IDENTIFICATION OF TRAITS FOR NITROGEN USE EFFICIENCY IN OILSEED RAPE *(BRASSICA NAPUS L.)*

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Als meus pares, l'Ajay, el Marcel i l'Avani

"In all science, error precedes the truth, and it is better it should go first than last."

Hugh Walpole

"Un dia uri jabe."

A & B

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ABSTRACT

Oilseed rape (*Brassica napus* L.) receives high inputs of Nitrogen (N) fertiliser while N uptake and N harvest index are low. This results in high residual soil N which leaches to water bodies and contributes to greenhouse emissions. Such negative environmental impact could be reduced by better understanding the genetic basis of N metabolism in oilseed rape and designating relevant traits for varietal selection towards high nitrogen use efficiency (NUE) at low N fertiliser inputs. In this study the doubled haploid population (TNDH) from a cross between the Chinese semi-winter variety Ningyou7 and the UK winter variety Tapidor was analysed for N physiology and Quantitative Trait Locus (QTL) mapped for relevant traits. Quantitative Trait Loci were mapped in two N treatments over two consecutive field trials for architectural traits such as plant height, foot length, pod number and chlorophyll content in bracts and leaves; yield and yield component traits such as plant biomass, seed yield, harvest index and N metabolism (seed, plant and total N concentration, N uptake, utilisation and use efficiencies and N harvest index). A larger number of QTL were detected at High N than at Low N. In total 49 QTL were detected at High N versus 44 in Low N during 2005/06, while in 2007/07, 72 versus 62 QTL were detected at High and Low N respectively. Most QTL for different traits were treatment specific. Novel QTL for agronomic traits specific at Low N were identified. The correlations between traits were also studied through QTL co-localisations, particularly for relationships between seed yield, N uptake and N use efficiency. Seven chromosomal regions are discussed for potential candidate genes. Additionally, QTL reproducibility, interval mapping and composite interval mapping, QTL x environment interactions and phenotypic plasticity in oilseed rape are also discussed.

KEY WORDS: BRASSICA NAPUS L., QUANTITATIVE TRAIT LOCI, NITROGEN, STRESS, VARIATION, AGRONOMIC TRAITS.

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LIST OF ACRONYMS

AFLPAmplification Fragment Length Polymorphism.
BAC Bacterial Artificial Chromosome.
BN Branch Number trait.
CB Chlorophyll in Bracts trait.
CIM Compsite Interval Mapping.
CL Chlorophyll in Leaves trait.
cM centiMorgan.
DH Doubled Haploids.
EST Expressed Sequence Tag.
FDAS Flowering trait in Days After Sowing.
FL Foot Length trait.
HI Harvest Index
IM Interval Mapping.
Kb Kilobase.
LOD Log Odds Ratio.
MAS Marker Assisted Selection.
MCIM Multi-trait Composite Interval Mapping
MIM Multiple Interval Mapping.
N Nitrogen.
NP Plant Nitrogen concentration trait.
NHI Nitrogen Harvest Index trait.
NUE Nitrogen Use Efficiency trait.
NUpE Nitrogen Uptake Efficiency trait.
NUtE Nitrogen Utilisation Efficiency trait.
PN Pod Nitrogen trait (chaff N concentration).
QTL Quantitative Trait Locus.
RAPD Random Amplified Polymorphic DNA.
RFLP Restriction Fragment Length Polymorphism.
SNP Seed Number per Pod trait.
SNPs Single Nucleotide Polymorphisms.
SSR Simple Sequence Repeats.
STN Stem Nitrogen concentration trait.
SY Seed Yield trait.
SN Seed Nitrogen (also NS: Nitrogen in Seed).
TL Total Length (total plant height trait).
TN Total Nitrogen concentration trait.
TW Total Weight (Total plant biomass trait).

GLOSSARY

AFLP PCR based marker. Not based on arbitrary priming of oligo's, but amplification of specifically selected restriction fragments (see SRFA).

BAC Cloning vector for large DNA fragments.

CIM Like simple interval mapping, this method evaluates the possibility of a target QTL at multiple analysis points across each inter-locus interval. However, at each point it also includes in the analysis the effect of one or more markers elsewhere in the genome. These markers, also called background markers, have previously been shown to be associated with the trait and therefore are each presumably close to another QTL (a background QTL).

cM A unit of measure of genetic recombination frequency. One cM is equal to a 1% chance that a marker at one genetic locus will be separated from a marker at another locus due to crossing over in a single generation. In oilseed rape, 1 cM is equivalent, on average, to 54 thousand base pairs. The centimorgan is named after the pioneering (and Nobel Prize winning) geneticist Thomas Hunt Morgan.

DH A progeny of doubled haploids derived from a heterozygous or F1 individual can serve as a mapping population. Making a DH is faster than RIL; DHs have a better resolution than a F2 progeny (no heterozygosity); DHs can be maintained infinitely.

EST PCR based marker. Highly specific oligos (16-20-mers) are designed by using sequence information of a cDNA. The locus represents a functional gene and is located in an actively transcribed region of the genome.

IM This method evaluates the association between the trait values and the expected genotype of a hypothetical QTL (the target QTL) at multiple analysis points between each pair of adjacent marker loci. The analysis point that yields the most significant associations may be taken as the location of a putative QTL.

LOD A statistical measure indicating the significance of linkage. The $\log_{10}$ of the Odds Ratio. The Odds Ratio is the probability (given H_0) divided by the probability (given H_a = unlinked).

MAS A breeding strategy applying indirect selection.

MIM It uses multiple marker intervals simultaneously to fit multiple putative QTL directly in the model for mapping QTL. The MIM model is based on Cockerham's model for interpreting genetic parameters and the method of maximum likelihood for estimating genetic parameters. With the MIM approach, the precision and power of QTL mapping could be improved. Also, epistasis between QTL, genotypic values of individuals, and heritabilities of quantitative traits can be readily estimated and analyzed.

NHI Ratio of seed N yield to total above ground biomass.

NUE The product of multiplying NUpE by NUtE.

NUpE The ratio of total above ground N to total amount of N fertilizer applied.

NUtE The ratio of seed yield to total above ground N in the plant.

QTL Single locus from a series of polygenes which are involved in a quantitative trait.

RAPD A PCR product that is obtained from genomic DNA using a single or a combination of typically 10-mer oligonucleotides. Alleles are visualized by the fragments that are amplified, separated on agarose gels and stained with EtBr. RAPDs show dominant inheritance. Variation is based on the position and orientation of primer-annealing sites and the interval they span.

RFLP A DNA fragment used to probe Southern blots of restricted genomic DNA from different strains of the same species. This results in the visualisation of variation in the size and/or number of detected restriction fragments generated from the different strains. The detected length variation is based on DNA sequence variation caused by insertions, deletions or changes in restriction sites.

SNPs Polymorphism based on a nucleotide substitution. Used as a marker diagnostic for a specific trait. Often mentioned in connection with a technique which allows the specific recognition of the SNP.

SSR Synonymous to STR or micro satellite repeats, in particular the dinucleotide repeats $(AC)_n$ $(AG)_n$ $(AT)_n$.

(http://www.plantbreeding.wur.nl/UK/acronyms.html)

CHAPTER 1. INTRODUCTION

Sustainable crop production is based on protecting the environment and human health, whilst maintaining the yield, quality of crops and economic sustainability. In intensive agricultural practises, cultivars have been bred according to their ability to respond to high inputs of nitrogen (N) fertiliser. Because of the negative environmental impacts derived from the use of inorganic N, resource-efficient crops are required (Gilland, 2006, Mosier, 2002).

1.1. IMPORTANCE AND USE OF OILSEED RAPE IN THE WORLD AND IN UK

Oilseed rape (*Brassica napus L.*) is one of the most important oilseed crops in the world (Fig 1.1) with major areas of production in Canada, China, EU and India. The two major Brassica species produced are *Brassica napus* L. and *B. rapa* L. (Raymer, 2002). Oilseed rape in terms of production is currently the second most important oilseed crop worldwide behind soybean.

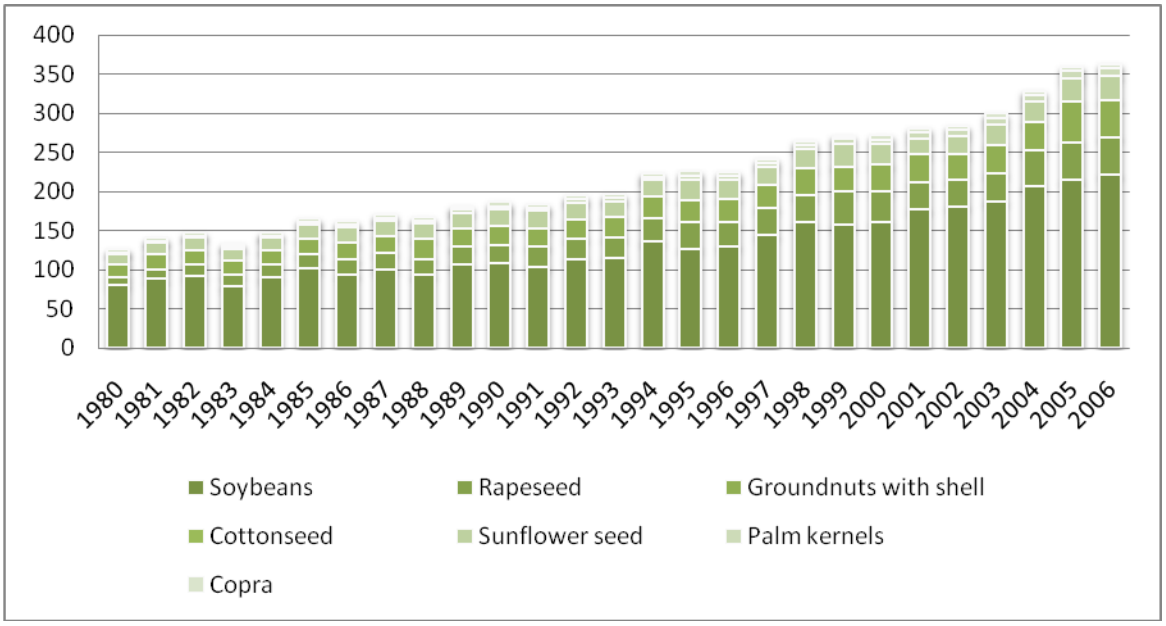


Figure 1.1. World oilseed production (source FAOSTAT 2008)

Oilseed rape in the UK, in terms of area is currently the third most important agricultural crop grown. Between 2000 and 2006, there has been an overall increase in production (Fig 1.2) that positively correlates with an increase in area. The economic value of oilseed rape in the UK reached a maximum of £423M in 2003 and declined to a low of £263M in 2005, almost as low as it was at the beginning of 2000, having recovered in 2006 with an intermediate value of £307M.

Oilseed rape yield has remained steady around 3-3.5 t ha^{-1}, and has remained at this level for the last 20 years, because the increase in production has been due to an increase in the cultivated area, rather than an increase in yield. Yield variations occur as a result of weather conditions, crop establishment, weed burden, pest attack and disease control. The production of oilseed rape in the UK serves the domestic demand with 100% of its use in this country.

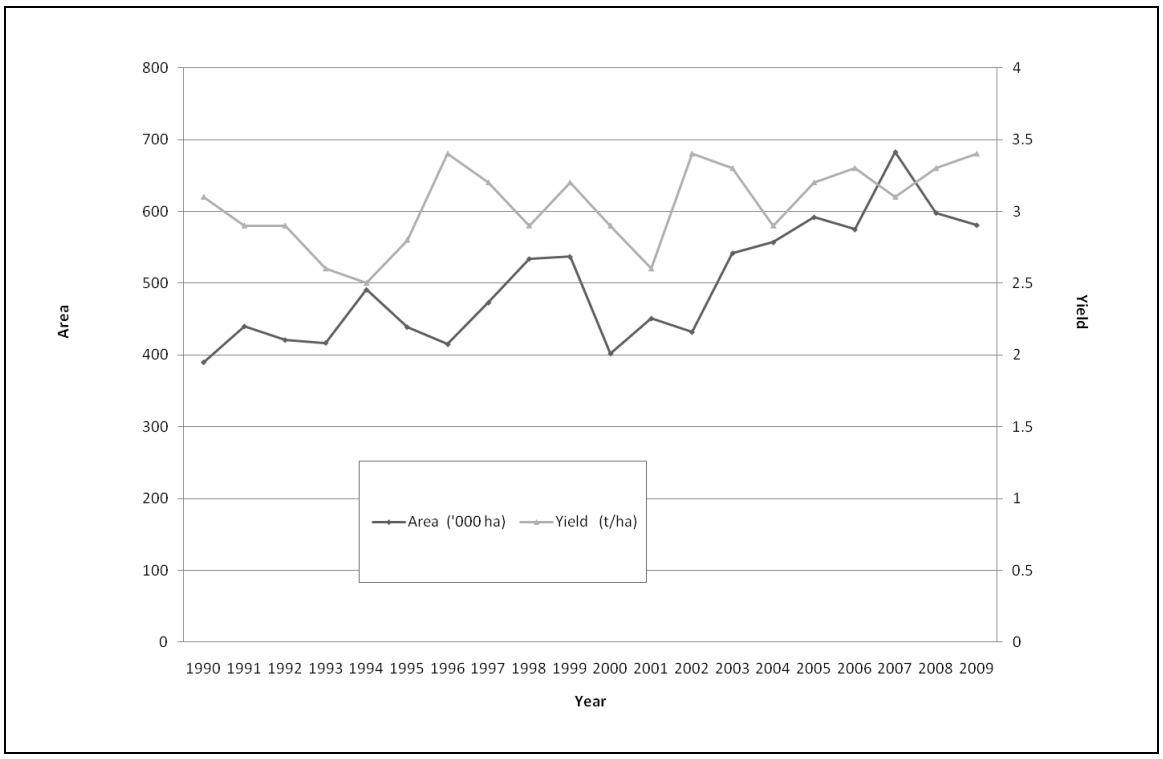


Figure 1.2. Oilseed rape area and yield in the UK (source DEFRA 2008)

The price of oilseed rape in the UK is around 300£/t (August 2010). It provides a reliable yield and gross margin to farmers of typically £510 per hectare for average yields of 3.2 tonnes per hectare, but can be as high as £800 per hectare for higher yields. Potential product losses due to management practices and pests would reduce this margin. Pest control of black-grass has become an important factor in maintaining production in rotation crops, as it can be transmitted from wheat to oilseed rape and back. If black-grass populations cannot be controlled in rape, following crops in the rotation system would be further affected (ADAS, 2009).

1.2. OILSEED RAPE USES

Oilseed rape is grown for different commercial uses, but mainly cultivated to produce vegetable oil both for human consumption and for industry (Schjoerring, 1995). Varieties with low content of glucosinolates have been grown for many years now (canola), as well as some GM varieties (generally herbicide tolerance). The crop is considered to have a lot of future potential, both for increasing oil content and modifying both fatty acid and protein composition. Seed oil content is usually around 42% and protein content 35%. Oilseed rape has been recently used to produce renewable industrial feedstocks such as composites and plastics from the oil and meal components e.g. polyurethanes, vegetable oil-based lubricants and hydraulic fluids (NNFCC, 2009).

Oilseed rape is also grown for biodiesel production; oil from crushed seed is mixed with the alcohol methanol and a catalyst and can be used directly in most engines without modification of the engine. Biodiesel from oilseed rape was thought to be very profitable for large scale biofuel production (a 60% of the oilseed rape production was destined for biofuels in Europe in 2007). However since 2009, the 20p/l duty relief on biodiesel produced from oilseed rape has been withdrawn and other biofuels have been gaining more importance such as bio-ethanol from wheat and sugar beet.

The by-product of oil extraction is the protein meal, extensively used in temperate regions (e.g. in Canada and the European Union) as an animal feed in substitution for soybean. Compared to soybean, oilseed rape has a lower proportion of lysine, but provides a much higher proportion of the amino acids cysteine and methionine.

Future estimates predict a substantial increase in the production of oil crops, both for oil and protein meal consumption. It is also predicted that production will increase in developing countries, as the main exporters on the world market retain a greater part of the crop for a diverse range of uses (FAO, 2002).

1.3. NITROGEN FERTILISER AND ENVIRONMENTAL IMPACTS OF OILSEED RAPE IN THE WORLD AND IN THE UK

Fertiliser is applied in agriculture to replace the soil nutrients that crops use to grow and is generally required when the soil and environment cannot supply enough nutrients not to limit plant growth. Nitrogen is the element required in greatest quantity by plants to support growth.

Phosphorus and nitrogen are key nutrients included in growth and need to be restored in the form of fertiliser to produce optimal yields. The nitrogen in the soil is usually not maintained because of plant absorption, run off, and microbial denitrification. Moreover, there is a seasonal variation in N concentration, in addition to habitat heterogeneity (Jackson and Caldwell, 1993), which makes necessary fertiliser application to ensure a high production level in agriculture. Crop plants generally prefer a mixture of ammonium and nitrate as fertiliser, requiring a higher quantity of ammonium than the concentration available in the soil (Crawford and Glass, 1998). Current levels of N fertiliser application to oilseed rape in the UK are about 200 kg ha^{-1} (Goodlass et al., 2003).

An estimate of the predicted N fertiliser consumption globally in 2050 was put at 134 Mt. with current global consumption at 90.9 Mt. in 2005 (Gilland, 2006).

Over recent decades, world production of crops has increased due to N fertiliser application, pesticides, and the use of improved varieties (Bacon, 1995). Despite plant breeders developing varieties with potentially higher yields, oilseed rape has a stable yield of around 3 t ha^{-1} in the UK since 1980s. The fertiliser use on oilseed rape in Great Britain has fallen from in excess of 250 kg N ha^{-1} in the early 1980s to 191 kg N ha^{-1} in 2006 with a low of 179kg kg N ha^{-1} in 1994 (British Survey of Fertiliser Practise, 2006). The fall has largely been caused by a switch in the method of arable crop support from production to area based subsidies. Explanations for this are a reduction in use of nitrogen fertiliser and other inputs due to an increase in costs and also a relatively low market price for oilseed rape, especially in recent years. It is justified to say, then, that an improvement in yield by breeders has allowed lowering of nitrogen fertiliser use while still maintaining yields (Berry and Spink, 2006).

Winter oilseed rape is one of the most profitable UK arable crops as it plays a significant role in agriculture as the major break crop in intensive cereal rotations. However, it is the second most important crop in terms of potential N leaching and greenhouse gas emissions (Teiwes et al., 1996), and excessive N-fertilization and other management practices can potentially lead to high nitrate leaching losses (Di and Cameron, 2002).

The further expansion of Nitrate Vulnerable Zones (NVZ) in England covered a total of 55% of the land area in 2002, and this was further increased to 62% in 2010. This increase highlights the further need to minimise the environmental implications of inefficient use of fertiliser N (DEFRA, 2007).

Adequate practises to fertilise crops with nitrogen are: application in the correct weather conditions (to avoid leaching losses in the form of NO_3^- due to high rainfall), at the appropriate stage in crop growth (so that when plants have high demand nitrogen is taken up quickly) and at the correct dose. Ammonia volatilization as NH_3 is affected by the type of fertiliser (it is especially high when urea is used as a fertiliser), soil pH and application method (Bouwman et al., 1997). A recent study investigating differences between different greenhouse gas emissions, showed that application of manure fertiliser, and poultry manure in particular, produced more NO and N_2O than urea fertiliser (Akiyama and Tsuruta, 2003). A reduction in the emissions of nitrous oxide (N_2O) from fertiliser use is a key target area in reducing the greenhouse gas emissions and the impact on climate change. Nitrates in the soil can be converted to, a greenhouse gas that is 300 times more potent than CO_2 molecule for molecule. Nitrous oxide emissions from soil account for 6% of the global greenhouse gas emissions (Aldhous, 2008). Reducing the fertiliser N inputs to agricultural crops by having greater Nitrogen Use Efficiency (NUE) will cut emissions of nitrous oxide in direct proportion.

A matter of concern is the rise of gas prices, which has also influenced the economics of growing oilseed rape and other crops as it has translated into a rapid increase in the price of N fertiliser (more than 50% of the price of N fertiliser is attributed to the price of gas used in manufacture, the Hober-Bosch process).

Since more effort is being put into developing new sources of energy from plants to substitute fossil fuels e.g. biofuels, more N fertiliser is required to produce optimal yields in certain plants. As N fertiliser is synthesised from fossil fuels, a target to reduce environmental pollution and maintain crop yields would be to produce crops having a high yield with low N fertiliser requirement (Hirel et al., 2007).

Improving N-efficiency of winter oilseed rape will reduce the potential for environmental pollution and improve economic returns (Grant and Bailey, 1993). In terms of the environment, the crop should receive optimum doses of N to ensure yield development and to avoid subsequent N-leaching from the soil (Aufhammer et al., 1994, Barlóg and Grzebisz, 2004, Behrens et al., 2001, Shepherd and Sylvester-Bradley, 1996).

Oilseed rape research has mostly been on yield and oil content or composition improvements, for example, with the introduction of hybrids, double low ‘00’ varieties (low erucic acid and low glucosinolates, destined for food) and HEAR varieties (with high erucic acid, destined for industrial uses).

1.4. NITROGEN PARAMETERS AND DEFINITIONS

The definitions used for N derived traits in this study are based on Moll et al. (1982). Nitrogen use efficiency (NUE) is defined as the grain (seed) produced per unit of available soil N supply. It can be split into two components, namely N uptake efficiency (NUpE) and N utilisation efficiency (NUtE). Nitrogen uptake efficiency is the efficiency with which N is taken up from the soil and N utilisation is the efficiency with which the absorbed N is converted into yield (Moll et al. 1982). Nitrogen Harvest Index (NHI) is defined as the ratio of N present in the seed to total plant N content. It is a measure of N translocation efficiency. Nitrogen-HI is analogous to HI (Harvest Index), which is the ratio of seed to total plant biomass.

Table 1.1. Nitrogen definitions for Nitrogen Uptake Efficiency (NUpE), Nitrogen Utilisation Efficiency (NUtE), Nitrogen Use Efficiency (NUE) and Nitrogen Harvest Index (NHI).

Variable	Definition	Units
Gw	Seed weight	$g\ m^{-2}$
Ns	N supply (fertiliser + residual)	$g\ m^{-2}$
Nt	Total N in plant	$gN\ m^{-2}$
Ng	N in seed	$gN\ m^{-2}$
Nt/Ns	NUpE	$gN\ g^{-1}$
Gw/Nt	NUtE	$g\ gN^{-1}$
Gw/Ns	NUE	
Ng/Nt	NHI	

Nitrogen uptake efficiency was calculated in $gN\ g^{-1}$ and NUtE was calculated in $g\ gN^{-1}$. Both NUE and NHI are unit-less, even though NHI is usually expressed as percentage (Table 1.1).

1.5. NITROGEN UPTAKE AND ASSIMILATION

Winter oilseed rape is typically sown in late August or early September. The plant then overwinters and flowers in spring. It is generally harvested in July and up to August. Winter oilseed rape is usually higher yielding than spring oilseed rape, averaging 3.7 t ha and generally under 2.5 t ha^{-1} respectively.

Flea beetle, cabbage stem beetle and pollen beetle are some of oilseed rape's most common pests. They are easily controlled by an autumn spray of insecticide. Also cabbage seed weevil needs to be controlled at early flowering, but control is generally not required. However, the biggest threats for oilseed rape are fungal diseases, which have to be treated preventively, before symptoms are visible.

The cycle of winter oilseed rape lasts about 320 days, comprising:

- The autumnal stage from sowing to early winter. This stage conditions to a great extent the implantation of the crop and its root system; leaf and flower initiation, which are critical for leaf development and yield potential, also take place at this time.
- The vegetative rest period, lasting about 2-3 months according to region and ending when the daily average temperature is regularly >5 °C.
- Vegetation regrowth to flowering, lasting about 2 months and defined by a very active accumulation of dry matter. This is the essential period for the absorption of mineral elements. Leaf development and leaf area per unit of soil area are key factors in determining yield.
- Flowering (about 220 days after sowing) to knotting. This stage can last for about 3 weeks and is marked by high competition for C supply between different types of organs (flowers on the main stem, newly forming young pods and seeds in pods).
- The pod-filling period, when the 1 000-seed weight is determined. At maturity the total biomass produced can reach 12-14 t/ha, of which about 30 % is in the seeds, 40 % in pod walls and peduncles, 20 % in stems and 10 % in roots.

Oilseed rape has a large early demand for nitrogen by April up to 130 kg ha^{-1}, rising to 230 kg ha^{-1} by June (Fig. 1.4). The later fertilizer can be applied, the more benefit will be gained; however, shortage of nitrogen in the plant will accelerate stem elongation reducing total yield potential. By the end of November a good performing crop could take up 70kg ha^{-1} or more of N fertilizer, making this the most effective crop at utilising nitrogen from crop residues. Through February and March, as demand for nitrogen increases and before fertilisers are applied, is when soil nitrogen supply should be assessed. After petal fall, to maximise yield, the pods need

to be provided with adequate nitrogen for photosynthesis, and to supply nitrogen to the developing seed for protein. By this stage the crop should have taken up over 200 kg ha^{-1} N (ADAS, 2010).

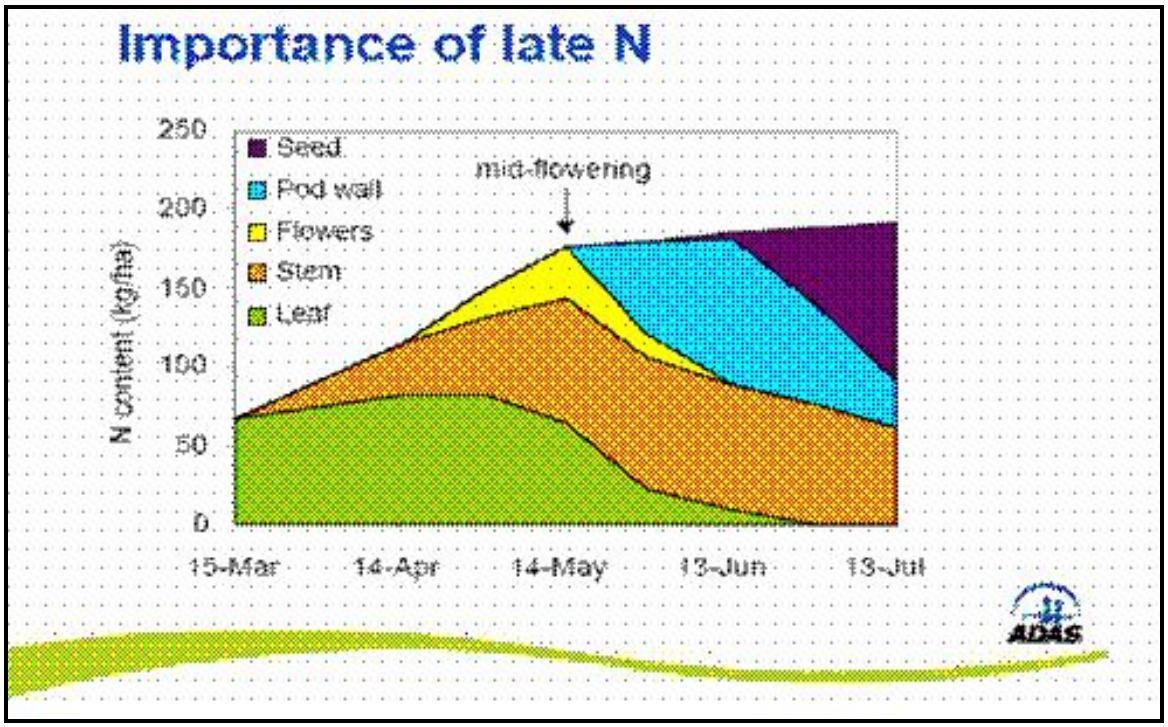


Figure 1.3. Nitrogen fertiliser demand during the different developmental stages of oilseed rape, for the different plant parts.

Nitrogen is the most consumed element in plants, therefore limiting growth (Crawford and Glass 1998). Nitrogen uptake both in NO_3^- and in NH_4^+ forms generally happens at lower than the plant's uptake potential, except under certain circumstances, i.e. low N availability, when uptake is maintained at high rate. Although average NH_4^+ concentration in the soil is often 10-1000 times lower than that of NO_3^-, the difference in soil concentration does not necessarily reflect the uptake ratio of each N source. It is suggested that uptake rates drop when plants reach N sufficiency due to possible osmotic difficulties or ion toxicities (Glass, 2003) who also suggested that to improve NUE, complementary strategies should be approached, considering that plants with high NO_3^- and NH_4^+ influx would induce the down regulation of N transporters more rapidly.

1.5.1. NITRATE ASSIMILATION

ENZYMES

The first steps in the nitrate (NO_3^-) assimilation pathway are the transport of nitrate from soil into the plant cells, followed by the reduction to nitrite (NO_2^-) by nitrate reductase, which is then translocated to the chloroplast where it is reduced to ammonia (NH_3) by nitrite reductase (Meyer and Stitt, 2001). A NO_3^- dissimilatory route is accepted in bacteria, but there is no consensus about NO_3^- anaerobic respiration in plant cells as yet (Lawlor et al., 2001).

Nitrate reductase (NR) which converts NO_3^- to NO_2^-, has been the centre of many studies since first isolated in 1953 by Evans and Narson (1953). There are three forms of NR enzyme present in nature, the most common in plants being NADH-specific NR. The localisation of NR is still not elucidated, but it is thought to be the cytosol (Solomonson and Barber, 1990). It is suggested NR has broad substrate specificity, being able to reduce chlorate to chlorite, nitrate to nitrite (Solomonson and Barber, 1990), and nitrite to nitrogen oxide (Yamasaki et al., 1999) thereby having a general contribution to plant metabolism. Nitrite reductase enzyme (NiR) is located in the chloroplasts of green leaves and in the plastids of roots. A NiR extraplastidic form has been found in mustard cotyledons (Schuster and Mohr, 1990).

METABOLIC ENGINEERING

Several studies have centred on NR, being of major interest are the ones focusing on mutants affecting apoenzyme genes (*nia* mutants) as they have been able to provide evidence to characterise NR activity, function as well as chromosome location. Findings in Arabidopsis revealed that one gene belonging to the NADH-specific form of NR (*Nia2*) was responsible for 90% of NR activity (Wilkinson and Crawford, 1993). A recent study by Lian et al. (2006) in rice analysed up and down regulation of genes after minutes to hours of plant stress under low nitrogen conditions, revealing that genes involved in plant N uptake and assimilation were barely affected by low N conditions. Another study in tobacco plants found a cultivar with high NUE and it had in addition to the highest NR activity levels, the lowest N and NO_3^- contents (Ruiz et al., 2006). Fan et al. (2007) compared two rice cultivars having different NUE after 24 hours without N supply. They found that the transcription of the *Nia* gene decreased after changing N conditions in both cultivars, but NR activity was higher in one, maintaining NO_3^- assimilation and keeping higher NUE.

As *nia* mutants have been produced for NR enzyme, transgenic plants with a deficiency in NiR enzyme (*nii*) have been produced for barley (Duncanson et al., 1993) and tobacco *Nicotiana tabbacum* (Vaucheret et al., 1992). Such mutants were characterised by increased nitrite accumulation.

REGULATION

Nitrate Reductase at transcriptional level is regulated by nitrate, hormones such as cytokinins, light, glutamine and sugars (Campbell, 1999, Stitt et al., 2002). Post-translational regulation of NR is by light. Regulation of NiR occurs, at the transcriptional level, by nitrate and light, and in a majority of cases it is co-regulated with NR (Lawlor et al., 2001). Post-transcriptional regulation of NiR is by the source of nitrogen (Crete et al., 1997), a mechanism different from NR regulation, and stating a secondary mechanism of regulation of the nitrate assimilation pathway.

TRANSPORTERS

The main transporters and enzymes involved in the N assimilation pathway from the uptake to protein synthesis are well characterised in higher plants. The first step of the cycle is nitrate or ammonium uptake, carried out by both nitrate and ammonium high and low affinity transporters, as reported by vonWiren et al., (1997). Nitrate is actively transported inside the plant by low and high affinity proton symporters. Nitrate uptake from the soil needs energy despite soil concentrations being high, it is higher at higher soil concentrations, the efflux is passive and it is inducible (Aslam et al., 1996) and nitrate selectable (Grouzis et al., 1997).

Roots account for three well characterised nitrate transport mechanisms: Constitutive High Affinity Transport Systems (CHATS), High Affinity Transporters (HATS), and Low Affinity Transporters (LATS) the latter being mediated by both constitutive and nitrate inducible transport systems (Crawford and Glass, 1998). The main difference between the two high affinity transport systems is the pathway capacity, whilst CHATS are a low capacity system, HATS are a high capacity high affinity pathway that can be induced by NO_3^- or NO_2^- concentrations hours after exposure (Crawford and Glass, 1998). The different transport mechanisms function additively, with the total N uptake being a sum of the three fluxes at high N concentrations (Li et al., 2007). There are two families of high affinity (HATS) nitrate transporters in plants: the NRT1 and the NRT2. The first one broadly works in N uptake; it is inducible and constitutive, whereas the second family is specifically NO_3^- inducible and has high affinity for NO_3^- uptake from soil

(Kronzucker et al., 1997, Chapin et al., 1993). Several studies have attempted to characterise the regulatory mechanisms of the NRT2 transporter family in Arabidopsis, finding that two genes *AtNTR2.1* and *AtNTR2.2* were strongly up-regulated in plants that had been under low nitrate conditions, increasing the nitrate influx (Okamoto et al., 2003). Li et al. (2007) classified these two genes in the HATS transport system, where *AtNTR2.2* has only a small contribution, but increases when *AtNTR2.1* is disrupted.

A NO_3^- efflux mechanism which recycles absorbed NO_3^- from the cell cytoplasm also exists. This mechanism has a minor role in NO_3^- net uptake from the soil and it is not well characterised in the literature due to technical difficulties in the labeling protocol, making its function still speculative (Lawlor et al., 2001). It is known that NO_3^- efflux is inducible by NO_3^- (Aslam et al., 1996), it is saturable and selective for NO_3^- (Lawlor et al., 2001) and that a passive protein-mediated efflux system exists in roots (Grouzis et al., 1997).

REGULATION

Regulation of transport systems is by NO_3^- induction, feedback regulation, by light, sugars and regulation at whole plant level by signaling molecules i.e. NO. Latest findings suggest two transcription factors HY5 and HYH are key to obtain high expression of NR in Arabidopsis (Jonassen et al., 2008). The same paper corroborated the importance of sucrose in NR activity for actively growing photosynthetic seedlings and rosette leaves.

1.5.2. AMMONIA ASSIMILATION

Ammonia assimilation consists of three complementary routes: primary assimilation from the soil in the form of NH_4^+, re-assimilation of NH_3 from amino acid catabolism and re-assimilation of ammonia released from seed germination (Lea et al., 1990).

Ammonium is the preferred source of N in plants despite its lower concentration in the soil, and its uptake happens at very high rates. It has to be mixed with NO_3^- as NH_4^+ supplied exclusively can lead to poor root and shoot development and it is toxic at high concentrations, which is why it is assimilated straight away (von Wiren et al., 2000). NH_4^+ is accumulated in the vacuole and the cytosol, also in the apoplasm (Nielsen and Schjoerring, 1998).

ENZYMES

Enzymes involved in ammonia assimilation in higher plants are Glutamate synthetase (GS), Glutamate synthase (GOGAT), Asparagine synthetase (AS) and Glutamate dehydrogenase (GDH).

Ammonia is metabolised into amino acids through the GS/GOGAT pathway, which is the primary route for assimilation of N in plants (Temple et al., 1998).

Glutamate Synthetase catalyses the conversion of glutamine from glutamate, using NH_3 as a substrate. Glutamate Synthetase has a GS_1 plastidic isoform located in plastids and a GS_2 cytosolic isoform found mostly in roots (Brangeon et al., 1989, Peat and Tobin, 1996). It can also be found in floral organs (Dubois et al., 1996) as well as in the phloem (Peat and Tobin, 1996, Sakurai et al., 1996, Dubois et al., 1996). The evidence that cytosolic GS is found mostly in the roots suggests it to have a main role in ammonia assimilation (Oaks and Hirel, 1985). The regulation of GS is generally at the transcriptional level, regulation also depending on protein stability and turnover (Temple et al., 1996, Ortega et al., 1999). The plastidic isoform (GS_1) has also been found to be regulated by light and sugars from photosynthesis (Migge et al., 1996, Migge et al., 1998). The cytosolic form (GS_2), on the other hand, is highly regulated by the developmental stage of the tissue where it is located (Marsolier et al., 1993).

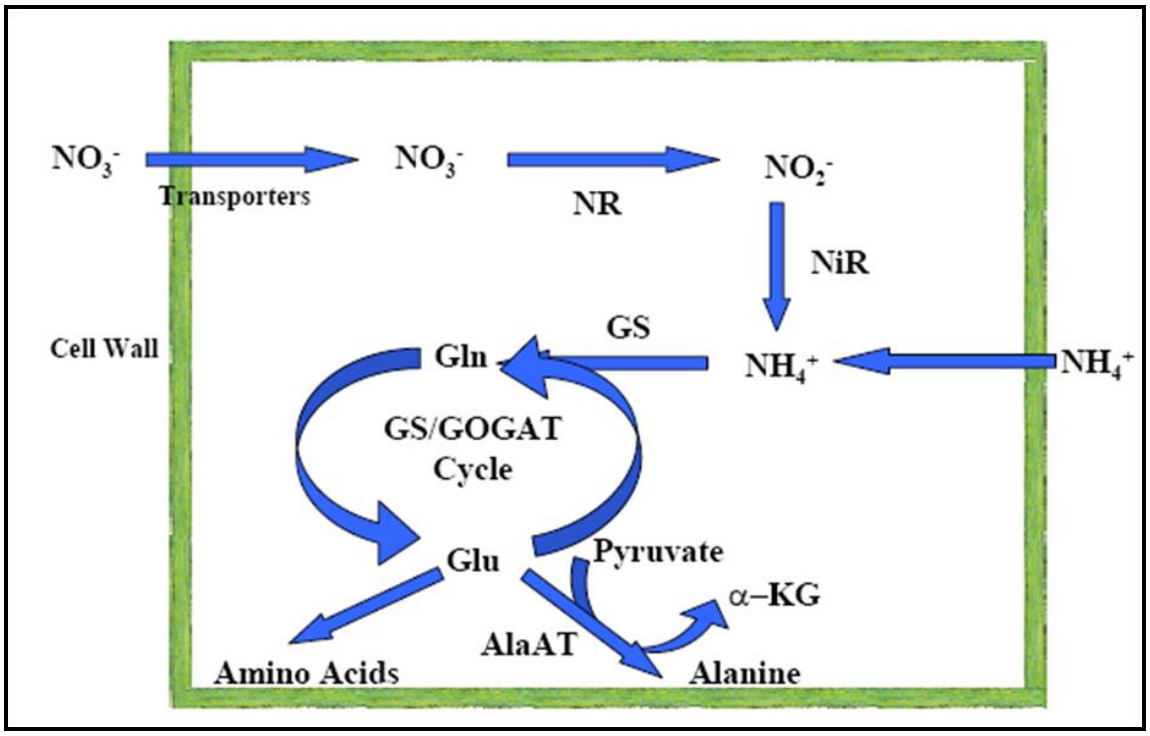


Figure 1.4. Key steps in the nitrogen assimilation pathway (Good, 2004).

The enzyme GOGAT catalyses the conversion of glutamine and 2-oxoglutarate to two molecules of glutamate. It exists in two forms: Fd-GOGAT catalyses the light dependent assimilation of NH_3

and NO_3^- reduction, as well as the assimilation of NH_3 from photorespiration. Arabidopsis has two genes coding for Fd-GOGAT, GLU1 in leaves and GLU2 in roots. NADH-GOGAT is the other form of the enzyme found in non-green tissues (vonWiren et al., 1997).

The GS/GOGAT system metabolises NH_3 into glutamine and back to glutamate, which is the starting point for N assimilation, as well as biosynthesis. The GS/GOGAT pathway is influenced by N conditions, light and developmental stage of the tissue where it is present (Hirel and Lea, 2001, Edwards et al., 1990, Thum et al., 2003). Both enzymes GS and NADH-GOGAT are highly present in roots as well as induced by NH_4^+, assigning them an important role in NH_4^+ primary assimilation (Ishiyama et al., 2003).

Aspartate aminotransferase/Asparagine transferase (AspAT) catalyses the reversible transamination of glutamate and oxaloacetate into aspartate. The enzymes transports equivalents from cytosol to chloroplasts, mitochondria, glyoxisomes and peroxysomes, and four isozymes have been identified for each compartment except glyoxisomes (Liepman and Olsen, 2003, Berkemeyer et al., 1998, Wadsworth, 1997).

Asparagine synthetase (AS) synthesises asparagine using glutamine and aspartate as substrates. Asparagine synthetase is located in vascular bundles and phloem (Nakano et al., 2000). Asparagine is an amino acid used for N storage and long distance transport in the plant, due to its high solubility (Sieciechowicz et al., 1988). Two genes coding for AS were isolated in 1990 in peas, *AS1* and *AS2* (Tsai and Coruzzi, 1990). A later study by Tsai and Coruzzi (1991) found that AS activity was stimulated by darkness and repressed by light in leaves in both genes, but AS2 expression in roots was constitutive. Further studies by Lam et al. (1996) and Lam et al. (1998) isolated three genes from Arabidopsis *AS1*, *AS2* and *AS3*. *AS1* in leaves was repressed by light and sucrose and activated by glutamine, glutamate or asparagine, whilst *AS2* activity was regulated reversely, explaining that the mechanism was to allow AS to synthesise asparagine both in light and darkness when necessary.

It has been suggested that the enzyme Glutamate dehydrogenase (GDH) catalyses the reversible deamination of glutamate producing ammonia and 2-oxoglutarate and liberating energy and C from amino acids (Miflin and Habash, 2002, MeloOliveira et al., 1996), but the GDH pathway is not universally agreed (Dubois et al., 2003).

METABOLIC ENGINEERING

A recent study on metabolic engineering showed transgenic potato plants with an improved net N assimilation. The experiments consisted of enhancing the well known Dof1 transcription factor implicated in general plant N and C metabolism (Yanagisawa et al., 2004). Other studies on the over-expression of GS did not show any enhancement in net N assimilation (Gallardo et al., 1999, Migge et al., 2000, Fuentes et al., 2001, Oliveira et al., 2002). Only recent reports showed transgenic plants over-expressing GS having improved assimilation of N under low N supply (Oliveira et al., 2002, Fuentes et al., 2001). Transgenic potato plants over-expressing Phosphoenol Pyruvate Carboxylase (PEPC) had higher amounts of amino acids but had severe growth defects as well (Rademacher et al., 2002). A recent study published on AtAMT1;1 in tobacco (Yuan et al., 2007) showed that plants over-expressing the ammonium transporter gene could take up 30% more ammonium from the soil, but this did not translate into better performance or improved N assimilation.

An unresolved question is which key enzymes are limiting in N metabolism thus restricting NUtE.

TRANSPORTERS

NH_4^+ transport from roots is a well known and an important source of N for the plant, but it is also important in transport to the shoot. There is a superfamily of high affinity ammonium transporters identified in nature as ammonium transporter/ methylammonium permease (MEP/AMT). No low affinity transport systems for NH_4^+ have been identified so far in plants. Members of the MEP/AMT family can be divided in to three subfamilies in plants, with a larger subfamily of genes AMT1. Genes from this subfamily have been isolated in Arabidopsis, such as AtAMT1;1 by Ninnemann et al. (1994). Most of the ammonium transporters are induced under low N conditions, as happens with NO_3^- (Gazzarrini et al., 1999), but the difference with those of NO_3^- is that NH_4^+ transporters are already up-regulated under low N supply, thus when re-supply of N happens, increased NH_4^+ transport is less noticeable than in NO_3^- transport (Glass, 2003). Transport is regulated by levels of N substrate, glutamine, feedback regulation, light and sugars from photosynthesis (Lawlor et al., 2001). The fact that light and N limitations do not affect the same AtAMT transporter suggests that different regulatory mechanisms operate for each of them (von Wiren et al., 2000).

1.5.3. N TRAFFIC AND STORAGE PROTEINS

Nitrogen traffic can be defined as the temporal and spatial pattern of N transfer and allocation occurring within the plant throughout its life- cycle (Lawlor et al., 2001), and it is highly related to NUE. Nitrogen Use Efficiency will be influenced by the speed and the effectiveness with which N is mobilised and relocated with storage to the needed parts. It has been noted that one of the major remobilisation processes affecting plant productivity and NUE is the relocation of N from senescent leaves and the rate of biomass loss by the plant (Garnier and Aronson, 1998).

When N is not limiting in the environment, concentrations in the plant can reach up to 100mM, stored mostly in the vacuole. In the cytoplasm, the concentration of NH_3 is kept constant (Miller and Smith, 1996).

N remobilisation is very important in plants that go through a dormancy period in winter to prepare for a major spring growth; i.e. in oilseed rape as remobilisation produces changes in many enzyme activities such as GS, GOGAT or GDH.

Vegetative Storage Proteins are found in many species and have been characterised as glycoproteins. Two isoforms of VSPs have been identified in tap roots of oilseed rape, mainly in cortical parenchyma close to the site of phloem transport. VSPs accumulate N during flowering from senescing leaves stimulated by methyl jasmonate and release it during seed filling, as well as grain filling (Rossato et al., 2001). The two proteins, napin and cruciferin, are regulated by absisic acid and methyl jasmonate (Wilen et al., 1991). In the presence of methyl jasmonate (synthesised by flowering) N uptake ceases, senescence starts, as well as N mobilisation from leaves into reproductive tissue, but in the case when the latter is not yet developed, the N is stored temporarily in the tap root and stem. The function of VSPs is not well characterised yet, but it is likely that a key function is as a temporary store of nitrogen between remobilisation from one tissue/organ to another, to be released as plant demands for N change. Accumulation of protein in oilseed rape is not directly correlated to N availability and is not induced by N uptake (Rossato et al., 2001).

1.5.4. INTERACTIONS BETWEEN C AND N METABOLISM

In addition to NO_3^- and NH_4^+ uptake from soils, it has been shown that plants can obtain N from amino acids, peptides and ureides (Nasholm et al., 2000), and the transport system for the organic nitrogen has been well characterised (Rentsch et al., 2007). A study in oilseed rape

(Tilsner et al., 2005) characterised three permeases as amino acid transporters in oilseed rape, but concluded these might not be neither the only ones nor the most important ones. Carbon skeletons required for the assimilation of ammonium into amino acids are provided by α-ketoglutarate and oxaloacetate showing the high linkage between carbon and nitrogen assimilation (Vance, 1997).

Leaf amino acid content is directly correlated to N supply (Foyer et al., 2003). In a situation where nutrients and water are not limiting for optimal plant growth it is carbon assimilation which is (Lawlor, 2002). Some studies have demonstrated that plants can uptake and assimilate more N under a high CO_2 atmosphere (Stitt and Krapp, 1999, Andrews et al., 2001). This evidence suggests that enzymes involved in N metabolism do not generally limit yield in crop plants (Andrews et al., 2004).

Few studies have recently used microarray technology to study in detail the Arabidopsis genome in response to N limitation. One study investigated long-term effects of N limitation showing an increase in protein degradation, down-regulation of genes involved in photosynthesis, protein synthesis, amino acids and chlorophyll, and up-regulation of genes for starch biosynthesis (Scheible et al., 2004). These results are opposite to those found in short-term response to N limitation (Wang et al., 2003). An exhaustive microarray in Arabidopsis analysed the carbon and nitrogen interactions in plants under a range of carbon and nitrogen treatments (Gutierrez et al., 2007). The study built a model for the whole metabolic network for Arabidopsis and identified regulatory pathways related to C, N and C/N interactions. The study showed that 78% of the nitrate inducible genes identified in a previous study (Wang et al., 2003), i.e. those involved in assimilation processes such as nitrate transport and nitrate reduction, were regulated by N interactions with C.

1.6. Nitrogen Uptake and Use in Oilseed rape

Oilseed rape has a low N harvest index due to the incomplete re-translocation of N because of early leaf abscission (Malagoli et al., 2005). Re-translocation occurs in reproductive stages of growth from the vegetative tissues into the seed. The result is an increased leaching of the absorbed N especially from the pod walls and leaves of senescing tissues. Nitrate losses contribute therefore to environmental pollution, which justifies the importance to select for efficient N use cultivars (Rossato et al., 2001).

Nitrogen Use Efficiency can be defined from a physiological point of view, as increased yield carbon production per unit of plant N over time; or from an agronomical point of view as protein DM yield per unit of plant N or N available to the crop (Gallais and Hirel, 2004).

Nitrogen use is important in oilseed rape, with respect to oil content and yield, with many publications analysing different relations and interactions between them (Dreccer et al., 2000a, Lisson et al., 2007). To increase seed yield and breed varieties more efficient in nitrogen use, three basic aspects have to be approached; efficient use of fertilizer, customised N application in relation to time and site, and selection of cultivar (Rathke and Diepenbrock, 2006). A review on yield suggested the use of pod number as a marker trait to select for high yield in oilseed rape, as well as seed number per pod determining pod length, which was considered an indirect trait to select for yield (Diepenbrock, 2000). Nitrogen harvest index (NHI) is also an important parameter defined as N in grain/ total N uptake at harvest, as it defines partitioning between the seed/grain and the rest of the plant. As grain protein content is negatively correlated with oil content, NHI has been negatively selected for (Hirel et al., 2007). Some lines have been found in wheat to have higher protein content than the one predicted from yield (Oury et al., 2003, Kade et al., 2005). In recent times, differences in NUE in spring canola were found resulting in increased harvest index. No differences between low and high N input were found for NUE, meaning that cultivars responding well at high N supply had comparable response at low N supply (Svecnjak and Rengel, 2005). A study in rice suggested that to select for varieties at low N input, but still producing high yield, the negative relationship between N uptake efficiency and both yield and NUE should be broken, and found evidence of that being possible (Borrell et al., 1998).

A range of projects have looked at different approaches to collect information related to N metabolism in oilseed rape by improving the agronomy of the plant (Leon, 1993, Leleu et al., 2000, Velasco and Mollers, 2000, Vos and vanderPutten, 1997). Studies have also focused their analysis on N leaching in a crop rotation (Arregui and Quemada, 2006, Sieling and Kage, 2006), with N leaching attributable to leaf senescence (Gombert et al., 2006), as well as nitrate uptake and partitioning (Etienne et al., 2007, Hocking et al., 1997, Malagoli et al., 2005, Rossato et al., 2001, Dejoux et al., 2000, Barlóg and Grzebisz, 2004). Other studies have concentrated on N metabolism from the photosynthesis perspective (Jensen et al., 1996, Krapp et al., 2005, Arntz et al., 2000a, Arntz et al., 2000b, Kappen et al., 1998) and photosynthesis of pods and leaves (Gammelvind et al., 1996, Mogensen et al., 1997, Muller et al., 2005). A study comparing wheat

and oilseed rape radiation use efficiency and NUE concluded that oilseed rape has higher assimilation of N during seed filling than wheat, with the system working close to optimal rates, but NUE was not optimal due to shading from the flowering canopy (Dreccer et al., 2000b). Other studies have tried to interpret the relationship between N protein and storage (Rossato et al., 2002 and Rossato et al., 2003), Rubisco enzyme activity (Ishimaru et al., 2001), and also leaf senescence (Etienne et al., 2007, Erley et al., 2007). The latter was a study in oilseed rape showing delayed leaf senescence when N uptake was high, and also finding that the most efficient line was able to adapt its photosynthetic rate during shading from the floral canopy. The study concluded that important leaf and root traits expressed under short term N deficiency conditions can be used to select varieties for improved nutrient use in the field. Data has also been collected about planting date and the effect on different traits related to nitrogen use either directly or indirectly i.e. flowering efficiency, oil content and seed weight (Adamsen and Coffelt, 2005). Planting methodology has also been shown to affect seed yield and water use efficiency. Buttar et al. (2006), and Hocking and Stapper (2001) studied a wheat canola rotation concluding that early sowing produced plants with higher nitrogen use and less N losses, thus obtaining better economic return from fertiliser use.

Other improvements in oilseed rape have been achieved through the development of 'restored' hybrid plants achieved by controlled crossing utilising male-sterile lines. These newly synthesised populations are more resistant to diseases than the conventional ones, apart from providing the advantages they were initially bred for. For example, semi-dwarfs (also called low biomass varieties) are hybrids that have an increased HI and are more efficient in N uptake (Sieling and Kage, 2008). They are also more manageable at harvest due to lower biomass and have more homogenous flowering times and maturity times. Contrary to other hybrid varieties where the main stem flowers before, in semi-dwarfs the main stem and other branches flower at the same time. They also give about 3% more higher yields (http://www.fwi.co.uk). In 2008, the HGCA list of recommended varieties for sowing, contained 27 conventional varieties, 20 hybrid varieties and 2 semi-dwarf hybrid varieties.

Previous studies have shown that the selection of varieties for NUE under different N supply leads to different results because of the genotype x nitrogen interaction. Consequently it is important to study genetic variation under both optimal and suboptimal N supply conditions (Rauna and Johnson, 1999). Yau and Thurling (1987a) found that in spring rape, cultivars with

the lowest yields at the lowest N supply generally responded producing higher yields than cultivars with a genetic high yield grown at high N supply.

Various studies have focused on physiological and agronomic aspects of nitrogen use of oilseed rape (Chamoro et al., 2002, Jensen et al., 1997, Tilsner et al., 2005, Kamh et al., 2005, Moroni et al., 1996, Seiffert et al., 2004, Sieling et al., 1998, Erley et al., 2007, Good et al., 2007, Svecnjak and Rengel, 2005, Svecnjak and Rengel, 2006). Fertiliser applications have been extensively studied as it is considered the main cause of leaching, with studies focusing on effects of liquid fertiliser (Rathke et al., 2005, Hocking and Stapper, 2001, Sieling and Beims, 2007, Holzapfel et al., 2007, Taylor et al., 1991, Malhi et al., 2007), others on effects of organic manure (Mooleki et al., 2004, Mooleki et al., 2002, Sieling et al., 1998, Wen et al., 2003).

An area that has attracted great interest is nutrient use efficiency, with the aim to maximise nutrient use to maintain and or maximise seed yield. Studies have mostly focused on nutrient deficiencies, fertiliser application to overcome soil deficiencies and leaching to minimise environmental pollution. Nutrients of interest studied in *Brassica* are N (Colnenne et al., 2002) and carbon (Aulakh and Aulakh, 2005), phosphorus (Aulakh and Pasricha, 1999, Malhi et al., 2002, Malhi et al., 2007) and sulphur (Fismes et al., 2000, McGrath and Zhao, 1996).

Malagoli et al. (2005) suggested NUE is not a heritable trait, thus to select for varieties with improved NUE, it is the NHI that should be improved, possibly by reducing leaf abscission N losses in spring with better translocation of N.

Hirel et al. (2007) very recently reviewed the improvement of NUE through quantitative genetic approaches and concluded that for oilseed rape, there is a shortage of data especially at low N fertilization supply.

1.7. Quantitative Trait Loci (QTL)

Quantitative trait loci (QTL) mapping consists of identifying (through linked genetic markers) the individual genetic factors influencing the value of a quantitative trait. Many agriculturally important traits e.g. grain yield are controlled by many genes and are known as quantitative traits. The most important QTL markers will be the ones that identify the genes involved in the expression of the desired character. However, most QTL basically detect regions of the DNA that are located physically close to such genes. The QTL approach becomes of particular interest when analysing complex systems like yield, drought stress and the metabolism networks e.g. N metabolism. The regions within genomes that contain genes associated with a particular trait

are known as Quantitative Trait Loci (QTL). A major breakthrough in the characterisation of quantitative traits which created an opportunity to select for QTL came with the development of molecular markers in the 1980s. Molecular markers are valuable tools for crop improvement in a number of species including oilseed rape.

In studies of the plant genome, the most commonly used molecular markers are: RFLP (restriction fragment length polymorphism), CAPS (cleaved amplified polymorphic sequences), STS (sequence-tagged sites), RAPD (random amplified polymorphic DNA), SCAR (sequence characterized amplified region), AFLP (amplified fragment length polymorphism), SSAP (or S-SAP, sequence-specific amplification polymorphism), SSR (single sequence repeats), ISSR (inter simple sequence repeats), and SNP (single-nucleotide polymorphism). Gupta et al. (1999) have proposed a classification of DNA markers, divided into three categories according to method of analysis: (1) Hybridization-based DNA markers (e.g., RFLP); (2) PCR-based DNA markers (e.g., CAPS, STS, RAPD, SCAR, AFLP, SSAP, SSR, ISSR); (3) DNA chip and sequencing-based DNA markers (SNP).

Benefits of QTL analysis are usefulness in identifying genes to clone; to test trait associations; identification of traits associated with stressed environments e.g. drought, salinity, nutrient, etc; for identification of new gene sources; and for monitoring introgression of genes into a breeding programme by using markers associated with genes (Salvi and Tuberosa, 2005).

1.7.1. Doubled Haploid populations: description and suitability for analysis

Complete evolution of a novel character requires the comparison of near isogenic lines (NIL); however, the use of DH lines can be integrated as an estimate of the potential of the character prior to generation of NIL, which is a lengthy process. Doubled Haploid (DH) populations in oilseed rape can be produced by regenerating plants by the induction of chromosome doubling, from either anther culture, or a more evolved technique i.e. microspore culture. The latter avoids contamination between the haploid material from the pollen microspores and the diploid anther wall, making the resulting cross of use for cell biology, microbiology and genetics (Forster et al., 2007). Doubled haploid populations are homozygous and have the benefit of being multiplied and reproduced without genetic change occurring, allowing for replicated trials across different locations and years. In plants, another advantage is the relatively short time needed to obtain a functional DH population, compared to acquisition of other populations such

as Backcross lines (Forster et al., 2007). It is estimated that nowadays half of the barley cultivated in Europe is produced from DH lines. Doubled haploid populations have been extensively used for the identification of genetic markers and the mapping of chromosomes.

Oilseed rape is a suitable option for QTL mapping and cloning purposes since dense genetic maps are currently available. Furthermore, the compatibility with the Arabidopsis genome allows the use of extensive mapping information currently accessible in the public domain, as well as information of genes directly related to N metabolism which are already positioned on the Arabidopsis physical map. Arabidopsis and oilseed rape (Brassica family) share common ancestry thus collinear sequences have been found between the 2 genomes. Arabidopsis genome has the advantages of being fully annotated and known, small, and with different genetic, genomics and proteomics resources (Arabidopsis Genome Initiative, 2000). As the *Brassica napus* genome is being sequenced, the alignment of this with Arabidopsis is a very powerful tool for fine mapping and gene identification.

In oilseed rape, to date, QTL analysis has only been used to study a limited number of traits, mainly related to seed oil content (Zhao et al., 2005, Burns et al., 2003), glucosinolates (Howell et al., 2003, Toroser et al., 1995) and disease resistance (Pilet et al., 2001, Zhao and Meng, 2003, Delourme et al., 2004). Very few studies concerning freezing tolerance (Teutonoco et al., 1995, Kole et al., 2002a) have been conducted and only Xu et al. (2001) have tried to resolve the genetic basis of nutrient efficiency with respect to the micronutrient boron.

The DH populations used in most of these studies were crosses between commercially bred varieties e.g. Victor, Tapidor and Darmor. Similar crosses between different ecotypes have been shown to broaden the genetic base due to the genetic distance between them. Quijada et al. (2004b) demonstrated that introducing germplasm from a spring variety to a winter variety can improve the seed yield as a consequence of earlier flowering in some DH lines.

An important consideration pointed out by Gallais and Coque (2005) is that selection of parental lines to study NUE has generally been conducted under high N conditions, therefore results of such investigations should be meticulously analysed. A suggestion to overcome the problem is to select lines from different areas of the world, thereby incorporating a wider genetic background into the population (Udall et al., 2004).

A study conducted by Zhao et al. (2005), showed that the recombination of positive alleles from European and Chinese plants was a powerful tool to increase the oil content in rapeseed,

considering that epistatic effects will cause an important influence. Moreover, the size of the population will ensure a high power of QTL mapping.

The TNDH population used in this project was produced from microspore culture as described in Qiu et al., (2006) and it has already been used to analyse oil content and erucic acid composition as part of the EU funded IMSORB project.

1.7.2. QTL FOR VARIOUS TRAITS IN OILSEED RAPE

OIL

Oil is the major product obtained from oilseed rape and the main reason for the crop to be cultivated. Various studies using QTL analysis have characterised different aspects of oil content and fatty acid composition in oilseed rape (Burns et al., 2003, Zhao et al., 2005, Wu et al., 2006, Delourme et al., 2006, Barker et al., 2007), also in *B. juncea* (Mahmood et al., 2003), as well as detailed characterisation of fatty acids of interest, e.g. oleic and linolenic acids (Hu et al., 2006), and erucic acid (Qiu et al., 2006), as well as tocopherols (Marwede et al., 2005). Other mapped QTL for seed quality, indirectly related to oil content, are seed colour (Fu et al., 2007a), husk proportion and lignin content (Fu et al., 2007b).

YIELD

Seed yield is one of the most valuable traits of crops, as it has been the principal target for plant breeders for many years. QTL for yield and yield components have been found in *Brassica napus* (Zhao et al., 2006, Quijada et al. 2006, Udall et al., 2006, Quijada et al., 2004a), and in *Brassica juncea* (Ramchiary et al., 2007). QTL have been also interpreted for yield in relation to heterosis (Shen et al., 2006). A study published by Gül (2002) found that there was no interaction between QTL for yield and N treatments.

PEST RESISTANCE

A very important cause of economical loss to crops is disease, and it is one of the main traits studied in oilseed rape. Major QTL have been identified in oilseed rape for blackleg resistance (Dion et al., 1995, Pilet et al., 1998b, Yang et al., 2006), Sclerotinia stem rot, caused by the fungus *Sclerotinia sclerotiorum* (Zhao et al., 2006), white rust (Kole et al., 2002b), turnip yellows virus (Dreyer et al., 2001), and light leaf spot (Pilet et al., 1998a).

FLOWERING TIME

Many studies have detected QTL for flowering time in *B. oleracea* (Axelsson et al., 2001, Camargo and Osborn, 1996, Kole et al., 2002a, Okazaki et al., 2007, Rae et al., 1999, Salathia et al., 2007) and in *B. rapa* (Teutonoco and Osborn, 1995, Ajisaka et al., 2001, Nishioka et al., 2005).

QTL FOR NUE IN OILSEED RAPE

No studies have been published so far, even though some genes have been identified considered to improve NUE in different crops including oilseed rape, i.e. alanine aminotransferase (AlaAT), and some root specific transcription factors.

1.8. QTL FOR NITROGEN USE EFFICIENCY IN DIFFERENT CROPS AND MODEL PLANTS

1.8.1. ARABIDOPSIS THALIANA

Nitrogen studies in Arabidopsis are extensive and due to the similarity between the Arabidopsis and the oilseed rape genomes, findings are of direct relevance for applications to oilseed rape. Nitrate has been studied from different points considering its function, as a main source of food for plants, but also as a signal molecule (Alboresi et al., 2005). The nitrogen metabolic pathway has been largely studied, as well as interactions between carbon and nitrogen cycles (Cross et al., 2006, Krapp et al., 2005), genes involving transport from roots (Chopin et al., 2007), and alterations in the metabolic pathway affecting uptake (Kawachi et al., 2006). Research has focused not only on gene characterisation with mutagens, but also in finding gene pools analysing natural variation with Quantitative Trait Loci, e.g. for: studying leaf senescence and stress response (Diaz et al., 2006), leaf senescence and longevity (Luquez et al., 2006), analysis of growth (El-Lithy et al., 2004), seed vitamin E levels (Gilland, 2006), water and anions in relation to nitrogen (Loudet et al., 2003a). Information about QTL studies conducted prior to 2004 can be found in the annual review by (Koornneef et al., 2004).

A study focused on QTL related to nitrogen and Nitrogen Use Efficiency in *Arabidopsis thaliana*, was conducted by Loudet et al., (2003b) with the aim to ascertain the genetic control of the regulatory pathways in nitrogen metabolism. The work failed to isolate new genes that could be involved in the regulation of the nitrogen cycle, suggesting that different alleles of those genes may cause subtle changes rather than strong global modifications.

1.8.2. MAIZE

Agrama et al. (1999) identified QTL for NUE in maize, showing correlation between ear number per plant and ear size as indicators of yield under limiting N fertilizer. Bertin and Gallais (2000), identified QTL for kernel size related to NUE and yield, and showed variability of NUE in response to both high N and/or low N, and concluded that limiting steps in N metabolism were different at high and low N conditions. Hirel et al. (2001) examined both genetic and physiological aspects of NUE showing that, with NUE, nitrate content, glutamate synthetase activity (GS) and yield were positively linked, but nitrate reductase was negatively correlated. Other QTL identified including germination efficiency in relation to N metabolism (Limami et al., 2002), root architecture QTL related to grain yield (Tuberosa et al., 2003, Coque and Gallais, 2006, Liu et al., 2008), the latter found a major QTL for average axial root length possibly useful as a marker for identifiying N use efficient varieties. More QTL identified are for grain yield, and traits related to yield e.g. kernel number, silking date and grain moisture at harvest (Moreau et al., 2004). A recent publication on maize describes the role of N uptake and N use related to grain filling under different N fertilization conditions (Uribelarrea et al., 2007).

1.8.3. WHEAT

Many studies have been published examining different aspects of N on QTL in wheat. Quantitative trait loci have so far been detected in relation to N uptake and early seed vigour (Barriere et al., 2006), root architecture (Laperche et al., 2006), and NUE as influenced by genotype and N fertiliser interactions (Laperche et al., 2007). In addition, genes coding for enzymes related to nitrogen metabolism have been sequenced to obtain a nitrogen genetic map (Boisson et al., 2005). Further work on N metabolism enzymes suggested glutamine synthetase (GS) activity as a possible marker to select wheat varieties for more efficient N use (Kichey et al., 2006).

Some studies in wheat have focused on grain N and protein content to characterise N remobilization (Martre et al., 2003). Kade et al. (2005) presented a study analysing the N uptake and translocation in a population containing the high protein content gene *hpc-B1*. The study concluded that differences in protein content were due to translocation efficiency more than to uptake, as total N and yield were not significantly different between lines at maturity. The study considered the contribution from N uptake at grain filling as part of the differences in protein content, but no difference between lines in relation to accumulation of N could be detected.

Oury et al. (2003) examined the relationship between grain protein and yield and concluded that lines could be found where grain protein content was higher than that predicted by the negative relationship with grain yield.

1.8.4. RICE

As one of the most important staple foods for human nutrition together with maize and wheat, recent studies have focused on improving NUE in rice, as well as characterising pathways involved in N metabolism. Quantitative trait loci studies have analysed N interactions with response to plant height (Fang and Wu, 2001), the relation between N content in flag leaf and concentration of Rubisco, and soluble protein (Ishimaru et al., 2001), chlorophyll content (Fang et al., 2004), low N tolerance at seedling stage (Lian et al., 2005), the relationship between grain yield and N uptake (Ju et al., 2006), and a major QTL on chromosome 2 for cytosolic glutamine synthetase and panicle number (Obara et al., 2004). Twenty single QTL and 58 pairs of epistatic loci have been identified for physiological nitrogen use efficiency, concentration of grain, straw, shoot, harvest index, grain yield, and straw yield both in high and low N treatments (Cho et al., 2007).

1.8.5. OTHERS

Lotus is another example that has been of interest to determine its NUE as well as the N metabolic route with a paper published on QTL for nitrate uptake and assimilation (Harrison et al., 2004). Other studies have indirectly studied N metabolism using *Populus* (Rae et al., 2007) who analysed QTL for biomass in relation to CO_2 levels.

1.9. THE HYPOTHESIS AND AIMS AND OBJECTIVES OF THE PRESENT STUDY

The following hypothesis was the basis for the investigations included in the present study:

Better NUE achieved by exploiting the genetic improvement potential of crops would not only improve sustainability of agricultural systems but also minimise the N fertiliser application-mediated adverse environmental impact of oilseed rape cultivation. Different genotypes of winter oilseed rape (TNDH lines in this case) have different nitrogen use efficiencies (NUE). Differences in NUE are due to differences in either nitrogen uptake efficiency (NUpE) or nitrogen utilization efficiency (NUtE) or a combination of the two. The magnitude of NUpE and NUtE are

affected by the level of N supply (High/Low) and their effects are independent of one another. Such independence posits that certain plant traits can be identified that are related specifically to NUpE and others to NUtE. Stable QTL for each such trait can be identified that are N treatment specific. Candidate genes can then be identified for the N derived traits that influence yield. With this hypothesis the specific objectives of this study were as follows:

- to identify traits related to different components of NUE in field experiments conducted over two years using different TNDH lines;
- to identify the nature of the relationships between traits under different N supply in the field (High and Low N treatments);
- to identify key loci involved in the expression of traits for differential responses to nitrogen supply using the Quantitative Trait Loci approach;
- to characterise the genetic basis of relevant traits for breeding varieties improved in NUE, particularly under low N conditions, by assessing the stability of identified QTL, their heritability and G x E interactions;
- to identify candidate genes related to the traits of interest through comparative genomics with Arabidopsis;
- to integrate QTL based information for indicating the genetic basis of N metabolism and enable genetic improvement of oilseed rape in terms for NUE.

CHAPTER 2. ANALYSIS OF TNDH PLANT PHYSIOLOGY

2.1. INTRODUCTION

One of the major interests of plant breeders has been to acquire a better knowledge of N uptake and metabolism to obtain varieties with improved NUE. To fulfil this objective many studies have tackled the subject following different approaches, focusing on the improvement of different traits. To design a successful and cost-efficient breeding program different aspects have to be taken into consideration, such as appropriate trait selection with phenotyping screens or markers, as well as appropriate selection of the parental lines and segregating population.

To increase breeding efficiency it is important to search for traits of indirect selection based on the correlation between primary and secondary traits. Therefore a choice of traits directly or indirectly related to N metabolism could serve as indirect selection criteria to improve N use efficiency in oilseed rape. Since NUE is defined as the product of N uptake efficiency and N utilisation efficiency, agronomic traits related to either/both of these two components should be studied (Kessel and Becker 1999).

An appropriate selection of traits is crucial to finding varieties with higher N use efficiency; however, the chances of success depend on the genetic variation for the trait in question. Thus narrow-sense heritability should be checked for each trait to determine whether the phenotype is a good reflection of the genotype, so that reproducibility can be assured (Buzza 1995).

Total plant biomass and other yield traits have been related to NUE in different studies. Svecnjak and Rengel (2006), investigated if differences in NUE in different canola cultivars were related to N uptake and partitioning and found a strong relationship between total plant biomass and NUE. Another study by Nyikako (2003) studied traits such as thousand seed weight (TSW), flowering interval, and plant height to indirectly select for improved grain yield and showed that improved N use efficiency was also found in canola.

Production of a new cultivar through traditional breeding methods takes usually more than a decade, in addition to which, it is very costly. The use of DH lines in indirect selection is promising where grain yield is to be correlated with agronomic traits. As genetically uniform populations they have been shown to produce better results for biomass and yield related traits when compared to conventional cultivars (Nyikako, 2003). A significant time saving in the production of a new cultivar is the most important contribution of DH lines since yield and other traits can be tested earlier than when using conventional methods (Kučera et al., 2002). The best way to investigate genetic correlations is therefore to grow

segregating populations of DH lines under various environments e.g. nitrogen, since these correlations may differ with different N supply (Gallais, 1984).

The TNDH population was chosen for two reasons: firstly, because the wide genetic base of the parents used provides an ideal opportunity to initiate fine mapping of QTL for NUE in *B. napus* and secondly, because of the availability of a genetic linkage map for this population as well as a supply of seed to conduct the trials. The TNDH population is known to express considerable variation for growth habit, canopy architecture, yield, and seed quality and its genetic variation in N Use Efficiency was analysed.

The aim of the first part of this thesis (Chapter 2) was to evaluate and analyse a number of physiological traits in response to differential nitrogen supply using the TNDH population derived from the Tapidor x Ningyou7 cross. Largely, the chapter aimed to identify traits related to different components of NUE in field experiments conducted over two years using different TNDH lines and to identify the nature of the relationships between traits under different N supply in the field (High and Low N treatments).

2.2. Materials and methods

2.2.1. Experiment 1. Field trial 2005/06

Description of site

The experimental site was located at Cockle Park Farm, Northumberland, UK (latitude 55:15:51N; longitude 1:41:08W). The field trial was carried out during the 2005/2006 growing season (Fig 2.1.) and the soil texture was characterised as a clay loam soil of the Dunkeswick series. The soil is described as a poor soil, with a tendency for infiltration and increased runoff.

The experimental site was part of a wheat- barley-oilseed rape crop rotation, with wheat the previous crop in the field used in 2005/2006. The field trial was established within a commercial oilseed rape crop in order to provide a typical environment for crop growth and minimise the risk of pigeon, insect damage, disease, etc.

Figure 2.1. Trial locations at Cockle Park Farm. Red rectangles and arrows showing exact location and approximate size of the trial site in the respective seasons.

DESCRIPTION OF PLANT MATERIAL

The *Brassica napus* population used was a DH population crossing Ningyou7 (a Chinese semi-winter variety) and Tapidor (a European winter variety), generated in vitro by microspore culture as described in Qiu et al. (2006). The TNDH population resulted in a total of 202 lines; of which, a subset of 188 lines had been identified with molecular markers and used for map construction. The 2 parental lines were originally selected for differences in architectural traits and seed oil composition. Of the 188 lines used for map construction, only 174 lines were fully genotyped and only these were sown for analysis.

EXPERIMENTAL DESIGN AND TREATMENTS

In the 2005/06 season, 188 F1 lines and the two parents Tapidor and Ningyou7 were grown in four randomised blocks with two nitrogen treatments i.e. High and Low N (Fig. 2.2.a).

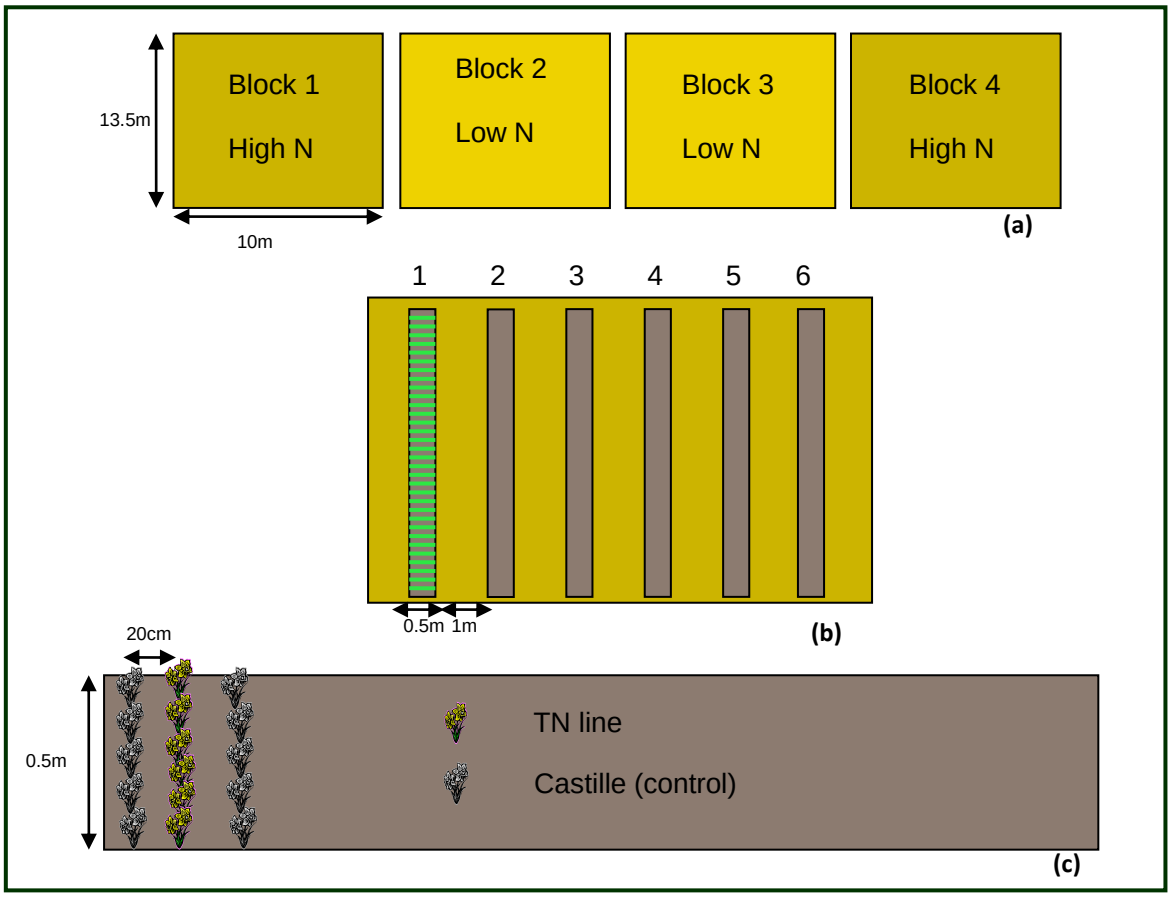


Figure 2.2. Experimental design of field trial in 2005/06. Four randomised blocks (**a**), two of which were treated at Low N fertiliser and the other two following standard High N fertiliser application. TNDH lines were distributed in six runs (**b**), alternating each TN line with a control variety Castille (**c**).

Two of the blocks were fertilised at a standard commercial agricultural rate of 200 kg N/ha and the other two blocks were fertilised under a low supply of 50 kg N/ha providing two replicated blocks for each High N treatment and two replicated blocks for each Low N treatment.

Each block consisted of six runs and within each run; each TNDH line was planted in a 0.5 metre row, 6 seeds per row, with 20cm between rows. Each TNDH line was interspersed by a 0.5m row of the control variety Castille (Fig 2.2.c) so that within each run there were 16 TNDH lines.

CROP MANAGEMENT

The field trial was sown by hand on the 30th and 31st of August 2005. Nitrogen fertiliser was applied on the 16th of March 2006 (50 kg N/ha to all blocks) with an additional 150 kg N/ha to High N blocks only on the 29th of March 2006. During the growing season, weed, insect and fungal disease control was carried out as for the commercial crop in each field. The trial received 3 slug pellet applications soon after sowing, and Katamaran (Metazachlor + quinmerac) for grass weed control. Caramba (metconazole) and Hallmark Zeon (lambda cyhalothrin) sprays were applied to combat light leaf spot and cabbage stem flea beetle respectively on 31st of October 2005. Hallmark was applied again on the 20th of April 2006 for pollen beetle control (Appendix 2 A).

