IDENTIFYING NOVEL VARIATION IN GRAIN YIELD, BIOMASS AND N-USE EFFICIENCY AND ITS PHYSIOLOGICAL AND GENETIC BASIS IN WHEAT

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DEDICATION

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LIST OF ABREVIATIONS

AGDM	Above-ground dry matter
A_{max}	Photosynthetic rate
ANI	added N interaction
ANOVA	analysis of variance
BPE	Biomass production efficiency
DAS	Days after sowing
DEFRA	Department of Environment, Food and Rural Affairs
Df	Degrees of freedom
DM	Dry matter
DURTOT	Total duration of the senescence
EM	Ears per m^2
FAO	Food and Agriculture Organisation of the UN
G	Genotype
GA	Green area
GE	Grains per ear
GM	Grains per m^2
GNC	grain nitrogen concentration
GPC	grain protein concentration
GS	Growth stage
GY	Grain yield
HGCA	Home Growth Cereal Authority
HI	harvest index
HN	High nitrogen treatment
IR	Intercepted radiation
K	Light extinction coefficient
LAI	leaf area index
LN	Low nitrogen treatment
N	Nitrogen
NDVI	Normalised Difference Vegetation Index
NHI	nitrogen harvest index
NO3-	Nitrate
NR	N remobilisation
NRE	N remobilisation efficiency
NUE	Nitrogen use efficiency
NUP	N-uptake
NUPE	N-uptake efficiency
NUTE	N-utilization efficiency
ONSEN	Onset of the rapid phase of senescence
PAR	Photosynthetically active radiation
PoA	Post-anthesis
QTL	Quantitative trait loci
RUBISCO	Ribulose 1,5-bisphosphate carboxylase/oxygenase
RUE	Radiation-use efficiency
SEN	Duration of the rapid phase of senescence
SPAD	Minolta SPAD-502 chlorophyll meter
TGW	Thousand grain weight

ABSTRACT

Wheat (*Triticum aestivum L*) is the most widely grown crop in the world and accounts for approximately 31% of global cereal consumption every year (FAO, 2012). Furthermore, wheat is the most traded product worldwide (Dixon et al. 2009). In UK, wheat is the largest arable crop, it is grown on about 1.8 M ha p.a. For wheat productivity and grain quality, nitrogen (N) is the most important fertilizer applied, and modern wheat culitvars are generally selected under high N supply. However, N fertilizer represents a significant cost of production and may have negative environmental impacts. N-use efficiency (grain DM yield / soil and fertilizer N available; NUE) can be divided into two main components: N-uptake efficiency (N uptake / soil and fertilizer N available; NUPE) and N-utilization.

efficiency (grain DM yield / NUP; NUTE).

The current study aims to identify novel variation for grain yield, above-ground dry matter (AGDM). NUE and NUE components in a panel of AE Watkins landraces compared to UK check cultivars in the glasshouse and field trials and in two Single Seed Descent (SSD) populations derived from the crosses between UK spring wheat Paragon and Watkins landrace line 481 (PxW481) and between Paragon and Watkins landrace line 141 (PxW141) in field experiments and to investigate the physiological and genetic bases of this variation.

Two glasshouse experiments (2011/12 and 2012/13) examining 17 genotypes (comprising five UK check cultivars and 12 Watkins landraces), and one field experiment (2011/12) examining a panel of 10 genotypes (comprising three UK cultivars and seven of the 12 Watkins landraces examined in the field) were carried out. In addition, three field experiments examining two SSD populations PxW481 (79 lines) (in 2011/12 and 2012/13) and PxW141 (97 lines) (in 2012/13) under two N treatments (HN=200 kg N/ha and LN=0 kg N/ha) were carried out. The glasshouse experiments were located at the University of Nottingham, School of Biosciences, Sutton Bonington Campus, UK and field experiments were located at the University of Nottingham experimental station at Bunny Park, Nottinghamshire, UK.

The genetic maps were presently constructed using 201 and 491 markers, including single nucleotide polymorphism (SNPs) and microsatellites (SSR) for PxW481 and PxW141, respectively. Grain yield (GY), yield components, AGDM, NUPE, NUTE, and physiological traits including leaf light-saturated photosynthesis rate (A_{max}), flag leaf chlorophyll content (SPAD) and the spectral reflectance index, Normalized Difference Vegetation Index (NDVI, indicative of canopy size, field experiments only) were measured in the experiments.

Genetic variation in grain yield was more closely associated with AGDM than with harvest index (HI) in the glasshouse and in the field under both HN and LN. For biomass production, the results showed that Paragon, Rialto, Xi19 and landrace W141 had higher AGDM than all other genotypes. In summary, UK check cultivars generally had higher grain yield (GY) compared to Watkins landraces and higher AGDM in the glasshouse under HN conditions. Similar results were found in the field under HN; however, under LN, the majority of Watkins lines maintained GY and AGDM than the UK cultivars compared to HN conditions.

With regard to N uptake at harvest, the Watkins landraces W040, W141 and W579 had similar NUP to Paragon and higher than Oakley in the glasshouse. In the field, in general, Watkins lines maintained relatively better NUPE under LN than the UK check cultivars.. A negative association amongst genotypes was found between NUTE and grain N concentration (%) in the glasshouse and in the field under HN and LN treatments, with the strongest association observed under LN conditions.

Variation for light-saturated leaf photosynthesis rate (A_{max}) was also observed amongst Watkins lines. In the glasshouse experiments, the UK cultivars, and landraces W141 and W352, generally had higher pre-anthesis A_{max} than other Watkins landrace lines. In the field experiment, landrace lines W352 and W141 had higher pre-anthesis A_{max} than Paragon under LN, whereas lines, W223 and W034 had higher values under HN. Genetic variation in GY and AGDM were overall better associated with post-athesis flag-leaf A_{max} than with pre-anthesis flag-leaf A_{max} in the field, and the associations with post-anthesis A_{max} tended to be stronger under HN rather than under LN conditions.

With regard to the two SSD mapping populations, both N and genotype had a significant effect on GY, AGDM, NUPE and NUTE for PxW481 in all three

experiments. For the PxW481 population, no SSD line had either higher GY or AGDM than Paragon under HN, while under LN, 13 lines had higher GY and two lines had higher AGDM than Paragon ($P< 0.05$). For the PxW141 population, three lines had higher grain yield under HN and three lines had higher GY under LN than Paragon. Five and 10 SSD lines under HN and LN, respectively, had higher AGDM than Paragon. The transgressive segregation for GY was due to either AGDM or HI, depending on the line. In both populations under both N treatments, genetic variation in NUE was more strongly associated with NUPE than with NUTE under HN and LN; however, the response of NUE to N availability was better associated with NUTE.

Averaging years for the PxW481 population under HN, no SSD line had higher NUPE than Paragon, while under LN L481-76 had higher NUPE than Paragon. For PxW141, no SSD line had higher NUPE than Paragon either under HN or under LN conditions. Seventeen SSD lines of the PxW481 population had significantly higher NUTE than Paragon under HN and 10 lines higher under LN conditions. For PxW141 under HN, L141-95 and L141-94 had higher NUTE than Paragon under HN, while under LN 12 SSD lines had higher values.

In both the PxW481 and PxW141 populations, genotype and N treatment had an effect on NDVI (canopy size) for all measurements pre and post-anthesis. Genetic variation in yield, biomass and NUPE were more closely associated with NDVI measured at around anthesis than at other measurement dates. In addition, the correlation amongst genotypes was stronger under LN than under HN conditions. Genetic variation for flag-leaf senescence parameters was also found in both mapping populations, and association with grain yield amongst genotypes was stronger under LN conditions than under HN conditions in both populations.

QTL analysis identified two major QTLs for grain yield on 6AL and 5A under HN and one major QTL on 2B under LN conditions in the PxW481 population. The QTL on 6AL co-located with a QTL for NUPE, while the one on 5A co-located with a putative QTL for AGDM. Simonds et al. (2014) reported a major stable QTL in the same region of 6AL in the Spark x Rialto DH population across several environments. Under LN conditions, the QTL on 2B co-located with a QTL for plant height.

For AGDM, a major QTL under both HN and LN was identified on chr 4B in the PxW481 population. This QTL co-located with a QTL for NUPE under both HN and LN conditions. In addition, a major QTL under HN for NUPE on 3BL (PxW481) co-located with QTLs for GY and AGDM. Another major QTL on 2A (PxW141), co-located with flag-leaf stay-green traits. This is consistent with the findings of Bogard et al. (2011) who reported two QTLs for NUPE under LN on 2A, associated with a delay in onset of flag-leaf senescence.

