
DB.226 (70)	Phureja	2	9.32	0.04	9.06	0.02	2.9	11.5	3.99	0.47	3.81	1.65	1.46	0.25	1.24	0.05	2.37	0.29	2.53	1.40
DB.337 (37)	Phureja	2	10.99	0.07	9.46	0.01	13.9	2.4	3.91	0.32	3.41	0.56	1.73	0.04	1.56	0.01	2.83	0.25	2.80	0.57
DB.375 (1)	Phureja	2	8.52	3.65	5.86	0.01	25.2	27.8	3.47	0.30	2.84	0.56	1.43	0.04	1.32	0.08	2.56	1.27	2.14	1.51
DB.378 (1)	Phureja	2	8.58	0.16	7.56	0.02	12.0	5.6	2.76	0.03	2.69	0.50	1.67	0.04	1.46	0.09	3.11	0.03	2.85	0.81
DB.384 (4)	Phureja	2	8.05	0.00	7.11	0.01	11.8	8.2	3.62	0.57	3.26	0.62	1.55	0.15	1.57	0.04	2.28	0.36	2.19	0.22
DB.520 (11)	Phureja	2	5.73	0.84	6.59	0.01	-10.2	65.6	3.46	0.59	3.60	0.08	1.24	0.04	1.31	0.15	1.75	0.54	1.82	1.52
PHU.951 (901)	Phureja	2	5.11	1.02	5.30	0.03	-5.4	17.6	3.33	0.43	2.93	0.67	2.09	0.47	1.77	0.13	1.60	0.51	1.86	0.85
12601 ab 1	Tuberosum	2	8.78	1.51	7.81	0.01	10.4	6.9	4.12	0.38	4.20	0.86	1.38	0.02	1.42	0.05	2.19	0.57	1.91	0.89
Ailsa	Tuberosum	2	12.19	2.38	11.87	0.02	0.8	19.0	4.13	0.33	3.66	0.62	1.90	0.15	1.60	0.03	3.02	0.82	3.30	1.22
Anya	Tuberosum	2	9.86	1.55	8.76	0.01	13.8	33.6	2.86	0.20	3.48	1.49	1.56	0.07	1.35	0.01	3.51	0.80	2.83	2.94
Brodick	Tuberosum	2	13.87	1.99	14.29	0.02	-4.4	19.4	3.71	0.23	3.81	0.22	1.75	0.02	1.62	0.06	3.79	0.78	3.76	0.61
Cara	Tuberosum	2	13.42	1.12	14.09	0.03	-4.9	3.4	4.32	0.08	4.06	0.86	2.40	0.12	1.80	0.08	3.11	0.31	3.55	1.44
Desiree	Tuberosum	2	13.09	1.90	12.97	0.02	-0.2	14.6	3.47	0.55	3.08	1.01	1.63	0.08	1.44	0.00	3.97	1.18	4.38	2.05
Estima	Tuberosum	2	14.63	1.00	12.74	0.01	12.9	1.2	2.87	0.76	3.04	1.46	1.20	0.03	1.25	0.11	5.59	1.84	4.51	2.68
Golden Millenium	Tuberosum	2	13.99	0.07	12.27	0.01	12.3	17.7	3.23	0.20	2.94	0.47	1.51	0.03	1.62	0.13	4.36	0.30	4.24	1.56
Home Guard	Tuberosum	2	11.63	2.07	11.20	0.03	2.7	11.2	2.52	0.25	2.57	0.18	1.38	0.01	1.25	0.03	4.74	1.30	4.34	0.76
Home Harvest	Tuberosum	2	13.34	2.77	10.16	0.02	21.4	23.5	3.55	0.26	3.33	0.48	1.47	0.00	1.54	0.16	3.83	1.06	3.08	0.81
Hermes	Tuberosum	2	15.96	1.24	11.45	0.02	26.9	35.1	3.98	0.69	4.14	0.78	1.50	0.12	1.33	0.13	4.19	1.04	2.74	0.40
Maris Piper	Tuberosum	2	16.82	0.79	14.53	0.01	13.2	15.8	3.63	0.23	3.52	0.50	1.74	0.01	1.37	0.10	4.66	0.51	4.14	0.22
Montrose	Tuberosum	2	14.40	2.88	11.98	0.01	14.1	26.9	3.37	0.42	3.21	0.33	1.59	0.14	1.68	0.13	4.45	1.41	3.76	0.73
Nadine	Tuberosum	2	19.12	0.05	15.44	0.02	19.2	4.8	2.92	0.49	3.25	1.25	1.52	0.02	1.57	0.00	6.74	1.12	4.96	2.16
Pentland Dell	Tuberosum	2	8.65	3.20	7.07	0.01	10.7	41.1	3.21	0.61	2.74	0.81	1.43	0.16	1.28	0.02	2.99	1.57	2.70	1.58
Pentland Squire	Tuberosum	2	16.28	2.08	13.79	0.01	13.1	35.4	3.76	0.36	3.77	0.61	1.71	0.02	1.56	0.03	4.43	0.98	3.66	0.03
Record	Tuberosum	2	12.17	1.12	11.64	0.02	4.1	7.6	4.27	0.20	4.01	0.85	1.51	0.13	1.56	0.06	2.87	0.40	2.95	0.93
Saxon	Tuberosum	2	15.77	0.76	14.40	0.02	8.2	18.5	2.84	0.64	2.86	0.87	1.75	0.08	1.56	0.09	5.91	1.60	5.11	1.02
Scarborough	Tuberosum	2	14.55	1.39	12.40	0.02	14.6	3.3	3.73	0.47	3.54	1.21	1.78	0.04	1.77	0.02	4.02	0.88	3.65	1.78
Stirling	Tuberosum	2	16.67	4.09	15.10	0.01	5.9	29.2	3.89	0.35	3.60	0.22	1.95	0.19	1.73	0.13	4.41	1.45	4.21	1.04
Tay	Tuberosum	2	12.54	1.58	11.54	0.02	6.4	25.8	3.76	0.37	3.45	0.83	1.63	0.09	1.31	0.48	3.41	0.75	3.39	0.73
Vales Everest	Tuberosum	2	17.04	1.81	14.61	0.02	13.4	16.9	3.60	0.10	3.43	0.36	1.80	0.06	1.43	0.01	4.72	0.37	4.27	0.52
Wilja	Tuberosum	2	16.27	3.27	14.20	0.02	11.4	12.9	3.08	0.50	3.34	2.14	1.70	0.26	1.66	0.05	5.60	1.97	4.94	4.28
*Mean +/- SE	Diploid Hybrid	2	7.90	2.18	7.16	1.01	5.17	12.25	3.93	0.10	4.02	0.39	2.40	0.40	2.01	0.29	2.0	0.49	1.8	0.12
	Phureja	7	8.04	0.77	7.28	0.59	7.17	4.61	3.51	0.15	3.22	0.16	1.59	0.10	1.46	0.07	2.4	0.21	2.3	0.16
	Tuberosum	23	13.96	0.56	12.36	0.47	9.83	1.63	3.51	0.10	3.44	0.09	1.64	0.05	1.51	0.04	4.2	0.22	3.8	0.17