SOIL MEASUREMENTS

Soil samples were taken on the 23rd of February 2006 before N fertiliser was applied. All blocks were sampled by bulking four soil cores from each block. Samples were taken at three depth intervals: 0-30, 30-60 and 60-90 cm. The samples were sent to a commercial laboratory for analysis of dry matter, NO_3^-, NH_4^+, and available N. Results summarising the soil analysis are shown in Table 2.1.

Table 2.1. Soil analysis results for experimental site prior to N treatments applied in 2006. Samples 1-4 correspond to the block numbers and N treatments described previously.

Block	Depth	Dry matter (w/w%)	NO_3^- (mg/kg)	NH_4^+ (mg/kg)	Available N (Kg N/ha)
1	0-30	74.9	2.12	0.06	8.7
	30-60	80.7	1.22	0.07	0.3
	60-90	84.1	0.04	0.04	0.35
	total				14.2
2	0-30	77.4	1.19	0.74	7.7
	30-60	81.4	0.05	0.05	0.4
	60-90	83.1	0.06	0.06	0.5
	total				8.6
3	0-30	77.4	1.35	0.51	7.5
	30-60	82.6	1.24	0.38	6.5
	60-90	82.3	0.3	0.06	1.4
	total				15.4
4	0-30	78.3	3.06	0.66	14.9
	30-60	80.9	0.06	0.06	0.5
	60-90	83.2	0.05	0.05	0.4
	total				15.8

PLANT MEASUREMENTS

CROP ESTABLISHMENT AND WINTER SURVIVAL

Establishment of the seedlings was measured on the 14th and 15th of November 2005 and germination rates calculated as percentage of germinated seedlings on these dates. Winter survival was recorded during the last week of February and was calculated as the percentage of germinated seedlings that were alive on the recorded date.

DETERMINATION OF LEAF CHLOROPHYLL CONTENT

A Soil Plant Analysis Diagnostic Meter (SPAD-502) was used to measure the chlorophyll content of leaves and bracts in SPAD units. Three readings were taken from the three youngest fully expanded leaves for each plant and the average reading was recorded for each plant. The same procedure was repeated for bract leaves on each plant. The variance in individual recordings could not be determined due to the data recording method. Readings were taken on the 8th and 11th of May 2006 (between 250 and 253 days after sowing) for Blocks 1 and 3 and on the 4th and 17th of May 2006 (245-258 days after sowing) for Blocks 2 and 4 respectively. At the time of recording the plant canopy was green without leaf loss (Blocks 1 and 3) and the first flower had already opened in most of the lines.

RECORDING OF FLOWERING TIME AND STEM CANKER

Flowering time was recorded as the number of days after the plants were sown (DAS) to the opening of the first flower on the terminal raceme. Flowering dates were recorded every second day from when the first TNDH lines started to flower and daily during peak flowering.

Stem canker was recorded in April/May 2006 at early flowering. Three plants of each TNDH line in Block 1, and 8 Castille control rows were measured (Appendix 5). The measuring of stem canker followed a severity scale scoring from 0 to 3, where 0 meant no presence of stem canker and 3 meaning maximum severity.

FINAL HARVEST MEASUREMENTS

At final harvest one plant from each line was removed from the field by cutting at the base of the stem and plants were dried by hanging in a polytunnel for determination of yield components and physiological parameters (Table 2.2). Traits analysed were architecture traits i.e. plant height, branch number, pod number, etc; flowering and yield traits such as seed yield, harvest index, seed number per pod, etc, and N traits such as seed N, N uptake efficiency (NUpE), N utilisation efficiency (NUtE), etc were determined.

To assess variability within each block and measure the environmental effects on plant growth, 1 sample from each run was taken from the control variety (Castille), and plant dry matter, seed yield and harvest index were determined.

Following all measurements, all plants were oven dried at 80^{o}C for 48h. Plants were then individually threshed to obtain seed and plant residue samples for analysis. Half of the separated seed was then sent to the John Innes Centre for oil content determination and the rest was ground for N determination as described below.

Oil content was measured by Near Infrared Spectroscopy (NIR) using the method described in Qiu et al. (2006).

Bird damage and pod shattering were assessed visually and estimated at 20% of seed loss in the most affected Blocks i.e. 2 and 4.

Determination of plant and seed nitrogen content

Dry plant material was milled with a medium hammer mill, using a 2mm diameter sieve. Each milled sample was sub-sampled and 0.25g used for N analysis. The N content was analysed using the Dumas' combustion principle with a LECO FP-428.

Seed was prepared for N content analysis by manually grinding 5g of seed in a pestle and mortar.

Nitrogen analysis in 2005/06 was carried out only using Blocks 1 and 3 for High and Low N respectively. Block 2 and 4 were discarded from analysis for 2 reasons: firstly, because growth in Blocks 2 and 4 had been affected by poor plant establishment producing weaker plants in comparison with Blocks 1 and 3. Consequently, stem canker and wind affected these blocks more severely. Due to these two factors, Blocks 2 and 4 were discarded because it would not have been possible to separate environmental effects from those caused by different N applications.

Table 2.2. List of characters both measured and determined in 2005/06 field trial.

Trait	Units	Description
Tot. plant height	cm	Taken from the base of the main stem to the tip of the terminal raceme, or tip of the longest branch.
Foot length	cm	Distance between base of the main stem and first branch.
Branch nº.		Primary branches only.
Fertile pod nº.		Terminal raceme and rest of branches separately
Total pod nº.		Total of terminal raceme and rest of branches separately
Pod fertility per plant	%	Ratio of number of fertile pods to number of total pods on whole plant
Pod fertility on terminal raceme	%	Ratio of number of fertile pods to number of total pods on terminal raceme
Biomass	g plant^{-1}	All plant parts weighed separately and summed
1000 seed wt.	g	
Seed number per pod		Total seed number divided by fertile pod number
Flowering	DAS	Days After Sowing
Chlorophyll	SPAD units	
Plant N concⁿ.	mg g^{-1}	
NUE		Multiplying NUpE by NUtE
NUpE	gN g^{-1}	Ratio of total above ground N to applied fertiliser amount and residual soil N
NUtE	g gN^{-1}	Ratio of SY to total above ground N
HI		ratio of seed yield to total above ground biomass
NHI		Ratio of seed N to total above ground N
Oil content	%	

The instrument used to determine total Nitrogen was a LECO FP-428 Total Nitrogen Analyzer (LECO Corporation, St. Joseph, MI, USA). The instrument operated on the Dumas method, where the sample was combusted at 950°C in an oxygen rich atmosphere. A 10ml portion of the combustion gas was scrubbed of water and carbon dioxide and passed through a hot copper column to convert the NO_x forms to N_2. The resulting nitrogen gas was then measured by thermal conductivity in a helium carrier. The instrument was previously calibrated with Ethylene Diamine Tetraacetic acid (EDTA) and the conditions for LECO analysis are listed in Table 2.3.

Table 2.3. Conditions for LECO FP-428 analysis.

Sample size	**25 mg (seed); 10mg (plant)**
Crucible	**LECO tin capsule**
Oxidation furnace temperature	**950 °C**
Reduction heater temperature	**750 °C**
Flow constants	**High, 20 s; high, 20 s; high, end**
Gases	**O_2, 99.99%; He, 99.99 %**

STATISTICAL ANALYSIS

Statistical analysis was performed using Genstat for Windows 9th Edition. (VSN International Ltd., 2006). The dataset was analysed for normality and data that deviated from normality was checked for common errors, logic checks (e.g. maximum 30 seeds per pod) and outliers, etc. Those values found to be outliers because of environmental effects other than genetic variability (i.e. an unexpected large number of plants having zero pods on the main stem attributed to frost or bird damage) were excluded from the analysis. The Linear Mixed Models (REML) was used for analysis of variance and performed on the 174 TN lines, across 2 treatments (High and Low N) and 2 replicates (one included Blocks 1,3 and the other one 2,4 as (High/Low N pairs) for all traits. For the nitrogen traits, the standard error could not be calculated due to lack of replication. Phenotypic correlations were calculated using Pearson Product Moment Correlation (Pearson's ρ) among traits using mean values combined across replications for each environment. Broad sense heritability (H^2) was calculated as the proportion of phenotypic variance due

to genetic factors. Since Broad sense heritability and narrow sense heritability (h^2) are equivalent for DH populations (Zhang and Zhou, 2006), it was determined using the following equation:

$$h^2 = V_A/V_P$$

where V_A represents the additive genetic variance and V_P represents the phenotypic variance.

Phenotypic correlations for the ratios of High/Low N for each trait were also calculated.

A multivariate Principal component analysis (PCA) was carried out with a standardised matrix of 174 TNDH lines by 23 traits, separately for High and Low N treatments.

2.2.2. Experiment 2. Field trial 2006/07

The second experiment was carried out during the growing season of 2006/07 within close proximity to the first experiment at Cockle Park Farm (Fig.2.1). The soil was again a clay loam soil of the Dunkeswick series. Winter barley was the previous crop grown and the trial was again located within a commercial oilseed rape crop.

Experimental design and treatments

In the 2006/07 experiment, 94 TNDH lines and the 2 parents were grown in four blocks, two for High N and two for Low N (Fig 2.3.). The 94 TNDH lines were a subset from the 188 lines sown the previous year, selected against early flowering (to avoid frost damage) and stem canker. Only 4 TNDH lines of the 94 had not been used for the first field trial: TN190, TN192, TN198 and TN200. The 94 TNDH lines were selected in the John Innes Centre and the population was labelled BnaTNDH_4, and was used in NOVORB LINK project, http://www.brassica.info/CropStore/populationslinked.php?pop=BnaTNDH). Within each block (20mx16m) there were 100 small plots (1mx0.8m) with each TNDH line allocated randomly to a small plot. Each small plot consisted of 4x1m rows of a TNDH line with 20cm between rows and 10 seeds sown per row. Plot areas were 1x0.6m, consisting of 40 plants per plot. Two of the blocks were treated with High N fertiliser at 150 kg N/ha and no fertiliser was applied to the other two blocks with Low N, i.e. treatments comparable to the previous field trial. A lower N fertiliser rate than the previous year was decided upon because of higher residual N in the soil (Table 2.4). The two middle rows were sampled at final harvest for yield, total biomass, HI and N related traits.

CROP MANAGEMENT

The second field experiment was sown by hand between the 4th and 6th of September 2006. Nitrogen fertiliser was applied to High N blocks only (50 kg N/ha) on the 30th of March 2007 with the remaining 100 kg N/ha to the same high N blocks on the 18th of May 2007. Pest and disease treatments were carried out according to commercial practise, including slug treatments, and plots were sprayed with Hallmark Zeon for cabbage stem flea beetle on the 31st of September 2006.

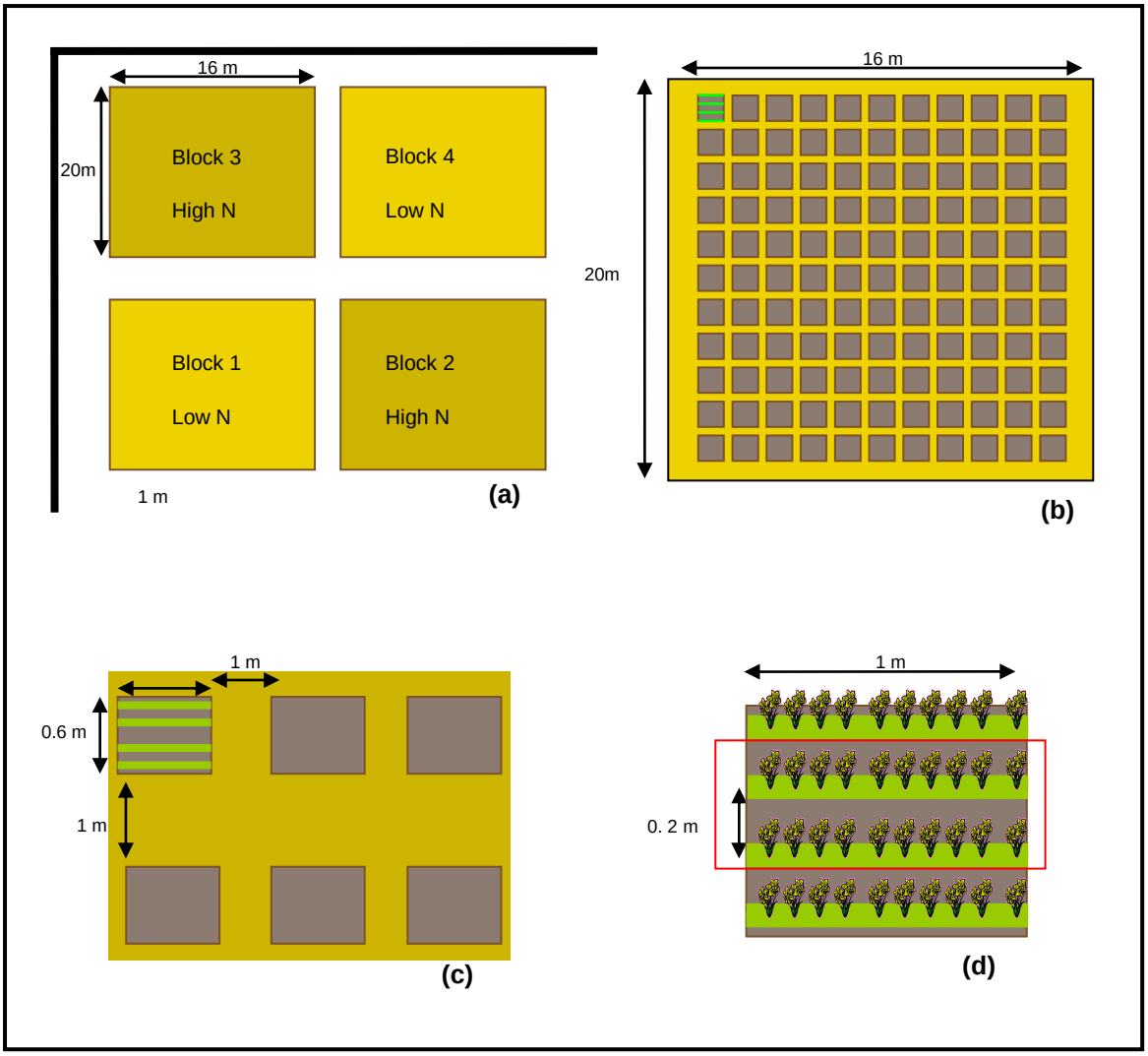


Figure 2.3. Experimental design for the field trial in 2006/07. (**a**) field location and distribution of N treatments, (**b**) distribution of the 94 TNDH lines, the 2 parental lines and 4 control plots (variety Castille) in a randomised design, (**c**) distribution and measurements of each block, and (**d**) plot dimensions consisting of 4 rows of 10 plants each.

Punch C (carbendazim and flusilazole) was sprayed for light leaf spot and phoma control on the 23rd of October and 29th November 2006, and Hallmark Zeon was applied on the 11th of April 2007 for pollen beetle control (Appendix 2 B).

SOIL MEASUREMENTS

On the 26th of February 2007 soil samples were taken, before fertiliser application. All blocks were sampled by bulking four soil cores from each block. Sampling followed the same procedure as in 2006, at three depth intervals: 0-30, 30-60 and 60-90 cm. The samples were sent to a commercial laboratory for analysis of dry matter, NO_3^-, NH_4^+, and available N. Results summarising the soil analysis are shown in Table 2.4.

Table 2.4. Soil analysis results for experimental site prior to N treatments applied in 2006. Sample A was a bulk of 8 sub-samples from the 2 blocks treated at High N after soil analysis and sample B was a bulk of 8 sub-samples from the 2 blocks treated at Low N.

Bulk sample	Depth	Dry matter (w/w%)	NO_3^- (mg/kg)	NH_4^+ (mg/kg)	Available N (Kg N/ha)
A	0-30	78.8	3.51	1.36	19.5
	30-60	83.7	1.3	1.07	9.5
	60-90	87.3	2.87	0.68	14.2
	total				43.2
B	0-30	78.5	2.65	0.77	13.7
	30-60	85	1.06	0.97	8
	60-90	87.9	1.71	0.57	9.1
	total				30.8

PLANT MEASUREMENTS

CROP ESTABLISHMENT

Germination rates were recorded during the second week of November 2006, as a percentage of the number plants emerged from the number of germinated seed in each block.

RECORDING OF FLOWERING TIME

Flowering time was recorded as the number of days after the plants were sown (DAS) to the opening of the first flower on the terminal raceme.

Final harvest measurements

At final harvest all plants from the 2 middle rows were removed from the field by cutting at the base of the stem and were bagged and stored in a greenhouse until yield and physiological parameters were analysed. Plants were then split into component parts of seed, support stem, and remainder i.e. pod bearing branches and hulls. Traits analysed in 2007 included all nitrogen traits i.e. seed and plant N concentrations, NUpE, NUtE, NUE and NHI together with seed yield, total biomass and harvest index. Seed samples were divided into 2 parts with one sent to the John Innes Centre in Norwich for oil content and protein determination by NMR (Nuclear Magnetic Resonance) spectroscopy.

Prior to N content determination, each plant fraction was milled separately as described in section 2.2.1 and 0.10g was used for analysis. Seed samples were ground for 30 sec. using a coffee grinder (Braun, model KSM2) and each seed sample was then sub-sampled with 0.25g used for N analysis by the LECO combustion method described previously

Table 2.5. List of characters measured and determined at final harvest in 2007.

Trait	Units	Description
Flowering	DAS	Days After Sowing
Biomass	g plant^{-1}	All plant parts separately and then summed
Plant N conc^{n}.	mg g^{-1}	All plant parts separately, measured by LECO
NUE		Multiplying NUpE by NUtE
NUpE	gN g^{-1}	Ratio of total above ground N to applied fertiliser amount and residual soil N
NUtE	g gN^{-1}	Ratio of SY to total above ground N
HI		ratio of grain yield to total above ground biomass
NHI		Ratio of grain N yield to total above ground N

STATISTICAL ANALYSIS

Data analysis was the same as previously carried out for the 2005/06 trial. Analysis of variance was performed using the Linear Mixed Model from Genstat for Windows 9th Edition, considering the 94 TNDH lines, the 2 treatments (High and Low N) and 2 Blocks paired, as Low and High N replicates respectively. Pearson's phenotypic correlation and broad-sense heritability were determined as described previously. A multivariate PCA analysis was carried out with a standardised matrix of 94 TNDH lines by 11 traits.

2.3. RESULTS

2.3.1. EXPERIMENT 1. FIELD TRIAL 2005/06

A wide range of values was observed in the TNDH lines for all traits measured in 2006 Differences between the parental lines Tapidor and Ningyou7 were small for most parameters. The mean values of the TNDH lines for the traits studied in 2006 and the parental lines Tapidor and Ningyou7 showed a marked difference between High and Low N treatments for most of the traits (Appendix tables 6-9). To be pointed out is that in 2006 Ningyou7 died after flowering at Low N treatment and the values shown on the histogram are only for Tapidor and the mean for the TNDH population.

Large differences in growth were observed between Blocks 1 and 3 and 2 and 4, consequently High N Block 1 was paired with Low N Block 3 and High N Block 4 with the Low N treatment in Block 2 for all analyses. Data for all blocks will be presented with the exception of N traits and histograms were only representing Block 1 as High N and Block 3 as Low N instead of averaging the 2 High N blocks and the 2 Low N blocks. The reason for that is no replication for the N traits was available.

YIELD AND YIELD COMPONENTS, TOTAL BIOMASS AND HARVEST INDEX

Higher values were observed at the High N treatment for TW and SY, and generally bigger plants were obtained in the High N treatment (Fig 2.4). Similar differences between High and Low N were observed for both Tapidor and Ningyou7. Yield traits such HI, TSW and SN/P did not show major differences for the TNDH lines across environments and were rather constant. Tapidor showed higher HI (0.26-0.27) at High N than at Low N (0.22-0.19), and was also higher than the highest value for Ningyou7 (0.19). Tapidor had higher SN/P at High N, while Ninyou7 presented higher SN/P at Low N supply. Ningyou7 had higher TSW values than those for Tapidor, but the trait did not show differences related to N treatment. For TW and SY Tapidor was very close to the average value for the TNDH population at High N, but not at the Low N treatment.

Total plant biomass was an average of 47.37 and 41.37 g plant^{-1} and SY was 12.12 and 10.48 g plant^{-1} for the population at High/Low N i.e. Blocks 1 and 3 respectively. Similar values were found for TW

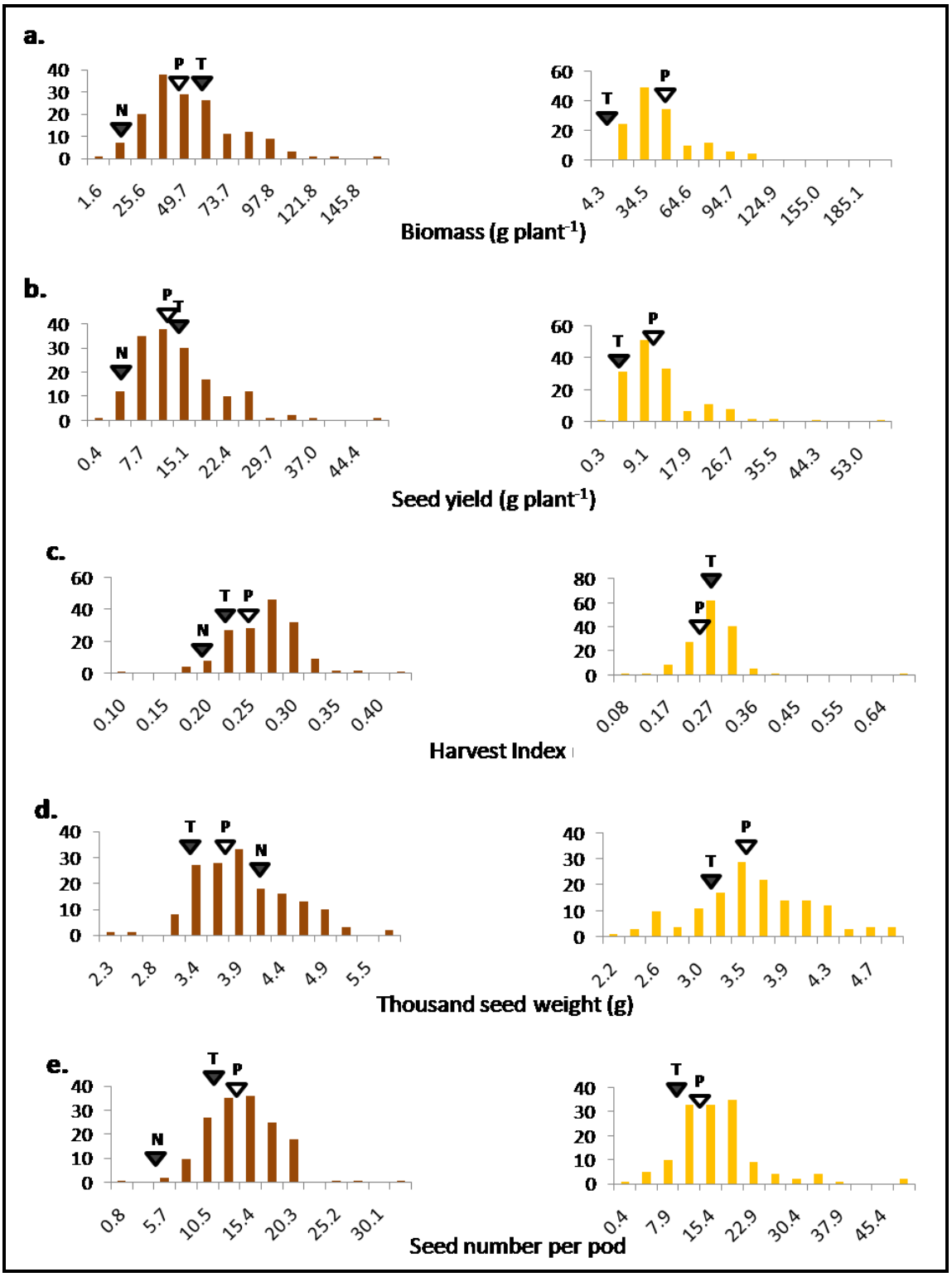


Figure 2.4. Frequency distribution of a) biomass, b) seed yield, c) harvest index, d) 1000 seed weight and e) seed number per pod in the 174 TNDH oilseed rape lines grown in 2005/06. Mean value for the TNDH lines ▽ as well as the parental lines, `Tapidor' and `Ningyou7' ▼ are shown at both High ■ and Low ■ nitrogen.

and SY for the High/Low Blocks 4 and 2, i.e. biomass of 31.7 and 20.49 g plant^{-1} and SY 8.63 and 5.69 g plant^{-1} respectively.

Pod number and pod survival ratios did not seem to follow a particular pattern at High or Low N treatment.

For both TW and SY little transgressive segregation was evident at both High and Low N treatments (Fig. 2.6). Harvest Index, TSW and SN/P showed transgressive segregation at both for higher and lower trait values.

NITROGEN AND NITROGEN DERIVED TRAITS AND OIL

Parental lines Tapidor and Ningyou7 showed little differences between them and to the average population for the nitrogen traits analysed. At High N for seed N. NUpE and NUE, Tapidor had the highest values and Ningyou7 the lowest with the population mean in between. For NHI, Tapidor inverted positions with the average value for the TN lines and Ningyou7 remained as having lower values than both Tapidor and the population mean. For both residual N and NUtE, Tapidor had lowest values followed by Ningyou7 and finally the average for the population.

All N related traits analysed presented very high variation showing a wider range at Low N than at High N. The range between the highest and the lowest values for NUE (g g^{-1}) was especially high at Low N with values differing by 164 g g^{-1}, but only by 44.5 g g^{-1} at High N. TNDH lines demonstrated transgressive segregation on higher values of all N traits in response to High N. Plant N concentration, NUtE and NHI also showed transgressive segregation for the lower values at High N. Mean values for plant N concentration (mg g^{-1}) were 5.27 for High N (Fig. 2.6) and 4.59 for Low N. The mean value for seed N concentration for Low N was 26.94, and higher than the mean at High N of 26.22 mg g^{-1}.

For NUpE and NUE mean values were higher at Low N than at High N. Mean value for NUpE at high N was 1.41 gN g^{-1} and the corresponding value for Low N was 3.58 units higher at 4.99 gN g $^{-1}$. Nitrogen Use Efficiency mean values were also much higher at Low N when compared to High N, being 11.42 High N and 40.2 at Low N in 2006 (Appendices 7-10 and 11-14).

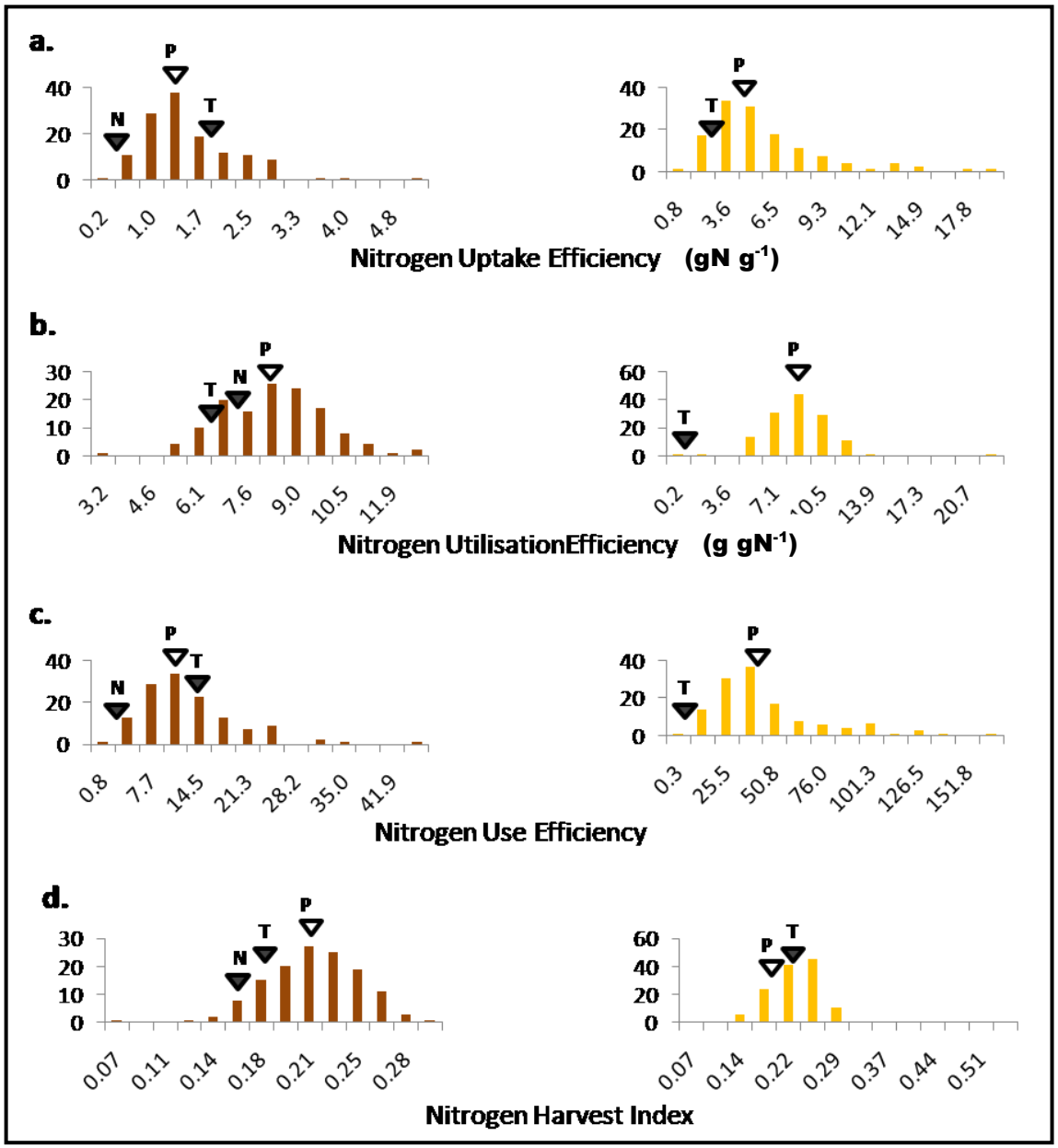


Figure 2.5. Frequency distribution of a) Nitrogen Uptake Efficiency, b) Nitrogen Utilisation Efficiency c) Nitrogen Use Efficiency and d) Nitrogen Harvest Index in the 174 TNDH oilseed rape lines grown in 2005/06. Mean values for the TNDH lines ▽ as well as the parental lines, `Tapidor' and `Ningyou7' ▼ are shown at both High ■ and Low ■ nitrogen.

Traits such as NUtE and NHI did not show clear differences between High and Low N treatments and were rather constant. With mean values for NUtE of 7.9 and 7.8 g g N^{-1} for High and Low N respectively the NHI was the same at 0.21 for both High and Low N treatments (Fig.2.8).

Many of the traits analysed at High N treatments in 2005/06 followed a normal distribution at $P<0.05$. The traits not showing a normal distribution were most of the traits at Low N. Data was not transformed as QTL software had an analysis option for data not following normality.

Ningyou7 had an oil content of 46.3%, which was much lower than Tapidor. Oil content followed a typical normal distribution at Low N. Oil content for Tapidor was 49.3% at Low N, significantly lower than at High N. Oil content analysis presented different distribution at High and Low N treatments. Frequency distribution at High N revealed two peaks, one at 53.1 % coinciding with the population mean and a second one at 55.5% coinciding with Tapidor (Fig. 2.6).

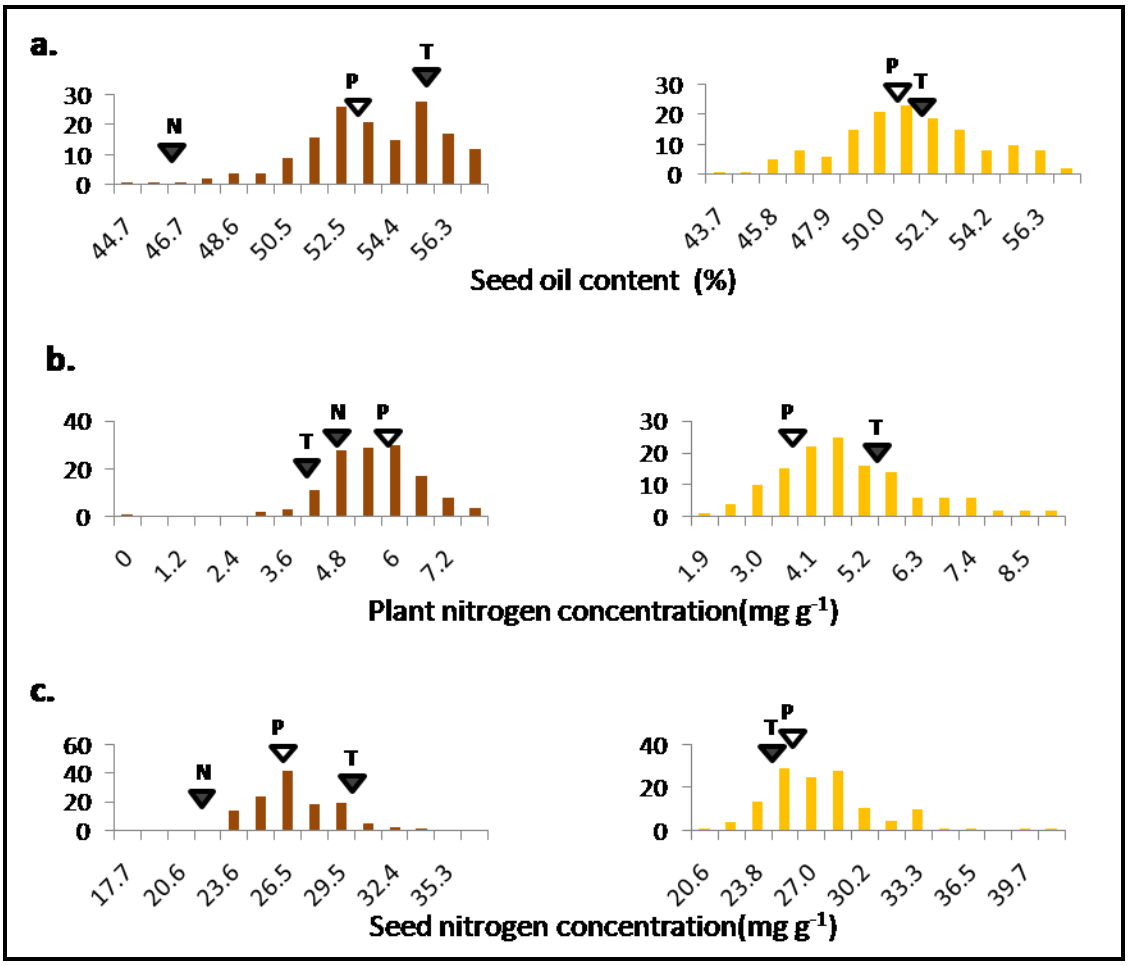


Figure 2.6. Frequency distribution of a) oil content, b) plant nitrogen concentration and c) seed N concentration in the 174 TNDH oilseed rape lines grown in 2005/06. Mean value for the TNDH lines ▽ as well as the parental lines, `Tapidor' and `Ningyou7 ▼ are shown at both High ■ and Low ■ nitrogen.

GERMINATION, FLOWERING AND CHLOROPHYLL CONTENT OF BRACTS AND LEAVES

Seed germination was an average of 65.5% of seed sown (Appendix 3). Only 13 lines of the 188 plus the 2 parental lines had germination rates below 50%, and most of these were around 45%, except for TN19 which had a very low germination of 20%. Other lines with germination rates below 50% were TN153 with 37.5% germination, TN61, 96, 133, 147 with 41.67%, TN27, 67, 105, 117, 132 and 160 with germination rate of 45.83%, and TN42 with 48% germination. Also 13 TNDH lines showed germination rates above 80%, and in particular TN126 had 100% germination. Other TN lines had very high germination rates, of 83.33% (TN4, 23, 41, 45, 69, 93, 125, 129, 167 and 171), 86.96% (TN149), 87.5% (TN185), and 90.91% (TN113). Germination in Blocks 2 and 4 was higher (71.81% and 71.29% respectively) than in Blocks 1 and 3 (61.60% and 58.02% respectively). Germination rates of Tapidor and Ningyou7 were 75% and 62% respectively, the first one being higher than the population mean and the latter slightly lower.

Winter survival ratio did not show major differences, and was around 65% in all blocks, ranging from 63.03% in Block 1 and 69.02% in Block 3. Winter survival for both Tapidor and Ningyou7 was 100%. Two TNDH lines were severely affected by winter conditions and had very low survival rates: TN19 had had a germination rate of 100% but only 20.83% of survival and TN153 37.5%. Other lines had survival rates between 40 and 50%.

Stem canker affected all blocks and controls, but with different intensity (Appendix 5). Quantitative analysis carried out on Block 1 showed that TNDH lines had an index of 1.979 and the control lines an index of 1.166 points when assessed using an index of 0-3 where 3 was severe infection. Stem canker was evident not as much by the presence of cankers but by lesions both at the base and higher up the stem.

Bird damage happened in the early maturing lines of all blocks, but was more pronounced in Block 4, with about 20% of the seed being lost from most lines in this block.

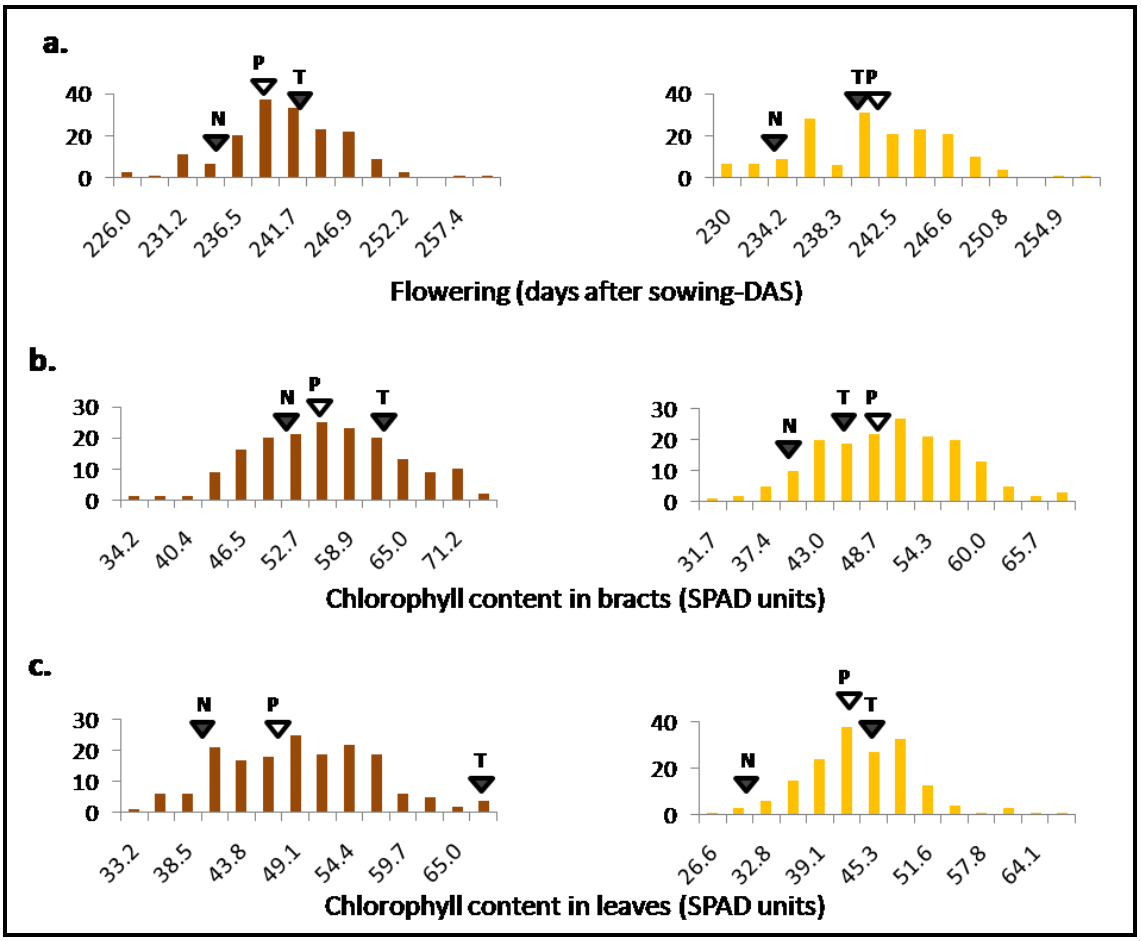


Figure 2.7. Frequency distribution of a) flowering, b) chlorophyll in bracts and c) chlorophyll in leaves in the 174 TNDH oilseed rape lines grown in 2005/06. Mean value for the TNDH lines ▽ as well as the parental lines, `Tapidor' and `Ningyou7' ▼ are shown at both High ■ and Low ■ nitrogen.

Relatively constant across environments was FDAS, which did not show any difference between High or Low N treatments. At High N the block averages for FDAS were 239.63 and 243.93, and 240.88 and 240.87 at Low N. However, high variation was found between the number of days between first and last TN line to flower in a block, showing marked transgressive segregation on both sides (Fig.2.7). Flowering duration ranged from 34 and 27 days at High N and 30 and 36 days at Low N, indicating that the flowering time differed by a minimum of 4 weeks between the first TNDH and the last one to flower in both N treatments, showing high variability between individual plants. The parental lines showed differences in flowering times. Ningyou7 was the first one to flower, at 234, 231, 233, and 247 days after sowing, whilst Tapidor flowered between 7 and 11 days later, at 242, 241, 240 and 258 in Blocks 1 to 4 respectively.

Chlorophyll in bracts (CB) and leaves (CL) was higher at High N than at Low N, and CB was always higher than CL. Mean values (SPAD units) for CB were 54.91 and 51.78 at High N, whereas at Low N mean values were 47.17 and 49.19. The mean values for CL were 48.26 and 44.89 at High and 40.29 and 42.43 at Low N. Frequency distribution for CL had 2 peaks, one coincided with the average population and the other one with Ningyou7. Tapidor and Ningyou7 also showed the same treatment response, showing higher values at High N than at Low N and bracts always had higher chlorophyll levels than leaves. Tapidor showed higher chlorophyll values than Ningyou7 in both N treatments as well as higher values than the average of the TNDH population at High N being 65.1 and 61.1 for CB at High N and 62.4 and 59.3 at Low N. There was transgressive segregation both for High and Low N in CB, as well as for CL in the Low N treatment. For High N there was no transgressive segregation for the higher CL values, but there was for the lower values (Fig. 2.7).

ARCHITECTURAL TRAITS

Architectural traits such as total length (TL), foot length (FL) and branch number (BN) presented higher values at High N (Fig 2.8) than at Low N. Both the parental lines had higher values for all 3 traits at High N than at Low N. For TL there was no transgressive segregation at the higher values as Tapidor had the highest TL amongst the lines at High N (Fig. 2.5). Frequency distribution for TL had 2 peaks, one allele from Ningyou7 and the other one coincided with the population mean which was close to the Tapidor allele. Both FL and BN did not follow a normal distribution and had transgressive segregation in both directions at both High and Low N. Foot length of Tapidor was very close to the population mean at Low N whereas is was not the case at High N.

Total plant height (TL) was 118.53 and 93.18 cm in Blocks 1 and 4 respectively (High N), which was slightly higher than 111.9 and 91.21 cm in the corresponding Blocks 3 and 2. The same pattern was observed for foot length in Blocks 1 and 3 which varied from 27.66 to 26.15 cm respectively and also branch number (BN) was higher in Block 1 (average of 6.8 branches/plant) than in Block 3 (6.0 branches per plant). However, foot length was higher in Block 2 (24.78 cm at Low N) than in Block 4 (14.91 cm at High N), whereas BN was higher in Block 4 (5.1 branches per plant) than in Block 2 (4.3 branches per plant).

The average TNDH population and Tapidor had higher values than Ningyou7 in all blocks and for all 3 traits. For example, Tapidor had a TL of 136.00 cm, FL of 47.00 cm and 8.0 branches per plant in Block 1, whereas Ningyou7 was 95.00 cm tall, FL was 6.00 cm and BN 3.0.

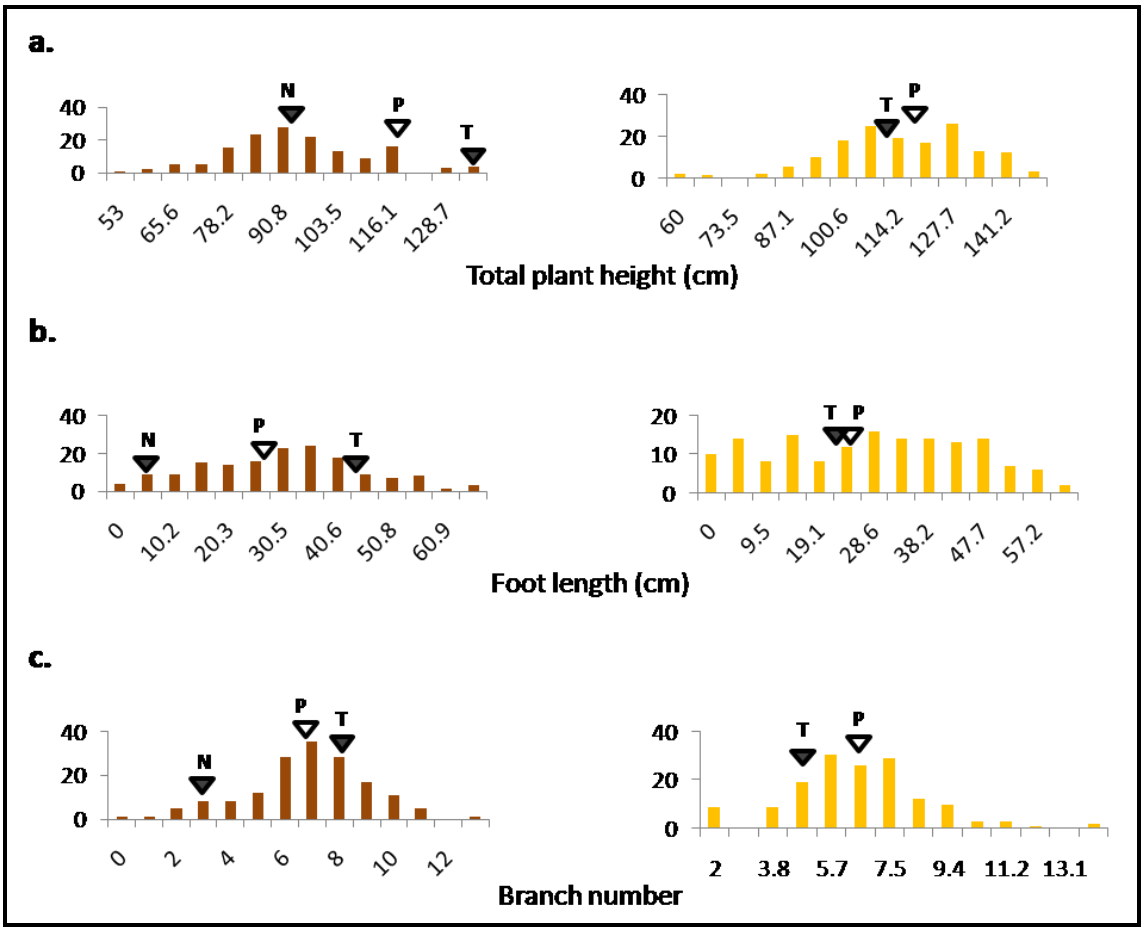


Figure 2.8. Frequency distribution of a) plant height, b) foot length and c) branch number in the 174 TNDH oilseed rape lines grown in 2005/06. Mean value for the TNDH lines ▽ as well as the parental lines, `Tapidor' and `Ningyou7' ▼ are shown at both High ■ and Low ■ nitrogen.

Analysis of Variance (ANOVA)

Analysis of variance was performed on all characters (except N traits due to lack of replication) analysed in 2005/06 using nitrogen treatment as a fixed variable, and line and the interactions of line and nitrogen and replicate and nitrogen as random variables. For the N traits the analysis was incomplete due to lack of replication; however, results are presented as an estimate.

In 2005/06, most of the traits showed significant differences for genotype, thus justifying the validity of the population choice for study. However, it was not the case for most of yield traits (except 1000-seed weight), also for branch number and pod counts.

For yield and yield derived traits, the analysis of variance showed genotypes did not show significant differences between one another for all yield traits except for 1000-seed weight (Table 2.6). The traits did not show significant differences in their response to nitrogen with the exception of harvest index.

Table 2.6. Results for the mixed model ANOVA, the Residual Maximum Likelihood (REML) for total above ground biomass (TW), seed yield (SY) and harvest index (HI), 1000-seed weight (TSW) and seed number per pod (SNP) in 2005/06. Levels of significance were ns,*, **, *** for no significance, <0.1, <0.01 and <0.001 respectively.

TW				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.430	1	0.43	0.511 ns
Random term	**component**	**s.e.**		**Z-test**
LINE	16.800	25.300		0.664 ns
LINE.NITROGEN	10.000	40.200		0.249 ns
NITROGEN.REP	167.700	171.200		0.980 ns
RESIDUAL	516.100	43.800		11.783
SY				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.600	1	0.6	0.437 ns
Random term	**component**	**s.e.**		**Z-test**
LINE	2.650	2.420		1.095 ns
LINE.NITROGEN	-0.900	3.770		-0.239 ns
NITROGEN.REP	8.420	8.760		0.961 ns
RESIDUAL	51.340	4.320		11.884
HI				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	1.300	1	1.3	0.254 ns
Random term	**component**	**s.e.**		**Z-test**
LINE	0.001	0.184		0.004 ns
LINE.NITROGEN	1.888	0.267		7.075 ***
NITROGEN.REP	0.015	0.020		0.746 ns
RESIDUAL	0.649	0.062		10.434
TSW				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.990	1	0.99	0.319 ns
Random term	**component**	**s.e.**		**Z-test**
LINE	0.160	0.025		6.371 ***
LINE.NITROGEN	0.010	0.016		0.601 ns
NITROGEN.REP	0.025	0.026		0.946 ns
RESIDUAL	0.191	0.017		11.369
SNP				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.050	1	0.05	0.823 ns
Random term	**component**	**s.e.**		**Z-test**
LINE	1.840	2.050		0.898 ns
LINE.NITROGEN	3.660	3.110		1.177 ns
NITROGEN.REP	1.590	1.840		0.864 ns
RESIDUAL	33.090	3.040		10.885

The analysis of variance for N traits was carried out without replication, for what some of the parameters analysed have no value (Table 2.7 and 2.8). The analysis is presented here as reference. The traits that showed significant genotypic differences were NUtE, NHI, plant nitrogen concentration and total plant N concentration.

Table 2.7. Results for the mixed model ANOVA, the Residual Maximum Likelihood (REML) for N uptake efficiency (NUpE), N utilisation efficiency (NUtE), N use efficiency (NUE) and N harvest index (NHI) in 2005/06. Levels of significance were ns,*, **, *** for no significance, <0.1, <0.01 and <0.001 respectively.

NUpE				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	1.180	1	1.18	0.277 ns
Random term	**component**	**s.e.**		**Z-test**
LINE	0.296	0.526		0.563 ns
NITROGEN.REP	5.395	aliased		
RESIDUAL	5.395	0.689		7.830
NUtE				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.010	1	0.01	0.918 ns
Random term	**component**	**s.e.**		**Z-test**
LINE	1.842	0.461		3.996 ***
NITROGEN.REP	2.416	aliased		
RESIDUAL	2.416	0.349		6.923
NUE				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.990	1	0.99	0.320 ns
Random term	**component**	**s.e.**		**Z-test**
LINE	35.700	43.000		0.830 ns
NITROGEN.REP	417.700	aliased		
RESIDUAL	417.700	54.300		7.692
NHI				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.000	1	0	0.998 ns
Random term	**component**	**s.e.**		**Z-test**
LINE	0.001	0.000		4.479 ***
NITROGEN.REP	0.001	aliased		
RESIDUAL	0.001	0.000		6.849

Oil content results (Table 2.8) showed both significant genotypic responses as well as significant genotype*nitrogen interaction (P<0.1).

Table 2.8. Results for the mixed model ANOVA, the Residual Maximum Likelihood (REML) for percentage of oil content (OIL), plant N concentration (NP), seed N concentration (NS) and total N concentration (NT) in 2005/06. Levels of significance were ns,*, **, *** for no significance, <0.1, <0.01 and <0.001 respectively.

OIL				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.180	1	0.18	0.667 ns
Random term	**component**	**s.e.**		**Z-test**
LINE	3.339	0.517		6.458 ***
LINE.NITROGEN	-0.454	0.357		-1.272 *
NITROGEN.REP	1.967	2.003		0.982 ns
RESIDUAL	5.144	0.442		11.638
NP				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.190	1	0.19	0.665 ns
Random term	**component**	**s.e.**		**Z-test**
LINE	0.433	0.164		2.640 **
NITROGEN.REP	1.168	aliased		
RESIDUAL	1.168	0.161		7.255
NS				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.03	1	0.03	0.861 ns
Random term	**component**	**s.e.**		**Z-test**
LINE	0.513	0.910		0.564 ns
NITROGEN.REP	8.682	aliased		
RESIDUAL	8.682	1.153		7.530
NT				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.000	1	0	0.985 ns
Random term	**component**	**s.e.**		**Z-test**
LINE	1.550	1.130		1.372 *
NITROGEN.REP	10.110	aliased		
RESIDUAL	10.11	1.340		7.545

Results of variance (Table 2.9) for flowering, chlorophyll content in bracts and in leaves showed significant differences in genotype (Line). Moreover, chlorophyll in bracts also showed a significant difference for N*line interaction, showing a different response of different genotypes to the two N treatments.

Table 2.9. Results for the mixed model ANOVA, the Residual Maximum Likelihood (REML) for flowering (FDAS), chlorophyll in bracts (CB) and chlorophyll in leaves (CL) in 2005/06. Levels of significance were ns,*, **, *** for no significance, <0.1, <0.01 and <0.001 respectively.

FDAS				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.300	1	0.3	0.583 ns
Random term	**component**	**s.e.**		**Z-test**
LINE	8.700	1.660		5.241 ***
LINE.NITROGEN	0.090	1.480		0.061 ns
NITROGEN.REP	4.630	4.760		0.973 ns
RESIDUAL	21.770	1.680		12.958
CB				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	7.450	1	7.45	0.006 **
Random term	**component**	**s.e.**		**Z-test**
LINE	14.160	3.320		4.265 ***
LINE.NITROGEN	-5.070	3.960		-1.280 *
NITROGEN.REP	3.200	3.590		0.891 ns
RESIDUAL	51.770	4.710		10.992
CL				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	6.390	1	6.39	0.011 *
Random term	**component**	**s.e.**		**Z-test**
LINE	10.570	2.580		4.097 ***
LINE.NITROGEN	-1.360	2.890		-0.471 ns
NITROGEN.REP	3.990	4.260		0.937 ns
RESIDUAL	35.830	3.250		11.025

Architectural traits analysed in 2005/06 such as plant height and foot length showed significant differences for the genotypes as well as for their interaction with N treatment (Table 2.7). However,

branch number did not show any significant difference in the ANOVA analysis, neither for genotype nor for their interaction with nitrogen.

Table 2.10. Results for the mixed model ANOVA, the Residual Maximum Likelihood (REML) for plant height (TL), foot length (FL) and branch number (BN) in 2005/06. Levels of significance were ns,*, **, *** for no significance, <0.1, <0.01 and <0.001 respectively.

TL				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.070	1.000	0.07	0.794 ns
Random term	**component**	**s.e.**		**Z-test**
LINE	50.000	12.600		3.968 ***
LINE.NITROGEN	-23.500	15.300		-1.536 *
NITROGEN.REP	268.300	269.800		0.994 ns
RESIDUAL	238.900	19.700		12.127
FL				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.410	1	0.41	0.520 ns
Random term	**component**	**s.e.**		**Z-test**
LINE	37.700	10.000		3.770***
LINE.NITROGEN	-21.700	12.800		-1.695*
NITROGEN.REP	40.300	41.500		0.971 ns
RESIDUAL	194.300	16.200		11.994
BN				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.410	1	0.41	0.523 ns
Random term	**component**	**s.e.**		**Z-test**
LINE	0.029	0.174		0.167 ns
LINE.NITROGEN	-0.115	0.299		-0.385 ns
NITROGEN.REP	1.499	1.527		0.982 ns
RESIDUAL	4.285	0.354		12.105

Total number of pods and total of fertile pods on main raceme were analysed for variance and did not show significant responses or interactions with nitrogen. As with the yield traits, one possibility for these results was the environmental effects observed in Blocks 2 and 4.

Table 2.11. Results for the mixed model ANOVA, the Residual Maximum Likelihood (REML) for total pod number (TP), total fertile pods in main raceme (PMF) in 2005/06. Levels of significance were ns,*, **, *** for no significance, <0.1, <0.01 and <0.001 respectively.

TP				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.590	1	0.59	0.441 ns
Random term	**component**	**s.e.**		**Z-test**
LINE	-362.000	808.000		-0.448 ns
LINE.NITROGEN	974.000	1391.000		0.700 ns
NITROGEN.REP	13012.000	13126.000		0.991 ns
RESIDUAL	16737.000	1431.000		11.696
PMF				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	1.120	1	1.12	0.289 ns
Random term	**component**	**s.e.**		**Z-test**
LINE	-5.400	11.600		-0.466 ns
LINE.NITROGEN	15.500	21.300		0.728 ns
NITROGEN.REP	14.300	16.000		0.894 ns
RESIDUAL	180.800	20.700		8.734

NARROW-SENSE HERITABILITY

Narrow-sense heritability (h^2) was calculated for all the traits analysed in 2005/06 with the exception of N related traits (Table 2.12). Values ranged from between 0.0% for HI to 63% for flowering (FDAS). The most significant values apart from oil content were for foot length (FL) at 62% and both chlorophyll content in bracts and leaves at 48% and 55%, respectively. Oil content had a high heritability value of 57%. Traits with very low heritability were harvest index, branch number, 1000-seed weight and seed number per pod, all with values between 0 and 0.02. Both total above ground biomass and seed yield had values of 39% and 37% respectively.

Table 2.12. Narrow sense heritability (h^2) for traits analysed in 2005/06.

TRAIT	HERITABILITY
FDAS	0.63
CB	0.48
CL	0.55
TL	0.18
FL	0.62
BN	0.01
TP	0.28
PMF	0.26
TW	0.39
SY	0.37
HI	0.00
TSW	0.02
SNP	0.02
OIL	0.57

PHENOTYPIC CORRELATION ANALYSIS

For all 174 lines phenotypic correlations were performed at both High N and Low N respectively for all traits analysed. There were 58 *r* values statistically significant at the 5% level for both N treatments, with values ranging from 0.2 to 0.98. Moreover, there were 39 statistically significant correlations ($P<0.05$) which only appeared at either High or Low N treatment (Table 2.13).

The highest correlation values that were consistent across treatments were those between total weight and seed yield with values of 0.97 and 0.96 at High and Low N respectively.

There were also significant and very high positive correlations between NUpE and NUE (0.95 and 0.91) and between NUtE and NHI (0.86). Two major trait associations were found: one relating TW, SY, NUpE and NUE, all correlations between them were positive ($r>0.90$) and significant at the 1% level.

The other trait association showed positive correlations between HI, NHI, and NUtE, but negative with plant and seed N concentrations, most of them with a level of confidence of 1%. There was a significant ($P<0.01$) correlation between HI and plant N concentration ($r=-0.18$), but only for the High N treatment.

A strong negative correlation was found between FDAS and TW ($r=-0.16$ and -0.37 for High and Low N respectively), and FDAS and SY ($r=-0.16$ and -0.37 for High and Low N respectively), both at $P<0.05$ for High N and $P<0.01$ for Low N. Traits studied in 2005/06 such as CB, CL, plant architecture traits (e.g. plant height, branch number, etc), and yield traits (e.g. TSW, seed number peer pod, etc.) also showed interesting correlations. For example, plant height and branch number presented significant positive correlations to both NUpE and NUE and a significant negative correlation with foot length ($P<0.01$). Also positively correlated with NUpE and NUE were total number of pods per plant and % of pod fertility per plant. Thousand seed weight was positively correlated with NUtE ($r=0.23$ and 0.27, $P<0.01$ at High and Low N respectively), and seed number per pod was positively correlated with all NUE related traits, all with a level of significance of 1%. Chlorophyll in bracts was positively correlated with CL ($r=0.44$ and 0.51, $P<0.01$) at both High and Low N respectively. Both CB and CL were also positively correlated ($P<0.01$) with FDAS, but only in the High N treatment, CB was positively correlated with foot length ($r=0.25$ and 0.19), and negatively correlated with seed number per pod ($r=-0.18$ at High N only).

Oil content was positively correlated with HI at both High and Low N treatments ($r=0.38$ and 0.39, $P<0.001$); and with TL, %F, TW, SY and SN/P at High N only (all at $P<0.001$, except %F which was at $P<0.01$). Oil content was positively correlated at Low N only with TSW and CB ($P<0.01$), and negatively correlated with FDAS ($r=-0.23$, $P<0.01$). Oil content presented different correlations with all traits related to N. It was negatively correlated to seed ($r=-0.43$) and plant ($r=-0.20$) N concentrations, but only at Low N. Oil content was positively correlated with other N traits i.e. NUtE, NUE and NHI at both High and Low

N (all at *P*<0.001 except for NUE at Low N with *P*<0.01). Oil content was also positively correlated with NUpE at High N (*P*<0.001), but did not show any correlation at Low N for NUpE.

Other phenotypic correlations among traits not directly related to NUE were also observed in 2006. Total biomass was positively correlated with plant height, branch number, seed number per pod and total number of pods per plant and negatively correlated with foot length and FDAS. Seed yield followed the same correlation pattern as TW. Plant height, in turn, showed obvious positive correlations with foot length, branch number, TW, SY, total number of pods per plant and % of fertile pods per plant. Seed number per pod presented both positive and negative correlations with total plant height at High and Low N: at High N there was a positive correlation (r=0.37) significant at P<0.01 and at Low N a negative correlation (r=-0.25) with the same level of significance. The same happened between seed number per pod and foot length, both correlation coefficients shared similar values of 0.22 and -0.20 for High (positive correlation) and Low N (negative correlation) respectively, and the levels of significance were 1% and 5% correspondingly. The correlation between NUE and SY was 0.99 for the Low N treatment and 1.0 for the High N treatment.

All traits measured in Block 1 were correlated with stem canker (Appendix 6). The Pearson's correlation results indicated that none of the correlations between all traits and stem canker were significant, thus indicating stem canker would not explain the environmental effects observed in the different TNDH lines. However stem canker was only measured in Block 1, therefore there is no other data that would either confirm or refute this observation.

Table 2.13. Phenotypic correlations for all TNDH lines both at both High N and Low N for all traits analysed in 2005/06, *,** significant at 5% and 1% level, respectively. coefficient for High N are presented on the top line [a], and for Low N on the bottom line [b]. Traits are FL (foot length), BN (branch number), F-DAS (flowering), TP (total number o (% of fertility), TW (total above ground biomass), SY (seed yield), HI (harvest index), TSW (1000-seed weight), SN/P (seed number per pod), CB (chlorophyll content in (chlorophyll content in leaves), OIL (seed oil content), [NP] (plant N concentration), [NS] (seed N concentration), NUpE (N uptake efficiency), NUtE (N utilisation efficiency), efficiency) and NHI (N harvest index).

	TL	FL	BN	F-DAS	TP	%F	TW	SY	HI	TSW	SN/P	CB	CL	OIL	[NP]	[NS]	NUpE	NUtE	NUE
FL	**0.38**** [a]																		
	0.34** [b]																		
BN	**0.25****	-0.01^{ns}																	
	0.35**	0.13^{ns}																	
F-DAS	0.09^{ns}	0.14^{ns}	-0.10^{ns}																
	0.08^{ns}	0.10^{ns}	-0.14^{ns}																
TP	0.21*	0.00^{ns}	0.21*	-0.17*															
	0.44**	-0.14^{ns}	**0.61****	-0.15^{ns}															
%F	**0.27****	0.03^{ns}	0.186*	-0.08^{ns}	-0.17^{ns}														
	0.20*	0.02^{ns}	0.08^{ns}	-0.04^{ns}	0.02^{ns}														
TW	**0.52****	-0.17*	**0.50****	-0.16*	**0.27****	0.351**													
	0.19*	**-0.23****	**0.30****	**-0.37****	**0.41****	0.11^{ns}													
SY	**0.47****	-0.14^{ns}	**0.46****	-0.16*	**0.28****	0.31**	**0.97****												
	0.19*	-0.19*	**0.35****	**-0.37****	**0.42****	0.12^{ns}	**0.96****												
HI	0.09^{ns}	0.05^{ns}	0.10^{ns}	-0.13^{ns}	0.20*	-0.02^{ns}	**0.27****	**0.47****											
	-0.02^{ns}	0.06^{ns}	0.11^{ns}	-0.24**	0.05^{ns}	0.12^{ns}	0.21*	**0.41****											
TSW	0.05^{ns}	-0.04^{ns}	0.05^{ns}	-0.04^{ns}	0.10^{ns}	-0.01^{ns}	0.08^{ns}	0.10^{ns}	0.13^{ns}										
	0.14^{ns}	0.10^{ns}	0.19*	**-0.25****	0.10^{ns}	0.08^{ns}	0.18*	**0.22****	**0.28****										
SN/P	**0.37****	**0.22****	0.13^{ns}	0.08^{ns}	0.11^{ns}	-0.09^{ns}	**0.41****	**0.49****	**0.56****	**-0.24****									
	-0.25**	-0.20*	-0.21*	-0.12^{ns}	**-0.24****	-0.04^{ns}	**0.38****	**0.39****	**0.31****	-0.08^{ns}									
CB	0.05^{ns}	**0.25****	-0.05^{ns}	0.18*	0.16^{ns}	-0.11^{ns}	-0.04^{ns}	-0.01^{ns}	0.06^{ns}	0.09^{ns}	0.18*								
	0.01^{ns}	0.19*	0.16^{ns}	0.09^{ns}	0.09^{ns}	0.12^{ns}	0.11^{ns}	0.16^{ns}	**0.26****	0.06^{ns}	0.12^{ns}								
CL	0.08^{ns}	0.19*	-0.05^{ns}	**0.31****	-0.06^{ns}	-0.03^{ns}	-0.06^{ns}	-0.07^{ns}	-0.09^{ns}	-0.09^{ns}	0.14^{ns}	**0.44****							
	0.01^{ns}	0.05^{ns}	0.03^{ns}	0.06^{ns}	0.05^{ns}	0.11^{ns}	0.10^{ns}	0.10^{ns}	0.10^{ns}	-0.05^{ns}	0.15^{ns}	**0.51****							
OIL	**0.31****	0.10^{ns}	0.13^{ns}	-0.05^{ns}	0.10^{ns}	0.18*	**0.33****	**0.38****	**0.38****	0.04^{ns}	**0.42****	-0.02^{ns}	-0.05^{ns}						
	-0.05^{ns}	0.11^{ns}	0.06^{ns}	-0.23*	-0.06^{ns}	0.04^{ns}	0.05^{ns}	0.13^{ns}	**0.39****	0.20*	0.17^{ns}	0.22*	-0.05^{ns}						
[NP]	**-0.38****	-0.07^{ns}	-0.05^{ns}	0.01^{ns}	-0.13^{ns}	-0.03^{ns}	**-0.27****	**-0.26****	-0.18*	0.00^{ns}	**-0.23****	0.08^{ns}	0.11^{ns}	-0.15^{ns}					
	-0.06^{ns}	0.10^{ns}	-0.02^{ns}	-0.04^{ns}	-0.11^{ns}	-0.01^{ns}	-0.10^{ns}	-0.10^{ns}	-0.03^{ns}	-0.10^{ns}	-0.07^{ns}	0.01^{ns}	0.05^{ns}	-0.20*					
[NS]	0.03^{ns}	-0.09^{ns}	0.08^{ns}	0.01^{ns}	-0.03^{ns}	-0.13^{ns}	0.00^{ns}	0.00^{ns}	-0.02^{ns}	-0.13^{ns}	0.06^{ns}	-0.15^{ns}	-0.09^{ns}	-0.03^{ns}	0.05^{ns}				
	0.14^{ns}	-0.01^{ns}	0.09^{ns}	0.15^{ns}	0.12^{ns}	-0.06^{ns}	-0.02^{ns}	-0.08^{ns}	**-0.34****	-0.07^{ns}	**-0.24****	-0.14^{ns}	-0.16^{ns}	**-0.43****	0.15^{ns}				
NUpE	**0.50****	**-0.23****	**0.47****	-0.13^{ns}	**0.36****	**0.313****	**0.98****	**0.95****	**0.27****	0.10^{ns}	**0.38****	-0.04^{ns}	-0.13^{ns}	0.33**	-0.18*	0.18*			
	0.32**	**-0.28****	**0.45****	**-0.37****	**0.53****	0.20*	**0.96****	**0.91****	0.18*	0.18*	**0.31****	0.05^{ns}	0.08^{ns}	0.03^{ns}	-0.03^{ns}	0.14^{ns}			
NUtE	0.14^{ns}	0.10^{ns}	0.03^{ns}	-0.12^{ns}	0.21*	0.061^{ns}	**0.28****	**0.44****	**0.88****	**0.23****	**0.49****	0.11^{ns}	0.01^{ns}	**0.40****	**-0.36****	**-0.44****	0.18*		
	-0.06^{ns}	0.02^{ns}	0.04^{ns}	-0.26**	0.05^{ns}	0.13^{ns}	0.20*	**0.41****	**0.91****	**0.27****	**0.36****	**0.24****	0.13^{ns}	**0.49****	-0.20*	**-0.58****	0.10^{ns}		
NUE	**0.48****	-0.19*	**0.41****	-0.13^{ns}	**0.38****	**0.31****	**0.97****	**1.00*****	**0.47****	0.16^{ns}	**0.46****	-0.01^{ns}	-0.11^{ns}	**0.40****	-0.26**	0.00^{ns}	**0.95****	**0.44****	
	0.29**	**-0.27****	**0.48****	**-0.41****	**0.54****	0.20*	**0.93****	**0.99****	**0.45****	**0.23****	**0.38****	0.14^{ns}	0.11^{ns}	0.18*	-0.12^{ns}	-0.10^{ns}	**0.91****	**0.42****	
NHI	0.17^{ns}	0.06^{ns}	0.06^{ns}	-0.13^{ns}	0.22*	0.002^{ns}	**0.32****	**0.49****	**0.98****	0.15^{ns}	**0.58****	0.08^{ns}	-0.03^{ns}	**0.44****	**-0.37****	0.06^{ns}	**0.30****	**0.86****	**0.49****
	-0.01^{ns}	0.02^{ns}	0.15^{ns}	-0.22*	0.09^{ns}	0.11^{ns}	**0.25****	**0.47****	**0.97****	**0.29****	**0.34****	**0.30****	0.11^{ns}	**0.40****	**-0.20***	-0.31**	**0.20***	**0.86****	**0.47****

PRINCIPAL COMPONENTS ANALYSIS

Principal Component Analysis (PCA) was carried out to identify patterns in the data and express it in such a way as to highlight more visually similarities and differences than a large number of individual tests based on single trait ANOVA.

All observed traits were included in the analysis and High and Low N treatments were analysed separately. Only TSW and %FM clustered close to the centre, indicating these variables did not contribute to the variance among the samples. A PC model including all 23 variables is presented in Figures 2.9 and 2.10 for High and Low N respectively.

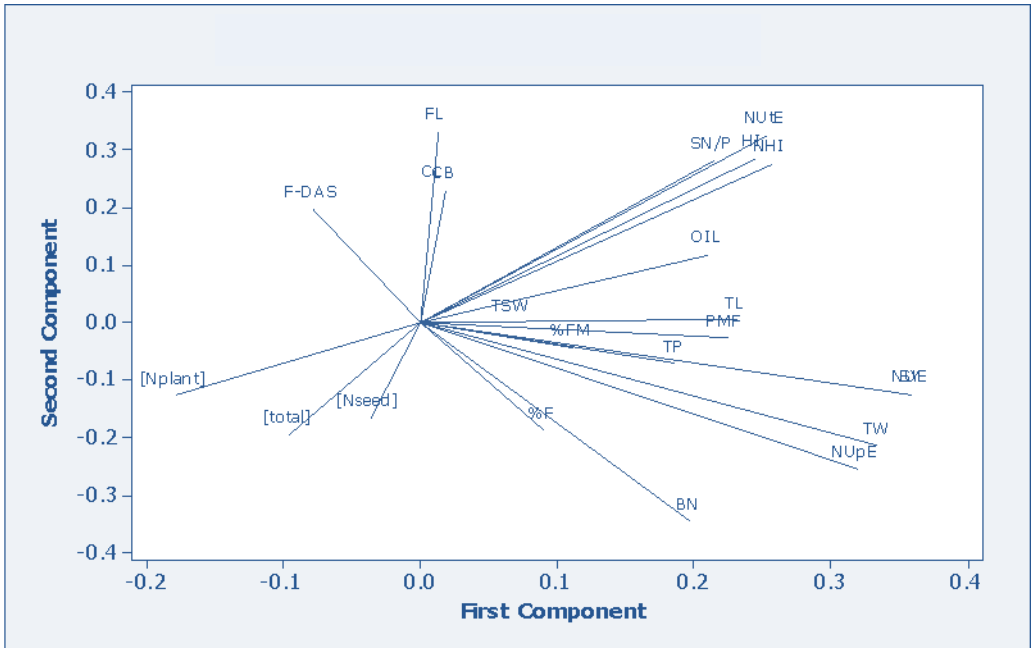


Figure 2.9. Loading plot of High N from 2005/06 data.

At High N, the first PC explaining 29.9% of the total variance was mainly related to a set of correlated variables: TW, SY (placed under NUE in the graph), NUpE and NUE. The second PC explained 13.3%, mainly related to FL, BN (negative), HI, SN/P, NUtE and NHI. The PC3 (not shown) accounted for 9.7% of the total variance was highly related to seed N concentration and total N concentration, and also related to SN/P and TSW. The 3 PCs explained 52.9% of the total variance and presented a pattern of correlated changes in different traits.

Traits could be classified in 4 major groups, according to the 4 quartiles: positive for both PC1 and PC2, negative for both PC1 and PC2, positive for PC1 only and positive for PC2 only. Traits positive for both

PC1 and PC2 were SN/P, NUtE, HI and NHI, values for PC1 were between 0.2 and 0.3, and similarly 0.2 to 0.3 for PC2. Oil content and TSW were in the same group, but had lower contribution to the 2 PCs, oil had values around 0.2 for both PCs and TSW close to 0.1, with almost no contribution to either PC due to its proximity to the centre of the graph.

Traits with positive contribution to PC1 and almost no association with PC2 (and strong association between them) were TL, PMF, TP, %F and BN with values around 0.2 for PC1 (except for %FM with 0.1) and values for PC2 between 0.1 and 0.0.

The group of traits with a major contribution to PC1 (values between 0.3 and 0.4) consisted of NUE, SY, TW and NUpE. Their values for PC2 were -0.1 for NUE and SY; and -0.2 for the rest. Branch number and %F also presented some contribution to PC1 and negative contribution to PC2, but with lesser importance than the previous traits.

The traits with major (positive) contribution to PC2 were FL (0.4) and CB and CL (0.25). The PC1 contribution of these traits was close to 0.

At Low N, PC1 accounted for 25.3% of the total variance and the associated traits were the same ones as for PC1 at High N. The second PC represented 17.2% and the traits were BN, TP, seed N concentration, total N concentration, NUpE (all negative) and NUtE (positive). The third PC (not shown) explained 9.7% of the total variance and the traits associated were TL, FL, TPM, and %FM. The 3 PCs explained 52.2% of the total variance.

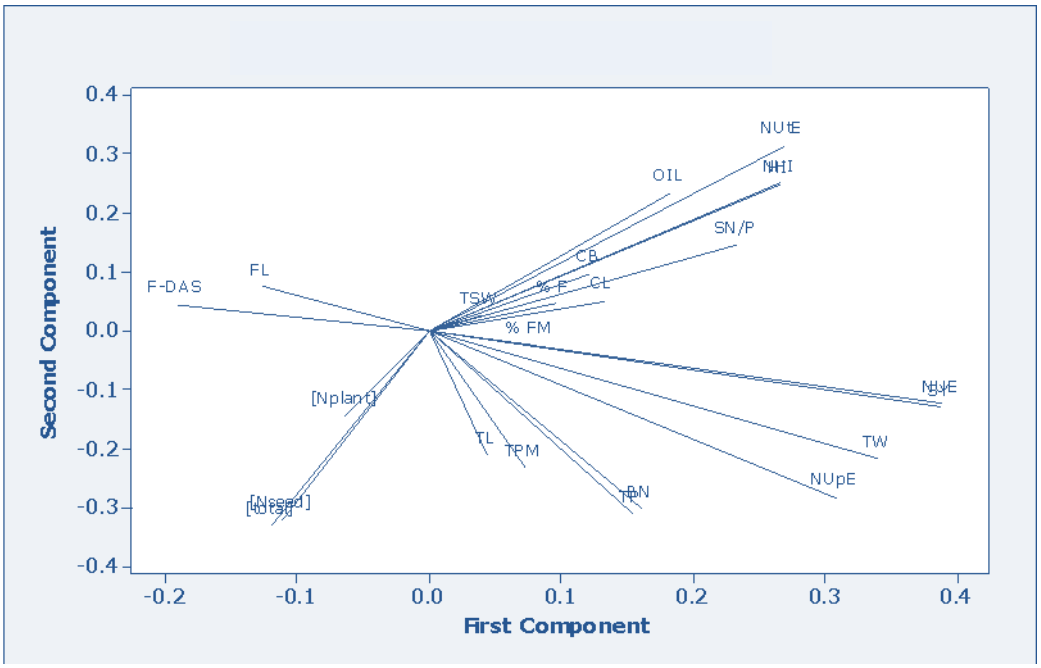


Figure 2.10. Loading plot of Low N from 2005/06 data.

Most of the traits at Low N exhibited a positive contribution to PC1, and were similarly divided into positive and negative contributions to PC2. The group of traits with higher positive contribution to PC1 were NUE, SY, NUpE and TW with values between 0.3 and 0.4 respectively.