With regard to NUTE, two major QTLs on 3B, co-locating with QTL for GY and HI, and on 1B, co-locating with a QTL for N grain concentration, were found for the PxW481 population under LN. In addition, three QTLs on 3A, co-locating with a QTL for TGW, and on 6D and on 7B, co-locating with QTLs for TGW, were identified.

Chapter 1: Introduction

1.1 Importance of wheat

Wheat (*Triticum aestivum* L) is a cereal grain originally from the fertile crescent in the near East and Ethiopia highlands (Gill and Friebe, 2002). The bread wheat crop is currently cultivated worldwide on 217 million hectares of land per annum and plays therefore a crucial role in global food security with total production of about 653 million metric tons per annum (FAO, 2010). It is the most widely grown crop in the world and accounts for about 31% of global cereal consumption every year (FAO, 2012) (Figure 1.1). Wheat provides 20% of the total calories of the world's population (Reynolds et al. 2011). It is the most important source of carbohydrate in a majority of countries and is the best of the cereal grains nutritionally as its starch and most of its proteins are easily digested. It also provides more nourishment for humans than any other food source. Wheat grain also contains vitamins, minerals, and lipids. In addition, bread wheat is a popular source of animal feed providing a valuable source of protein in animal diets, particularly in the industrial countries (e.g. 45 percent of total use in the EU) (FAO 2002). A low protein grain is often used by industry to make alcohol, paper additives and adhesives (B.C. Curtis 2002). Finally, wheat grain has also become also a biofuel crop (HGCA, 2010).

Several studies have predicted that due to predicted population increase to > 9 billion, increasing meat and dairy consumption and increasing biofuel consumption, crop production should increase by 60% to 110% by 2050 to meet demand (Godfray et al. 2010, Tilman et al. 2011, Foley, et al. 2011; Pingali 2006; UN DESA). Crop area is likely to increase only by 10% over the same period (The Land Commodities Global Agriculture and Farming Investment Report, 2009). Therefore, increasing grain yield per unit land area is required to achieve the global food security goal (Hulme, et al. 2013). Future area expansion for agriculture would incur a significant risk to remaining forests and savannah, so agricultural growth must rely more than ever on productivity gains through increased crop and animal yields (Fischer et al. 2014).

Since the current rate of genetic gain of grain yield of about 1% per year under optimal conditions (Miralles and Slafer, 2007; Foulkes al. 2010; Fischer and Edmeades, 2010) is lower than the predicted rate of demand for wheat (Rosegrant

and Cline, 2003; Singh and Trethowan, 2007; Foulkes et al. 2010; Hall and Richards, 2013), an acceleration in genetic yield potential improvement is required for global food security. In addition to these factors, environmental concerns associated with intensive agriculture (Montgomery, 2007 and WBCSD, 2008) and the impacts of climate change on grain yield associated with increased frequency of drought and heat stress (IPCC, 2007) reinforce the need for genetic improvement of yield potential.

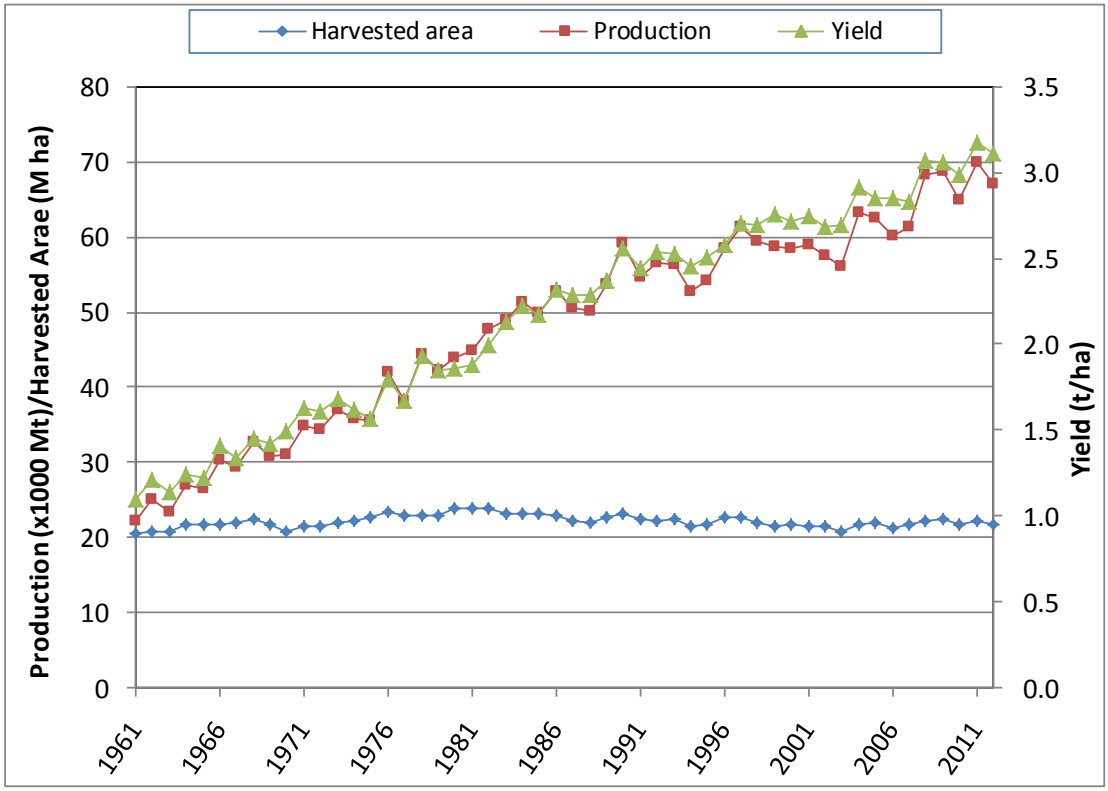


Figure 1. 1: Bread wheat grain production, grain yield per unit land area and harvested area in the world since 1960 (data from FAO 2013 web site: http://www.faostat.fao.org/**)**

In the UK, winter wheat is the most widely grown arable crop accounting for over 40% of tilled land in eastern countries of England (Foulkes et al. 2001; DEFRA 2012).

The UK currently produces around 15 million tonnes of wheat each year and around 20% of this is exported to countries around the world. About 40% of the national crop is used in animal feed rations going to chickens, cows and pigs. The balance of the crop is used for human consumption with wheat being used in literally thousands

of products and responsible for the daily production of 10 million loaves of bread (http://www.ukagriculture.com/).

In addition to the current 0.8 M tonnes of grain used by UK distillers for alcohol production, a potential market of some 2.5 million tonnes per year could be created in UK for bio-ethanol (Agriculture and Horticulture Development Board 2010). High starch/low protein grains are usually needed for bioethanol production. In order to minimise environmental impacts and maximize profitability, biofuel crops should be managed as for feed wheat with moderate nitrogen (N) rates (HGCA, 2010).

However, bioconversion of wheat straw to ethanol is becoming an attractive alternative to ethanol production from conventional grain crops, especially with wheat straw being a low commercial value, high volume agricultural by-product and grain prices continuously increasing. Similarly, using wheat straw as an energy source in the ethanol biorefinery has become a subject of interest in recent years, since using grain competes with grain crop for food (Ghayur et al. 2011).

1.2 Wheat genetics

With a global output of 681 million tonnes in 2011 (USDA, 2012), bread wheat accounts for 20% of the calories consumed by humans (FAO, 2011) and is an important source of protein, vitamins and minerals. It is an allohexaploid species (2n=6x=42); its genetics are more complicated than that of majority of other crop species (Hancock, 2004). It has three sets of seven diploid homozygous chromosomes called genomes (AABBDD) derived from three different diploid donor species (Zohary et al. 1969). The genome A originated from *Triticum urartu*, the genome D from *Triticum tauschii* (Gill and Friebe, 2002); howeverthe exact origin of the B genome is still unclear, but a closely related species or an ancestral relative of *Ae. speltoides* (S genome) has been suggested to be the likely donor. The process evolved between 2.5 and 4.5 million years ago in two stages: the cross between *Triticum urartu* (A genome) and *Aegilops speltoides* (B genome) produced *Triticum turgidum subsp.* (Emmer wheat, AB genome) and then a second cross about 8,000 years ago (Nesbitt, M. and Samuel, D., 1996) between *Triticum turgidum subsp. durum* (AB genome) and *Triticum tauschii* (D genome) resulted in *Triticum aestivum* L. (AABBDD) bread wheat carrying the three genomes A, B and D.