Table 5 Yields per plot of five plants (kg FW plot^{-1}), P concentration of diagnostic leaves ($[P]_{leaf}$), P concentration of tubers ($[P]_{tuber}$) and yield / $[P]_{leaf}$ quotients for genotypes cultivated either with (high P) or without (low P) P-fertilizer additions in 2009 and 2010. Yield loss for each genotype grown without P-fertilizer applications is expressed in percentage terms as (1-(yield unfertilised / yield fertilised)) x 100). Data are expressed as mean ± SE either for 2 years (for individual genotypes) or for n genotypes.

Chromosomal loci (QTL) affecting PUE, PUpE and PUtE

An existing tetraploid genetic mapping population of clones derived from a cross between the processing clone 12601ab1 and the table cultivar Stirling was grown in the field in 2009 and 2010 both with and without the application of P-fertilizer. Tuber yields and tuber mineral concentrations were determined at commercial maturity and mineral concentrations in diagnostic leaves were determined at canopy closure. These data, together with data obtained in previous studies of this population, were used to identify QTL for (a) tuber yield, (b) P concentrations in diagnostic leaves ($[P]_{leaf}$), and (c) tuber yield/$[P]_{leaf}$, and their responses to P-fertilisation. It was observed that consistent QTL affecting yield, $[P]_{leaf}$ and tuber yield/$[P]_{leaf}$ could be identified in experiments performed in different years and with contrasting P-fertilizer applications, and that major QTL for tuber yield, $[P]_{leaf}$ and PUtE on Stirling Linkage Group V at 68 cM co-locate with a QTL explaining about half the phenotypic variation in crop maturity in the same population. These data could be used to develop molecular markers for breeding for PUE in potato. These observations were reported in the publishable report of White *et al.* (2013).

Physiological Mechanisms Underpinning PUE, PUpE and PUtE

Eight genotypes (12601ab1, Ailsa, Cara, Home Guard, Maris Piper, Nadine, Pentland Dell, Stirling) with contrasting yields at low P input, yield responses to P-fertilizer addition, PUtE and $[P]_{leaf}$ were identified for physiological studies (Table 5). These genotypes were grown in the field in 2011 both with and without the application of P fertilizer. Significant positive relationships were observed between the size of the juvenile root system and early plant vigour (Figure 17), canopy development, P accumulation and final yield. These data were reported in the publishable report of White *et al.* (2012).