NUtE, HI, NHI and SN/P, closely followed by oil content formed the group with higher contribution to PC2, also positive. Values were between 0.2 and 0.35. A similarly strong negative contribution to PC2 with values close to -0.4 came from seed N and total N in plant, which as well had minor negative contribution to PC2 of -0.1.

In summary, nitrogen use efficiency (NUE) was influenced by both NUpE and NUtE at both High and Low N treatments. Apparently, more traits were influencing NUE at Low N than at High N, and more traits had a positive relationship with NUE than negative. Seed yield (SY), biomass (TW) and branch number (BN) were positively related to NUpE in both N treatments and foot length (FL) negatively related. Likewise, HI (harvest index), NHI (N harvest index) and seed number per pod (SN/P) were related to NUtE at both High and Low N. Other traits such as plant height (TL) and total pod number (TP) were linked to NUpE at Low N, but not directly at High N; similarly, oil content was linked to NUtE at Low N but not at High N. Chlorophyll content in bracts and leaves and % of pod fertility positively influenced NUtE at Low N only.

Table 2.14. A summary of the relationships between the main traits underlying NUE at High and Low N for the field trial carried out in 2005/06.Traits are classified in NUpE related traits, NutE related traits and Other (traits influencing NUE not related to the NUpE or NUtE directly).

	High N 2005/06			Low N 2005/06	
relationship	NUE			NUE	
POSITIVE	**NUpE**	**NUtE**	**Other**	**NUpE**	**NUtE**
	seed yield	HI	TSW	seed yield	HI
	biomass	NHI	plant height	biomass	NHI
	branch no.	seed no./ pod	total pod no.	branch no.	seed no./ pod
			oil	plant height	oil
				total pod no.	chlorophyll bracts
					chlorophyll Leaves
					% pod fertility
NEGATIVE	foot length		N seed	foot length	
				flowering	

2.3.2. EXPERIMENT 2. FIELD TRIAL 2006/07

A wide range of values were observed in the TNDH lines for all traits measured in 2007 (Appendix tables 11 to 14). Differences between the parental lines Tapidor and Ningyou7 were small for most of the parameters determined. Most of the traits analysed presented noticeable differences between High and Low N treatments. Ningyou7 died after flowering but before harvest in Block 2, meaning yield and N data for this parental line is not available.

For this field trial only 94 TNDH lines were evaluated and of these, 90 had been grown in the previous trial in 2005/06. Growth was generally homogeneous among all 4 blocks with the exception of a patch in Block 1 covering about 15 mini plots that was affected by poorer growth. Total biomass and plant size in general of the lines in the patch were lower in comparison to other lines in the block. Some lines in the block also had very low biomass but were not necessarily belonging to the affected patch. For this reason all data from Block 1 were included in the analysis. Plants in Block 4 looked abnormally large and healthy for a low N treatment, as they had biomass comparable to the high N treatments i.e. Blocks 2 and 3. In this experiment there were two independent replications, 2 for High and 2 for Low nitrogen treatments using High N blocks and Low N blocks as independent replications.

YIELD, TOTAL BIOMASS AND HARVEST INDEX

Yield traits analysed in 2007, such as TW, SY and HI were higher at High N than at Low N. Similar results were obtained for the parental lines Tapidor and Ningyou7 at both High and Low N, with an exception in Block 4 where TW, SY and HI values for Tapidor but also for the TNDH lines were abnormally high in the Low N treatment (Fig 2.11). Harvest index for Tapidor ranged from 0.35 in Block 4 to 0.27 in Block 2, and for Ningyou7 HI also fluctuated from 0.27 in Block 1 to 0.35 in Block 3.

Little difference was present between Tapidor and the population mean for TW, SY and HI at both High and Low N. Ningyou7 had lower values for both TW and SY, particularly at Low N, as differences between parents and the population mean were not that clear at High N. Almost no transgressive segregation was present at lower values for both TW and SY in both N treatments. On the other hand, the TNDH population demonstrated transgressive segregation for HI in both directions as well as for higher values of all traits at both High and Low N.

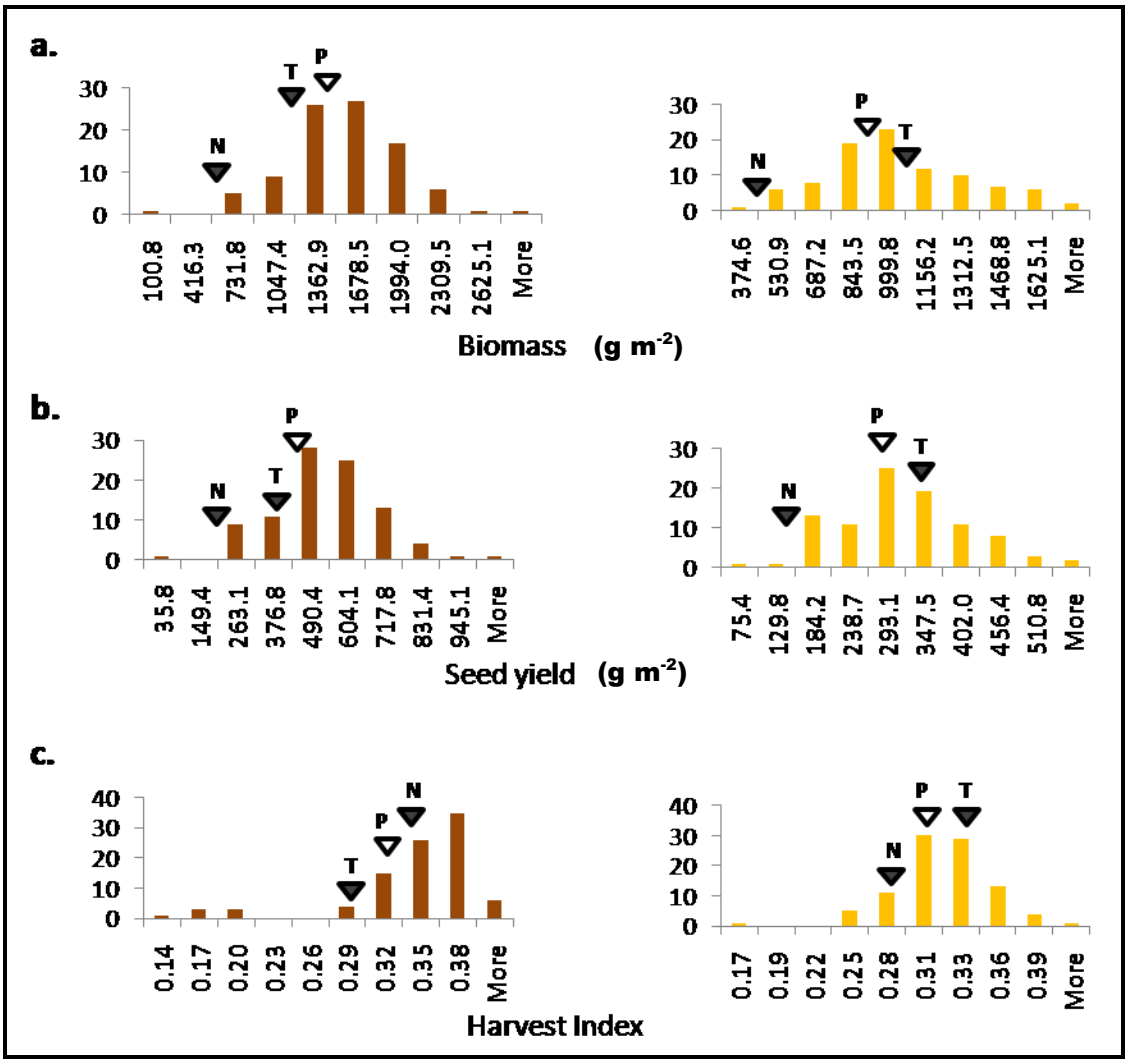


Figure 2.11. Frequency distribution of a) plant biomass, b) seed yield and c) harvest index in the 94 TNDH oilseed rape lines grown in 2006/07. Mean value for the TNDH lines ▽ as well as the parental lines, `Tapidor' and `Ningyou7' ▼ are shown at both high ■ and low ■ nitrogen treatments.

Mean values for TW were 1425 g m^{-2} at High N and 973 g m^{-2} at Low N. Seed yield followed the same trend as TW with higher values at High N, i.e. 482 g m^{-2} than at Low N 290.77 g m^{-2}. Harvest index also presented slightly higher values at High N 0.33 than at Low N i.e. 0.31.

NITROGEN AND NITROGEN DERIVED TRAITS

Tapidor had the highest values for NUpE and NUE when grown at High N (Fig. 2.12). For NHI there was no significant difference between Tapidor and Ningyou7 and between High and Low N treatments. The N derived traits analysed also presented very high variation for the TNDH lines showing a wider range at

Low N than at High N. For example, NUpE had a range varying from 0.74 to 5.10 gN g^{-1} at High N but varied from 0.39 to 17.32 gN g^{-1} at Low N, resulting in lines with the highest and lowest NUpE being from the Low N regime.

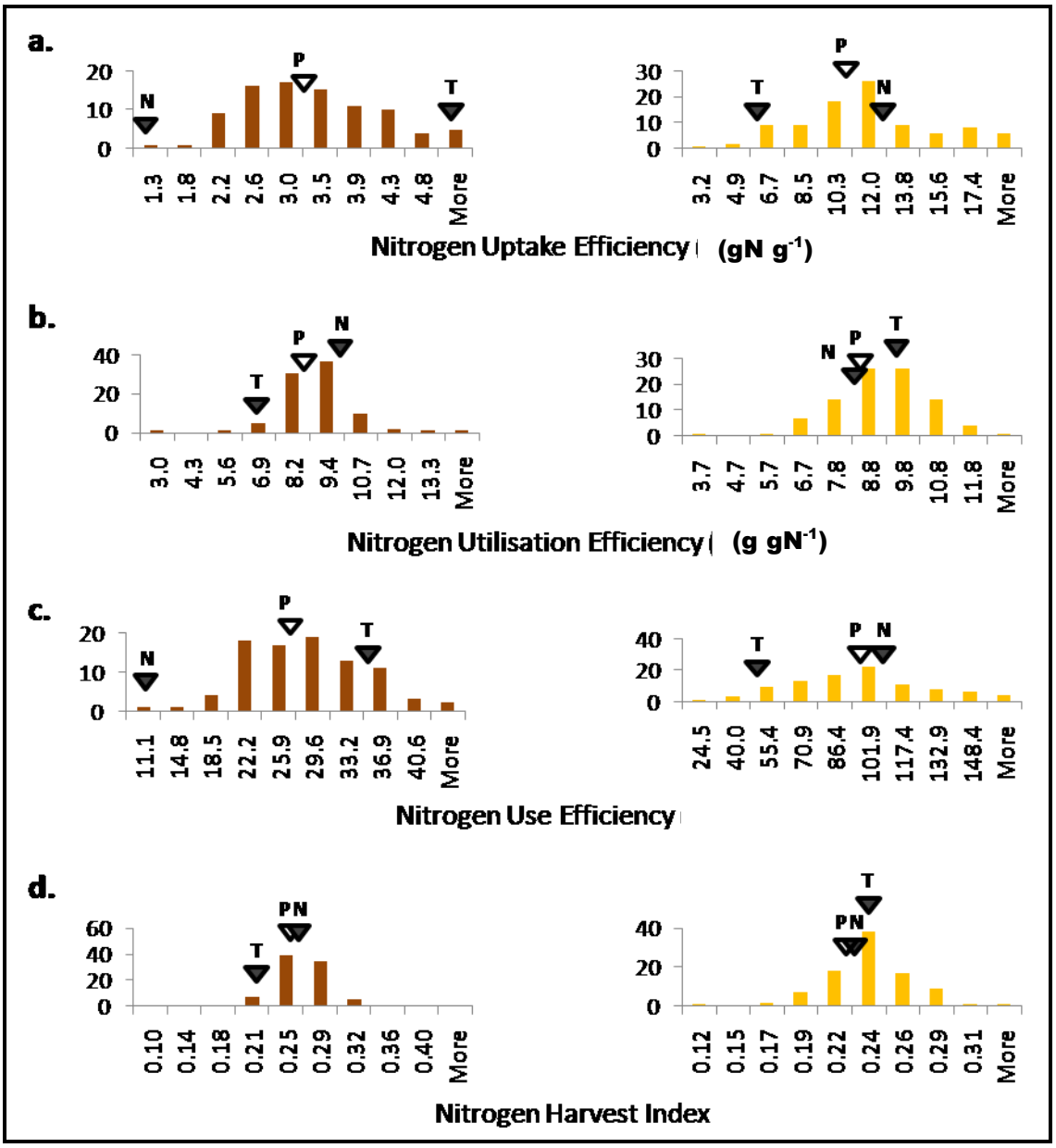


Figure 2.12. Frequency distribution of a) Nitrogen Uptake Efficiency, b) Nitrogen Utilisation Efficiency, c) Nitrogen Use Efficiency and d) Nitrogen Harvest Index in the 94 TNDH oilseed rape lines grown in 2006/07. Mean value for the TNDH lines ▽ as well as the parental lines, `Tapidor' and `Ningyou7' ▼ are shown at both High ■ and Low ■ nitrogen treatments.

Nitrogen Utilisation Efficiency (g gN^{-1}) values were much closer together with little difference between treatments in 2007, where NUpE varied by 4.36 gN g^{-1} between the highest and lowest values at High N to 16.93 gN g^{-1} at Low N.

Mean value for NUpE at high N was 2.66 gN g^{-1} and the corresponding value for Low N was 6.56 gN g^{-1} (Fig 2.12). NUE mean values were also 34.5 higher at Low N compared to High N, with values of 24.8 g g^{-1} and 59.3 at High and Low N respectively.

Mean values for NUtE were 9.5 g gN^{-1} for both treatments in 2007. For NHI, on the other hand it was 0.27 at High N and 0.24 at Low N.

Tapidor and Ningyou7 demonstrated different response to treatments. Ningyou7 presented very similar values to the mean of the population for all N derived traits at Low N and only for NHI were values similar between Ningyou7 and the population mean at High N. All traits presented transgressive segregation in both directions, with the exception of NUpE at High N. Also NUE did not show transgressive segregation for lower values at High N.

Most of the traits analysed at High N in 2007 followed a normal distribution at $P<0.05$. The traits that did not appear normally distributed were NutE and NHI for Low N in 2007. The QTL software used had an option to analyse non-normal data, thus data not following a normal distribution was not transformed.

Oil content results for 2006/07 were still under analysis at the time of writing the thesis.

Nitrogen traits analysed had a different response to High and to Low N i.e. stem N concentration showed little or no difference between the population mean and both the parents (Fig. 2.13), whereas marked differences were present for the same traits at Low N. Parent Ningyou7 had similar values to the average population for chaff N at both N levels and little or no difference for seed N at Low N. At High N, seed N concentration for Ningyou7 was much lower.

Mean values for N traits were higher at High N than at Low N, at High N measurements for stem, chaff and seed N concentration were 5.2, 6.4 and 29.9 mg g^{-1} respectively, whereas they were 3.9, 5.3 and 26.8 mg g^{-1} at Low N.

Most of the traits showed transgressive segregation in both directions except for stem N where it was evident for the lower values at Low N only and seed N for the higher values at High N only. More specifically, mean values for plant N concentration (mg g^{-1}) were 9.74 at high N and 7.52 at Low N with values being slightly higher than in the previous season. Mean values for seed N concentration were 28.27 mg g^{-1} and 25.84 mg g^{-1} at High N and Low N respectively (Fig. 2.13).

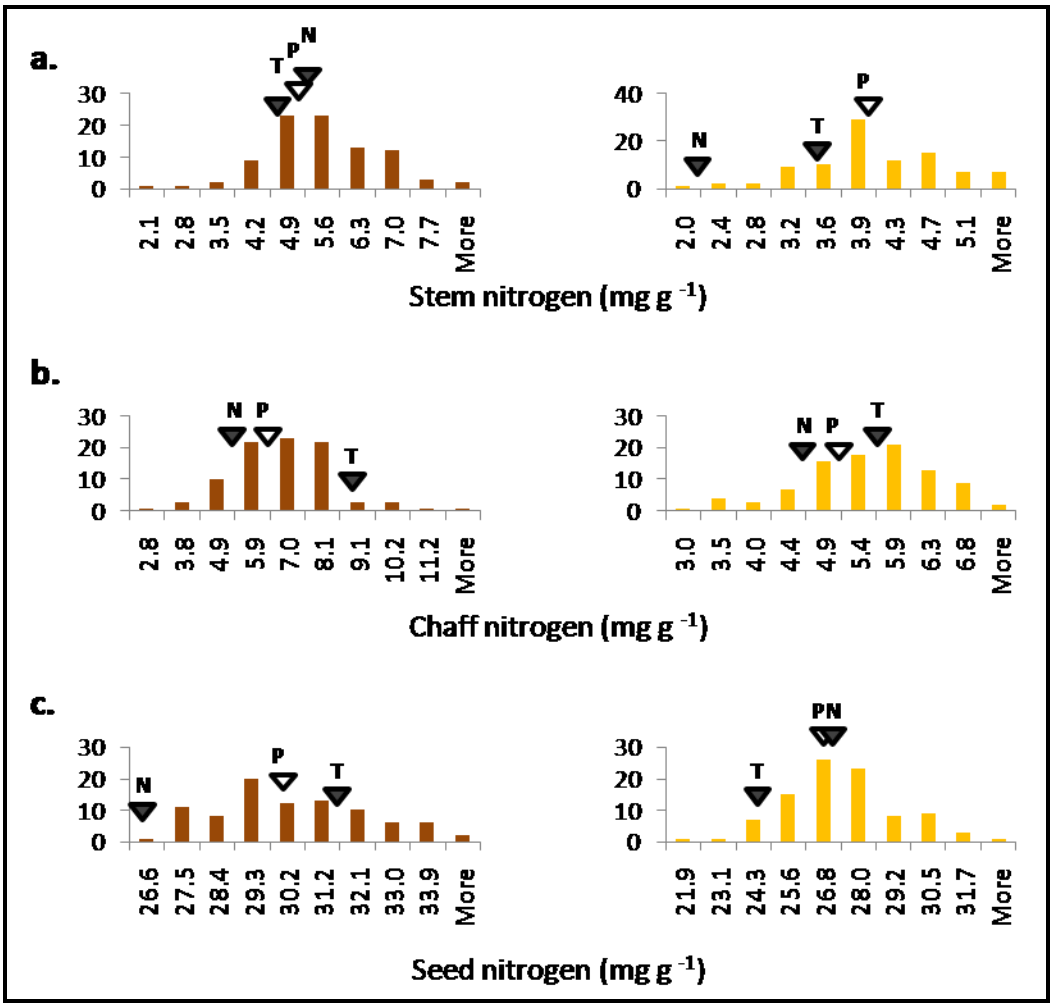


Figure 2.13. Frequency distribution of a) stem nitrogen concentration b) chaff N concentration and c) seed N concentration in the 94 TNDH oilseed rape lines grown in 2006/07. Mean value for the TNDH lines ▽ as well as the parental lines, `Tapidor' and `Ningyou7' ▼ are shown at both High ■ and Low ■ nitrogen treatments.

GERMINATION AND FLOWERING

Germination rates in 2006/07 (Appendix 4) were higher than those recorded in 2005/06. Germination rates were above 75% in all blocks, the highest was 90.57% in Block 2 and the lowest 76.92% in Block 1. Tapidor had a fairly constant germination rate, the lowest in Block 1 was of 77.5% and the highest in Block 3 was of 95%. Ningyou7 had higher germination rates in 2 blocks of 97.5%, another one was 90% but a very low 57.5% in Block 1. Some TNDH lines showed very high germination rates i.e. 100% for TN5, 7, 10,11, 19, 29, 51, 57, 78, 126, 152, 158, 163, 176, 177, and 181. Only 3 TNDH lines had germination rates below 50%: TN87 at 27.5%, TN115 at 30% and TN145 at 37.5%.

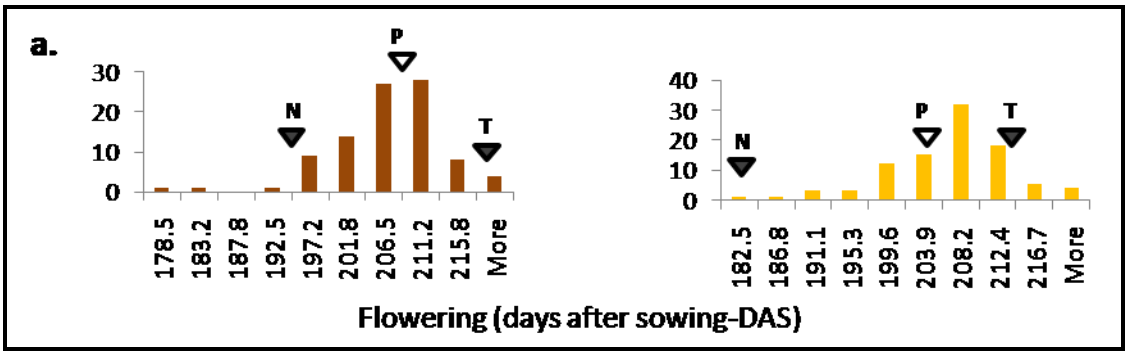


Figure 2.14. Frequency distribution of flowering in the 94 TNDH oilseed rape lines grown in 2006/07. Mean value for the TNDH lines ▽ as well as the parental lines, `Tapidor' and `Ningyou7' ▼ are shown at both high ■ and low ■ nitrogen treatments.

The FDAS trait (Fig 2.14) showed slight variation between High and Low N treatments. Average flowering date for all lines was 209 DAS at High N (Blocks 2 and 3) and 204 DAS at Low N (Blocks 1 and 4). Pronounced differences existed between FDAS for Tapidor and Ningyou7, particularly at Low N. Both showed a different response to treatment i.e. Tapidor flowered later at High N than at Low N and in both treatments after Ningyou7. The latter also showed shorter FDAS at Low N.

Analysis of Variance (ANOVA)

Analysis of variance was performed on all traits in 2006/07. The model was the same as in 2005/06, where N was considered a fixed factor and lines, line*nitrogen interaction and replicate*nitrogen interaction were random factors.

The REML analysis for yield traits gave similar results as in 2005/06 (Table 2.15). Both total above ground plant biomass and seed yield did not show significant genotype differences, or a different behaviour of different genotypes at High or Low N. However, harvest index did show different genotypic behaviour and different responses of these genotypes to the 2 N treatments.

A possible explanation would be because of high environmental effects (other than nitrogen) influencing the genotypes' response. It could also be possible that the TNDH population showed high plasticity and adaptability for these traits. That could be due to different architectural backgrounds of the parents Tapidor and Ningyou7, which could influence both total above ground plant biomass and yield under stress conditions such as nitrogen deficiency.

Table 2.15. Results for the mixed model ANOVA, the Residual Maximum Likelihood (REML) for total above ground plant biomass (TW), seed yield (SY) and harvest index (HI) in 2006/07. Levels of significance were ns,*, **, *** for no significance, <0.1, <0.01 and <0.001 respectively.

TW				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	1.800	1.000	1.8	0.179 ns
Random term	**component**	**s.e.**		**Z-test**
TN_line	2062.000	13771.000		0.150 ns
TN_line.NITROGEN	-6569.000	24248.000		-0.271 ns
NITROGEN.REP	137257.000	140185.000		0.979 ns
RESIDUAL	263764.000	28524.000		9.247
SY				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	3.790	1.000	3.79	0.052 *
Random term	**component**	**s.e.**		**Z-test**
TN_line	367.000	1574.000		0.233 ns
TN_line.NITROGEN	-412.000	2740.000		-0.150 ns
NITROGEN.REP	11815.000	12141.000		0.973 ns
RESIDUAL	29277.000	3179.000		9.209
HI				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	8.760	1	8.76	0.003 **
Random term	**component**	**s.e.**		**Z-test**
TN_line	0.00027	0.00011		2.333 **
TN_line.NITROGEN	-0.00049	0.00018		-2.726 **
NITROGEN.REP	0.00012	0.00015		0.808 ns
RESIDUAL	0.00247	0.00027		9.286

Nitrogen derived traits such as NUpE and NUE did show similar results as total above ground biomass and seed yield (Table 2.16). The traits did not show genotypic differences between lines or different response of the lines to High and Low N.

Table 2.16. Results for the mixed model ANOVA, the Residual Maximum Likelihood (REML) for N uptake efficiency (NUtE), N utilisation efficiency (NUtE), N use efficiency (NUE) and N harvest index (NHI) in 2006/07. Levels of significance were ns,*, **, *** for no significance, <0.1, <0.01 and <0.001 respectively.

NUpE				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	2.900	1	2.9	0.088*
Random term	**component**	**s.e.**		**Z-test**
TN_line	0.360	0.760		0.474ns
TN_line.NITROGEN	-0.120	1.240		-0.097ns
NITROGEN.REP	23.170	23.310		0.994ns
RESIDUAL	12.170	1.390		8.755
NUtE				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.010	1	0.01	0.927ns
Random term	**component**	**s.e.**		**Z-test**
TN_line	0.217	0.192		1.130ns
TN_line.NITROGEN	-0.520	0.320		-1.625*
NITROGEN.REP	1.681	1.728		0.973ns
RESIDUAL	3.794	0.433		8.762
NUE				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	4.080	1	4.08	0.043*
Random term	**component**	**s.e.**		**Z-test**
TN_line	24.700	52.800		0.468ns
TN_line.NITROGEN	32.900	83.800		0.393ns
NITROGEN.REP	1055.300	1064.400		0.991ns
RESIDUAL	761.300	87.600		8.691
NHI				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.950	1	0.95	0.329ns
Random term	**component**	**s.e.**		**Z-test**
TN_line	0.000	0.000107		2.523**
TN_line.NITROGEN	0.000	0.000153		-2.229*
NITROGEN.REP	0.000	0.000516		0.955ns
RESIDUAL	0.002	0.00022		8.802

For NUtE and NHI, on the other hand, they showed different response of the lines at different N treatment. Nitrogen harvest index also showed differences between genotypes, but NUtE did not.

Table 2.17. Results for the mixed model ANOVA, the Residual Maximum Likelihood (REML) for stem N concentration (STN), chaff N concentration (PN), seed N concentration (SN) and total N concentration (TN) in 2006/07. Levels of significance were ns,*, **, *** for no significance, <0.1, <0.01 and <0.001 respectively.

STN				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	1.350	1.000	1.35	0.245 ns
Random term	**component**	**s.e.**		**Z-test**
TN_line	-0.126	0.081		-1.556 *
TN_line.NITROGEN	-0.074	0.162		-0.457 ns
NITROGEN.REP	1.148	1.169		0.982 ns
RESIDUAL	1.705	0.194		8.789
PN				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.500	1	0.5	0.481 ns
Random term	**component**	**s.e.**		**Z-test**
TN_line	0.035	0.122		0.287 ns
TN_line.NITROGEN	-0.173	0.219		-0.790 ns
NITROGEN.REP	2.125	2.154		0.987 ns
RESIDUAL	2.319	0.269		8.621
SN				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	2.900	1.000	2.9	0.089 *
Random term	**component**	**s.e.**		**Z-test**
TN_line	-0.282	0.376		-0.750 ns
TN_line.NITROGEN	-0.362	0.704		-0.514 ns
NITROGEN.REP	2.678	2.770		0.967 ns
RESIDUAL	7.577	0.853		8.883
TN				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	1.590	1	1.59	0.207 ns
Random term	**component**	**s.e.**		**Z-test**
TN_line	-1.740	0.810		-2.148 *
TN_line.NITROGEN	-0.570	1.660		-0.343 ns
NITROGEN.REP	16.530	16.740		0.987 ns
RESIDUAL	17.470	2.000		8.735

Nitrogen traits such as seed and chaff N concentration did not show any differences between lines or in their response to N treatment (Table 2.17). Only stem N concentration showed different genotypes had different N concentrations in stem, even though no differences were found at High or Low N concentration. Total N concentration (the sum of stem, chaff and seed N) did also show different total N concentrations in different lines.

Flowering time showed different genotypes existed for the trait and those had different response at High or Low N treatment (Table 2.18). This result differs from the one in 2005/06, as previously no line and nitrogen interaction was detected.

Table 2.18. Results for the mixed model ANOVA, the Residual Maximum Likelihood (REML) for flowering time (FDAS) in 2006/07. Levels of significance were ns,*, **, *** for no significance, <0.1, <0.01 and <0.001 respectively.

FDAS				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0.010	1	0.01	0.932 ns
Random term	**component**	**s.e.**		**Z-test**
TN_line	14.100	5.4		2.611 **
TN_line.NITROGEN	-18.000	7.8		-2.308 *
NITROGEN.REP	-0.500	0.7		-0.714 ns
RESIDUAL	108.700	11.300		9.619

Analysis of variance was also performed with a combination of the two years' results. The model used for such analysis was nitrogen as fixed factor and random factors were: year, line, and the interactions nitrogen*year, nitrogen*year*replicate, nitrogen*line, year*line and year*nitrogen*line.

The ANOVA analysis was performed with 2 replicates from each year, 2 from the 2005/06 field trial and 2 from the 2006/07 field trial for all traits except N traits. For the N traits, only 1 replicate was used from 2005/06, and 2 from 2006/07. As mentioned before, N traits were not analysed for Blocks 2 and 4 in 2005/06.

For yield traits (Table 2.19), in accordance with previous results, total above ground biomass and seed yield showed no differences between lines, nitrogen treatments or years. That could still be explained by high variability in the plant performance in the field.

Table 2.19. Results for the mixed model ANOVA, the Residual Maximum Likelihood (REML) for total above ground biomass (TW), seed yield (SY) and harvest index (HI) combining years. Levels of significance were ns,*, **, *** for no significance, <0.1, <0.01 and <0.001 respectively.

TW				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	3.79	1	3.79	0.052*
Random term	**component**	**s.e.**		**Z-test**
YEAR	675350	1000364		0.675104ns
YEAR.NITROGEN	-43303	138025		-0.31373ns
YEAR.NITROGEN.REP	139176	141157		0.985966ns
LINE	773	11121		0.069508ns
NITROGEN.LINE	-1418	15507		-0.09144ns
YEAR.LINE	656	13671		0.047985ns
YEAR.NITROGEN.LINE	1451	20739		0.069965ns
RESIDUAL	171066	15428		11.08802
SY				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	1.79	1	1.79	0.181ns
Random term	**component**	**s.e.**		**Z-test**
YEAR	69843	107228		0.65135ns
YEAR.NITROGEN	2403	18654		0.12882ns
YEAR.NITROGEN.REP	11919	12139		0.981877ns
LINE	8	1235		0.006478ns
NITROGEN.LINE	-52	1711		-0.03039ns
YEAR.LINE	241	1521		0.158448ns
YEAR.NITROGEN.LINE	133	2286		0.05818ns
RESIDUAL	18967	1716		11.05303
HI				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	2.64	1	2.64	0.104ns
Random term	**component**	**s.e.**		**Z-test**
YEAR	0.002709	0.003987		0.679458ns
YEAR.NITROGEN	0.0001	0.000325		0.307692ns
YEAR.NITROGEN.REP	0.000127	0.00016		0.79375ns
LINE	0.000219	0.000133		1.646617*
NITROGEN.LINE	-0.000033	0.000149		-0.22148ns
YEAR.LINE	0.0001	0.000159		0.628931ns
YEAR.NITROGEN.LINE	-0.000564	0.000255		-2.21176*
RESIDUAL	0.0028	0.000281		9.964413

Table 2.20. Results for the mixed model ANOVA, the Residual Maximum Likelihood (REML) for N uptake efficiency (NUpE), N utilisation efficiency (NUtE), N use efficiency (NUE) and N harvest index (NHI) combining years. Levels of significance were ns,*, **, *** for no significance, <0.1, <0.01 and <0.001 respectively.

NUpE				
Random term	**component**	**s.e.**		
YEAR	-4.791	*		
YEAR.NITROGEN	11.271	*		
YEAR.NITROGEN.REP	20.873	*		
LINE	0.114	*		
NITROGEN.LINE	-0.338	*		
YEAR.LINE	0.221	*		
YEAR.NITROGEN.LINE	0.939	*		
RESIDUAL	8.991	*		
NUtE				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0	0	*	*
Random term	**component**	**s.e.**		**Z-test**
YEAR	0.344	0.333		1.033033 ns
YEAR.NITROGEN	-1.008	0.84		-1.2 ns
YEAR.NITROGEN.REP	0.98	0.839		1.168057 ns
LINE	0.17	0.204		0.833333 ns
NITROGEN.LINE	0.135	0.27		0.5 ns
YEAR.LINE	0.069	0.255		0.270588 ns
YEAR.NITROGEN.LINE	-0.896	0.423		-2.1182 *
RESIDUAL	4.166	0.452		9.216814
NUE				
Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	22.4	1	22.4	<0.001
Random term	**component**	**s.e.**		**Z-test**
YEAR	383.3	839.9		0.456364 ns
YEAR.NITROGEN	-443.6	955.1		-0.46445 ns
YEAR.NITROGEN.REP	1066.3	1073.6		0.9932 ns
LINE	-3.3	47.5		-0.06947 ns
NITROGEN.LINE	-48.3	63.5		-0.76063 ns
YEAR.LINE	30.9	67.9		0.455081 ns
YEAR.NITROGEN.LINE	123.8	96.9		1.277606 *
RESIDUAL	575.8	61.7		9.332253
NHI				
Random term	**component**	**s.e.**		
YEAR	0.001188	*		
YEAR.NITROGEN	-0.001893	*		
YEAR.NITROGEN.REP	0.000437	*		
LINE	0.000159	*		
NITROGEN.LINE	-0.000147	*		
YEAR.LINE	0.000064	*		
YEAR.NITROGEN.LINE	-0.000263	*		
RESIDUAL	0.00191	*		

Harvest index, did show different genotypes had different HI values and they would also have a different response to high and low N depending on the year as well. This result would indicate that the year had an important factor influencing the lines' response to N treatment.

For N derived traits, the analysis combining the 2 field trials was performed with 3 replications, 1 from 2005/06 and 2 from 2006/07 field trial. The analysis could not be performed for NUpE and NHI, due to smaller datasets for these 2 traits. For NUtE, the analysis could be performed, but there were not enough degrees of freedom to calculate the N factor. However, the results would show that the TN lines response to N would be influenced by the year. The same response was observed from NUE.

For flowering, the interactions of nitrogen*year*replicate, nitrogen*line and year*line were the more significant ones with P<0.001 (Table 2.21). The year*nitrogen*line interaction was also significant but with P<0.01. Results indicate that lines had different flowering times and that these were influenced by the nitrogen treatment. However, the year factor had an influence on the results, particularly in the nitrogen and line interaction and also on the replicates and the nitrogen treatment.

Table 2.21. Results for the mixed model ANOVA, the Residual Maximum Likelihood (REML) for flowering time (FDAS) combining years. Levels of significance were ns,*, **, *** for no significance, <0.1, <0.01 and <0.001 respectively.

FDAS

Fixed term	**Wald statistic**	**d.f.**	**Wald/d.f.**	**chi pr**
NITROGEN	0	0	*	*

Random term	**component**	**s.e.**	**Z-test**
YEAR	624.75	883.88	0.706827 ns
YEAR.NITROGEN	-0.29	0.39	-0.74359 ns
YEAR.NITROGEN.REP	-0.22	0.02	-11 ***
LINE	8.77	3.19	2.749216 **
NITROGEN.LINE	0.34	0.03	11.33333 ***
YEAR.LINE	2.03	0.18	11.27778 ***
YEAR.NITROGEN.LINE	-14.38	5.12	-2.80859 **
RESIDUAL	83.24	7.37	11.29444

NARROW-SENSE HERITABILITY

Table 2.22. Narrow sense heritability (h^2) for traits analysed in 2006/07.

TRAIT	HERITABILITY
FDAS	0.51
TW	0.63
SY	0.76
HI	0.00
SN	0.12
PN	0.04
STN	0.10
TN	0.67
NUpE	0.43
NUtE	0.00
NUE	0.74
NHI	0.00

In 2007, values for h^2 (Tables 2.22) ranged from 0.0% for HI, NUtE and NHI to 76% for SY. Other traits with high heritability were NUE (74%), total N (67%) and total above ground biomass (63%). As in 2005/06, FDAS had high heritability (51%). Some traits had very low heritability, i.e. seed N, chaff N and stem N concentrations. These results contrast with the high heritability of total N, meaning that maybe total N concentration in the plant can be inherited, but not the concentration in any part of the plant.

2.4.6 PHENOTYPIC CORRELATION ANALYSIS

For the phenotypic correlations at High and Low N there were 27 *r* values statistically significant at the 5% level for both N treatments, with absolute values ranging from 0.22 to 0.94 (Table 2.23.).

Moreover, there were 21 other statistically significant correlation coefficients at the 5% level present in one of the treatments i.e. either High N or Low N.

The highest correlation values were those between total biomass and seed yield of 0.947 and 0.945 at High and Low N respectively.

High correlations were also present between NUpE and NUE and between NUtE and NHI, in addition to which seed N, plant N, pod N and total plant N showed high correlations between themselves. Seed N did not correlate with stem N at High N, but showed a significant correlation (P<0.01) at Low N. Seed N correlated with pod N at High N (r=0.414, P<0.01 and at Low N (r=0.215, P<0.05).Traits can be grouped into 2 major associations: one between TW, SY, NUpE and NUE, all giving significant positive correlations (P<0.01). The other trait association showed positive correlations between HI, NHI, NUtE, and total plant concentration but negative correlations (P<0.01) between chaff, stem, seed and total N. There was no correlation between HI and stem and chaff N concentration, but a strong negative correlation was observed between HI and total plant N.

Table 2.23. Phenotypic correlations for both high N and low treatments between characters in 2006/07. Correlation coefficients at High N presented on top line [a] and Low N the bottom line [b]. Traits are TW (total above ground biomass), HI (harvest index), SY (seed yield), F-DAS (flowering), TSW (1000-seed weight), SN/P (seed number per pod) (chlorophyll content in bracts), CL [NS] (seed N concentration), [STN] (stem N concentration), [NP] (pod N concentration), [TotalN] (Total above ground N concentration), NUp uptake efficiency), NUtE (N utilisation efficiency), NUE (N use efficiency) and NHI (N harvest index).

	TW	HI	SY	F-DAS	[SN]	[STN]	[PN]	[TotalN]	NUpE	NUtE	NUE
HI	**0.454**** [a]										
	-0.222* [b]										
SY	**0.947****	**0.571****									
	0.945**	0.041 ns									
F-DAS	0.096 ns	0.074 ns	0.071 ns								
	0.073 ns	-0.041 ns	0.021 ns								
[SN]	-0.111 ns	**-0.312****	-0.162 ns	0.186 ns							
	-0.302**	**-0.406****	**-0.409****	-0.167 ns							
[STN]	-0.076 ns	-0.024 ns	-0.104 ns	-0.109 ns	0.224*						
	-0.248*	-0.165 ns	**-0.315****	0.056 ns	**0.341****						
[PN]	0.094 ns	-0.203 ns	0.032 ns	0.246 ns	**0.414****	**0.38****					
	-0.057 ns	-0.122 ns	-0.093 ns	**0.414****	0.215*	**0.311****					
[TotalN]	-0.045 ns	**-0.271****	-0.111 ns	0.176 ns	**0.811****	**0.617****	**0.792****				
	-0.305**	**-0.378****	**-0.412****	0.033 ns	**0.886****	**0.623****	**0.573****				
NUpE	**0.786****	-0.015 ns	**0.696****	0.066 ns	0.116 ns	0.027 ns	**0.36****	0.231*			
	0.751**	**-0.327****	**0.643****	0.16 ns	-0.01 ns	0.092 ns	0.223*	0.092 ns			
NUtE	0.127 ns	**0.513****	**0.332****	-0.144 ns	**-0.584****	**-0.428****	**-0.609****	**-0.732****	-0.107 ns		
	-0.063 ns	**0.875****	0.156 ns	-0.027 ns	**-0.701****	**-0.402****	**-0.355****	**-0.734****	**-0.309****		
NUE	**0.815****	0.267*	**0.844****	-0.017 ns	-0.193 ns	-0.227*	-0.028 ns	-0.194 ns	**0.849****	**0.372****	
	0.777**	-0.014 ns	**0.797****	0.113 ns	**-0.309****	-0.109 ns	0.059 ns	-0.233*	**0.892****	0.044 ns	
NHI	0.101 ns	**0.476****	**0.32****	-0.097 ns	-0.267*	**-0.414****	**-0.551****	**-0.525****	-0.08 ns	**0.931****	**0.356****
	-0.236*	**0.945****	0.004 ns	-0.133 ns	**-0.345****	**-0.322****	**-0.344****	**-0.451****	**-0.401****	**0.896****	-0.093 ns

In 2007, plant N concentration was calculated from the summation of seed, stem and chaff components which resulted in a significant correlation (P<0.01) between chaff N and stem N concentrations as well as between chaff N and seed N concentrations at High (p<0.01) and Low (p<0.05) N respectively. There was also a positive correlation between chaff N concentration and flowering time at Low N (r=0.414, P<0.01).

Principal Components Analysis

Principal Component Analysis (PCA) was carried out using all observed traits in the analysis, with High and Low N treatments analysed separately. A PC model including all 23 variables is presented for High and Low N respectively.

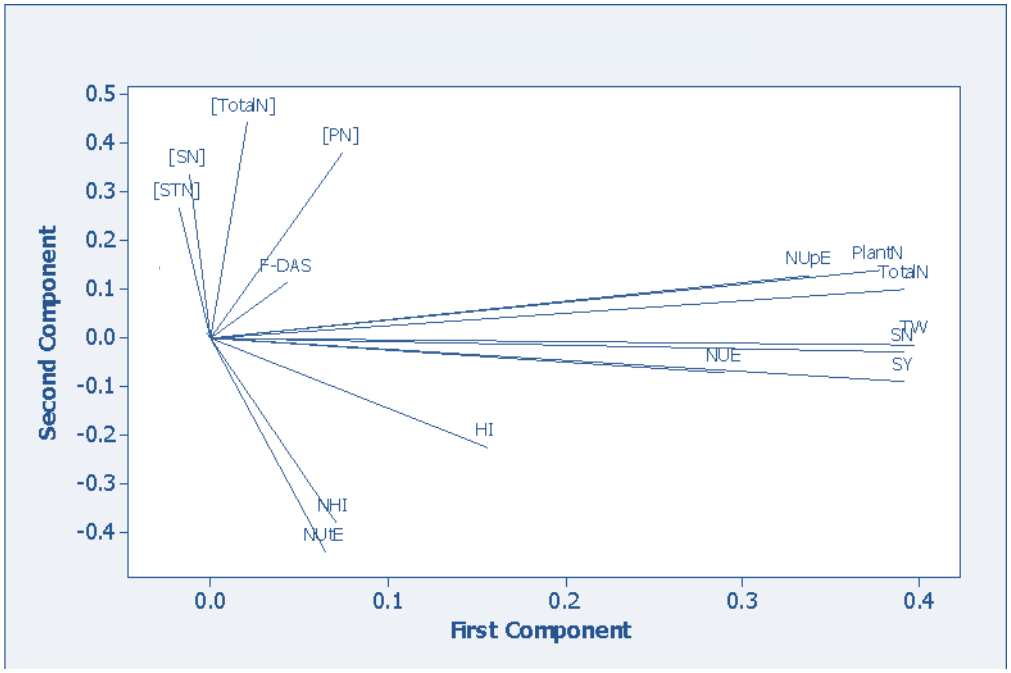


Figure 2.15. Loading plot of High N from 2006/07 data.

At High N (Fig 2.15) the first PC explained 33.5% of the total variance and was mainly related to a set of correlated variables i.e. all traits for N concentration (negative), NUtE and NHI (positive). The second PC explained 24.9% and was, mainly related to TW, SY, NUpE and NUE. The PC3 (not shown) accounted for 12.5% of the total variance was highly related to FDAS, (negative) and stem N concentration. The 3 PCs explained 70.9% of the total variance and presented a pattern of correlated changes in different traits.

A large group of variables clustered in the same direction strongly contributed to PC1 (0.4) i.e. NUpE, plant N and total N concentration grouped at PC1=0.4, PC2=0.1, whereas TW, seed N concentration, SY were at PC1=0.4, PC2≈-0.1 and closely followed by NUE (with PC1 of 0.3). All the variables had PC2 values very close to 0, meaning they had little to no contribution to this component.

The values influencing PC2 were total N (0.5) and chaff N (0.4) and stem N and seed N (0.3) concentrations. Negatively influencing PC2 were NHI and NUtE with values around -0.4.

At Low N (Fig 2.16) PC1 accounted for 35.1% of the total variance and the associated traits were TW, SY, NUpE, NUE and negatively to NHI. The second PC represented 30.6% and the traits associated were seed N concentration, total N concentration (all negative) and NUtE (positive). The third PC (not shown) explained 14.2% of the total variance and FDAS was very strongly associated (negative), and chaff N concentration was also related but less strongly. The 3 PCs explained 79.9% of the total variance.

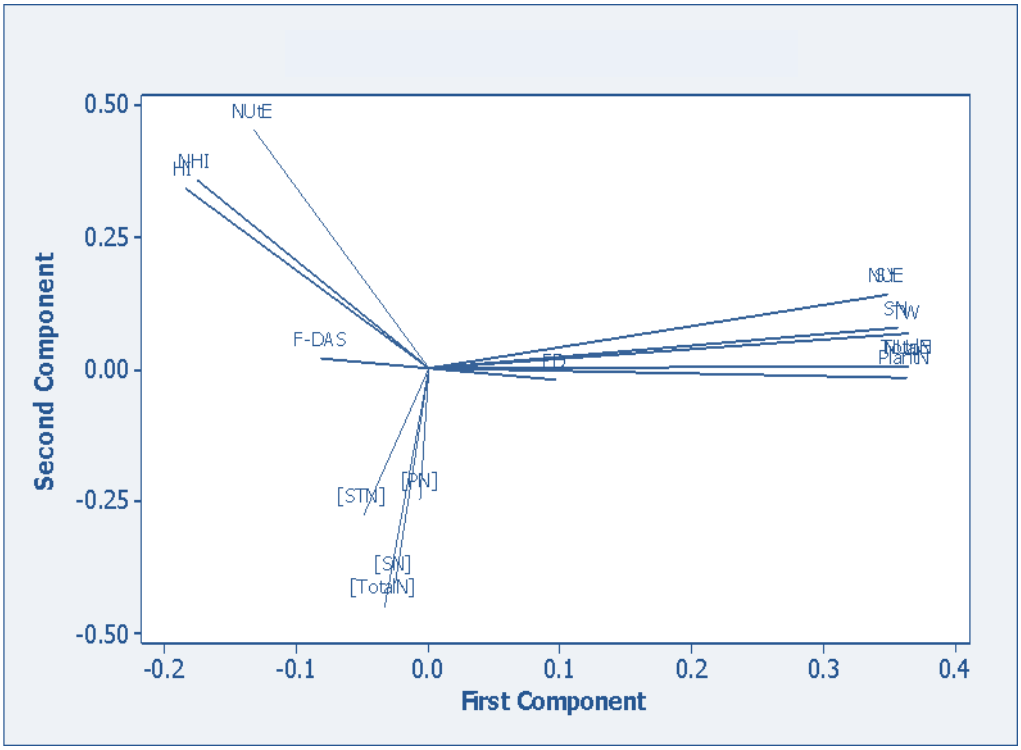


Figure 2.16. Loading plot of Low N from 2006/07 data.

The same group of traits as for High N positively contributed to PC1 but were even more clustered than before, with values around 0.4 for all traits. Nitrogen utilisation efficiency was the main trait to positively contribute to PC2 with 0.5, followed by HI and NHI around 0.3. Total N concentration and seed

N concentration were the 2 main traits negatively contributing to PC2, with values close to -0.5. Stem and chaff also contributed to PC2 with negative values around -0.25.
Flowering-DAS almost had no contribution to either PC1 or PC2, as values for both were close to 0.

Nitrogen use efficiency (NUE) was primarily influenced by NUpE and NUtE at High N but apparently, only by NUpE at Low N. Most of the traits had a positive relationship with NUE, with the exception of stem N concentration (STN). Both plant biomass (TW) and seed yield (SY) were positively related to NUpE, whereas HI (harvest index) and NHI (N harvest index) were related to NUtE. Stem N, which was directly related to NUtE, was negatively related to NUE.

Table 2.24. A summary of the relationships between the main traits underlying NUE at High and Low N for the field trial carried out in 2006/07. Traits are classified in NUpE related traits, NutE related traits and Other (traits influencing NUE not related to the NUpE or NUtE directly).

	High N 2006/07		Low N 2006/07	
relationship	**NUE**		**NUE**	
POSITIVE	**NUpE**	**NUtE**	**NUpE**	**Other**
	biomass	NHI	biomass	seed N
	seed yield	HI	seed yield	
NEGATIVE		stem N		

2.4. DISCUSSION

2.4.1. VARIABILITY

Two field trials were grown during the 2005/06 and 2006/07 seasons, each year using 2 blocks at High N and 2 blocks at Low N. It was assumed that N fertilisation was the major cause for variation across environments and affecting different traits. However, other factors i.e. soil structure and texture, drainage etc could have influenced differences between High and Low N treatments, particularly at Low

N when plants were growing under stress. The effect of these factors was considered as random across experiments.

Seed for the 2005/06 field trial was grown in China. When compared to the control variety Castille, seeds were generally very small and for most lines were immature i.e. not fully rounded and of a red/brown colour rather than black. This is likely to be the main reason for the lower germination rate observed in 2005/06 as germination conditions (e.g. temperature and moisture) were not limiting. Seed used in the 2006/07 field trial was grown in the UK, where seed size, shape and colour were more comparable to the control variety Castille and homogeneous between the different TNDH and parental lines. This resulted in much improved germination and establishment.

Other factors to consider were experimental design, number of replications and plot size. For the first field trial, lines were grown in single rows (6 plants per row), whereas in the second field trial lines were grown in blocks of 4 rows with 10 plants per row. The use of different strategies with different precision, most probably affected estimates for variance components (Falconer 1989, Hallawer and Miranda 1988). Generally, a wider between-row distance than 35 cm and lower seed density would influence yield traits. Plants sown in narrower rows are taller with lesser branches than at wider spacing (Sincik et al., 2010). The total number of pods per plant would also be higher at narrow spacing even though the number of pods in the main raceme would decline. Therefore seed yield would higher than for oilseed rape grown in wider rows. In 2005/06, when plants were sown with 6 plants per row, seed yield per plant was 11.3 g plant^{-1} at High N and 7.15 g plant^{-1} at Low N. In 2006/07, seed yield per plant was 13.3 g plant^{-1} at High N and 7.71 g plant^{-1} at Low N. In 2005/06 seeding rate was 60 seed m^{-2} and in 2006/07, plants were sown according to commercial practises at 50 seeds m^{-2}. The seeding rate was higher in the 2005/06 field trial to counteract the potential edge effects associated with rows, rather than mini-plots used in the following year. In the mini-plots, the plants in the centre which were later sampled for analysis were not subjected to an edge effect. Therefore, despite using different field designs; seed yield did not differ significantly between the two years.

Growth abnormalities were observed in both the 2005/06 and 2006/07 field trials. In 2005/06, Blocks 2 and 4 were sown one day after Blocks 1 and 3, when land had to be worked again (power harrowed) and in the opposite direction to the original way of working the land. These blocks were much slower to germinate than Blocks 1 and 3 and it appears that the additional cultivation significantly reduced the moisture availability in the soil thus reducing germination and early growth. From a slow start Blocks 2 and 4 were never able to achieve the growth levels associated with Blocks 1 and 3 and always seemed to be catching up. The fact that 2 and 4 were much smaller and very different than Blocks 1 and 3 meant

that in effect there were 2 individual experiments each comparing High versus Low N treatments but neither experiment had any replication.

In addition, Blocks 2 and 4 were sown at 90º to 1 and 3 and seemed to be more affected by wind direction later in the growing season together with weak plants due to poor growth and stem canker. When this population was grown in other parts of the UK in the same season the whole experiment was severely affected with stem canker and the plant would fall flat on the floor (Colin Morgan, pers. comm.). The parent Ningyou7 has very low resistance to stem canker and although high levels of the disease are not generally seen in northern parts of England, infection levels were very severe as recorded in this experiment.

For the first field trial, there were a high number of TNDH lines grown and plants were grown as single half meter rows due to lack of available seed. The final analysis was carried out on a single plant with high heterogeneity evident between plants. Selection was based on an average plant. In 2006/07 a smaller number of TNDH lines were sown but this time in small plots. That analysis was carried out using the two central 1m rows, resulting in a much greater homogeneity between harvested plants which were then bulked to determine seed yield, total biomass etc.

The amount of seed available for the second field trial in 2006/07 permitted a design which reduced the variability encountered in 2005/06 i.e. in the second year an average of 20 plants from 2 rows were used for analysis whilst the first year only 1 plant out of a maximum of 6 was harvested. Plant growth was more homogeneous in 2006/07 with the exception of a patchy area in Block 1 which showed much reduced plant growth. The area covered 15 plots approximately where many of them were totally affected and some of them were partially affected with lesser growth. These lines were not discarded from further analysis because, despite a visual effect on growth, analysis of biomass and seed yield recordings showed the whole of Block 1 had lower recordings compared to those of Block 4. Analysis also showed Block 4 had higher mean recordings than expected for a Low N block, as results were closer to the ones obtained from Blocks 2 and 3 (i.e. High N blocks).

Tapidor was more stable across years within the same N treatment than Ningyou7 e.g. Tapidor had total biomass of 58.8 g plant^{-1} at High N in 2005/06 and 66.95 mg g^{-1} in 2006/07 and Ningyou7 had 11.2 and 27 mg g^{-1} respectively. Similar values were recorded for seed yield and harvest index, for Tapidor seed yield was 12.9 and 19.9 mg g^{-1} and harvest index 0.22 and 0.35 in 2005/06 and 2006/07 respectively. For Ningyou7, seed yield was 2.2 and 9.55 mg g^{-1} and harvest index 0.19 and 0.29 for the first and second field trials respectively. This variability across years being higher for Ningyou7 could be due to it being grown in a different climate as it was bred and selected for in China where it is classified as a semi-

winter variety (Shi et al 2009). The variety Tapidor was bred and selected for under UK conditions and as expected behaved more uniformly in general when grown under UK conditions. These results would mean that TNDH lines closer genetically to Ningyou7 may present similar variability to the parental line, and lines closer to Tapidor would be more stable as well when grown in a northern European environment. From the data it was evident that Ningyou7 was much earlier flowering than Tapidor and therefore in general it flowered too early for UK conditions i.e. in 2005/06 Ningyou7 flowered at 234 DAS; and in 2006/07 Ningyou7 flowered at 195 and 172 DAS at High and Low N, respectively. At that time of middle March and beginning of April in the North-east of England the risk of frost and low temperatures is high. In 2005/06 substantial frost damage was evident in the early flowering lines e.g.TN2, 9, 35, 68, 104, 143, etc., thus causing abortion of terminal racemes and of many early formed pod sites.

Analysis of variance (ANOVA) was performed to assess variability. The analysis showed high variability among TNDH lines as well as different responses of oilseed rape at High and Low N. However, the ANOVA analysis also showed that TNDH lines behaviour was highly influenced by environmental conditions, thus considering all blocks analysed as independent experiments. The analysis of variance showed different traits had different responses for different genotypes and some of them showed different responses under different N treatments. One of the explanations for that could be the capacity of adaptation of the TNDH population (containing semi-spring background) to winter conditions. Another possible explanation would be that part of the variability observed could be due to high variability among replicates.

The fact that the replicate blocks responded like independent experiments could also be explained by the sampling method, as no internal replication was performed neither for the first nor for the second field trial. Consequently, variability because of sampling was increased.

The control variety Castille also reinforced the idea of variability in the Blocks. In 2005/06, plant biomass ranged from 54.40 to 99.73 g/plant and the mean for these samples was 81.16 g/plant in Block 1 (High N). In Block 3 (Low N) of the same year, 6 control plants were analysed and total biomass data ranged from 78.51 to 126.42 g/plant, the mean for these samples was 92.86 g/plant. These results indicate that the control variety had relatively high variation for total plant biomass, moreover, it responded better at Low N than at High N, with increased plant biomass. Similar results were obtained for seed yield and harvest index, but seed yield was similar at High and Low N and harvest index was lower at Low N. Data for N traits i.e. seed, plant and total N concentrations and NUpE and NHI did not show marked

differences between High and Low N treatments, except for NUpE. Values for NUpE were between 4.69 and 17.32 g m^{-2} at High N and 32.68 and 51.61 g m^{-2} at Low N (Appendix 14).

In 2006/07, data was bulked and only two control samples were analysed consisting of 20 plants each. Nitrogen data from all blocks did not present major differences between High and Low N treatments except for Block 1, which always had the lowest values for N concentration traits (Appendix 14). Control data from Block 2 showed behaviour expected from a High N Block. Blocks 3 and 4 had some contradictory results related to High and Low N treatments; for example, one of the controls analysed in Block 3 had the lowest total N value of all blocks and Block 4 had the highest values among all blocks. However, NUpE values did agree with the expected results for High and Low N treatments, and Blocks 2 and 3 had 10 times lower values than Blocks 1 and 4.

2.4.2. Sources of variation

Genotypic variation

Eight out of the 16 traits analysed in 2005/06 showed statistically significant variation existed between TNDH lines i.e. FDAS, HI, TSW, CB, CL and oil (P<0.001) foot length at P<0.01 and seed number per pod at P<0.05. These results meant that for 8 traits there was enough genetic variation to detect the genetic mechanisms controlling these traits. The remaining traits did not show significant variation between TNDH lines indicating limited genetic variation existed between lines, but most likely because the error component was highly significant. Presumably, such high error was attributed to the variance for High and Low N being calculated together (and not separately as reported for maize by Bäzinger and Laffite, 1997), thus adding up the error component.

To further assess genetic variability, heritability was calculated for all traits in both years. Heritability values were particularly low for HI and NHI, as well as NUtE. Contradicting Yau and Thurling (1987) results, showing that NUE trait was not genetically transmitted in oilseed rape, the heritability found in 2006/07 was of 74%. The heritability for NUpE was lower than NUE (0.46), but still higher than NUtE. For seed yield and total above ground biomass, large differences were found for heritability in the 2 years. In 2005/06 TW and SY had heritability values of 0.39 and 0.37 respectively, in 2006/07 the heritability values for these traits increased to 0.63 and 0.76, respectively.

Phenotypic variation

TNDH lines demonstrated transgressive segregation in both directions for most of the traits and environment combinations studied, indicating that the 2 parental lines did not represent the lowest and

highest values of the traits. The most valid hypothesis to date to explain transgressive segregation is the interaction of complementary gene action (Grant, 1975; Vega & Frey, 1980). Moreover, different species or different parental lines are often fixed for sets of alleles with opposing effects, resulting in transgressive segregation in hybrids (deVicente & Tanksley, 1993).

YIELD AND YIELD DERIVED TRAITS

Yield traits commonly analysed in both years such as TW, SY and HI demonstrated transgressive segregation only in the positive direction and they were more influenced by Tapidor than Ningyou7. These results indicate that the 2 parental lines were very appropriate to enhance breeding for such traits, as the TNDH lines presented higher values than the parents, but not lower. Thousand seed weight and seed number per pod showed transgressive segregation in both directions; however transgression was higher at higher values. Both traits were predominantly influenced by Tapidor. Harvest Index and 1000-seed weight presented values for the parental lines very close to the population mean, indicating 1 allele regulation of the trait was present in both parents, and that either of them could be the dominant one.

For traits like 1000 seed weight and seed number per pod very high heritability i.e. 0.987 and 0.972 respectively was reported by Zhang and Zhou (2006), but heritability for 1000 seed weight was reported as low at 0.34 (Yu et al. 1998) and even lower results were obtained for this trait on the TNDH study (h^2=0.02).

Zhang and Zhou (2008) studied agronomic and seed quality traits in *Brassica napus* and concluded that to improve seed yield plants with more pods per plant and higher thousand seed weight should be selected to obtain higher seed yield. They also found high trait heritability for plant height and 100 seed weight and concluded that early selection should be stringent for these traits. Complementary gene interaction was observed for number of pods per plant, pod length and seed yield, meaning selection should be based on these traits altogether.

Low h^2 values can also be explained by the high variability within blocks and across years, agreeing with the theory that TNDH population was highly influenced by environmental conditions for yield and N related traits. However, very high values for yield and NUE were found in 2006/07.

NITROGEN AND NITROGEN DERIVED TRAITS

Seed N concentration and plant N concentration had little or no transgressive segregation in the negative direction in 2005/06; with similar results obtained the following year for seed N. Plant N showed transgressive segregation in 2006/07 the split into chaff N and stem N could have influenced

this as both traits demonstrated transgressive segregation in both directions. However, both trials 2005/06 and 2006/07 showed little difference between the mean value for the TNDH population and the parental values for plant N or chaff and stem N respectively, thus indicating that the inclusive allele responsible for most of the trait variability was present in both parents. Chaff N in 2006/07 was slightly more influenced by Ningyou7 than Tapidor, meaning that one of the alleles responsible for this trait may most probably come from this parent.

Nitrogen derived traits such as NUpE and NUE behaved similarly in both years. Analyses from the first field trial showed clear transgressive segregation for both traits in both directions, whereas for the second year there was no transgressive segregation for NUpE and only NUE showed segregation in the positive direction only. There are some differences between years for NUtE and NHI: for the first field trial the population mean was higher than the parents, favouring selection and indicating heterosis. In the second year, the mean value was very closely related to Ningyou7 but was never higher. Results for NUtE and NHI in particular would suggest the TNDH population is an appropriate choice for studying further the genetic control of these traits. Heritability values were very high for NUE in 2006/07, and moderate for NUpE, suggesting the traits would be favourable for selection. However, NUtE and NHI had very low heritability (h^2=0).

OIL CONTENT

Oil content demonstrated transgressive segregation for the higher values only, possibly due to adaptation mechanisms towards improving the trait. The histogram presented 2 peaks one bell-shaped peak corresponding with the population mean, indicating 2 or more alleles were influencing the trait and the other one corresponding with Tapidor, explained by 1 or 2 alleles. Narrow sense heritability for oil content in 2005/06 was very high (0.57) compared with those for yield, architectural and N related traits. The TNDH population had very high heritability for seed oil content in 2005/06 in contrast with the 0.153 from Zhang and Zhou (2006) indicating the potential of this population in studying the control of oil content in oilseed rape.

CANOPY TRAITS

Flowering presented a similar pattern in both years showing transgressive segregation in both directions. The trait seemed more influenced by Tapidor than Ningyou7 both at High and Low N (as the population mean was closer to Tapidor than Ningyou7), particularly in the first year and presumably because the environment was more favourable for the Tapidor cultivar. For the second field trial, the TNDH population mean was closer to Tapidor than Ningyou7, but not as much as for the first year.

Possible reasons were that seed was grown in the UK which is where Tapidor grows best, but also because the lines sown were selected based on eliminating the very early flowering lines, (population named BnaTNDH_4, used in NOVORB LINK project, http://www.brassica.info/CropStore/populationslinked.php?pop=BnaTNDH). In selecting this subset, lines were selected to avoid frost damage and those known to have increased resistance to stem canker, therefore possibly biasing the population towards Tapidor.

In Long et al. (2007), broad-sense heritability was reported as high as 0.77 and 0.9 for the TNDH population and an offspring population (RC-F_2) respectively when grown in China.

Chlorophyll in bracts and leaves also showed negative transgressive segregation, but only CB showed positive transgressive segregation as well. Little differences were found between the parental lines and the mean population for chlorophyll content in bracts, but higher variation was found for CL. That could possibly indicate that CB is more constant and CL more variable and adaptable to different conditions. Heritability for CL was higher (0.55), in comparison to the 0.35 found in maize (Bäzinger and Laffite 1997).

Both parents showed different behaviour at High and Low N for architectural traits i.e. plant height, foot length and branch number, where Tapidor presented higher values than Ningyou7 for all 3 traits. Architectural traits i.e. TL, FL and BN studied only in 2005/06 presented transgressive segregation in both directions. The average of the TNDH population was more influenced by Tapidor in all 3 traits. Interestingly, plant height presented two peaks in the distribution, one corresponding to Ningyou7 and the other one to the population mean, which was very much closer to Tapidor. That meant one allele would explain the peak for the population mean and 2 or more alleles influencing the trait would explain the bell shape of the peak on Ningyou7. These traits had low narrow sense heritability values in general, i.e. 0.18 for plant height. These results contrast with those of Zhang and Zhou (2006) and Yu et al. (1998) who found that heritability for plant height in an oilseed rape DH population was 0.927 and 0.68 respectively when grown in China. They found the lowest heritability for number of branches of 0.172 (Zhang and Zhou, 2006) in accordance to the results obtained in 2005/06 when heritability was 0.01.

2.4.3. Trait response under High versus Low N

Parental variation

Parental lines Tapidor and Ningyou7 showed different responses at High and Low N for the different traits analysed. Flowering presented a similar structure across years and treatments; Ningyou7 was the

parental line to flower earlier and Tapidor the one to flower later (Ningyou7 is semi-winter and Tapidor winter) as described by Long et al. (2007). For the second field trial there was a clear response to N treatment, showing early flowering under low N conditions as reported in Roux et al., (2005) that late flowering varieties would do so to accumulate more nutrients and have higher yields at a later stage. Both Tapidor and Ningyou7 had a significant response to N treatment for both CB and CL, with much lower values at Low N. A typical characteristic of Ningyou7 was the yellow-green colour of its leaves, whereas Tapidor exhibited a much darker green colour. This was confirmed by the chlorophyll readings from leaves, confirming Ningyou7 had lower chlorophyll concentration than Tapidor. In bracts, the difference between chlorophyll readings was not so large but Tapidor was still higher than Ningyou7.

Both parents showed different behaviour at High and Low N for architectural traits i.e. plant height, foot length and branch number, where Tapidor presented higher values than Ningyou7 for all 3 traits. Both for TL and BN the population was more influenced by Tapidor than Ningyou7. Tapidor had higher values than the average of the TNDH lines at High N, particularly for TL where Tapidor had the highest value amongst all lines analysed. However, Tapidor had lower trait values than the average at Low N for architectural traits. Ningyou7 had lower values than the average population at High N for TL, FL and BN traits.

All yield traits analysed were very much influenced by Tapidor, as for most of the traits the population mean was very close to the Tapidor value for the trait i.e. TW, SY, HI, and TSW and SN/P in 2005/06. A similar trait response to N treatment was present at both High and Low N. At High N, Ningyou7 had lower values than Tapidor and the mean for the TNDH lines, except for TSW in 2006 and for HI in 2007 where Ningyou7 had higher values. At Low N, Tapidor had higher values for yield traits than the population mean and Ningyou7 only in 2007, in 2006 Ningyou7 died and Tapidor had lower values than the average for the population.

Tapidor had higher oil content than Ningyou7 both at High and Low N, however, oil content was slightly lower at Low N and the value was closer to the population mean. Ningyou7 had very low oil content almost the lowest of all the lines studied. Transgressive segregation in the positive direction for oil content was larger at Low N.

Plant N concentration remained rather constant both at High and Low N in both years. Tapidor and Ningyou7 had similar values at High N meaning little difference existed for the trait in 2006. In 2007 when the trait was split into chaff and stem N, Tapidor showed higher values for chaff N, whereas stem N remained constant. Stem N values for Ningyou7 dropped considerately at Low N and chaff N dropped only moderately, thus indicating a higher efficiency in N translocation to the seed for Ningyou7.These

findings are in accordance with those of Malagoli et al. (2005) in winter oilseed rape. They observed progressive nitrogen mobilisation from bottom leaves and stem to upper parts; in accordance with findings of Schjoerring et al. (1995) in *Brassica napus* as well. They also suggested the stem would work as a buffer organ, storing N from taproot and lower leaves for remobilisation later on during pod filling. Tapidor also had higher seed N than Ningyou7 in both years at High N, but lower than both Ningyou7 and the average of the TNDH lines at Low N. Ningyou7 did not seem to be influenced by N treatment for this trait and remained rather constant These results are in accordance with the NUpE and NUE analysis which indicated Ningyou7 is more efficient at Low N, whereas Tapidor is more efficient at High N. This reflects the breeding and selection regime of the 2 varieties i.e. Tapidor bred and selected under High N regime (UK) and Ningyou7 under a lower N regime (due to reduced N inputs in China). Ningyou7 also had higher values for NUtE and NHI at High N in 2007 only, even though both parents and the population mean were very close together for these traits. Tapidor showed little or no response at High or Low N for N derived traits in 2007, thus indicating Tapidor would not be an efficient variety for studying N related traits under Low N conditions.

YIELD AND YIELD COMPONENT TRAITS

The TNDH lines did not show a different response at high or Low N for yield traits, with the exception of harvest index. The traits did not show different response between the different genotypes, except 1000-seed weight and HI. The TNDH lines showed different responses to N treatment in plant height and foot length. As there is a strong positive relationship between total above ground biomass and plant height, it could be possible that lines did actually have different responses under High and Low N but it could not be found in the analysis. That would reflect very high variability in the plant responses under different circumstances, and would suggest plants exhibited very different behaviour in the different replications carried out.

The correlations of seed yield with days to beginning of flowering showed a negative correlation at both N levels in 2005/06. These same results were also found by PCA analysis and were fully in agreement with the findings in oilseed rape by Kessel (2000), suggesting that early maturing genotypes will profit from the soil available N to produce yield not only when soil N supply is limited but also under conditions of high N supply.

Strong positive correlation between number of pods per plant and seed yield was found at High and Low N treatments both by Pearson's and PCA, as in Zhang and Zhou (2008), who analysed a newly

synthesised *B. napus* DH population under normal agricultural practises in China (F1 analysed in 2002 and F2 in 2003/04) .

NITROGEN AND NITROGEN DERIVED TRAITS

Nitrogen and N derived traits did not show a response to N treatment. Only NUtE and NHI did in 2006/07. Moreover, most of the traits did not show genotypic differences between lines. However, in the case of N concentration traits in different parts of the plant (i.e. seed, stem, and chaff) did not show differences, but total N did. As suggested before, it is possible that total plant N is genetically regulated; but the seed, stem and chaff N concentrations are not. These results would be in accordance with the fact that NUtE did not appear as a heritable character for the population.

No significant correlation was found between yield and seed N as in a previous study by Kessel (2000), and it is therefore concluded that the N efficient genotypes will have more N in the seed at harvest, regardless of the N level.

Nitrogen use efficiency is defined as the grain produced per unit of available soil N supply and it can be split into two components, namely N uptake (the efficiency with which N is taken up from the soil) and N utilisation (the efficiency with which the absorbed N is converted into yield) according to Moll et al. (1982). Nitrogen Uptake and Use Efficiency mean values were higher at Low N than at High N, meaning TNDH lines were more efficient in N uptake and use at Low N regime than at High N, where N was not limiting, contradicting findings by Svecnjak and Rengel (2005). They studied spring canola and found no significant differences between NUE at High or Low N treatments. However, findings from Lemaire et al. (2007) analysed different crops and suggested that both wheat and canola maintained the resource use efficiency (N uptake per unit LAI) at Low N supply, whereas the N content per unit leaf area dropped at High N supply, due to the heterogeneous distribution of N in the canopy.

Traits such as NUpE, NUE and NHI did show higher values in 2007 than in 2006, NUE was higher due to an increase in NUpE, as NUtE remained rather constant across years.

There were significant and very high positive correlations between NUpE and NUE both at High and Low N treatments, meaning NUpE was an important component of NUE in explaining the genetic variation. This is in accordance with results reported by Moll et al. (1982) for High soil N conditions and Ortiz-Monasterio et al. (1997) for Low soil N conditions.

No significant correlation between N uptake and N concentration was found for seed N and plant N concentration at High N by either Pearson's or PCA. These results are in accordance with Malagoli et al. (2005) who suggested possible explanations could be associated with complex enzyme regulations at

the transcription level and also interactions with the carbon metabolic pathway. However, a strong positive correlation between NupE and chaff N concentration was found at both High and Low N supply. They also suggested that genotypes with a higher level of N mobilization maintained a relatively similar constant NUpE.

Nitrogen utilisation efficiency is considered to be an essential physiological parameter contributing to improving NUE as identified by Isfan (1993). Both experiments exhibited positive relationship between NUE and NUtE at High N treatment, but there was no obvious correlation at Low N, suggesting that the main factor contributing to higher NUE at Low N was NUpE. As neither NUpE nor NUE showed line*nitrogen interaction, that meant the traits had similar response at both treatments, therefore, the selection could be carried out at High N only.

Ortiz-Monasterio et al. (1997) defined NUtE as a combination of HI and BPE (total above ground biomass produced per unit of N absorbed) and showed that new wheat cultivars had improved HI rather than improved BPE. Earlier studies on spring wheat and barley grown in the Nordic region showed that improvement in NUtE was achieved through reduced plant height and enhanced yields via higher HI (Ortiz et al., 1998 and 2002). In results presented here, both phenotypic correlation and PC analyses concluded that NUtE was highly correlated with HI and NHI; hence NUtE could be improved by significant improvements in HI. Further improvements in seed yield and in NUtE would be attained by both breeding for varieties with higher HI and by increasing the total above ground biomass while maintaining the HI at the current level.

A very strong correlation was found between seed yield and NUE at both N levels, for all years (0.99 in 2005/06 and 0.84 in 2006/07 at High N), agreeing with findings that genes from the N pathway i.e. GS1 (Glutamine Synthase) was found to be increased in mutants with affected kernel yield in maize (Martin et al. 2006).

Both NUE and seed yield are highly correlated with root traits, such as root biomass, as is NUpE. It has been suggested that NUE can be improved by an improvement in the root system. For example, a study in wheat proved that plant root biomass contributed to 65% of the variation observed for total plant N (Ehdaie et al., 2010). And higher total N concentration in plants has been shown in higher NUE genotypes. Brady et al (1993) showed important correlations between plant root biomass and N uptake efficiency. Another correlation from the same study showed an increase of seed yield by increasing root biomass. In this occasion, plant root biomass explained a 53% of the variation for grain yield in wheat.

Results from PCA agreed with those of Grami and La Croix (1977) who working with spring rapeseed reported a direct relationship between N uptake and seed N content, thus concluding that selection for high seed N content leads to improved N uptake and translocation efficiency.
Malagoli et al. (2005) suggested that to increase NHI and NUE higher N mobilisation from source tissue, particularly lower leaves, would be the ideal scenario for then obtain higher pod N.

OIL CONTENT

Oil content analysis show the trait has a different response at High or Low N, and it has a high heritability as well, thus making it a good trait for selection.

Oil content in seed had a strong negative correlation with seed N concentration at Low N, and also at High N but this was not statistically significant at either N level. Using PCA analysis a strong negative correlation could be identified at both High and Low N. Strong negative correlations between oil and protein content have been found in oilseed rape and other species in a number of studies (Zhao et al., 2008; Hirel et al., 2007). A strong positive correlation was identified by PCA for TSW and oil content.

CANOPY TRAITS

Architectural traits had different responses at High and Low N treatments (plant height and foot length), except for branch number which did not.

Plant height had a large amount of positive correlations amongst traits, most of them at both High and Low N, i.e. with foot length, branch number, number of pods per plant, plant biomass, and seed yield. Most of the traits' correlations were influenced by plant size, the bigger the plant the higher the trait values were. A strong positive correlation was found between plant height and branch number, these results are contrasting with Zhang and Zhou (2006), who identified a strong negative correlation. All correlations were confirmed by PCA analysis, also confirming a positive correlation for TL and BN at High N; however it was weaker at Low N.
Flowering showed different genotypes responded differently at High or Low N, only in 2006/07. Probably the selection of a sub-population reduced part of the variability in the experiment. Flowering did negatively correlate with total above ground biomass and seed yield at Low N in 2005/06, but the correlations were not repeated in the following field trial. In 2005/06 it also negatively correlated with NUpE, NUE and NHI, only at Low N; and with positively correlated chlorophyll content in bracts and leaves at High N only. None of the correlations was subsequently found in 2006/07/

Chlorophyll content in bracts did show a different response to N treatment, whereas chlorophyll in leaves did not.

Chlorophyll in bracts was found to be positively correlated with the amount of chlorophyll in leaves, both at High and Low N. Chlorophyll in bracts was also strongly positively correlated with harvest index, NUtE, NHI and oil content but only at Low N. Bract chlorophyll concentration correlated with flowering time and seed number per pod at High N only, and with foot length at both N treatments, but showed a stronger relationship at High N. Chlorophyll in leaves was positively correlated with foot length and flowering at High N only. It had no correlation with seed yield which contradicted the findings of Bäzinger and Laffite (1997) who found positive correlation between CL and SY in maize. Chlorophyll correlations were confirmed by PCA analysis, showing strong relationship between them and FL. According to PCA analysis neither CB nor CL correlated to FL, but supported the findings via Pearson's correlation analysis confirming a correlation between them at both N regimes.

CHAPTER 3. QUANTITATIVE TRAIT LOCI ANALYSIS (QTL) OF AGRONOMIC TRAITS USING THE TNDH POPULATION

3.1. INTRODUCTION

The genus Brassica differs in genome structure with different species which are combinations of the A, B and C genomes. ***Brassica rapa*** (syn. campestris); is the diploid ancestor of the A genome, *B. nigra* of the B genome and ***B. oleracea*** of the C genome. ***Brassica napus*** developed from ancestral genomes of *B.rapa* and *B. oleracea* is a mixture of both the A and C genomes.