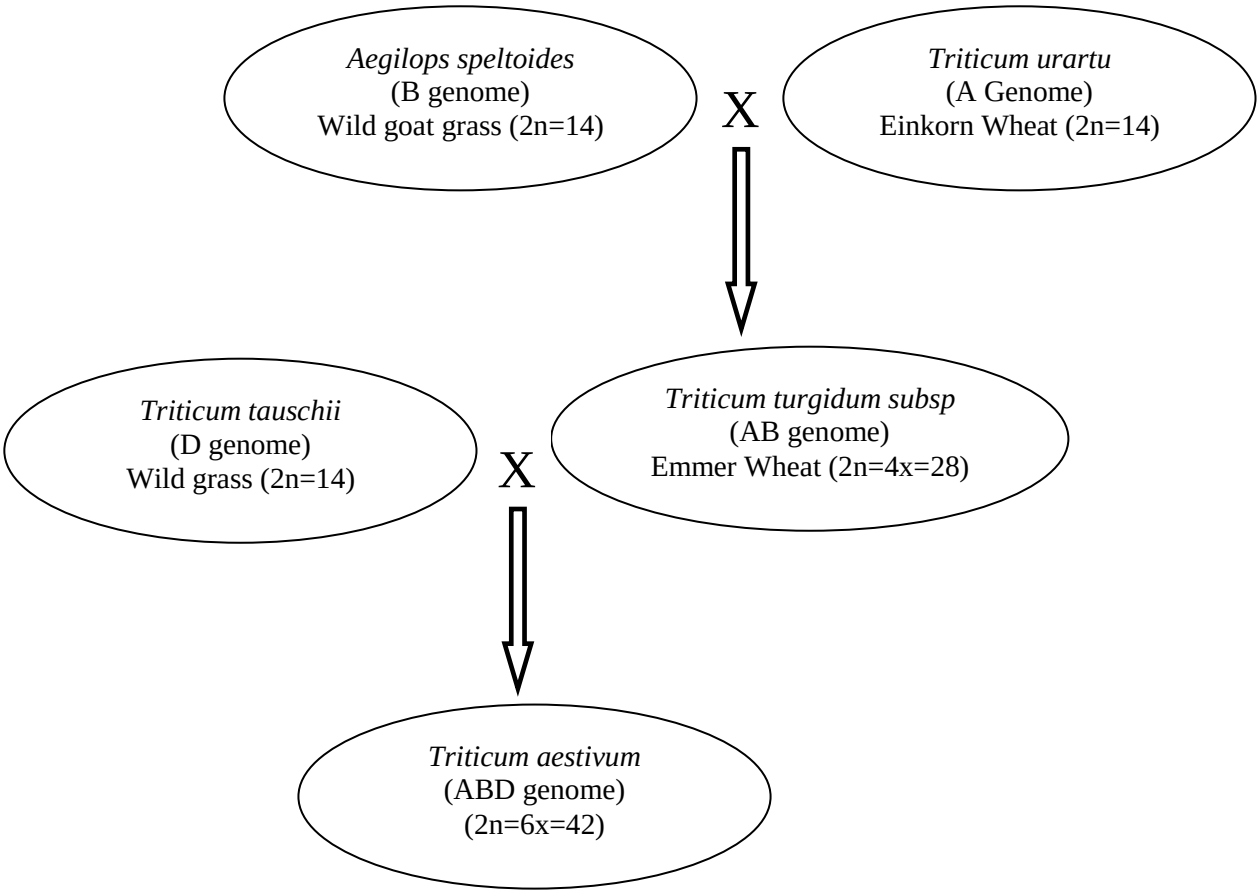


Figure 1. 2: Hybridization process of creating polyploid wheat

1.3 Yield potential and its limitations

Serious concerns about the environmental impacts of fertilisers emphasize the need to improve nutrient-use efficiency and climate change is leading to increased frequency of heat and water stress making wheat production more unpredictable. To solve these two problems, grain yield potential of cultivars should be increased but without commensurate increases in N and water inputs (Slafer and Araus, 2007; Foulkes et al. 2011). Some progress has been made in the last two decades in a number of research areas to improve biomass production and photosynthetic capacity through genetic interventions, mainly by increased carbon fixation rate, spike fertility, and leaf stay-green and other physiological traits (Parry et al. 2003a, 2007; Long et al. 2006; Zhu et al. 2008; Reynolds et al. 2009).

During the Green Revolution (1960s and 1970s) novel high-yielding wheat cultivars were produced carrying semi-dwarfing genes. The main phenotypic effect of these genes is a 10-20 cm decrease in plant height (Hellwell et al. 2000). This characteristic of reduced plant height prevents the varieties from lodging before harvest and allows higher N fertiliser inputs. Additionally the reduced stature of the semi-dwarf cultivars leads to the reduction in the competition for assimilate from the growing stem during stem elongation and consequently greater spike dry weight at anthesis, more grain per spike, grain per m^2, and higher harvest index (HI, proportion of above gounr dry matterin the grain) (Fischer and Stockman, 1986). Thus, genetic gains in yield potential in the last decades have resulted mainly from increased partitioning of biomass to the grain (improved harvest index) rather than above-ground biomass (Calderini et al. 1999a, Brancourt-Hulmel et al. 2003; Acreche et al. 2008). Austin (1980) estimated that HI has a hypothetical upper limit in wheat (0.62). In addition to that, limited genetic progress in HI was observed since 1990. The highest values reported worldwide are typically ca. 0.55 for winter wheat (Reynolds et al. 1999; Shearman et al. 2005) and 0.50 for spring wheat (Foulkes et al. 2011). However, increasing biomass production is an alternative for increasing yield potential, as HI is approaching its maximum limit.

Therefore, improvement of breeding programs focused on yield potential requires improved physiological understanding of the traits limiting the performance of genotypes as well as factors influencing stability across target environments, e.g. N availability. More effective approaches to crop improvement require knowledge of selection criteria for the important traits limiting yield potential including biomass and photosynthetic capacity for application in plant breeding.

Cereals are frequently affected by abiotic stresses which constrain their growth and productivity. Water availability is the single greatest abiotic stress factor that limits crop growth worldwide (Araus et al. 2002). Genetic increases in yield potential, however, are not only associated with enhanced yields in favourable environments but also under abiotic stress, e.g. drought (Trethowan et al. 2002; Araus et al. 2002; Slafer and Araus, 2007) and nitrogen deficiency (Ortiz-Monasterio et al. 1997; Abeledo et al. 2003). These gains are significant because further large increases in the area under irrigation are not expected, and intensive agriculture threatens land deterioration in those areas already irrigated (Araus, 2004).

Since a comprehensive understanding of the genetic basis of adaptation among elite cultivars is not yet fully achieved, plant improvement will continue to rely on selection for crop phenotype in addition to selection according to molecular markers. To avoid stress phenological patterns of crops should be optimised (Ludlow and Muchow, 1990). The development of a more extensive root system is another physiological mechanism for minimizing the occurrence of water and/or nutrient stress through accessing deeper soil levels when drought and nutrient deficiencies occur (Lopes and Reynolds, 2010) allowing higher photosynthetic efficiency and transpiration rates matching evaporative demand (Amani et al. 1996). The potential beneficial impact of physiological breeding in Australia (Richards, 2006; Rebetzke et al. 2009) and trait-based breeding at CIMMYT Mexico (Reynolds et al. 2012) have been demonstrated in wheat breeding programs.

1.4 Nitrogen fertiliser use by wheat and environmental impacts

1.4.1 Function of N in wheat plants

Crops need nutrients from the soil in order to grow. However, in general, the soil and environment cannot supply enough nutrients, thus fertilisers are applied to supplement the soil nutrients taken up by plants. Nitrogen is the most important nutrient required by plants. While the plant root is the major organ involved in N uptake, the whole plant utilises N. In plants, N is particularly important for leaf photosynthesis. Indeed, in wheat leaves more than 75% of the total N is in photosynthetic proteins (soluble and in thylakoids) (Field and Mooney, 1986; Evans, 1989). Nitrogen is needed for both fixing CO_2 (Ribulose 1,5-Bisphosphate Carboxylase/Oxygenase; RubisCO) and harvesting the light (chlorophyll and associated proteins) (Evans, 1989). Chlorophyll, a light harvesting pigment, can account for 60-85% of the thylakoid protein and each chlorophyll molecule contains four atoms of N (Heldt, 1997). The chlorophyll-protein complex can comprise between 20 and 60% of photosynthetic N, depending on irradiance (Hikosaka and Terashima, 1995). RUBISCO is a large molecule (560 kDa) and is the most abundant soluble protein in plant tissue. It is located in the chloroplast stroma. RUBISCO has two enzymic activities. It catalyses the carboxylation of Ribulose 1,5-Bisphosphate (RUBP), fixing CO_2 to form two molecules of 3-phosphoglycerate

(PGA) within the Calvin cycle. It also catalyses the oxygenation of RUBP by O_2, to form phospho-glycollate and PGA (Heldt, 1997). RUBISCO, however, has a low catalytic rate and a poor affinity for CO_2 (Evans, 1989) and so is required in large amounts to maintain adequate carboxylation rates for growth. Proteases, peroxidases, some inhibitors (i.e. trypsin and chymotrypsin) and elements of replication and transcription of DNA (deoxyribonucleic acid) also require N during their formation process.