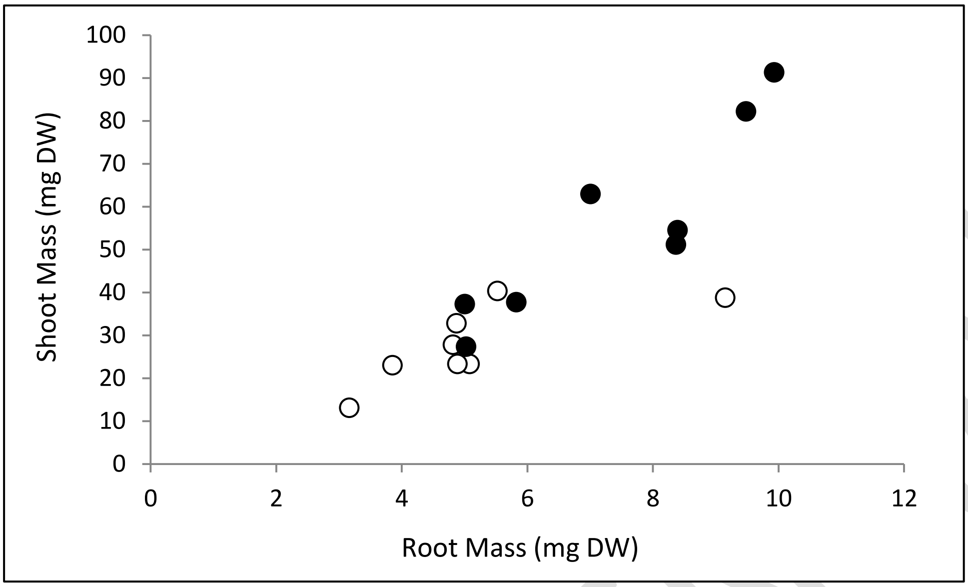

Figure 17 The relationship between root mass and shoot mass of eight Tuberosum genotypes three weeks after emergence. Data show means of four individual plants grown with (closed circles) or without (open circles) P-fertilizer application.

In 2012, the same eight genotypes were grown for two weeks after emergence in pots in the glasshouse with and without P fertilizer additions, and detailed assessments of root system architectures were made on juvenile plants. The nomenclature developed by Wishart *et al.* (2013) was adopted. Relationships were observed between various aspects of root system architecture and root biomass, phosphorus uptake, shoot growth and PUE in the field. For example, there was a significant correlation between the length of stolon roots of plants grown in pots without P-fertilizer addition and the mass of roots three weeks after emergence of plants grown in the field without P-fertilizer addition (Figure 18).

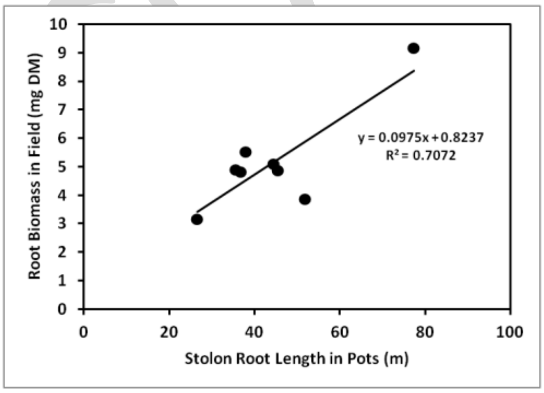

Figure 18 The relationship between the combined length of stolon roots of plants grown for two weeks after emergence in pots in the glasshouse without P fertilizer addition and the biomass of roots three weeks after emergence of plants grown in the field without P-fertilizer addition for eight Tuberosum genotypes. Data show means of three (glasshouse experiment) or four (field experiment) individual plants.

To obtain further insights into the physiology of PUE in potato, gene expression was investigated in roots of four of these genotypes (12601ab1, Pentland Dell, Maris Piper, Stirling) grown in flowing nutrient solutions containing either low (25 µM) or sufficient (250 µM) phosphorus concentrations. Gene expression in roots differed between genotypes, and changed with P supply. Sixty-two genes differed in their expression between roots of P-replete and P-starved plants of all genotypes. These genes represent a common transcriptional response to P-starvation, and might be used to diagnose P-starvation in potato (c.f. Hammond *et al.*, 2011). A specific transcriptional response associated only with roots of genotypes with high yields in the absence of P-fertilizer application in the field was also identified. Fifty-nine genes differed in their expression between roots of P-replete and P-starved plants of the high-yielding genotypes Maris Piper and Stirling, but not between roots of P-replete and P-starved plants of the low-yielding genotypes 12601ab1 and Pentland Dell. Since the expression of these genes is correlated with high yields at low P inputs, they might provide candidates for understanding the differences in yield responses of potato genotypes to P-supply. This information is reported in the publishable report of Davis *et al.* (2012).

The Effects of N-fertilizer Management on PUE, PUpE and PUtE

Finally, a theoretical study was undertaken to explore the relationships between the use efficiencies of nitrogen (N) and phosphorus (P) by the potato crop. It was concluded that, for a given combination of P and N availability to a crop, the rankings of genotypes for NUE and PUE will be identical. However, selecting simultaneously for greater N and P uptake efficiencies, or greater N and P utilization efficiencies, is not so simple because these traits vary independently among potato genotypes. Nevertheless, many plant traits can benefit both NUE and PUE because they serve to increase tuber yield. Such traits include, for example, accelerated juvenile root growth, production of an extensive root system with low tissue density and long root hairs, greater root penetration of strong subsoils, accelerated canopy development, greater photosynthetic efficiency and greater harvest index. These insights are discussed in the publishable report of White *et al.* (2014).