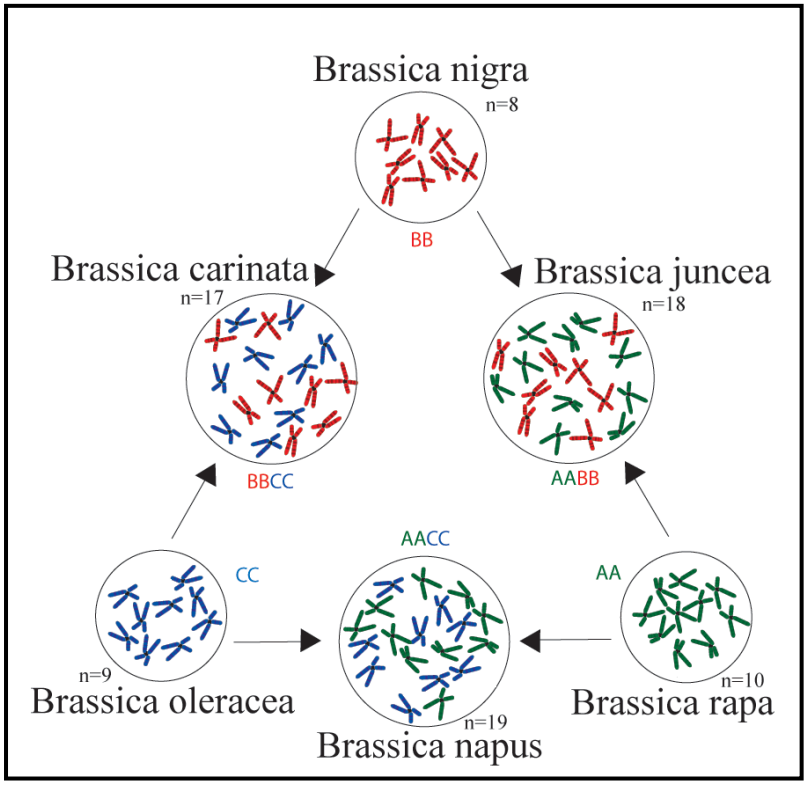


Figure 3.1. Brassica genome origins according to "Triangle of U" theory.

Brassica napus is an amphidiploid which has 19 pairs of chromosomes (n=19), 10 from the A genome and 9 from the C thus named A1 to A10 and C1 to C9. Current projects funded to sequence both the *Brassica* A and C genomes are at different stages of completion (Bancroft personal communication).

Many agronomic traits of interest to breeders are quantitative; hence most crop breeding programs concentrate on such traits (Paterson et al. 1991). Quantitative traits are generally polygenic traits

governed by more than one gene whose functions are very much influenced by environmental conditions. Such environmental effects reduce the heritability for the trait, thus making them difficult to study.

To enhance trait selection, breeders have to rely on phenotypic data, being very time and resource consuming, as well as of variable effectiveness. Since the development of molecular marker technology in the 1990s, the use of QTL for mapping and identification of agronomic traits has been increasingly used (Dudley, 1993). The use of molecular markers has provided insights into the genomic location of individual QTL as well as gene function and interactions, thus broadening knowledge of the genetic basis of traits of interest (Lander and Botstein, 1989; Tanksley et al., 1989).

Molecular markers are useful tools to aid breeding strategies, both to gather information on the genetic basis of the traits of interest and to assess genetic diversity. The selection process, i.e. marker-assisted selection (MAS), is based on the genotype and is therefore not influenced by the environment. Many marker families have been developed with different characteristics and benefits; starting with Restriction Fragment Length Polymorphism markers (RFLP), evolving to Random Amplified Polymorphic DNA (RAPDs) and Amplified Fragment Length Polymorphisms (AFLPs), and finally to Simple Sequence Repeats (SSR), Single Nucleotide Polymorphisms (SNPs), Diversity Array Technology (DArT) markers and Microsatellites. One of the important functions of molecular markers is the construction of a linkage map, which allows determination of the location of markers linked to genes of particular interest. Restriction Fragment Length Polymorphism markers were the first ones developed in plants and used to construct genetic maps (Helentjaris et al. 1985 and Helentjaris et al. 1986).

Choosing an appropriate population is critical to the final detection of QTL. The first Doubled Haploid population (DH) to be produced was reported in *Datura stramonium* (Blakeslee et al., 1922). Most of the advantages of a DH population rely in the fact that homozygous lines can be produced after the first generation and in a large number, thus making the technique efficient and cost-effective. Homozygous lines allow replication, and therefore, data for QTL analysis is more robust. The main disadvantages are linked to homozygosis, as undesired characters are difficult to eliminate and narrow selection (Pink et al. 2008).

Different studies have been reported using the TNDH population, i.e. QTL have been detected for seed oil and erucic acid content (Qiu et al. 2006) and yield (Shi et al., 2009); moreover, markers associated with boron efficient genes have been localised (Zhao et al. 2008).

The aim of this chapter was to identify key loci involved in the expression of traits for differential responses to nitrogen supply using the Quantitative Trait Loci (QTL) approach and to characterise the

genetic basis of relevant traits for breeding varieties improved in NUE, particularly under low N conditions, by assessing the stability of identified QTL, their heritability and G x E interactions.

3.2. MATERIALS AND METHODS

3.2.1. PLANT MATERIAL

The population used for QTL analysis was the TNDH population described in Chapter 2. In 2005/06, 174 TNDH lines were analysed (one plant was sampled from each line in each block) and in 2006/07, 94 TNDH lines were studied (a bulk of 20 plants was sampled from each line for analysis).

3.2.2. STATISTICAL ANALYSIS AND QTL MAPPING

Statistical analyses of variance, phenotypic correlations and narrow sense heritability were analysed and described in Chapter 2.

The QTL analysis was performed using WinQTL Cartographer version 2.5 (http://statgen.ncsu.edu) on data collected from Blocks 1 and 3 (at High and Low N respectively) from the first field trial in 2005/06, and from Blocks 1, 2, 3 and 4 independently for the second field trial (High N Blocks were 2 and 3; and Low N Blocks were 1 and 4) in 2006/07. In 2006/7, the QTL analysis was carried out separately for Blocks 1-4 because, when the QTL analysis was carried out for the average values across the two replicates, no QTL were detected. Therefore a separated analysis approach was adopted.

To perform QTL analysis a process of 6 steps had to be completed to upload the relevant files. For the first step the following parameters were set: number of chromosomes, trait number, and binary trait number, sample size, missing trait value, cross type and marker genotype table. The number of chromosomes or linkage groups was set to 19, as per the number of chromosomes present in oilseed rape.

The traits were organised as architectural traits, yield traits, nitrogen traits and nitrogen derived traits and the number of traits was set prior to each analysis according to the grouping.

Other traits of binary value were always set to 0. Individual number or sample size was set to 174 for the first trial and to 94 for the second year according to the size of the population used in the 2 years.

All TNDH lines were included in the QTL analysis and those lines where some or all of the data was missing were also considered with the symbol "\" for missing trait value. The cross type was "Ri0"

corresponding to DH populations in WinQTL Cartographer. The marker genotype table was completed as follows: the symbol for AA parent was A, for aa parent was B and for missing value was U.

The next 2 steps consisted of providing information on the genetic map. The first file uploaded for this step contained information on the numbers of markers per chromosome. The second one was a 2 step upload with information on marker names first and marker positions second (in cM), both classified in chromosome order.

The fourth step organised the number of files to be uploaded in step 5. The fifth step consisted of uploading genotypic information as well as trait (phenotypic) information.

The final step is the creation of a source file that can be used to perform all QTL analysis such as single marker analysis i.e. interval mapping (IM), composite interval mapping (CIM) and multiple interval mapping (MIM).

MAP AND MOLECULAR MARKERS

The map used for QTL analysis was the latest version sharing alignment with the *Arabidopsis* genome as described by Shi et al. (2009). The newly developed map was in accordance with previous maps used for the TNDH population (Qiu et al. 2006, Long et al. 2007) and covered the 19 chromosomes of *Brassica napus* with an average distance of 2.7cM between markers.

The final map contained 786 markers of which 277 were used as anchor markers with the *Arabidopsis* genome, according to Parkin et al. (2005).

Only molecular markers present in both markers position file and markers genotypes file were included in the QTL analysis, as the WinQTL Cartographer does not allow mismatching data.

QTL ANALYSIS WITH WINQTL CARTOGRAPHER SOFTWARE

Quantitative trait loci analysis was firstly performed by Single Marker Analysis to overview the general QTL distribution and then QTL were detected by Interval Mapping and Composite Interval Mapping (Zeng, 1994) using WinQTL Cartographer (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm).

The first analyses with single marker (SMA) and Interval Mapping (IM) were run using the default parameters from the program (for IM walking speed was 2cM, number of control markers 5, window size 10cM). The second round of analyses was run at 1cM walking speed whilst window size and number of control markers were kept as the default value. For CIM analysis the number of control markers, window size and walking speed were set at 5, 10cM and 1cM respectively. The threshold LOD score (logarithm of the odds ratio) considered to detect a meaningful QTL for the TNDH population was set at

>2.5 with *P<0.05*, and a lower LOD score of >2.0 at *P<0.5* (with 1000 permutation analysis test) was also considered for QTL with a more subtle effect as described by Shi et al. (2009). Multiple interval mapping (MIM) with the program default parameters was used when 2 or more QTL were detected on the same chromosome either/both by IM or CIM.

Multi trait analysis was also performed with WinQTL Cartographer (using IM) to analyse QTL by environment interactions and trait associations and for this a joint LOD score of 2 and above was considered significant (*P<0.5*). Estimate values for the square of the partial correlation coefficient (R^2) to calculate phenotypic variation and additive effect were obtained from WinQTL Cartographer output files. The default genetic distance of 5 cM was used to define a QTL in each treatment.

To study the QTL x Environment interactions, multiple trait analysis using CIM (MCIM) from WinQTL Cartographer was used with the default parameters from the program and only the walking speed was changed from 2 to 1cM. The analysis allowed for a simultaneous analysis of one particular trait at both High and Low N treatments, performed with the module JZmapqtl available in WinQTL Cartographer.

3.3. RESULTS

Analysis of variance (Chapter 2) showed considerable genotype x environment interaction and as a consequence the 2 field trials were analysed separately for QTL mapping.

3.3.1. EXPERIMENT 1. FIELD TRIAL 2005/06.

YIELD AND YIELD COMPONENT TRAITS

Interval mapping results for TW (total above ground biomass) detected 2 QTL at High N only, both on chromosome 19 with LOD scores of 2.38 and 2.68 respectively (Table 3.1). Composite interval mapping detected 6 QTL at High N, 5 on chromosome 19 and 1 on 1 with LOD scores ranging from 2.30 to 4.64. The 2 QTL from IM explained 13% of the phenotypic variation and 3 QTL from CIM (confirmed as independent QTL by MIM) accounted for 20.3% of the phenotypic variation of the TNDH population.

Table 3.1. Putative QTL and related marker position influencing total above ground plant biomass (TW) in the 2005/06 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0 at High and Low N.

Trait	QTL	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
TW	IM								
	TW_19_5_H06	High N	19	5	43.97	171.66	0.061	2.38	44.0
	TW_19_10_H06	High N	19	10	57.32	186.83	0.069	2.68	57.3
	CIM								
	TW_1_1_H06	High N	1	1	0.01	163.13	0.050	2.30	0.0
	TW_19_2_H06	High N	19	2	16.39	-165.80	0.051	2.21	
	TW_19_3_H06	High N	19	3	29.17	-176.52	0.055	2.31	29.2
	TW_19_8_H06	High N	19	8	49.78	234.29	0.095	4.13	
	TW_19_9_H06	High N	19	9	57.21	243.50	0.103	4.64	57.2
	TW_19_11_H06	High N	19	11	59.05	165.94	0.052	2.23	
	TW_1_24_L06	Low N	1	24	56.63	180.66	0.056	2.62	56.6
	TW_1_28_L06	Low N	1	28	58.29	160.95	0.044	2.08	
	TW_1_36_L06	Low N	1	36	63.92	169.57	0.049	2.31	
	TW_1_38_L06	Low N	1	38	65.65	159.84	0.044	2.06	65.7
	TW_7_46_L06	Low N	7	46	109.34	-191.47	0.058	2.74	109.3

Composite interval mapping also detected four QTL on chromosome 1 and one on 7 at Low N; three of these (TW_1_24_L06, TW_1_38_L06 and TW_7_46_L06) were confirmed by MIM, with LOD scores of 2.06, 2.64 and 2.74 respectively. The 3 QTL together explained 15.8% of the phenotypic variation of the population.

Two and three QTL were identified for seed yield (Table 3.2) at High N by IM and CIM respectively. The QTL identified by IM presented LOD scores of 2.09 and 2.21 and accounted for 11.1% of the phenotypic variation, whilst the 3 QTL from CIM had LOD scores of 3.74, 3.81 and 2.16 and collectively accounted for 22.9% of the phenotypic variation.

At Low N, 6 and 9 QTL were identified for SY using IM and CIM respectively, but only 1 and 3 were confirmed by MIM, classifying the rest as ghost QTL. The QTL identified by IM on linkage group 6 had a LOD score of 2.16 and accounted for 5.6% of the phenotypic variation. The other 3 QTL from CIM were on chromosomes 6 and 7, and had LOD scores of 2.79, 2.31 and 3.03. Together they accounted for 19% of the phenotypic variation of the TNDH population.

Only QTL at High N were detected for HI using IM. Seven out of the 11 QTL detected were also confirmed by MIM. The LOD scores of these ranged from 2.12 to 3.06 and collectively explained 51% of the phenotypic variation of the population.

Table 3.2. Putative QTL and related marker position influencing seed yield (SY) in the 2005/06 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0 at High and Low N.

Trait	QTL	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
SY	IM								
	SY_19_5_H06	High N	19	5	43.97	45.24	0.054	2.09	44.0
	SY_19_10_H06	High N	19	10	57.32	47.82	0.057	2.21	57.3
	SY_6_24_L06	Low N	6	24	63.46	-50.50	0.055	2.13	
	SY_6_27_L06	Low N	6	27	65.57	-50.42	0.055	2.13	
	SY_6_30_L06	Low N	6	30	66.23	-52.00	0.057	2.24	
	SY_6_33_L06	Low N	6	33	67.12	-51.31	0.057	2.23	
	SY_6_35_L06	Low N	6	35	68.41	-51.01	0.056	2.17	
	SY_6_37_L06	Low N	6	37	69.6	-50.53	0.056	2.16	69.6
	CIM								
	SY_19_8_H06	High N	19	8	49.78	63.06	0.089	3.74	49.8
	SY_19_10_H06	High N	19	10	57.32	62.41	0.088	3.81	57.3
	SY_19_14_H06	High N	19	14	67.11	46.64	0.052	2.16	67.1
	SY_6_22_L06	Low N	6	22	60.68	-49.04	0.052	2.26	
	SY_6_24_L06	Low N	6	24	63.46	-53.29	0.061	2.79	63.5
	SY_6_27_L06	Low N	6	27	65.57	-54.04	0.063	2.87	
	SY_6_30_L06	Low N	6	30	66.23	-57.30	0.069	3.23	
	SY_6_33_L06	Low N	6	33	67.12	-53.71	0.062	2.90	
	SY_6_35_L06	Low N	6	35	68.41	-53.94	0.062	2.87	
	SY_6_37_L06	Low N	6	37	69.6	-52.24	0.060	2.73	
	SY_6_38_L06	Low N	6	38	73.84	-54.75	0.064	2.31	73.8
	SY_7_46_L06	Low N	7	46	109.34	-55.62	0.065	3.03	109.3

When using CIM to analyse HI (Table 3.3), 8 and 10 QTL were detected at High and Low N respectively. Of these, 4 and 6 were confirmed by MIM at High and Low N respectively. The LOD scores for the QTL detected at High N were from 2.23 to 3.74 and the 4 QTL explained 27.2% of the phenotypic variation. At Low N, the LOD scores were from 2.14 to 4.74 and the 6 QTL jointly explained 36.9% of the phenotypic variation of the TNDH population. One QTL detected on chromosome 7 HI_7_25_L06 had a particularly high LOD score of 4.74 and explained 10.3% of the phenotypic variation of the population.

Table 3.3. Putative QTL and related marker position influencing harvest index (HI) in the 2005/06 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0 at High and Low N.

Trait	QTL	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
HI	IM								
	HI_7_28_H06	High N	7	28	70.9	0.02	0.062	2.45	70.9
	HI_7_30_H06	High N	7	30	73.69	0.02	0.065	2.54	
	HI_9_41_H06	High N	9	41	79.39	0.02	0.086	3.06	79.4
	HI_9_42_H06	High N	9	42	82.43	0.02	0.078	2.79	
	HI_9_44_H06	High N	9	44	85.98	0.02	0.071	2.80	
	HI_9_46_H06	High N	9	46	91.11	0.02	0.073	2.89	91.1
	HI_9_56_H06	High N	9	56	128.38	0.03	0.106	2.12	128.4
	HI_10_8_H06	High N	10	8	10.77	-0.02	0.056	2.18	
	HI_13_37_H06	High N	13	37	124.52	-0.02	0.055	2.13	124.5
	HI_13_42_H06	High N	13	42	136.3	-0.02	0.064	2.50	136.3
	HI_17_5_H06	High N	17	5	35.41	0.02	0.061	2.33	35.4
	CIM								
	HI_7_25_H06	High N	7	25	66.88	0.02	0.046	2.25	66.9
	HI_7_29_H06	High N	7	29	72.94	0.02	0.068	3.38	72.9
	HI_9_41_H06	High N	9	41	80.39	0.02	0.080	3.61	80.4
	HI_9_44_H06	High N	9	44	85.98	0.02	0.057	2.85	
	HI_9_46_H06	High N	9	46	91.11	0.02	0.060	2.99	
	HI_9_48_H06	High N	9	48	93.78	0.02	0.045	2.23	
	HI_13_41_H06	High N	13	41	135.88	-0.03	0.078	3.74	135.9
	HI_17_5_H06	High N	17	5	35.41	0.02	0.058	2.86	
	HI_6_24_L06	Low N	6	24	64.46	-0.02	0.044	2.14	64.5
	HI_6_28_L06	Low N	6	28	65.97	-0.02	0.046	2.24	
	HI_6_33_L06	Low N	6	33	67.12	-0.02	0.047	2.28	
	HI_6_35_L06	Low N	6	35	68.41	-0.02	0.044	2.15	
	HI_7_21_L06	Low N	7	21	54.16	-0.03	0.050	2.34	54.2
	HI_7_25_L06	Low N	7	25	66.88	0.05	0.103	4.74	66.9
	HI_7_26_L06	Low N	7	26	69.78	0.05	0.119	5.22	
	HI_7_30_L06	Low N	7	30	73.69	0.04	0.060	2.74	73.7
	HI_7_46_L06	Low N	7	46	109.34	-0.03	0.065	3.11	109.3
	HI_97_52_L06	Low N	9	52	116.17	0.02	0.047	2.17	116.2

Six QTL were identified for TSW (Table 3.4) using CIM and, of these, 2 were also detected using IM i.e. TSW_4_2_H06 and TSW_9_55_H06 and confirmed by MIM. The LOD scores for IM QTL at High N were 2.26 and 2.22 and for CIM were from 2.09 to 3.29. The phenotypic variation explained by the 2 IM QTL was 11.5% and the combined variation explained by the 6 CIM QTL was 28.8%.

At Low N, QTL for TSW were detected on chromosome 1 with IM and on 1 and 4 with CIM. The 1 QTL from IM confirmed with MIM had a LOD score of 2.26 and explained 6.2% of the phenotypic variation of the population, and the 2 from CIM had LOD scores of 2.15 and 2.06, and together explained 10.1% of the phenotypic variation of the population.

Quantitative trait loci for SNP were detected at High N only using IM (Table 3.5). Five QTL were also detected using MIM as individual QTL, with LOD scores ranging from 2.08 to 2.24. These QTL together accounted for 28% of the phenotypic variation of the TNDH population. Using CIM 4 QTL were detected for SNP at High N, 3 of which were confirmed by MIM. The 3 QTL had LOD scores of 2.11, 2.15 and 2.48 and collectively accounted for 15.2% of the phenotypic variation of the population.

Table 3.4. Putative QTL and related marker position influencing 1000-seed weight (TSW) in the 2005/06 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0 at High and Low N.

Trait	QTL	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
TSW	IM								
	TSW_4_2_H06	High N	4	2	2.14	-0.29	0.058	2.26	
	TSW_4_4_H06	High N	4	4	11.85	-0.29	0.061	2.37	
	TSW_9_55_H06	High N	9	55	120.48	0.29	0.057	2.22	120.5
	TSW_1_38_L06	Low N	1	38	65.65	0.33	0.059	2.31	
	TSW_1_40_L06	Low N	1	40	67.68	0.34	0.061	2.35	
	TSW_1_41_L06	Low N	1	41	69.72	0.34	0.062	2.26	
	TSW_7_46_L06	Low N	7	46	109.34	-2.26	0.059	2.30	
	CIM								
	TSW_4_2_H06	High N	4	2	2.14	-0.32	0.069	3.29	2.1
	TSW_4_3_H06	High N	4	3	11.37	-0.31	0.066	3.10	11.4
	TSW_4_7_H06	High N	4	7	16.97	-0.28	0.051	2.18	17.0
	TSW_5_37_H06	High N	5	37	100.24	-0.29	0.057	2.27	100.2
	TSW_9_55_H06	High N	9	55	125.48	0.26	0.045	2.09	125.5
	TSW_12_25_H06	High N	12	25	123.92	-0.28	0.053	2.43	
	TSW_1_35_L06	Low N	1	35	63.79	0.30	0.047	2.02	
	TSW_1_38_L06	Low N	1	38	65.65	0.33	0.057	2.49	
	TSW_1_40_L06	Low N	1	40	67.68	0.35	0.064	2.78	
	TSW_1_41_L06	Low N	1	41	69.72	0.32	0.054	2.15	69.7
	TSW_4_4_L06	Low N	4	4	11.85	-0.30	0.047	2.06	11.9

Two QTL were detected at Low N by both CIM and MIM, with LOD scores of 2.35 and 3.81, on chromosomes 1 and 7 respectively. The 2 QTL together explained 13.7% of the phenotypic variation of the population.

Additive effects were mostly influenced by Tapidor for TW, both at High and Low N, whereas for seed yield, additive effects were dictated by Tapidor at High N and by Ningyou7 at Low N. A similar effect was observed for HI, but not as clearly divided as for SY. For TSW the opposite effect was in evidence, with

Ningyou7 mostly influencing the trait at High N and Tapidor at Low N. There was not a clear pattern for SNP, though Tapidor seemed to have a stronger additive effect both at High and Low N.

Table 3.5. Putative QTL and related marker position influencing seed number per pod (SNP) in the 2005/06 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0 at High and Low N.

Trait	QTL	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
SNP	IM								
	SNP_1_9_H06	High N	1	9	21.09	1.37	0.056	2.19	21.1
	SNP_1_19_H06	High N	1	19	46	1.38	0.058	2.24	46.0
	SNP_13_37_H06	High N	13	37	124.52	-1.32	0.055	2.16	124.5
	SNP_13_39_H06	High N	13	39	126.44	-1.30	0.054	2.05	
	SNP_13_42_H06	High N	13	42	136.3	-1.32	0.055	2.14	136.3
	SNP_18_20_H06	High N	18	20	64.59	1.33	0.056	2.08	64.6
	CIM								
	SNP_1_19_H06	High N	1	19	46	1.27	0.048	2.26	
	SNP_3_1_H06	High N	3	1	3.01	1.46	0.059	2.11	3.0
	SNP_7_1_H06	High N	7	1	0.01	1.32	0.045	2.15	0.0
	SNP_18_20_H06	High N	18	20	64.59	1.31	0.054	2.48	64.6
	SNP_1_38_L06	Low N	1	38	65.65	1.94	0.046	2.10	
	SNP_1_40_L06	Low N	1	40	67.68	2.05	0.052	2.35	67.7
	SNP_7_46_L06	Low N	7	46	109.34	-2.74	0.085	3.81	109.3

NITROGEN AND NITROGEN DERIVED TRAITS

Quantitative trait loci for NUpE (Table 3.6) were only identified at Low N by IM and/or CIM, with no QTL present at High N. Most of the QTL were found on chromosome 1, but others were also identified on chromosomes 4, 7, and 16 (by CIM only). Three QTL on chromosome 1 were commonly identified by IM and CIM and later confirmed by MIM i.e. NUpE_1_25_L06, NUpE_1_33_L06 and NUpE_1_40_L06. The LOD scores for these QTL were 2.01, 2.26, 3.14 and 3.04, 3.69, 4.60 by IM and CIM respectively. The phenotypic variation of these QTL was 19% and 24.1% by IM and CIM respectively. Three QTL were identified for NUpE at Low N using CIM. The QTL on chromosome 4 had a LOD score of 2.05, the one on chromosome 7 a LOD score of 2.85 and the one on chromosome 16 a LOD score of 2.83. All the 6 QTL identified with CIM collectively accounted for 40.7% of the phenotypic variation of the TNDH population.

Table 3.6. Putative QTL and related marker position influencing Nitrogen uptake efficiency (NUpE) in the 2005/06 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0 at High and Low N.

Trait	QTL	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
NUpE	IM								
	NUpE_1_25_L06	Low N	1	25	57.14	0.82	0.052	2.01	
	NUpE_1_29_L06	Low N	1	29	59.49	0.82	0.053	2.07	
	NUpE_1_33_L06	Low N	1	33	62.62	0.86	0.058	2.26	
	NUpE_1_38_L06	Low N	1	38	65.65	0.97	0.073	2.87	
	NUpE_1_40_L06	Low N	1	40	67.68	1.01	0.080	3.14	
	NUpE_1_41_L06	Low N	1	41	69.72	0.97	0.074	2.80	
	CIM								
	NUpE_1_25_L06	Low N	1	25	57.14	0.92	0.066	3.04	57.1
	NUpE_1_29_L06	Low N	1	29	59.49	0.93	0.067	3.11	
	NUpE_1_33_L06	Low N	1	33	62.62	1.02	0.080	3.70	62.6
	NUpE_1_36_L06	Low N	1	36	64.92	1.04	0.083	3.73	
	NUpE_1_38_L06	Low N	1	38	65.65	1.10	0.093	4.37	
	NUpE_1_40_L06	Low N	1	40	67.68	1.12	0.097	4.60	67.7
	NUpE_1_41_L06	Low N	1	41	69.72	1.03	0.082	3.60	
	NUpE_4_3_L06	Low N	4	3	3.37	-0.74	0.042	2.06	3.4
	NUpE_7_46_L06	Low N	7	46	109.34	-0.90	0.059	2.86	109.3
	NUpE_16_2_L06	Low N	16	2	12.52	0.93	0.066	2.83	12.5

In contrast to NUpE, only QTL at High N were identified for NUtE (Table 3.7), with the exception of one identified at Low N. Four QTL were identified using CIM, on chromosomes 1 (1), 2 (1) and 7 (2) and one of these NUtE_17_8_H06 was also identified with IM. The LOD scores for these QTL were 2.38, 2.29, 5.83 and 2.95. The QTL on chromosome 17 with a LOD score of 5.83 was also identified by IM with a LOD score of 2.05. The 4 QTL identified by CIM explained 30.6% of the phenotypic variation of the TNDH population.

One QTL was detected on A5 at Low N for NUtE using CIM. The QTL had a LOD score of 2.26 and accounted for 5.1% of the phenotypic variation of the population.

For NUE, three QTL at High N were detected using CIM of which two were identified on chromosome 7 and one on chromosome 19 (Table 3.8). The respective LOD scores were 2.79, 2.57 and 3.00, and the 3 QTL together explained 18.6% of the phenotypic variation of the population.

Table 3.7. Putative QTL and related marker position influencing nitrogen utilisation efficiency (NUtE) in the 2005/06 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0 at High and Low N.

Trait	QTL	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
NUtE	IM								
	NUtE_17_7_H06	High N	17	7	44.98	0.87	0.053	2.03	
	NUtE_17_8_H06	High N	17	8	47.12	0.90	0.058	2.05	
	CIM								
	NUtE_1_7_H06	High N	1	7	15.68	0.85	0.051	2.38	15.7
	NUtE_12_24_H06	High N	12	24	108.21	-0.86	0.052	2.29	108.2
	NUtE_17_6_H06	High N	17	6	41.33	1.38	0.100	4.25	
	NUtE_17_7_H06	High N	17	7	44.98	1.71	0.130	5.72	
	NUtE_17_8_H06	High N	17	8	46.12	1.76	0.140	5.83	46.1
	NUtE_17_10_H06	High N	17	10	52.78	1.51	0.083	3.52	
	NUtE_17_14_H06	High N	17	14	65.4	-1.19	0.063	2.95	65.4
	NUtE_5_13_L06	LowN	5	13	49.82	1.77	0.051	2.26	

Thirteen QTL were identified both by IM and CIM for NUE at Low N, mostly on chromosome 1, but also one each on chromosomes 6 and 7. Two QTL were confirmed by MIM on chromosome 1, with LOD scores of 2.61 and 3.71. The latter was also identified by IM with a LOD score of 2.49. The QTL for NUE on 7 had a LOD score of 2.64 and the one on chromosome 16 a LOD score of 2.43. The four QTL identified by CIM jointly explained 24.8% of the phenotypic variation of the TNDH population.

Five QTL were identified for NHI at High N using CIM, and one of these on chromosome 17 was also identified by IM. The QTL identified had LOD scores ranging from 2.16 for the QTL on chromosome 7 to 4.71 for the QTL on chromosome 17. All these QTL together explained 36.8% of the phenotypic variation of the TNDH population.

Table 3.8. Putative QTL and related marker position influencing nitrogen use efficiency (NUE) in the 2005/06 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0 at High and Low N.

Trait	QTL	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
NUE	IM								
	NUE_1_29_L06	LowN	1	29	59.49	7.15	0.054	2.10	
	NUE_1_33_L06	LowN	1	33	62.62	7.25	0.055	2.15	
	NUE_1_38_L06	LowN	1	38	65.65	7.92	0.066	2.59	
	NUE_1_40_L06	LowN	1	40	67.68	8.26	0.072	2.80	
	NUE_1_42_L06	LowN	1	42	70.56	7.85	0.065	2.49	
	CIM								
	NUE_7_35_H06	High N	7	35	89.36	-2.78	0.060	2.79	89.4
	NUE_7_37_H06	High N	7	37	94.41	-2.34	0.055	2.57	94.4
	NUE_19_9_H06	High N	19	9	55.21	2.39	0.071	3.00	55.2
	NUE_19_10_H06	High N	19	10	57.32	2.37	0.070	3.00	
	NUE_19_11_H06	High N	19	11	59.05	1.94	0.054	2.26	
	NUE_1_25_L06	Low N	1	25	57.14	7.36	0.056	2.61	57.1
	NUE_1_29_L06	Low N	1	29	59.49	7.86	0.065	3.02	
	NUE_1_33_L06	Low N	1	33	62.62	8.19	0.070	3.26	
	NUE_1_38_L06	Low N	1	38	65.65	8.92	0.083	3.92	
	NUE_1_40_L06	Low N	1	40	67.68	8.91	0.083	3.90	
	NUE_1_42_L06	Low N	1	42	70.56	8.76	0.080	3.71	70.6
	NUE_7_46_L06	Low N	7	46	109.34	-7.54	0.055	2.64	109.3
	NUE_16_2_L06	Low N	16	2	13.52	7.43	0.057	2.43	13.5

Four QTL were identified using CIM at Low N for NHI and were confirmed by MIM with LOD scores ranging from 2.13 to 2.65 (Table 3.9). The 4 QTL explained 21.7% of the phenotypic variation of the population.

Additive effects were from Tapidor only for NUpE at Low N, and mostly the same situation occurred for NUtE at High N, with 2 exceptions (NUtE_12_24_H06 and NUtE_17_14_H06) where additive effects were from Ningyou7. For NUE, most additive effects were from Tapidor at both High and Low N, with few exceptions at both High and Low N. The same was found for NHI where most of the additive effects were influenced by Tapidor with few occasions at both High and Low N when Ningyou7 was responsible.

Table 3.9. Putative QTL and related marker position influencing nitrogen harvest index (NHI) in the 2005/06 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0 at High and Low N.

Trait	QTL	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
NHI	IM								
	NHI_7_28_H06	High N	7	28	70.9	0.02	0.053	2.06	
	NHI_17_7_H06	High N	17	7	44.98	0.02	0.055	2.12	
	NHI_17_8_H06	High N	17	8	47.12	0.02	0.061	2.18	
	NHI_1_38_L06	Low N	1	38	65.65	0.02	0.058	2.25	
	NHI_1_40_L06	Low N	1	40	67.68	0.02	0.059	2.30	
	NHI_1_42_L06	Low N	1	42	72.56	0.03	0.069	2.29	
	NHI_7_46_L06	Low N	7	46	109.34	-183.07	0.054	2.10	
	NHI_14_34_L06	Low N	14	34	129.45	187.04	0.060	2.14	
	NHI_14_35_L06	Low N	14	35	137.78	186.58	0.058	2.10	
	CIM								
	NHI_1_7_H06	High N	1	7	15.68	0.02	0.049	2.32	15.7
	NHI_7_37_H06	High N	7	37	94.41	-0.02	0.043	2.16	94.4
	NHI_17_6_H06	High N	17	6	40.33	0.03	0.088	3.71	40.3
	NHI_17_7_H06	High N	17	7	44.98	0.04	0.105	4.66	
	NHI_17_8_H06	High N	17	8	46.12	0.04	0.111	4.71	46.1
	NHI_17_9_H06	High N	17	9	50.31	0.04	0.088	3.60	
	NHI_17_10_H06	High N	17	10	53.78	0.04	0.077	3.39	53.8
	NHI_1_33_L06	Low N	1	33	62.62	0.02	0.053	2.45	62.6
	NHI_1_36_L06	Low N	1	36	64.92	0.02	0.046	2.05	
	NHI_1_38_L06	Low N	1	38	65.65	0.02	0.052	2.41	
	NHI_1_40_L06	Low N	1	40	67.68	0.02	0.053	2.43	67.7
	NHI_1_42_L06	Low N	1	42	72.56	0.02	0.052	2.07	
	NHI_7_18_L06	Low N	7	18	48.98	-0.03	0.048	2.01	
	NHI_7_19_L06	Low N	7	19	50.58	-0.03	0.050	2.15	
	NHI_71_20_L06	Low N	7	20	53.11	-0.03	0.064	2.65	53.1
	NHI_7_21_L06	Low N	7	21	54.16	-0.03	0.060	2.64	
	NHI_9_52_L06	Low N	9	52	116.17	0.02	0.047	2.13	116.2

Quantitative trait loci for NPLANT (Table 3.10) were detected on chromosome 14 at Low N and on 7 at High N. Of the 8 QTL detected at High N using CIM, only 4 were confirmed by MIM, LOD scores of these ranged from 2.05 to 3.82 and together they accounted for 27.8% of the phenotypic variation of the population.

At Low N, only one QTL was detected on chromosome 14 named NP_14_14_L06, with LOD scores of 2.46 and 3.07 using IM and CIM respectively. That QTL alone explained 6.3 and 5.2% of the phenotypic variation by IM and CIM respectively.

Table 3.10. Putative QTL and related marker position influencing NPLANT (plant nitrogen concentration not including seed N concentration) in the 2005/06 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0 at High and Low N.

Trait	QTL	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
NPLANT	IM								
	NP_14_8_L06	Low N	14	8	33.87	-0.05	0.053	2.05	
	NP_14_14_L06	Low N	14	14	50.82	-0.06	0.063	2.46	50.8
	CIM								
	NP_7_23_H06	High N	7	23	64.01	0.07	0.069	3.10	
	NP_7_25_H06	High N	7	25	66.88	0.08	0.074	3.57	66.9
	NP_7_26_H06	High N	7	26	68.78	0.07	0.068	2.94	
	NP_7_28_H06	High N	7	28	70.9	0.08	0.075	3.62	
	NP_7_29_H06	High N	7	29	72.94	0.08	0.066	2.98	
	NP_7_31_H06	High N	7	31	78.61	0.09	0.082	3.82	78.6
	NP_7_37_H06	High N	7	37	94.41	-0.08	0.079	3.49	94.4
	NP_7_46_H06	High N	7	46	109.34	0.07	0.043	2.05	109.3
	NP_14_14_L06	Low N	14	14	50.82	-0.05	0.052	2.40	

Only 1 QTL was detected for NSEED on chromosome 17 at High N using CIM (Table 3.11). This QTL had a LOD score of 2.53 and explained 6.1% of the phenotypic variation of the population.

Three QTL were identified for NSEED at Low N using IM, and 2 of these were also detected by CIM. The LOD scores of these QTL were 2.32 on chromosome 1, 3.01 on chromosome 7 and 2.58 on chromosome 18. The 3 QTL collectively explained 20.2% of the phenotypic variation of the population. The same QTL on 7 and 18 were identified using CIM with LOD scores of 3.57 and 3.31 respectively and jointly accounted for 14.6% of the phenotypic variation.

Table 3.11. Putative QTL and related marker position influencing seed nitrogen concentration (NSEED) in the 2005/06 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0 at High and Low N.

Trait	QTL	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
NSEED	IM								
	NS_1_33_L06	Low N	1	33	62.62	0.28	0.055	2.13	
	NS_1_38_L06	Low N	1	38	65.65	0.29	0.061	2.37	
	NS_1_40_L06	Low N	1	40	68.68	0.29	0.060	2.32	68.7
	NS_7_19_L06	Low N	7	19	50.58	-0.30	0.065	2.47	
	NS_7_20_L06	Low N	7	20	53.11	-0.31	0.068	2.55	
	NS_7_22_L06	Low N	7	22	54.99	-0.33	0.076	3.01	55.0
	NS_18_9_L06	Low N	18	9	32.29	0.28	0.052	2.01	
	NS_18_11_L06	Low N	18	11	39.71	0.31	0.066	2.58	
	CIM								
	NS_17_7_H06	High N	17	7	43.98	0.29	0.059	2.46	
	NS_17_8_H06	High N	17	8	47.12	0.29	0.061	2.53	47.1
	NS_7_20_L06	Low N	7	20	53.11	-0.46	0.082	3.56	
	NS_7_21_L06	Low N	7	21	54.16	-0.47	0.078	3.57	54.2
	NS_18_11_L06	Low N	18	11	39.71	0.32	0.068	3.31	39.7

The same QTL pattern was found for total plant N concentration (NTOTAL,) as for NSEED (Table 3.12). The same QTL were identified by IM and CIM at High and Low N. The LOD scores for QTL found using IM were slightly higher for Total N, but the opposite happened for QTL identified using CIM, where LOD scores for Total N were slightly lower than those for NSEED.

Additive effects from both Tapidor and Ningyou7 influenced total N concentration (NTOTAL), with no evident pattern. Similarly, alternate additive effects were present for both seed N content and total N at Low N; however, only additive effects from Tapidor were present at High N.

Table 3.12. Putative QTL and related marker position influencing total plant nitrogen concentration (NTOTAL) in the 2005/06 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0 at High and Low N.

Trait	QTL	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
NTOTAL	IM								
	NT_1_33_L06	Low N	1	33	62.62	0.32	0.054	2.10	
	NT_1_38_L06	Low N	1	38	65.65	0.34	0.061	2.38	
	NT_1_40_L06	Low N	1	40	68.68	0.34	0.060	2.33	68.7
	NT_7_19_L06	Low N	7	19	50.58	-0.34	0.059	2.25	
	NT_7_20_L06	Low N	7	20	53.11	-0.35	0.062	2.30	
	NT_7_22_L06	Low N	7	22	54.99	-0.37	0.071	2.79	55.0
	NT_18_11_L06	Low N	18	11	39.71	0.36	0.066	2.58	39.7
	CIM								
	NT_17_7_H06	High N	17	7	44.98	0.32	0.050	2.21	
	NT_17_8_H06	High N	17	8	46.12	0.33	0.053	2.22	46.1
	NT_7_21_L06	Low N	7	21	54.16	-0.53	0.075	3.43	
	NT_18_9_L06	Low N	18	9	32.29	0.33	0.052	2.52	
	NT_18_11_L06	Low N	18	11	39.71	0.36	0.064	3.07	

OIL CONTENT

The analysis shows 2 QTL were detected for oil content using IM, one on chromosome 4 at High N and one on 5 at Low N (Table 3.13). These QTL were also detected using CIM and confirmed by MIM analysis.

Table 3.13. Putative QTL and related marker position influencing oil content (OIL) in the 2005/06 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0 at High and low N.

Trait	QTL	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
OIL	IM								
	OIL_4_4_H06	High N	4	4	11.85	-3.54	0.049	1.90	
	OIL_5_6_L06	Low N	5	6	28.54	5.00	0.063	2.48	
	CIM								
	OIL_4_4_H06	High N	4	4	11.85	-3.37	0.043	2.03	11.9
	OIL_9_29_H06	High N	9	29	67.62	3.53	0.047	2.09	67.6
	OIL_1_35_L06	Low N	1	35	63.79	4.37	0.048	2.19	
	OIL_1_38_L06	Low N	1	38	65.65	4.66	0.054	2.52	
	OIL_1_40_L06	Low N	1	40	67.68	4.84	0.059	2.68	
	OIL_1_41_L06	Low N	1	41	69.72	4.44	0.049	2.09	69.7
	OIL_5_6_L06	Low N	5	6	28.54	5.44	0.074	3.42	28.5
	OIL_5_9_L06	Low N	5	9	37.98	4.46	0.050	2.15	

The QTL named OIL_4_4_H06 had a LOD score of 1.9 and 2.03 from IM and CIM respectively, and the QTL at Low N OIL_5_6_L06 had a LOD score of 2.48 and 3.42 from IM and CIM respectively.

FLOWERING AND CHLOROPHYLL CONTENT

Results showed the presence of one minor QTL at High N for FDAS (Table 3.14) at the beginning of chromosome 3 (linkage group 13), both using IM and CIM with LOD scores of 1.91 and 1.92 respectively.

Table 3.14. Putative QTL and related marker positions influencing flowering (FDAS) in the 2005/06 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0 at High and Low N.

Trait	QTL	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
FDAS	IM								
	FDAS_13_1_H06	High N	13	1	0.01	8.25	0.049	1.91	
	CIM								
	FDAS_13_1_H06	High N	13	1	0.01	7.82	0.044	1.92	
	FDAS_9_3_L06	Low N	9	3	7.53	9.68	0.051	2.26	7.3

The QTL on chromosome 3, confirmed using MIM, accounted for 4.9 and 4.4% of the total variance by IM and CIM respectively. Another QTL was identified for FDAS at Low N only using CIM, with a LOD score of 2.26 which accounted for 5.1% of the variance.

Ten QTL were identified for CB (Table 3.15) at High N using IM and 8 using CIM, with LOD scores from 2.04 to 2.91 using IM and from 2.14 to 3.40 using CIM. Several QTL identified by either IM (7) or CIM (5), were confirmed using MIM. Only 1 QTL was commonly identified by both methods (i.e. CB_2_20_H06, with LOD of 2.04 and 3.32 by IM and CIM respectively). The other 2 QTL identified by IM only were also confirmed using MIM, whilst the remaining 3 QTL from either IM or CIM were considered ghost QTL (artefact occurring due to real QTL in surrounding intervals). Quantitative trait loci identified by IM and CIM collectively explained 50.9 and 30% of the phenotypic variation for CB at High N in the population respectively. At Low N, 4 QTL were identified using both IM and CIM, 2 of which were common to both 2 methods i.e. CB_2_4_L06 and CB_17_2_L06. Of these QTL, 3 identified by both methods were confirmed using MIM and explained 19% and 19.4% of the phenotypic variation in the TNDH population by IM and CIM respectively.

Table 3.15. Putative QTL and related marker positions influencing chlorophyll content of bracts (CB) in the 2005/06 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0 at High and Low N.

Trait	QTL	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
CB	IM								
	CB_1_41_H06	High N	1	41	69.72	2.81	0.070	2.42	69.7
	CB_1_42_H06	High N	1	42	77.56	2.58	0.059	2.20	77.6
	CB_2_20_H06	High N	2	20	68.86	2.60	0.056	2.04	68.9
	CB_2_22_H06	High N	2	22	73.27	3.35	0.098	2.67	
	CB_2_23_H06	High N	2	23	75.36	3.20	0.091	2.51	75.4
	CB_6_39_H06	High N	6	39	74.39	3.14	0.083	2.88	74.4
	CB_6_42_H06	High N	6	42	78.62	2.58	0.056	2.17	
	CB_6_45_H06	High N	6	45	91.27	3.03	0.074	2.91	91.4
	CB_9_57_H06	High N	9	57	131.13	3.01	0.073	2.69	131.1
	CB_9_59_H06	High N	9	59	137.74	2.67	0.060	2.34	
	CB_1_11_L06	Low N	1	11	22.32	2.42	0.054	2.10	
	CB_2_4_L06	Low N	2	4	18.92	-2.76	0.071	2.63	18.9
	CB_2_10_L06	Low N	2	10	46.11	-2.48	0.058	2.26	46.1
	CB_17_2_L06	Low N	17	2	7.29	2.55	0.061	2.36	7.3
	CIM								
	CB_2_17_H06	High N	2	17	64.09	2.38	0.045	2.14	64.1
	CB_2_20_H06	High N	2	20	68.86	3.05	0.072	3.32	
	CB_2_21_H06	High N	2	21	71.07	3.18	0.076	3.40	71.1
	CB_2_24_H06	High N	2	24	76.12	2.60	0.053	2.50	76.1
	CB_6_H06	High N	6						78.6
	CB_6_H06	High N	6						91.3
	CB_9_56_H06	High N	9	56	129.38	3.10	0.079	2.62	129.4
	CB_17_3_H06	High N	17	3	7.68	2.35	0.047	2.33	9.9
	CB_17_4_H06	High N	17	4	9.88	2.35	0.047	2.33	
	CB_1_11_L06	Low N	1	11	22.32	2.18	0.041	2.05	22.3
	CB_2_4_L06	Low N	2	4	18.92	-2.21	0.043	1.97	
	CB_17_2_L06	Low N	17	2	7.29	3.13	0.089	4.24	7.3
	CB_17_4_L06	Low N	17	4	34.88	2.65	0.064	3.00	34.9

Four QTL were identified for CL (3.16) by IM at High N and 8 by CIM. Of these, 3 QTL identified by IM and 5 by CIM were confirmed by MIM, meaning the other ones were ghost QTL. The 2 QTL on linkage group 1 were commonly identified by the 2 methods. The 3 QTL from IM collectively accounted for 19.5% of the phenotypic variation and the 5 QTL from CIM for 26.7% of the phenotypic variation. Six and 9 QTL were detected for CL at Low N, of which 2 and 7 identified by IM and CIM respectively were confirmed using MIM analysis. Two QTL on chromosome 16 were identified by IM and CIM i.e. CL_16_22_L06 and CL_16_25_L06. The 2 QTL from IM represented 10.4% of the phenotypic variation of the population and the 7 QTL identified with CIM accounted for 46.3% of the variation.

Table 3.16. Putative QTL and related marker positions influencing chlorophyll content of leaves (CL) in the 2005/06 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0 at High and Low N.

Trait	QTL	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
CL	IM								
	CL_1_41_H06	High N	1	41	69.72	2.35	0.060	2.06	69.7
	CL_1_43_H06	High N	1	43	78.93	2.22	0.054	2.07	78.8
	CL_2_23_H06	High N	2	23	75.36	2.73	0.081	2.05	75.4
	CL_9_16_H06	High N	9	16	38.98	2.21	0.053	2.06	
	CL_1_11_L06	Low N	1	11	22.32	2.31	0.064	2.52	
	CL_1_17_L06	Low N	1	17	37.6	2.18	0.057	2.21	
	CL_1_18_L06	Low N	1	18	39.11	2.20	0.057	2.16	
	CL_13_1_L06	Low N	13	1	1.01	2.73	0.066	2.09	
	CL_16_22_L06	Low N	16	22	67.15	-2.13	0.051	1.99	
	CL_16_25_L06	Low N	16	25	71.75	-2.13	0.053	2.05	
	CIM								
	CL_1_41_H06	High N	1	41	69.72	2.60	0.071	3.03	
	CL_1_43_H06	High N	1	43	78.93	2.67	0.074	3.48	
	CL_7_13_H06	High N	7	13	27.57	2.26	0.049	2.33	27.6
	CL_9_8_H06	High N	9	8	23.68	2.19	0.048	2.28	23.7
	CL_9_14_H06	High N	9	14	32.97	2.09	0.043	2.05	33.0
	CL_14_29_H06	High N	14	29	105.98	-2.27	0.054	2.09	106.0
	CL_14_31_H06	High N	14	31	115.82	-2.66	0.073	3.27	115.8
	CL_14_33_H06	High N	14	33	124.96	-2.16	0.047	2.10	
	CL_1_10_L06	Low N	1	10	22.12	2.15	0.052	2.47	22.1
	CL_1_14_L06	Low N	1	14	33.6	1.95	0.045	2.03	33.6
	CL_16_5_L06	Low N	16	5	25.61	-4.21	0.056	2.56	25.6
	CL_16_7_L05	Low N	16	7	40.53	5.37	0.086	4.00	40.5
	CL_16_20_L06	Low N	16	20	61.98	-3.08	0.085	3.28	62.0
	CL_16_22_L06	Low N	16	22	67.15	-2.89	0.081	3.83	67.2
	CL_16_25_L06	Low N	16	25	72.75	-2.51	0.066	2.91	
	CL_16_26_L06	Low N	16	26	75.54	-2.36	0.060	2.75	
	CL_16_28_L06	Low N	16	28	78.39	-2.28	0.058	2.68	78.4

All QTL detected for flowering, chlorophyll content in bracts and leaves were influenced by Tapidor as explained by the positive values for additive effect.

ARCHITECTURAL TRAITS

The results showed only one QTL was present for TL (Table 3.17) at High N which was localised on chromosome 17 (linkage group 17) and had LOD scores of 2.05 and 3.37 with IM and CIM respectively. Many more QTL were detected from both IM and CIM for TL at Low N, all on chromosome 1. Of the 3 QTL detected using IM, none were confirmed by MIM, indicating they were all ghost QTL. Seven QTL

were identified using CIM, but only 3 were confirmed by MIM as independent QTL, i.e. TL_1_22_H06; TL_1_35_H06 and TL_1_41_H06 with LOD scores of 2.04, 3.81 and 2.61 respectively. The 3 QTL together explained 17.4% of the total phenotypic variation for the TNDH population.

Table 3.17. Putative QTL and related marker position for plant height (TL) in the 2005/06 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0 at High and Low N.

Trait	QTL	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
TL	IM								
	TL_17_4_H06	High N	17	4	9.88	8.25	0.050	2.05	
	TL_1_30_L06	Low N	1	30	60.05	9.23	0.054	2.09	
	TL_1_32_L06	Low N	1	32	62.08	9.20	0.053	2.06	
	TL_1_34_L06	Low N	1	34	63.48	9.52	0.057	2.22	
	CIM								
	TL_17_4_H06	High N	17	4	9.88	9.89	0.070	3.37	
	TL_1_22_L06	Low N	1	22	52.9	8.56	0.045	2.04	53.0
	TL_1_25_L06	Low N	1	25	57.14	9.00	0.050	2.26	
	TL_1_30_L06	Low N	1	30	60.05	9.71	0.059	2.66	
	TL_1_35_L06	Low N	1	35	63.79	11.77	0.083	3.81	63.9
	TL_1_38_L06	Low N	1	38	65.65	9.99	0.061	2.77	
	TL_1_40_L06	Low N	1	40	68.68	8.96	0.049	2.18	
	TL_1_41_L06	Low N	1	41	69.72	10.11	0.062	2.61	69.7

Four QTL were identified for foot length (FL) (Table 3.18) at High N using IM, compared to 17 QTL identified using CIM. Three QTL were commonly identified by the 2 methods and all 3 were confirmed by MIM. Seven of the 17 QTL detected by CIM were confirmed by MIM, presenting LOD scores from 2.02 to 3.44. These QTL jointly accounted for 37.1% of the phenotypic variation of the population.

At Low N, 9 QTL were identified on chromosome 9 by CIM and 2 of these were detected by IM as well (FL_9_56_L06 and FL_9_56_L06). One other QTL was detected on chromosome 10 by CIM only. Four QTL on chromosome 9 (including those commonly detected by the 2 analyses) were confirmed by MIM as well as the QTL on chromosome 10. The 2 QTL detected by IM presented LOD scores of 3.74 and 2.48 respectively and the 5 QTL from CIM presented LOD scores ranging from 2.03 to 3.68. The 2 QTL identified by IM explained 20.1% of the phenotypic variation and the 5 QTL from CIM 35.8%.

All QTL for plant height (TL) and branch number (BN) presented additive effects from the parental line Tapidor, whereas QTL for FL were almost equally influenced by both Tapidor and Ningyou7.

Table 3.18. Putative QTL and related marker position for foot length (FL) in the 2005/06 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0 at High and Low N.

Trait	QTL	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
FL	IM								
	FL_3_1_H06	High N	3	1	2.01	4.37	0.069	2.36	
	FL_9_56_H06	High N	9	56	128.38	5.77	0.131	2.66	
	FL_9_58_H06	High N	9	58	137.28	4.36	0.072	2.84	
	FL_19_2_H06	High N	19	2	13.39	-4.11	0.067	2.27	
	FL_9_56_L06	Low N	9	56	129.38	6.77	0.138	3.74	
	FL_9_59_L06	Low N	9	59	137.74	4.59	0.063	2.48	
	CIM								
	FL_3_1_H06	High N	3	1	3.01	4.84	0.082	3.37	3.0
	FL_3_2_H06	High N	3	2	5.72	4.69	0.076	3.15	
	FL_6_23_H06	High N	6	23	62.53	-4.34	0.070	3.44	62.6
	FL_6_24_H06	High N	6	24	64.46	-3.84	0.055	2.69	
	FL_6_28_H06	High N	6	28	65.97	-3.76	0.052	2.56	
	FL_6_31_H06	High N	6	31	66.68	-3.71	0.051	2.48	
	FL_6_34_H06	High N	6	34	67.53	-3.58	0.047	2.29	
	FL_6_38_H06	High N	6	38	73.84	-4.04	0.061	2.16	73.8
	FL_9_56_H06	High N	9	56	129.38	4.55	0.074	2.35	129.4
	FL_9_57_H06	High N	9	57	137.13	3.95	0.056	2.78	
	FL_9_60_H06	High N	9	60	139.21	3.71	0.049	2.38	
	FL_16_9_H06	High N	16	9	42.54	-3.35	0.043	2.11	42.6
	FL_16_12_H06	High N	16	12	43.8	-3.53	0.048	2.39	
	FL_16_15_H06	High N	16	15	44.78	-3.53	0.048	2.39	
	FL_16_18_H06	High N	16	18	46.43	-3.50	0.047	2.36	
	FL_16_19_H06	High N	16	19	58.85	-3.35	0.041	2.02	58.9
	FL_19_2_H06	High N	19	2	12.39	-3.77	0.053	2.43	12.4
	FL_9_24_L06	Low N	9	24	62.82	-6.15	0.071	2.59	62.8
	FL_9_27_L06	Low N	9	27	66.21	-7.04	0.076	3.59	
	FL_9_30_L06	Low N	9	30	67.88	-6.90	0.067	3.03	68.0
	FL_9_33_L06	Low N	9	33	68.35	-6.12	0.056	2.61	
	FL_9_36_L06	Low N	9	36	69.51	-6.79	0.071	3.35	
	FL_9_56_L06	Low N	9	56	129.38	6.58	0.122	3.68	129.4
	FL_9_59_L06	Low N	9	59	137.74	4.18	0.049	2.28	137.7
	FL_10_30_L06	Low N	10	30	66.98	4.03	0.049	2.03	67.0

Six QTL were detected for BN at High N using IM, whereas only 4 were detected by CIM, of which 2 were common to both analyses i.e. BN_3_44_H06 and BN_3_47_H06. Four out of 6 QTL (LOD scores from 2.16 to 2.68) from IM and 2 of CIM (LOD scores 3.60 and 2.41) were confirmed by MIM. The 4 IM QTL explained 19.8% of the phenotypic variation of the TNDH population and the 2 from CIM 13%.

Nine QTL were detected for BN at Low N using CIM, 2 of which were also detected by IM and confirmed by MIM. Two more QTL from CIM were also confirmed by MIM making a total of 4 out of 9 QTL confirmed. The LOD scores were 1.91 and 1.98 for IM QTL and ranged from 2.20 to 2.78 for CIM QTL.

The 2 QTL identified by IM accounted for 12.6% of the phenotypic variance of the population, whilst the 4 QTL from CIM jointly accounted for 24.7% of the phenotypic variation of the population.

Table 3.19. Putative QTL and related marker position for branch number per plant (BN) in the 2005/06 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0 at High and Low N.

Trait	QTL	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
BN	IM								
	BN_3_30_H06	High N	3	30	51.72	0.74	0.061	2.16	51.7
	BN_3_41_H06	High N	3	41	73.08	0.67	0.056	2.18	
	BN_3_44_H06	High N	3	44	75.32	0.74	0.068	2.68	75.4
	BN_3_47_H06	High N	3	47	77.12	0.68	0.056	2.19	
	BN_14_20_H06	High N	14	20	62.19	-0.72	0.063	2.47	62.3
	BN_15_10_H06	High N	15	10	62.17	0.73	0.066	2.55	62.2
	BN_1_42_L06	Low N	1	42	70.56	0.67	0.049	1.91	
	BN_9_56_L06	Low N	9	56	127.38	0.83	0.077	1.98	
	CIM								
	BN_3_44_H06	High N	3	44	75.32	0.80	0.077	3.60	
	BN_3_47_H06	High N	3	47	77.12	0.85	0.086	4.01	
	BN_3_48_H06	High N	3	48	83.16	0.66	0.052	2.38	
	BN_15_6_H06	High N	15	6	31.41	0.68	0.053	2.41	31.4
	BN_1_30_L06	Low N	1	30	60.05	0.67	0.049	2.20	60.0
	BN_1_34_L06	Low N	1	34	63.48	0.75	0.062	2.78	
	BN_1_38_L06	Low N	1	38	65.65	0.75	0.061	2.72	
	BN_1_40_L06	Low N	1	40	67.68	0.72	0.057	2.52	
	BN_1_42_L06	Low N	1	42	70.56	0.75	0.061	2.74	70.6
	BN_9_52_L06	Low N	9	52	116.17	0.73	0.054	2.32	116.2
	BN_9_54_L06	Low N	9	54	118.85	0.69	0.049	2.10	
	BN_9_55_L06	Low N	9	55	124.48	0.76	0.061	2.44	
	BN_9_56_L06	Low N	9	56	127.38	0.86	0.083	2.50	127.4

QTL SUMMARY FOR 2005/06

Results for traits analysed in 2005/06 identified a higher number of QTL at Low N than at High N, by both IM and CIM when all traits were considered together. Analysis of each QTL by IM and CIM showed a large number of traits with a higher number of QTL at Low N i.e. CB, SY, TSW, NP, NS, NT, NUpE, NUE, NHI. Traits that had a larger number of QTL at High N, commonly identified by both methods, included FL, FDAS, HI, SNP and NUtE. All N and N derived traits except NUtE had a higher number of QTL at Low N.

Table 3.20. Summary of major QTL identified at High N in 2005/06 field trial. QTL in the same line were co-localised in the same marker. Traits' acronyms are: TL (plant height), FL (foot length), BN (branch number), FDAS (flowering days after sowing), CB (chlorophyll in bracts), CL (chlorophyll in leaves), TW (total above ground plant biomass), SY (seed yield), HI (harvest index), TSW (1000 seed weight), SNP (seed number per pod), SN (seed N), TN (total N), NUpE (N uptake efficiency), NUtE (N utilisation efficiency), NUE (N use efficiency), NHI (N harvest index).

CHROMOSOME	Marker	TRAIT (LOD score)			
1	1	TW (2.3)			
	7	NUTE (2.38)	NHI (2.32)		
	9	SNP (2.19)			
	19	SNP (2.26)			
	41	CB (2.42)	CL (3.03)	CL (3.48)	
2	17	CB (2.14)			
	21	CB (3.4)	CL (2.05)		
3	1	FL (3.37)	SNP (2.11)		
	30	BN (2.16)			
	44	BN (2.68)			
4	4	TSW (3.29)	OIL (2.03)		
5	37	TSW (2.27)			
6	23	FL (3.44)			
	38	FL (2.16)	CB (2.88)		
	45	CB (2.91)			
7	1	SNP (2.15)			
	13	CL (2.33)			
	25	NP (3.57)	HI (2.25)		
	29	HI (3.37)	NP (3.82)		
	37	NP (3.49)	NUE (2.79)	NHI (2.16)	
	46	NP (2.05)			
9	8	CL (2.27)			
	29	OIL (2.09)			
	41	HI (3.61)			
	46	HI (2.99)			
	56	FL (2.35)	TSW (2.09)	HI (2.12)	CB (2.69)
12	24	NUTE (2.29)	TSW (2.43)		
13	37	SNP (2.16)			
	41	HI (3.74)	SNP (2.14)		
14	14	NP (2.46)			
	20	BN (2.47)			
	31	CL (3.27)			
15	10	BN (2.55)			
16	9	FL (2.11)			
17	4	TL (3.37)	HI (2.86)	CB (2.33)	
	8	NT (2.22)	NUTE (5.83)	NHI (4.71)	
	10	NHI (3.39)			
	14	NUTE (2.95)			
18	20	SNP (2.48)			
19	5	TW (2.38)	SY (2.09)	FL (2.43)	
	9	NUE (3.00)	SY (3.81)	TW (2.68)	

Table 3.21. Summary of major QTL identified at Low N in 2005/06 field trial. QTL in the same line were co-localised in the same marker. Traits' acronyms are: TL (plant height), FL (foot length), BN (branch number), FDAS (flowering days after sowing), CB (chlorophyll in bracts), CL (chlorophyll in leaves), TW (total above ground plant biomass), SY (seed yield), HI (harvest index), TSW (1000 seed weight), SNP (seed number per pod), SN (seed N), TN (total N), NUpE (N uptake efficiency), NUtE (N utilisation efficiency), NUE (N use efficiency), NHI (N harvest index).

CHROMOSOME	Marker	Trait (LOD score)								
1	10	CL (2.47)	CB (2.10)							
	24	TW (2.62)	TL (2.04)	NUpE (3.04)	NUE (2.61)					
	30	BN (2.20)								
	33	NHI (2.45)	NUpE (3.70)	TL (3.81)						
	38	NUE (3.92)	TW (2.06)	TSW (2.49)	SNP (2.10)					
	41	OIL (2.68)	NT (2.33)	NUpE (4.60)	NHI (2.43)	TSW (2.78)	SNP (2.35)	TL (2.61)	BN (2.74)	NUE (3.71) NHI (2.07)
2	4	CB (2.63)								
	10	CB (2.26)								
4	4	TSW (2.06)								
5	6	OIL (3.42)								
	13	NUtE (2.26)								
6	24	SY (2.79)	HI (2.14)							
	30	SY (3.23)								
	35	SY (2.90)	HI (2.15)							
	38	SY (2.31)								
7	21	NS (3.57)	NT (3.43)	HI (2.34)	NHI (2.65)					
	25	HI (4.74)								
	30	HI (2.74)								
	46	NUpE (2.86)	NUE (2.64)	NHI (2.10)	TW (2.74)	SY (3.03)	HI (3.11)	TSW (2.30)	SNP (3.81)	
9	3	FDAS (2.26)								
	24	FL (2.59)								
	30	FL (3.03)								
	52	BN (2.32)	NHI (2.13)	HI (2.17)						
	56	FL (3.68)	BN (2.50)							
	59	FL (2.28)								
16	2	NUpE (2.83)	NUE (2.43)							
	7	CL (3.40)								
	22	CL (3.83)								
	28	CL (2.68)								
17	2	CB (4.24)								
18	11	NS (3.31)	NT (3.07)							

Other traits i.e. TL, BN, CL and OIL presented the same number of QTL at both High and Low N, but not necessarily the same QTL. Only TW did not identify any common QTL from both IM and CIM, even though 2 QTL for the trait were identified by IM at High N and 6 and 5 QTL by CIM at High and Low N respectively.

The trait with a highest number of QTL identified by both IM and CIM methods at High N was HI with 4, and the trait with highest number of QTL identified by both methods at Low N was SY with 6.

Despite a larger number of QTL being identified at Low N, the additive effects of the QTL were larger at High N, indicating that a lower number of QTL at High N had a greater effect on the phenotypic variation of the population.

COMPARISON OF QTL POSITIONS FOR THE DIFFERENT TRAITS ANALYSED IN 2005/06

Results showed all traits presented QTL localised on one or more chromosomes (Figs. 3.6-3.10), all chromosomes had QTL with the exception of chromosomes 8, 10 and 11 where no QTL were identified for traits analysed in the 2005/06 season.

Many QTL were identified on chromosome 1 at Low N, particularly at the bottom end, between 67 and 79cM. The traits with common QTL in that area of the chromosome were branch number (BN), plant height (TL), total above ground plant biomass (TW), 1000 seed weight (TSW), seed number per pod (SNP), total plant N concentration (TN), seed N concentration (SN), nitrogen uptake efficiency (NUpE), nitrogen use efficiency (NUE) and nitrogen harvest index (NHI). At High N, QTL for the chlorophyll traits i.e. CB and CL (bracts and leaves respectively) were found partially overlapping the same area of chromosome 1, between 68.7 and 87.7 cM. Additional QTL for CB and CL were found further up chromosome 1 (19.1 to 27.6 cM and 31.6-50.3 cM) at Low N, as well as QTL for SNP on the same mapping interval, but at High N. Only QTL for CB and CL were localised on chromosome 2 at both High and Low N. One section where QTL for both chlorophyll traits were found was on the lower end of the chromosome between 74.3 and 81.5 cM at High N. Meanwhile at Low N 2 QTL both spanning large areas were located at the top end of chromosome 2.

Three QTL were identified for foot length (FL) at different mapping intervals on chromosome 3 at High N one of which was co-localised with a QTL for SNP and another one partially with BN.

Quantitative trait loci for TSW were co-localised at the upper end of chromosome 4, both at High (with oil content) and Low N (with NUpE).

Three different QTL were identified at 3 different locations on chromosome 5 (Fig. 3.7). The QTL for TSW at High N was localised at the bottom end of the chromosome from 86.0 to 103.9 cM while QTL for OIL and NUtE at Low N were localised in the upper regions from 23.2 to 39.3 cM and 49.1 to 53.4 cM respectively.

On chromosome 6 (Fig 3.7), 2 QTL were found for FL at High N, one was found at the same location as seed yield (SY) but at Low N, whereas the other QTL was found in the same interval as CB at High N and SY again at Low N. Of the other QTL on chromosome 6 three were identified for CB at High N, and 1 QTL for harvest index (HI) at Low N localised from 65.6 to 69.2 cM.

Three regions (1 at High N and 2 at Low N) on chromosome 7 (Fig 3.8) were identified with many co-locations of QTL for different traits. At High N, a section between 61.0 and 96.6 cM contained 2 QTL for HI, 3 for plant N concentration (PN), 2 for NHI and 1 for NUE. Within that section PN, HI and NHI were found at the same QTL interval i.e. 70.0 to 70.9 cM and NP, NUE and NHI at another interval i.e. 89.3 – 93.1 cM. A QTL for HI was found at both High and Low N within the same interval.

At Low N, a QTL interval between 49.6 and 54.1 cM included QTL for TN, SN, HI and NHI and another QTL cluster at the lower end of chromosome 7 included almost all yield traits analysed (TW, SY, SNP and HI) as well as QTL for NUpE and NUE. Other QTL localised on chromosome 7 were SNP and CB both at High N and each in the upper region of the chromosome.

On the upper region of chromosome 9 (Fig 3.8) a QTL for flowering was identified at Low N, and just below this region 2 QTL for CL were found at High N. On the central area of the chromosome, a QTL for oil content was found at High N starting at 66.2 cM, at the same location as a QTL for FL at Low N. Another QTL for FL was found at Low N a further 7 cM down the chromosome. At the lower end of chromosome 9 (126.9 – 139.6 cM), a QTL for FL was also found at both High and Low N. At High N it was found in the same region as QTL for CB, HI, and TSW and at Low N as QTL for HI and NHI. Two additional QTL were detected for HI at High N between 73.4 and 93.1 cM.

Only one QTL for NUtE was found on chromosome 12 at High N (Fig. 3.8), at marker interval OI10H02-em18me6-220 between 96.3 and 109.9 cM.

A QTL for FDAS was identified at the beginning of chromosome 13 at High N (Fig. 3.9), on the same location as a QTL for CB at Low N. At the bottom end of the chromosome a QTL was found for HI overlapping with a QTL for SNP, both at High N.

Three QTL were identified on chromosome 14 (Fig. 3.9), 2 at High N and 1 at Low N, all at different locations. At High N, QTL were for CB and BN and at Low N for PN.

On chromosome 15 (Fig 3.9), 2 QTL were also detected for BN 30 cM apart one from the other hand, both at High N.

At the beginning of chromosome 16 (Fig. 3.9), a QTL for NUpE was found at the same location as a QTL for NUE, both at Low N. The latter slightly overlapped with a QTL for CL also at Low N. Two more QTL were found for CL at Low N at 40.5-42.1 cM and the other lower down the chromosome at 59.0-73.5 cM. A QTL for BN identified at High N (53.8 to 66.3 cM) partially overlapped with the latter QTL for CL at Low N.

All QTL on chromosome 17 were identified on the upper half of the chromosome (Fig. 3.9), many at High N compared to only 1 at Low N. In the region between the beginning of the chromosome and 37.3 cM,

QTL were found for TL, CB and HI at Low N, and for CB at High N. The QTL for CB was in almost the exact location at both High and Low N. Just below this region, QTL for NUtE, NHI, NS and NT were found at High N, between 43 and 50.8 cM. Another QTL for NHI was found at High N from 64.1 to 69.6 cM.

On chromosome 18 (Fig. 3.10) one QTL was found for SNP at High N, at the base of the chromosome, and one QTL for NS at High N, around the middle region of the chromosome.

Quantitative trait loci on chromosome 19 (Fig. 3.10) were all detected within the same region (between 11.4 and 59.1 cM) and at High N. A QTL for FL was detected from 11.4 to 38.9 cM, on the same exact location as a QTL for TW. A QTL for SY partially overlapped these 2 QTL and was also co-localised with QTL for TW and NUE.

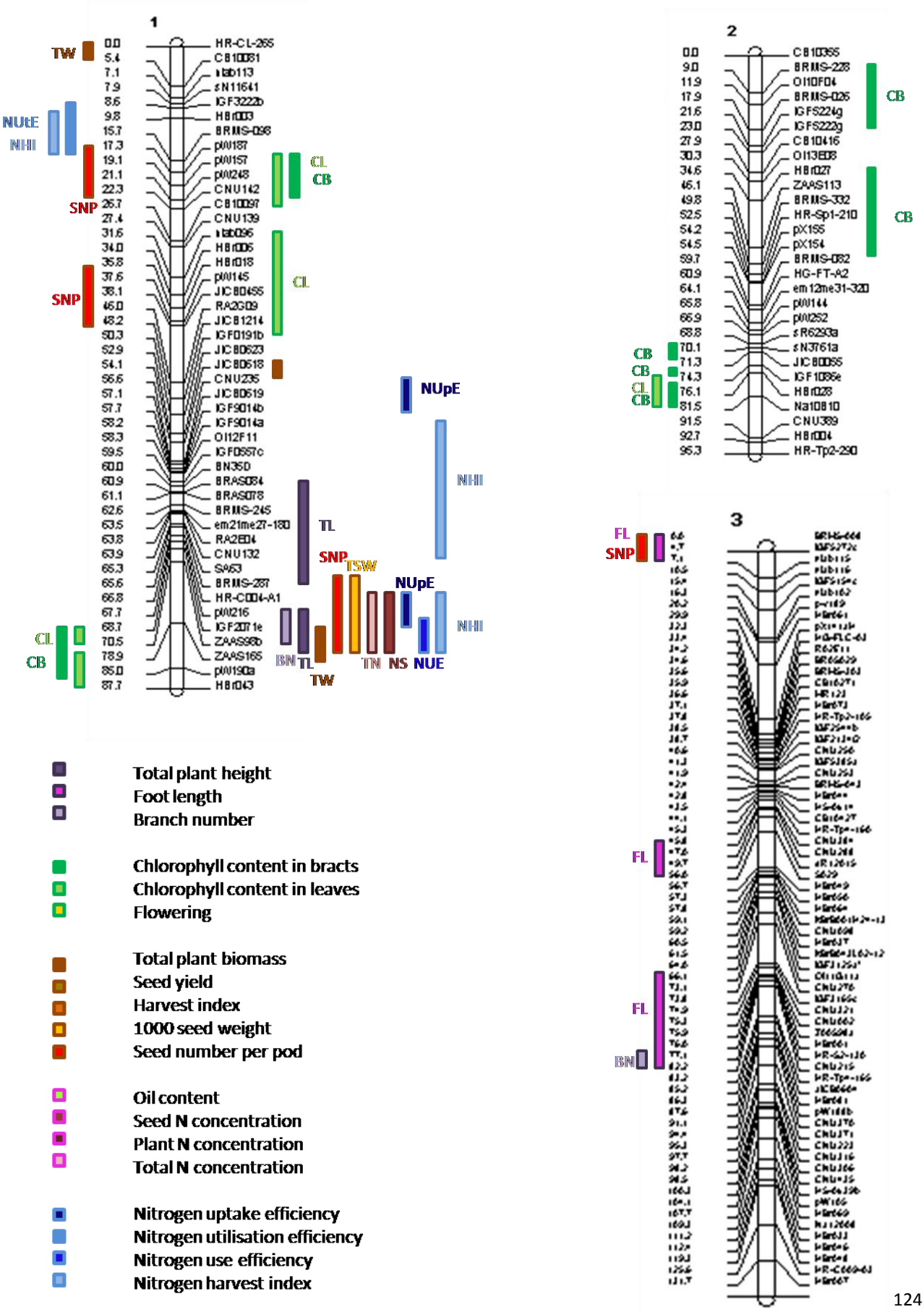


Figure 3.2. Quantitative trait loci positions for the traits analysed in 2005/06, on chromosomes 1 to 3. The QTL on the left are for High N and on the right are for Low N.

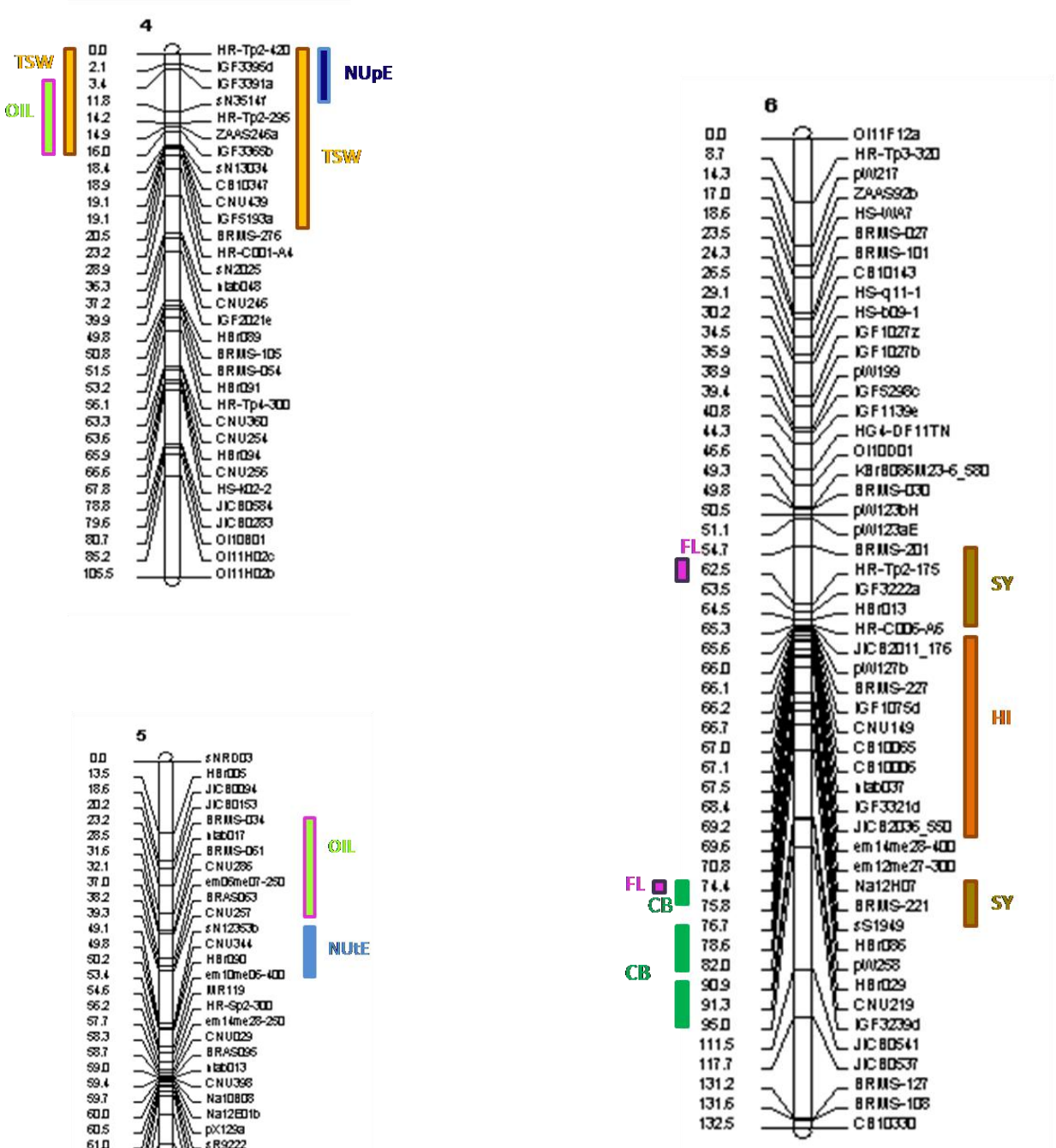


Figure 3.3. Quantitative trait loci positions for the traits analysed in 2005/06, on chromosomes 4 to 6. The QTL on the left are for High N and on the right are for Low N.