During grain filling, nitrogen is transported from different vegetative plant organs to the grains (N remobilisation). This process is linked to the senescence of the yield-forming leaves during the grain filling stage. In the grains, N is important for as a constituent of gluten content (allowing the viscoelasticity of wheat dough) (Shewry, 2009).

Nitrogen and phosphorus, key nutrients included in growth process, need to be restored to the soil to achieve high yields. Nitrogen is very mobile in the soil, and is not maintained because of plant absorption, leaching, surface run off and denitrification. Furthermore, there is soil heterogeneity in addition to a seasonal variation in soil N mineralization (Jackson and Caldwell, 1993), necessitating N fertiliser application to produce optimal yields. As N fertiliser, wheat plants generally prefer a mixture of ammonium and nitrate (Crawford and Glass, 1998). Global N consumption worldwide in 2005 was estimated to be 90.9 Mt (Heffer and Prud'homme, 2006). In the UK, over the last 25 years, between 180 - 190 kg ha^{-1} of N fertiliser was applied on average to winter wheat crops (British Survey Fertiliser Practice, 2013). Recent projections state that global N demand for N fertilisers could be between 107 and 171 Mt in 2050 (Millennium Ecosystem Assessment, 2005). Other scenarios suggested that global fertiliser N consumption could be between 110 and 140 Mt. In fact the long term projection is subject to large uncertainty because it depends on the ability to improve N-use Efficiency (NUE = grain yield DM /N supply from soil and fertiliser) in new cultivars (Stanley et al. 2004). In addition, growing global demand for grain crops has generated higher crop prices and increased demand for fertilisers. In addition, the demand for N fertiliser is increasing annually mainly in South and East Asia and Africa (FAO, 2011). Therefore improving NUE in new released cultivars is a necessity in wheat production.

1.4.2 N fertiliser usage and management

Nitrogen (N) is one of the most complex elements regarding all of the potential processes and forms involved in the N cycle. However, it is an element essential for life. The historical perspective on the N cycle and world patterns for N use show that N use continues to increase (Keeney and Hatfield 2001). In the last 20 years largest increases in N fertiliser use are observed in developing countries while developed countries have fairly consistent levels of N use (FAO, 2013). Figure 2 shows the nitrogen fertiliser consumption between 1961 and 2012 in five regions in the world (Data from http://faostat.fao.org/faostat). Asia (mainly China) has shown a linear increase in N fertiliser use since 1976 levelling off since about 1996, while in Europe a significant decrease was observed and in the Americas there was no significant change in annual N use since 1978 (FigureFigure 1.3).

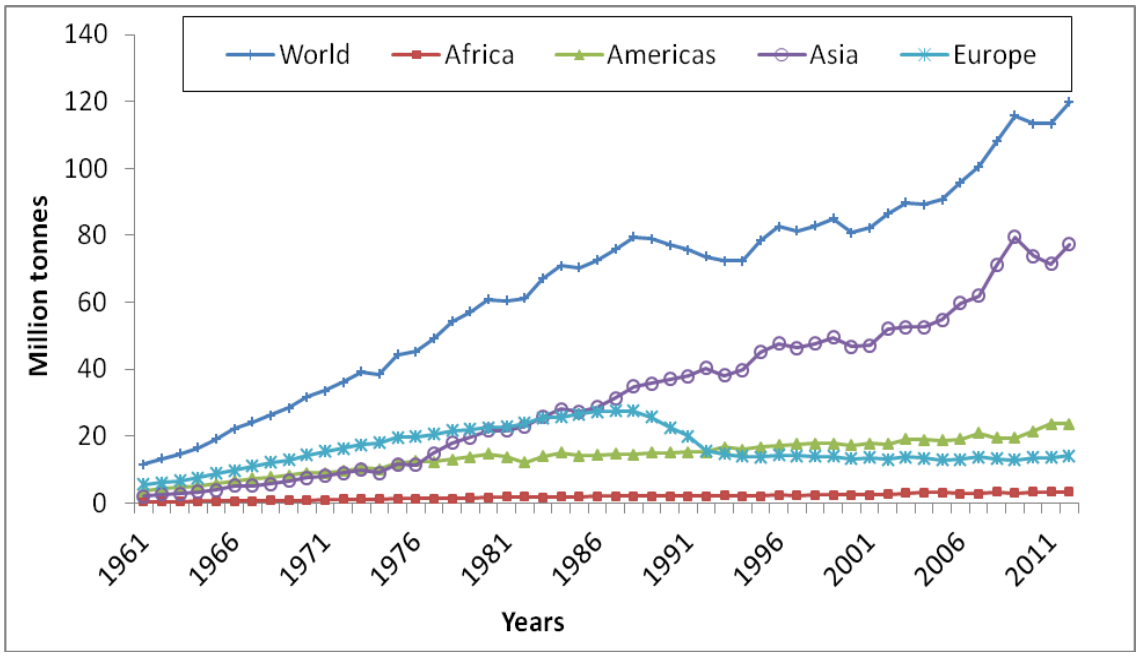


Figure 1.3: N fertiliser consumption from 1961 to 2012 in the most important regions and in the world (Data source is **http://faostat.fao.org/faostat).**

Improved crop N management will also play a crucial role in stabilizing and increasing crop yields (Reynolds and Tuberosa, 2008). Indeed losses of N have been reduced while grain yield was increased by optimized agronomic N management. This has been achieved by optimising soil and irrigation management, N fertilisation monitoring (quantities and timing of application) and by taking into account seasonal and environmental variation in soil N supply and climate.

For UK wheat, solid ammonium nitrate granules (AN: about 34% N) is the main form of straight N application. Alternative forms of soil-applied fertiliser N are: Urea (U: 46% N), combination of AN and U, calcium ammonitrate (CAN: 27% N), compound (NPK) fertiliser and ammonium sulphate (AS: 21% N). Fertiliser price, quality (spreading ease and efficiency) and efficiency of crop recovery are the most important factors affecting N fertiliser choice.

N already in the soil as part of the organic matter may partly fulfil crop N requirement and the remainder has to be supplied by N fertiliser. However, it is very difficult to accurately estimate the N mineralisation from organic matter. Previous cropping and manuring history have a large influence on N availability in the soil. The need for fertiliser N is high in a continuous cereal rotation. The timing of N applications will depend on the total N rate and the crop's stage of growth. All N should generally be applied by the springtime. In the UK, N is typically applied in three splits to winter wheat: the first split (1/4) is applied to encourage tiller development and survival (GS 25: End February and beginning of March). The main split (1/2) is applied when the crop is growing rapidly (GS 30/31: End March and beginning of April). Finally, the third split (1/4) is applied to achieve canopy required for optimum yield or to help improve grain protein levels in bread making varieties.

In order to optimize N management, two nitrogen terms, 'nitrogen supply' and 'nitrogen losses' should be considered. Nitrogen supply can be from the soil, organic manures, atmosphere and fertiliser. Nitrogen losses may be by leaching, run-off, ammonia volatilisation and denitrification (DEFRA RB209). To make accurate decisions on fertiliser timings and amounts, the soil proprieties in the surface and subsoil of the field should be considered. Soil type is usually related to soil texture, which is defined by the proportion of sand, silt and clay sized mineral particles in the soil and can be determined by laboratory soil analysis or by extrapolated regional soil maps (DEFRA RB209).

Some spectral reflectance indices were developed such as NDVI (Normalised Difference Vegetation Index) used to assess crop nitrogen status in cereals (Aparicio, et al. 2000). Rapid screening techniques (e.g. SPAD light transmission indicating chlorophyll content and NDVI spectral reflectance index indicating canopy area and

N uptake) are methods to estimate leaf/canopy N status during the season in order to optimise N fertiliser inputs (Li et al. 2009). Indeed, spectro-radiometers estimating NDVI have been demonstrated to successfully estimate soil N availability and yield potential hence optimum N amount for wheat grown in the USA (Raun et al. 2001; Raun et al. 2005; Raun et al. 2002).