Summary

In summary, this work has demonstrated genetic variation in PUE, PUpE and PUtE among potato genotypes and identified QTL for these traits in a tetraploid genetic mapping population (12601ab1 x Stirling). Agronomic PUE was correlated with PUpE rather than with PUtE and maturity class was observed to be a dominant factor affecting PUE. One trait associated with improved PUE was rapid juvenile root growth. Rapid juvenile root growth accelerated P acquisition, canopy formation and the accumulation of tuber biomass, which can result in greater yields at final harvest. Glasshouse screening of juvenile root systems correlated well with field assays allowing a "rapid, high-throughput" screen for root traits linked with final tuber yield. Differential expression of genes at high and low P supply was used to elucidate possible candidate genes affecting PUE.

The results suggest that potato genotypes with greater PUE can be developed within a particular maturity class by screening for accelerated juvenile root development. The correlation between glasshouse root growth and field performance means that relatively inexpensive, rapid and higher-throughput assays can be used to screen varieties for juvenile root growth, which could result in savings of time and money in future breeding projects. Further studies should be conducted to validate juvenile root growth as a trait which can be used to select for PUE and yield improvement. In addition, although both PUE and NUE might benefit from accelerated juvenile root development, future studies should investigate the relationships between P nutrition and N nutrition to identify traits that will contribute to greater PUpE, NUpE, PUtE and NUtE of potato crops.

3.6 Integration of breeding and agronomic innovations to improve NUE

Background

Within the NUE-Crops project, studies have been made on genetic improvements in NUE of four main agricultural crops. However, what society needs to improve resource utilization, protect the environment and for feeding an increasing world population is not just nutrient use efficient crops, but nutrient use efficient agriculture.

Breeding improved crops is only one element in developing a more nutrient use efficient agriculture. Crops interact with the environmental conditions and crop management where they are grown; nutrient use efficiency is the outcome of this interaction. High nutrient use efficiency (NUE) is not a characteristic of the crop genotype, but is only achieved as an outcome of the crop genotype (G) interacting with its environment (E) and crop management (M). Within NUE-Crops SP5 we studied these GxExM interactions through field experiments with cropping systems, crop rotation and crop management, as well as in model simulation studies allowing a broader range of conditions to be tested.

The goal of these studies was to understand how variable NUE interacts with the crop growth conditions as defined by the environment and crop management. The main focus was on nitrogen use efficiency (NitUE), though P balance and water use efficiency (WUE) were also studied.

The main questions addressed were, how do genotypes with different NitUE perform under different cropping conditions, how may the effect be improved, how can these questions be studied/evaluated. We also wanted to compare the extent of effects obtained through breeding approaches to effects of agricultural management and crop rotation measures.

Results

WP5.1 and 5.2: Interactions between crop genotypes, cropping system and crop management were studied in the Nafferton factorial systems trial at Newcastle University. Effects of growing wheat and potato genotypes assumed to have different Nit-UE were studied using organic or conventional cropping strategies for crop protection and fertilization, and applying different levels of fertilization.

The results showed the agronomic factors having much stronger effects than genotypes on wheat yield and Nit-UE. The rotational position of the crop had a very strong effect, and when wheat was grown after a three-year clover grass ley, high yields of winter wheat was achieved and little or no N fertilizer response found.

In most situations N fertilization did increase the yields and reduce Nit-UE, as it is commonly found. These effects of N fertilization were much stronger than the differences among the genotypes studied. Only in one comparison, it was found that the modern high yielding wheat variety was better able to respond to higher N fertility than a genotype with long straw breed for organic farming.

Model simulations of the organic vs. the conventional cropping systems, showed much stronger N deficits in the conventional systems, while the organic system showed a small N deficit, the conventional system showed a deficit of more than 160 kg N ha^{-1} $year^{-1}$, i.e. quite dramatic system differences compared to differences which can be seen among varieties.

Differences were larger between the two potato varieties were compared, than among the wheat varieties. The late maturing variety had higher yield and Nit-UE than the early variety. Higher yield is generally related to high Nit-UE, so this result is not so surprising, and selecting late maturing varieties allow the crop more time to produce a higher yield. This trait is important in the potato crop, where early and lower yielding varieties are grown for various reasons, while in most other crops the optimal maturity time is more fixed for various reasons.

Several examples of interaction between crop management, crop rotation and genotype were shown, indicating that for optimal Nit-UE, genotypes may need to be adapted to the specific conditions of the system they are to be grown in.

Calculating the P balance showed a significant P deficit in the organic system, illustrating the potential for such P budgeting to show weaknesses which need to be addressed to make the system sustainable in the longer term.