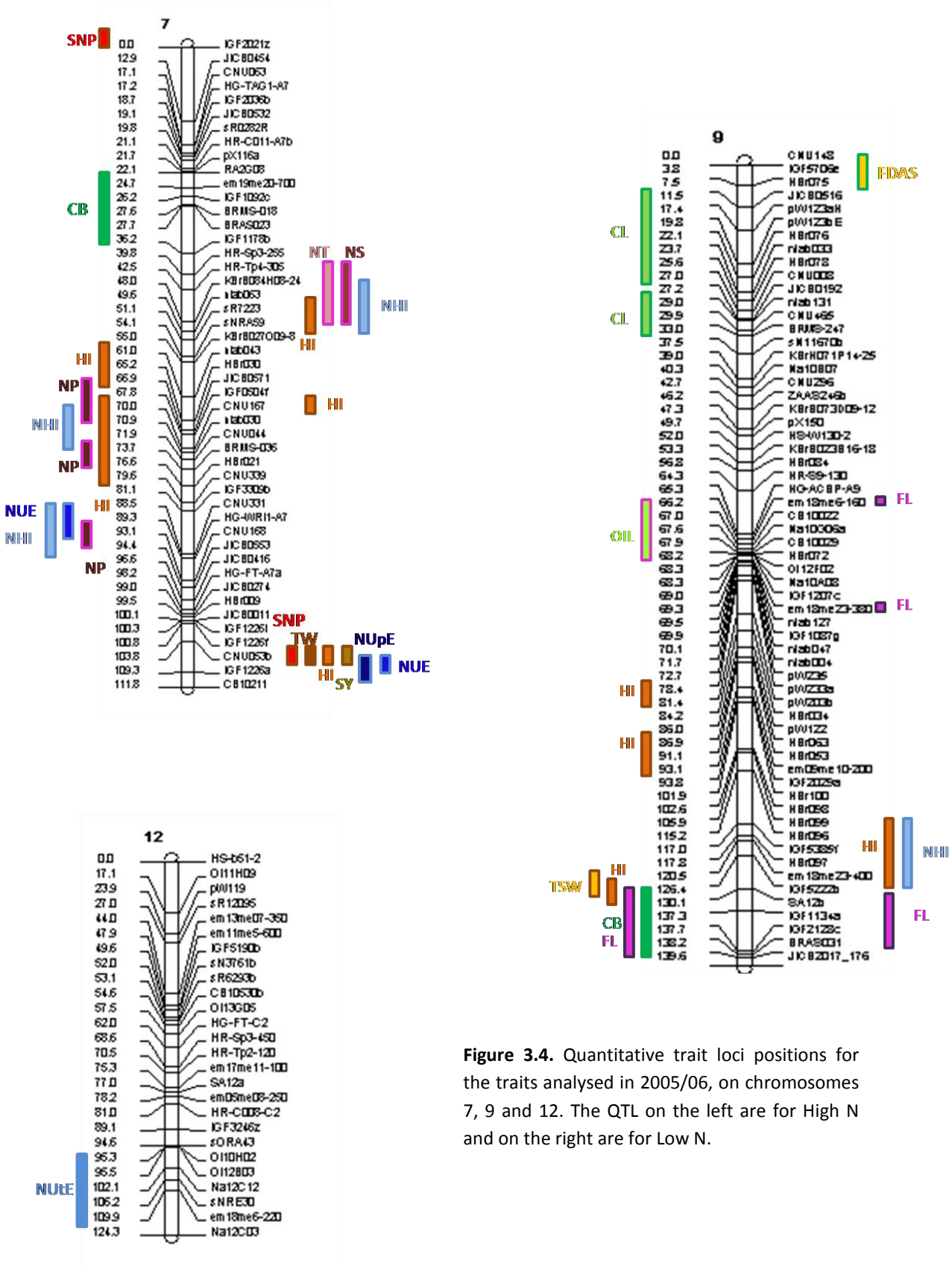


Figure 3.4. Quantitative trait loci positions for the traits analysed in 2005/06, on chromosomes 7, 9 and 12. The QTL on the left are for High N and on the right are for Low N.

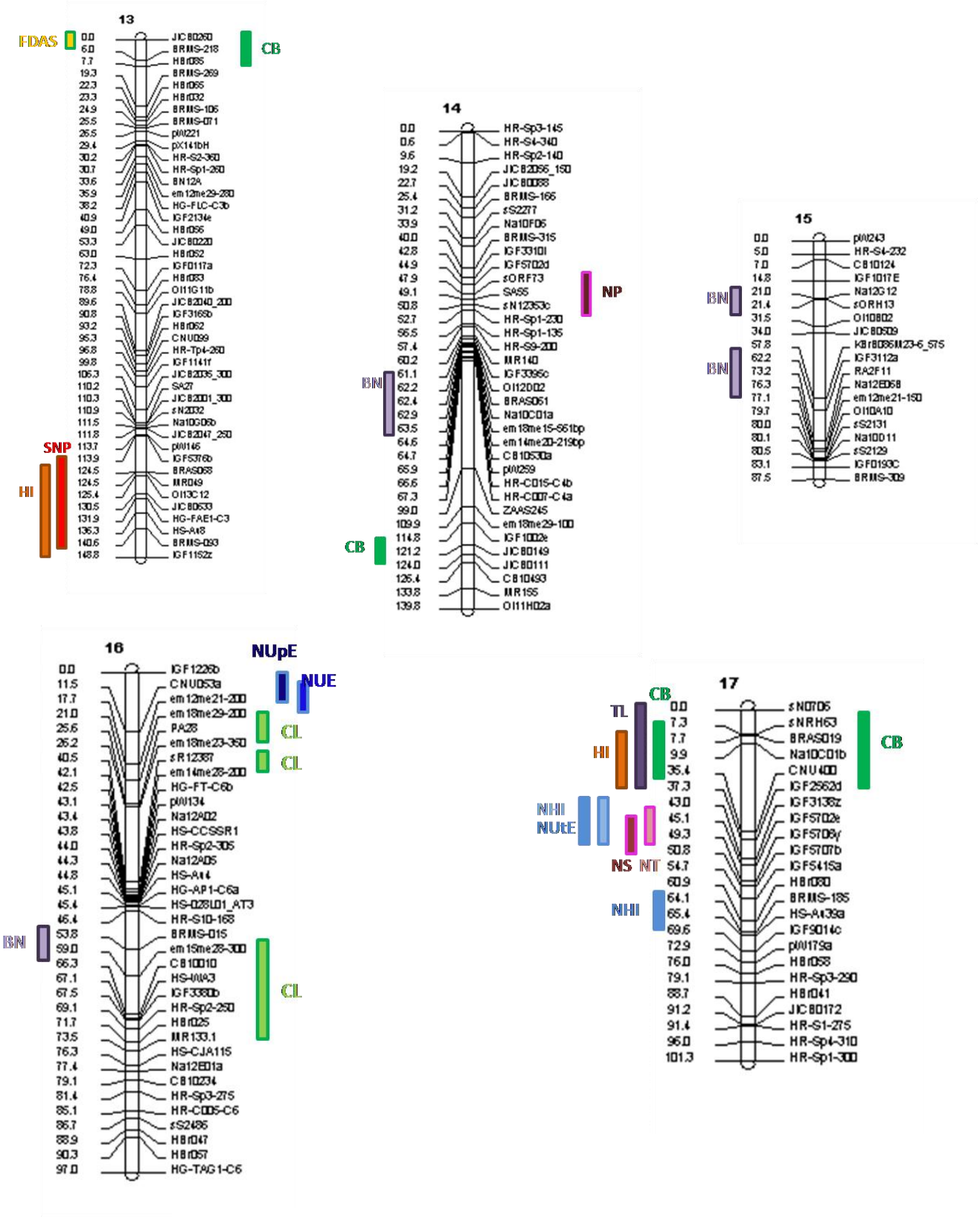


Figure 3.5. Quantitative trait loci positions for the traits analysed in 2005/06, on chromosomes 13 to 17, from left to right and top to bottom. The QTL on the left are for High N and on the right are for Low N.

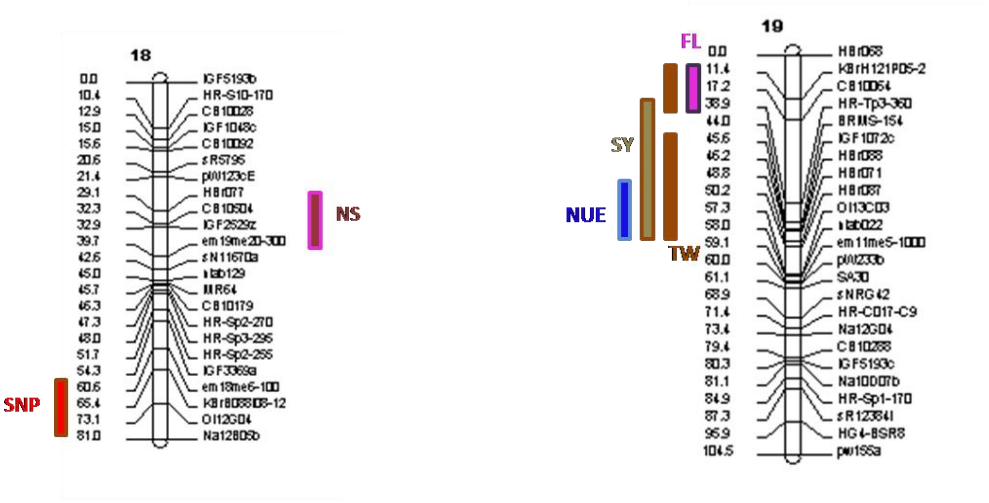


Figure 3.6. Quantitative trait loci positions for the traits analysed in 2005/06, on chromosomes 18 and 19, from left to right. The QTL on the left are for High N and on the right are for Low N.

QTL x Environment interactions

The QTL x Environment analysis was performed by CIM using the Jzmapqtl module from WinQTL Cartographer to assess the significance of the QTL across High and Low N treatments in 2005/06. Quantitative trait loci with LOD scores above 1.8 were considered significant (ref). The resulting LOD scores for High N, Low N and joint LOD scores were assessed (Appendix 21). According to that, 3 different types of QTL were identified: QTL with LOD scores higher than 1.8 at High N, Low N and joint; QTL with LOD score above 1.8 in either High or Low N or for the joint LOD score and finally, QTL with a significant LOD score for the joint analysis only. The first result would refer to conservative QTL, i.e. not influenced by N treatment, the second result would mean the QTL had an interaction with the N treatment as signified by LOD scores above 1.8 and the third result would indicate there was an interaction between the QTL and the environment, but not necessarily due to N treatment.

Most QTL identified for all traits presented significant QTL x N interaction, either at High or Low N. All traits also presented QTL with significant LOD score for the joint analysis only. Only 6 QTL were identified for both N treatments simultaneously in 5 traits i.e. QTL for foot length: FL_19_2_06, chlorophyll in bracts: CB_1_41_06, 2 QTL chlorophyll in leaves: CL_1_43_06 (same QTL as for CB) and CL_1_31_06, seed N concentration: SN_18_11_06 and for total N concentration: TN_7_22_06.

3.3.2. EXPERIMENT 2. FIELD TRIAL 2006/07.

Results for QTL of the traits studied in 2006/07 are presented according to trait groups i.e. flowering, yield and nitrogen. For each group of traits, QTL have been identified at both High N (Bocks 2 and 3) and Low N (Blocks 1 and 4).

YIELD AND YIELD COMPONENTS

HIGH N QTL

A number of QTL were detected for total above ground plant biomass (TW) HI and seed yield (SY) from both IM and CIM at High N.

Most of the QTL identified for total above ground plant biomass (Table 3.22) were found in Block 2, both by IM (5 out of 7) and CIM (3 out of 5). All 5 QTL identified by IM from Block 1 were localised on chromosome 7, four of which were confirmed by MIM with LOD scores between 3.07 and 4.15.

Table 3.22. Putative QTL and related marker position influencing total above ground plant biomass (TW) at High N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
TW	**IM**									
	TW_7_16_R2H07	2	High	7	16	40.79	-251.13	0.132	2.80	
	TW_7_17_R2H07	2	High	7	17	44.47	-292.99	0.178	3.29	44.5
	TW_7_19_R2H07	2	High	7	19	50.58	-296.63	0.185	4.02	50.6
	TW_7_22_R2H07	2	High	7	22	56.99	-300.71	0.188	4.15	57.0
	TW_7_26_R2H07	2	High	7	26	67.78	-260.60	0.138	3.07	67.8
	TW_1_17_R3H07	3	High	1	17	37.60	238.96	0.104	2.26	37.6
	TW_1_18_R3H07	3	High	1	18	40.11	251.67	0.116	2.16	
	CIM									
	TW_7_17_R2H07	2	High	7	17	46.47	-332.97	0.225	5.56	46.6
	TW_7_19_R2H07	2	High	7	19	50.58	-339.28	0.235	6.54	
	TW_7_22_R2H07	2	High	7	22	57.99	-370.00	0.272	7.71	58.0
	TW_1_14_R3H07	3	High	1	14	31.60	263.83	0.124	3.42	31.6
	TW_1_18_R3H07	3	High	1	18	38.11	295.49	0.150	4.23	38.1

These QTL accounted for 68.9% of the phenotypic variance of the population for TW. Three of these QTL were also identified by CIM i.e. TW_7_17_R2H07, TW_7_19_R2H07 and TW_7_22_R2H07, together accounting for 55.1% of the variation.

The 2 QTL identified from Block 3 by IM were localised on chromosome 1, and only 1 was confirmed by MIM (TW_1_17_R2H07) with a LOD score of 2.26 which represented 10.4% of the phenotypic variance. The other QTL (TW_1_18_R2H07) with a LOD score of 4.23 was also found when analyzing data by CIM but was not confirmed by MIM. Another QTL identified by CIM from Block 3 was TW_1_14_R2H07 localised on chromosome 1, with a LOD score of 3.42. Both QTL from CIM analysis explained 27.4% of the phenotypic variation of the population.

Table 3.23. Putative QTL and related marker position influencing harvest index (HI) at High N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
HI	IM									
	HI_10_3_R3H07	3	High	10	3	5.50	-0.04	0.096	2.07	5.5
	HI_10_10_R3H07	3	High	10	10	12.58	-0.05	0.110	2.30	12.6
	CIM									
	HI_7_22_R2H07	2	High	7	22	60.99	-0.06	0.100	2.73	61.0
	HI_16_3_R2H07	2	High	16	3	18.71	-0.03	0.088	2.24	18.7
	HI_8_2_R3H07	3	High	8	2	7.69	0.04	0.103	3.26	7.7
	HI_10_3_R3H07	3	High	10	3	6.50	-0.05	0.103	2.68	6.5
	HI_10_6_R3H07	3	High	10	6	8.58	-0.04	0.079	2.24	
	HI_10_10_R3H07	3	High	10	10	12.58	-0.07	0.170	5.06	12.6
	HI_10_12_R3H07	3	High	10	12	17.52	-0.04	0.083	2.13	

Two QTL were identified using IM for harvest index on chromosome 10 i.e. HI_10_3_R307 and HI_10_10_R307 with LOD scores of 2.07 and 2.30 respectively and both were from Block 3. These same QTL were again identified using CIM with LOD scores of 2.68 and 5.06 respectively. An additional 3 QTL were identified from Block 3 and 2 from Block 2 by CIM. The latter QTL were detected on chromosomes 7 and 16, and had LOD scores of 2.73 and 2.24 respectively. Most of the QTL were confirmed by MIM,

except for HI_10_6_R3H07 and HI_10_12_R3H07 on chromosome 10 from Block 3 which had been identified by CIM. Phenotypic variation for harvest index QTL was 20.6% by IM and 18.8% for the 2 QTL from Block 2 and 27.6% for the 3 QTL from Block 3. The QTL from Block 2 on chromosome 7 (HI_7_22_R207), had been previously identified for TW.

Table 3.24. Putative QTL and related marker position influencing seed yield (SY) at High N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
SY	**IM**									
	SY_7_16_R2H07	2	High	7	16	41.79	-75.51	0.099	2.08	
	SY_7_17_R2H07	2	High	7	17	44.47	-89.76	0.141	2.49	
	SY_7_19_R2H07	2	High	7	19	50.58	-91.36	0.147	3.09	50.6
	SY_7_22_R2H07	2	High	7	22	57.99	-101.17	0.179	3.91	59.0
	SY_7_24_R2H07	2	High	7	24	66.20	-85.86	0.129	2.69	
	SY_7_26_R2H07	2	High	7	26	67.78	-86.46	0.128	2.83	67.8
		2	High	9						114.9
	SY_1_14_R3H07	3	High	1	14	31.60	84.50	0.103	2.24	31.6
	SY_1_17_R3H07	3	High	1	17	37.60	93.05	0.124	2.72	37.6
	SY_1_18_R3H07	3	High	1	18	40.11	96.59	0.133	2.53	
	CIM									
	SY_4_7_R2H07	2	High	4	7	15.97	67.73	0.066	2.25	16.0
	SY_7_19_R2H07	2	High	7	19	50.58	-114.34	0.220	5.73	50.6
	SY_7_20_R2H07	2	High	7	20	52.11	-111.05	0.207	5.44	
	SY_7_22_R2H07	2	High	7	22	60.99	-135.46	0.290	8.48	61.0
	SY_7_24_R2H07	2	High	7	24	66.20	-124.49	0.245	6.66	
	SY_7_27_R2H07	2	High	7	27	70.01	-124.26	0.238	6.68	70.0
	SY_7_28_R2H07	2	High	7	28	71.90	-116.04	0.205	5.55	
	SY_14_3_R2H07	2	High	14	3	9.56	65.14	0.073	2.28	9.6
	SY_16_28_R2H07	2	High	16	28	77.39	70.04	0.071	2.39	77.4
	SY_16_30_R2H07	2	High	16	30	81.40	77.32	0.091	3.13	
	SY_1_14_R3H07	3	High	1	14	31.60	90.72	0.113	3.42	31.6
	SY_1_17_R3H07	3	High	1	17	37.60	96.11	0.123	3.75	37.6
	SY_5_15_R3H07	3	High	5	15	53.45	-84.87	0.093	2.79	53.4
	SY_5_18_R3H07	3	High	5	18	57.73	-90.19	0.104	3.21	
	SY_5_20_R3H07	3	High	5	20	58.70	-82.40	0.087	2.65	
	SY_5_22_R3H07	3	High	5	22	59.38	-82.55	0.088	2.66	59.4
	SY_5_24_R3H07	3	High	5	24	60.04	-74.33	0.072	2.16	
	SY_5_28_R3H07	3	High	5	28	63.50	-84.27	0.092	2.70	
	SY_5_29_R3H07	3	High	5	29	67.10	-86.08	0.096	2.45	67.1
	SY_8_1_R3H07	3	High	8	1	0.01	80.85	0.089	2.80	0.0
	SY_8_2_R3H07	3	High	8	2	11.69	82.09	0.095	2.35	11.7
	SY_17_3_R3H07	3	High	17	3	7.68	69.37	0.067	2.12	7.7

Seven and 10 QTL were identified for seed yield at High N from Block 2 by IM and CIM respectively (Table 3.24). Of these, 3 and 6 from IM and CIM respectively were confirmed by MIM. Three QTL on chromosome 7 were identified by the 3 different analyses IM, CIM and MIM i.e. SY_7_19_R207,

SY_7_22_R207 and SY_7_26_R207. These QTL had LOD scores of 3.09, 3.91, 2.83 when analysed by IM and 5.73, 8.48 and 6.68 when analysed using CIM.

Other QTL from Block 2 for SY were identified on chromosomes 4, 14 and 16 using CIM only. The 3 QTL identified for SY by IM from Block 2 accounted for 45.4% of phenotypic variation of the population, whereas the 6 QTL identified using CIM accounted for 95.8% of the phenotypic variation for the TNDH population.

Negative additive effects for the QTL found on chromosome 7 indicated that alleles were derived from Ningyou7, and from Tapidor for other QTL on chromosomes 4, 14 and 16.

Quantitative trait loci SY_7_17_R207, SY_7_19_R207, SY_7_22_R207 and SY_7_26_R207 were co-localised with the QTL for TW at High N, and QTL 7_22_R207 was identified for the 3 yield traits i.e. TW, HI and SY, analysed at High N.

Three QTL were identified for SY from Block 3 using IM and 12 using CIM. Two QTL from IM were also identified by CIM and MIM (SY_1_14_R307 and SY_1_17_R307). Six other QTL identified by CIM were also confirmed by MIM. The LOD scores ranged from 2.12 on chromosome 17 to 3.42 on chromosome 1. The 2 QTL from IM accounted for 22.7% of the phenotypic variation for the TNDH population and the 8 QTL from CIM jointly accounted for 76.4% of the phenotypic variation of the TNDH population.

The QTL identified on chromosomes 1, 8 and 17 had positive additive effects, indicating alleles derived from Tapidor and the QTL on chromosome 5 showed negative additive effects therefore being derived from Ningyou7.

Low N QTL

Four QTL were detected for total above ground plant biomass (TW) at Low N from Block 1 using CIM. One of these QTL was also identified by IM (TW_11_31_R1H07) with LOD scores of 1.96 and 3.35 by IM and CIM respectively. Two additional QTL on chromosomes 7 and 18 were identified by CIM with respective LOD scores of 3.23 and 2.20. The 3 QTL together explained 30.7% of the phenotypic variance of the population for total above ground plant biomass at Low N.

Additive effects were positive for the QTL on chromosome 11 and negative for QTL on chromosomes 7 and 18.

Table 3.25. Putative QTL and related marker position influencing total above ground plant biomass (TW) at Low N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
TW	**IM**									
	TW_11_31_R1L07	1	Low	11	31	58.11	119.98	0.091	1.96	58.1
	TW_4_8_R4L07	4	Low	4	8	18.42	177.72	0.124	2.72	18.4
	TW_4_12_R4L07	4	Low	4	12	20.51	172.91	0.121	2.66	
	TW_4_13_R4L07	4	Low	4	13	25.18	177.96	0.129	2.28	25.2
	TW_4_19_R4L07	4	Low	4	19	50.83	143.65	0.094	2.04	
	TW_4_20_R4L07	4	Low	4	20	52.50	153.29	0.108	2.21	52.5
	TW_9_39_R4L07	4	Low	9	39	71.70	-153.08	0.105	2.28	71.7
	CIM									
	TW_7_46_R1L07	1	Low	7	46	109.34	-148.27	0.113	3.23	109.3
	TW_11_31_R1L07	1	Low	11	31	58.11	137.52	0.118	3.35	58.1
	TW_11_32_R1L07	1	Low	11	32	62.22	124.07	0.095	2.64	
	TW_18_18_R1L07	1	Low	18	18	51.69	-111.19	0.076	2.20	51.7
	TW_4_8_R4L07	4	Low	4	8	18.42	179.71	0.118	3.62	18.4
	TW_4_12_R4L07	4	Low	4	12	20.51	173.87	0.111	3.40	
	TW_4_13_R4L07	4	Low	4	13	24.18	175.25	0.115	2.98	
	TW_9_24_R4L07	4	Low	9	24	57.82	-144.69	0.089	2.11	57.8
	TW_9_39_R4L07	4	Low	9	39	71.70	-151.94	0.096	2.62	71.7
	TW_14_12_R4L07	4	Low	14	12	47.88	-147.63	0.088	2.73	47.9

Six QTL were detected both by IM and CIM in Block 4 and of these 3 on chromosome 4 and 1 on chromosome 9 were common (TW_4_8_R2L07, TW_4_12_R2L07, TW_4_13_R2L07, and TW_9_39_R2L07), with LOD scores of 2.21 to 2.28 from IM and 2.11 to 3.63 from CIM. The 3 QTL identified by IM accounted for 46.6% of the phenotypic variance and the 4 from CIM for 39.1%.

For harvest index, only 2 QTL were found by IM at Low N, both on chromosome 7 and from Block 1 (HI_7_18_R1L07 and HI_7_20_R1L07). These 2 QTL were also detected by CIM but only HI_7_18_R1L07 was confirmed by MIM. Out of the 8 QTL found by CIM in Block 1 only 3 were confirmed by MIM i.e. HI_3_61_R1L07 with a LOD score of 2.13, HI_7_15_R1L07 with a LOD score of 2.49 and HI_7_18_R1L07 with a LOD score of 2.74. The cumulative phenotypic variation for these 3 QTL was 24.4% of the

population. The additive effect values were very low for all the QTL being derived from Ningyou7 for the QTL on chromosome 3 and from Tapidor for QTL on chromosome 7.

Eleven QTL were found for HI from Block 4, all by CIM and 6 of them were also confirmed by MIM. The LOD scores for these QTL ranged from 2.28 to 5.85, with 3 QTL having LOD scores well above 4 i.e. HI_2_16_R1L07 with LOD score of 4.65, HI_14_17_R1L07 with a LOD score of 5.85 and HI_14_21_R1L07 with a LOD score of 4.55. The 3 QTL jointly accounted for 80.5% of the phenotypic variation of the TNDH population for HI at Low N.

Table 3.26. Putative QTL and related marker position influencing harvest index (HI) at Low N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
HI	IM									
	HI_7_18_R1L07	1	Low	7	18	47.98	0.02	0.115	2.53	48.0
	HI_7_20_R1L07	1	Low	7	20	51.11	0.02	0.111	2.42	
	CIM									
	HI_3_61_R1L07	1	Low	3	61	107.72	-0.02	0.073	2.13	107.7
	HI_3_64_R1L07	1	Low	3	64	112.42	-0.03	0.101	3.06	
	HI_3_65_R1L07	1	Low	3	65	120.31	-0.02	0.083	2.26	
	HI_7_15_R1L07	1	Low	7	15	36.19	0.02	0.082	2.49	36.2
	HI_7_16_R1L07	1	Low	7	16	39.79	0.02	0.082	2.49	
	HI_7_18_R1L07	1	Low	7	18	47.98	0.02	0.089	2.74	48.0
	HI_7_20_R1L07	1	Low	7	20	51.11	0.02	0.081	2.46	
	HI_18_22_R1L07	1	Low	18	22	80.14	0.03	0.120	3.45	
	HI_2_16_R4L07	4	Low	2	16	62.95	-0.03	0.176	4.65	63.0
	HI_2_17_R4L07	4	Low	2	17	65.09	-0.03	0.173	4.60	
	HI_2_19_R4L07	4	Low	2	19	66.88	-0.03	0.158	4.37	
	HI_2_22_R4L07	4	Low	2	22	71.27	-0.02	0.092	2.28	71.3
	HI_14_11_R4L07	4	Low	14	11	45.88	-0.02	0.140	3.19	45.9
	HI_14_12_R4L07	4	Low	14	12	48.88	-0.02	0.113	2.73	
	HI_14_17_R4L07	4	Low	14	17	57.43	-0.03	0.220	5.85	57.4
	HI_14_21_R4L07	4	Low	14	21	62.43	-0.03	0.177	4.55	62.4
	HI_14_23_R4L07	4	Low	14	23	63.48	-0.03	0.177	4.58	
	HI_14_26_R4L07	4	Low	14	26	65.91	-0.03	0.184	4.76	
	HI_17_7_R4L07	4	Low	17	7	42.98	-0.02	0.104	2.99	43.0

One QTL i.e., TW_14_12_R4L07 was found both for TW and HI at Low N which was also detected by MIM for TW. Two QTL for HI on chromosome 7 i.e., HI_7_18_R1L07 and HI_7_20_R1L07 were also co-localised with TW and SY at High N.

One QTL was detected for seed yield at Low N using IM in Block 1, whereas 8 different ones were found by CIM in the same block. The QTL from IM had a LOD score of 2.26 and accounted for 10.4% of the

phenotypic variation of the population. The 5 QTL from CIM confirmed by MIM had LOD scores between 2.08 and 5.41 and jointly accounted for 50.9% of the phenotypic variation.

Table 3.27. Putative QTL and related marker position influencing seed yield (SY) at Low N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
SY	**IM**									
	SY_11_31_R1L07	1	Low	11	31	58.11	36.01	0.104	2.26	58.1
	SY_4_8_R4L07	4	Low	4	8	18.42	55.48	0.119	2.61	18.4
	SY_4_12_R4L07	4	Low	4	12	20.51	51.88	0.107	2.34	
	SY_4_13_R4L07	4	Low	4	13	25.18	56.14	0.126	2.24	
	SY_4_19_R4L07	4	Low	4	19	50.83	49.00	0.108	2.36	
	SY_4_20_R4L07	4	Low	4	20	52.50	49.62	0.111	2.37	
	SY_9_39_R4L07	4	Low	9	39	71.70	-49.26	0.107	2.33	71.7
	SY_18_1_R4L07	4	Low	18	1	0.01	48.93	0.106	2.27	0.0
	CIM									
	SY_2_26_R1L07	1	Low	2	26	91.56	-36.13	0.066	2.08	91.6
	SY_2_27_R1L07	1	Low	2	27	94.73	-44.23	0.087	2.66	
	SY_9_3_R1L07	1	Low	9	3	10.53	60.51	0.119	3.52	10.5
	SY_9_8_R1L07	1	Low	9	8	23.68	-74.31	0.179	5.41	23.7
	SY_9_10_R1L07	1	Low	9	10	27.00	-69.97	0.121	3.59	
	SY_9_11_R1L07	1	Low	9	11	28.24	-70.78	0.137	3.68	
	SY_16_15_R1L07	1	Low	16	15	44.78	32.54	0.070	2.35	44.8
	SY_16_19_R1L07	1	Low	16	19	53.85	33.25	0.075	2.54	53.8
	SY_18_4_R1L07	4	Low	18	1	0.01	75.42	0.159	4.53	0.0

For Block 4, seven QTL were found for SY at Low N using IM and SY_18_4_R1L07 on chromosome 18 at 0.01cM was also detected by CIM. Out of the 7 QTL only 3 were commonly detected by MIM, on chromosomes 4, 9 and the previously mentioned 18. The LOD scores for these QTL were 2.61, 2.33 and 2.27 respectively and these explained 33.2% of the phenotypic variation for SY at Low N.

A number of QTL for TW and SY at Low N were co-localised and of these 5 were on chromosome 4, 1 on chromosome 9 and one on chromosome 18 in Block 4; and one on chromosome 11 in Block 1.

NITROGEN AND NITROGEN DERIVED TRAITS

HIGH N QTL

Results showed 2 QTL were detected for seed N at High N by IM both in Block 2, one on chromosome 9 and the other one on 14, the latter was also found by CIM. These 2 QTL had LOD scores of 2.30 and 2.70 respectively. They jointly accounted for 23.1% of the phenotypic variation of the TNDH population.

Three QTL were identified from Block 2 by CIM on chromosomes 4, 11 and 14. The QTL had LOD scores of 2.43, 2.68 and 2.06 respectively and together explained 23.9% of the phenotypic variation. All additive effects had a negative sign indicating alleles derived from Ningyou7.

Quantitative trait loci for NUpE at High N were identified on chromosome 7 (4) and 9 (3) in Block 2 and on chromosome 13 (1) in Block 3 by IM. The 4 QTL on 7 were also detected by MIM, and of these 3 were also identified by CIM. The LOD scores for these QTL were between 2.33 and 2.99, all with a negative additive effect. Only one QTL of the 3 detected on chromosome 9 by IM was also detected by MIM, the LOD score of which was 2.07. The 4 QTL on chromosome 7 together with the one on chromosome 9 accounted for 83.6% of the phenotypic variation of the TNDH population. The QTL found on chromosome 13 from Block 3 had a LOD score of 2.29 and explained 10.5% of the phenotypic variation of the population.

Five QTL were identified by CIM from Block 2 and 6 from Block 3, and were confirmed by MIM. The 5 QTL from Block 2 were found on chromosomes 4, 7 and 17, with LOD scores between 2.37 and 5.75. Three QTL on chromosome 7 (NUPE_7_17_R2H07, NUPE_7_19_R2H07 and NUPE_7_22_R2H07) had been previously identified in close proximity to a QTL for TW and SY at High N and for HI at Low N. The QTL for SY had been detected at High N 7cM down chromosome 17 from the QTL NUpE_17_1_R2H07.

No QTL were detected for NUtE at High N using IM. However, 3 QTL from Block 2 and 8 from Block 3 were detected by CIM, from which, only 2 and 3 respectively were confirmed by MIM from Blocks 2 and 3. The 2 QTL identified in Block 2 were found on chromosome 6, with LOD scores of 2.15 and 2.51 respectively, and together accounted for 20.5% of the phenotypic variation of the TNDH population.

Table 3.28. Putative QTL and related marker position influencing N uptake efficiency (NUpE) at High N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
NUPE	IM									
	NUPE_7_17_R2H07	2	High	7	17	45.47	-0.64	0.138	2.45	45.6
	NUPE_7_19_R2H07	2	High	7	19	50.58	-0.64	0.139	2.95	50.6
	NUPE_7_22_R2H07	2	High	7	22	56.99	-0.64	0.139	2.99	57.0
	NUPE_7_26_R2H07	2	High	7	26	67.78	-0.57	0.107	2.33	67.8
	NUPE_9_51_R2H07	2	High	9	51	114.88	-0.59	0.115	2.49	
	NUPE_9_54_R2H07	2	High	9	54	119.85	-0.54	0.098	2.07	119.9
	NUPE_9_55_R2H07	2	High	9	55	121.48	-0.54	0.100	2.05	
	NUPE_13_29_R3H07	3	High	13	29	106.30	-0.48	0.105	2.29	106.3
	CIM									
	NUPE_4_9_R2H07	2	High	4	9	18.90	0.50	0.073	2.37	18.9
	NUPE_7_17_R2H07	2	High	7	17	44.47	-0.79	0.202	4.99	44.5
	NUPE_7_19_R2H07	2	High	7	19	50.58	-0.76	0.188	5.50	50.6
	NUPE_7_22_R2H07	2	High	7	22	59.99	-0.79	0.194	5.75	60.0
	NUPE_17_1_R2H07	2	High	17	1	1.01	0.66	0.133	3.04	1.0
	NUPE_4_5_R3H07	3	High	4	5	14.20	0.42	0.065	2.07	14.2
	NUPE_11_27_R3H07	3	High	11	27	53.88	-0.42	0.079	2.39	53.9
	NUPE_13_29_R3H07	3	High	13	29	106.30	-0.42	0.079	2.50	106.30
	NUPE_15_11_R3H07	3	High	15	11	76.21	-0.43	0.082	2.49	76.20
	NUPE_15_18_R3H07	3	High	15	18	83.07	-0.43	0.081	2.49	83.10
	NUPE_19_17_R3H07	3	High	19	17	73.39	0.46	0.096	2.98	73.40

The 3 QTL identified in Block 3 were localised on chromosomes 9 (2) and 19 (1), with LOD scores of 4.00, 4.01 and 3.10 respectively. The 3 QTL jointly explained 38.1% of the phenotypic variation of the population. Additive effects for all detected QTL were negative except for the QTL on chromosome 19 which showed a positive additive effect.

Table 3.29. Putative QTL and related marker position influencing N utilisation efficiency (NUtE) at High N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
NUTE	CIM									
	NUTE_6_38_R2H07	2	High	6	38	73.84	-0.94	0.112	2.15	73.9
	NUTE_6_43_R2H07	2	High	6	43	82.06	-0.85	0.093	2.51	82.1
	NUTE_7_46_R2H07	2	High	7	46	111.34	1.82	0.132	2.96	
	NUTE_3_64_R3H07	3	High	3	64	117.42	-1.86	0.137	3.98	
	NUTE_3_65_R3H07	3	High	3	65	121.31	-1.89	0.138	4.00	121.3
	NUTE_9_1_R3H07	3	High	9	1	3.01	-1.90	0.129	3.76	
	NUTE_9_2_R3H07	3	High	9	2	5.79	-2.03	0.143	4.01	5.8
	NUTE_9_3_R3H07	3	High	9	3	9.53	-2.23	0.157	4.40	
	NUTE_9_4_R3H07	3	High	9	4	12.51	-2.27	0.148	4.32	
	NUTE_9_6_R3H07	3	High	9	6	20.78	-2.07	0.117	2.46	
		3	High	19	10	57.32	1.55	0.100	3.10	57.3

Results identified many QTL for NUE from Block 2 at High N, both by IM and CIM, whereas only 1 QTL was detected from Block 3 by CIM. Five QTL were detected by IM on Block 2, four on chromosome 7 and 1 on 9, of which 3 and 1 were confirmed by MIM. The 3 QTL on chromosome 7 were NUE_7_19_R2H07 with LOD score of 2.45, NUE_7_22_R2H07 with LOD score of 3.25 and NUE_7_26_R2H07 with LOD score of 2.41. The QTL on chromosome 9 had a LOD score of 2.20. The 4 QTL jointly accounted for 48.2% of the phenotypic variation of the population for NUE. All QTL detected by IM had negative additive effect reflecting an influence from Ningyou7.

Seven QTL were identified by CIM for NUE from Block 2, and 6 were confirmed by MIM. The 6 QTL were identified on chromosomes 4 (1), 7 (3), 11(1) and 17 (1). The LOD scores for these QTL ranged from 2.16 to 5.49 and the 6 QTL jointly explained 69.7% of the phenotypic variation of the TNDH population. The QTL NUE_7_22_R2H07 with a LOD score of 5.49 represented 18.6% of the phenotypic variation of the population. The 3 QTL on chromosome 7 had been identified by for NUE as well as for NUpE by IM at High N and also for TW and SY.

Table 3.30. Putative QTL and related marker position influencing N use efficiency (NUE) at High N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
NUE	IM									
	NUE_7_19_R2H07	2	High	7	19	50.58	-4.45	0.118	2.45	50.6
	NUE_7_22_R2H07	2	High	7	22	58.99	-5.04	0.151	3.25	59.0
	NUE_7_24_R2H07	2	High	7	24	66.20	-4.21	0.106	2.20	
	NUE_7_26_R2H07	2	High	7	26	67.78	-4.35	0.110	2.41	67.8
	NUE_9_51_R2H07	2	High	9	51	114.88	-4.18	0.103	2.20	114.9
	CIM									
	NUE_4_9_R2H07	2	High	4	9	18.90	3.57	0.066	2.16	18.9
	NUE_7_19_R2H07	2	High	7	19	50.58	-5.23	0.158	4.44	50.6
	NUE_7_22_R2H07	2	High	7	22	59.99	-5.76	0.186	5.49	60.0
	NUE_7_25_R2H07	2	High	7	25	66.88	-4.74	0.126	3.64	
	NUE_7_27_R2H07	2	High	7	27	70.01	-4.76	0.126	3.64	70.0
	NUE_11_1_R2H07	2	High	11	1	0.01	-3.52	0.071	2.29	0.0
	NUE_17_1_R2H07	2	High	17	1	0.01	4.20	0.090	2.84	0.0
	NUE_8_2_R3H07	3	High	8	2	10.69	4.32	0.092	2.30	10.7

The QTL identified by CIM from Block 3 was on chromosome 8, with a LOD score of 2.30 and accounted for 9.2% of the phenotypic variation of the population. All QTL detected by CIM, both from Block 2 and 3 had negative additive effect except QTL on chromosomes 4 and 17 from Block 2 and on chromosome 8 from Block 3.

Results showed no QTL were detected by IM for NHI (Table 3.31), however, 2 QTL both on chromosome 11 were found in Block 2 and 13 were identified in Block 3 by CIM. The 2 QTL on chromosome 11 had LOD scores of 2.78 and 2.17, respectively, and jointly explained 18.2% of the phenotypic variation for this trait. Eleven of the 13 QTL found in Block 3 by CIM were also confirmed by MIM and were localised on chromosomes 1 (1), 3 (3), 6 (1), 9 (2), 12 (3) and 14 (1). The LOD scores for these QTL ranged from 2.01 to 3.62, with the highest ones above 3 on chromosomes 3, 9 and 12. The 6 QTL with LOD scores above 2.5 together explained 65.6% of the phenotypic variation of the population for NHI at High N.

Additive effects were found to be very weak (close to 0) but slightly towards the negative sign with 8 negative and 6 positive effects.

Table 3.31. Putative QTL and related marker position influencing harvest index (NHI) at High N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
NHI	CIM									
	NHI_11_1_R2H07	2	High	11	1	0.01	-0.03	0.100	2.78	0.0
	NHI_11_3_R2H07	2	High	11	3	6.54	-0.02	0.082	2.17	6.5
	NHI_1_42_R3H07	3	High	1	42	70.56	0.03	0.060	2.07	70.6
	NHI_3_51_R3H07	3	High	3	51	86.30	-0.04	0.078	2.29	86.3
	NHI_3_58_R3H07	3	High	3	58	98.47	-0.05	0.118	3.60	98.5
	NHI_3_59_R3H07	3	High	3	59	103.35	-0.04	0.090	2.63	
	NHI_3_60_R3H07	3	High	3	60	107.06	-0.05	0.120	3.62	107.1
	NHI_6_38_R3H07	3	High	6	38	71.84	0.04	0.112	2.15	71.8
	NHI_9_1_R3H07	3	High	9	1	0.01	-0.04	0.103	3.11	0.0
	NHI_9_2_R3H07	3	High	9	2	4.79	-0.04	0.096	2.81	
	NHI_9_3_R3H07	3	High	9	3	9.53	-0.04	0.102	2.89	9.5
	NHI_12_17_R3H07	3	High	12	17	79.24	0.04	0.099	2.85	79.2
	NHI_12_18_R3H07	3	High	12	18	86.01	0.04	0.114	3.05	86.0
	NHI_12_22_R3H07	3	High	12	22	101.50	0.03	0.072	2.18	101.5
	NHI_14_3_R3H07	3	High	14	3	9.56	0.03	0.066	2.01	9.6

The 3 QTL on chromosome 9, at marker positions 1, 2, and 3 were also found co-localised with QTL for NUtE, total plant N (TN) and stem N while the QTL at marker positions 1 and 3 were co-localised with QTL for pod N concentrations. The QTL on chromosomes 6 and 11 were co-localised with QTL for NUtE, and NUE respectively in Block 2.

Two QTL were detected for SN on chromosome 8 in Block 3, with respective LOD scores of 3.99 and 2.49, the first one had positive additive effect and the last one negative, both of similar magnitude. Two more QTL were detected by CIM in Block 3, on chromosomes 12 and 19, with LOD scores of 2.01 and 2.08. The 4 QTL jointly explained 40.9% of the phenotypic variation of the TNDH population.

Table 3.32. Putative QTL and related marker position influencing seed N concentration (SN) at High N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
SN	IM									
	SN_9_52_R2H07	2	High	9	52	115.17	-0.32	0.106	2.30	115.2
	SN_14_35_R2H07	2	High	14	35	133.78	-0.37	0.125	2.70	133.8
	CIM									
	SN_4_14_R2H07	2	High	4	14	29.92	-0.36	0.074	2.43	29.9
	SN_11_3_R2H07	2	High	11	3	6.54	-0.31	0.096	2.68	6.5
	SN_14_35_R2H07	2	High	14	35	133.78	-0.28	0.069	2.06	133.8
	SN_8_1_R3H07	3	High	8	1	7.01	6.57	0.148	3.99	7.0
	SN_8_4_R3H07	3	High	8	4	25.80	-5.27	0.087	2.49	25.8
	SN_12_8_R3H07	3	High	12	8	51.99	3.42	0.070	2.01	52.0
	SN_19_1_R3H07	3	High	19	1	8.01	4.19	0.104	2.08	8.0

Many QTL were identified for STN from Block 2 (4 by IM and 10 by CIM), compared with only 4 identified by CIM from Block 3. Results from IM identified 2 QTL on each of chromosomes 9 and 14; one QTL from each chromosome was also identified by CIM i.e. STN_9_52_R2H07 and STN_14_34_R2H07. The LOD scores of the 4 QTL ranged from 2.11 to 3.16 and the QTL together accounted for 50.1% of the phenotypic variation of the population.

Seven out of the 10 QTL identified by CIM in Block 2 were also confirmed by MIM, with LOD scores ranging from 2.00 to 4.10. They jointly explained 68.5% of the phenotypic variation of the TNDH population. All additive effects were negative indicating alleles derived from Ningyou7 for STN.

Table 3.33. Putative QTL and related marker position influencing stem nitrogen concentration (STN) at High N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
STN	IM									
	STN_9_15_R2H07	2	High	9	15	37.46	-0.08	0.097	2.11	37.5
	STN_9_52_R2H07	2	High	9	52	116.17	-0.08	0.108	2.20	116.2
	STN_14_32_R2H07	2	High	14	32	121.19	-0.09	0.142	3.13	121.2
	STN_14_34_R2H07	2	High	14	34	129.45	-0.10	0.154	3.16	129.4
	CIM									
	STN_2_1_R2H07	2	High	2	1	8.01	-0.08	0.088	2.81	8.0
	STN_9_50_R2H07	2	High	9	50	102.58	-0.07	0.064	2.00	102.6
	STN_9_52_R2H07	2	High	9	52	115.17	-0.08	0.097	3.14	115.2
	STN_9_54_R2H07	2	High	9	54	119.85	-0.08	0.104	3.09	
	STN_9_55_R2H07	2	High	9	55	121.48	-0.08	0.100	2.94	
	STN_13_36_R2H07	2	High	13	36	123.94	-0.09	0.112	3.48	
	STN_13_39_R2H07	2	High	13	39	126.44	-0.10	0.140	4.10	126.4
	STN_13_41_R2H07	2	High	13	41	131.88	-0.08	0.105	3.27	131.9
	STN_14_31_R2H07	2	High	14	31	119.82	-0.08	0.098	2.85	119.8
	STN_14_34_R2H07	2	High	14	34	133.45	-0.08	0.093	3.03	133.4
	STN_7_31_R3H07	3	High	7	31	76.61	0.67	0.089	2.63	76.6
	STN_79_1_R3H07	3	High	9	1	0.01	-0.62	0.074	2.19	0.0
	STN_9_3_R3H07	3	High	9	3	10.53	-0.64	0.075	2.16	10.5
	STN_19_9_R3H07	3	High	19	9	57.21	0.75	0.109	3.16	57.2

Four QTL were identified for STN in Block 3, 1 on chromosome 7, 2 on 9 and 1 on 19, with LOD scores between 2.16 and 3.16 (Table 3.33). They accounted for 34.7% of the phenotypic variation of the TNDH population. Additive effects from Tapidor were present for QTL on chromosomes 7 and 19 and Ningyou7 was predominant for the QTL on chromosome 9.

Results showed 1 QTL was detected for pod N concentration by IM on chromosome 14 and 10 QTL were detected by CIM from Block 2. Seven of the 10 QTL detected by CIM were localised on chromosomes 2, 4, 8 and 13. The LOD scores for these QTL ranged from 2.10 to 3.62 and together accounted for 59.2% of the phenotypic variation. Additive effects were all positive except for the QTL found on chromosome 13.

One QTL was also found for PN (Table 3.34) using IM from Block 3 on chromosome 3 with a LOD score of 2.09 and a negative additive effect. This QTL was also found by CIM with a LOD score of 2.01. Four other QTL were found for PN by CIM on the exact chromosomal locations as previously identified for STN: one on chromosome 7, 2 on 9 and one on 19. The LOD scores for these QTL ranged from 2.03 to 4.19 and the 5 QTL together accounted for 49.4% of the phenotypic variation for the TNDH population.

Table 3.34. Putative QTL and related marker position influencing chaff N concentration (PN) at High N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
PN	IM									
	PN_14_35_R2H07	2	High	14	35	133.78	-0.10	0.100	2.16	133.8
	PN_3_66_R3H07	3	High	3	66	127.60	-0.90	0.104	2.09	127.6
	CIM									
	PN_2_26_R2H07	2	High	2	26	91.56	0.10	0.071	2.10	91.6
	PN_4_2_R2H07	2	High	4	2	2.14	0.09	0.090	2.62	2.1
	PN_4_4_R2H07	2	High	4	4	11.85	0.11	0.121	3.62	11.8
	PN_4_6_R2H07	2	High	4	6	15.95	0.11	0.105	3.05	
	PN_4_9_R2H07	2	High	4	9	18.90	0.09	0.073	2.13	18.9
	PN_8_14_R2H07	2	High	8	14	66.22	0.10	0.098	2.97	66.2
	PN_8_16_R2H07	2	High	8	16	67.51	0.10	0.097	2.96	
	PN_8_19_R2H07	2	High	8	19	73.10	0.09	0.071	2.03	73.1
	PN_13_30_R2H07	2	High	13	30	110.22	-0.08	0.068	2.36	110.2
	PN_13_33_R2H07	2	High	13	33	111.54	-0.08	0.069	2.39	
	PN_3_66_R3H07	3	High	3	66	125.60	-0.75	0.065	2.01	125.6
	PN_7_31_R3H07	3	High	7	31	76.61	0.78	0.076	2.38	76.6
	PN_9_1_R3H07	3	High	9	1	0.01	-1.02	0.142	4.19	0.0
	PN_9_3_R3H07	3	High	9	3	10.53	-1.02	0.136	3.96	10.5
	PN_19_17_R3H07	3	High	19	17	76.39	0.73	0.075	2.03	76.4

Analysis of TN by IM identified 2 QTL from Block 2, one on chromosome 9 and one on chromosome 14, with corresponding LOD scores of 2.34 and 3.06 respectively. They together accounted for 24.9% of the phenotypic variation of the TNDH population. The QTL on chromosome 14 was also identified by CIM. Both QTL had also been identified for SN and STN and the latter also for PN.

Composite interval mapping detected 6 QTL in Block 2 and 4 in Block 3 for TN at High N, all QTL were also identified by MIM except for TN_9_2_R3H07 from Block 3. For Block 2, three QTL were found on

chromosome 4, and one each on 6, 11 and 14. Two QTL had the highest LOD scores i.e. TN_4_4_R2H07 with 4.53 and TN_14_35_R2H07 with 4.67. All 6 QTL jointly explained 68.2% of the phenotypic variation of the TNDH population for TN. Two QTL on chromosome 4 i.e. TN_4_4_R2H07 and TN_4_4_R2H07 had previously been detected for yield traits at High and Low N. Of the 4 QTL in Block 3, three were found on chromosome 9 and one on chromosome 7, with LOD scores as high as 3.84. Out of the 4 QTL, 3 were also detected by MIM and jointly accounted for 31.1% of the phenotypic variation. Additive effects were all influenced by Ningyou7 as explained by the negative sign.

Table 3.35. Putative QTL and related marker position influencing total N concentration in plant (TN) at High N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
TN	IM									
	TN_9_52_R2H07	2	High	9	52	115.17	-0.47	0.108	2.34	115.2
	TN_14_35_R2H07	2	High	14	35	133.78	-0.56	0.141	3.06	133.8
	CIM									
	TN_4_4_R2H07	2	High	4	4	11.85	0.77	0.150	4.53	11.9
	TN_4_7_R2H07	2	High	4	7	17.97	0.92	0.156	3.87	18.0
	TN_4_14_R2H07	2	High	4	14	30.92	-0.51	0.070	2.06	30.9
	TN_6_4_R2H07	2	High	6	4	16.97	0.43	0.071	2.20	17.0
	TN_11_12_R2H07	2	High	11	12	29.10	-0.41	0.076	2.39	29.1
	TN_14_35_R2H07	2	High	14	35	133.78	-0.60	0.159	4.67	133.8
	TN_7_31_R3H07	3	High	7	31	76.61	4.83	0.068	2.04	76.6
	TN_9_1_R3H07	3	High	9	1	0.01	-5.90	0.108	3.05	0.0
	TN_9_2_R3H07	3	High	9	2	4.79	-6.06	0.111	3.03	
	TN_9_3_R3H07	3	High	9	3	10.53	-6.79	0.135	3.84	10.5

Low N QTL

Results showed four QTL were in Block 4 for NUpE both by CIM and MIM. The QTL were localised on chromosomes 4, 14 and 15 (2) with LOD scores ranging between 2.02 and 3.53. The 4 QTL together explained 41.0% of the phenotypic variation for the population. All QTL had positive additive effect

except for the one on chromosome 14. Of the 2 QTL identified on chromosome 15 (NUpE_15_13_R4L07) had also been identified for the same trait by IM and was co-localised with the QTL for TN at Low N.

Table 3.36. Putative QTL and related marker position influencing N uptake efficiency (NUpE) at Low N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
NUPE	IM									
	NUPE_15_13_R4L07	4	Low	15	13	78.09	2.11	0.098	2.05	78.1
	CIM									
	NUPE_7_40_R1L07	1	Low	7	40	98.98	-1.24	0.075	2.23	99.0
	NUPE_7_44_R1L07	1	Low	7	44	102.82	-1.24	0.076	2.19	
	NUPE_7_45_R1L07	1	Low	7	45	107.76	-1.41	0.094	2.62	107.7
	NUPE_7_46_R1L07	1	Low	7	46	110.34	-1.44	0.096	2.60	
	NUPE_11_18_R1L07	1	Low	11	18	35.79	-2.24	0.084	2.23	35.8
	NUPE_11_20_R1L07	1	Low	11	20	36.32	-2.21	0.080	2.19	
	NUPE_11_24_R1L07	1	Low	11	24	37.45	-2.21	0.080	2.18	
	NUPE_11_29_R1L07	1	Low	11	29	55.75	1.32	0.074	2.12	55.8
	NUPE_11_31_R1L07	1	Low	11	31	58.11	1.60	0.107	3.15	
	NUPE_11_32_R1L07	1	Low	11	32	62.22	1.41	0.083	2.41	
	NUPE_4_17_R4L07	4	Low	4	17	47.88	1.86	0.072	2.02	47.9
	NUPE_14_8_R4L07	4	Low	14	8	33.87	-2.02	0.088	2.55	33.9
	NUPE_15_13_R4L07	4	Low	15	13	77.09	2.42	0.126	3.53	77.1
	NUPE_15_18_R4L07	4	Low	15	18	83.07	2.42	0.124	3.49	83.1

Results (Table 3.37) indicated 5 QTL for NUtE found by IM all on chromosome 7, but only 2 of these were confirmed by MIM i.e. NUtE_7_16_R1L07 (LOD score 2.78) and NUtE_7_18_R1L07 (LOD score 3.51). The 2 QTL jointly explained 28.3% of the phenotypic variation of the population. Additive effects were positive for both QTL.

Table 3.37. Putative QTL and related marker position influencing N utilisation efficiency (NUtE) at Low N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
NUTE	IM									
	NUTE_7_9_R1L07	1	Low	7	9	21.75	0.87	0.097	2.12	
	NUTE_7_11_R1L07	1	Low	7	11	25.71	0.89	0.102	2.21	
	NUTE_7_16_R1L07	1	Low	7	16	39.79	0.98	0.126	2.78	39.8
	NUTE_7_18_R1L07	1	Low	7	18	47.98	1.10	0.157	3.51	48.0
	NUTE_7_20_R1L07	1	Low	7	20	51.11	1.08	0.153	3.43	
	CIM									
	NUTE_2_26_R1L07	1	Low	2	26	91.56	-0.91	0.067	2.04	91.6
	NUTE_2_27_R1L07	1	Low	2	27	94.73	-1.10	0.085	2.59	
	NUTE_7_16_R1L07	1	Low	7	16	39.79	1.19	0.157	4.42	39.8
	NUTE_7_18_R1L07	1	Low	7	18	47.98	1.36	0.214	6.30	48.0
	NUTE_7_20_R1L07	1	Low	7	20	51.11	1.39	0.210	6.17	
	NUTE_7_22_R1L07	1	Low	7	22	58.99	1.24	0.135	3.53	59.0
	NUTE_7_35_R1L07	1	Low	7	35	89.36	-0.94	0.087	2.82	89.4
	NUTE_2_12_R4L07	4	Low	2	12	52.50	-0.76	0.072	2.05	52.5
	NUTE_9_19_R4L07	4	Low	9	19	46.21	0.90	0.098	2.62	46.2
	NUTE_9_23_R4L07	4	Low	9	23	55.29	1.05	0.136	3.31	55.3

Seven QTL were identified for NUtE in Block 1 by CIM, of which 5 were also confirmed by MIM as independent QTL. The QTL were identified on chromosomes 2 and 7 and had LOD scores from 2.04 to 6.30. The QTL NUtE_7_18_R1L07 explained 21% of the phenotypic variation of the TNDH population, and all 5 QTL together explained 66% of the phenotypic variation. The QTL on chromosome 2 had negative additive effect whereas the QTL on chromosome 7 had positive additive effect.

The QTL found on chromosome 7 at markers 18 and 22 had been previously identified for various traits from Block 2 at High N i.e. TW, SY, NUpE and NUE.

Table 3.38. Putative QTL and related marker position influencing N use efficiency (NUE) at Low N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
NUE	IM									
	NUE_11_31_R1L07	1	Low	11	31	58.11	11.23	0.094	2.02	
	NUE_16_19_R1L07	1	Low	16	19	53.85	11.86	0.094	2.03	53.9
	CIM									
	NUE_9_3_R1L07	1	Low	9	3	10.53	19.89	0.120	3.45	10.5
	NUE_9_8_R1L07	1	Low	9	8	23.68	-23.95	0.172	5.10	23.7
	NUE_9_10_R1L07	1	Low	9	10	27.00	-22.61	0.117	3.41	
	NUE_9_11_R1L07	1	Low	9	11	28.24	-22.72	0.130	3.43	
	NUE_16_12_R1L07	1	Low	16	12	43.80	10.03	0.062	2.01	
	NUE_16_15_R1L07	1	Low	16	15	44.78	10.88	0.072	2.35	44.8
	NUE_16_19_R1L07	1	Low	16	19	53.85	11.15	0.078	2.56	53.9
	NUE_10_29_R4L07	4	Low	10	29	63.92	-16.42	0.092	2.50	63.9
	NUE_10_30_R4L07	4	Low	10	30	65.98	-15.55	0.082	2.26	
	NUE_14_4_R4L07	4	Low	14	4	22.18	-15.89	0.086	2.31	
	NUE_14_5_R4L07	4	Low	14	5	23.72	-15.74	0.083	2.30	
	NUE_14_7_R4L07	4	Low	14	7	31.23	-18.49	0.117	3.38	31.2
	NUE_14_9_R4L07	4	Low	14	9	42.01	-18.45	0.116	2.97	42.0
	NUE_19_4_R4L07	4	Low	19	4	40.91	16.62	0.085	2.00	
	NUE_19_7_R4L07	4	Low	19	7	46.23	16.65	0.092	3.12	46.2
	NUE_19_8_R4L07	4	Low	19	8	49.78	17.10	0.096	3.06	
	NUE_19_11_R4L07	4	Low	19	11	58.05	16.21	0.087	2.96	58.0
	NUE_19_15_R4L07	4	Low	19	15	68.93	14.61	0.068	2.24	68.9

Results showed 2 QTL on chromosomes 11 and 16 for NUE in Block 1, detected by IM. The QTL had LOD scores of 2.02 and 2.03 respectively, and the additive effect was positive for both of them. The QTL on chromosome 11 had previously been detected for other traits such as TW, SY and NUpE at Low N treatment and for FDAS at High N, with the peak 3cM down chromosome 11 but linked to marker 32 instead of 31.

Seven QTL were detected for NUE in Block 1 by CIM (Table 3.38) and of these 4 were confirmed by MIM. The QTL were detected on chromosomes 9 and 16 and the LOD scores for these QTL ranged between 2.35 and 5.10. The 4 QTL jointly explained 44.2% of the phenotypic variation of the TNDH population for NUE. Additive effects were positive for all QTL on chromosome 16 and one positive and one negative for the QTL on chromosome 9. The QTL on chromosome 9, marker 8 had the strongest and only negative additive effect and highest LOD score of 5.10 accounting for 17.2% of the phenotypic variation of the population.

An additional 11 QTL were detected for NUE by CIM on Block 4, six of which were confirmed by MIM. The QTL were identified on chromosomes 10 (1), 14 (2) and 19 (3), with LOD scores between 2.24 and 3.38, and the 6 QTL together accounted for 41.7% of the phenotypic variation. Additive effects were negative (Ningyou7 alleles) for QTL on chromosomes 10 and 16 and positive (Tapidor) for all QTL on chromosome 19. Two QTL identified for NUE on chromosome 14 at markers 7 and 9 (NUE_14_7_R4L07 and NUE_14_8_R4L07), were localised before and after a QTL for NUpE identified at marker 8 (NUPE_14_8_R4L07). The QTL on chromosome 19 at marker 15 (NUE_19_15_R4L07) was in the exact same location as the QTL for pod N concentration PN_19_15_R4L07.

Two QTL were identified using interval mapping in Block 1, both on chromosome 7 but only 1 was confirmed by MIM as an independent QTL. The LOD score was 2.43, and explained 11.1% of the phenotypic variation.

Five QTL were detected by CIM in Block 1, all on chromosome 7 and 4 of them were confirmed by MIM (NHI_7_18_R4L07 had also been identified by IM). The LOD scores were between 3.27 and 5.38, and the 4 QTL together explained 58.4% of the phenotypic variation of the population.

Table 3.39. Putative QTL and related marker position influencing N harvest index (NHI) at Low N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
NHI	IM									
	NHI_7_18_R1L07	1	Low	7	18	47.98	0.02	0.111	2.43	48.0
	NHI_7_20_R1L07	1	Low	7	20	51.11	0.02	0.108	2.35	
	CIM									
	NHI_7_16_R1L07	1	Low	7	16	39.79	0.03	0.139	3.60	39.8
	NHI_7_18_R1L07	1	Low	7	18	47.98	0.03	0.197	5.34	48.0
	NHI_7_20_R1L07	1	Low	7	20	51.11	0.03	0.198	5.38	
	NHI_7_22_R1L07	1	Low	7	22	60.99	0.03	0.138	3.60	61.0
	NHI_7_34_R1L07	1	Low	7	34	88.56	-0.02	0.114	3.27	88.6
	NHI_2_10_R4L07	4	Low	2	10	46.11	-0.02	0.076	2.12	46.1
	NHI_9_19_R4L07	4	Low	9	19	46.21	0.03	0.128	3.43	46.2
	NHI_9_21_R4L07	4	Low	9	21	51.74	0.02	0.088	2.25	
	NHI_9_23_R4L07	4	Low	9	23	54.29	0.03	0.122	3.04	54.3
	NHI_9_25_R4L07	4	Low	16	25	71.75	-0.04	0.122	3.16	71.8

Five QTL were identified in Block 4 by CIM, on chromosomes 2, 9 and 16, four of which were confirmed by MIM. The LOD scores of these QTL ranged from 2.12 to 3.43 and the 4 together explained 44.8% of the phenotypic variation of the TNDH population for NHI. Additive effects were influenced by Ningyou7 for the QTL on chromosomes 2 and 16 and by Tapidor for the QTL on chromosome 9. The QTL on chromosome 9 was co-localised with QTL for pod N and total N concentration at marker 21 and 23, as well as for NUtE on marker 19. The QTL on chromosome 7 was co-localised with many other traits, particularly the ones linked to markers 18 and 22 (TW, SY, NUpE and NUE at High N; and NUtE at Low N).

The 3 QTL in Block 4 were identified on chromosomes 3, 12 and 18 and respective LOD scores were 2.12, 2.07 and 3.10. The 3 QTL jointly explained 26.3% of the phenotypic variation of the population for SN at High N. Additive effects were negative for the QTL on chromosome 4 in Block 1 and positive for all the QTL in Block 4.

Results showed one QTL was detected for seed N concentration (SN) at Low N for Block 1 and 3 for Block 4 by CIM and all were confirmed by MIM (Table 3.40). No QTL were detected by IM for this trait. The QTL from Block 1 was detected on chromosome 4, with a LOD score of 2.04 and accounted for 6.7% of the phenotypic variation of the population for this trait. A QTL for pod N at High N was detected 2cM down the same chromosome for Block 2, and other N related QTL were localised further down the chromosome between markers 4 and 12.

Table 3.40. Putative QTL and related marker position influencing seed N concentration (SN) at Low N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
SN	CIM									
	SN_4_6_R1L07	1	Low	4	6	14.95	-0.16	0.067	2.04	14.9
	SN_3_66_R4L07	4	Low	3	66	125.60	2.48	0.076	2.12	125.6
	SN_12_12_R4L07	4	Low	12	12	61.98	2.30	0.075	2.07	62.0
	SN_12_25_R4L07	4	Low	12	25	123.92	-1.94	0.050	1.56	
	SN_18_12_R4L07	4	Low	18	12	42.62	2.82	0.112	3.10	42.6

Four QTL for STN were identified by IM and confirmed by MIM (Table 3.41) from Block 1 on chromosomes 1 (2), 3 (1) and 18 (1). The respective LOD scores ranged from 2.15 to 3.26 and the 4 QTL

jointly explained 58.5% of the phenotypic variation for STN. The QTL on chromosome 18 explained 18.9% of the phenotypic variation by itself with a LOD score of 2.48. Additive effects were very close to 0 and equally divided between Tapidor and Ningyou7.

Composite Interval Mapping identified more QTL than IM for stem nitrogen, 8 from Block 1 and 4 from Block 4, of which 6 and 3 respectively were also confirmed by MIM. Quantitative trait loci from Block 1 were found on chromosomes 1, 3, 9, 14 and 17 with LOD scores between 2.24 and 3.86, four of which had LOD scores of 3.49 and above. The 6 QTL jointly explained 62.9% of the phenotypic variation of the TNDH population for STN at Low N. All QTL effects were influenced by Tapidor except for the QTL on chromosomes 1 and 14 which were influenced by Ningyou7. All QTL had very low additive values i.e. relatively close to 0.

Four QTL were identified for stem N in Block 4 by CIM but only 3 were confirmed by MIM, one on each of chromosomes 6, 9 and 18. The respective LOD scores were 3.76, 2.40 and 2.50, and the 3 QTL together accounted for 30.6% of the phenotypic variation for STN at Low N. All QTL had positive additive effects (influenced by Tapidor).

Table 3.41. Putative QTL and related marker position influencing stem N concentration (STN) at Low N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
STN	IM									
	STN_1_33_R1L07	1	Low	1	33	62.62	-0.04	0.110	2.39	
	STN_1_36_R1L07	1	Low	1	36	64.92	-0.04	0.137	2.91	64.9
	STN_1_43_R1L07	1	Low	1	43	81.93	-0.04	0.160	3.26	81.9
	STN_3_10_R1L07	1	Low	3	10	33.45	0.04	0.099	2.15	33.5
	STN_18_1_R1L07	1	Low	18	1	3.01	0.05	0.189	2.48	3.0
	CIM									
	STN_1_43_R1L07	1	Low	1	43	82.93	-0.04	0.113	3.49	82.9
	STN_3_9_R1L07	1	Low	3	9	33.28	0.03	0.076	2.49	33.3
	STN_9_52_R1L07	1	Low	9	52	115.17	0.04	0.108	3.50	115.2
	STN_9_54_R1L07	1	Low	9	54	117.85	0.04	0.115	3.86	
	STN_9_55_R1L07	1	Low	9	55	123.48	0.03	0.088	2.24	
	STN_9_56_R1L07	1	Low	9	56	127.38	0.04	0.129	2.27	127.4
	STN_14_1_R1L07	1	Low	14	1	0.01	-0.04	0.116	3.73	0.0
	STN_17_22_R1L07	1	Low	17	22	96.01	0.03	0.087	2.93	96.0
	STN_6_46_R1L07	4	Low	6	46	95.05	0.70	0.136	3.76	95.0
	STN_9_22_R1L07	4	Low	9	22	51.97	0.51	0.083	2.40	52.0
	STN_18_8_R1L07	4	Low	18	8	29.10	0.54	0.087	2.50	29.1
	STN_18_10_R1L07	4	Low	18	10	35.91	0.51	0.073	2.05	

Interval mapping results for chaff N (PN) showed 2 QTL were identified for Block 4 on chromosomes 6 and 9 (Table 3.42). The 2 QTL had LOD scores of 2.04 and 2.10 respectively and together accounted for 24.2% of the phenotypic variation of the TNDH population for PN at Low N. The additive effects were positive showing influence from Tapidor. These same 2 QTL on both chromosomes 6 and 9 had also been identified for stem N at Low N.

Interval mapping results for chaff N (PN) showed 2 QTL were identified for Block 4 on chromosomes 6 and 9. The 2 QTL had LOD scores of 2.04 and 2.10 respectively and together accounted for 24.2% of the phenotypic variation of the TNDH population for PN at Low N. The additive effects were positive showing influence from Tapidor. These same 2 QTL on both chromosomes 6 and 9 had also been identified for stem N at Low N.

Table 3.42. Putative QTL and related marker position influencing chaff N concentration (PN) at Low N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
PN	IM									
	PN_6_15_R4L07	4	Low	6	15	41.84	0.93	0.153	2.04	41.8
	PN_9_21_R4L07	4	Low	9	21	49.74	0.72	0.099	2.10	49.7
	CIM									
	PN_6_34_R1L07	1	Low	6	34	67.53	0.04	0.083	2.34	67.5
	PN_6_39_R1L07	1	Low	6	39	74.39	0.04	0.073	2.05	74.4
	PN_6_47_R1L07	1	Low	6	47	113.48	-0.07	0.173	4.42	113.5
	PN_9_21_R4L07	4	Low	9	21	49.74	0.69	0.086	2.39	49.7
	PN_13_5_R4L07	4	Low	13	5	22.26	-1.43	0.114	3.26	22.3
	PN_19_15_R4L07	4	Low	19	15	69.93	0.70	0.092	2.55	69.9

Six QTL were identified for PN by Composite Interval Mapping, 3 from Block 1 and 3 from Block 3. All QTL from Block 1 were detected on chromosome 6, with LOD scores of 2.34, 2.05 and 4.42. The 3 QTL jointly explained 32.9% of the phenotypic variation of the population.

Three QTL were also identified by CIM from Block 4, on chromosomes 9, 13 and 19. The LOD scores for these QTL were 2.39, 3.26 and 2.55, respectively, and jointly accounted for 29.2% of the phenotypic variation of the TNDH population for PN at Low N. Both QTL on chromosome 9 and 19 had positive additive effects and the one on chromosome 13 had a negative additive effect. The QTL PN_9_21_R4L07 had also been identified for STN on marker 22 instead of 21, about 2cM down chromosome 9.

Results showed only one QTL was detected for total N concentration by CIM from Block 1 (Table 3.43). The QTL was found on chromosome 2 and had a LOD score of 2.44, phenotypic variation was 7.1% for this QTL. The QTL had positive additive effect indicating the origin of the alleles was Tapidor.

Eight QTL were found for TN by CIM in Block 4, but only 5 were confirmed as independent QTL by MIM. The 5 QTL were identified on chromosomes 9, 15, 16 (2) and 17, with LOD scores between 2.03 and 3.12. The 5 QTL explained 46.5% of the phenotypic variation of the population. All additive effects were positive (from Tapidor) except for the QTL on chromosome 16. The QTL identified on chromosome 9 was on the same marker position as a QTL for PN.

Only one QTL for nitrogen uptake efficiency was found by IM, in Block 4. The QTL was detected on chromosome 2 and had a LOD score of 2.05. The QTL explained 7.1% of the phenotypic variation of the population.

Table 3.43. Putative QTL and related marker position influencing total plant N concentration (TN) at Low N in the 2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
TN	CIM									
	TN_2_20_R1L07	1	Low	2	20	68.86	0.20	0.071	2.44	68.9
	TN_9_21_R4L07	4	Low	9	21	50.74	3.96	0.096	2.42	50.7
	TN_9_22_R4L07	4	Low	9	22	52.97	3.69	0.085	2.21	
	TN_9_23_R4L07	4	Low	9	23	55.29	4.07	0.103	2.54	
	TN_15_13_R4L07	4	Low	15	13	79.09	4.20	0.116	3.12	79.1
	TN_15_17_R4L07	4	Low	15	17	82.51	3.92	0.101	2.72	
	TN_16_23_R4L07	4	Low	16	23	67.55	-4.39	0.086	2.37	67.5
	TN_16_26_R4L07	4	Low	16	26	73.54	-4.42	0.089	2.40	73.5
	TN_17_22_R4L07	4	Low	17	22	97.01	-3.69	0.078	2.03	97.0

Ten QTL were found from Block 1 by CIM, however, only 4 were confirmed by MIM on chromosomes 7 and 11. The LOD scores of these QTL ranged from 2.12 to 2.62, and together explained 32.7% of the phenotypic variation of the population. All the QTL presented negative additive effects except for chromosome 11 which had positive additive effects.

One QTL on chromosome 7, marker position 45 was also found for TW at Low N but linked to marker 46 instead. Two more QTL on chromosome 11, at markers 31 and 32 were also found for TW and only one at marker 31 for SY.

FLOWERING

HIGH NITROGEN QTL

Overall results showed a larger number of QTL were detected for flowering using CIM (16), whereas only 5 were detected by IM (Table 3.44). Of these, MIM confirmed 9 QTL from CIM and 4 QTL from IM.

Table 3.44. Putative QTL and related marker position influencing flowering (FDAS) at High N in the2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
FDAS	IM									
	FDAS_18_2_R2H07	2	High	18	2	10.43	2.96	0.096	2.02	10.4
	FDAS_19_16_R2H07	2	High	19	16	72.40	3.16	0.110	2.26	72.4
	FDAS_19_17_R2H07	2	High	19	17	74.39	3.11	0.106	2.09	
	FDAS_19_22_R2H07	2	High	19	22	88.28	3.35	0.122	2.64	88.3
	FDAS_9_56_R3H07	3	High	9	56	128.38	-4.82	0.192	2.49	128.4
	CIM									
	FDAS_1_2_R2H07	2	High	1	2	5.44	-4.56	0.143	4.40	5.4
	FDAS_1_6_R2H07	2	High	1	6	12.81	-4.17	0.115	3.33	12.8
	FDAS_2_23_R2H07	2	High	2	23	75.36	4.06	0.174	2.62	75.4
	FDAS_11_26_R2H07	2	High	11	26	48.41	-3.56	0.131	3.22	48.4
	FDAS_11_27_R2H07	2	High	11	27	53.88	-3.26	0.111	3.37	53.9
	FDAS_11_32_R2H07	2	High	11	32	62.22	-3.10	0.098	3.05	
	FDAS_15_13_R2H07	2	High	15	13	77.09	2.41	0.061	2.23	
	FDAS_15_15_R2H07	2	High	15	15	80.01	2.64	0.072	2.58	
	FDAS_15_17_R2H07	2	High	15	17	81.51	2.52	0.067	2.27	
	FDAS_15_18_R2H07	2	High	15	18	87.07	2.40	0.060	2.03	
	FDAS_19_17_R2H07	2	High	19	17	74.39	3.25	0.115	3.30	74.4
	FDAS_19_19_R2H07	2	High	19	19	80.27	3.33	0.121	3.60	80.3
	FDAS_1_22_R2H07	2	High	19	22	87.28	3.51	0.134	4.15	87.3
	FDAS_19_23_R2H07	2	High	19	23	96.92	3.30	0.116	3.26	
	FDAS_9_56_R3H07	3	High	9	56	129.38	-3.84	0.115	2.56	129.4
	FDAS_9_60_R3H07	3	High	9	60	139.21	-3.16	0.076	2.15	

Three QTL were common to the 3 methods, 2 on chromosome 19 from Block 2 (FDAS_19_17_R207L and FDAS_19_22_R207L) and 1 on chromosome 9 from Block 3 (FDAS_9_56_R307L). No common QTL were detected in Blocks by any of the methods. For flowering, most of the QTL identified were from Block 2.

The 2 QTL on chromosome 19 had LOD scores of 2.09 and 2.64 from IM and 3.30 and 4.15 from CIM respectively. These QTL jointly accounted for 22.8% of the phenotypic variation of the TNDH population when analysed by IM and 24.9% of the phenotypic variation when analysed by CIM. Positive additive effects for these QTL indicated that favourable alleles originated from Tapidor.

The QTL found in Block 3 on chromosome 9 had LOD scores of 2.49 and 2.56 by IM and CIM respectively. Phenotypic variation was 19.2% from IM and 11.5% from CIM, and the additive effect result indicated that favourable alleles originated from Ningyou7.

Low N QTL

At Low N, 8 QTL were identified by IM all from Block 1 and 5 of these were confirmed by MIM (Table 3.45). In all 11 QTL (8 from Block 1, and 3 from Block 4) were detected by CIM.

Table 3.45. Putative QTL and related marker position influencing flowering (FDAS) at Low N in the2006/07 field trial. Interval mapping and CIM were performed using WinQTL Cartographer, with a LOD threshold of 2.0.

Trait	QTL	Block	Treatment	Linkage group	Marker	Position (cM)	Additive effect	R^2	LOD score	MIM position (cM)
FDAS	IM									
	FDAS_3_27_R1L07	1	Low	3	27	45.30	3.33	0.098	2.12	45.3
	FDAS_11_17_R1L07	1	Low	11	17	34.57	-3.24	0.103	2.25	
	FDAS_11_19_R1L07	1	Low	11	19	36.14	-3.29	0.104	2.26	36.1
	FDAS_11_24_R1L07	1	Low	11	24	37.45	-3.19	0.100	2.17	
	FDAS_11_25_R1L07	1	Low	11	25	40.89	-3.22	0.101	2.05	
	FDAS_17_21_R1L07	1	Low	17	21	94.44	3.99	0.141	2.11	94.4
	FDAS_19_16_R1L07	1	Low	19	16	71.40	3.24	0.103	2.25	71.4
	FDAS_19_22_R1L07	1	Low	19	22	92.28	3.19	0.100	2.04	92.3
	CIM									
	FDAS_4_13_R1L07	1	Low	4	13	23.18	3.32	0.070	2.13	23.2
	FDAS_4_16_R1L07	1	Low	4	16	37.23	5.26	0.114	3.56	37.2
	FDAS_4_19_R1L07	1	Low	4	19	50.83	-6.16	0.165	5.01	50.8
	FDAS_4_22_R1L07	1	Low	4	22	56.12	-4.51	0.115	3.33	56.1
	FDAS_11_17_R1L07	1	Low	11	17	34.57	-2.89	0.081	2.64	34.6
	FDAS_11_20_R1L07	1	Low	11	20	36.32	-2.78	0.074	2.41	
	FDAS_11_24_R1L07	1	Low	11	24	37.45	-2.77	0.074	2.41	
	FDAS_11_26_R1L07	1	Low	11	26	45.41	-2.72	0.073	2.14	45.4
	FDAS_10_27_R4L07	4	Low	10	27	55.70	-3.22	0.087	2.27	55.7
	FDAS_10_29_R4L07	4	Low	10	29	63.92	-4.01	0.130	3.26	63.9
	FDAS_10_30_R4L07	4	Low	10	30	65.98	-3.85	0.123	3.03	

Multiple Interval Mapping confirmed 8 of the 11 QTL detected in total. From IM, one QTL was found on chromosomes 3, 11, and 17, and 2 were found on chromosome 19. The LOD scores for these QTL ranged from 2.04 to 2.26. From CIM, 4 QTL were identified on chromosome 4, two on chromosome 11 (from Block 1) and 2 on chromosome 10 (from Block 4) with LOD scores between 2.13 and 5.01.

The 5 QTL from IM accounted for 54.6% of the phenotypic variation and the 8 QTL from CIM for 83.5% of the phenotypic variation of the population for FDAS (61.8% if using the 6 QTL from Block 1 only).

Four QTL identified by IM had positive additive effects i.e. alleles from Tapidor and 1 had a negative effect, whereas of the 6 QTL identified from CIM (Block 1) 4 had negative and 2 had positive additive effects. Two QTL were commonly detected at both High and Low N for flowering: one on chromosome 11 (FDAS_11_26_H07) and the second on chromosome 19 (FDAS_19_22_H07) detected in both Blocks 1 and 2.

QTL SUMMARY FOR 2006/07

Results identified a large number of QTL at High N for the data collected from the 2006/07 field trial. However, a larger number of QTL were identified for Block 2 than for Block 3 by all methods, i.e. by IM 29 and 8 in Blocks 2 and 3 respectively, by CIM 57 and 46 respectively, and by both IM and CIM 17 and 8 in Block 2 and 3 respectively.