Under the EU Nitrates Directive in England and Wales, the area covered by Nitrate Vulnerable Zones (NVZs) was increased from 55% to 70% between 2002 and 2009 (www.defra.gov.uk). The NVZs are areas of land which drain to waters where nitrate concentrations exceed, or are likely to exceed, the 50 mg l^{-1} level dictated by the EC Surface Water Abstraction Directive (1975). In 1996, the UK government designated 66 NVZs in England. Aiming to reduce the loss of nitrate from agricultural lands, the Government introduced an action programme to change farming practices within these NVZs to minimise N-leaching from the soil the crop. According to the action programme, the crops should receive optimum doses of N according to the guidelines set out in fertiliser manual RB209 (DEFRA, 2010). Adequate N fertilisation practises are related to application of the correct N dose at the correct crop developmental stage and weather conditions.

1.4.3 Environmental impacts of N fertilisers

N fertilisers may have negative environmental impacts through: (i) nitrate leaching into ground water (health concerns associated with elevated nitrate levels in drinking water), (ii) nitrate release into rivers and lakes causing eutrophication which is harmful to aquatic plant and animal species and (iii) denitrification of nitrate by soil bacteria that releases nitrous oxide (N_2O) into the atmosphere contributing to the greenhouse effect and global warming (DEFRA RB209 fertiliser recommendations). Reducing N_2O emissions from N fertiliser use would contribute to the reduction of the global warming impact of greenhouse gas emissions, since the impact of N_2O on global warming is 300 times more than that of CO_2 per molecule. N_2O contributes 6% of global greenhouse emissions (Aldhous, 2008). The main cause of agricultural N_2O emissions is the application of synthetic N fertiliser to arable soils. Ninety percent of N_2O emissions are from three sources: direct emissions from soils (42%), indirect emissions from soils (33%) and emissions from pasture range and paddock manures (15%) (Defra 2011). Therefore, improved NUE in new cultivars will help in

reducing N input and limit emissions of N_2O in direct proportion as well as improve economic returns.

Wheat is cultivated in temperate regions worldwide; therefore development of sustainable agricultural systems that are more economically efficient with reduced environmental impacts requires breeding wheat with improved NUE. Many studies reported genetic variation for NUE in wheat (VanSanford and Mackown, 1986; Dhugga and Waines, 1989; Van Sanford et al. 1991; Ortiz-Monasterio et al. 1997; and Le Gouis and Pluchard, 1996). These investigations indicated that NUE is genetically controlled and a heritable trait and genetic improvement is feasible to reduce N requirements hence N applications. This breeding goal, however, requires improved understanding of the genetic control of NUE and it stability across environments and related traits in wheat under different N availability. Indeed, physiological and molecular studies on N uptake and assimilation pathways reveal the high complexity of N use in plants (Crawford and Forde, 2002).

1.5 Nitrogen-use efficiency and components

Modern cereal cultivars grown in high yield potential environments require large amounts of N fertiliser; and they are selected under non-limiting N conditions (Foulkes et al. 2009). For wheat plant productivity and grain quality, nitrogen (N) is the most important fertiliser applied. However, N fertiliser represents a significant cost of production for the growers and has an effect on the environment as mentioned above.

Nitrogen-use efficiency (NUE) is defined as grain dry matter yield per unit of N available (from the soil and/or fertiliser) and is divided into two components: (i) N-uptake efficiency (above-ground N uptake/ N available; NUPE) and (ii) N-utilization efficiency (grain DM / above-ground N uptake; NUTE) (Moll et al. 1982). NUPE is linked to the ability of the crop to capture available N from the soil and is principally determined by root-associated traits such as root length density (RLD, root length per unit soil volume) at depth. Total N-uptake may also be affected by the capacity for N accumulation in the stem (Foulkes et al. 2009; Hawkesford 2012). NUTE reflects the functionality of the above-ground biomass and is determined by photosynthetic rate, canopy architecture, and canopy longevity (Hawkesford 2012). Shanahan et al. (2008) reported that cereal production is generally characterised by

low NUE. In fact, worldwide fertiliser N-uptake efficiency was estimated to be about 33% (Raun and Johnson, 1999).

There is an increasing need to reduce fertiliser N inputs in current cereal systems whilst maintaining acceptable grain yields and adequate grain protein contents. Breeding to develop new wheat varieties which can use N more efficiently can be achieved by exploiting new genetic resources with wider variation like landraces to improve uptake and utilisation in order to lower crop fertiliser N requirements without compromising yields. The improvement of fertiliser N use through the identification and selection of new genetic resources and physiological traits linked to efficient use of N is now a high priority in wheat production systems (http://www.wheatisp.org and http://www.breedwheat.fr).

1.6 Use of wheat relatives and landraces in wheat breeding

Many years ago, the National Research Council (1972) scientist raised an alarm about genetic erosion. The genetic erosion is the term given to describe the phenomenon of narrowing of the germplasm by crops improvement processes (Harlan, 1972). In most regions wheat production is replaced by modern cultivars; landraces are only cultivated by farmers in very limited areas (Moragues et al. 2006a). This landrace replacement by homogeneous cultivars has resulted in a significant loss of genetic variation in resistance to biotic stresses. Thus drastic crop loss can be caused by a simple mutation in a disease and insect or by an environmental change. Improvement of new cultivars by introgressing genes and traits from landrace population is one of the strategies to improve yield and yield stability in agricultural system mainly reduced input levels.

Feldman and Sears, (1981) reported that landraces and wild relatives of crop plants are very important sources to broaden genetic base. These landraces are rich in agronomic traits that should be characterized (Murphy and Withcomebe 1981). Many studies conducted on durum wheat (Gökgöl, 1939; Zhukovsky et al. 1951; Kün, 1988; Damania et al. 1996; Zencirci and Kün, 1996) revealed that there is a great amount of variation in terms of agronomic and grain quality traits and disease resistance in wheat relatives.

In wheat breeding programs, genetic variation often comes from landraces. For instance, Rht dwarfing genes came from Japanese wheat "Norin 10", derived from landrace Shiro Daruma (Kihara, 1982). The two genes RhtB1 (Rht1) and RhtD1 (Rht2) affected directly yield by reducing hight and lodging. The same landrace diversity was used to introduce the resistance to some diseases, such as stem rust, leaf rust, Russian wheat aphid (Skovmand and Rajaram, 1990; Skovmand et al. 1994) and also for some abiotic stresses like heat (Hede et al. 1999; Skovmand et al. 2001).

In the International Maize and Wheat Improvement Center (CIMMYT) wheat breeding programme, exotic wheat relatives have been used to increase the wheat diversity through inter-specific hybridization and crossing with landraces (Reynolds et al. 2007). Crossing conventional lines can achieve new transgressive segregation leading to better performance, however, crossing more adapted cultivars with landraces accessions is required to increase allelic diversity by introducing new genes and new gene combinations. Such accessions may complement existing stress-adaptive process by providing novel alleles.

In barley, landraces have been used for some time for breeding (Ceccarelli, 1984), their use in bread wheat still limited and physiology of wheat landrace needs to be more investigated (Reynolds et al. 2007).

1.7 Mapping populations: development and uses for QTL discovery

Within a given plant species several different types of segregating populations may be utilized for mapping genes. One of the most important types of population is Recombinant Inbred Lines (RILs). RILs are developed from inbreeding of individual F2 plants following an initial outcrossing to develop an F1 plant. The population consists of a series of homozygous lines, each containing a unique combination of chromosomal segments from the original parents (Collard et al. 2005). For developing RILs by the Single Seed Descent (SSD) method, usually six to eight generations of self-polination are required. Single seed descent is a high-throughput method to achieve homozygosity while often practising minimal selection (Goulden 1939). The method consists of selfing a random sample of F_2-derived plants in each generation and advancing only one seed per plant in up to 3 generations per year. This process

attempts to represent each genotype present in the F_2 regardless of merit (no selection).

Linkage analysis with molecular genetic markers is a very powerful tool in the genetic analysis of quantitative traits (van Ooijen, 1999) such as grain yield, NUE and many other quantitative traits. These traits are often called complex or quantitative traits in contrast to Mendelian traits and the relation between genotype and phenotype cannot be observed directly. The regions within genomes that contain genes associated with a particular quantitative trait are known as Quantitative Trait Loci (QTLs). The genetics of NUE in the wheat plant is complex since NUE is a quantitative trait. However, its understanding can be achieved by exploiting high density molecular linkage maps and carrying out a QTL analysis. Successful NUE QTL studies have been reported in Arabidopsis (Loudet et al. 2003) and in rice (Lian et al. 2005; Obara et al. 2004). In wheat also several QTLs for yield and yield components are mapped (Quarrie et al. 2005, Bogard et al. 2010). By testing 214 European winter wheat cultivars in eight environments, 333 genomic regions associated with 28 traits related to NUE using genome-wide association were identified (Cormier et al. 2014).