WP5.3: Comparing genotypes for silage maize production under different tillage systems at FiBL did not show any large differences among the maize varieties. Soil N supply and crop response to N fertilization varied strongly among the experiments, in one case being so high that little response to fertilizer N application was seen. High Nit-UE was found in a tall variety which has previously been shown to have low Nit-UE when grown for grain production. This shows an example of Nit-UE not being a crop trait as such, but being a "trait" of the crop and how we grow and use it. When grown for seeds, the vegetative plant material is a waste product, but when harvested for silage, it is part of the yield. Thus, Nit-UE of genotypes with a high fraction of vegetative material will be evaluated differently depending on how we harvest and use them.

Studies of the root system were also made, but gave inconclusive results. Root measures using the capacitance method correlated to root biomass in one year, but not in the other. Significant effects on mycorrhizal colonization of the roots were seen, but the effects were not consistent across environments.

Combined studies of Nit-UE and WUE of maize varieties, and their interaction with fertilization and soil tillage were made at ShanDong Agricultural University in China. Also PUE and KUE were studied. One important conclusion was that there were significant genotype x tillage x fertilization interactions for Nit-UE but not for yield. It was therefore concluded that breeding for improved Nit-UE depends on the environment where the selection is done, different growth conditions will lead to selection of different genotypes. It is concluded that in order to improve Nit-UE in crop production, management and genotypes must be improved simultaneously.

The studies within WP5.1, WP5.2 and WP5.3 all find that the Nit-UE is lower when organic fertilizers are used, as much of their N content is not immediately available to the crop. It must be concluded that when comparing mineral and organic fertilizers the effect in following years must be taken into account. The organic matter and the N from the organic fertilizers not utilized by the first crop, will add to soil quality and to Nit-UE in the succeeding crops.

WP5.4: At the experiments at University of Copenhagen more detailed studies on Nit-UE of six varieties of wheat and three varieties of winter oilseed rape were made. Growth, root growth, N uptake, litter loss, yield and Nit-UE were studied. Root development was studied, using the minirhizotrons method allowing repeated measurements and estimates of root depth development. Little is known about variety differences in root growth under field conditions, though it may obviously affect Nit-UE as well as WUE.

For winter oilseed rape the litter loss during growth was studied as well, as high litter loss during growth is a main reason for its relatively low Nit-UE. We got detailed data for the litter loss, but did not see systematic differences among varieties, and the semi-dwarf genotype did not show reduced litter loss as hypothesized. Winter oilseed rape showed deeper rooting than winter wheat, reaching c. 2.5m depth, roughly 1m more than the winter wheat. Within winter wheat we saw a tendency for stronger and deeper root growth with the variety 'Hereford' compared to six other varieties. The variability of root measurements is high, so this result is not highly significant in statistical terms, but it is large enough to be significant in functional terms

WP5.5: The topic of WP5.5 at University of Copenhagen was model simulation studies of the interactions between genotype, environment and management also studied in the field experiments of SP5. The advantage of model simulation being that much more comprehensive "studies and measurements" can be made, the disadvantage being that the conclusions are less certain than if based on direct measured data.

The simulations confirmed the general findings from the other WPs of SP5. The effect of genotypes was smaller than the effects of management factors such as N fertilization, sowing date, rotational position, or including cover crops into the rotations. We compared e.g. genetic improvement in root depth of wheat to the effect of a cover crop or to earlier sowing of winter wheat to increase its autumn N uptake. The cover crop and the earlier sowing both reduced N leaching loss by 30 to 60 kg N ha^{-1}, while assuming that we could increase the root depth of the wheat crop by 20% only reduced the losses by c. 5 kg N ha^{-1}. While the effects of the two management options are well documented and can be used here and now, increasing root depth development rates by 20% will take years of breeding effort to achieve.

The model simulations allowed us to work with many years of weather data, studying effects of year to year variations in N leaching loss and N availability. Examples of such yearly variation were seen in several of the field experiments of SP5, but with the model simulations we could study their significance in more detail. It is clear from the results that the year to year variation should be a main target for improving cropping system Nit-UE. If farmers just apply standard amounts of N fertilizer to their crops, years with high natural N availability will lead to a waste of N fertilizer and increased risk of N losses.

General conclusions from SP5

It is clear from experiments as well as from the model simulation studies that the value of different traits related to crop Nit-UE depend on the conditions under which the crops are grown. Some results even indicate that Nit-UE may be more dependent on the growth conditions than yield. Several results from SP5 illustrate the strong effect of rotational position, location and year to year variation in N availability for crops. In some cases so much N was available in the soil that no crop N response was seen. If farmers apply standard N fertilizer rates in such situations, the Nit-UE will be poor. Understanding, predicting and utilizing such variation for variable N fertilizer application across years and fields is probably the most important opportunity for improving Nit-UE.

Improvements in Nit-UE depend on growing conditions and on the traits in question. As an example, increased rooting depth is mainly valuable when N is available in deeper soil layers. This again depends on the cropping history on the field, previous fertilization, soil type and surplus precipitation. The dependency on surplus precipitation makes the value of deep rooting depend on yearly weather variation. In similar ways, the effect of other Nit-UE related traits depend on the conditions.