The method that detected the most QTL was CIM both in Block 2 and Block 3. Nitrogen harvest index had a large number of QTL, with a total of 11 in Block 3 identified by CIM. Finally, the traits which had a highest number of QTL detected by both methods were TW and NUpE, both with 3 QTL detected by both IM and CIM in Block 2 and 1 QTL detected by both methods in Block 3. However, TW was the trait that was explained by the highest proportion of the phenotypic variation of the population at 73.2%.

Despite the same treatment and the same plant material being used in Blocks 2 and 3, no QTL were co-localised in both blocks.

Results at Low N showed a higher number of QTL detected in Block 1 compared to Block 4, particularly for IM. Results for CIM showed a higher number of QTL detected than by IM in both blocks (45 in Block 1 and 44 in Block 4). A higher number of QTL detected by both IM and CIM was found in Block 1 (9), compared to a total of 5 QTL in Block 4. Like at High N, a higher number of QTL was detected by CIM than by IM, both in Block 1 and 4. Nitrogen use efficiency was the trait with a higher number of QTL identified in both blocks with a total of 10; four of these were in Block 1 and 6 in Block 4.

Table 3.46. Summary of major QTL identified at High N in 2006/07 field trial. QTL in the same line were co-localised in the same marker. Traits' acronyms are: FDAS (flowering days after sowing), TW (total above ground plant biomass), SY (seed yield), HI (harvest index), SN (seed N), STN (stem N), PN (chaff N), TN (total N), NUpE (N uptake efficiency), NUtE (N utilisation efficiency), NUE (N use efficiency), NHI (N harvest index).

CHROMOSOME	Marker	Trait (LOD score)				
1	2	FDAS (4.40)				
	17	SY (3.75)	TW (4.23)			
	42	NHI (2.07)				
2	1	STN (2.81)				
3	65	NUTE (3.40)	PN (2.09)	NHI (3.62)		
4	4	PN (3.62)	TN (4.53)	NUPE (2.07)	SY (2.25)	TN (3.87)
	9	NUPE (2.37)	NUE (2.16)	SN (2.43)	TN (2.06)	
5	18	SY (3.21)				
	28	SY (2.70)				
6	38	NHI (2.15)	NUTE (2.51)			
7	22	HI (2.73)	SY (8.48)	TW (7.71)	NUE (5.49)	NUPE (5.75)
	31	PN (2.38)	STN (2.63)	TN (2.04)		
	46	NUTE (2.96)				
8	1	SY (2.80)	HI (3.26)	SN (3.99)	NUE (2.30)	
	14	PN (2.97)				
9	1	NHI (3.11)	PN (4.19)	STN (2.19)	NUTE (4.40)	TN (3.84)
	15	STN (2.11)				
	52	STN (3.14)	SN (2.30)	TW (2.34)	NUPE (2.49)	FDAS (2.56)
10	3	HI (2.68)				
	10	HI (5.06)				
11	1	NHI (2.78)	NUE (2.29)	SN (2.68)		
	12	TN (2.39)				
	27	NUPE (2.39)	FDAS (3.37)			
12	18	NHI (3.05)				
13	33	PN (2.39)	STN (4.10)	NUPE (2.50)		
14	3	SY(2.28)				
	35	PN (2.16)	STN (3.16)	SN (2.70)	TN (4.67)	
15	18	NUPE (2.49)				
16	3	HI (2.24)				
	30	SY (3.13)				
17	1	NUE (2.84)	NUPE (3.04)	SY (2.12)		
19	9	STN (3.16)				
	22	FDAS (4.15)	NUPE (2.98)			

Total above ground plant biomass (TW) was the trait with the highest number of QTL commonly identified by both IM and CIM with a total of 3, one in Block 1 and 2 in Block 4. At 37.1% the QTL for NUtE explained the highest proportion of the phenotypic variation of the population at Low N.

As previously found for High N, no QTL were commonly identified in Blocks 1 and 4, even though N treatment and plant material were the same.

Table 3.47. Summary of major QTL identified at Low N in 2006/07 field trial. QTL in the same line were co-localised in the same marker. Traits' acronyms are: FDAS (flowering days after sowing), TW (total above ground plant biomass), SY (seed yield), HI (harvest index), SN (seed N), STN (stem N), PN (chaff N), TN (total N), NUpE (N uptake efficiency), NUtE (N utilisation efficiency), NUE (N use efficiency), NHI (N harvest index).

CHROMOSOME	Marker	Trait	LOD score				
1	36	STN (2.91)					
1	43	STN (3.49)					
2	10	NHI (2.12)	NUTE (2.05)				
2	16	HI (4.65)					
2	22	HI (2.28)	NUTE (2.59)	SY (2.66)	TN (2.44)		
3	9	STN (2.49)					
3	64	HI (3.06)	SN (2.12)				
4	10-22	NUPE (2.02)	SN (V)	SY (2.61)	TW (3.62)	FDAS (5.01)	
6	15	PN (2.04)					
6	34	PN (2.34)					
6	46	STN (3.76)	PN (4.42)				
7	20	NHI (5.38)	NUTE (6.30)	HI (2.74)			
7	34	NHI (3.27)	NUTE (2.82)				
7	45	NUPE (2.62)	TW (3.23)				
9	8	NUE (5.10)	SY (5.41)				
9	21	PN (2.39)	STN (2.40)	TN (2.54)	TW (2.11)	NUTE (3.31)	NHI (3.43)
9	39	SY (2.33)	TW (2.62)				
9	54	STN (3.86)					
10	29	FDAS (3.26)	NUE (2.50)				
11	18	NUPE (2.23)	FDAS (2.64)				
11	24	NUPE (2.18)	FDAS (2.41)				
11	31	SY (2.26)	TW (3.35)	NUPE (3.15)	NUE (2.02)		
13	5	PN (3.26)					
14	1-26	HI (5.85)	TW (2.73)	STN (3.73)	NUPE (2.55)	NUE (2.38)	
15	13	TN (3.12)	NUPE (3.53)				
16	19	SY (2.54)	NUE (2.56)				
16	25	NHI (3.16)	TN (2.40)				
17	7	HI (2.99)					
17	22	STN (2.93)	TN (2.03)	FDAS (2.11)			
18	1	STN (2.48)	SY (4.53)				
18	12	SN (SN)	STN (2.50)				
18	18	TW (2.20)	HI (3.45)				
19	7-15	NUE (3.12)	PN (2.55)	FDAS (2.25)			

When comparing High and Low N treatments, a higher number of QTL were detected at High N and these were particularly associated with Block 2 and overall, the phenotypic variation was higher at High N.

COMPARISON OF QTL POSITIONS FOR THE DIFFERENT TRAITS ANALYSED IN 2006/07

Results showed more QTL identified at High N than at Low N on chromosome 1 organised in 3 delimited regions (Fig. 3.11). Two QTL for flowering (FDAS) were identified one after the other at the beginning of the chromosome, at High N. At High N, 4 QTL were identified, 2 for total above ground plant biomass (TW) and 2 for seed yield (SY) both on the same exact location on the chromosome, one after the other. At the bottom of chromosome 1, a QTL was detected for nitrogen harvest index (NHI) at High N, from 67.7 to 78.9 cM. On the same region at Low N, 2 QTL were identified for stem N concentration (STN) the 2 together occupied an area from 60.0 to 85.5 cM.

On chromosome 2, more QTL were found at Low N than at High N (Fig. 3.11). A QTL for STN was localised right at the top of the chromosome, at High N. Also at High N, 2 QTL were detected close to the end of the chromosome, one for FDAS (which was partially co-localised with a QTL for total N concentration (TN) at Low N) and a QTL for chaff N (PN) which was partially co-localised with QTL for SY and NUtE at Low N. An additional QTL for NUtE was also found at Low N, 46 cM above the previously mentioned. Just above which a QTL, for NHI was identified at Low N.

On chromosome 3 (Fig. 3.12), a QTL for STN was identified on the upper region of the chromosome at Low N, and further down on the central region, a QTL for FDAS was also identified at Low N. Close to the bottom of chromosome 3, three QTL for NHI were identified one after the other and all at High N. Immediately after these, a region with 4 QTL, 2 at High N and 2 at Low N was detected. At High N, a QTL for NUtE partially overlapped with a QTL for PN. At Low N a QTL for HI was found on the same exact location as the QTL for NUtE while a QTL for SN was on the same exact location as the QTL for PN.

Quantitative trait loci on chromosome 4 were detected only in the upper and middle regions of the chromosome from 0 to 64 cM both at High and Low N (Fig. 3.12). On the uppermost region (0 to 16 cM) QTL for SY, PN, TN and NUpE were identified overlapping at High N and on the same region at Low N a QTL for SN was found. Between 18.4 and 19.1 cM, QTL for NUpE, NUE, TN all overlapped with the SY QTL mentioned previously at High N, whereas on the same area at Low N QTL for TW, SY and FDAS were detected. A QTL for SN was found at the same exact location as a QTL for TN (from 23.2 to 37.2 cM),

partially overlapping with QTL for NUpE and NUE at High N and with 2 QTL for FDAS at Low N. Additional QTL identified at Low N were for NUpE, SY and 2 for FDAS located between 39.9 and 63.6 cM.

Only one QTL was detected on chromosome 5 (Fig. 3.12) for SY at High N, from 37.0 to 38.2 cM.

Quantitative trait loci for PN, STN, TN, NUtE and NHI were widespread on chromosome 6 (Fig. 3.13) at both High and Low N. At High N, 3 QTL were identified: one for TN at the top of chromosome 6 and 1 for NHI and 2 for NUtE between 69.6 and 82.0 cM. Four QTL for PN were identified at Low N, covering different regions of chromosome 6. One of these QTL localised from 74.4 to 76.7 cM was partially co-localised with a QTL for NUtE identified at High N. Another QTL for PN from 95 to 117.7 cM also partially overlapped with a QTL for STN at Low N.

The QTL identified on chromosome 7 were contained in 2 delimited regions (Fig. 3.13). The first region with the major number of QTL clustered covered from 27.6 cM to 79.6, with a sub-region from 48 to 51.1 cM with the highest concentration of QTL both at High and Low N. Quantitative trait loci were organised in 2 groups: QTL for TW, SY, NUpE at High N and QTL for HI, NUtE and NHI were found in the same region at Low N. Quantitative trait loci for STN, PN and TN were detected on the same exact location, partially overlapping with QTL for NUpE and NUE. Two more QTL for NUtE and NHI were found at the same exact location from 88.5 to 89.3 cM at Low N and right at the bottom of the chromosome QTL for NUpE and NUtE were co-located with a smaller QTL for HI.

On chromosome 8 (Fig. 3.13) QTL for 4 traits i.e. SY, HI, SN and NUE were clustered at the beginning of the chromosome, from 0 to 22.3 cM at High N. A second QTL for SN was also identified immediately below this cluster also at High N. Two QTL for PN were detected 1 around the central region and the other one close to the bottom end, both at High N. No QTL were detected in 2006/07 on chromosome 8 at Low N.

Many QTL were detected on chromosome 9, both at High and Low N (Fig. 3.14). Quantitative trait loci for 5 traits (STN, PN, TN NUtE and NHI) were identified on the same location at the beginning of the chromosome (0 to 17.4 cM), at High N. At the same location QTL for SY and NUE were detected at Low N. Two additional QTL were identified for both SY and NUE (23.7 to 29.9 cM) at Low N and a QTL for STN was localised at 33.0 – 37.5 cM at High N. Another QTL cluster for the same traits (STN, PN, TN, NUtE and NUE) was found between 40.3 and 60.3 cM but at Low N. Quantitative trait loci for TW and SY were found at the same position on the central region of the chromosome at Low N. At the bottom of chromosome 9 (between 106 and 120.5 cM) another cluster of QTL were identified for traits such as SN, STN, TN and NUE, at High N, and two QTL for STN at Low N. Immediately after that, a QTL for FDAS was detected also at High N which coincided with one of the QTL for STN at Low N.

Five QTL for 3 different traits were identified on chromosome 10 (Fig. 3.14). Two QTL for HI were detected one after the other at the top end of the chromosome, at High N. At the bottom end of the chromosome, 2 QTL for FDAS were found one after the other, this time at Low N. A QTL for NUE also at Low N was detected overlapping with both of these FDAS QTL.

Almost all areas of chromosome 11 were covered by different QTL, either at High N, Low N or both at the same time (Fig. 3.14). On the upper end of the chromosome, QTL for SN, NUE and NHI were found on the same location at High N. Following down the chromosome at High N, a QTL for TN was localised and right after the end of that one QTL for FDAS and NUpE were present at Low N. Close to the end of these QTL, again QTL for FDAS and NUpE were identified but in the High N treatment. Finally, QTL for TW, SY and NUpE were identified on the same location at Low N, at the end of chromosome 11.

Two QTL for SN were identified on chromosome 12, one starting where the other one ended, at High N and Low N respectively (Fig. 3.14). Close to the end of the lower SN QTL, 2 QTL for NHI were identified one after the other, at High N.

Three QTL at High N and one at Low N were detected on chromosome 13 (Fig. 3.15). Starting at 99.8 cM QTL for NUpE, PN and STN were identified one after the other at High N. A QTL for TN was present close to the top of chromosome 13 at Low N.

Right at the top of chromosome 14 (0 to 9.6 cM), QTL for SY and NHI at High N and for STN at Low N were found (Fig. 3.15). Quantitative trait loci for NUpE and NUE were detected on the same location between 25.4 and 40 cM at Low N, and a second QTL for NUE was found right after these also at Low N. At the lower end of chromosome 14, QTL for SN, STN, PN and TN were detected at High N.

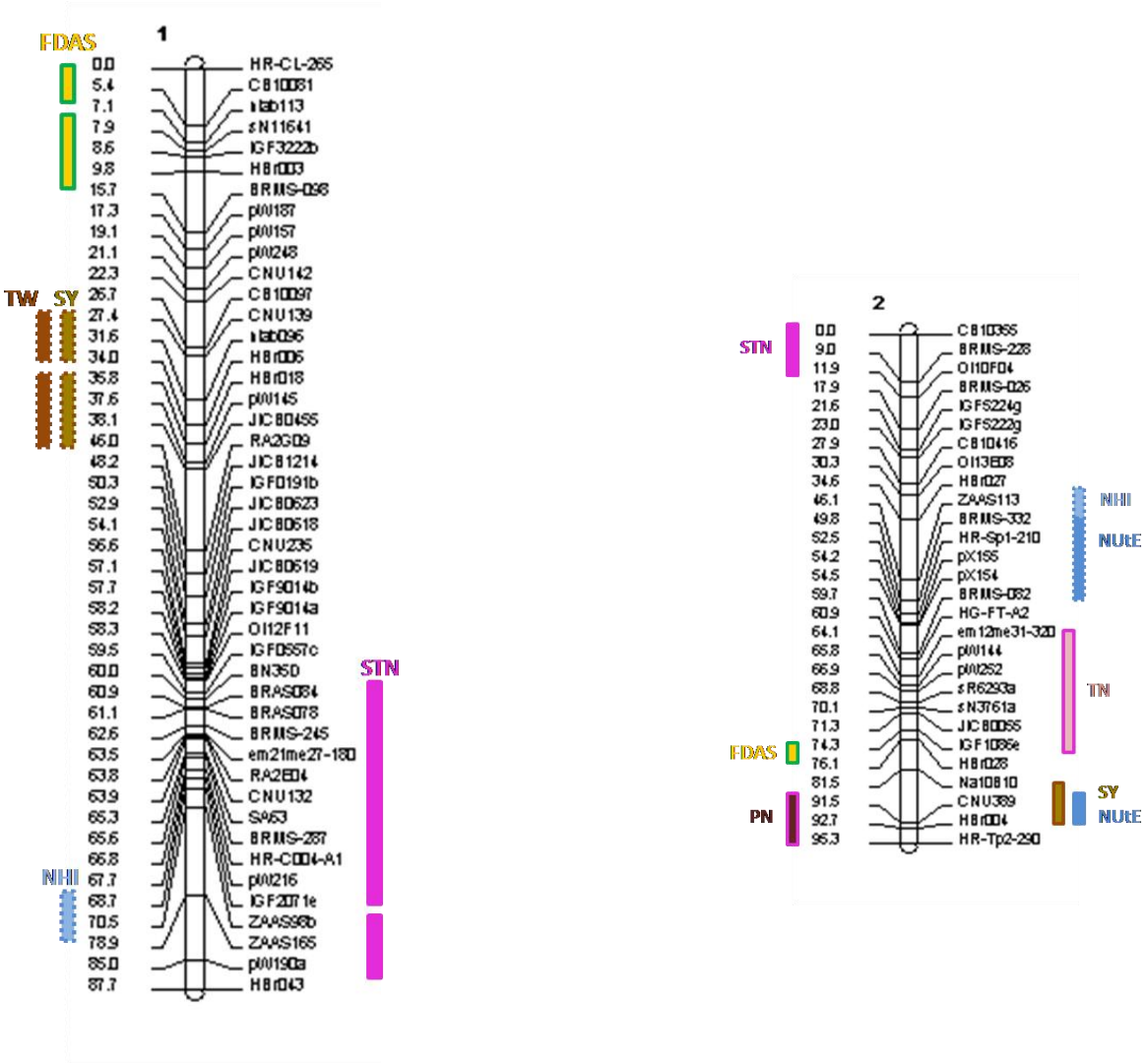
Three QTL for NUpE were found on chromosome 15, 1 at High N and 2 at Low N (Fig.3.15). The QTL at High N covered from 77.1 to 79.7 cM and the 2 at Low N were localised one after the other from 76.3 cM to the end of the chromosome. Two more QTL were found on chromosome 15, one for NUtE from 14.8 to 21.4 cM and one for TN overlapping with the QTL for NUpE, all at Low N.

Only one QTL was found at High N on chromosome 16, for SY, from 79.1 to 85.1 cM (Fig. 3.15). Another QTL for SY was found at Low N, coinciding with a QTL for NUE from 43.8 to 59 cM. Further down the chromosome, close to the end of these 2 QTL were QTL for TN and NHI which were co-localised.

Quantitative trait loci on chromosome 17 were found at the upper end of the chromosome at High N and at the lowermost region at Low N (Fig. 3.16). The 3 QTL found at High N were for NUpE and NUE (on the same location) and SY which covered a larger area. The QTL found at Low N were for FDAS, STN and TN, with different degrees of overlapping between them.

One QTL for FDAS was found at High N, right at the top of chromosome 18 (Fig. 3.1516). On the same location at Low N, QTL for SY and STN were also found. Another QTL for STN was detected further down also at Low N, from 20.6 to 39.7 cM and another QTL for SY was also detected from 48 to 54.3 cM. In between these 2 QTL, a QTL for SN was detected, all at Low N.

Many QTL for different traits were detected on chromosome 19, covering almost all regions of the chromosome (Fig. 3.16). At the top of the chromosome a QTL for SN was found at High N. Further down the chromosome, between 48.8 and 59.1 cM, QTL for STN and NUtE were co-localised at High N with a QTL for NUE at Low N. A second QTL for NUE at Low N was found starting just after the first one and co-localised with a QTL for PN (60 to 71.4 cM), both overlapped with QTL for FDAS (68.9 to 80.3 cM at High N and 71.4 – 79.4 cM at Low N). The QTL for FDAS identified at the bottom of chromosome 19 was co-localised with QTL for FDAS and NUpE identified at High N. Two more QTL for FDAS were detected at Low N (between 80.3 and 70.5 cM), one after the other. An area around 71.4 cM contained QTL for FDAS and NUpE at High N and for FDAS, PN and NUE at Low N.



Flowering

Total plant biomass
Seed yield
Harvest index

Seed N concentration
Stem N concentration
Chaff N concentration
Total N concentration

Nitrogen uptake efficiency
Nitrogen utilisation efficiency
Nitrogen use efficiency
Nitrogen harvest index

QTL from Blocks 2 (left of the chromosome) and 1 (right)

QTL from Blocks 3 (left of the chromosome) and 4 (right)

Figure 3.7. Quantitative trait loci positions for the traits analysed in 2006/07 on chromosomes 1 and 2. The QTL on the left are for High N (Blocks 2 with continuous border and Block 3 dashed border) and on the right for Low N (Block 1 continuous border and Block 4 with dashed border).

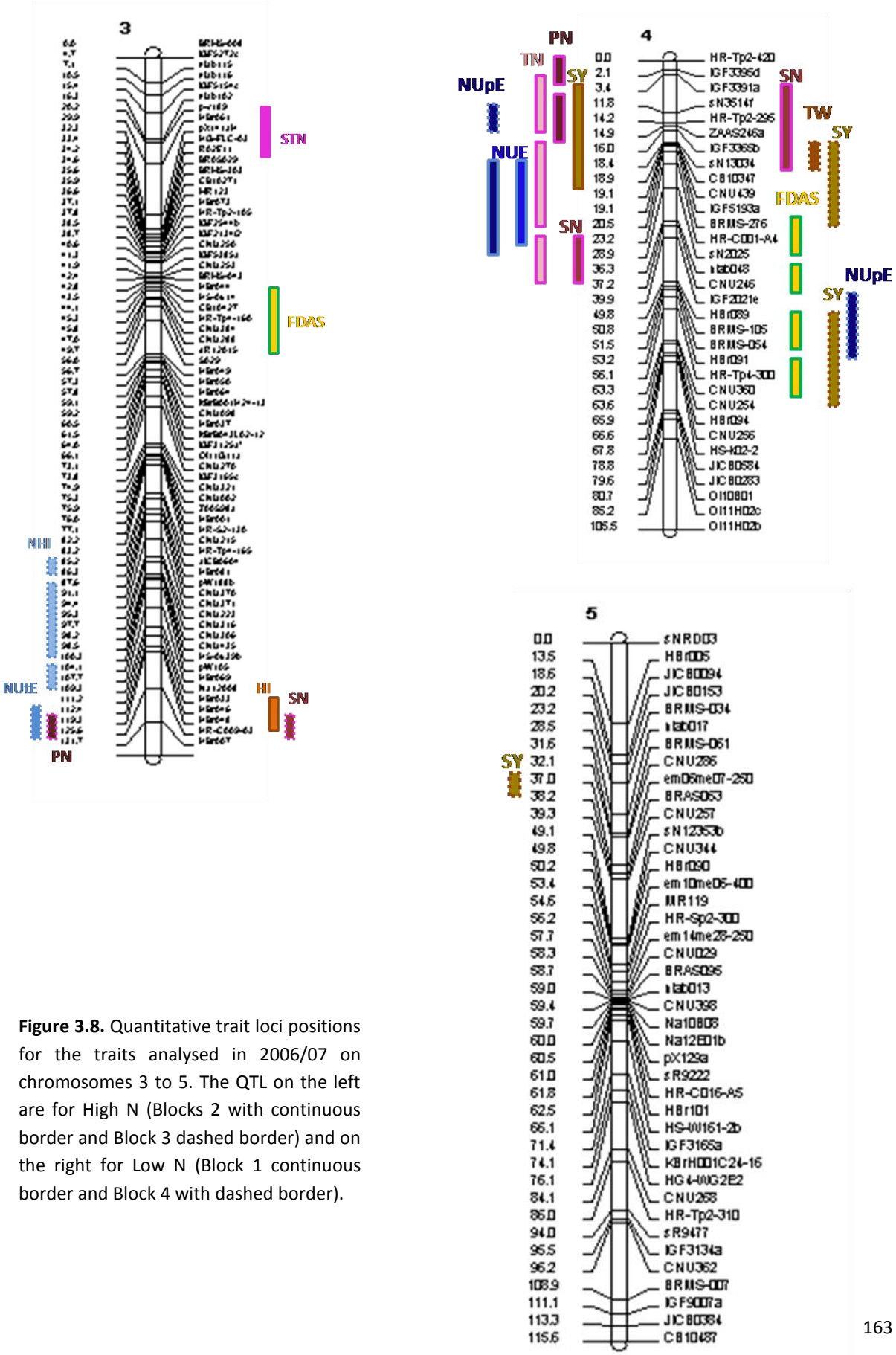


Figure 3.8. Quantitative trait loci positions for the traits analysed in 2006/07 on chromosomes 3 to 5. The QTL on the left are for High N (Blocks 2 with continuous border and Block 3 dashed border) and on the right for Low N (Block 1 continuous border and Block 4 with dashed border).

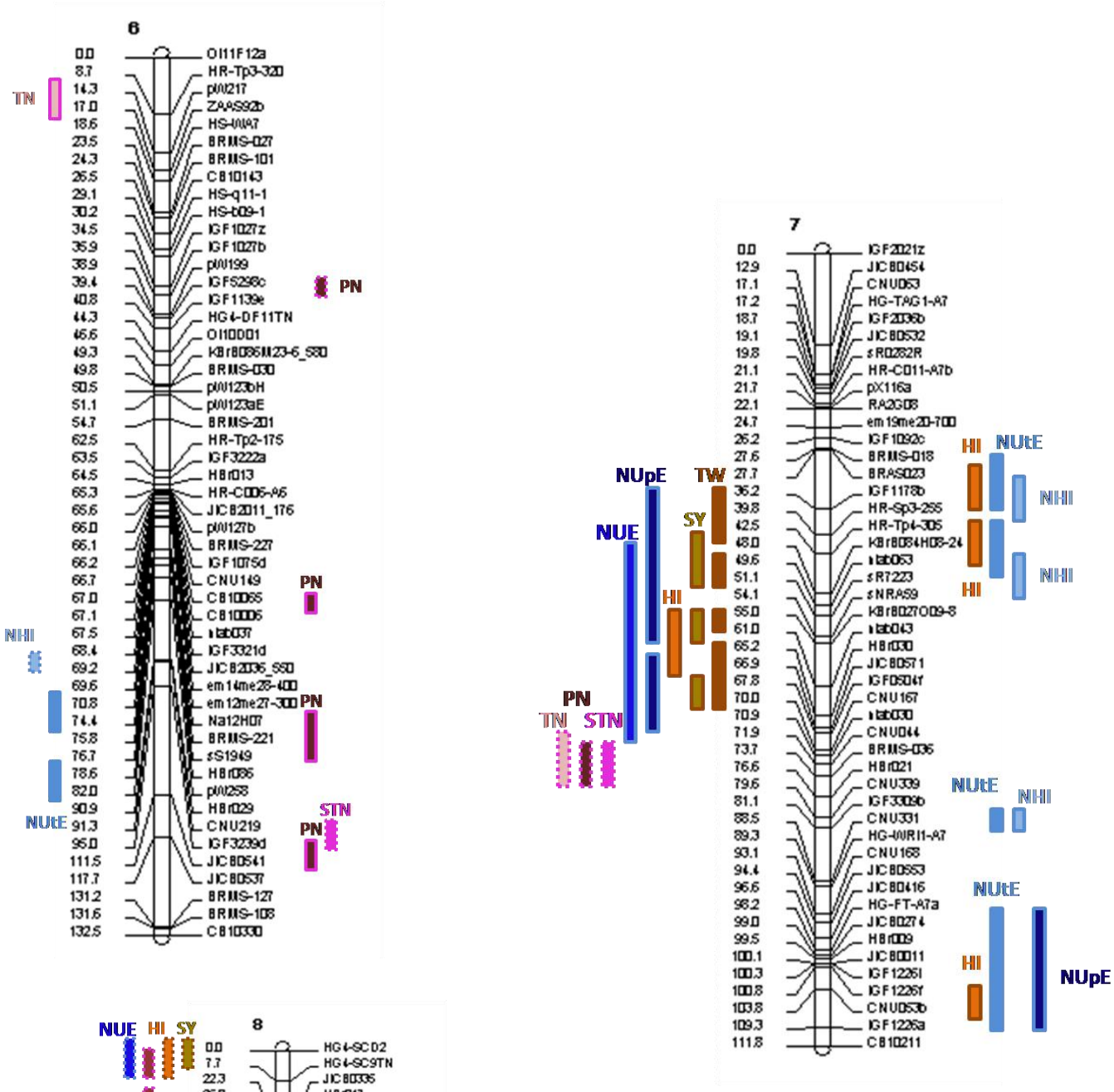


Figure 3.9. Quantitative trait loci positions for the traits analysed in 2006/07 on chromosomes 6 to 8. The QTL on the left are for High N (Blocks 2 with continuous border and Block 3 dashed border) and on the right for Low N (Block 1 continuous border and Block 4 with dashed border).

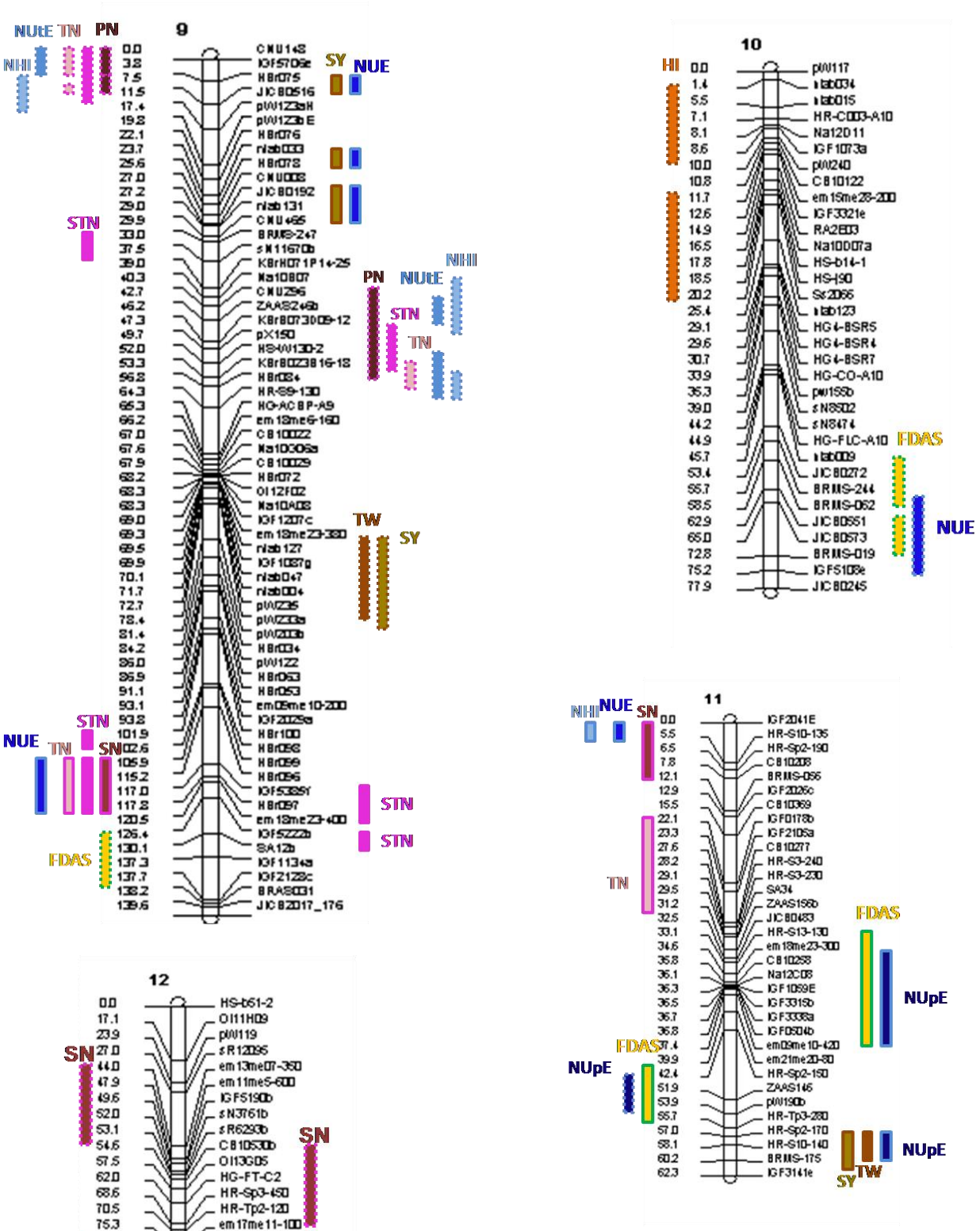


Figure 3.10. Quantitative trait loci positions for the traits analysed in 2006/07 on chromosomes 9 to 12. The QTL on the left are for High N (Blocks 2 with continuous border and Block 3 dashed border) and on the right for Low N (Block 1 continuous border and Block 4 with dashed border).

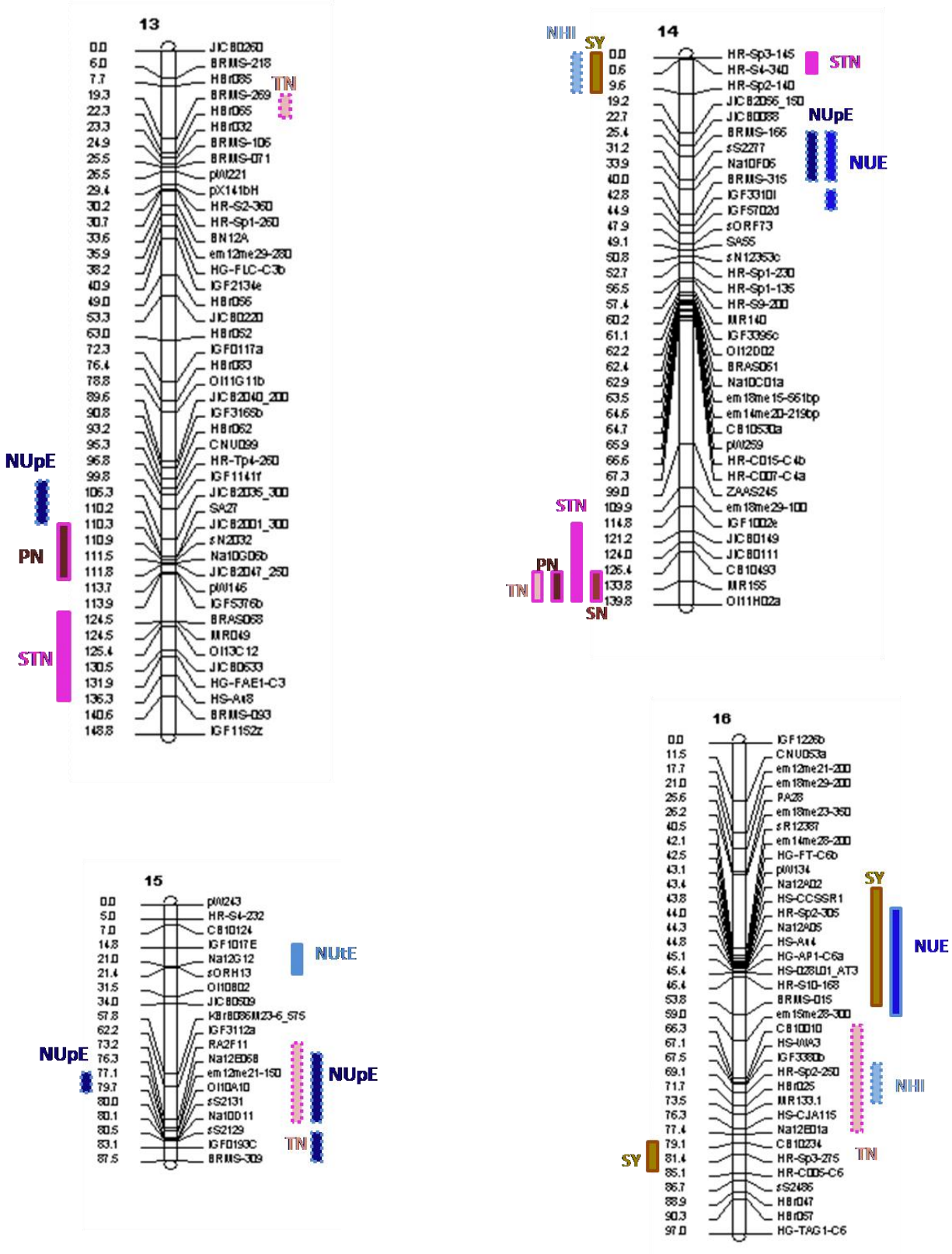


Figure 3.11. Quantitative trait loci positions for the traits analysed in 2006/07 on chromosomes 13 to 16. The QTL on the left are for High N (Blocks 2 with continuous border and Block 3 dashed border) and on the right for Low N (Block 1 continuous border and Block 4 with dashed border).

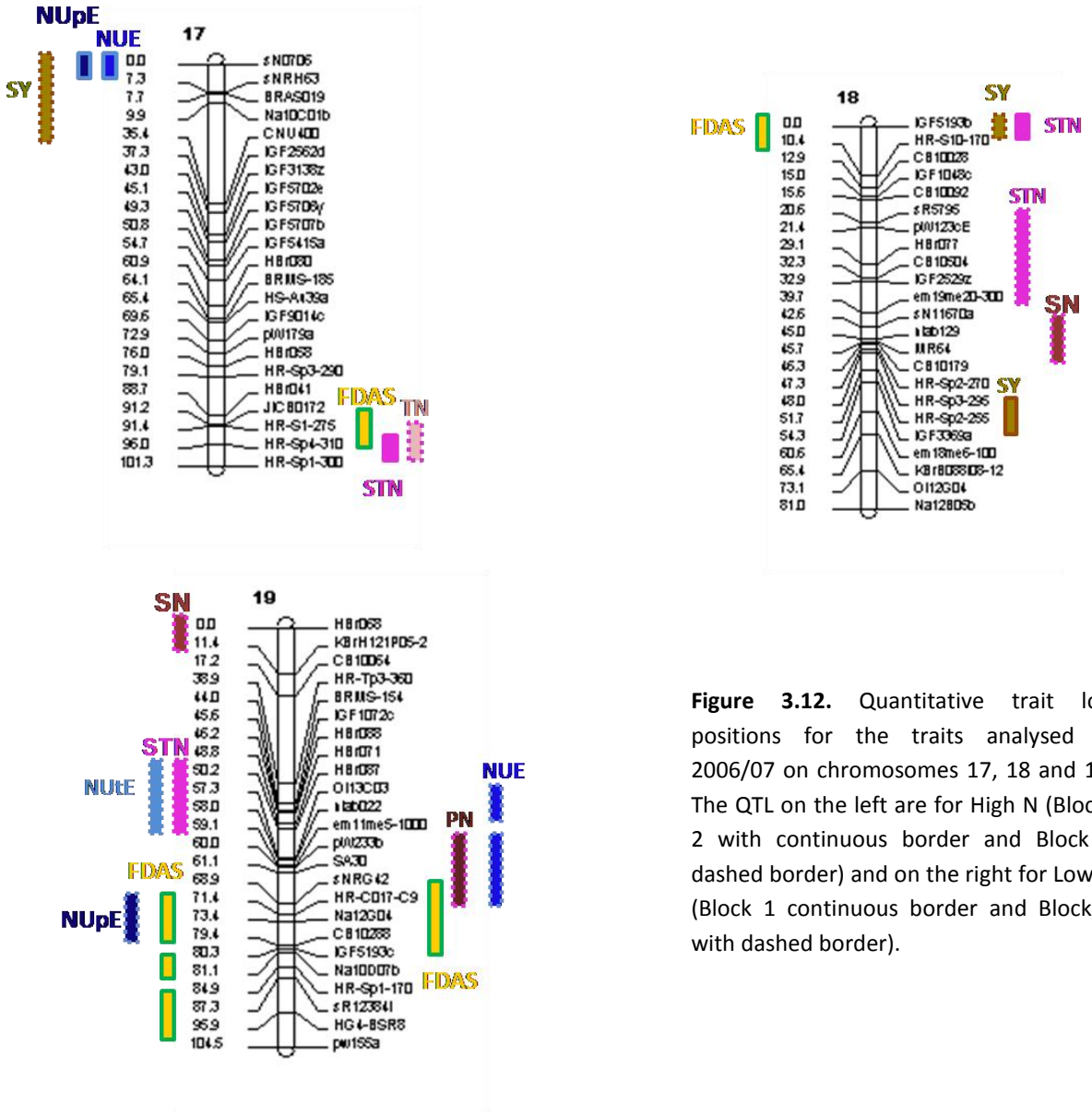


Figure 3.12. Quantitative trait loci positions for the traits analysed in 2006/07 on chromosomes 17, 18 and 19. The QTL on the left are for High N (Blocks 2 with continuous border and Block 3 dashed border) and on the right for Low N (Block 1 continuous border and Block 4 with dashed border).

QTL x Environment interactions

The QTL x Environment analysis was performed by CIM using the Jzmapqtl module from WinQTL Cartographer to assess the significance of the QTL across High and Low N treatments in 2006/07. For this analysis Blocks were paired randomly High N/ Low N, hence Block 1 (Low) paired with Block 2 (High) and Block 4 (Low) with Block 3 (High). Following the same criterion as for 2005/06, QTL with LOD scores above 1.8 were considered significant and the resulting LOD scores for High N, Low N and joint LOD score were assessed (Appendix 22). Quantitative trait loci were classified as having environmental

effects according to the significance of the LOD scores: those QTL with LOD scores higher than 1.8 at High N, Low N and joint; then QTL with LOD score above 1.8 at either High or Low N and for the joint LOD score and finally, QTL with a significant LOD score for the joint analysis only. As in 2005/06, in the second field trial most of QTL identified for all traits presented significant QTL x N interactions, either with High or Low N. Again, all traits presented QTL with significant LOD scores for the joint analysis only. Only 1 QTL was identified for both SN and NUpE: QTL SN_3_66_07 and NUPE_3_65_07. A total of 19 QTL were commonly identified in analysis of Blocks 1-2 and Blocks 3-4. The QTL were identified for FDAS, HI, TN, NUtE and NHI with 1 QTL each trait, 2 QTL for PN, 3 QTL for STN, 4 for NUE and 5 common QTL for NUpE. These QTL that were identified in both pairs of Blocks would sometimes have a different environment interaction, for example it would be a QTL detected at High N in analysis of Blocks 1-2 and the same QTL would have an interaction at Low N in the analysis of Blocks 3-4. The 3 remaining traits i.e. TW, SY and SN did not identify common QTL within the 2 sets of Blocks.

Table 3.48. Summary of common QTL identified both in 2005/06 and 2006/07 at High and Low N treatments. Traits in RED are QTL detected in 2005/06 and in BLUE QTL detected in 2006/07.

Chromosome number	Marker number	Traits at High N	Traits at Low N
1	36		TW, STN
	38		SN, TN, NUpE, NHI
	40	NHI	NUE
	42		
	43		STN
2	20	CB	TN
	22		HI
	23	CB, CL, FDAS	
	24	CB	
	26	PN	NUtE
3			
4	2	PN, TSW	
	3	TSW	NUpE
	4	OIL, NT, PN	TSW
	5		NUpE
	6	PN	SN
	7	SY, NT, TSW	
5	13		NUtE
	15	SY	
6	24	FL	SY, HI
	33		SY, HI
	34	FL	PN
	35		SY, HI
	38	FL, NUtE, NHI	SY
	39	CB	PN
7	20	SN, SY	HI, NHI, NUtE
	21	SN	HI, SN, NUtE
		TW, HI, SY, NUpE,	
	22	NUE	SN, TN, NUtE
	25	Npl	HI
	26	SY, NUpE, NUE	
	30		HI
	31	Npl, STN, PN, TN	
	35	NUE	NUtE
	37	Npl, NUE, NHI	
			TW, HI, SY, SNP, TSW, NUpE, NUE, NHI, TW, NUpE,
	46	Npl, NUtE	NUE
8			
		STN, PN, TN, NUtE,	
9	3	NHI	FDAS, SY, NUE
	39		TW, SY
	41	HI	
	51	NUpE, NUE	
	52	SN, STN, TN	BN, HI, NHI, STN
	54	STN, NUpE	
	55	TSW	
			FL, BN, HI, NHI,
	56	FL, HI, FDAS	STN
	57	CB	

Chromosome number	Marker number	Traits at High N	Traits at Low N
10			
11			
12	22	NHI	
	24	NUtE	
	25	TSW	SN
13	1	FDAS	CL
	5		PN
	37	HI, SNP	
	39	SNP, STN	
	41	HI, STN	
	42	HI, SNP	
14			
15	10	BN	
	11	NUpE	
	13		TN, NUpE
	15	FDAS	
	17		TN
	18	NUpE	NUpE
16	19	FL	SY, NUE
	20		CL
	22		CL
	23		TN
	24		
	25		CL, NHI
	26		TN
	28	SY	CL
17	1	NUpE, NUE	
	3	CB, SY	
	4	TL	CB
	5	HI	
	6	NHI	
	7	NUtE	HI
		SN, NT, NUtE,	
	8	NHI	
	10	NHI	
	14	NUtE	
18	10		STN
	11		SN, TN
	12		SN
	18		TW
	20	SNP	
19	1	SN	
	2	FL	
	3	TW	
	5	SY	
	7		NUE
	8	SY	
	9	TW, NUE, STN	
	10	TW, SY, NUtE	NHI
	11		NUE
	14	SY	NHI
	15		PN, NUE
	16		FDAS
	17	FDAS, PN, NUpE	

3.4. DISCUSSION

Quantitative trait loci reproducibility and stability (the occurrence of QTL for the same trait at the same location) are key factors when analysing QTL. Most QTL studies analysing different crops are designed for many years at the same locations or in few years (i.e. 2 or more) in many locations, to achieve some QTL reproducibility. Many studies have found that about half of QTL appeared repeatedly in different locations, treatments or years, and most studies found only few QTL were specific to a particular stress in wheat, (Quarrie et al., 2005), maize (Messmer et al., 2009) and rice (Wan et al., 2006).

In the present study stability of the QTL detected was low for all traits, within High N blocks (2 and 3) or Low N blocks (1 and 4) analysed in 2007, despite the same treatment and the same plant material being used and a high number of QTL detected for many of the traits. However, reproducibility was found when 2005/06 data was evaluated together with data from 2006/7. Quantitative trait loci were identified in the same location for both field trials at both High and Low N for 4 different traits i.e. HI, Plant N concentration, NUpE and NHI; but not for SY. At High N, a QTL for plant N concentration co-localised with QTL for stem N and chaff N concentration (the trait was split as in the 2 components in 2007). For HI, 2 QTL on chromosome 7 at different intervals were found, both in 2005/06 and 2006/07 at Low N. The same happened with a QTL for NHI on chromosome 7 and one more for NUpE on chromosome 7 (which in turn was at the same location as the previously mentioned QTL for HI) both at Low N. These results indicate that despite a strong influence of the environment on QTL it was still possible to obtain QTL which were consistent across years. Moreover, clusters of QTL for the same traits were repeatedly detected in different years, even though they occurred in different locations and even on different chromosomes.

Considering an estimate by Long et al. (2007) that in 15 cM of the oilseed rape genome there will be approximately 1800 genes and believing some of these could respond differently to different environments, QTL mapped from different environments at overlapping intervals were referred as two different QTL.

However, little similarity of QTL locations for the two blocks within N treatments was found in 2006/07. One possible explanation is that many QTL were multi-peak instead of one single peak and comprised broad distances of 15 cM or sometimes more. These QTL were considered as one when 1 peak only was above the LOD 2 threshold. If 2 or more peaks were above the threshold and at least 5cM apart, it was considered as more than one QTL. It is then when MIM was used to decide if all peaks belonged to real QTL. Because of the multi peak nature of the QTL it could be that some QTL were moved up or down the chromosome, thus considered as different QTL altogether. A larger number of QTL were detected by

Single Marker Analysis than either IM or CIM. Occasional QTL coincidences detected by SMA were later not found by IM and/or CIM. For example, a QTL for seed N was detected on chromosome 8 by SMA in Blocks 2 and 3, but it was only detected in Block 3 by CIM. When looking at CIM results from Block 2, the QTL on chromosome 8 had a LOD score of 0.94 and 1.18 by IM, well under the threshold level and was not considered as a QTL.

In any case, QTL for different traits shared the same peaks at the same confidence intervals within the same block.

3.4.1. Comparison of QTL in 2005/06 and 2006/07

Different QTL numbers and also confidence intervals were detected in 2005/06 and 2006/07. Only a relatively small portion of all the QTL detected in the first field trial was reproduced in the second field trial, mostly because of environmental variability and differences in plant density. A small number of QTL were identified for most of the traits as predicted by Gilliland et al. (2006), finding that rarely more than 10 QTL are detected in one experiment, despite the traits analysed being regulated by multiple genes. However, 10 or more QTL were detected for FL, HI, NUpE and NHI by either IM or CIM in 2005/06 and for STN, PN, NUpE and NHI in 2006/07.

Yield and yield related traits

Total above ground plant biomass (TW)

Nine QTL on chromosomes 1, 7 and 19 were identified for TW in 2005/06, equally distributed between High and Low N. In the second field trial, 9 QTL were identified, 3 QTL on chromosomes 1 and 7 were detected at High N and 6 at Low N (chromosomes 4, 7, 9, 11, 14, 18).

Harvest index (HI)

Quantitative trait loci for HI were identified on 8 different chromosomes i.e. 1, 6, 7, 8, 9, 10, 13 and 17 and 12 different chromosomal regions, distributed at High and Low N and in 2005/06 and 2006/07. A region on chromosome 7 (from 42.5 to 67.8 cM) was of particular interest in terms of QTL reproducibility as QTL for HI at both High and Low N were identified at semi-overlapping confidence intervals in both field trials. In addition the QTL at High and Low N were also partially overlapping. In a lower region of chromosome 7 (100.8-103.8 cM), QTL for HI were detected at the same confidence interval at High N, both in 2005/06 and 2006/07. A previous study from a DH population from a cross between Express and the re-synthesised variety R53, detected QTL for HI on chromosomes 5, 6, 10, 12 13, 17 and 19 (Radoev et al., 2008). Two of the QTL identified in Radoev et al. (2008), on chromosomes

13 and chromosome 17 were identified within the same confidence intervals as HI_13_41_H06 and HI_17_5_H06 respectively, potentially narrowing the region of these QTL and increasing the capability for identifying candidate genes for this trait on both chromosomes 13 and 17.

SEED YIELD (SY)

Twenty-five QTL for SY were identified at both High and Low N, distributed on 14 chromosomes in both field trials. None of the QTL identified in either year or treatment was reproduced at the same interval. Only 4 QTL for SY were identified in 2005/06 (1 at High N and 3 at Low N) with the remainder detected in 2006/07 and almost equally distributed between High (10 QTL) and Low N treatments (11 QTL). These results contradict those of Bertin and Gallais (2001), who studied a maize RIL population for yield and yield related traits and concluded more QTL were found at High N, but also that QTL at Low N were a subset of QTL at High N, indicating QTL for both treatments were co-localised.

Some studies detected QTL for SY in oilseed rape on chromosomes not identified in this work i.e. on chromosomes 2, 3, 7, 10, 11, 13, 15 and 16 (Quijada et al., 2006), on chromosomes 5 (99.4-137), 6 (82.6-96.6), 10 (20.3-29.1), 12 (34.2-35.7), 13 (133-158), 17 (0-16.1), 19 (0-27.5) (Radoev et al., 2008) and on 4 different regions of chromosome 2 in the TNDH population (Shi et al., 2009). A QTL detected on chromosome 17 (ST_17_3_R3H07) was co-localised with the one identified on the same chromosome by Radoev et al. (2008). All QTL are widespread in the genome and in response to the different N treatments, as expected from a polygenic trait, because of many genes intervening directly or indirectly in the regulatory mechanisms of seed yield (Shi, 2009; Thurling, 1991).

SEED NUMBER PER POD (SNP)

Quantitative trait loci for SNP were identified on 5 chromosomes i.e. 1, 3, 7, 13 and 18; in 8 different genomic regions at High N, but in only 2 regions at Low N in 2005/06. Radoev et al. (2008) analysed 250 lines of a doubled-haploid population from a cross between the cultivar "Express" and the resynthesized line "R53, in field experiments at four locations in Germany and found QTL on chromosome 5, 11 and 19. Another study in oilseed rape DH population by Gül (2002) detected QTL for SNP on chromosomes 15 and 18 at both High and Low N, at the same confidence interval on chromosome 15 as was found in this study. Gül (2002) also identified QTL on chromosome 18 but in different regions for each treatment and concluded that despite SNP presenting different responses to N treatment (i.e. significant GxN), there was no QTLxN interaction.

THOUSAND SEED WEIGHT

Four genomic regions contained QTL for TSW on 4 different chromosomes i.e. 1, 4, 5 and 9, three at High and two at Low N. Previous studies had identified QTL on chromosome 1 (Radoev et al. 2008) and 5 (Radoev et al. 2008, Gül 2002) in *B.napus* and Radoev et al. (2008) identified additional QTL on chromosomes 3, 7, 11, 12, 13, 16 and 19. Still, 1 QTL on chromosome 5 detected at both at High and Low N was reported for the first time in this study. Detection of a QTL at both High and Low N was reported in the paper by Gül (2002), where 3 out of 4 QTL were co-localised at both High and Low N and presented high QTLxN interaction, whereas only one QTL seemed to be independent of N treatment.

NITROGEN AND NITROGEN DERIVED TRAITS

NITROGEN UPTAKE EFFICIENCY (NUPE)

Quantitative Trait Loci for NUpE were identified on 4 different chromosomes 1, 4, 7 and 16 at 5 different locations in 2005/06. More QTL were detected in 2006/07, a total of 9 at High N and 7 at Low N, compared with only 5 identified in 2005/06. One QTL on chromosome 7 was identified at the same exact location in both years at Low N but the confidence interval detected in 2006/07 was larger than in the previous year. Most of the QTL detected were treatment specific and were only present at either High or Low N. Only one QTL was commonly identified at both High and Low N, however, the confidence interval was shorter at High N, possibly indicating only gene/s in that smaller portion had an effect on both treatments, or just meaning that the gene/s of interest were in the smaller QTL area only but not specific to that treatment.

NITROGEN UTILISATION EFFICIENCY (NUTE)

Five QTL were identified for NUtE at High (1 QTL) and Low N (4 QTL) in 2005/06 and 5 and 9 QTL respectively, in 2006/07. Again a larger number of QTL were detected in the second year, however, more QTL were identified at Low than at High N in both years. A possible explanation would be that the lower the supply of available N in the soil, the lower the N concentration in the plant, thus the more the gene/s involved in mechanisms for N transportation, relocation etc. for N would be activated as stress response to become more efficient, translating in the form of an increased number of QTL detected at Low N.

These results contradict those from Gallais and Hirel (2004), who detected more QTL at High than at Low N for N uptake and grain yield whereas it was the reverse for grain protein and N utilisation in maize.

NITROGEN USE EFFICIENCY (NUE)

Quantitative trait loci for NUE were identified on 2 genomic regions at High N (chromosomes 7 and 19) and 3 at Low N (chromosomes 1, 7 and 9) in 2005/06 and on 7 genomic regions at High N (chromosomes 4, 7, 8, 9, 11 and 17) and 8 genomic regions (chromosomes 9, 10, 11, 14, 16 and 19) at Low N in 2006/07. Only on one occasion was a QTL detected in both years found to be co-localised, yet it was at High N in 2005/06 and at Low N in 2006/07. A similar number of QTL were detected at High and Low N in both years.

Quantitative trait loci detected at Low N carried alleles from Ningyou7, the more N efficient parent as concluded in the previous chapter, suggesting gene/s providing higher NUE to the plant were present in Ningyou7. Identification of candidate genes should therefore start with those QTL for NUE at Low N that are independent of QTL at High N for the same trait and that have a high additive effect with negative sign (allele from Ningyou7).

NITROGEN HARVEST INDEX (NHI)

Nine QTL were detected on 5 different chromosomes in 2005/06 and 16 QTL were detected for 2006/07 on 11 different chromosomes. Quantitative trait loci were widely distributed and no QTL reproduction was encountered on QTL for the same N treatment. Similar to results for NUE, almost the same number of QTL were found at either High or Low N in both years (5 and 4 at High and Low N respectively, in 2005/06 and 8 at both High and Low in 2006/07). One QTL on chromosome 1 was found to be co-localised across years: at Low N in 2005/06 (NHI_1_42_L06) and at High N (NHI_1_42_R307) in 2006/07.

In all N and N derived traits analysed there was a higher number of QTL detected in 2006/07 when compared to the previous year. Moreover, in the first field trial, most of the QTL for these traits were identified at Low N, whereas a higher proportion of QTL for the N concentration traits and NUpE was detected at High N in the second field trial. Possibly, the reason is that a lower number of QTL were detected in 2005/06. Since QTL were identified at different positions for each treatment this indicates that no optimal genotype was found for both high and Low N treatments, thus the selection of genotypes for a particular trait should be conducted under the specific treatment for which that trait was selected (Agrama et al., 1999; Bertin and Gallais, 2000; Rauh et al, 2002). This suggests that sets of

genes are differentially expressed according to the N level in the plant (Bertin et al 2001). Therefore, a genotype with high NUE at Low N should be selected by growing it under Low N conditions

SEED NITROGEN CONCENTRATION (NS)

Quantitative Trait Loci for seed N were identified on 12 chromosomes (1, 3, 4, 7, 8, 9, 11, 12, 14, 17, 18 and 19) and in 16 different genomic regions, 4 were identified from the first field trial and 12 were identified from the second field trial. In 2005/06, 3 out of the 4 QTL for the trait were found at Low N, however, the opposite was found in 2006/07, with 8 out of 12 QTL identified at High N. Results from Bertin and Gallais (2001) found more QTL for grain protein content at Low N in a study with 99 RIL of a maize population crossed to a tester.

PLANT NITROGEN CONCENTRATION (NP)

Three QTL were identified for plant N in 2005/06 distributed on 2 chromosomes i.e. 7 and 14, 2 at High N and 1 at Low N, whereas in 2006/07 19 QTL were identified at High N for both chaff (10) and stem (9) N and 16 at Low N (5 for chaff and 11 for stem), located on chromosomes 1, 2, 3, 6, 7, 9, 13, 14, 17, 18 and 19 for STN and on 2, 3, 4, 6, 7, 8, 9, 13, 14 and 19. These results indicate that the number of QTL increases as the trait is split into components, allowing for more specific QTL identification, but also amplifying the area to search for candidate genes. Only one QTL was detected at the same location for plant N in 2005/06 and for both chaff and stem N in 2006/07 and this was at High N. Moreover, 1 QTL co-localised for chaff and stem at High N with 2 QTL at Low N in 2006/07, suggesting some of the gene/s regulating both chaff and stem N are common, but many others may respond at different times or the gene/s involved may be different. More QTL were detected for plant N at High N than at Low N in both years (grouping QTL for chaff and stem together). Stem N had nearly the same number of QTL at High and at Low N i.e. 9 and 11 respectively. Two QTL were detected at the same confidence interval, one for stem N at both High and Low N and another one partially overlapping for stem N at High N with chaff N at Low N, possibly due to different control mechanisms for different N treatments.

TOTAL PLANT NITROGEN CONCENTRATION (NT)

In 2005/06 QTL were detected on chromosomes 1, 7 and 17 and in 2006/07 on 2, 4, 6, 7, 9, 11, 13, 14, 15, 16 and 17 but none were co-localised.

OIL CONTENT

Three QTL for oil content were identified in 2005/06 on chromosomes 4 and 9 at High N and on 5 at Low N. The QTL on chromosome 4 partially co-localised with a QTL identified by Qiu et al. (2006) using the same TNDH population at High N, however the QTL identified on chromosome 9 between x and x cM, was located just below the one detected on the same chromosome by Qiu et al. (2006), probably increasing the potential region to search for candidate genes. Other QTL identified by Qiu et al (2006) were on chromosomes 1, 2, 3, 6, 8, 10, 12, 13 and 17. Another study by Delourme et al. (2006) in oilseed rape identified QTL on chromosomes 1, 2, 6, 10, 13, 15 and 16.

The QTL identified at the lower end of chromosome 1 (63.9-69.7 cM) by CIM at Low N should be also taken into consideration as it was detected by MCIM as well on the same location, and co-localised with most of the traits analysed in 2005/06.

A QTL identified on chromosome 5 (23.2 to 39.3 cM) at Low N has not previously been reported. The QTL had LOD score of 2.48 and 3.42 by IM and CIM respectively and the phenotypic variation was 6.3 and 7.4% respectively.

ARCHITECTURAL TRAITS

FLOWERING TIME (FDAS)

More QTL for flowering time were identified in 2006/07 than in 2005/06. The QTL were distributed on 2 chromosomes i.e. 9 and 13 at 2 genomic regions in 2005/06, one at High N and one at Low N respectively. In 2006/07, QTL were identified on 6 linkage groups corresponding to 11 genomic regions at High N and to 4 chromosomes and 7 genomic regions at Low N. A study in wheat at CYMMIT by Reynolds (pers. comm.), noted that when lines with very different flowering times are grown together, the flowering dates have a tendency to adjust to the early flowering lines, thus suggesting lines should be sown in the field grouped according to known/predicted flowering times (i.e. early flowering, mid-flowering, late flowering lines).

Quantitative trait loci for FDAS were not consistent across replicates or across years. However, QTL on chromosomes 1, 3, 10, 18 and 19 were also detected by Long et al. (2007), who studied flowering time in the TNDH population in 11 different environments both spring and winter. They found 41 QTL spread on chromosomes 1, 2, 3, 5, 9, 10, 12, 16, 17, 18 and 19. Seven of these QTL coincided totally or partially with QTL identified in the 2006/07 field trial: on chromosome 1 qFT1_1 co-localised with FDAS_1_6_R2H07 at High N, on chromosome 3 qFT3_5 with FDAS_3_27_R1L07 at Low N, on 10 qFT10_6

and close to qFT10_7 with FDAS_10_27_R4L07 at High N, on chromosome 18 qFT18_1 with FDAS_18_2_R2H07 at High N and finally, on chromosome 19 qFT19_3 with FDAS_19_17_R2H07 and FDAS_19_16_R1L07 at High and Low N, respectively, and qFT19_4 with FDAS_19_22_R2H07.

Other studies analysing QTL for FDAS in oilseed rape also identified QTL on the same chromosomes e.g. Delourme et al. (2006) studied an oilseed rape population (two populations of 445 and a 242 doubled haploids (DH) derived from the crosses "Darmor-bzh" x "Yudal" (DY) and "Rapid"" x "NSL96/25" (RNSL), respectively) and detected QTL for FDAS on chromosomes 1 (2), 2, 4 and 19, but also QTL on chromosomes 6, 12, and 17. Another publication from Quijada et al. (2006) in hybrid spring rape identified QTL for FDAS on chromosomes 3, 6, 10, 11 and 12, of which QTL on 3, 10 and 11 were commonly identified on the same chromosomes, but always positioned at an above confidence interval.

CHLOROPHYLL CONTENT IN BRACTS (CB) AND LEAVES (CL)

The data for CB and CL was collected at the beginning of flowering for many TNDH lines but at a time when some of the earlier lines were already in flower. Despite some of the lines being at a different developmental stage when the data was recorded, it is unlikely to have affected the QTL analysis in a major way as data were compared between High and Low N treatments and chlorophyll content has been shown to increase proportionally to N fertiliser supply independent of developmental stage (Rostami et al., 2008). Another consideration is that the TNDH lines would be at different developmental stages for different lines, but at a similar developmental stage for the same line analysed at High N and at Low N. Quantitative Trait Loci were detected on 9 chromosomes and occupied 19 different genomic regions. A study to identify QTL for chlorophyll in wheat at different growth stages concluded more than half of the QTL detected (10 out of 17) were expressed at more than 2 growth stages (Zhang et al., 2009).

Only on 3 occasions were QTL for chlorophyll content in bracts and in leaves found at the same confidence interval, twice at High N and once at Low N. On 8 occasions QTL for CB were found independently of CL (only one at Low N), and 3 times QTL for CL were found, 2 of which were at Low N. These results suggest different mechanisms are involved in the regulation of chlorophyll in different parts of the plant, but more interestingly at different N treatments when recorded at flowering time.

TOTAL PLANT HEIGHT (TL)

Three QTL distributed on 2 chromosomes were identified for plant height, two of them on chromosomes 1 and 2 were identified at Low N and one on chromosome 17 was detected at High N. On

chromosome 1, two QTL were identified at Low N and on chromosome 17 one QTL was detected at High N. The QTL on chromosome 17 (TL_17_4_H06) was found to partially overlap with a QTL for plant height identified by Chen et al. (2007), between 6.1 and 9.4 cM analysing a population of 258 DH lines of a cross between the canola variety Quantum and a resynthesized *B. napus* line No.2127-17, and a fixed immortalized F-2. Other QTL found by Chen et al. (2007) were on chromosomes 3 (70.3-77.2 cM), 4 (19.5-24.1), 16 (48.6-56.8), 13 (143.3-151.6) and 14 (0-8.8). Regardless of 3 QTL being found for TL, the two QTL at Low N are newly identified and have not yet been reported. A previous study of a rice DH population also detected more QTL for TL at Low N and only one was co-localised in both High and Low N treatments (Fang and Wu, 2001), suggesting the expression of QTL for plant height was induced under Low N conditions.

FOOT LENGTH (FL)

Ten QTL were detected for foot length in 4 chromosomal areas but only 3 were detected at Low N. This is the first time QTL for FL have been reported. The trait has not been considered of major interest previously, even though interesting correlations with more agronomically important traits were found e.g. strong positive correlations with TL, CB and CL (the latter at High N only) and strong negative correlations with TW, NUpE and NUE at both treatments and with SY at Low N only. These relationships will be further discussed later in the Clustering of QTL section.

These results indicate a higher number of QTL identified for the trait under non-stressed conditions.

BRANCH NUMBER (BN)

Five chromosomes 1, 3, 14, 15 and 16 contained QTL for BN with QTL distributed in 6 different genomic regions, 5 at High N and 1 at Low N. The QTL on chromosome 15 BN_15_6_H06 was co-localised with a QTL detected on chromosome 16 at approximately 60.5 cM by Chen et al. (2007) when analysing a *B.napus* DH population and F2 lines (of a cross between a canola variety Quantum and a resynthesized *B. napus* line No.2127-17). They also identified QTL on chromosomes 5, 7, 11, 13 and 14, which were identified at different confidence intervals, leading to the conclusion that 5 new QTL were identified for BN in this study.

Table 3.49. Summary of distribution of QTL on chromosomes at High and Low N in both 2005/06 and 2006/07, using both IM and CIM.

	2005/06		2006/07	
TRAIT	High	Low	High	Low
TL	17	1	/	/
FL	3, 6, 9, 16, 19	9, 10	/	/
BN	3, 14, 15	1, 9	/	/
FDAS	13	9	1, 2, 9, 11, 15, 18, 19	3, 4, 10, 11, 17, 19
CB	1, 2, 6, 9, 17	1, 2, 17	/	/
CL	1, 2, 7, 9, 14	1, 13, 16	/	/
TW	1, 19	1, 7	1, 7	4, 7, 9, 11, 14, 18
HI	7, 9, 10, 13, 17	6, 7	7, 8, 10, 16	2, 3, 7, 14, 17, 18
SY	19	6, 7, 9	1,4,5, 7, 8, 14, 16, 17	2, 4, 9, 11, 16, 18
TSW	4, 5, 9	1, 4, 7	/	/
SNP	1, 3, 7, 13, 18	1, 7	/	/
OIL	4, 9	1, 5	/	/
SN	17	1, 7, 18	4, 8, 9, 11, 12, 14, 19	3, 4, 12, 18
PlantN	7	14	/	/
STN	/	/	2,7, 9, 13, 14, 19	1, 3, 6, 9, 14, 17, 18
PN	/	/	2, 3, 4,7, 8, 9, 13, 14, 19	6, 9, 13, 19
TN	17	1, 7, 18	4, 6, 7, 9, 11, 14	2, 9, 15, 16, 17
NUPE		1, 4, 7, 16	4, 7, 9, 11, 13, 17	4, 7, 11, 14, 15
NUTE	1, 12, 17	5	3, 6,7, 9	2, 7, 9
NUE	7, 19	1, 7, 16	4, 7, 8, 9, 11, 17	9, 10, 11, 14, 16, 19
NHI	1, 7, 17	1, 7, 9	1, 3, 6, 9, 11, 12, 14	2, 7, 9, 16

3.4.2. CLUSTERING OF QTL AND TRAIT ASSOCIATIONS

Quantitative trait loci were found at the same location for some of the traits at High N and Low N. Different traits had a tendency to cluster together on the same region of the chromosome, indicating that either some gene/s involved in different traits shared regulatory mechanisms or even the same gene/s. In some cases genes were clustered according to phenotypic correlations (analysed in Chapter 2) such as yield QTL together or NUpE and NUE also co-localising in the same region.On other occasions,

and generally in this study, co-location of QTL did occur less than expected from the phenotypic correlations according to previous studies (Chen et al., 2007; Yue et al., 2009) suggesting clusters represented the same results as those found in phenotypic correlations.

HIGH NITROGEN

An expected QTL cluster was composed by TW and SY, because of the high correlation values between the 2 traits observed in Chapter 2. Important relationships were those found between NUpE and SY (4 QTL) and NUE and SY (5 QTL), 3 of them were common for the 3 traits (SY-NUpE-NUE), probably the region on the chromosomes 4 and 7 where these QTL were found contains gene/s related to N assimilation (Senthilvel, 2008). These QTL would be a starting point to look for candidate genes that are at the same time involved in N uptake or N use efficiency and yield, with the objective of producing N efficient varieties and maintaining high yields. All NUpE, NUtE and NHI traits had 4 QTL at the same location as one of the traits for N concentration i.e. seed, stem, chaff or total N. Quantitative trait loci for HI and SNP were detected at the same location on chromosome 13, together with STN suggesting that genes involved in both traits are different.

Quantitative trait loci for BN were co-localised with FL on chromosome 3 and with NUpE on chromosome 15. Oil content QTL were found together with TSW, SY, TN, chaff N, and NUpE. That would allow selection for a high yielding variety with high oil content in conjunction with N uptake efficiency.

At High N, flowering (FDAS) was found at the same location as CB twice on chromosomes 9 and 13, and FDAS and NUpE were also found co-localised twice on chromosomes 11 and 19, in accordance with a study in barley that found QTL for FDAS related to gene/s related to N protein content (See et al., 2002). Flowering QTL were also found co-localised with TW on one occasion and with NUtE and NHI on another. Quantitative trait loci for FDAS did not map together with QTL for TL as reported in a previous study in maize, suggesting regulatory mechanisms are different for oilseed rape (Quijada et al., 2006) Chlorophyll in bracts (CB) and CL were found co-localised on 2 occasions only, one with NHI and another one FDAS. These results indicated certain independence between the 2 chlorophyll traits. Chlorophyll in bracts was found in other QTL clusters related to both yield and architectural traits e.g. CB-TSW-HI-FL to N related traits CB-STN or many different traits e.g. CB-TW-NUpE or CB-TL-HI-SY-NUpE-NUE. Also QTL for CL were found together with N related traits CL-STN and CL-STN-NHI. Interestingly, QTL for CB were detected at the same location as HI, twice one time together with FL on chromosome 9 and another time together with TL on chromosome 17, suggesting a relationship between chlorophyll in bracts, plant

height and foot length or harvest index could indicate that co-localisation of QTL gene/s for C/N metabolism and related to photosynthesis.

LOW NITROGEN

Quantitative Trait Loci for SY and NUE were detected at the same confidence interval on 3 occasions, twice on chromosome 9 and once on 16. The same relationship was found between NUpE and NUE on chromosomes 1, 7 and 14. Clusters of QTL for TW, SY and NUpE, Hi and NHI and SY and NUE were identified by Zhao et al. (2007) in rice. In this study they grouped the traits into 3 categories influencing yield i.e. NUpE and biomass, NUE and HI and NHI. They concluded that genotype was the main factor controlling grain yield thus it is possible to develop a variety that uses N efficiently at Low N without compromising grain yield. On four occasions QTL for NUtE co-localised with QTL for NHI on chromosomes 2, 7 and 9, an indication of sharing of part of the genetic background between these 2 traits. No QTL co-localisation was found for NUE and NUtE at Low N, meaning that the gene/s controlling the genetic mechanisms of these traits are mostly different at Low N, so to increase NUE at Low N, NUpE is the component to be targeted.

Two clusters shared partly the same traits: HI-NUtE-NHI-NT-NS and HI-NUtE-NUpE-NUE-SY-TW-SNP indicating that common genes between NUtE and HI are related to N concentration and NHI on one hand and to yield and yield components on the other hand. The first ones were associated with NHI and the second ones with TW, SY and SNP. Sabouri et al. (2009), in a study of a rice population, also determined associations between HI, SNP (seed number per panicle) and SY.

Both plant height (TL) and branch number (BN) were detected in one cluster of many traits: TL-BN-TW-SNP-TSW-TN-SN-STN-NUpE-NUE-NHI. Total above ground plant biomass (TW) was found co-localised with QTL for SY and TSW but also with NUpE, NUE and only once with N concentration, suggesting that N concentration was independent of total above ground plant biomass. Quantitative trait loci for HI and NHI were also found clustered four times, of these, 3 occasions were together with N concentration traits and one with NUtE, probably explained by the close relationship between the 2 traits.

Similarly to High N, QTL for flowering were found at the same location as QTL for NUpE on two chromosomes i.e. 4 and 11 and also with NUE on chromosomes 9, 10 and 19. At one of the QTL clusters formed by FDAS and NUpE, a QTL for SY was also present, and the same happened with the FDAS-NUE cluster. The co-location of QTL for FDAS-NUpE-SY and also FDAS-NUE-SY at Low N is very important for the screening of N efficient varieties, as flowering is supposedly a very highly heritable trait thus a safe choice for selection. Flowering was also co-localised once with PN and another time with STN and TN. Quantitative trait loci for CB and CL were found at the same location at High N, only on one occasion

confirming the genetic independence of the traits when measured just before flowering time. One QTL detected for CB was found at the same location as one for NUtE and another one for CB at the same location as NHI. Foot length was correlated with STN and a second time with TW and SY.

3.4.3. QTL x ENVIRONMENT INTERACTIONS

The MCIM (Multiple trait Composite Interval Mapping) analysis identified QTL x environment interactions for both field trials. The analysis was run for the same trait at both High and Low N treatments simultaneously and a joint LOD score showing the level of environmental interaction was calculated. The QTL detected in only one of the environments indicated the presence of environmental interaction and that was the most common result for both 2005/06 and 2006/07 analysis. Six QTL were commonly detected at both High and Low N treatments in 2005/06 and only 1 in 2006/07, indicating QTL were independent from N treatment. Interestingly, when the traits were analysed individually by CIM in 2005/06, 4 QTL were detected at both N treatments, and none of those coincided with the 6 detected by MCIM at both High and Low N. These results are in accordance with those from Cao et al. (2001) and Yadav et al. (2002) who showed that QTL x environment interactions were not always detected in all environment combinations, but only in a subset. Moreover, changes of significance of the QTL effect were also encountered.

Despite the findings from the present study a QTL detected in both N environments could still have interaction with environment as suggested by Yan et al., (1998), as some of these QTL detected both at High and Low N treatment were not detected in both 2005/06 and 2006/07 trials, or even in the comparison between Blocks 1-2 and 3-4 in 2006/07.

Many QTL were commonly detected by MCIM between 2005/06 and 2006/07 and also between the 2 subsets analysed from 2006/07. The trait with more QTL commonly detected was NUpE with 9 and the least was TW with 1 only. Four QTL were detected for both the years and in the same year: one for STN, 2 for NUpE and 1 for NUE. In many commonly detected QTL it happened that the QTL (either from the same year or across years) would be present only at High N on one occasion and at Low N only on another occasion. A possible explanation would be because of the changes in magnitude of the QTL effects, converting in undetected QTL those with LOD scores below 1.8 in one of the environments. No QTL for SY were commonly found either between Blocks 1-2 and 3-4 Blocks or when compared across years, probably because SY is regulated by many genes and shows a significant genotype x environment interaction.

Quantitative trait loci with strong environmental effects at Low N would be of interest for traits such as NUpE, NUtE, NUE and NHI, in particular those QTL having strong additive effects as well, for the selection of varieties with high efficiency of N (Gül, 2002).

3.4.4. Comparison of IM QTL and CIM QTL

Quantitative trait loci were studied using IM analysis, CIM analysis and finally MIM. Interval mapping and CIM were used for QTL detection and MIM was used to decide, in those circumstances when 2 QTL occurred on the same chromosome, if it was 2 different QTL or only one QTL altogether.

When using IM and CIM with this set of data, CIM always identified at least one QTL per trait, and for each detected QTL the CIM results would generally present a higher LOD score and higher R^2 values than IM for the same QTL, agreeing with reports that say CIM has higher sensitivity and accuracy than IM (Zeng, 1994; Li et al., 1999; Ledeaux et al., 2006). It was also observed that the same QTL was not identified at the same exact location by both IM and CIM and was generally shifted a few markers up or down between the two methods of analysis. Two general concerns have been reported about IM analysis: when 2 or more QTL are present at one particular interval, the QTL position obtained by IM analysis can be shifted as it only considers the occurrence of one QTL per interval. A second point to consider is that only 2 markers are used at a time to detect QTL positions, being really inefficient (Doerge, 1997). On the other hand, the use of background markers by CIM has to be very well balanced, as too many background markers could generate artificial QTL and too few markers could translate in to lower powers of detection (Zeng, 1994). Both IM and CIM have important advantages but also some disadvantages to consider when analysing QTL, in both occasions potentially resulting in QTL that either do not exist at the indicated location or that the QTL detected does not have a relationship with the trait being analysed. Multiple interval analysis was performed using CIM result files as a model, to discern between QTL found in close proximity to each other. The advantages of MIM, apart from reducing possible biased results obtained from the other methods, are the possibility to calculate phenotypic, genotypic and environmental variance and the derived heritability in the broad sense. Moreover, MIM analysis can also study QTL effects such as epistasis.

In the present study no epistatic effects were found with MIM analysis for any trait analysed between 2 detected QTL, however the possibility that higher-order epistasis (between multiple QTL) exists should be considered as metabolic pathways such as N have many interacting genes involved in regulation of different quantitative traits (MCMullen et al., 1998).

CHAPTER 4. MAPPING CANDIDATE GENES FOR AGRONOMIC TRAITS USING THE TNDH POPULATION

4.1. INTRODUCTION

Quantitative Trait Loci (QTL) analysis facilitates the association of a phenotypic characteristic with a DNA region. It is in this DNA region where the genes responsible for that particular phenotype may be found and analysed. Even though the number of times that individual genes have been identified from a QTL is small, there are many examples of quantitative traits in which single genes have major effects and their molecular basis has been studied (Roff, 2007). One reason for this discrepancy is that many QTL map to relatively large regions of the genome in length, and these regions often contain multiple loci that influence the same trait. Another reason is that identifying the actual loci that affect a quantitative trait involves demonstrating causality using techniques like positional cloning followed by targeted gene replacement. Positional cloning is a method of gene identification in which a gene for a specific phenotype is identified, with only its approximate chromosomal location (but not the function) known, also known as the candidate region (Clee et al., 2006). Targeted gene replacement is a technique in which a cloned piece of DNA with a gene of interest is modified to allow further identification (Sullivan et al, 1997).