The identification of QTL with agronomic interest is a first step to develop new markers to use in indirect selection of genetic determinant of a trait of interest using marker-assisted selection (MAS). MAS, after marker development, is a simpler method compared to the phenotypic screening, allowing selection at seedling stage with increased reliability (no environmental effects).

1.8 Aims and Objectives of Project

A major world agriculture challenge is to optimise the trade-off between enhancing grain yield and the negative environmental impacts associated with fertiliser N use in intensive agricultural systems. The breeding of new N-efficient varieties of wheat will be critical in meeting these challenges and concerns, whilst maintaining increases in grain production to ensure food security.

To meet these challenges, new traits must be identified exploiting new sources of genetic diversity for these traits and selection methods developed including molecular markers to allow new wheat varieties to be bred requiring lower N inputs whilst maintaining, or ideally increasing, grain yields. This increase in the crop NUE

should be achieved, through an increase in both the crop N-Uptake efficiency (NUPE) and crop N-utilisation efficiency (UTE; grain DM yield/ crop N uptake). The overall aims of the project aims are to:

- Quantify the variation between elite UK winter wheat varieties and AE Watkins wheat landraces for grain yield, biomass, NUE, NUE components and related physiological traits in glasshouse and in field experiments under high and low N conditions.
- Compare between genotype performance in the glasshouse and in the field with regard to genetic variation in grain yield, biomass, NUE and components and their physiological determinants at the crop, plant and leaf scale.
- Identify novel physiological traits determining genetic variation in biomass and NUE and NUE components in two wheat SSD mapping populations developed from crosses between Paragon (UK spring wheat variety) and two AE Watkins landraces (PxW481 and PXW141) under high N and low N conditions.
- Increase the understanding of the physiological processes determining genetic variation in biomass production, NUE and N partitioning within the plant canopy and responses to N availability.
- Identify quantitative trait loci (QTL) for NUE, biomass and underlying traits in the two SSD mapping populations PxW481 and PxW141, under high N and low N conditions.

Chapter 2: Literature review

2.1 Yield potential, NUE and use of physiological traits in breeding

The yield potential of wheat crops is determined by many factors, genetic, environmental and socioeconomic. Yield potential may be defined as the yield of a cultivar grown under environmental conditions to which it is adapted and with the optimal agronomic conditions, i.e. when pests, diseases, lodging, weeds and other stresses are effectively controlled (Evans, 1993). The average farm yield (when a cultivar grows under less favourable conditions in the presence of one or more yield limiting factors) will be less than the potential yield. The difference between the potential yield and the farm yield is called the yield gap (Fischer and Edmeades, 2010).

While significant genetic yield gain has been mainly achieved empirically based on yield *per se* (Evans, 1993), without the aid of physiological tools (Rajaram and van ginkel, 1996; Reynolds 2002; Reynolds et al. 1999; Foulkes et al. 2007; Fischer, 2011), breeders, as well as physiologists, generally agree that multidisciplinary research will be needed to realize acceleration in genetic gain in wheat yield (Jackson et al. 1996; Reynolds et al. 2012a). In addition, grain yield has a relatively low heritability and the selection for yield potential *per se* may not be efficient as there is a high genotype x environment interaction (Jackson et al. 1994; Slafer, 2005). Therefore trait-based breeding may improve the efficiency of wheat breeding (Reynolds et al. 2001). There is an urgent need to develop new and more efficient wheat breeding methodologies to complement existing breeding techniques, as well as to identify new traits, which will drive faster yield gains (Reynolds, 2002, 2012).

Several studies agreed that physiological selection traits have the potential to improve rates of genetic yield gains in wheat. Fisher et al. (1998) demonstrated an association between a number of physiological traits, including flag-leaf stomatal conductance and photosynthesis rate and yield potential in the high-yielding irrigated environment in NW Mexico. Under unfavourable wheat-growing regions in CIMMYT, other physiological traits like canopy temperature depression were effective in selecting wheat genotypes with improved yield performance cultivars under water-stressed environments (Reynolds et al. 1996a; Araus et al. 2002, 2003;

Olivares-Villegas et al. 2007; Mohammadi et al. 2012). In Australia, physiological selection traits like higher transpiration efficiency, early vigour and reduced tillers per plant, were introduced into a wheat breeding programme for improved performance under drought (Richards et al. 1996).

Globally, wheat is the leading source of vegetable protein in human food, having a higher protein content than other major cereals, i.e. maize or rice (FAO, 2010). However a major global challenge for wheat nutrition is to increase grain yield under low N inputs whilst maintaining, or ideally increasing, grain protein content. High levels of N supply result in a higher grain protein content, but decreased efficiency of N utilization is realized as grain N concentration increases and grain yield remains stable (Ortiz-Monasterio et al. 1997). In wheat, the improvement of nutrient use efficiency in general and NUE specifically in cropping systems can be achieved through two main strategies: i) by adopting more efficient crop management practices (such as nutrient rate, timing, source, and placement); and ii) by breeding more N-use efficient cultivars. Both are important for wheat production in all environments (Ortiz-Monasterio et al. 2012).

Reports have shown that about 50% of applied N fertiliser is usually recovered in the above-ground crop under average high-potential field conditions, and efficient timing and placement of N could increase recovery of applied N up to 60 or to 70% (Legg and Meisinger, 1982). Appropriate N application timing and rates are critical to achieve a synchrony between N supply and crop demand in time and space (Shanahan et al. 2008) and may indicate considerable opportunities for improving NUE in systems where best management practises are not already in place (Dhugga and Waines, 1989; Blankenau et al. 2002). Sylvester-Bradley et al. (1998) suggested that the highest demand on N is during leaf expansion and the optimal timing for N application should coincide with the appearance of the first large leaf on the main stem which is around GS31.

Breeding for more N-efficient wheat cultivars is the alternative approach to improve NUE. Identification and selection of desirable traits which increase NUE components (NUPE and/or NUTE) can accelerate wheat breeding progress and complement empirical selection for yield under low N conditions (Araus et al. 2002).

These physiological traits can be at the canopy, leaf, or biochemical level and can be identified through physiological studies.

The identification of Quantitative Trait Loci (QTL) using molecular marker mapping approaches and appropriate mapping populations is also an alternative for developing selection criteria for improved NUE cultivars since NUE is a complex trait (Hirel et al. 2007). The QTL can then be used to develop molecular markers useful to screen large numbers of lines for traits underlying NUE in marker-assisted selection.

The main aim of this chapter is to review the literature regarding the physiological bases of the efficiency of the use of N by wheat crops. and to provide information on QTL and gene discovery studies carried out to on NUE traits in wheat to date.

2.2 Wheat developmental growth

2.2.1 Wheat vernalisation and photoperiod

Two types of wheat are differentiated by their response to vernalisation (Flood and Halloran, 1986):

- Winter wheat: For this type a period of cool weather (3 - 7°C) is required in order to flower. It has a strong response to vernalisation and is very resistant to frost in the early stages of growth. The vernalisation requirements of winter types may be fully substituted by short days at no vernalizing temperatures between 21° and 16°C (Evans, 1987). Winter wheat does not flower until the completion of a cool period. The double-ridge stage is not reached until chilling requirements are met, and the vegetative phase is prolonged generating a higher number of leaves in the main shoot (Mossad et al. 1995).
- Spring wheat: This type of wheat does not or has lower response to vernalisation and very sensitive to frost.

For spring types usually temperature between 7° and 18°C for 5 to 15 days are required for floral induction, while between 0° and 7°C for 30 to 60 days are required for winter genotypes (Evans et al. 1975). In winter genotypes, vernalisation

stimulates the cell division processes facing the inhibitory mechanism occurring at high temperatures.

Photoperiod sensitivity is defined as the physiological reaction of organisms to the length of day or night. Most cultivated wheats do not require a particular day length to flower; however they flower faster as the day-length increases (Evans et al. 1975; Major and Kiniry, 1991).