To advance our understanding of the effects and many interactions, we need more studies on effects of genotypes with different Nit-UE on the crop itself, the soil, and effects in the following years. Due to the cost, it is not realistic to do this with a broad range of genotypes. The work within SP5 illustrates this, even though the focus was on genotype effects, the studies made included only two to six genotypes.

Studies should be made using a few contrasting genotypes, though preferably at least four genotypes. The results from such studies should be used to advance our understanding of the effects, but also to parameterize and validate crop simulation models. With such input to the models, they can then be used to simulate the effects of more subtle genotypic differences. The output from such studies can be used for evaluating the value and optimal application of genotypes with improved Nit-UE traits, and for prioritizing what traits to breed for.

Many aspects of knowledge need to be developed, but especially on root growth and function, and interaction with soil organisms, we need to develop methods. The functioning of the root system is obviously important for Nit-UE and WUE, but our knowledge about genotypic differences in root system development and function is very limited, and we need method development in order to advance our ability to breed for improved root systems, and for understanding the functionality and the breeding goals we should aim for.

Field studies as well as model simulations repeatedly showed agronomy to have much larger effects on Nit-UE than genotype. However, the methods are so different in nature and application, that direct comparison makes little sense. Even though it is clear that at least in the short term we can achieve much larger improvements through improved agronomy, we cannot conclude that agronomy should be prioritized over genetic improvement of crops. The value of breeding for improved Nit-UE is much larger than indicated by the studies made here.

Breeding advances tend to be durable and to accumulate over the years, whereas the agronomy based improvements need to be repeated year after year. Good agronomy will always be important for nutrient use efficiency, but breeding offer important opportunities as well. The effect of breeding is more easily spread on large agricultural areas when farmers start to grow improved varieties instead of older ones. Even though the effect in kg N ha^{-1} is typically small, the total effect can become large, as the improved genotypes are grown on large areas.

The conclusion must be that we need both agronomy and breeding, they often solve different aspects of the Nit-UE problems, and often they need to be combined to obtain the best results.

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4. Impact, dissemination activities and exploitation of results

4.1 Socio-economic impact and wider societal implications of the NUE-CROPS project

The NUE-CROPS project grew out of recognition within the agricultural research community and wider society that there is a need to improve efficiency of resource use in food production. The prospect of a steadily growing world population relying on finite resources (fossil fuels and minerals) to supply the fertilizer industry places future food security at risk. Increases in crop yields achieved during the last century have sustained the rapidly growing world population, but it is widely acknowledged that these increases have been accompanied by less efficient use of fertilizer inputs. This is the "law of diminishing returns" that all crop nutritionists know becomes increasingly evident as crop yields approach their maximum potential. The NUE-CROPS project aims to address the challenge of reducing the environmental impact of crop production, while maintaining or improving current yield and quality levels and increasing sustainability and competitiveness. The development of crop varieties that are more efficient at taking up and utilizing crop nutrients (specifically N and P) is one of a suite of strategies that the agricultural research sector will need to address to meet the future food security demands of society. Integrating these improved varieties into efficient crop production systems will be another essential piece of the puzzle.

The fact that within this project we have identified potential molecular markers that can be used to assist breeders to select for more NUE crops is one major socio-economic impact of the research. The molecular markers will allow the more rapid selection of NUE varieties. Once released to the agricultural sector, NUE varieties should be able to produce yields equivalent to non-NUE varieties, with less fertilizer input. When used in this way, environmental impacts of crop production can be greatly reduced. This includes reductions in soil nitrate levels during critical periods when leaching is a risk. In the case of P, more PUE varieties will be able to produce acceptable yields when soil P indices are lower than currently possible. This will reduce the risk of P runoff to water bodies since in soils with high P indices P can become soluble and move into drainage water to become a pollutant.

Reductions in fertilizer use that may accompany the introduction of more NUE varieties can also lead to reductions in air pollution from fertilizer manufacture, and reductions in emissions of GHG during manufacture of nitrogen fertilizers. This is an additional societal benefit that can result from the development of NUE varieties. The manufacturing process of ammonium nitrate fertiliser is associated with a significant release of Green House Gases (GHGs) (approx. 2.38 kg CO2 equivalents of CO2, CH4 and N2O per kg of ammonium nitrate produced) into the atmosphere. A 10 to 20% reduction in N fertiliser use in Europe would therefore result in an annual reduction of greenhouse gas emissions of approximately 2.6 to 5.2 M tonnes CO2 equivalents, making a significant contribution to the GHG emission reduction targets of Europe. These calculations do not include reductions in N_2O emissions due to denitrification after application of N-fertilisers to soil, which are more difficult to estimate. These emissions are expected to also decline, due to a reduction in the quantities of excess nitrate-N present in the soil when more Nit-UpE crops are combined with agronomic innovations.