The common approaches used to identify candidate genes are generally conditioned on existing information about the genome sequencing and location of genes, as well as their function (Zhu and Zhao, 2007).Therefore, the most common approach to identify individual genes within a QTL starts with the previous identification of possible (or known) candidate genes using classical reverse genetics or bioinformatics. A functional relationship between the candidate gene and the QTL must then be demonstrated by gene cloning techniques, like functional complementation or deletion mapping. While functional complementation is based on the addition of complementary DNA of the gene of interest to produce a different phenotype, deletion mapping identifies the gene function by locating the mutations within.

The aims of this chapter are to identify candidate genes related to the traits of interest through comparative genomics with Arabidopsis and to integrate QTL based information for indicating the genetic basis of N metabolism and enable genetic improvement of oilseed rape in terms of NUE.

4.2. MATERIALS AND METHODS

4.2.1. PLANT MATERIAL

The population used for QTL analysis was the total N concentrationDH population described in Chapter 2. In 2005/06, 174 total N concentrationDH lines were analysed (one plant was sampled from each line in each block) and in 2006/07, 94 total N concentrationDH lines were studied (a bulk of 20 plants was sampled from each line for analysis), as described in Chapter 3. The selection of chromosomal regions for further candidate gene analysis was based on the heritability information in Chapter 2, but mostly on the QTL information in Chapter 3.

4.2.2. CANDIDATE GENE IDENTIFICATION BY COMPARATIVE GENOMICS

The QTL regions identified using WinQTL Cartographer analysis were then compared to orthologous regions of the *Arabidopsis thaliana* genome already identified by synteny mapping of the 2 genomes.

Candidate genes were identified using 2 approaches: from QTL to candidate gene and forward, and from candidate gene to QTL. For the first approach, markers associated to the QTL were localised in bacterial artificial chromosome (BAC) libraries of the *B. rapa* genome (www.brassica.bbsrc.ac.uk) where comparative results with *Arabidopsis* indicating potential candidate genes were displayed. When no *B.rapa* correspondence was found (the genome of *B.napus* is not fully sequenced, thus alignment with *B.rapa* may not provide any results) alignment with the Arabidopsis genome was performed. Then the orthologous regions between Arabidopsis and *B. napus* where the genes were localised were identified using a comparative map (Parkin et al., 2005); and finally the presence or absence in that particular region of *B. napus* was assessed (Long et al., 2009). When the QTL of interest would not include a marker present in alignment with Arabidopsis genomes (as in Parkin et al., 2005), a direct comparison between the markers' locations in the Qiu map and the Parkin map was carried out, and the closest marker in the Parkin map was used for comparison with Arabidopsis.

For the second approach, genes of interest were identified and localised in the *Arabidopsis* genome or *B. rapa* BACs.

After the list of candidate genes from Arabidopsis was extracted for each candidate region, all the genes within the 1Mb intervals were classified according to function. The genes were assigned a putative function using Pfam (pfam.sanger.ac.uk/) and InterPro (www.ebi.ac.uk/interpro/) databases and functionally categorized as represented in pie charts.

4.3. RESULTS

Candidate genes were identified in 8 regions of interest on chromosomes 1, 4, 7 (2), 9, 15, 16 and 19. The regions on the different chromosomes were selected depending on what traits shared common QTL in the same interval of confidence. The candidate genes identified for each chromosomal segment were within 1Mb of chromosome in the Arabidopsis genome. The eight QTL candidate regions were matched with all Arabidopsis pseudo-chromosomes (At1 to At5). The candidate region on chromosomes 1 of oilseed rape was aligned with chromosome At4 of Arabidopsis. The candidate region on chromosome 4 and 9 of oilseed rape were aligned with the same region on At2 from Arabidopsis. The region of chromosome 9 being was 2 Mb bigger, thus taking a larger space of the Arabidopsis chromosome from the upper side. Both candidate regions on chromosome 7 were aligned with At1, one superposed regions as well on the chromosome. Finally, candidate region on chromosome 16 was aligned with At3 and the region on chromosome 19 was aligned with At5. The region on chromosome 15 did not align with Arabidopsis as there were no common markers within the region of interest. However, parallel alignment of the 2 chromosomes showed the region aligned with chromosome 1 of Arabidopsis.

Candidate genes identified in each region were classified in different functional categories: cell wall modifying genes, metabolism related genes, cell defence and rescue genes, genes involved in signalling, GPRs (glycine rich proteins) and unidentified or unknown function. The genes that were not classified in any of these categories were considered of miscellaneous function. Most of the proteins of miscellaneous function were different kinds of transporters not specifically associated with metabolism and also cell organs such as ribosomal units etc., all with general functions, sometimes with multiple functions. Glycine Rich Protein genes were not included in the graphic representation as their contribution to the total was 0%.

4.3.1. IDENTIFICATION OF CANDIDATE GENES

CHROMOSOME 1

The first region to be analysed by comparative genomics with Arabidopsis was on chromosome 1, between 66.8 and 73.41 cM. In that region QTL for total above ground plant biomass, seed number per pod, 1000-seed weight, branch number, plant height, seed N concentration, total N concentration, NUpE, NUE and oil from the first field trial and QTL for stem N concentration from the second field trial were co-localised at Low N. The region was aligned between 13.78 and 7.59 Mb of chromosome At4 of

Arabidopsis. In that QTL region, 1278 genes were found within, of which, 28% were classified as miscellaneous, 24% as metabolism genes and 23% as of undetermined function. The rest of the genes were classified into signalling 17%, cell defence and rescue 7% and cell wall modifying 1% (Fig.4.1).

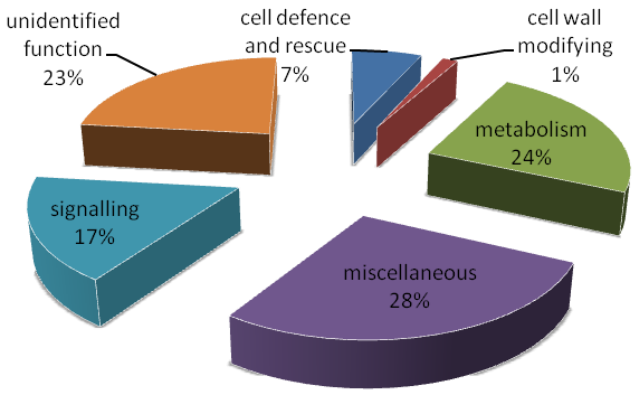


Figure 4.1. Functional cataloguing of proteins from chromosome At4, corresponding to chromosome 1 OSR candidate region.

Six genes relevant to nitrogen metabolism were identified in the QTL region in alignment with Arabidopsis (Table 4.1). Two of these genes, the APG8A and the NLP7 were found to be responsive to Low N conditions as N starvation response mechanism in plants. The APG8A is a gene with its highest expression in flowers. The gene is activated during dark-induced carbon starvation. It is predominantly found in the cytoplasm independent of the N status of the plant. Concanamycin A is the protein that accumulates in the central vacuole as punctuate structures that resemble autophagic bodies, localization that becomes more abundant during N starvation conditions. The NLP7 gene functions as a transcription factor. It modulates nitrate sensing and metabolism (Castaings et al., 2009).

Two other genes identified were transporter genes i.e. the AAT1 acting as amino acid transporter of the CAT subfamily; and the NRT1.5 nitrate oligopeptide transporter from the POT family protein. The holoenzime Aspartate aminotransferase 1 (AAT1) is localised in the mitochondria and most probably related to cytosolic transport of aspartate/asparagine compounds. The NRT1.5 is directly related to N uptake and its mutants have affected the root to shoot nitrate transport (Lin et al., 2008).

Another gene identified was the AT4G18810.1, is a transcription repressor involved in regulation of N utilisation, located in chloroplasts and vacuoles. The last gene identified was an aminotransferase class I and II family protein, which is expressed in roots.

Three genes were identified in relation to yield traits total above ground plant biomass (TW), seed yield (SY) and harvest index (HI). One of the genes, the UGE2 is involved in general plant growth and cell wall biosynthesis. Together with isoforms UGE1 and UGE5 it has been shown to influence root growth and cell wall galactose content by affecting galactan content. It was also related to shoot growth, together with UGE4 (Rosti et al., 2007). Another gene of interest is the ARK3, mostly expressed in roots, but also in shoots. It is thought to be involved in processes of transition of growth patterns. Finally, the RHS15 is a transporter of general substrate expressed in root hair only.

Table 4.1. List of genes of interest identified at the QTL on chromosome 1 of oilseed rape, aligned with pseudo-chromosome At4 from *Arabidopsis thaliana*, from 7.59Mb to 13.78Mb.

NITROGEN (NupE, NUE, SN, STN, TN)		
Locus	**Name**	**Description**
10322438	AT4G18810.1:	binding / catalytic/ transcription repressor involved in regulation of nitrogen utilization, metabolic process.
11270045	AAT1:	member of the cationic amino acid transporter (CAT) subfamily of amino acid polyamine choline transporters. Mediates efficient uptake of Lys, Arg and Glu in a yeast system.
11517353	AT4G21680.1:	proton-dependent oligopeptide transport (POT) family protein that functions in transporter activity. BEST *Arabidopsis thaliana* protein match is: NRT1.5 (NITRATE TRANSPORTER 1.5); nitrate transmembrane transporter/ transporter.
11655589	APG8A:	Encodes APG8, a component of autophagy conjugation pathway, delivered to the lumens of vacuole under nitrogen-starvation condition.
12307126	AT4G23590.1:	aminotransferase class I and II family protein; functions 1-aminocyclopropane-1-carboxylate synthase activity, pyridoxal phosphate binding, transferase activity, transferring nitrogenous groups, transaminase activity, catalytic activity.
12479752	NLP7:	Encodes NIN Like Protein 7 (NLP7). Mutants of NLP7 show features of nitrogen-starved plants and are tolerant to drought stress.
YIELD (TW, SY, HI)		
Locus	**Name**	**Description**
11388925	ARK3:	putative receptor-like serine/threonine protein kinases that is similar to Brassica self-incompatibility (S) locus. Expressed in root and shoot. Expression limited at the root-hypocotyl transition zone and at the base of lateral roots as well as in axillary buds and pedicels.
12431277	UGE2:	protein with UDP-D-glucose 4-epimerase activity. Involved in growth and cell wall carbohydrate biosynthesis.
12920927	RHS15:	transporter, putative;major facilitator superfamily MFS-1, general substrate transporter.
OIL		
Locus	**Name**	**Description**
12900430	OLEO1:	Encodes oleosin1, a protein found in oil bodies, involved in seed lipid

		accumulation.
13473516	ATS1:	Encodes caleosin, a 27-kDa protein found within seed lipid bodies. Catalyze hydroperoxide-dependent mono-oxygenation reactions. Require calcium for peroxygenase activity. Probably deeply buried in lipid droplets or microsomes.
13487457	AT4G26790.1:	GDSL-motif lipase/hydrolase family protein; acting on ester bonds, carboxylesterase activity, lipid metabolic process.

Three more genes were related to oil: the oleosin 1 (OLEO1), caleosin (ATS1) and a GDSL-motif lipase. Oleosin 1 is shown to regulate the size and morphology of the oil bodies, but also facilitate the access of triacylglycerides (TAG) during germination. The ATS1 gene is a caleosin involved in lipid storage. It is involved in different sorting pathways than oleosins, using small vesicles, and it is mostly involved in lipid trafficking, membrane expansion and oil bodies' biogenesis. A GSDL-motif lipase hydrolase was also identified within the QTL, involved in hydrolysis of ester bonds.

CHROMOSOME 4

The second region of interest was between 49.8 and 51.5 cM on chromosome 4, with QTL for FDAS, seed yield and NUpE detected in 2006/07 at Low N only. The QTL interval was aligned between 10.79 and 12.90 Mb of Arabidopsis chromosome At2. In that region, 680 genes were identified classified in miscellaneous (30%), unidentified (27%), metabolism (21%), signalling (15%), cell defence and rescue (6%) and cell wall modifying (1%) (Fig. 4.2).

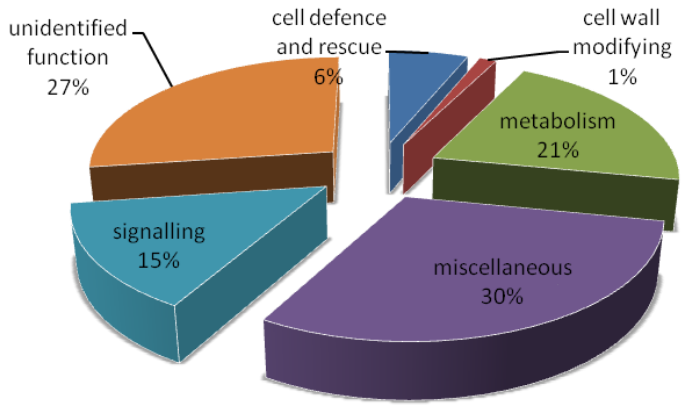


Figure 4.2. Functional cataloguing of proteins from chromosome At2, corresponding to chromosome 4 OSR candidate region.

The nitrate transporter NTP2 was found in the area of interest related to a QTL for N uptake efficiency (Table 4.2). It has been identified as the nitrate transmembrane transporter NRT1.1. The transporter participates in the signalling pathway involved in development of lateral roots towards soil areas richer in nitrate. The signalling cascade is not activated when nitrate supply is uniformly spread (Remans et al., 2006).

Table 4.2. List of genes of interest identified at the QTL on chromosome 4 of oilseed rape, aligned with pseudo-chromosome At2 from Arabidopsis thaliana, from 10.79Mb to 12.90Mb.

NITROGEN (NUpE)		
Locus	**Name**	**Description**
11347147	AT2G26690.1:	nitrate transporter (NTP2);involved in response to jasmonic acid stimulus and response to wounding.
YIELD (SY)		
Locus	**Locus**	**Description**
12262013	PRS:	Encodes a homeodomain containing protein that regulates lateral axis-dependent development of Arabidopsis flowers and is required for cell proliferation.
11462067	GRV2:	GRV2 mutants result in a reduction in gravitropic response in hypocotyls and shoots but do not affect root gravitropism and mutants are defective in amyloplast sedimentation.
11977944	FRA8:	involved in secondary cell wall biosynthesis. Mutants have irregular xylem formation, reduced cellulose levels and plants are smaller than normal siblings.
FLOWERING		
Locus	**Locus**	**Description**
11059035	ELF3:	novel nuclear protein that is expressed rhythmically and interacts with phytochrome B to control plant development and flowering through a signal transduction pathway. Core component of the circadian clock regardless of light conditions.
12226091	RAP2.7:	RELATED TO AP2.7 (RAP2.7); has transcription factor activity and is involved in organ morphogenesis, regulation of transcription, DNA-dependent, vegetative to reproductive phase transition.

In this region, three candidate genes were identified in relation to a seed yield QTL: PRS, involved in the regulation of lateral axis development; GRV2, involved in gravitropic response in hypocotyls and shoots; and FRA8, involved in cell wall biosynthesis and related to xylem, cellulose and general growth. The "pressed flower" (PRS) is a homeobox gene that functions independently of the determinations of floral organ identity and floral meristem size. It is expressed in a restricted number of L1 cells at the lateral

regions of flower primordia, floral organ primordia, and young leaf primordia. The gravitropism defective 2 (GRV2) genes GRV2/RME-8 function in vesicle trafficking from the multivesicular body/pre-vacuolar compartment to the tonoplast. A defect in the system might be caused by a general defect in vacuolar morphology that affects correct amyloplast sedimentation (Silady et al., 2008). The fragilefibre 8 (FRA8) is involved in xylan synthesis and related to cellulose depositions in second cell wall formation (Zhong et al., 2005).

Two genes were found to be related to flowering: ELF3 and RAP2.7. The early flowering gene 3 (ELF3) is a transcription factor that is shown to help in the regulation of the circadian clock together with COP1 (Yu et al., 2008). The RAP2.7 gene matches with protein TOE1, already identified previous study in the same TNDH population as involved in flowering.

CHROMOSOME 7

On chromosome 7, two regions were considered of interest, due to high number of QTL, particularly seed yield and NUpE and NUE. The first region considered on chromosome 7 region was between 51.1 and 93.1 cM. I was aligned between 24.16 and 30.24 Mb on Arabidopsis' chromosome At1.

In the second interval, QTL for total above ground plant biomass, seed yield, harvest index, NUpE and NUE were identified in 2005/06 and harvest index, NUpE and NUtE in 2006/07, all at Low N. Additive effects were relatively low for all traits except for total above ground plant biomass (-148.26 for the QTL detected in 2006/07) and alleles were from Ningyou7 (negative). That region was aligned between 27.28 and 30.24 Mb of chromosome At1.

Because of the two interval matching superposed regions on the Arabidopsis chromosome 1, they were treated as one region altogether for analysis, considering the biggest interval only. In that area of At1 then, 1074 genes were identified. The genes were classified as 34% miscellaneous proteins, 25% genes of unidentified function, 18% metabolism, 14% signalling, 7& cell defence and rescue and 2% cell wall modifying (Fig. 4.3).

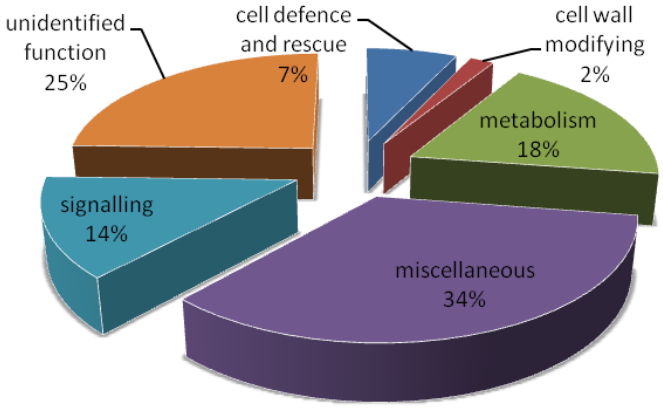


Figure 4.3. Functional cataloguing of proteins from chromosome At1, corresponding to chromosome 7 OSR candidate region.

In that QTL region on chromosome 7, three candidate genes were identified in relation to NUpE, NUtE and NUE. One of the genes, the NIA1 is involved in 15% of the nitrate reductase (NR) activity in shoots and responsible for the cytokinin induced increase of NR activity (Yu et al., 1998). The nucleotide transporter 1 (NTT1) was also found in this region. The NTT1 acts as a proton-dependent adenine importer. Another gene identified was an aminotransferase class I and II family protein, which is involved asparagine catabolic process and glutamate catabolic process to oxaloacetate.

Table 4.3. List of genes of interest identified at the QTL on chromosome 7 of oilseed rape, aligned with pseudo-chromosome At1 from *Arabidopsis thaliana*, from 24.16Mb to 30.24Mb.

NITROGEN (NUpE, NUE, NUtE)		
Locus	**Name**	**Description**
29235803	NIA1:	Encodes the cytosolic minor isoform of nitrate reductase (NR). Involved in the first step of nitrate assimilation, it contributes about 15% of the nitrate reductase activity in shoots.
30208499	AT1G80360.1:	aminotransferase class I and II family protein; asparagine catabolic process, biosynthetic process, glutamate catabolic process to oxaloacetate, aspartate transamidation.
YIELD (SY, HI)		
Locus	**Name**	**Description**
27659673	PIN1:	auxin efflux carrier involved in shoot and root development. Mutants have an inflorescence meristem that does not initiate any flowers, resulting in the formation of a naked inflorescence stem. PIN1 is involved in the determination of leaf shape by actively promoting development of leaf margin serrations.

29347581	PGM:	PHOSPHOGLYCERATE/BISPHOSPHOGLYCERATE MUTASE (PGM); has intramolecular transferase activity, phosphotransferases, catalytic activity; involved in response to nitrate.
30191856	NTT1:	NUCLEOTIDE TRANSPORTER 1 (NTT1).

One of the genes related to yield; the PIN1 encodes an auxin efflux carrier involved in shoot and root development. Loss of function severely affects organ initiation, pin1 mutants are characterised by an inflorescence meristem that does not initiate any flowers, resulting in the formation of a naked inflorescence stem. That is the result of a reduced ability to transport auxin IAA through the stem. PIN1 is involved in the determination of leaf shape by actively promoting development of leaf margin serrations. In roots, the protein mainly resides at the basal end of the vascular cells, but weak signals can be detected in the epidermis and the cortex (Jones et al., 2005).

Another gene identified,the plastidic phosphoglyceratemutase (PGM) is an important factor affecting carbon flux in triacylglycerol accumulation in oilseed plants. It is most likely through its essential role in starch synthesis. The induction of this gene by low nitrate is very strong in shoots and to a lower extent in roots (Wang et al., 2003). The NTT1 transporter is an adenylate translocator from the cytosol. Together with the glucose-6-phosphate/phosphate translocator (GPT) they showed increased yield and starch in tubers, i.e. potato (Zhang et al., 2008).

CHROMOSOME 9

A fifth region of interest included between 105.9 and 120.5 cM of chromosome 9. Traits with QTL at High N were TSW, seed N concentration, stem N concentration, total N concentration and NUE, and at the same location at Low N were harvest index, N harvest index and stem N concentration.

The region was aligned with chromosome At2 of Arabidopsis, from 9.18 to 13.14 Mb. In that region, 1618 genes were identified. Most of the genes were classified as miscellaneous (27%), an equal number was classified as unidentified and metabolism (23%), 18% of the genes were classified as signalling genes, 6% were classified as cell defence and rescue genes and 3% of the genes were classified as cell wall modifying (fig. 4.4).

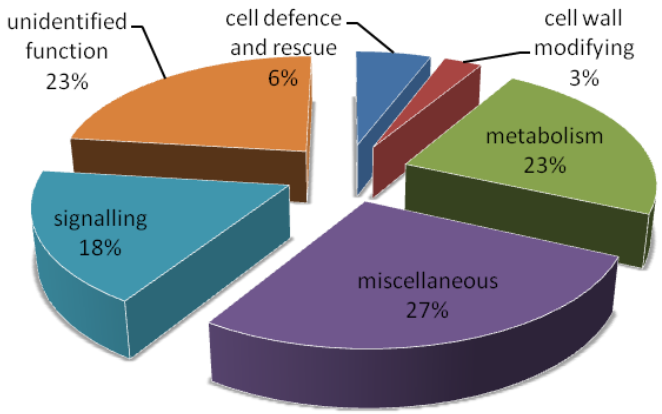


Figure 4.4. Functional cataloguing of proteins from chromosome At2, corresponding to chromosome 9 OSR candidate region.

Four genes were found of interest in the QTL region on chromosome 9: the aspartate aminotransferase (AAT), the GCN5- related N-acetyltransferae (NAGS1), the glutamine dumper family protein (AtGDU4) and the nitrate transporter (NTP2). The AAT enzyme plays a key role in the regulation of carbon and nitrogen flux in all organisms. In eukaryotes, along with malate dehydrogenase, it works as the malate/aspartate shuttle. It may also regulate the supply of glutamate for the C-5 pathway and the biosynthesis of tetrapyrroles, notably chlorophyll (Schultz et al., 1998).

The NAGS1 is an enzyme involved in arginine/ornitine synthesis pathway from glutamate. The GDU family proteins are involved in export mechanisms in plants. These genes specifically stimulate amino acid export and that they potentially act as regulators of amino acid exporters. Also the NTP2 was found in this region, as in the QTL region on chromosome 4, as 2Mb of chromosome At2 were shared between the two QTL.

Six genes were found as potential candidates for yield traits on chromosome 9 QTL. One of the genes, the VHA-A2 is required for efficient nitrate storage. Mutants contained 80% less nitrate in the vacuoles, the NR activity was increased by 90% and they contained more glutamine than controls (Krebs et al., 2010). The GRF1 is a growth regulating factor expressed in root, shoot and flower. It is involved in stem elongation and cell expansion in leaves and cotyledon tissues. Mutants result in smaller leaves indicating the role of the gene in leaf development (Kim et al., 2003).

The curly leaf gene (CLF) is involved in leaf morphogenesis and cell fate. The gene is necessary for stable repression of a floral homeotic gene and encodes a protein with homology to the product of the Polycomb-group gene *Enhancer of zeste* (Goodrich et al., 1997).

Table 4.4. List of genes of interest identified at the QTL on chromosome 9 of oilseed rape, aligned with pseudo-chromosome At2 from *Arabidopsis thaliana*, from 9.18Mb to 13.14Mb.

NITROGEN (NUE, NHI, SN, STN, TN)		
Locus	**Name**	**Description**
9457810	AAT:	ASPARTATE AMINOTRANSFERASE (AAT); FUNCTIONS IN: L-aspartate:2-oxoglutarate aminotransferase activity, pyridoxal phosphate binding, transferase activity, transferring nitrogenous groups, catalytic activity.
9749869	NAGS1:	GCN5-related N-acetyltransferase (GNAT) family protein / amino acid kinase family protein; involved in arginine biosynthetic process, amino acid biosynthetic process, metabolic process.
10559384	AtGDU4:	Encodes a member of the GDU (glutamine dumper) family proteins involved in amino acid export.
11347147	AT2G26690.1:	nitrate transporter (NTP2) has transporter activity and is involved in response to jasmonic acid stimulus, response to wounding.
YIELD (HI, TSW)		
Locus	**Name**	**Description**
9162620	VHA-A2:	Vacuolar proton ATPase subunit VHA-a isoform 2. Localized in the tonoplast. Required for efficient nutrient storage but not for sodium accumulation.
9728756	AtGRF1:	Growth regulating factor encoding transcription activator. One of the nine members of a GRF gene family. containing nuclear targeting domain.
9955553	CLF:	Similar to the product of the Polycomb-group gene Enhancer of zeste. Involved in the control of leaf morphogenesis. Mutants exhibit curled, involute leaves. AGAMOUS and APETALA3 are ectopically expressed in the mutant.
10566898	COL3:	Positive regulator of photomorphogenesis that acts downstream of COP1 but can promote lateral root development independently of COP1 and also function as a daylength-sensitive regulator of shoot branching.
10933061	MOT1:	high-affinity molybdate transporter. Mutant has reduced concentrations of molybdate in roots and shoots, and reduced shoot and root length when growing on Mo-limited medium.
11462067	GRV2:	GRV2 mutants result in a reduction in gravitropic response in hypocotyls and shoots but do not affect root gravitropism and mutants are defective in amyloplast sedimentation.

Another gene, the COL3 is a positive regulator of photomorphogenesis that acts downstream of COP1 but can promote lateral root development independently of COP1 and also function as a day length-sensitive regulator of shoot branching (Datta et al., 2006). The molybdate transporter (MOT1) is required for efficient uptake and translocation of molybdate and for normal growth under conditions of

limited molybdate supply (Tomatsu et al., 2007). Finally, the gravitropism defective 2 (GVR2) gene was also found and described in the same location on chromosome 4.

CHROMOSOME 15

In the region on chromosome 15, between 77.1 and 79.3 cM, QTL for BN at High N and total N concentration at Low N, but also NUpE in both N treatments were found. That region did not successfully align with the Arabidopsis genome through common markers, as no common markers were present in the region of interest. Therefore, the approach used was a linear comparison of the TNDH map of chromosome 15 with the similar map of chromosome 15 published in 2005 (Parkin et al., 2005). In the map in Parkin et al. (2005), the region between 77.1 and 79.3 cM was aligned with the region between 6 and 7 Mb of pseudo-chromosome At3 of Arabidopsis. The difference between this alignment and the one used for the other chromosomes is that the first is not considered a synteny block (Long et al., 2009). They describe a synteny block as a region from the TN map with at least 3 closely linked homologous loci within a particular segment of the Arabidopsis genome.

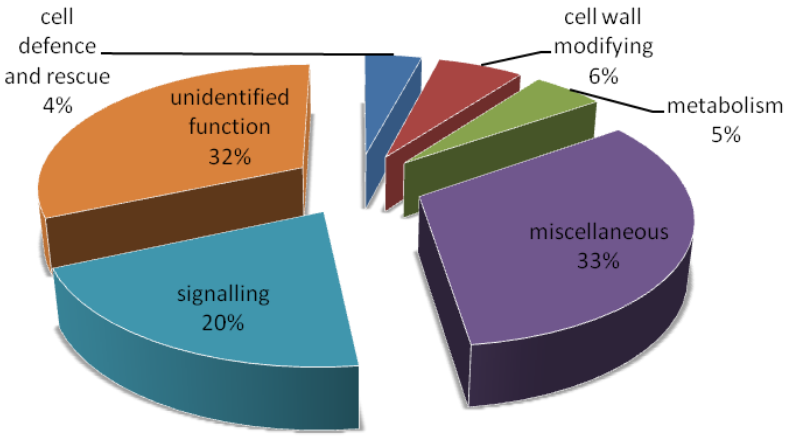


Figure 4.5. Functional cataloguing of proteins from chromosome At3, corresponding to chromosome 15 OSR candidate region.

After the alignment of the oilseed rape and Arabidopsis genomes, 147 genes were identified in the region of interest. Of these, 45 were of unidentified function (31%) and 47 were of miscellaneous function (32%). The rest of the genes were classified as signalling 20%, cell wall modifying 6%, metabolic function 5%, cell defence and rescue 4%, and GRPs 2% (Fig. 4.5).

Within the genes with metabolic function, a glutamate ammonia ligase, synonym to glutamine synthetase, was identified (Table 4.5). Also a pyridoxal-dependent DC was identified, enzyme involved in aminoacid biosynthesis.

Table 4.5. List of genes of interest identified at the QTL on chromosome 15 of oilseed rape, aligned with chromosome 3 from *Arabidopsis thaliana*, from 6Mb to 7Mb in At3.

NITROGEN (NUpE, TN)
ATGSKB6; copper ion binding / glutamate-ammonia ligase
YIELD (BN)
pyridoxal-dependent decarboxylase family protein

CHROMOSOME 16

On chromosome 16 a region between 53.8 and 59 cM was selected for further analysis. Within the interval, QTL for branch number at High N and chlorophyll content in leaves, seed yield and NUE at Low N were identified. The region was aligned with chromosome At3 of Arabidopsis between 19.51 and 22.26 Mb. In that region, 1043 genes were identified.

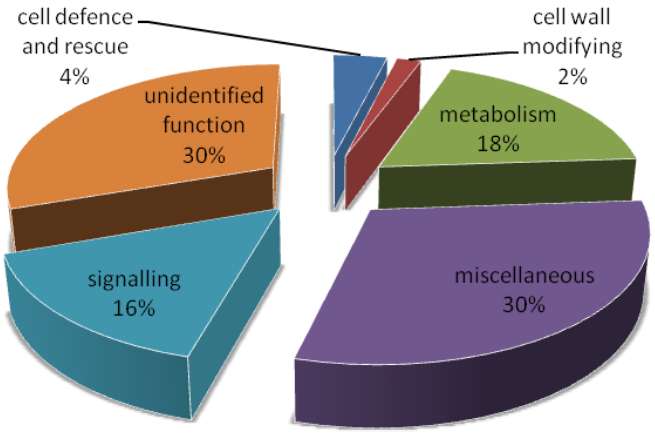


Figure 4.6. Functional cataloguing of proteins from chromosome At3, corresponding to chromosome 16 OSR candidate region.

They were classified as: miscellaneous (30%), the same as genes of undetermined function (30%), metabolism (18%), signalling (16%), cell defence and rescue (4%) and cell wall modifying (2%), (Fig. 4.6).

The candidate genes found in that QTL in relation to N were six, two of them related to chlorophyll: the HCF109 and GUN4. As HCF109 affects photosystems I and II and the plastid NAD(P)H dehydrogenase complex by specifically controlling the stability of only these distinct transcripts (Meurer et al., 1996). The GUN4 gene is a regulator for chlorophyll synthesis. It participates in plastid-to-nucleus signaling by regulating Mg-Proto synthesis or trafficking (Larkin et al., 2003).

Other genes identified related to N metabolism were a glutamate-ammonia ligase, a di- and tri-peptide and microRNA. The glutamate-ammonia ligase is also referred to as glutamine synthetase (GS). Glutamine synthetase plays an essential role in the metabolism of nitrogen by catalysing the condensation of glutamate and ammonia to form glutamine. The di- tri-peptide transporter is a gene involved in long-distance transport of di- and tripeptides and has higher affinity for Ala-Ala and Ala-Lys than for Ala-Asp (Dietrich et al., 2004).

The gene miR393 targets transcripts that code for a basic helix-loop-helix (bHLH) transcription factor. The miR393/*AFB3* mutant showed that both primary and lateral root growth responses to nitrate were altered. Therefore, miR393/*AFB3* is a unique N-responsive module that controls root system architecture in response to external and internal N availability in *Arabidopsis* (Vidal et al., 2010).

Table 4.6. List of genes of interest identified at the QTL on chromosome 16 of oilseed rape, aligned with pseudo-chromosome At3 from *Arabidopsis thaliana*, from 19.51 Mb to 22.26Mb.

NITROGEN (CL, NUE)		
Locus	**Name**	**Description**
19706894	AT3G53180.1:	catalytic/ glutamate-ammonia ligase; involved in nitrogen compound metabolic process, N-terminal protein myristoylation, nitrogen fixation, metabolic process, glutamine biosynthetic process.
20045663	PTR1:	di- and tri-peptide transporter that recognizes a variety of different amino acid combinations. PTR1 plays a role in dipeptide uptake in the roots.
20691647	MIR393B:	microRNA that targets several TIR1/AFB family members and one bHLH family member. Specifically cleaves AFB3 transcripts, controlling AFB3 mRNA accumulation in roots in response to nitrate exposure.
21166403	AT3G57190.1:	peptide chain release factor, putative; release factor activity. BEST Arabidopsis thaliana protein match is: HCF109 (HIGH CHLOROPHYLL FLUORESCENT 109).
21948717	GUN4:	GUN, genomes uncoupled, is necessary for coupling the expression of some nuclear genes to the functional state of the chloroplast. Although required for chlorophyll accumulation under normal growth conditions, GUN4 is not essential for chlorophyll synthesis.

YIELD (SY, BN)		
Locus	**Name**	**Description**
19587821	VSR1:	Encodes the Vacuolar Sorting Receptor-1 (VSR-1)/Epidermal Growth Factor Receptor-like protein1 (VSR-1/ATELP1). Binds vacuolar targeting signals. Involved in sorting seed storage proteins into vacuoles.
19616021	AtGRF4:	Growth regulating factor encoding transcription activator. Involved in leaf development and expressed in root, shoot and flower.
19956656-	UPP:	Encodes UPP, a plastidial uracil phosphoribosyltransferase (UPRT) involved in uracil salvage. Loss-of-function mutation causes dramatic growth retardation, a pale-green to albino phenotype, abnormal root morphology and chloroplastic disorders.
20091976	RGD3:	ROOT GROWTH DEFECTIVE 3 (RGD3).
20114684	WRI1:	WRINKLED1 encodes transcription factor of the AP2/ERWEBP class. Protein involved in the control of storage compound biosynthesis in Arabidopsis. Mutants have wrinkled seed phenotype.
20254725	AMP1:	glutamate carboxypeptidase. Various alleles show-increased cotyledon number and rate of leaf initiation, show transformation of leaves to cotyledons, altered flowering time and photomorphogenesis and an increased level of cytokinin biosynthesis.
21283338	ADPG1:	Encodes ADPG1, a polygalacturonase protein involved in silique and anther dihiscence. Loss of function mutations have reduced seed set, indehiscent fruit and reduced pollen shedding.
21506613	GIS:	Putative transcription factor,regulates aspects of shoot maturation in Arabidopsis thaliana.
21944178	FTA:	Encodes the alpha-subunit shared between protein farnesyltransferase and protein geranylgeranyltransferase-I. Involved in shoot and flower meristem homeostasis, response to ABA and drought and regulates leaf cell shape.

Nine candidate genes were found to be directly or indirectly related to yield. Five of the genes had transcription factor activity. For example, the GRF4 is involved leaf development and expressed in root, shoot and flower (Kim and Lee, 2006). The WRI1 is involved in the regulation and synthesis of storage compound accumulation during seed development in Arabidopsis, related to germination and stablishment of the next generation. Mutants have wrinkled seed phenotype, which is related to defective seed oil accumulation (Cernac et al., 2006). Also transcription factor GIS regulates aspects of shoots maturation in Arabidopsis (Gan et al., 2006). And the RGD3, which is transcription factor required for neoformation of the shoot apical meristem (Tamaki et al., 2009). Also the Vacuolar Sorting Receptor-1 (VSR-1)/Epidermal Growth Factor Receptor-like protein1 (VSR-1/ATELP1) is involved sorting seed storage proteins into vacuoles through vacuolar signals (Otegui et al., 2006).

Another candidate gene, UPP, is involved in plastid biogenesis and starch accumulation suggesting that uracil salvage is of major importance for plant development. Mutants would suffer from growth

retardation, showing a pale-green to albino phenotype, with abnormal root morphology and chloroplastic disorders (Mainguet et al., 2009).

An enzyme related to glutamate was the glutamate carboxypeptidase (AMP1). It is expressed with higher expression in roots, stems, inflorescences, and siliques. It is suggested the enzyme regulated a small signaling molecule that acts to regulate a number of aspects of plant development, in particular the size of the apical meristem (Helliwell et al., 2001).

Another candidate gene for yield, the dehiscence zone polygalacturonase1 (ADPG1), is a polygalacturonase protein involved in silique and anther dihiscence. Loss of function mutations have reduced seed set, indehiscent fruit and reduced pollen shedding (Ogawa et al., 2009). One last gene is the farnesyl transferase α subunit (FTA), which is involved in shoot and flower meristem homeostasis, response to ABA and drought and regulates leaf cell shape. Conditional and specific down-regulation of FTA in canola using the *AtHPR1* promoter driving an RNAi construct resulted in yield protection against drought stress in the field (Wang et al., 2009).

CHROMOSOME 19

The last region considered of interest was on chromosome 19, between 57.3 and 59.1 cM. The traits with QTL present in that region were total above ground plant biomass, seed yield, stem N concentration and NUtE at High N and NUE at both N treatments. The region was aligned between 14.68 and 17.96 Mb of chromosome At5 of Arabidopsis.

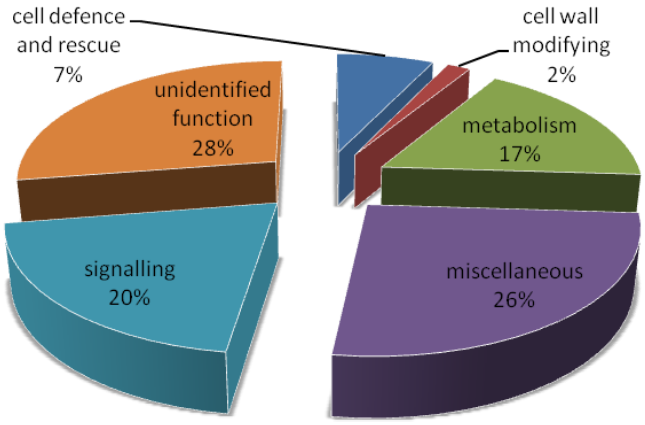


Figure 4.7. Functional cataloguing of proteins from chromosome At5, corresponding to chromosome 19 OSR candidate region.

In that region 1058 genes were identified. Most of the genes were classified as unidentified (28%) and then as miscellaneous (26%), followed by signalling (20%). The rest of the genes were classified as metabolism (17%), cell defence and rescue (7%) and cell wall modifying (2%), (Fig 4.7).

Five candidate genes were found in relation to QTL for NUE, NUtE and stem N concentration. One of the genes, the glutamine synthetase, plays an essential role in the metabolism of nitrogen by catalysing the condensation of glutamate and ammonia to form glutamine. The gene was also identified in the previous QTL on chromosome 16. Another gene was the glutamine dumper, previously identified on chromosome 9. The GDU family proteins specifically stimulate amino acid export and that they potentially act as regulators of amino acid exporters. Two more transporters were identified, the LHT1 And the ATCLC-A.

Table 4.7. List of genes of interest identified at the QTL on chromosome 19 of oilseed rape, aligned with pseudo-chromosome At5 from *Arabidopsis thaliana*, from 14.58 Mb to 17.96Mb.

NITROGEN (NUE, NUtE, STN)		
Locus	**Name**	**Description**
14933336	GSR 1:	cytosolic glutamine synthetase, the enzyme has high affinity with substrate ammonium.
15526114	AtGDU7:	member of the GDU (glutamine dumper) family proteins involved in amino acid export.
16280283	AT5G40645.1:	nitrate-responsive NOI protein, putative; is involved in the response to nitrate.
16323682	LTH1:	Encodes LHT1 (lysine histidine transporter), a high-affinity transporter for cellular amino acid uptake in both root epidermis and leaf mesophyll.
16381346	ATCLC-A:	voltage-dependent chloride channel member, also functions as a NO3-/H+ exchanger that serves to accumulate nitrate nutrient in vacuoles. Mutants have reduced nitrate uptake capacity in high nitrate environment and exhibit hypersensitivity to chlorate.
YIELD (TW, SY)		
Locus	**Name**	**Description**
15821442	SGR4:	member of SNARE gene family, is involved in vesicle transport.
16127725	MYB23:	Encodes a MYB gene that, when overexpressed ectopically, can induce ectopic trichome formation.
16173393	OLEO2:	Encodes oleosin2, a protein found in oil bodies, involved in seed lipid accumulation.
17756264	CRA1:	12S seed storage protein. Protein is tyrosine-phosphorylated and its phosphorylation state is modulated in response to ABA in Arabidopsis thaliana seeds.
17832779	AT5G44265.1:	Encodes a Protease inhibitor/seed storage/LTP family protein.

Another gene encodes for NOI, a nitrate responsive protein which has conserved functions in regulation of plant defence responses and that these are targets for at least AvrRpt2, AvrRpm1, and AvrB (Kim et al., 2005). And finally, the ATCLC-A voltage-dependent chloride channel member, also functions as a NO3-/H+ exchanger that serves to accumulate nitrate nutrient in vacuoles. Mutants have reduced nitrate uptake capacity in high nitrate environment and exhibit hypersensitivity to chlorate. Although the cellular localization of AtCLC-a remains unclear, it is suggested that AtCLC-a plays a role in controlling the intracellular nitrate status (Geelen, 2000).

Five candidate genes were found to be related to yield QTL. The gene SGR4 is a member of SNARE gene family and its mutant alleles such as sgr4/zig are defective in the shoots response to gravity resulting in a zigzag growth pattern of the stem. It is involved in gravity perception and/or gravity signal transduction for the shoot gravitropic response and in protein trafficking to lytic vacuoles (Yamauchi et al., 1997). Another gene is the MYB23, MYB which can induce ectopic trichome formation when expressed ectopically. The gene is expressed in leaves, stems, flowers, seeds and roots and quite strongly in trichomes. Involved in regulation of trichome initiation and trichome branching. It is therefore involved in reinforcing cell fate decisions and ensure robust establishment of the cell type pattern in the Arabidopsis root epidermis (Kang et al., 2009).

Another gene involved related to yield is the oleosin 2, important for lipid accumulation in seeds and size of oil bodies (Sliloto et al., 2006). Finally, 2 seed storage proteins were identified: CRA1, encoding a 12S storage protein and a LTP family protein.

4.4. DISCUSSION

Eight QTL regions were targeted in this study on chromosomes 1, 4, 7 (2), 9, 15, 16 and 19 for a first insight into potential candidate genes. The QTL regions were selected on the basis of the quantitative traits identified and on which N treatment the QTL were detected for these traits. In addition, the 9 QTL regions were selected according to some reproducibility either across replicates, years or methods of analysis and also considering additive effects. The QTL heritability was also assessed, but not considered as the main factor for selection as additive heritability values were between 0.1 and 0.4 for most traits (Appendix 12-13). The traits of interest considered were all N derived traits at Low N, particularly NUpE, NUE and N harvest index and also oil, seed yield and harvest index to maintain these qualities and use them for selection. According to this selection, candidate genes under these QTL would be expected to influence N metabolism in general under stress conditions as well as C/N interactions.

The classification of genes by function did provide an insight into the QTL structure and most of the QTL regions had similar distribution in functions. Still a large number of gene functions are not determined, sometimes because it has not been studied or due to other limitations. A large number of proteins was found to belong to the miscellaneous category, most of which were different types of transporters and proteins related to nuclear elements, translation, transcription and structure. Generally there was a similar amount of transcription factors and metabolism related proteins. Large number of transcription factors could be as a result of targeting regions in the chromosomes that could potentially be very active in transcription. A large number of metabolic proteins could be explained by the fact that N traits and yield traits are mostly related to C and N metabolisms, which are present in the QTL regions analysed.

Candidate genes were identified for the different traits analysed. Traits of major interest such as NUE and seed yield were the ones selected for candidate gene's identification. However, little is known in the mechanisms of N metabolism; therefore, even though genes were identified to be involved in the N cycle, it is difficult to determine to what extent and what is the level of importance. Moreover, little or no studies have been published of genes related to N starvation thus its identification is more difficult for that QTL study.

4.4.1 Candidate genes identified by chromosome

Chromosome 1

The first region to be analysed by comparative genomics with Arabidopsis was on chromosome 1, between 66.8 and 73.41 cM. In that region QTL for total above ground plant biomass, seed number per pod, 1000-seed weight, branch number, plant height, seed N concentration, total N concentration, NUpE, NUE and oil from the first field trial and QTL for stem N concentration from the second field trial were co-localised at Low N. In this region, 4 genes related to N metabolism were identified: directly related with N uptake QTL was the nitrate transporter NRT1.5; APG8 and NLP7 induced under N deficiency and AT4G18810.1 a transcription regulator for the N pathway. For the oil QTL, OLEO1 and a caleosin were identified, thought to be very relevant genes in seed oil composition. For example APG8 is supposed to have an important role in autophagic recycling, especially during situations that require substantial nitrogen and carbon mobilization (Doelling et al, 2002). The NIN like 7 protein (NLP7) would be related to all N QTL identified. It is an important element of the nitrate signal transduction pathway and regulatory protein specific for nitrogen assimilation in non-nodulating plants (Castaings, 2009). The

mechanism of operation is not defined yet, so it could possibly regulate the N uptake as well as influence in the N concentration of the plant, thus increasing N use efficiency.

CHROMOSOME 4

The second region of interest was between 49.8 and 51.5 cM on chromosome 4, with QTL for FDAS, seed yield and NUpE detected in 2006/07 at Low N only. In this QTL a very important gene was identified to be related to N uptake, the transporter NRT1.1. The transporter is activated and induces lateral root development towards rich N areas in the soil. The transporter would not be activated when nitrate is uniformly spread, confirming a detection mechanism during N deficiency (Remans et al., 2006), which makes it a very good candidate to improve N uptake and use efficiency. A candidate gene found in relation to yield is FRA8. The gene is involved in secondary cell wall biosynthesis, and plants without this gene fully functional are smaller than control plants. This gene, therefore, is indirectly involved with seed yield. Two genes were related to flowering: the ELF3 influences the circadian clock and the RAP2.7 is related to TOE1, a gene known to influence flowering time (Okamuro et al., 1997) and previously identified as such in the same QTL location in the TNDH population (Colin Morgan, unpublished results).

CHROMOSOME 7

On chromosome 7, two regions were considered of interest, due to high number of QTL, particularly seed yield and NUpE and NUE. The first region considered on chromosome 7 was between 51.1 and 93.1 cM. In the second interval, QTL for total above ground plant biomass, seed yield, harvest index, NUpE and NUE were identified in 2005/06 and harvest index, NUpE and NUtE in 2006/07, all at Low N. All the genes identified were between 27.28 and 30.24 CM, both QTL candidate areas were considered as one. One candidate gene was identified for N, the NIA1. The enzyme is directly related to N uptake and is involved in the first step for N assimilation in leaves. It is responsible for 15% of the nitrate reductase (NR) activity in shoots as well as for the cytokinin induced increase of NR activity (Yu et al., 1998). The gene could be related to N uptake and utilisation efficiency as well as NUE. A candidate gene identified possibly for harvest index and seed yield is the phosphoglycerate/bisphosphoglycerate (PGM). The enzyme is shown to participate in C metabolism influenced by the nitrate status of the plant. It also has an important role in starch biosynthesis. This gene, therefore, is a very good candidate to improve both N efficiency and yield at the same time.

CHROMOSOME 9

A fifth region of interest included between 105.9 and 120.5 cM of chromosome 9. Traits with QTL at High N were 1000-Seed weight, seed N concentration, stem N concentration, total N concentration and NUE, and at the same location at Low N were harvest index, N harvest index and stem N concentration. Two important candidate genes were identified in this QTL: one is the NRT1.1 already described in chromosome 4 section. The coincidence in NRT1.1 in two QTL regions one for NUpE at Low N and one for NUE at High N may indicate that NRT1.1 could be influencing some of these traits at High and Low N differently. The other gene is the aspartate aminotransferase (AAT). AAT enzyme plays a key role in the regulation of carbon and nitrogen flux in all organisms possibly related to N harvest index and possibly also to harvest index and 1000-seed weight. It is also involved in the biosynthesis of chlorophyll. The VHA-a2 could be a candidate gene for QTL for N concentration in seed, stem and total plant in general, due to the involvement of the gene with nitrate accumulation in vacuoles.

CHROMOSOME 15

The region on chromosome 15 between77.1 and 79.3 cM was chosen because of the occurrence of QTL for branch number at High N and total N concentration at Low N, but also NUpE in both N treatments. A relevant finding in this region was the glutamate ammonia ligase. The protein is one of the key enzymes (with Gltp) in the secondary pathway for glutamate biosynthesis from ammonia, and whose expression is regulated by the nitrogen source and by the amino acid limitation. Another enzyme identified in the same region is pyridoxal-dependent DC. It is primarily involved in the biosynthesis of amino acids and amino acid-derived metabolites, but is also found in the biosynthetic pathways of amino sugars, and it is probably related to total N concentration QTL to some extent.

CHROMOSOME 16

On chromosome 16 a region between 53.8 and 59 cM was selected for further analysis. Within the interval, QTL for branch number at High N and chlorophyll content in leaves, seed yield and NUE at Low N were identified. In that QTL, the gene glutamate ammonia ligase was also identified, as in chromosome 15. The gene could be related to NUpE (in chromosome 15) and NUE traits at low N. Another gene related to N metabolism identified was MIR393B, which controls root architecture, by controlling the expression of AFB3 (auxin receptor) in roots, influenced by internal and external nitrate levels (Vidal et al., 2010). This is also a possible candidate for NUE QTL. A candidate gene for chlorophyll content in leaves is GUN4, as the gene is required for chlorophyll accumulation under normal conditions, even though it could be over-expressed in N limiting conditions. A candidate gene for branch number could be GIS, involved in shoot maturation in Arabidopsis. The ADPG1 gene could possibly be

responsible for the QTL for seed yield indentified, as the gene is involved in seed number. Both GRF4 and UPP genes are involved at different levels in plant growth. UPP could be involved in a general phenotype under Low N conditions as it causes growth retardation, a pale-green to albino phenotype, abnormal root morphology and chloroplastic disorders, all of which could be attributed to N-starved plants.

CHROMOSOME 19

The last region considered of interest was on chromosome 19, between 57.3 and 59.1 cM. The traits with QTL present in that region were total above ground plant biomass, seed yield, stem N concentration and NUtE at High N and NUE at both N treatments. A candidate gene identified on chromosome 19 and commonly found in the last two QTL is glutamate ammonia ligase, also called glutamine synthetase. The gene is most probably involved in NUE at Low N and also related to the NUE component NUpE. Another gene identified in relation to N is the AtCLC-a, involved in nitrate accumulation in vacuoles. The gene could be related with the NUE component NUtE at High N. It could be related to stem N concentration QTL identified in the region. Two genes were identified as possible candidates for total above ground biomass and seed yield: MYB23 and OLEO2 respectively. MYB23 is related to trichome formation, initiation and branching; and OLEO2 is involved in oil bodies accumulation in seeds, important for all oilseed plants and related to seed yield.

4.4.2. SUMMARY OF CANDIDATE GENES AND QTL TRAITS

Eight QTL regions were analysed and candidate genes relevant to N metabolism were identified in each region. Eleven genes were considered of interest and directly related to the improvement of N use efficiency in oilseed rape. Of these, six were considered the most relevant genes and the ones to be considered for further analysis (Table 4.8). The selection of genes was partly conditioned by the data available. Lack of literature on candidate genes as well as on key steps of the N metabolic pathway meant that some genes were not considered and/or discarded.

The final genes are NLP7, At4G18810.1, PGM, NTP2, miR393b and GSR1. Four of these genes are involved in regulation mechanisms in relation to nitrate as NLP7, At4G18810.1 and miR393b and nitrate transporter NRT1.1 (gene NTP2). The reason for choosing transcription factors and regulatory genes is influences by the response of Dof1 transcription factor and its improvement of NUE in oilseed rape plants (Yanagisawa, 2004). As other candidate genes such as assimilation or reduction enzymes failed to improve NUE when mutants were generated (Vincent et al., 1997), this transcription factor did generate viable plants with increased NUE. Because of the nature of transcription factors that regulate more than

one gene for the same response, it is easier to modify a metabolic pathway like the nitrogen cycle (Yaganisawa, 2004). Another gene suggested for further analysis is the GSR1 because it was common to 3 different QTL. However, some studies have shown that plants transformed with the enzyme do not show improved NUE, even though the plants have the enzyme over-expressed (Oliveira et al., 2002). Probably the glutamine synthetase (GSR1) should be over-expressed with an appropriate promoter. And finally, the last gene suggested for further experiments is the PGM. This enzyme is related to carbon metabolism and responds to nitrogen level in the plant, therefore it is an ideal candidate to improve NUE and yield simultaneously (Wang et al., 2003).

Table 4.8. Arabidopsis genes controlling several traits investigated in our experiments were collected from the TAIR website and the published papers. The genes in bold letters are the ones selected for further analysis.

Chromosome	Gene name	Description	Reference
Chr1	APG8A:	component of autophagy conjugation pathway expressed under nitrogen-starvation condition.	J Biol Chem. 277(36):33105-14
Chr1	**NLP7:**	**NIN Like Protein 7 (NLP7). Modulates nitrate sensing and metabolism.**	Plant J. 57(3):426-35
Chr1	**AT4G18810.1:**	**binding / catalytic/ transcription repressor involved in regulation of nitrogen utilization.**	TAIR website
Chr7	**PGM:**	**intramolecular transferase activity, in response to nitrate.**	Plant Physiol. 132:556-567
Chr9	AAT:	functions in L-aspartate:2-oxoglutarate aminotransferase activity.	Plant J. 7(1): 61-75
Chr9	**NTP2**	**nitrate transporter NRT1.1.**	PNAS 103 (50): 19206–19211
Chr9	VHA-A2:	Vacuolar proton ATPase subunit VHA-a isoform 2	PNAS 107 (7): 3251–3256
Chr16	UPP:	plastidial uracil phosphoribosyltransferase (UPRT) has major role in plant development.	Plant J. 60: 280–291
Chr19	**MIR393B:**	**microRNA controlling AFB3 mRNA accumulation in roots in response to nitrate exposure.**	PNAS 107 (9): 4477-4482
Chr15, 16,19	**GSR 1:**	**cytosolic glutamine synthetase, the enzyme has high affinity with substrate ammonium.**	Euphytica 151(3): 291-302
Chr19	ATCLC-A:	functions as a NO3-/H+ exchanger that serves to accumulate nitrate nutrient in vacuoles.	Plant J. 21(3):259-67

The information gathered on candidate genes related to nitrogen and NUE would be the first step towards breeding plants with improved NUE. After candidate genes identification, mutants should be generated to assess the real effect and improvement they would produce on transformed plants. That process would take up to 9 months, after that, if some genes do show an improved response to NUE, the generation of plants with improved NUE could be entered for field testing.

CHAPTER 5. GENERAL DISCUSSION

The combination of physiological and quantitative genetic approaches using molecular markers in this study allowed for the identification of key loci involved in the expression of specific traits, to enable the selection of genotypes for increased Nitrogen Use Efficiency in the oilseed rape plant.

A main hypothesis was formulated, which was that better NUE achieved by exploiting the genetic improvement potential of crops would not only improve sustainability of agricultural systems, but also minimise the N fertiliser application-mediated adverse environmental impact of oilseed rape cultivation. Different genotypes of winter oilseed rape (TNDH lines in this case) have different nitrogen use efficiencies (NUE). Differences in NUE are due to differences in either nitrogen uptake efficiency (NUpE) or nitrogen utilization efficiency (NUtE) or a combination of the two. The magnitude of NUpE and NUtE are affected by the level of N supply (High/Low) and their effects are independent of one another. Such independence posits that certain plant traits can be identified that are related specifically to NUpE and others to NUtE. Stable QTL for each such trait can be identified that are N treatment specific. Candidate genes can then be identified for the N derived traits that influence yield. From this hypothesis, six objectives were studied and are discussed in the following text.

5.1. PARENT AND TNDH POPULATION PERFORMANCE

The parental lines Tapidor and Ningyou7 exhibited different responses to High and Low N for some traits and similar responses to certain other traits. For example, flowering pattern remained similar across years and treatments. Ningyou7 flowered earlier than Tapidor and in general it flowered too early for UK conditions. The leaves of Ningyou7 were characteristically yellow-green as opposed to the darker green Tapidor leaves. This was confirmed by the higher chlorophyll amount in the leaves of Tapidor. Similarly, the bracts of Tapidor contained more chlorophyll than those of Ningyou7 although the difference was not as pronounced as in the leaves.

For yield and yield related traits Ningyou7 had lower values than Tapidor, except for TSW in 2006 and for HI in 2007 where Ningyou7 had higher values. As Tapidor was bred and selected under UK conditions, it behaved generally in a more uniform way when grown in the northeast of England. On the other hand, Ningyou7 was more variable across years, possibly because of it being grown in a different climate as it was bred and selected for in China where it is classified as a semi-winter variety (Shi et al 2009). These results would mean that TNDH lines genetically closer to Ningyou7 may present similar

variability to the parental line, and lines closer to Tapidor would be more stable when grown in a northern European environment.

Both parents showed different behaviour at High and Low N for architectural traits i.e. plant height, foot length and branch number, where Tapidor was taller, had increased foot length and produced more branches than Ningyou7. For most of the yield traits analysed, the population mean was very close to the Tapidor parental value for the trait. A similar trait response to N treatment was present at both High and Low N. At High N, Ningyou7 had lower values for yield and yield related traits compared to Tapidor and to the mean for the TNDH lines, except for TSW in 2006 and for HI in 2007 where Ningyou7 had higher values. At Low N, Tapidor had higher values for yield traits compared to the population mean and to Ningyou7 in 2007. In 2006 Ningyou7 did not survive and Tapidor had lower values than the average for the population. The 2 parental lines were an effective target to enhance breeding for yield traits because the TNDH lines exhibited higher values than the parents in both years.

The oil content was higher in Tapidor than in Ningyou7 independent of High or Low N. However, in both Tapidor and Ningyou7 the oil content was slightly lower at Low N. This result is in accordance with earlier studies which found seed oil concentration decreased with decreased N fertiliser application (Taylor et al. 1991). Plant N concentration remained relatively constant both at High and Low N in both years. Both parents had similar values for plant N concentration at High N in 2006. In 2007, the trait was split into chaff and stem N and Tapidor showed higher values for chaff N, whereas stem N remained constant. Ningyou7 had much lower N concentration in stem at Low N and chaff N dropped only moderately, thus indicating a higher efficiency in N translocation to the seed for Ningyou7. Tapidor had higher seed N than Ningyou7 in both years at High N, but lower than both Ningyou7 and the average of the TNDH lines at Low N. Ningyou7 did not seem to be influenced by N treatment for this trait. A similar response at High and Low N was seen with NUpE and NUE traits, which indicated Ningyou7 is more efficient at Low N and Tapidor is more efficient at High N. Values for NUtE and NHI at High N were very similar for both parents and for the population mean, however Ningyou7 had higher N in the second field trial. Tapidor showed little or no response to N for NUpE, NUtE, NUE and NHI traits in 2007, thus indicating Tapidor would not be an efficient variety for studying N related traits under Low N conditions.

To summarise, the performance analysis of the parental lines under High and Low N conditions for the different traits reflected that Tapidor was superior for most of the traits related to plant biomass and yield e.g. branch number; whereas it showed no evident response to N for N derived traits such as NUpE, NUtE, NUE and NHI. On the other hand, Ningyou7, had a poorer performance from a productivity point of view, but had a higher response to N treatment for the N derived traits showing better potential

to adapt at varying N conditions. Ningyou7 was the parental line that had a major influence on QTL for N, particularly those for NUpE and NUE. With the parents exhibiting wide variation for many of the traits analysed, this was reflected in wide phenotypic diversity in the TNDH population, facilitating the ability to detect representative QTL.

The TNDH population proved suitable to grow under Northern UK conditions for some of the traits like oil content. For this trait, the population demonstrated potential for further improvement under these conditions as TNDH lines showed positive transgressive segregation only. Other traits showing positive transgressive segregation at Low N were Plant N concentration, HI and NHI in the first field trial. In the second field trial, stem N concentration showed negative transgressive segregation and NHI showed positive transgressive segregation, whereas HI showed transgressive segregation in both directions. However, most TNDH lines demonstrated transgressive segregation in both directions for most of the traits and environment combinations studied, indicating that the 2 parental lines did not represent the lowest and highest values of the traits, presumably because of complementary gene action (Grant, 1975; Vega & Frey, 1980; Xu et al., 1998), or because of the 2 parental lines being fixed for sets of alleles with opposing effects, resulting in transgressive segregation (deVicente & Tanksley, 1993).

Some cultivated varieties of different species i.e. rice have lost some beneficial traits in the breeding process (Sakai and Itoh, 2010) to become more suitable for commercial purposes, and it has been suggested that crossing these varieties with wild types would be beneficial to adapt to some stresses (Morgan et al., 2004). By crossing two varieties with such different adaptive backgrounds as the Tapidor and Ningyou7, it has also been observed that some additional beneficial traits would be transferred thus improving adaptation.

5.2. TRAITS INTERACTION AND QTL ANALYSIS

The specific objectives formulated under this section were:

- to identify traits related to different components of NUE in field experiments conducted over two years using different TNDH lines;
- to identify the nature of the relationships between traits under different N supply in the field (High and Low N treatments);
- to identify key loci involved in the expression of traits for differential responses to nitrogen supply using the Quantitative Trait Loci approach.

Nitrogen Use Efficiency was calculated as the product of NUpE by NUtE, where NUpE is the ratio of total above ground N to applied fertiliser and NUtE the ratio of seed yield to total above ground N. The component that most influenced NUE was NUpE, whereas NUtE was a more constant element and less subject to variability or to changes in N treatment. This close relationship between NUE and NUpE was also observed in the correlations, where both traits had a very similar relationship with other traits analysed. Conversely, a previous study in maize (Bertin and Gallais, 2000) suggested that both NUpE and NUtE influenced NUE, but the former at High N and the latter at Low N. The other trait to be highly correlated with both NUpE and NUE was seed yield. Results with similar relationships between NUE, NUpE and SY were found with the QTL analysis, where QTL for the 3 traits co-localised on many occasions. Other studies to find correlations between NUE and yield were Bertin and Gallais (2000) and Hirel et al. (2001) both in maize. However in both of these studies, limiting steps in N metabolism were found not to be dependent on N treatment. The NUpE-NUE correlation is a very important indicator for trait selection, suggesting that NUE can be improved by increasing NUpE as it was most influenced by this trait. The relationship between both NUpE and NUE with SY suggests that there is a close relationship between carbon and N metabolism, particularly in the case of mechanisms regulating NUE. Strong trait correlations present between yield traits and N traits i.e. between seed yield and NUE suggest strong interactions between C and N metabolic pathways at the regulatory level, thus suggesting not only that improvement of NUE is possible while maintaining the desired high yields, but also that by selecting for varieties with higher NUE, higher seed yields could also be achieved.

Two main traits were positively correlated with NUpE: total above ground plant biomass and seed yield (as earlier discussed). These same traits were also positively correlated at Low N with NupE. On the other hand, NUtE was positively correlated with harvest index and N harvest index at High N but no such correlation existed at Low N. Stem N concentration was negatively correlated with NUtE at High N, but again no correlation existed for NUtE at Low N. On the other hand, seed and total N concentrations were negatively correlated with NUtE at Low N, but not at High N. These results would suggest similar trait relationships would exist for NUE at High and Low N treatment, whereas different ones would exist for NUtE, depending on N availability.

Data from the two field trials identified associations between architectural, agronomic and nitrogen related traits in response to both High (standard agricultural conditions) and Low N (stressed N conditions) fertiliser regimes and QTL were detected according to these correlations.

As previously reported by Long et al. (2007) and Quijada et al. (2006), QTL were mapped in the same chromosomal regions for highly correlated traits. For example, total plant biomass was detected at the

same location as seed yield. Also N Uptake Efficiency co-localised with N Use Efficiency and seed yield. In addition, N Utilization Efficiency co-localised with N Harvest Index and finally, seed N concentration with total N concentration. The Pearson's value for all the correlations was positive and above 0.8. Few QTL for total plant biomass coincided with either N Uptake Efficiency and/or N Use Efficiency, despite all traits being highly correlated between them and also with seed yield. An explanation could be that the genes regulating these traits are independent and coincidence of QTL can be explained by the fact that some genes are linked and/or by the pleiotropic effect of genes (Long et al., 2007).

Traits showed few QTL with large effects, like flowering time, plant height, oil content and NUE, values of these were between 3 and 10. Two traits which showed very large additive effects were total plant biomass and seed yield, with values above 100 for TW and close to 100 for SY. Moreover, some N related traits i.e. seed N and total N concentrations, NUpE, and particularly NUE, had higher additive effects at Low N but only in the second field trial. This means that the average phenotype that would be produced by substituting an allele from one parental line (i.e. Tapidor) by the other (Ningyou7) would have a more pronounced phenotypic change at Low N than at High N for these particular traits.

It is possible to apply MAS to small effect or even low reproducibility QTL, however, the costs may be higher as more QTL need to be analysed (Tsonev et al., 2009).

5.3. QTL VARIABILITY AND STABILITY

To assess QTL reproducibility in this study, the following objective was proposed:

- to characterise the genetic basis of relevant traits for breeding varieties improved in NUE, particularly under low N conditions, by assessing the stability of identified QTL, their heritability and G x E interactions.

More QTL were detected in 2006/07, despite the population being cut down to almost half, from 174 to 94 lines, as other studies suggested that strong year x genotype interaction is present, particularly for physiological traits (Fontaine et al., 2009). Traits with a major number of QTL in 2006/07 were flowering, seed yield and most of the nitrogen traits, except NUtE, which had more QTL in the second field trial but not very different from the first field trial. Other traits that shared similar QTL number in both years were total plant biomass and harvest index.

Reproducibility of QTL proved to be low in this study, both within the same year and across years, mainly due to a high degree of plasticity and adaptability present in oilseed rape (Julien et al., 2009). However, QTL were detected for all traits with a certain degree of reproducibility meaning the TNDH population is suitable for QTL analysis for traits related to N and NUE.

Many QTL were detected for the traits studied in both field trials, with generally more QTL found in 2006/07 than in 2005/06. With the exception of the lower region of chromosome 4, and some parts of chromosomes 5, 6 and 13, QTL were widespread and were located in different areas of all chromosomes. Additionally, QTL for architectural, agronomic and nitrogen related traits were detected and positioned at respective regions on the chromosomes under both High and Low N regimes.

A study by Fontaine et al. (2009) analysed QTL for glutamine synthetase, glutamate dehydrogenase and other nitrogen-related traits. They used a DH population of winter wheat (cross between Arche x Recital which are both bread-making varieties) for 3 field trials sown in Northern France during the 2004, 2006 and 2007 growing seasons. They detected QTL for all traits analysed and also studied correlations between physiological and agronomic traits in relation to NUE and also detected candidate genes in 6 chromosomal regions. They experienced high environmental variability in QTL detection between the 3 years, and many QTL were identified in one year only. They attributed a high year component to the genotypic variation but discarded population size as having a major effect on the data, as more than 100 random lines were repeated across years. They therefore considered the 3 years of experiments as independent replicates. However, QTL were commonly detected in 2 different years, indicating that those QTL were accurate. That confirms high variability occurs across years, thus suggesting QTL trials should preferably be highly replicated within one year, otherwise to increase the number of field trials over different years as suggested by Quarrie et al., (2005) and Fontaine et al., (2009). Some traits, such as yield in wheat (Quarrie et al., 2005), have shown a large interaction between genotype and environment explains the different number of QTL in different experiments. In the Fontaine et al (2009) study in wheat, they reported that QTL for physiological traits detected in one year, co-localised with QTL for agronomic traits detected in a different year, concluding these regions have to be further analysed as they are key to understanding the flexibility of the plant in adapting to a particular environment.

In addition to the phenotypic plasticity, there was variability in growth between blocks that would partly explain a high degree of variability within the data. This natural variability within oilseed rape will have been increased within the TNDH population from the selection of the parents used i.e. a European winter variety and a Chinese semi-winter variety. Moreover, big QTL clusters were identified in different

years, indicating high influence of the environment in QTL expression. These results suggest that more replicates would be desirable when studying QTL in oilseed rape. One of the principal factors influencing the results was the variability experienced with the TNDH population. Limited seed availability in 2005/06 influenced the sampling method, with the result of only one plant being harvested in the first field trial whereas 20 plants were bulked in the second field trial, thus having a more reliable dataset for the second field trial.

Quantitative trait loci for different traits were not detected on the same locations at High N and at Low N. This would suggest that selection of varieties for improved NUE should be carried out under Low N conditions, contrary to other studies in the literature suggesting that traits can effectively be selected at High N treatment without losing any valuable data (Gallais and Bertin, 2004). They detected more QTL at High N than at Low N for traits related to vegetative development, NUpE, grain yield and yield related traits in maize and noted that QTL at Low N were the same as QTL detected at High N for those traits, thus they concluded that QTL at Low N were not treatment specific. A recent paper by Gallais et al. (2008) questioned if the selection of a maize population for yield traits i.e. grain yield and kernel protein content, etc. should be at High N or at Low N. They observed lower heritability thus higher environmental variance at Low N, and therefore that there was a higher efficiency using indirect selection (selecting at High N for lower N-input varieties).

Quantitative trait loci were identified at different regions for different N levels with IM and CIM analyses (Interval Mapping and Composite Interval Mapping respectively). Further analysis of N variation in QTL identification was studied by MCIM (Multiple trait Composite Interval Mapping), to assess QTL x N interactions. When comparing analysis from either IM or CIM with MCIM, the results agreed that the number of N specific QTL was higher than the number of conservative QTL (indifferent of the treatment). Analysis performed by MCIM showed only 6 QTL were commonly identified for both treatments, and that nitrogen specific QTL were the majority of them. The MCIM results showed that other QTL were not influenced by N treatment but by other environmental factors.

Quantitative Trait Loci for some traits were identified at the same location in different trials across years, for example, for flowering on chromosome 19, bract chlorophyll content on chromosome 17, harvest index on chromosomes 7 and 9, stem N concentration on chromosome 9, NUpE on chromosomes 4, 7 and 15, NUE on chromosome 19 and NHI on chromosomes 1 and 7.

The major QTL consensus was on chromosome 7, where 3 different QTL for HI were identified at the same exact locations in 2005/06 and in 2006/07 and for the same N treatment (1 at High N and 2 at Low N). On the same chromosome 7, a QTL for NUpE at Low N was commonly identified in both 2005/06 and

2006/7. The same was found for NHI, when a QTL on chromosome 7 was both identified in 2005/06 and 2006/07 at Low N.

5.4. CANDIDATE GENES

The following objective was set to study candidate genes possibilities:

- to integrate QTL based information for indicating the genetic basis of N metabolism and enable genetic improvement of oilseed rape in terms for NUE;
- to identify candidate genes related to the traits of interest through comparative genomics with *Arabidopsis*.

The chosen method of integrating QTL information was through the candidate gene approach. Eight chromosomal regions with QTL of interest were further investigated and nitrogen related candidate genes were identified. The candidate genes were identified on chromosomes 1, 4, 7, 9, 15, 16 and 19 of oilseed rape. These corresponded with the pseudo-chromosomes At4, At2, At1, At2, At3, At3 and At5 of *Arabidopsis thaliana*, respectively. More than 20 candidate genes were identified from all QTL related to different traits analysed. The final list was brought down to six genes thought to be the more relevant candidates for NUE improvement in oilseed rape. The final genes are NLP7, At4G18810.1, PGM, NTP2, miR393b and GSR1. Four of these genes are involved in regulation mechanisms in relation to nitrate as NLP7, At4G18810.1 and miR393b and nitrate transporter NRT1.1 (gene NTP2). The NLP7 gene functions as a transcription factor and modulates nitrate sensing and metabolism (Castaings et al., 2009). The At4G18810.1 is a transcription repressor involved in regulation of N utilisation, located in chloroplasts and vacuoles (TAIR website). This gene has not been given a name indicating no work related to the gene has been published yet. The NTP2 is also called NRT1.1 is a very relevant gene for NUpE, as it induces lateral root development towards rich N areas in the soil. Most importantly, the transporter would not be activated when nitrate is uniformly spread, confirming a detection mechanism during N deficiency (Remans et al., 2006). The miR393b controls root architecture, by controlling the expression of AFB3 (auxin receptor) in roots, influenced by internal and external nitrate levels (Vidal et al., 2010). The GSR1 gene is most probably involved in NUE at Low N and also related to the NUE component NUpE and it was identified in 3 different QTL for NUE. The enzyme is the product of multiple genes with complex promoters that ensure the expression of the genes in an organ- and tissue-specific manner and in response to a number of environmental variables affecting the nutritional status of the cell. Its activity

is also regulated post-translationally. Therefore, GS and plant nitrogen metabolism is best viewed as a complex matrix continually changing during the development cycle of plants (Miflin and Habash, 2002). Such complexity could explain the difficulty of improving NUE by solely using this gene. That is why plants with the enzyme over-expressed did not show improved NUE (Oliveira et al., 2002).

The phosphoglycerate/bisphosphoglycerate, also called PGM, is a gene belonging to C metabolism and related to starch biosynthesis, but influenced by the nitrate levels of the plant. The gene is highly induced in shoots and to a lower extent in roots under N starvation (Wang et al., 2003).

CHAPTER 6. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1. SUMMARY

Oilseed rape is, in terms of production, currently the second most important oilseed crop worldwide behind soybean. The major areas of productivity are Canada, China, EU and India. In the UK, in terms of area, it is currently the third most important agricultural crop grown, steadily increasing since year 2000. Oilseed rape is grown for different commercial uses, but mainly cultivated to produce vegetable oil both for human consumption and for industry, although some has been grown for biodiesel production. Winter oilseed rape is one of the most profitable UK arable crops, particularly as the major break crop in intensive cereal rotations. However, it is the second most important crop in terms of potential N leaching and greenhouse gas emissions (Teiwes et al., 1996). Excessive N-fertilization and other management practices can potentially lead to high nitrate leaching losses, and adequate fertilising practices are fundamental. Therefore, a better understanding of the genetic basis of nitrogen metabolism, and particularly of NUE, using the QTL approach is an important objective in the improvement of oilseed rape commercial varieties.

The *Brassica napus* population used in this study was the TNDH population, a cross of Ningyou7 (a Chinese semi-winter variety) and Tapidor (a European winter variety), generated *in vitro* by microspore culture as described by Qiu et al. (2006). For this study, 188 TNDH lines were sown in 2005/06 and the population BnaTNDH_4, a subset of 94 TNDH lines, was sown in 2006/07 at the experimental station at Cockle Park Farm, Northumberland, UK. The 94 TNDH lines were a subset from the 188 lines sown the previous year, selected against early flowering (to avoid frost damage) and stem canker.

The map used is the one described in Long et al., (2007). A total of 344 markers, including SSR, RFLP, SNP, MS-AFLP (methylation sensitive-AFLP), and STS markers, were added to the basic linkage map generated with the TN DH population by Qiu et al. (2006). The new linkage map contained 621 markers, spanning 2060 cM with an average interval of 3.3cM between markers. The analysis performed using SMA, IM (IM and CIM and the latter was used to study QTL x environmental interactions.

6.2. CONCLUSIONS

In conclusion, the study of the Tapidor x Ningyou7 doubled haploid population under High N and Low N allowed identification of traits for indirect selection of genotypes with improved response to nitrogen use under low N conditions without compromising yield. In addition, the quantitative trait character

and identification of the underlying loci was elaborated for nitrogen metabolism in oilseed rape at High N and for the first time at Low N. The specific conclusions of this study may be summed up as follows:

- Both NUpE and NUtE are important components influencing oilseed rape NUE. However, NUpE has more influence on NUE than NUtE, as the latter remains less affected by N treatment.
- NUpE is related to total above ground biomass and seed yield under both N treatments; whereas NUtE shares different relationships depending on N treatment. This raises the question of whether large plant size/greater growth rate is the result of efficient recovery of fertiliser N or whether large plants are able to capture a greater amount of soil N.
- Heritabilities for NUE and NUpE were above 50%, whereas NUtE and NHI were 0% under these conditions in this study; therefore, the improvement of NUE by improving mechanisms related to NUpE may potentially be inherited through breeding and could be stable in future generations.
- Despite high variability observed in oilseed rape plants and low QTL reproducibility across years, some QTL proved to be stable and reproducible either within this study or in comparison with other studies in oilseed rape, some of which used the same TNDH population.
- More than 20 genes were identified by syntheny studies with *Arabidopsis*, as potentially responsible for traits of interest. Of these, six genes could be more relevant in relation to NUE and recommended for further analysis to improve NUE in oilseed rape.
- Four out of the six candidate genes predicted to be more relevant to NUE were involved in regulatory mechanisms, such as NLP7, At4G18810.1 and miR393b and nitrate transporter NRT1.1.

6.3. RECOMMENDATIONS

The present study depicts the complexity of N metabolism in oilseed rape. In this study, the TNDH population was used in the North east of England to identify traits related to NUE and N metabolism for selection in the future of varieties exhibiting higher fertiliser use efficiency. Traits were identified at both High and Low N treatments and QTL for these traits were also identified at both N treatments. During this time, this same population has been grown in different locations in China for other flowering, architectural and yield traits. The TNDH population has also been grown in the Midlands, UK, for the

same flowering, architectural and yield traits. To complement this work, it would be interesting to grow the TNDH population in the UK as a spring variety and study if it would have a better performance for example, in flowering. Further to these projects the TNDH population could be analysed in different locations like the ones in China for N traits, particularly for NUE and observe whether the same varieties have a better performance in both parental locations.