Bernier et al. (1993) reported that the photoperiod is usually sensed by mature leaves and not by meristems. For floral initiation, a single leaf could be enough to sense the photoperiod. Once the vegetative period ends, floral initiation starts and the reproductive stage begins (double-ridge). The shorter the length of the day, the longer the spikelet primordia production phase is (Major, 1980; Boyd, 1986) and the bigger the flag leaf (Mossad et al. 1995); however Evans et al. (1975) showed that longer days advance floral initiation. After initiation, the spikelet primordia are at a rate that is also dependent on day length in those genotypes sensitive to photoperiod (Stefany, 1993). With shorter days, the period from double ridge to terminal spiklet extends increasing the period to terminal spikelet and the number of spikelets per spike.

Vernalisation and photoperiod constitute the basic processes of the adaptation of wheat to various environments. Knowledge and genetic manipulation of them provides significant tools for adaptation and yield (Acevido et al. 2002).

2.3 Source and sink

For wheat growth, the plant life cycle can be simplified into two main phases: i) the pre-anthesis phase, when the plant forms its canopy area and root system, and young developing leaves and root behave as sink, and ii) the phase after anthesis, when senescing leaves are sources of carbohydrates for the grain (Good et al. 2004)

Sink organs are those plant parts utilizing assimilate produced by others called source organs (Gardener et al. 1985). An organ can be defined as source or sink depending on the direction of assimilates. If the organ produces assimilate (i.e. mature leaves photosynthesizing) it called source organ, whereas if the organ consumes assimilate products it is called a sink organ. For example, leaves can be considered sink once young, because they consume sugar, but become source when

they start photosynthesis and send assimilate to other organs (Hay and Walker, 1989).

Both the accumulation of assimilate (sink) or supply of assimilate (source) can limit grain yield (Evans et al. 1970; Suwignyo et al. 1995; Wardlaw, 1990). During early grain-filling source limitation of photosynthate may be more important while during late grain-filling, when there is less demand from competing sink limitation may be more important, (Fischer, 1975; Herzog, 1986; Martinez-Carrasco and Thorne, 1979)

2.3.1 Growth and light interception

The green area index (GAI) is defined as the ratio of green area (lamina, stem and attached sheth and ear) of one side of the canopy area to the ground area. It is a useful tool in canopy management. Increased GAI leads to an increase in solar radiation interception as the area receiving radiation is increased. However, when the light interception increases up to the point when all the incident radiation is intercepted by the canopy, the increase in green area of the upper leaf lamina will not increase light interception, but has a maintenance respiration cost, thus decreasing above-ground biomass (Gardner et al. 1985). As the radiation is transmitted through the canopy layers, both the quantity and the quality of the intercepted radiation are changed (attenuation). The light attenuation between canopy layers is explained by the light extinction coefficient (K) using Beer's law of absorption (Monsi and Sakei, 1953) (equation 2.1). K is defined as a parameter that describes the efficiency of light interception for the canopy of a terrestrial ecosystem. A low K indicates that much radiation can reach the bottom of the canopy. Where, a high K indicates that only a little radiation can penetrate into the lower leaf layers of the canopy (Monsi and Saeki, 1953; Campbell, 1986).

$$k = -\ln(I_i/I_0)/L \qquad \text{Equation 2.1}$$

Where k is the light extinction coefficient of the canopy, I_i is the PAR below the i layer of leaves, I_o is the photosynthetically active radiation (400-700 nm; PAR) above the canopy and L is the green area index of the canopy to the i layer of leaves. Consequently, GAI and leaf inclination affect the amount of radiation transmitted through the canopy (Gardner et al. 1985), and reducing the extinction coefficient (more vertical leaf attitude) would optimize the distribution of the intercepted

radiation within the canopy, hence, radiation-use efficiency through reducing light saturation of the upper leaves (Araus et al. 1993).

2.3.2 Growth and radiation use efficiency

Biomass accumulation is facilitated by the capture of photosynthetically active radiation (PAR, 400-700 nm), which is then used in photosynthesis. The effectiveness of the use of radiation is quantified as the radiation-use efficiency (RUE, g MJ^{-1}) defined as the amount of above-ground dry matter produced per unit of PAR intercepted (Monteith, 1977).

Radiation-use efficiency can be affected by many factors including nutrient imbalances (Sinclair and Horie, 1989) and temperature (Goyne et al. 1993). Total crop biomass is linearly related to the amount of light intercepted (Kiniry et al. 1989; Monteith and Moss, 1977) although little variance has been observed for RUE as a result of LAI (Sinclair and Muchow, 1999). Photosynthesis activity is thus affected by canopy architecture. Vertical leaves allow more light penetration to the lower levels of the canopy, in addition to more light reflection to the canopy leading to optimized photosynthesis with reduced light saturation of photosynthesis (Stoskopf, 1981 and Duncan, 1971). Araus et al. (1993) reported that cultivars with erecte leaves are more efficient in radiation-use. In addition, higher RUE is associated with smaller, thicker flag leaves (Shearman et al. 2005).

Many studies have explored variation in canopy traits of bread wheat and their influence over RUE. Calderini and Slafer, (1998) found that pre-anthesis RUE did not differ widely in cultivars released between 1920 and 1990; however, they reported a variation for post anthesis RUE. In Australia studies showed an increase of RUE between modern and old cultivars (Yanusa et al. 1993; Siddique et al. 1989b). Similar results were reported by Shearman et al. (2005) in UK. Resulting from poorer canopy architecture and radiation distribution, there was a decline in RUE in the presence of semi-dwarf alleles (Miralles and Slafer, 1997). Muurinen and Peltonen-Sainio, (2006) also found that, at lower altitudes, RUE differed between old and modern cultivars; and ancient wheat species had increased RUE. Furthermore, spelt and emmer wheat purportedly perform favourably in low-input conditions (Troccoli and Codianni, 2005) and have been observed to display increased RUE

when compared to their modern counterparts (Muurinen and Peltonen-Sainio, 2006). Sparkes (2010) also investigated a range of ancient wheat characteristics and compared them to modern wheat; spelt was found to have the greatest RUE (1.81 MJ m^{-2}).

It is stated that increased RUE usually leads to a rise wheat biomass (Han et al. 2008; Li et al. 2009) therefore increasing RUE may be central to the improvement of modern bread wheat yields through biomass improvement. Furthermore, Reynolds et al. (2000) states that to date, breeding has not substantially improved RUE. Reynolds et al. (2000), suggested new physiological avenues to achieve this including: photosynthetic metabolism, canopy photosynthesis, determination of grain number and size (sink strength), vascular transport of water, nutrients and assimilates, respiratory costs and buffering of these processes to environmental fluxes (Gaju 2007).

2.3.3 Photosynthetic capacity

In C3 plants like wheat, photosynthesis is the process used to convert light energy, normally from the sun into chemical energy in the form of organic carbon from water (taken from the soil) and carbon dioxide (CO_2, fixed from the air). The C3 plants use only Calvin cycle for fixing CO_2 from the air. In the first step, CO_2 reacts with ribulose bisphosphate (RuBP) to produce two 3-carbon molecules of 3-phosphoglyceric acid (3-PGA). The second stage (light independent reactions through the Calvin cycle) begins with carbon fixation, whereby RUBISCO (ribulose-1, 5-bisphosphate carboxylase) incorporates CO_2 from the air into organic molecules in the chloroplasts by conjugating it to a five carbon sugar (ribulose bisphosphate) (RuBP) (Beadle and Long, 1985; Campbell and Reece, 2002; Langdale et al. 1988). Then the Reduction phase, results to the production of complex carbohydrates. In the final steps of the Calvin cycle, ATP is used to rearrange carbon products to regenerate the CO_2 acceptor, RuBP. It is this concluding step that enables the cycle to continue.

The genetic progress in RUE can be achieved by Increasing the carboxylase activity of the Rubisco (ribulose 1-5 bisphosphate carboxylase oxgenase) and decreasing its oxygenase activity (Austin, 1999) and improving the photosynthetic efficiency

through increasing the activity of Rubisco (Reynolds et al. 2009). Many avenues to increase Rubisco's catalytic activity were proposed by Parry et al. (2007 and 2011): increasing Rubisco amount in the chloroplast, maintaining Rubisco activity, accelerating RuBP regeneration, increasing the CO_2 cocentration at Rubisco catalytic site and finally decreasing the energy lost to photorespiration. Several authors also suggested introducing C4 mechanism into C3 plant in order to accelerate the rate of yield gain (Reynolds et al. 2009 and Parry et al. 2011, Murchie et al. 2009).

The flag leaf is a predictor of source capacity as the main source of carbon during grain filling (Austin and Edrich, 1975; Evans et al. 1970). Flag leaf parameters (CO_2 exchange rate, RuBP carboxylation efficiency, chlorophyll concentration and leaf area) describe photosynthesis capacity which depends itself on source size and activity during grain filling through feedback and upregulation processes (Herzog, 1986).