An alternative scenario in the future may be that the development of NUE varieties does not result in reductions in fertilizer use, but instead, leads to production of higher yields at current levels of N and P input. In this scenario, the development of NUE varieties will therefore help society to address the ongoing challenge of growing demand for food from a rapidly growing world population. At the farm level, the development of NUE-CROPS may improve profitability due to the reduction in input costs and/or higher yields at current input levels.

4.2 Main dissemination activities of the NUE-CROPS project

The original Plan for Use and Dissemination of Foreground (PUDF) within the NUE-CROPS project was outlined in section B3.2 of the Technical Annex. This plan focussed on a programme of training and dissemination activities (WPs 6.1 and 6.2) designed to have a 'multiplier' effect with respect to the impact expected of the NUE-CROPS project ("to reduce the environmental impact of crop production leading to more efficient and sustainable farming").

A major activity within the NUE-CROPS project was the planning and delivery of five early stage researcher workshops. These activities were described in deliverables (D2, D4, D6, D8 and D10) submitted for WP6.1 and proved to be very successful. Topics covered included: methods and strategies in molecular breeding, application of "omics" technologies in crop breeding, methods for studying molecular, physiological and morphological mechanisms of N and P use efficiency in plants, nutrient budgeting and modelling, and farmer-participatory approaches to crop breeding. The workshops were designed to address the human resource limitations within the crop breeding and agronomy sector, and were particularly effective at bringing together experts as well as ESRs from a range of disciplines, thus developing inter-disciplinary capacity within the sector.

Training of early stage researchers within the NUE-CROPS project is also evident from the number of PhD theses completed during the project. A total of 17 PhD theses have been listed as direct outputs of the NUE-CROPS project (with one further thesis in preparation).

The development of a new cohort of researchers who are able to work at multiple scales (from the laboratory using molecular techniques, through to the field scale) and with an understanding not only of molecular breeding techniques, but also agronomy and modelling, will be essential if the industry is to move forwards towards a genuinely more nutrient use efficient system of crop production.

The PUDF also highlighted the development of a project website with regular updates on project activities. The website has been developed and is linked to the coordinator's existing dissemination website (www.nefg-organic.org). This website uses a scrolling news-screen approach to disseminating new and interesting project results. News items are created by members of the project team and posted on a regular basis. Readers are encouraged to "like" the news item on Facebook and to "tweet" about the news item as well. In this way, the impact of the news item is effectively multiplied. The main project page is still maintained and continues to be used as a vehicle for disseminating information about project activities and results.

The outcome of the NUE-CROPS stakeholder conference (held as a joint meeting of the EUCARPIA section on organic and low-input agriculture in September 2013) has already been reported (see WP6.1 D3). An additional deliverable consisting of the book of abstracts from this meeting has been submitted to the Commission. In addition, a special issue of the journal Euphytica (from the EUCARPIA meeting) is now in press and includes four papers that are direct outputs of the NUE-CROPS project.

The second NUE-CROPS event was held in Brussels in April 2014 and was designed specifically to appeal to policy makers. The report from that event, which includes comments and input from invited participants, provides a useful summary of the key findings of the NUE-CROPS project as well as ideas for future directions for research. This report has been submitted to the Commission as WP6.1 D6).

The preferred avenue for dissemination of scientific results by project partners is via publication in refereed scientific journals. NUE-CROPS activities resulted in the publication of 26 articles in peer-reviewed journals. Among these was a publication in the prestigious journal Nature Genetics (impact factor=35) on "The genome of the mesopolyploid crop species *Brassica rapa*" (Wu et al. 2014). Other high profile papers included an article in the Journal of Experimental Botany (impact factor=5.542) on "A new physical interpretation of plant root capacitance" (Dietrich et al. 2012) and one in Annals of Botany answering the question: "Can root electrical capacitance be used to predict root mass in soil?" (Dietrich et al. 2013); both of these papers advanced the state-of-the-art knowledge on the use of electrical capacitance meters to measure root system traits. Further high impact publications on the Brassica research were published in BMC Genomics (Wang et al. 2011) and BMC Plant Biology (Wu et al. 2012). Publications on the use of proteomics and transcriptomics methods to identify possible genes involved in NUE were published in PLoS One (Tetard-Jones et al. 2014) and Molecular Breeding (Rempelos et al. 2013; Tetard-Jones et al. 2013). Publications in agronomy journals are by their nature usually lower impact, due to the more limited audience for this research, nevertheless, the NUE-CROPS project resulted in a publication in the European Journal of Agronomy on "Morphological and physiological characteristics of corn roots from cultivars with different yield potentials" (Qi et al. 2012) as well as publications in Agricultural Water Management (Jia and Liu 2014), the Journal of the Science of Food and Agriculture (Hildermann et al. 2010; Bonte et al. 2014) and the Netherlands Journal of Agricultural Science (Lammerts van Bueren et al. 2010). The breadth of research outputs from the NUE-CROPS project is demonstrated by the broad range of journals included on the list of peer-reviewed publications, including Ecological Modelling (Khan et al. 2013), Potato Research (Khan et al. 2013), and Euphytica (various authors in the 2014 Special Issue on the NUE-CROPS conference).