Probably, the next step in this project would be to select for segregating markers for the traits of interest from the identified QTL. The selection of markers would allow for the identification of those lines that are positive for the QTL of interest e.g., NUE, yield, oil, etc. Once this has been achieved, these lines could be used for the development of Near Isogenic Lines (NILs). The selection of markers would also allow Marker Assisted Selection (MAS) breeding. Breeding oilseed rape through marker assisted selection can potentially generate new varieties in shorter time and in a more cost-effective manner. That would depend on the kind of markers that have been selected or from which QTL those markers have been selected.

Another future step would be to clone the candidate genes identified as potentially the more relevant to improve NUE in oilseed rape, with the aim of generating transgenic lines more efficient in N use. Once the lines were produced, analyses would be carried out to determine whether the new lines have higher NUpE and/or NUE. If the results are positive, the next step would be to start breeding for new varieties by developing NILs with the genes of interest and to perform Marker Assisted Selection (MAS). The new varieties should go through about two generations of field tests and then to clear biosafety and regulatory issues. Finally, the new varieties could be released. Because this is a very long process, it would take around 10 to 12 years from the candidate genes to the release of the new variety and broad adoption. The longest step would be the regulatory issues and biosafety requirements, which currently take between 6 and 8 years to complete.

Another possible approach for developing new lines would be to develop QTL-NILs, from the lines that contain the QTL of interest with the objective of fine mapping the QTL regions with the trait/s of interest (i.e. NUpE, NUE, seed yield, oil content). To start the breeding process the lines would be backcrossed and used for MAS selection. The lines carrying the traits of interest would go for experimental and field trials and finally, the varietal lines with the desired NUE increase would be released as new varieties. This process would take about 20 years to accomplish, as it has done for some rice varieties like the drought resistant Sub1 (Zeigler. 2009).

Results suggested that the key to improving NUE is to improve N uptake. Thus a more in depth study of oilseed rape root morphology; root functioning and root specific N enzymes and N transporters would

be very valuable. More information on genes that are related to N metabolism is desirable to improve NUE in oilseed rape. Despite candidate genes being identified as related to N metabolism to a greater or lesser degree, the specific function or the mechanisms in which they operate are not very well defined. Therefore, whilst their relationship with N metabolism is certain, their relationships with NUpE, NUE, or their effects on these traits, are not clearly identified. For that reason, further studies of the gene functions and relationships in the N metabolism are desirable.

Results have pointed out the strong relationship between carbon and nitrogen metabolisms and some even suggesting that the control of N metabolism is interrelated with C metabolism. Further work should be centred on providing a better understanding of the interaction of these two metabolic pathways in relation to nitrogen use efficiency. Moreover, efforts should also be placed on assessing oilseed rape response mechanisms under nitrogen deficiency stress in relation to other nutrient deficiencies, but more importantly in relation to water deficiency.

Future research in QTL mapping should focus on developing more powerful statistical tools for identifying QTL and to assess different QTL interactions with the environment as well as epistasis.

This is the first study to identify QTL for Nitrogen Use Efficiency in oilseed rape under two N regimes. Both IM and CIM identified QTL, which will allow future studies to move towards the potential for breeding varieties of oilseed rape with improved efficiency in nitrogen use.

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APPENDIX

Appendix 1. Summary of meteorological data: **A)** monthly means of temperature in °C, **B)** radiation in total hours and **C)** rainfall in mm, for the Northeast of England, between January 1929 and August 2010. Average data for winter (WIN), spring (SPR), summer (SUM), autumn (AUT) and annual averages (ANN) are also presented for each year. Data for the first field trial (season 2005/06) is shaded in green and data for the second field trail (season 06/07) is shaded in purple.

A. Temperature (degree C)

Year	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	WIN	SPR	SUM	AUT	ANN
1970	2.3	1.7	2.9	5.6	11.3	14.4	14.2	15.2	13.4	9.6	5.9	3.6	3.65	6.59	14.55	9.65	8.37
1971	3.5	4	4.4	6.5	10.2	11	15.5	15	13.2	10.3	5.1	5.8	3.96	7.04	14.55	9.56	8.69
1972	2.9	3.1	5.2	7.4	9.7	11.2	14.1	14.4	10.8	9.6	5.2	4.2	3.83	7.45	13.67	8.52	8.13
1973	3.6	3.6	5.9	5.9	10	14.1	14.7	14	13	8.2	4.5	3.5	4.23	7.26	13.09	8.54	8.5
1974	4.6	4.6	4.5	6.1	9.9	12.2	13.9	14.8	11.1	6.8	5.3	6.5	5.16	6.82	14.52	7.71	8.33
1975	5.3	3.5	3.8	7.3	8.1	13	16	14.2	12.2	8.9	5	4.5	4.3	6.4	13.45	8.71	8.79
1976	4.5	3.8	3.8	6.9	10.5	15.5	16.6	17.5	12.2	9.7	4.9	1.1	1.92	7.06	15.49	8.93	8.78
1977	1.4	3.3	5.8	6.2	9	11.2	14.4	15.7	12.2	10.6	5.3	5	2.63	6.99	15.91	9.38	8.24
1978	1.8	0.9	5.8	5.2	10	12.5	13.4	14.1	13	11	7.4	2.6	0.65	7.02	13.25	10.47	8.18
1979	-1.1	0.4	3.5	6.5	8.9	13	14.8	13.9	12.4	10.1	5.5	4.4	3.38	6.29	13.29	9.36	7.74
1980	1.5	4.4	3.5	7.6	9.4	12.9	13.5	13.8	13.7	7.8	5.8	4.7	3.54	6.84	13.92	9.12	8.29
1981	3.5	2.4	6.4	6.5	10.4	12.5	14.6	14.6	13.7	7	6.5	-1	1.4	7.77	13.68	9.06	8.16
1982	1.4	4	5.2	7.7	10.3	13.7	15.1	15.1	13.2	9.2	6.5	3	3.2	7.72	14.07	9.65	8.7
1983	5.4	1	5.7	5.5	8.8	12.7	17.3	14.8	12.5	9.3	6.5	4.7	3.11	6.66	14.55	9.45	8.83
1984	2	2.6	3.8	6.8	8.6	13.1	15.4	16	12.5	10.1	7.1	4.3	2.17	6.39	15.32	9.9	8.54
1985	0.5	1.8	3.8	7.3	9.5	11.2	14.9	16	13.4	10.4	2.9	5	2.04	6.85	14.87	8.93	7.9
1986	2.2	-1.4	4.2	4.8	10.4	13.1	14.8	13.7	10.7	9.7	6.7	4.7	2.63	6.48	13.3	9.04	7.76
1987	0.3	2.9	3.2	9	8.7	11.4	14.7	12.4	12.4	8.5	5.7	5.1	4.38	6.92	13.46	8.88	8.06
1988	4	4	5	7.1	10.2	13.2	14	14.5	12.3	9.4	4.6	6.4	5.64	7.43	13.55	8.77	8.75
1989	5.5	4.9	6.1	5.4	11.3	13.2	16.5	14.6	13.4	10.7	5.7	3.8	5.08	7.65	13.93	9.94	9.37
1990	5.4	6.2	7.6	7.1	11.1	12.7	15.2	15.4	12	10.8	6	3.8	2.48	8.62	15.06	9.61	9.61
1991	2.1	1.4	6.9	6.9	9.9	11.2	16.4	17	13.3	9.3	5.5	3.8	3.89	7.91	15	9.4	8.64
1992	3.1	4.8	6.3	7.7	11.8	14.7	15.1	16.2	12.2	6.7	5.9	2.8	3.88	8.64	14.66	8.26	8.81
1993	4.5	4.5	5.7	8.2	10	13.4	14.3	14.4	11.4	7.5	3.7	3.8	3.16	7.94	14.74	7.55	8.39
1994	3.7	1.8	6.4	7.3	9.1	13.6	16.6	13.4	11.6	9	8.5	5.1	4.36	7.59	13.72	9.65	8.97
1995	3	5.1	4.3	7.7	10.5	12.5	17	14.6	12.6	11.9	6.8	1.5	2.26	7.47	14.94	10.48	9.2
1996	3.3	2	3.1	7.4	8.1	13.1	15.3	17.1	12.6	10.4	4.6	2.4	3.28	6.2	15.59	9.24	8.19
1997	2	5.6	7.4	8	10.1	12.6	15.6	15.7	12.8	9	7.5	5	5.36	8.47	14.69	9.77	9.45
1998	4.2	7	6.9	6.9	11.4	13.1	14.6	17.5	13.5	9.2	4.7	4.4	4.38	8.41	15.29	9.15	9.25
1999	4.5	4.2	6.5	8.5	11.7	12.8	16.3	14.8	14.8	9.9	7	3.2	4.2	8.9	14.17	10.58	9.57
2000	4.3	5.1	6.7	7.1	10.6	13.6	13.9	15	13.7	9.3	5.9	4.4	3.38	8.13	14.71	9.62	9.19
2001	2.5	3.3	3.8	6.5	11.2	12.7	15.9	15.6	12.4	12.5	6.7	3	4.31	7.19	14.36	10.53	8.89
2002	4.4	5.7	6.5	8.2	11.1	13.6	14.9	15.8	13.3	8.7	7	4.6	3.88	8.6	14.83	9.67	9.55
2003	3.9	3	6.6	8.7	11.4	15.1	16.6	16.3	13.5	8.4	7.2	3.9	4.27	8.89	14.96	9.7	9.61
2004	4.2	4.7	5.7	8.8	11.1	14.4	14.8	16.5	13.6	9.7	6.9	4.6	4.54	8.54	16.08	10.04	9.58
2005	5.1	3.8	6.3	7.8	10.4	14.4	15.6	16.4	14.1	12	5.7	3.8	3.7	8.17	15.2	10.6	9.55
2006	3.6	3.6	3.8	7.4	10.9	14.8	18.4	15.2	15.8	11.9	7.3	5.2	5.41	7.39	15.07	11.68	9.87
2007	5.9	5.1	6.3	10.2	10.8	13.6	14.8	15.2	13.2	10.2	6.8	4	4.7	9.06	16.13	10.04	9.66
2008	5.4	4.7	5.2	7	11.6	13.3	15.7	14.9	13	8.9	6.1	3.1	3.15	7.94	14.43	9.34	9.17
2009	2.7	3.7	6.4	8.9	11.2	13.5	15.6	15.8	13.6	10.5	7.3	2.1	1.52	8.83	14.95	10.48	9.34
2010	0.8	1.6	5.5	8.2	9.8	14.2	16.3	16.2						7.84	15.1		

B. Radiation (total hours)

Year	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	WIN	SPR	SUM	AUT	ANN
1970	31.2	96.6	117.5	134.9	177.1	241.2	143.1	168.9	126	99.8	54.5	44.2	143	346	553.3	280.2	1434.9
1971	33	65.8	84.4	105.7	219.6	123.6	206.7	124.9	151.8	116.6	69.5	34	104.4	429.5	455.2	338	1335.7
1972	36.8	33.7	114.8	112.1	157.6	148	153.2	177.2	103.6	88.5	64.4	32.6	164.5	409.7	478.3	256.5	1222.4
1973	36	95.8	114.6	138.5	167.5	209.5	131.9	166.2	126.7	76.8	88.3	54	170.7	384.5	507.6	291.8	1405.9
1974	54.2	62.4	88.8	118.5	198.4	176.4	151.2	172.1	123.1	75.5	57.6	53.7	139	420.6	499.7	256.1	1331.9
1975	41.8	43.5	95.2	115.3	187.4	243.4	182.4	227.4	134.6	90.8	72.7	53.9	147.9	405.8	653.2	298.1	1488.4
1976	48	45.9	98.5	123.1	130.4	240.2	255.5	226.3	83.8	66.9	65	53.8	159.7	397.9	722	215.7	1437.3
1977	50.5	55.4	86.5	157.2	209.3	163.2	166.9	149.5	100.3	92.7	79.1	38.5	134.2	352	479.6	272.1	1349.1
1978	45.8	49.9	113.5	96.1	192.5	164.2	138.8	108.2	123.4	86.2	71.5	21.3	127	453	411.2	281.1	1211.3
1979	50.1	55.6	86.1	107.8	163.2	183.2	165.9	147.8	161.4	93.8	71	47.9	128.2	402	497	326.2	1334
1980	46.4	33.9	83.9	159.2	218.1	146.6	135.5	131.3	127.6	104.4	49.7	45.9	160.9	357.2	413.4	281.7	1282.4
1981	51.9	63.1	63.9	134	167.2	164.9	158.9	170.1	153.9	132.5	57.7	46.3	156.3	461.1	494	344	1364.4
1982	53.5	56.5	145.1	162.1	223.3	123.5	166.4	158.4	149.9	59.8	54.1	44.8	167.9	365.1	448.3	263.8	1397.5
1983	58.6	64.5	84.2	121.6	107.6	160.1	196.2	188.3	111	109.6	43.7	43.7	158.3	530.5		264.2	1289.1
1984	59.5	55.1	48.1	199.3	177.9	181	212.4	313.4	98.5	107.6	44.7	47.7	156.7	425.3	544.6	250.8	1420.5
1985	38	70.9	110.3	110.9	136.5	166	164.1	188.6	116	103.7	84.8	34.6	157.3	357.6	582	304.5	1282
1986	59.9	62.8	123.8	112.8	197.4	197.7	162.5	146.2	184.4	123.2	76.5	48.9	164	434.1	476.3	384.1	1468.7
1987	48	67.1	97.2	144.1	175.3	103	154.7	118.7	157	96.8	49.5	26.8	181.1	416.6	479	303.3	1245.1
1988	47.1	107.2	96.3	112.5	182.1	178	143.6	125.7	142	92.3	88.6	53.2	210.3	390.9	383.4	322.9	1423.6
1989	61	96.1	113.5	121.8	251.8	227.4	245.9	180.7	128.8	91.3	75.7	28.7	159	487.1	502.3	295.9	1660.5
1990	58.2	72.1	131.6	195.1	212.3	114	235.3	218.2	123.2	85.9	51.6	41.4	163.9	539	691.6	260.7	1529.6
1991	73.2	49.3	66.7	147.8	127.6	142.5	185	209	166.4	75.7	57.2	48.5	184.8	342	558.3	299.3	1335.7
1992	58.8	77.5	89.6	119.3	231.6	193.7	161.6	195.9	109.4	84.7	66.4	36.7	128.7	440.6	523.3	260.5	1403.2
1993	43.4	48.6	110.8	103.9	161.7	175.1	166	173.8	88.9	92.7	41.4	48.3	172.5	376.4	529.1	223	1242
1994	62.7	61.5	123.3	168.3	156.3	208.6	203.1	161.2	110.5	94.9	56.5	56	196.8	447.9	502.4	262	1466.8
1995	54.3	86.6	152.7	165.9	193.4	180.9	219.1	165	124.7	125.9	56.4	35.2	129.5	512	576.7	307	1643.8
1996	14.1	80.1	44.9	121.2	183.6	222	208	248.8	111	112.6	83.6	41.1	166.6	349.7	648.8	307.2	1399.7
1997	41.7	83.8	132.1	131.5	215.6	128	205	177.4	156.4	110.9	37.4	31.8	159.4	479.2	607.4	304.8	1460
1998	43.4	84.2	80	114.7	176.3	128.7	145.8	185.6	93.6	109.6	67.2	38.9	197.7	371	518.7	270.4	1247.8
1999	62.7	96.1	105.1	148.7	161.5	160	211.6	165.4	158	104.6	62.8	64.7	236.1	415.2	439.9	325.4	1474.6
2000	75.3	96.1	121.4	129.3	190.2	157.3	127.9	138.8	99.9	100.5	61.1	52.3	219.6	440.9	510.4	261.5	1399.2
2001	73.1	94.2	109.9	124.5	247.7	165.1	173.5	187.8	97.2	109.1	60	65.8	188.8	482.2	473.1	266.3	1490.9
2002	43.5	79.5	121.5	190.6	167.6	162	154.6	170.7	129.7	90.3	49.2	29.8	187.5	479.7	509.3	269.2	1365.6
2003	56.8	100.9	171.3	192.7	186.8	212	179.8	147.3	162.1	129.8	65.2	61.6	199.2	550.7	463.9	357.1	1726.7
2004	45.8	91.8	104.9	116.3	209.8	177.9	163.7	207.7	155.8	92.3	49.2	62.3	191.3	431	599.6	297.3	1428.4
2005	56.4	72.6	66.3	151.8	219.7	185	169.9	158.6	155.2	59	97.8	58.5	166.6	437.7	500.1	312	1477
2006	44.1	64.1	93	171.3	195.8	204.1	295	184.9	162.3	90.4	95.6	62.1	211.7	460.1	539.8	348.2	1617.4
2007	73.2	76.4	137.7	200.2	160.1	134.8	171.7	139.6	148.4	117.5	71.4	43.2	206	497.9	638.8	337.3	1533.3
2008	48.6	114.2	134.6	131.9	211.3	178.2	185	198.9	104.2	126.2	66.2	56.6	168.8	477.9	505.4	296.6	1467.4
2009	53	59.1	162.1	158.5	229.7	191.1	189.3	110.4	136.5	87.3	73.2	65.2	172.5	550.2	473.5	297	1578.4
2010	54	53.3	123.8	169.5	204.3	215.3	151.3	173.4						497.5	553.8		

C. Rainfall (mm)

Year	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	WIN	SPR	SUM	AUT	ANN
1970	82.4	58.6	47.4	73.6	16.5	29.2	75.9	47.7	37.2	51	108.4	57.2	145.9	137.6	171	196.5	701.1
1971	64.6	24.1	61.7	50.9	42.5	61.4	43.8	63.6	19.7	40	74.7	29.7	176.5	155.1	168.8	134.4	628.9
1972	93	53.8	64.1	47.8	61.1	68.4	47.4	115.9	34.4	17.2	73.4	56	112.1	173	221.1	125	641.3
1973	33	23.2	14.9	65.5	66.3	55.7	102	24.6	60	46.4	27.3	50.2	188.1	146.7	140.5	133.7	603.2
1974	78.2	59.6	45.8	18.8	24.4	44.8	73.3	58.8	78.8	101.2	80.6	60.6	156.2	89	216.4	260.6	732.5
1975	72.1	23.5	62.8	64.6	49.4	27.7	59.6	66.4	64.6	26.8	49.5	45.9	137.9	176.7	184.5	140.9	600.7
1976	64.9	27.1	34.9	24.5	82.2	11.4	24.3	54.2	156	138.1	37.7	71.5	266.7	141.7	141.5	331.8	691.8
1977	95.3	99.8	58.4	43	50.6	75.4	19	19.1	28.3	46.7	84.6	69.3	242.7	152.1	54.8	159.7	732.7
1978	97.9	75.5	52.7	42.2	44	57.6	75.7	62	56.2	20.5	43.7	175.7	284.2	138.9	156.5	120.4	819.5
1979	61	47.5	116.9	48	109.4	32.5	31.9	77.7	35.8	64.6	75.8	121.7	269.9	274.3	211	176.2	830.5
1980	77.2	71	90.2	12.3	26.4	123.4	49.6	85.4	36.7	102.6	80.8	54.9	142.6	129	149.8	220.1	826.8
1981	40.1	47.6	122.8	70.5	58.9	36.5	54.8	101.8	102.9	92	59.2	57.8	150.3	252.1	274.7	254.1	785.7
1982	69	23.5	69.9	13.5	35.4	128.7	27.5	42.6	54.3	83.6	85.4	67.7	173.6	118.8	134	223.3	732.3
1983	59.8	46	59	98.3	97.6	33.9	37.5	73.8	77.9	56.2	38.7	105.1	257.8	254.9	230	172.9	735.5
1984	111.3	41.4	70	13.6	43.1	42.9	22.4	25.3	100.9	58.2	128.8	42	128.2	126.7	96.8	287.9	733.7
1985	78.3	7.9	65.2	61.1	71.1	61.1	77.3	59.2	51.8	31.9	78.7	83.6	204.3	197.4	124.5	162.4	761.3
1986	83.5	37.1	55.9	92.7	87.2	35.4	39.5	93.3	21	58.4	66.5	105.7	195.8	235.8	231.6	145.9	801.3
1987	42.5	47.6	82.3	59.9	41.9	93.4	72.4	118.3	57.9	113.4	61.6	47	201.2	184.1	193.2	233	800.6
1988	101	53.3	74.2	31.4	54	31.1	129.5	80.7	45.9	76.8	57.2	34.4	114.9	159.6	246.5	179.9	751.7
1989	25	55.5	57.7	63.1	16.7	56.2	28.7	63	22.8	60.1	37.7	79.5	260.3	137.5	223.6	120.5	552.7
1990	83.9	97	20	20.8	35.5	69.4	29.8	49.8	40.9	72.9	53.5	98.5	243.8	76.3	134.7	167.3	666.5
1991	62.7	82.7	57.5	38	16	62	36.4	44.4	47.5	54.6	76	49.6	121.7	111.5	143.5	178.1	605.9
1992	38	34.1	82.3	68.1	27.8	26.5	81.2	23	90.2	72.8	84.2	59.6	152.5	178.2	121.4	247.1	752.5
1993	75.7	17.2	16.9	99.8	79.4	41.4	58.9	87.7	111.7	75	67.3	101.3	257	196.1	195.4	253.9	813.5
1994	95.7	60	60.4	52.4	37	26.6	49	69	86.5	63.4	68.9	95.9	279.2	149.8	169.4	218.9	755.5
1995	104.4	78.9	50.6	25.9	42.8	25	24.7	59.5	97	33.1	80	69.9	180.9	119.3	135.2	210.1	640.8
1996	41.1	69.8	24.6	39	41.9	24	40.6	8.5	23	53.7	97	79.7	179.1	105.6	58.3	173.7	598.5
1997	15	84.3	23.6	17.5	60.4	144.2	61.2	63.9	23	43.2	72.9	95.4	203.7	101.5	128.5	139	704.7
1998	93.6	14.7	68.8	118.9	52.8	105.8	58.6	64.1	57.7	114.2	71.2	59.3	186.2	240.5	269.5	243	862.6
1999	94.3	32.6	89.5	56.4	53.8	81.7	22.1	47	76.7	66.2	53.3	92.2	192.8	199.6	211.4	196.2	801.7
2000	47.5	53	30.6	135	72.3	57.4	56.3	82.8	109.8	119	147.1	85.9	226.1	237.8	186.6	375.8	972.6
2001	44	96.2	52.4	87.7	25.7	56.4	59	58.9	94.4	92	50.9	48.9	211.6	165.8	172.6	237.2	786.1
2002	58.1	104.6	40.1	31.9	62.4	54.1	92.2	78.6	31.1	123.9	107	109.1	216.2	134.4	194	262	903
2003	82.8	24.3	27.9	31.7	67.5	84.1	53.1	88.6	43.8	46.6	58.5	77.1	236.4	127.1	234.9	148.9	614.7
2004	106.9	52.4	41.9	84	30.1	73.3	81.4	17.4	35.9	126.4	35.4	37.9	161.1	155.9	154.6	197.7	872.3
2005	61.7	61.5	48	77.9	43.6	50	77.9	166.6	62	89.3	71.9	45.6	124.2	169.5	321.4	223.3	752.4
2006	29.1	49.5	85.9	38.7	96.2	18.7	30.3	63	73.2	82.9	67.1	88.7	244.7	220.8	190.9	223.2	767.9
2007	84.3	71.7	39.3	10	77.1	181.8	113.6	107.7	40.2	31	61	71.8	242.2	126.3	156.6	132.2	825
2008	137.1	33.3	75.8	77.3	25.5	75.9	106.2	43.2	108.2	74.2	67.3	59.7	171.2	178.5	338.6	249.7	951.5
2009	61.8	49.6	31.3	29.6	51.9	55.4	133.5	111	31.4	61.8	147.5	87.4	228.7	112.8	293	240.7	803.6
2010	69.5	71.7	72.3	23.6	22.4	44.3	62.7	62.3						118.3	251.2		

Appendix 2. Summary of management inputs applied: name of product, use, timing of application and rate for A) growing season 2005/06 and B) growing season 2006/07.

A. Field Inputs 2005/06

Name of product	Use	Timing	Dose	Application rate	Comments
NPK fertiliser	Feriliser	16/03/2006	1	50 kg/ha	To all Blocks
		29/04/2006	2	150 kg/ha	To High N Blocks
Slug pellets	Slug pest control	07/09/2005	3	3.75 kg/ha	To all Blocks
Katamaran	Grass weed control	07/09/2005	1	2.5 L/ha	To all Blocks
Caramba	Light leaf spot control	31/10/2005	1	1 L/ha	To all Blocks
Hallmark zeon	Cabbage stem flea beetle	31/10/2005	1	35 mL/ha	To all Blocks
	Pollen beetle control	20/04/2006	1	40 mL/ha	To all Blocks

B. Field Inputs 2006/07

Name of product	Use	Timing	Dose	Application rate	Comments
NPK fertiliser	Feriliser	30/03/2007	1	50 kg/ha	To High N Blocks
		18/05/2007	2	100 kg/ha	To High N Blocks
Slug pellets	Slug pest control	13/09/2006	3	3.75 kg/ha	To all Blocks
Punch C	Light leaf spot control and phoma	23/10/2006	1	0.4 L/ha	To all Blocks
		29/11/2006	1	0.4 L/ha	To all Blocks
Hallmark zeon	Cabbage stem flea beetle	31/09/2006	1	35 mL/ha	To all Blocks
	Pollen beetle control	11/04/2007	1	40 mL/ha	To all Blocks

Appendix 3. Average % germination rate for all Blocks in 2005/06.

Line	%	Line	%	Line	%	Line	%	Line	%	Line	GR%
Ningyou7	62.50	**TN31**	75.00	**TN64**	66.67	**TN97**	64.00	**TN130**	70.83	**TN163**	70.83
Tapidor	75.00	**TN32**	62.50	**TN65**	70.83	**TN98**	56.00	**TN131**	54.17	**TN164**	62.50
TN1	69.57	**TN33**	66.67	**TN66**	53.85	**TN99**	58.33	**TN132**	45.83	**TN165**	70.83
TN2	62.50	**TN34**	75.00	**TN67**	45.83	**TN100**	66.67	**TN133**	41.67	**TN166**	62.50
TN3	70.83	**TN35**	56.00	**TN68**	66.67	**TN101**	70.83	**TN134**	54.17	**TN167**	83.33
TN4	83.33	**TN36**	68.18	**TN69**	83.33	**TN102**	70.83	**TN135**	58.33	**TN168**	66.67
TN5	60.00	**TN37**	58.33	**TN70**	60.00	**TN103**	69.23	**TN136**	62.50	**TN169**	60.71
TN6	58.33	**TN38**	70.83	**TN71**	66.67	**TN104**	62.50	**TN137**	75.00	**TN170**	79.17
TN7	75.00	**TN39**	58.33	**TN72**	76.00	**TN105**	45.83	**TN138**	58.33	**TN171**	83.33
TN8	66.67	**TN40**	75.00	**TN73**	75.00	**TN106**	50.00	**TN139**	69.57	**TN172**	58.33
TN9	75.00	**TN41**	83.33	**TN74**	75.00	**TN107**	79.17	**TN140**	50.00	**TN173**	50.00
TN10	50.00	**TN42**	48.00	**TN75**	72.00	**TN108**	79.17	**TN141**	58.33	**TN174**	76.92
TN11	59.09	**TN43**	50.00	**TN76**	78.26	**TN109**	68.00	**TN142**	70.83	**TN175**	66.67
TN12	52.00	**TN44**	75.00	**TN77**	60.00	**TN110**	75.00	**TN143**	79.17	**TN176**	70.83
TN13	66.67	**TN45**	83.33	**TN78**	50.00	**TN111**	66.67	**TN144**	66.67	**TN177**	60.00
TN14	54.17	**TN46**	68.00	**TN79**	58.33	**TN112**	75.00	**TN145**	70.83	**TN178**	64.00
TN15	79.17	**TN47**	62.50	**TN80**	70.83	**TN113**	90.91	**TN146**	75.00	**TN179**	66.67
TN16	62.50	**TN48**	66.67	**TN81**	58.33	**TN114**	58.33	**TN147**	41.67	**TN180**	70.83
TN17	58.33	**TN49**	58.33	**TN82**	62.50	**TN115**	58.33	**TN148**	66.67	**TN181**	75.00
TN18	62.50	**TN50**	72.00	**TN83**	56.00	**TN116**	54.17	**TN149**	86.96	**TN182**	77.27
TN19	20.83	**TN51**	50.00	**TN84**	75.00	**TN117**	45.83	**TN150**	62.50	**TN183**	75.00
TN20	79.17	**TN52**	58.33	**TN85**	75.00	**TN118**	54.17	**TN151**	79.17	**TN184**	57.69
TN21	54.17	**TN53**	64.29	**TN86**	66.67	**TN119**	70.83	**TN152**	79.17	**TN185**	87.50
TN22	52.17	**TN54**	66.67	**TN87**	79.17	**TN120**	73.91	**TN153**	37.50	**TN186**	66.67
TN23	83.33	**TN55**	69.23	**TN88**	60.00	**TN121**	66.67	**TN154**	79.17	**TN187**	66.67
TN24	66.67	**TN56**	58.33	**TN89**	66.67	**TN122**	75.00	**TN155**	70.83	**TN188**	75.00
TN25	50.00	**TN57**	56.00	**TN90**	56.52	**TN123**	54.17	**TN156**	50.00		
TN26	75.00	**TN58**	73.91	**TN91**	70.83	**TN124**	79.17	**TN157**	75.00		
TN27	45.83	**TN59**	50.00	**TN92**	62.50	**TN125**	83.33	**TN158**	79.17		
TN27	66.67	**TN60**	58.33	**TN93**	83.33	**TN126**	100.00	**TN159**	72.00		
TN28	69.57	**TN61**	41.67	**TN94**	79.17	**TN127**	68.00	**TN160**	47.83		
TN29	58.33	**TN62**	76.00	**TN95**	56.00	**TN128**	79.17	**TN161**	54.17		
TN30	79.17	**TN63**		**TN96**	41.67	**TN129**	83.33	**TN162**	64.00		

Appendix 4. Average % germination rates for all Blocks in 2006/07.

TN line	%	TNDH line	%	TNDH line	%
Ningyou7	90	**126**	100	**129**	95
Tapidor	80	**51**	100	**135**	97.5
1	95	**57**	100	**138**	92.5
3	90	**60**	90	**140**	95
4	97.5	**63**	95	**141**	72.5
5	100	**64**	97.5	**142**	97.5
6	97.5	**66**	97.5	**144**	97.5
7	100	**69**	90	**145**	37.5
8	60	**72**	90	**147**	97.5
10	100	**75**	92.5	**149**	97.5
11	100	**76**	95	**152**	100
12	90	**77**	95	**157**	90
16	65	**78**	100	**158**	100
17	90	**81**	95	**159**	97.5
18	95	**82**	92.5	**160**	82.5
19	100	**85**	87.5	**161**	90
24	97.5	**87**	27.5	**163**	100
28	95	**88**	92.5	**170**	92.5
29	100	**89**	70	**171**	92.5
30	77.5	**90**	97.5	**172**	97.5
31	80	**93**	97.5	**173**	77.5
32	92.5	**97**	80	**174**	90
33	95	**98**	95	**175**	95
34	97.5	**103**	97.5	**176**	100
38	85	**108**	97.5	**177**	100
39	85	**109**	82.5	**178**	97.5
40	92.5	**112**	92.5	**180**	92.5
44	97.5	**114**	97.5	**181**	100
45	90	**115**	30	**190**	85
47	92.5	**121**	97.5	**192**	90
48	95	**124**	95	**198**	92.5
49	95	**128**	90	**200**	90

Appendix 5. Stem canker table results from Block 1 on 3 different plants P1, P2 and P3 in 2005/06.

Lines	P1	P2	P3	A	Lines	P1	P2	P3	A	Lines	P1	P2	P3	A	Lines	P1	P2	P3	A	Lines	P1	P2
NY7	2	1	1	1.33	**TN41**	3	2	3	2.67	**TN81**	1	2	1	1.33	**TN121**	1	1	1	1.00	**TN161**	1	2
T	1	1	1	1.00	**TN42**	2	1	1	1.33	**TN82**	1	1	2	1.33	**TN122**	1	2	1	1.33	**TN162**	3	2
TN3	1	2	2	1.67	**TN43**	2	2		2.00	**TN83**	2	2	2	2.00	**TN123**	2	1	1	1.33	**TN163**	1	2
TN4	2	2	2	2.00	**TN44**	2	1	1	1.33	**TN84**	2	2	2	2.00	**TN124**	3	3	3	3.00	**TN164**	3	3
TN5	2	2	2	2.00	**TN45**	2	2	2	2.00	**TN85**	1	1	1	1.00	**TN125**	2	2	3	2.33	**TN165**	2	3
TN6	3	3	3	3.00	**TN46**	3	2	3	2.67	**TN86**	3	2	2	2.33	**TN126**	2	2	2	2.00	**TN166**	1	2
TN7	1	2	2	1.67	**TN47**	3	2	3	2.67	**TN87**	3	3	3	3.00	**TN127**	1	1	1	1.00	**TN167**	2	3
TN8	2	2	1	1.67	**TN48**	2	2		2.00	**TN88**	1	1	1	1.00	**TN128**	1	1	2	1.33	**TN168**	1	1
TN9	3	1	1	1.67	**TN49**	1	small		1.00	**TN89**	2	2	2	2.00	**TN129**	3	2	3	2.67	**TN169**	4	4
TN10	2	2	2	2.00	**TN50**	1	1	1	1.00	**TN90**	2	3	3	2.67	**TN130**	2	3	3	2.67	**TN170**	3	3
TN11	2	1	2	1.67	**TN51**	2	1	1	1.33	**TN91**	2	1	2	1.67	**TN131**	1	2		1.50	**TN171**	1	2
TN12	3	3	2	2.67	**TN52**					**TN92**	2	3		2.50	**TN132**	4	3	3	3.33	**TN172**	3	2
TN13	1	2	2	1.67	**TN53**	2	2		2.00	**TN93**	1	2	1	1.33	**TN133**	2	2	2	2.00	**TN173**	1	1
TN14	2	3	3	2.67	**TN54**	2	1	2	1.67	**TN94**	2	3	2	2.33	**TN134**	1			1.00	**TN174**	3	2
TN15	1	1	1	1.00	**TN55**	3	3	2	2.67	**TN95**	1	1		1.00	**TN135**	3	2	1	2.00	**TN175**	2	2
TN16	1	2	1	1.33	**TN56**	1	1	1	1.00	**TN96**	2	3	2	2.33	**TN136**	3	2	2	2.33	**TN176**	1	2
TN17	1	2	3	2.00	**TN57**	2	2		2.00	**TN97**	3	3	2	2.67	**TN137**	1	2	2	1.67	**TN177**		
TN18	3	2	2	2.33	**TN58**	2			2.00	**TN98**	2	2	2	2.00	**TN138**	2	2	2	2.00	**TN178**	3	3
TN19	2			2.00	**TN59**	2	2	2	2.00	**TN99**	2	2	2	2.00	**TN139**	2	2	1	1.67	**TN179**	1	1
TN20	1	2	2	1.67	**TN60**	1	2	3	2.00	**TN100**	3	2	2	2.33	**TN140**	2			2.00	**TN180**	2	2
TN21	3	2	2	2.33	**TN61**	1			1.00	**TN101**	1	1	1	1.00	**TN141**	1	1	1	1.00	**TN181**	3	3
TN22	1	1	2	1.33	**TN62**	2	2	1	1.67	**TN102**	2	3	2	2.33	**TN142**	2	2	2	2.00	**TN182**	2	1
TN23	2	2	3	2.33	**TN63**	2	2	2	2.00	**TN103**	3	3	3	3.00	**TN143**	3	3	2	2.67	**TN183**	3	2
TN24	2	2	2	2.00	**TN64**					**TN104**	2	2	3	2.33	**TN144**	2			2.00	**TN184**	3	2
TN25	2			2.00	**TN65**	2	2	2	2.00	**TN105**	2	1	2	1.67	**TN145**	3	2	2	2.33	**TN185**	1	2
TN26	3	3	2	2.67	**TN66**	2	2	3	2.33	**TN106**	1	1		1.00	**TN146**	2	1	1	1.33	**TN186**		
TN27	2			2.00	**TN67**	1	1	1	1.00	**TN107**	2	2		2.00	**TN147**	1	2	1	1.33	**TN187**	2	3

TN28	3			3.00	**TN68**	2	1	2	1.67	**TN108**	3	3	2	2.67	**TN148**	1	1	2	1.33	**TN188**	2	2
TN29	3	2	3	2.67	**TN69**	2	3	3	2.67	**TN109**					**TN149**	1	2		1.50	**control**	1	2
TN30	1	1	1	1.00	**TN70**	3	2	3	2.67	**TN110**	2	3	3	2.67	**TN150**	2	2	2	2.00	**control**	1	2
TN31	2	3		2.50	**TN71**					**TN111**	1	2	1	1.33	**TN151**	1			1.00	**control**	1	1
TN32	2	2	3	2.33	**TN72**	3	3	2	2.67	**TN112**	2	2	2	2.00	**TN152**	2	1	1	1.33	**control**	1	2
TN33	2	2	2	2.00	**TN73**	3			3.00	**TN113**	2			2.00	**TN153**	2	2	3	2.33	**control**	1	1
TN34	2	2		2.00	**TN74**	2	2	1	1.67	**TN114**	1	1		1.00	**TN154**	2	2	1	1.67	**control**	1	2
TN35	2	1	1	1.33	**TN75**	2	2	2	2.00	**TN115**	3			3.00	**TN155**	3	2		2.50			
TN36	2	2	1	1.67	**TN76**	3	3	3	3.00	**TN116**	2	2	3	2.33	**TN156**	1	2	2	1.67			
TN37	2			2.00	**TN77**	1	2	2	1.67	**TN117**	2	2	1	1.67	**TN157**	3	1	2	2.00			
TN38	2	1		1.50	**TN78**	2	1	1	1.33	**TN118**	3	2	2	2.33	**TN158**	1			1.00			
TN39	2	2	2	2.00	**TN79**	2	2	2	2.00	**TN119**	2	2	2	2.00	**TN159**	4	3	3	3.33			
TN40	2	1		1.50	**TN80**	1	2	1	1.33	**TN120**	2	2	3	2.33	**TN160**	3	3	2	2.67			

Appendix 6. Pearson's correlation between all traits in Block 1 analysed in 2005/06 and stem canker measurements taken from Block 1.

	CANKER	
TL	0.02	ns
FL	0.12	ns
BN	-0.02	ns
F-DAS	-0.03	ns
TP	0.05	ns
%F	0.00	ns
TW	-0.02	ns
SY	0.02	ns
HI	0.11	ns
TSW	-0.03	ns
SN/P	0.06	ns
CB	0.13	ns
CL	-0.04	ns
OIL	0.03	ns
[Nplant]	-0.07	ns
[Nseed]	0.12	ns
[total]	0.08	ns
NUpE	0.04	ns
NUtE	0.10	ns
NUE	0.07	ns
NHI	0.18	ns

Appendix 7. Summary table for Block 1 (High N), traits from 2005/06.

BLOCK1	TL	FL	BN	F-DAS	TP	%F	PMF	%FM	TW	SY	HI	TSW	SN/P	CB	CL	OIL	[Nplant]	[Nseed]	[total]	Nplant	Nseed	total	NUpE	NUtE	NUE	NHI
Mean	118.5	27.7	6.8	239.6	56.8	65.6	40.5	81.2	47.4	12.1	0.25	3.8	13.4	54.9	48.3	53.1	5.3	26.2	31.5	1172	317	1490	1.4	8.0	11.4	0.21
Standard Error	1.2	1.2	0.2	0.4	1.3	0.8	1.1	1.1	2.0	0.6	0.00	0.0	0.3	0.6	0.6	0.2	0.1	0.2	0.2	54	17	71	0.1	0.1	0.6	0.00
Standard Deviation	15.6	14.6	2.2	5.6	14.9	9.8	12.4	12.8	25.5	7.3	0.04	0.6	4.2	7.9	7.3	2.5	1.1	2.6	2.9	627	202	820	0.8	1.6	7.0	0.04
Range	83	66	13	34	102	53.6	63	78.6	156.3	47.6	0.33	3.4	31.7	40.1	34.4	12.6	7.8	19.1	19.0	3950	1354	5257	5.0	9.5	44.5	0.23
Maximum	155	66	13	260	120	94.5	66	100	157.9	48.0	0.42	5.7	32.5	74.3	67.6	57.3	7.8	36.8	41.7	4120	1374	5494	5.2	12.7	45.3	0.30
Count	160	160	160	171	134	159	135	135	159	160	160	160	157	171	171	157	133	133	133	133	133	133	133	133	133	133
NINGYOU7	**95**	**6**	**3**	**234**	**133**	**73.7**	**0**	**0**	**11.2**	**2.2**	**0.19**	**4.1**	**5.4**	**52.5**	**62.3**	**46.3**	**4.7**	**22.8**	**27.5**	**257**	**49**	**307**	**0.3**	**7.0**	**2.0**	**0.16**
TAPIDOR	**136**	**47**	**8**	**242**	**474**	**67.1**	**54**	**84.4**	**58.8**	**12.9**	**0.22**	**3.4**	**12.1**	**62.4**	**65.1**	**55.5**	**4**	**30.1**	**34.1**	**1615**	**389**	**2004**	**1.9**	**6.4**	**12.2**	**0.19**

Appendix 8. Summary table for Block 2 (Low N), traits from 2005/06.

BLOCK2	TL	FL	BN	F-DAS	TP	%F	TM	%FM	TW	SY	HI	TSW	SN/P	CB	CL	OIL
Mean	91.2	24.8	4.3	240.9	165.2	64.4	43.9	72.9	20.5	5.7	0.64	3.8	12.1	47.2	40.3	53.3
Standard Error	1.3	1.3	0.1	0.4	7.0	1.5	1.2	1.7	1.0	0.5	0.23	0.1	0.4	0.8	0.6	0.3
Standard Deviation	15.9	15.2	1.6	5.5	83.7	18.1	12.1	17.0	12.6	6.6	2.81	0.6	4.7	9.0	6.4	3.1
Range	82	61	7	30	404	88.64	58	92.1	78.6	71.0	29.76	3.0	26.3	65.3	33.1	21.0
Maximum	135	61	8	259	432	88.89	71	100	78.6	71.0	29.85	5.6	26.5	66.9	56.1	59.1
Count	146	146	146	171	145	146	99	100	146	146	145	135	134	112	112	156
NINGYOU7	**93**	**1**	**4**	**331**	**280**	**78.9**	**0**	**0**	**41.9**	**8.0**	**0.19**	**4.0**	**7.2**	**45.0**	**35.3**	
TAPIDOR	**100**	**40**	**6**	**241**	**250**	**72.4**	**53**	**77.4**	**24.5**	**6.5**	**0.26**	**3.5**	**7.5**	**51.0**	**39.1**	**49.3**

Appendix 9. Summary table for Block 3 (Low N), traits from 2005/06.

BLOCK3	*TL*	*FL*	*BN*	*F-DAS*	*TP*	*%F*	*TPM*	*%FM*	*TW*	*SY*	*HI*	*TSW*	*SN/P*	*CB*	*CL*	*OIL*	*[Nplant]*	*[Nseed]*	*[total]*	*Nplant*	*Nseed*	*total*	*NUpE*	*NUtE*	*NUE*	*NHI*
Mean	111.9	26.2	6.0	240.3	305.7	67.1	50.3	71.3	41.4	10.5	0.25	3.5	14.7	49.2	42.4	50.7	4.6	26.9	31.5	955	264	1220	5.0	7.9	40.3	0.21
Standard Error	1.4	1.3	0.2	0.4	15.6	1.1	1.6	1.9	2.4	0.7	0.00	0.0	0.6	0.6	0.5	0.2	0.1	0.3	0.3	55	17	71	0.3	0.2	2.6	0.00
Standard Deviation	17.0	16.7	2.3	5.2	193.5	13.5	16.7	19.4	28.7	8.1	0.06	0.6	7.6	7.2	6.4	2.9	1.4	3.4	3.9	631	200	818	3.3	2.4	29.4	0.05
Range	88	62	12	27	1462	90.7	93	94.2	195.8	57.1	0.61	2.8	48.7	36.8	40.6	13.7	7.1	20.7	24.1	3678	1186	4535	18.4	22.2	164.2	0.48
Maximum	148	62	14	257	1541	91.4	104	94.4	200.2	57.4	0.69	5.0	49.1	68.5	67.2	57.4	9.0	41.3	48.6	3769	1200	4730	19.2	22.4	164.4	0.55
Count	153	153	153	169	153	153	109	109	146	148	146	148	139	170	170	142	131	131	131	131	131	131	132	132	131	131
NINGYOU7	died	d	d	233	d	d	d	d	d	d	d	d	d	46.4	44.8	d	d	d	d	d	d	d	d	d	d	d
TAPIDOR	102	24	5	240					20.8	5.7	0.27	3.2		39.2	28.6	51.8	5.5	25.3	30.8	496	144	640	2.6	0.2	0.5	0.23

Appendix 10. Summary table for Block 4 (High N), traits from 2005/06.

BLOCK4	TL	FL	BN	F-DAS	TP	%F	TM	%FM	TW	SY	HI	TSW	SN/P	CB	CL	OIL
Mean	93.2	14.9	5.0	243.9	237.6	68.2	45.4	73.2	0.1	8.6	0.26	3.8	14.1	51.8	44.9	52.0
Standard Error	1.3	0.9	0.2	0.4	11.6	1.0	1.5	1.9	0.0	0.6	0.00	0.1	0.7	0.7	0.6	0.2
Standard Deviation	16.7	11.2	2.0	5.8	147.6	13.3	13.8	17.5	1.8	7.0	0.05	0.6	8.0	7.2	6.4	2.8
Range	122	59	14	36	1465	65.69	59	86.8	219.4	64.8	0.26	4.6	35.7	35.0	43.2	14.2
Maximum	132	59	14	260	1465	89.90	78	98.0	222.2	64.8	0.37	6.1	37.2	69.2	64.9	57.7
Count	162	162	162	168	162	162	85	85	65306	141	140	145	119	115	114	141
NINGYOU7	79	0	2	247	171	49.7	0	0	5.5	0.6	0.11	4.4	0.8			48.2
TAPIDOR	84	11	5	258	310	64.5	0	0	32.9	4.6	0.14	2.3	6.4	59.3	61.6	55.0

Appendix 11. Summary table for Block 1 (Low N), traits from 2006/07.

BLOCK1	*TW*	*HI*	*SY*	*F-DAS*	*FD*	*[SN]*	*[STN]*	*[PN]*	*[TotalN]*	*SN*	*PlantN*	*TotalN*	*NUpE*	*NUtE*	*NUE*	*NHI*
Mean	612	0.31	182.4	204.7	20.1	25.8	3.3	4.3	33.4	464	156	2020	6.6	9.5	59.4	0.24
Standard Error	41	0.01	11.4	1.0	0.8	0.3	0.1	0.1	0.4	29	11	137	0.4	0.2	3.7	0.00
Standard Deviation	392	0.06	109.6	10.0	7.6	2.8	0.9	1.0	3.6	278	104	1306	4.2	2.2	35.7	0.05
Range	1508	0.37	447.0	68.0	51.0	17.4	3.8	5.6	20.6	1202	452	5216	16.9	12.1	145.1	0.30
Maximum	1545	0.51	457.8	232.0	58.0	34.6	5.5	7.2	43.7	1227	461	5335	17.3	15.9	148.6	0.42
Count	92	92	92	94	94	91	91	91	91	91	91	91	91	91	91	91
NINGYOU7	**452**	**0.27**	**139.5**	**164**	**41**	**27.7**	**1.5**	**3.7**	**32.9**	**647**	**218**	**2824**	**9.2**	**8.3**	**75.8**	**0.23**
TAPIDOR	**858**	**0.31**	**233.5**	**216**	**16**	**22.2**	**2.3**	**4.7**	**29.2**	**310**	**101**	**1319**	**4.3**	**10.6**	**45.3**	**0.23**

Appendix 12. Summary table for Block 2 (High N), traits from 2006/07.

BLOCK2	*TW*	*HI*	*SY*	*F-DAS*	*FD*	*[SN]*	*[STN]*	*[PN]*	*[TotalN]*	*SN*	*PlantN*	*TotalN*	*NUpE*	*NUtE*	*NUE*	*NHI*
Mean	1578	0.33	522.8	205.6	24.4	31.0	6.0	7.4	44.3	1628	540	7030	3.6	7.6	27.0	0.2
Standard Error	61	0.00	21.6	1.0	0.7	0.3	0.2	0.2	0.5	69	23	288	0.2	0.2	1.1	0.0
Standard Deviation	572	0.05	201.9	9.5	6.5	2.5	1.7	2.0	4.4	629	209	2641	1.4	1.4	10.2	0.0
Range	2939	0.33	1005.3	50.0	48.0	12.1	9.2	9.1	19.1	2897	1100	13757	7.1	8.2	52.0	0.3
Maximum	3336	0.49	1114.8	226.0	45.0	38.2	12.5	12.6	55.4	3278	1221	15347	7.9	11.1	57.7	0.4
Count	87	87	87	94	94	84	84	83	84	84	84	84	83	83	83	83
NINGYOU7	died	d	d	182	29	d	d	d	d	d	d	d	d	d	d	d
TAPIDOR	1509	0.27	413.5	219	25	35.2	6.4	11.2	52.8	1456	651	7966	4.1	5.2	21.4	0.2

Appendix 13. Summary table for Block 3 (High N), traits from 2006/07.

BLOCK3	*TW*	*HI*	*SY*	*F-DAS*	*FD*	*[SN]*	*[STN]*	*[PN]*	*[TotalN]*	*SN*	*PlantN*	*TotalN*	*NUpE*	*NUtE*	*NUE*	*NHI*
Mean	1373	0.35	489.3	203.9	24.7	28.3	4.4	5.3	37.7	1358	376	5116	2.7	9.5	24.9	0.27
Standard Error	67	0.00	23.7	1.1	0.7	0.3	0.1	0.2	0.5	59	18	231	0.1	0.3	1.1	0.01
Standard Deviation	615	0.04	216.2	10.8	7.0	2.5	1.0	1.3	4.1	492	149	1929	1.0	2.2	9.3	0.05
Range	4311	0.30	1563.3	66.0	45.0	12.7	5.2	8.4	26.6	2303	680	8430	4.4	17.7	46.0	0.49
Maximum	4512	0.46	1612.5	223.0	48.0	34.8	7.5	9.8	52.1	2474	763	9859	5.1	20.7	48.6	0.60
Count	84	83	83	94	94	70	68	68	70	70	70	70	68	68	68	68
NINGYOU7	540	0.35	191	208	24	26	5.3	5.7	37	497	150	1999	1.0	9.6	9.9	0.25
TAPIDOR	1168	0.33	381.4	222	17	28.1	3.7	6.4	38.2	1072	339	4463	5.8	8.5	49.4	0.24

Appendix 14. Summary table for Block 4 (Low N), traits from 2006/07.

BLOCK4	*TW*	*HI*	*SY*	*F-DAS*	*FD*	*[SN]*	*[STN]*	*[PN]*	*[TotalN]*	*SN*	*PlantN*	*TotalN*	*NUpE*	*NUtE*	*NUE*	*NHI*
Mean	1342	0.30	403.0	204.8	23.6	27.8	4.8	6.4	38.9	1065	396	5021	16.3	7.8	125.4	0.21
Standard Error	45	0.00	14.4	1.1	0.7	0.3	0.1	0.2	0.4	36	13	165	0.5	0.2	4.3	0.00
Standard Deviation	429	0.04	138.4	10.5	6.8	2.7	1.1	1.5	3.8	332	124	1522	4.9	1.5	39.4	0.03
Range	2501	0.23	808.0	46.0	38.0	17.9	5.8	6.7	21.3	1858	662	8127	26.4	9.0	189.5	0.19
Maximum	2968	0.39	925.0	222.0	48.0	36.9	8.1	10.2	50.6	2243	799	9933	32.2	12.3	227.5	0.32
Count	92	92	92	94	94	86	85	86	85	86	85	85	85	85	85	85
NINGYOU7	**455**	**0.31**	**158.5**	**181**	**45**	**27**	**3**	**5.9**	**35.9**	**1179**	**394**	**5124**	**16.6**	**8.5**	**141.8**	**0.23**
TAPIDOR	**1427**	**0.35**	**436.8**	**210**	**21**	**27.6**	**4.7**	**7**	**39.3**	**437**	**135**	**1789**	**5.8**	**8.9**	**51.5**	**0.24**

Appendix 15. Nitrogen analysis data of control variety Castille analysed in 2005/06 and 2006/07. For the first field trial, 6 control plants were sampled and analysed and for the second field trial 2 bulks of 20 plants were sampled and analysed.

	seed N	stem N	pod N	total N	NUpE	NHI
2005/06						
block1	3.18	0.54	\	3.7	17.32	0.85
block1	2.74	0.6	\	3.3	12.63	0.82
block1	2.62	0.48	\	3.1	13.64	0.85
block1	2.44	0.46	\	2.9	10.35	0.84
block1	2.37	0.63	\	3.0	10.71	0.79
block1	2.23	0.49	\	2.7	9.71	0.82
block3	2.14	0.53	\	2.7	32.68	0.80
block3	2.18	0.49	\	2.7	51.61	0.82
block3	2.05	0.55	\	2.6	42.57	0.79
block3	2.41	0.43	\	2.8	36.99	0.85
block3	2.26	0.44	\	2.7	32.99	0.84
block3	2.46	0.48	\	2.9	35.29	0.84
2006/07						
block1	1.95	0.39	0.39	3185.9	1034.39	0.71
block1	2.18	0.42	0.34	2257.9	733.09	0.74
block2	2.53	0.44	0.75	5289.1	273.76	0.68
block2	2.47	0.54	0.97	4191.3	216.94	0.62
block3	2.86	0.38	0.6	1933.4	100.07	0.74
block3	2.71	0.41	0.69	3463.3	179.26	0.71
block4	2.66	0.49	0.74	6937.8	2252.54	0.68
block4	2.42	0.49	0.55	8624.9	2800.30	0.70

Appendix 16. Additive heritability for the QTL identified by MIM for the different traits analysed in 2005/06.

TRAIT	High N	Low N
	Block 1	Block 3
FDAS	0.05	0.04
TL	0.05	0.06
FL	0.32	0.22
BN	0.13	0.12
CB	0.26	0.13
CL	0.21	0.24
TW	0.14	0.04
SY	0.06	0.08
HI	0.27	0.24
TSW	0.16	0.10
SNP	0.21	0.11
OIL	0.09	0.12
NS	0.05	0.14
NP	0.16	0.06
NT	0.05	0.13
NUPE	0.03	0.23
NUTE	0.21	0.04
NUE	0.04	0.16
NHI	0.12	0.12

Appendix 17. Additive heritability for the QTL identified by MIM for the different traits analysed in 2006/07.

TRAIT	High N		Low N	
	Block 2	Block 3	Block 1	Block 4
FDAS	0.51	0.13	0.36	0.07
TW	0.19	0.10	0.22	0.26
SY	0.38	0.13	0.38	0.25
HI	0.14	0.22	0.13	0.25
SN	0.19	0.31	0.08	0.23
STN	0.41	0.25	0.46	0.21
PN	0.30	0.37	0.17	0.22
TN	0.42	0.16	0.02	0.28
NUPE	0.37	0.37	0.15	0.23
NUTE	0.09	0.32	0.35	0.15
NUE	0.38	0.08	0.32	0.22
NHI	0.06	0.47	0.21	0.20

Appendix 18. Quantitative trait loci summary for oilseed rape traits analysed in 2005/06.

Trait	Number of QTL identified						Accumulative % explained variation	
	IM		CIM		Both methods			
	High N	Low N	High N	Low N	High N	Low N	High N	Low N
TL	1	3	1	7	1	1	5.0	5.7
FL	4	2	17	8	3	2	20.9	17.1
BN	6	2	4	9	2	2	16.3	14.4
FDAS	1	0	1	1	1	0	4.4	\
CB	10	4	9	4	1	3	7.2	17.3
CL	4	6	8	9	2	2	14.5	14.7
TW	2	0	6	5	0	0	\	\
SY	2	6	3	9	1	6	8.8	37.7
HI	11	0	8	10	4	0	25.5	\
TSW	3	4	6	5	1	3	6.9	17.5
SNP	6	0	4	3	2	0	10.2	\
OIL	1	1	2	6	1	1	4.3	7.4
NPLANT	0	2	8	1	0	1	\	5.2
NSEED	0	8	2	3	0	2	\	15.0
NTOTAL	0	7	2	3	0	1	\	6.4
NUpE	0	6	0	10	0	3	\	24.3
NUtE	2	0	7	1	2	0	27.0	\
NUE	0	5	5	8	0	5	\	38.1
NHI	3	6	7	10	2	3	21.6	15.7
TOTAL	56	62	100	112	23	35	NA	NA

Appendix 19. Quantitative trait loci summary for traits analysed in 2006/07 at High N treatment.

Trait	Number of QTL identified						Accumulative % explained variation	
	IM		CIM		Both methods			
	Block2	Block3	Block2	Block3	Block2	Block3	Block2	Block3
FDAS	3	1	8	1	2	1	24.9	11.5
TW	4	1	3	2	3	1	73.2	15
HI	0	2	2	3	0	2	\	27.3
SY	4	2	6	3	2	2	51.0	23.6
SN	2	0	3	4	1	0	6.9	\
STN	4	0	7	4	2	0	19.0	\
PN	1	1	7	5	0	1	\	6.5
TN	2	0	6	3	1	0	15.9	\
NUpE	5	1	5	6	3	1	58.4	7.9
NUtE	0	0	2	3	0	0	\	\
NUE	4	0	6	1	2	0	34.4	\
NHI	0	0	2	11	0	0	\	\
TOTAL	29	8	57	46	17	8	NA	NA

Appendix 20. Quantitative trait loci summary for traits analysed in 2006/07 at Low N treatment.

Trait	Number of QTL identified						Accumulative % explained variation	
	IM		CIM		Both methods			
	Block1	Block4	Block1	Block4	Block1	Block4	Block1	Block4
FDAS	5	0	6	2	2	0	17.5	\
TW	1	4	3	4	1	2	11.8	24.1
HI	1	0	3	6	1	0	8.9	\
SY	1	3	5	1	0	1	\	15.9
SN	0	0	1	3	0	0	\	\
STN	4	0	6	3	1	0	11.3	\
PN	0	2	3	3	0	1	\	8.6
TN	0	0	1	5	0	0	\	\
NUpE	0	1	4	4	0	1	\	12.6
NUtE	2	0	5	3	2	0	37.1	\
NUE	1	0	4	6	1	0	7.8	\
NHI	1	0	4	4	1	0	19.7	\
TOTAL	16	10	45	44	9	5	NA	NA

Appendix 21. QTL x environment analysis for traits studied in 2005/06 at both High and Low N simultaniously. Multiple trait CIM was performed using WinQTL Cartographer, with a LOD threshold of 1.8, A. Plant height; B. Foot length; C. Branch number; D. Flowering; E. Chlorophyll in Bracts; F. Chlorophyll in Leaves; G. Total plant biomass; H. Seed yield; I. Harvest index; J. Oil content; K. 1000-seed weight; L. Seed number per pod; M. Seed N concentration; N. Plant N concentration; O. Total N concentration; P. N Uptake Efficiency; Q. N utilisation efficiency; R. N Use Efficiency and S. N harvest index. The QTL names correspond to chromosome_marker. Grey, brown and blue shadding indicate the occurrence of the QTL at High N, Low N or both respectively, analysed by CIM analysis.

A.

QTL	HIGH	LOW	JOINT
19_1	1.08	1.45	2.27
18_1	0.95	0.78	1.84
17_21	2.00	0.38	2.56
3_44	0.95	0.87	2.05
3_1	2.36	0.10	2.38
1_41	0.80	2.60	3.01
1_35	0.40	3.80	4.02
1_24	0.37	2.24	2.50

B.

QTL	HIGH	LOW	JOINT
19_2	1.90	1.94	2.23
17_9	1.65	0.02	1.85
10_30	0.59	1.73	2.02
9_56	1.63	2.67	3.97
9_36	0.20	3.40	3.73
9_27	0.01	3.49	3.50
3_7	2.14	0.19	2.20
3_1	2.70	0.74	3.30

C.

QTL	HIGH	LOW	JOINT
15_10	2.49	0.03	2.49
14_28	1.39	0.81	2.38
9_55	1.57	1.24	2.45
3_44	2.05	0.10	2.21
3_30	2.08	0.21	2.32
1_35	/	3.11	3.11

D.

QTL	HIGH	LOW	JOINT
13_1	1.97	1.32	2.67
9_12	0.86	1.81	2.20
9_3	0.79	2.58	2.86
7_36	0.85	2.19	2.50

E.

QTL	HIGH	LOW	JOINT
17_2	0.26	2.40	2.46
14_28	1.53	0.41	2.41
9_56	1.81	0.37	3.15
9_2	0.12	2.66	2.66
7_36	0.60	1.83	2.20
6_43	3.08	0.19	3.24
6_39	3.26	0.22	3.46
2_22	2.63	0.28	2.73
2_8	0.61	1.37	2.42
1_41	1.83	1.80	3.09
1_31	0.99	1.91	2.61

F.

QTL	HIGH	LOW	JOINT
16_4	0.37	3.47	4.33
14_31	1.77	0.08	2.12
13_1	1.66	2.02	3.02
9_56	1.64	1.49	3.07
7_22	1.49	0.42	2.08
3_10	1.81	0.16	2.22
2_23	2.27	0.40	2.45
1_43	2.47	2.27	4.17
1_31	2.34	1.91	3.74

G.

QTL	HIGH	LOW	JOINT
19_9	2.68	0.03	2.72
9_56	1.65	0.69	2.29
7_46	0.04	2.03	2.09
6_33	0.05	2.64	2.73
1_44	0.44	2.12	2.34
1_36	0.21	3.10	3.19
1_24	0.05	3.15	3.18

H.

QTL	HIGH	LOW	JOINT
19_10	2.20/		2.20
19_3	0.43	1.45	2.06
9_56	1.98	1.04	2.91
6_30	0.06	2.97	2.98
6_24	0.14	2.78	2.84
3_30	0.53	2.58	2.79
1_44	0.70	2.75	3.23
1_39	1.12	1.99	2.90
1_24	0.15	2.17	2.24

I.

QTL	HIGH	LOW	JOINT
17_5	2.33	0.58	2.58
13_42	2.49	0.08	2.49
13_37	2.13	0.36	2.25
10_19	1.90	0.02	2.12
10_8	2.17	0.28	2.24
9_56	2.12	1.17	2.95
9_41	2.20	0.05	2.22
7_37	1.15	1.63	2.26
7_28	2.09	1.50	3.02
7_21	0.11	2.14	2.16
6_28	0.33	2.22	2.37
3_30	1.10	0.51	1.88
1_41	0.91	1.79	2.45

J.

QTL	HIGH	LOW	JOINT
9_55	1.51	1.15	2.37
5_11	0.13	2.07	2.14
1_40	0.60	2.82	2.82
1_34	0.16	2.41	2.47

K.

QTL	HIGH	LOW	JOINT
10_4	1.86	0.07	1.86
9_55	2.30	0.54	2.46
7_37	1.57	0.64	1.82
6_33	0.78	0.74	1.92
5_3	1.52	0.20	1.94
4_4	2.32	1.55	3.28
1_40	1.41	2.68	2.94
1_35	0.92	2.22	2.78

L.

QTL	HIGH	LOW	JOINT
18_21	2.04	0.09	2.20
18_8	1.91	0.71	2.22
13_42	2.14	0.63	2.33
13_37	2.16	0.42	2.25
10_16	0.39	1.07	1.96
10_7	1.50	0.12	1.98
7_46	0.00	2.97	3.14
7_1	1.92	0.65	2.02
6_19	1.52	0.06	1.84
3_1	1.78	0.19	2.14
1_41	1.42	1.69	2.49
1_30	0.84	1.57	1.99
1_7	1.97	0.24	2.67

M.

QTL	HIGH	LOW	JOINT
19_2	0.18	1.98	2.01
18_11	1.82	3.41	4.60
17_8	1.20	0.94	2.28
7_22	1.57	2.50	4.00
1_40	0.13	2.02	2.04
1_33	0.05	2.13	2.13
1_7	1.91	0.31	2.57

N.

QTL	HIGH	LOW	JOINT
18_11	0.99	2.47	3.08
14_14	0.12	2.31	2.34
10_6	1.92	0.61	2.24
7_31	2.08	0.05	2.06
7_22	2.66	0.95	3.59
2_24	2.18	0.03	2.21
2_12	1.97	0.23	2.12
1_34	0.15	1.82	1.88
1_7	1.64	0.34	2.17

O.

QTL	HIGH	LOW	JOINT
19_2	0.16	1.94	1.96
18_11	1.70	3.41	4.50
7_31	1.83	0.21	1.97
7_22	1.84	2.68	4.19
1_40	0.21	2.09	2.15
1_33	0.11	2.15	2.16
1_7	1.81	0.76	3.07

P.

QTL	HIGH	LOW	JOINT
19_20	1.81	0.33	1.83
7_46	0.11	2.18	2.53
7_36	2.13	0.34	3.09
7_23	1.11	0.65	2.01
7_7	0.81	1.00	2.28
4_3	0.21	1.93	1.94
1_40	0.29	4.42	4.43
1_6	1.33	1.26	3.08

Q.

QTL	HIGH	LOW	JOINT
18_8	1.16	1.51	2.19
15_5	0.46	1.23	1.86
12_25	1.57	0.64	2.25
10_30	0.02	1.96	1.98
10_23	2.48	0.09	2.51
7_30	2.45	0.28	2.47
7_20	1.45	0.01	2.33
5_13	0.25	1.62	2.01

R.

QTL	HIGH	LOW	JOINT
17_5	0.73	1.37	1.81
10_24	2.10	0.46	2.27
9_56	1.20	1.45	2.25
7_46	0.31	2.18	2.95
7_36	0.99	0.80	2.40
7_7	0.27	1.45	2.12
3_7	0.68	1.76	1.94
1_42	1.38	3.48	4.07

S.

QTL	HIGH	LOW	JOINT
18_11	1.04	1.51	2.20
10_23	1.90	0.03	1.90
7_37	0.43	1.79	2.12
7_31	2.54	0.11	2.63
7_20	0.45	4.13	5.27
6_22	1.71	0.36	1.82
5_13	0.22	1.48	2.02

Appendix 22. QTL x environment analysis for traits studied in 2006/07 at both High and Low N simultaniously. Multiple trait CIM was performed using WinQTL Cartographer, with a LOD threshold of 1.8, A. Total plant biomass; B. Harvest index; C. Seed yield; D. Flowering; E. Seed N concentration; F. Stem N concentration; G. Chaff N concentration H. Total N concentration; I. N Uptake Efficiency; J. N utilisation efficiency; K. N Use Efficiency and L. N harvest index. The QTL names correspond to chromosome_marker. Grey, brown and blue shadding indicate the occurrence of the QTL at High N, Low N or both respectively, analysed by CIM analysis.

A.

	BLOCKS 1/2				BLOCKS 3/4			
QTL	HIGH	LOW	JOINT		QTL	HIGH	LOW	JOINT
14_3	0.86	0.74	2.04		9_39	0.69	2.94	3.46
11_31	0.14	2.52	2.81		9_24	1.03	2.64	3.47
7_45	0.54	3.20	3.27		6_43	0.02	1.79	1.82
7_22	4.01	0.01	4.18		5_24	1.10	0.60	1.88
7_17	3.11	0.01	3.29		4_21	0.14	2.30	2.37
6_3	1.79	0.04	1.82		4_7	0.27	3.34	3.52

B.

	BLOCKS 1/2				BLOCKS 3/4			
QTL	HIGH	LOW	JOINT	QTL	HIGH	LOW	JOINT
7_22	1.61	1.32	3.29	14_23	0.60	2.15	2.58
7_16	1.07	2.80	3.23	14_17	0.67	2.27	2.76
6_43	1.41	1.03	3.03	10_10	3.00	0.67	3.30
6_39	1.65	0.78	2.91	10_3	2.29	0.28	2.52
6_28	0.92	0.93	2.23	6_34	1.57	0.71	2.22
3_64	0.08	2.09	2.20	2_24	1.23	1.18	2.53
				2_19	1.04	3.67	4.80

C.

	BLOCKS 1/2				BLOCKS 3/4			
QTL	HIGH	LOW	JOINT	QTL	HIGH	LOW	JOINT
16_18	0.21	1.94	2.01	18_1	0.18	2.27	2.35
11_31	0.14	1.87	2.17	17_17	0.70	1.86	2.45
9_3	0.03	3.45	3.74	14_28	1.35	1.08	2.14
7_22	4.27	0.29	4.29	4_21	0.15	2.78	2.91
7_17	2.60	0.17	2.60	4_17	0.61	2.04	2.48
2_27	0.16	2.66	2.67	3_2	0.94	1.23	1.83
				1_16	2.02	0.23	2.17

D.

	BLOCKS 1/2				BLOCKS 3/4			
QTL	HIGH	LOW	JOINT	QTL	HIGH	LOW	JOINT
19_22	2.64	2.03	2.68	14_28	1.23	0.84	2.17
18_21	0.53	1.62	1.82	10_29	0.79	2.95	4.30
17_21	0.19	1.98	2.73	9_56	2.45	0.42	3.26
13_38	0.23	0.44	3.06	4_15	0.02	1.82	1.84
13_31	0.15	0.69	3.53	3_66	2.57	0.28	2.61
11_26	1.73	1.63	1.93				
9_3	1.76	0.70	2.02				
6_15	0.20	0.49	2.79				
5_8	0.70	0.05	1.81				
4_16	1.13	3.99	4.44				
4_13	1.39	2.47	2.47				
2_26	2.04	0.93	2.09				

E.

	BLOCKS 1/2				BLOCKS 3/4			
QTL	HIGH	LOW	JOINT		QTL	HIGH	LOW	JOINT
14_35	2.62	0.07	2.62		18_12	0.01	1.80	1.81
9_55	2.07	0.00	2.11		12_7	0.78	1.41	2.46
9_52	2.44	0.15	2.49		9_4	1.62	0.28	2.16
8_5	1.62	0.13	1.90		7_31	1.60	0.57	1.84
1_11	0.14	2.50	2.70		4_9	2.04	0.76	3.34
1_8	0.27	2.88	3.38		4_5	1.55	0.61	2.62
					3_66	2.24	2.24	3.92
					3_7	0.57	2.27	2.53

F.

	BLOCKS 1/2				BLOCKS 3/4			
QTL	HIGH	LOW	JOINT		QTL	HIGH	LOW	JOINT
19_12	0.81	1.35	2.62		19_10	1.48	0.70	2.44
19_7	0.54	2.02	2.83		11_26	1.44	0.12	1.83
18_1	0.02	2.23	2.24		10_8	0.04	1.84	1.84
16_33	0.36	0.13	1.82		9_4	2.45	0.52	3.17
14_34	3.55	0.04	3.68		7_30	1.55	1.16	2.46
14_1	0.06	1.67	1.80		6_46	0.02	2.51	2.64
13_36	1.10	0.97	1.95		6_16	1.78	0.44	1.99
9_51	2.47	1.72	3.81		3_62	0.20	1.87	1.97
9_20	1.14	0.67	2.53		3_52	0.21	1.16	2.14
9_15	1.70	1.06	1.98		3_40	0.14	2.72	2.73
6_40	0.23	0.25	2.22		3_33	0.00	2.65	2.68
4_31	2.04	0.34	2.52		3_28	0.00	2.32	2.34
3_10	0.08	3.32	3.37		1_36	1.55	0.27	2.01
2_26	0.98	1.46	2.36					
1_43	0.17	3.27	3.40					
1_16	0.61	1.50	2.27					

G.

	BLOCKS 1/2				BLOCKS 3/4			
QTL	HIGH	LOW	JOINT		QTL	HIGH	LOW	JOINT
17_1	1.64	0.59	1.89		19_16	1.26	1.23	2.62
16_1	1.35	1.02	1.88		13_5	0.63	2.74	3.29
15_10	1.40	0.60	1.96		10_11	0.30	2.05	2.40
14_35	2.15	0.21	2.32		10_7	0.77	1.62	2.47
8_17	2.15	0.01	2.15		10_3	1.11	1.35	2.59
6_39	0.13	1.92	1.99		9_21	0.42	1.70	2.25
6_34	0.69	2.29	3.17		9_3	2.59	0.23	2.74
6_22	1.08	1.94	3.26		7_30	2.59	0.02	2.71
6_2	0.58	1.02	2.03		7_24	1.97	0.11	2.14
5_31	0.84	1.63	2.23		6_16	2.01	0.02	2.01
5_5	0.98	1.24	2.19		4_9	1.77	0.01	1.78
4_25	0.39	2.41	2.79		4_5	1.54	0.33	1.83
4_5	2.53	0.43	3.01		3_65	2.13	0.08	2.31

H.

	BLOCKS 1/2				BLOCKS 3/4			
QTL	HIGH	LOW	JOINT		QTL	HIGH	LOW	JOINT
17_17	0.00	0.68	1.95		19_16	0.90	0.77	1.94
14_35	2.54	1.64	2.55		16_16	2.03	0.84	2.53
11_26	1.78	2.93	2.93		15_18	1.04	1.91	2.59
11_17	1.64	2.30	2.32		15_14	1.68	2.20	3.39
9_56	1.15	0.41	2.00		11_27	1.33	0.77	2.14
9_52	1.69	0.44	2.05		9_23	0.11	2.67	2.71
8_22	2.36	0.84	2.57		9_4	1.56	0.49	2.40
8_14	2.31	1.12	2.37		6_16	2.95	0.17	2.95
7_22	2.45	0.48	3.29		4_9	2.10	0.65	3.18
7_17	1.82	0.50	2.19		4_5	2.65	0.26	3.23
4_27	1.53	0.26	2.18		3_65	1.53	2.09	3.27
3_8	1.28	0.38	2.50		3_33	0.18	2.88	3.20
					3_28	0.20	2.40	2.77

I.

	BLOCKS 1/2				BLOCKS 3/4			
QTL	HIGH	LOW	JOINT	QTL	HIGH	LOW	JOINT
16_19	0.59	1.68	1.86	19_17	1.31	0.52	2.16
15_10	1.14	0.56	1.85	16_12	1.42	1.11	2.26
14_14	0.71	0.92	1.94	16_7	1.05	1.25	2.05
11_32	1.17	0.91	2.33	15_14	1.43	2.24	3.28
9_55	2.16	0.31	2.65	14_4	1.67	0.06	1.92
9_52	2.81	0.16	3.17	13_29	2.04	0.41	2.47
7_45	0.24	2.43	2.46	11_27	1.63	0.89	2.77
7_40	0.18	2.15	2.19	7_31	2.01	0.61	2.37
7_22	3.81	0.15	4.05	7_26	1.50	1.02	2.11
7_17	2.47	0.10	2.65	4_17	0.49	2.86	3.32
1_2	1.45	0.18	1.80	4_9	2.37	0.40	2.99
				4_5	2.97	0.08	3.17
				3_65	2.01	2.36	3.80
				1_36	1.75	1.48	3.33
				1_28	0.59	1.66	2.28
				1_18	1.22	1.31	2.22
				1_12	1.65	1.38	3.12
				1_6	2.25	0.82	3.00

J.

	BLOCKS 1/2				BLOCKS 3/4			
QTL	HIGH	LOW	JOINT	QTL	HIGH	LOW	JOINT
7_28	1.20	1.12	2.60	19_17	0.73	0.87	2.52
7_22	1.51	2.04	4.22	19_10	1.31	0.23	2.03
7_20	0.24	4.08	4.91	16_17	2.06	0.81	2.34
7_17	0.90	4.21	4.31	15_14	1.85	0.35	1.89
7_1	1.57	0.27	2.04	12_9	1.92	0.27	1.92
6_43	1.81	0.97	3.12	11_31	0.40	0.74	1.91
6_39	1.35	0.55	2.18	9_43	2.04	0.77	2.22
3_4	0.29	1.45	1.98	9_23	0.49	2.88	2.89
2_27	0.03	4.82	4.82	9_3	2.34	0.15	2.35
				8_2	2.67	0.51	3.86
				6_38	1.63	0.92	1.84
				6_16	2.21	0.04	2.42
				3_64	3.82	0.31	3.83
				1_36	2.38	0.16	2.38

K.

	BLOCKS 1/2				BLOCKS 3/4			
QTL	HIGH	LOW	JOINT	QTL	HIGH	LOW	JOINT
16_18	0.51	2.11	2.38	19_17	1.04	0.72	2.32
14_13	0.62	0.36	1.89	16_17	1.61	0.89	2.05
11_31	0.63	1.77	2.06	15_14	1.48	1.10	2.09
9_55	2.32	0.03	2.32	14_8	0.03	2.94	3.00
9_52	2.52	0.00	2.60	13_29	1.69	1.02	2.78
9_3	0.14	2.60	2.82	10_29	0.49	3.50	3.58
7_36	1.27	1.20	2.16	7_35	0.67	1.81	2.19
7_28	1.76	0.10	2.56	4_17	0.53	3.03	3.79
7_22	3.69	0.03	3.78	4_8	2.13	0.09	2.47
4_21	2.56	0.25	2.63	3_64	3.16	0.05	3.19
2_27	0.13	2.70	2.89	1_36	1.67	1.12	2.50
				1_18	2.25	0.46	2.47

L.

	BLOCKS 1/2				BLOCKS 3/4			
QTL	HIGH	LOW	JOINT	QTL	HIGH	LOW	JOINT
8_22	1.81	0.06	2.02	19_9	1.26	0.26	1.86
7_26	0.87	1.39	2.54	16_18	2.22	1.12	2.78
7_22	1.35	2.66	4.56	12_10	2.46	0.02	2.47
7_17	0.65	3.72	3.91	12_5	1.15	0.96	1.94
7_1	1.48	0.29	1.96	10_29	0.05	1.83	1.83
6_43	1.85	0.61	2.79	9_43	1.90	0.81	2.30
4_27	0.47	1.43	2.19	9_21	1.77	2.23	3.44
3_6	0.43	1.73	2.61	9_4	2.40	0.16	2.74
2_27	0.00	2.17	2.17	8_3	0.80	1.45	2.68
				7_45	1.23	1.66	2.63
				6_38	1.92	0.40	2.01
				3_64	2.82	0.24	2.87