In addition several papers generated during the project period are in preparation or currently under review. This includes six publications by partners in China (CAAS) on results of long-term fertilizer input and irrigation trials, five of which will be submitted to Soil and Tillage Research, with the sixth targeting Applied Soil Ecology. A high profile publication in Theoretical and Applied Genetics is anticipated from partners in Bulgaria on QTL for NUE in maize. Studies for publication in agronomy journals frequently take longer to conduct and publish due to the need for at least two years of field data collection. We are now anticipating two papers to be submitted to the European Journal of Agronomy and a third to the journal Plant and Soil, by partners at the University of Copenhagen on winter wheat growth and oilseed rape leaf litter loss. We also expect two more papers on ecophysiological modelling and genetic variation in potato growth characteristics to be submitted to Field Crops Research by partners at Wageningen University.

The scientific community is also reached through presentations at conferences and publication of results in conference proceedings. During the NUE-CROPS project articles on research activities were published in 12 conference proceedings. Many more fellow scientists were reached via oral or poster presentations at conferences. A total of 173 presentations were made at academic conferences and meetings about NUE-CROPS project outputs.

The wider public was reached through a range of activities including: oral presentations to the wider public (45 events), articles in the popular press (8), and exhibits at stakeholder events. These activities may target the farming community, often through attendance at agricultural exhibitions and through farmer field days at research facilities. Schools are also included in the wider public, with school visits to research facilities being hosted, and a “Teacher Pack” being produced as an additional deliverable from the NUE-CROPS activities at Newcastle University.

Use a diversity of delivery mechanisms NUE-CROPS partners have very effectively reached a large number of stakeholders from a wide range of interest groups. Foreground generated by the project that has not yet been published will continue to be released through a range of routes in the coming years. The plan for this is outlined in the following section of this report.

4.3 Exploitation of results from the NUE-CROPS project

The exploitable foreground generated by the NUE-CROPS project fell into one of four categories: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, or Exploitation of results through EU policies.

Several partners reported that they had advanced the knowledge on the importance of genotype by management interactions in NUE and the importance of the site and weather (i.e. year to year variations). For example, researchers at FiBL in Switzerland noted the interaction between management i.e. tillage and fertilizer rate, as well as variety choice, and expect to use that knowledge to advise federal offices (for agriculture and environment) on fertilizer guidelines, recommendations to improve soil fertility, and inoculants. The identification of recommended varieties of wheat and maize for specific environments and management conditions is a commercially exploitable result of the NUE-CROPS work conducted by FiBL that will benefit German, Swiss and Austrian plant breeders. Researchers at Copenhagen University also improved their understanding of the interactions between genotype traits important for NitUE and environment and management. This could also have implications for future policy developments in the area of reductions in N losses from leaching, and will be available to the scientific and policy community when published within the next six months.

Researchers at Wageningen University in collaboration with commercial potato breeders Agrico, advanced the knowledge on the use of crop models to generate parameters that can be used in QTL mapping; this will benefit stakeholders involved with agronomy and crop physiology research. At the same time, they developed innovative methods of evaluating NUE; for example the quadrant approach they used for assessing NitUE of potato varieties is transferable to other crops, and presents an alternative method of evaluation of NUE when resources are limited and it is not possible to conduct a large trial with many rates of N application. The publication of this work will allow broad dissemination of this approach and may have a large impact on how NUE is studied in the future.

Knowledge advancement in the field of crop modelling was also achieved by researchers at the University of Copenhagen. It has often been assumed that there is some genetic variation in the rooting potential of different varieties of wheat; however, it has rarely been documented. The state-of-the-art methods used at UCoP allowed varieties of wheat to be compared and clear evidence was collected about the differences in rooting characteristics between varieties. This information can be used in crop models that have a rooting component, to design NUE systems. The information will be available to the wider scientific public as soon as it is published (likely within the next six months).

UCoP also contributed to the advancement of knowledge in the modelling of oilseed rape growth, through collection of specific data about litter loss rates among different varieties. This may be of interest to plant breeders as they work to develop more efficient oilseed rape varieties, and will be available once published in the next six months.

Researchers at Newcastle University used their long-term field trials to study the potential of including a grass/clover ley within the rotation as a source of N. Specifically, they were able to demonstrate that growing a grass/clover ley for three years prior to wheat can allow the farmer to obtain a high yield without the need for additional fertilizer N. This outcome may have relevance for policy makers in future as they develop recommendations for strategies to reduce external N inputs to cropping systems. Results will be published in the upcoming Special Issue of the journal Euphytica.

Several partners identified potentially commercially exploitable knowledge that had been generated by the project. The details of this knowledge are confidential, but broadly speaking relate to the identification of molecular markers that can be used in breeding for NUE and in some cases, development of new varieties expected to be released in the next few years.