Photosynthesis rate per unit area should be improved after canopy architecture, light interception and photosynthetic duration have been improved (Long et al. 2006; Raines, 2006; Parry et al. 2007). Theoretically, the photosynthetic energy conversion efficiency of C3 plants is about 4.6% (Zhu et al. 2008), which is three times higher than the value in the field leaving substantial room to increase photosynthetic energy conversion efficiency (Reynolds et al. 2000; Zhu et al. 2008).

Parry et al. (2011) reported that significant improvement in RUE is still possible by increasing photosynthesis in wheat. The study by Fischer et al. (1998) on spring wheat cultivars released between 1962 and 1988 by CIMMYT showed that the increase in grain yield was associated with an improvement in light-saturated flag leaf photosynthesis rate (A_{max}). In addition, evidence for increased yield in response to CO_2 enrichment (Ainsworth and Long, 2005), and the positive relationship between photosynthesis and biomass (Kruger and Volin, 2006) support these results. However, selecting for yield based on A_{max} was not successful in the 1990's (Reynolds et al. 2000). Thus, despite photosynthesis being the primary determinant of plant productivity, previous research has seldom provided good evidence for a strong positive relationship between the rate of photosynthesis per unit leaf area and yield (Driever et al. 2011). Most previous studies assessing variation in wheat have involved limited measurements on small sets of germplasm, (Fischer et al. 1981,

1998; Blum, 1990; Watanabe et al. 1994; Reynolds et al. 2000; Xue et al. 2002; Chytyk et al. 2011; Sadras et al. 2012).

Semi-dwarf genotypes tend to have smaller flag leaves and contain more Rubisco per unit leaf area than tall genotypes (Pyke and Leech, 1985). Rubisco, is an enzyme that generally constitutes about 50% of the total soluble leaf protein and it is involved in RuBP carboxylation efficiency (Van Oosten et aI., 1995) which may limit photosynthesis and could be increased in dwarf lines.

Several studies indicate that tall wheat cultivars have lower maximum CO_2 exchange rates (a measure of net photosynthetic rate) and chlorophyll concentrations than dwarf ones (Morgan et al. 1990; Kulshrestha and Tsunoda, 1981). Photosynthetic capacity can be influenced by plant height (which in some cases is associated with grain sink size) (Bonnett and Incoll, 1993 a; Ehdaie and Waines, 1994; Gent, 1995; Wardlaw, 1990).

Decreases in canopy photosynthesis and biomass accumulation result from a reduction in height decreasing in light interception in wheat (Gent, 1995). Gent reported that in early developmental stages, tall wheat lines had 20% more light interception, canopy photosynthesis and biomass than semi-dwarf isolines. After spike emergence, no differences were observed among height classes in terms of photosynthesis and light interception. However, Rawson and Evans (1971) reported that dwarf wheat cultivars have lower leaf photosynthetic rates in single leaf than the taller ones. In addition, other cultivars containing dwarfing genes were found to have higher respiration rates per unit biomass than taller genotypes (Gent and Kiyomoto, 1985).

2.3.4 Senescence and stay-green traits

Stay-green traits allow genotypes a prolonged carbon fixation potential during grain-filling through longer period of CO_2 assimilation from the air (Thomas and Howarth, 2000). They described the types of stay-green: i) late senescence initiation, ii) slower senescence rate, iii) chlorophyll degradation, iv) rapid death and v) enhanced leaf greenness. Hay and Walker (1989) showed that photosynthesic activity increases linearly during leaf expansion before declining when the leaf reaches its full size. Photosynthesis is usually negatively affected by senescence mainly under water and nutrient-limited conditions (Gardner et al. 1985). The net assimilation rate (the rate

of photosynthesis minus respiration losses; NAR) is also affected by senescence as the reduction in photosynthesis in senesced leaves is higher than the reduction in respiration rate (Milthorpe and Moorby, 1979; Stoskopf, 1981).

Stay-green phenotypes have been described most extensively in monocotyledonous species. Grain yield was positively affected by stay-green trait in rice mutants (Fu and Lee, 2008; Yoo et al. 2007). Similar results were reported on sorghum (Borrell and Hammer, 2000). Borrell and Hammer found that the stay-green phenotype was directly associated with grain yield and genotypes carrying the stay-green trait had higher leaf nitrogen concentration (N per leaf area) and could absorb the majority of the nitrogen required for grain-filling from the soil (Borrell and Hammer, 2000) or translocate it from the stem (van Oosterom et al. 2010).

In contrast, another study on maize suggested that stay-green cultivars had higher biomass and took up more N, while no effect on grain yield was observed and grain N concentration was lower. Similarly, in Canada, extended leaf longevity was associated with a higher source-to-sink ratio, higher grain yield (Rajcan and Tollenaar, 1999a) and elevated grain N content from increased N uptake (Rajcan and Tollenaar, 1999b).

Spano et al. (2003) reported that durum wheat mutants stayed green for longer, continued photosynthesizing and had higher grain yield and seed weight. The stay-green characteristics were then validated using gene expression. However, grain N content was lower in some of the mutants supporting the idea that the maintenance of N in the photosynthetic machinery might be limiting to N remobilisation to the grain. In China, wheat lines with a wheat-rye chromosome translocation were developed which showed a functional stay-green phenotype combined with increased grain yield and total biomass of up to 25% when grown in the field (Chen et al. 2010; Luo et al. 2006). In the United States a study on spring wheat revealed that the effect of stay-green can depend on the environmental conditions (Blake et al. 2007). The study was on two sets of RILs, one of them showed a positive correlation between stay-green and yield, grain size and TGW under irrigated and unirrigated conditions, while in the second population the stay-green phenotype had an effect only on grain size, TGW and grain N content under unirrigated treatment. Naruoka et al. (2012) studied another spring wheat mapping population and found that stay-green

genotypes had lower grain yield under cool and well-watered conditions, however, they maintained yield under dry and hot environments.

2.3.5 Stem carbohydrate storage

In the pre-anthesis period, plants use mainly the stems to store surplus assimilate which is relocated to the ear shortly before and after anthesis (Fischer and Hillierislambers, 1978). In addition, Austin et al. (1977) showed that, during grain filling, stems can lose about 30% of their dry mass as carbohydrates are remobilized to the grains. Austin et al. (1980) and by Herzog, (1986) reported that stem carbohydrate reserves make an increased contribution to the final grain mass. They added that this contribution can reach 50% under stressed conditions. In taller wheat, when photosynthesis is not adequate, assimilate stored in stems can act as a buffer. Nevertheless, under non-stress conditions at anthesis, the stems of dwarf cultivars contained lower proportion of flag-leaf assimilates than the taller wheat. According to Rawson and Evans (1971) there were no differences between dwarf and tall cultivars in the stem reserves utilizating stem carbohydrate reserves.

2.3.6 Grain partitioning

Dwarfing genes which have been used to reduce plant height have substantially increased harvest index and lodging resistance, thus increasing wheat yields (Peng et al. 2000, Foulkes et al. 2011). Higher grain yields and HI of the dwarf modern cultivars reduce competition from the developing stem because a reduction in height leads to a reduction in stem mass (Rawson and Evans, 1971). To identify further mechanisms to increase HI and elucidate limitations to grain yield, the relationship between photosynthetic capacity, stem mass and plant height could be an issue. Sink strength, grain number, and size are partially under genetic control (Fischer and HillieRis Lambers, 1978; Thornley, 1979; Walpole and Morgan, 1970).

2.4 Plant growth and N availability

Within plants, seeds, leaves and roots contain the maximum N concentration in organic and inorganic forms. Nitrogen deficiency leads leaves to lose their green colour, to decrease their area and photosynthetic capacity. Many studies reported that N application increases total biomass, grain yield and yield components (tillers, shoots and grain number) in wheat (Barraclough et al. 1989; Latiri-Souki et al. 1998;

Mengel and Kirkby, 2001; Jiang et al. 2004; Cossani et al. 2009). N fertiliser therefore plays an important role in increasing photosynthesis rate and efficiency (Lawlor et al. 1988) as well as leaf cell number and volume by increasing N uptake. N also plays a key role in wheat nutrition because of its importance in protein and nucleic acid synthesis (Clark, 1983). N uptake and distribution in plants and crops involves many aspects of growth and development. Thus, the relationship between N and biomass accumulation in crops relies on the reciprocal regulation of multiple crop physiological process.

Nitrogen helps in improving photosynthetic capacity by increasing radiation interception though the increases in canopy green area (Lawlor, 1995). Nitrogen supply has a large effect on leaf growth and increases the leaf area of plants and therefore photosynthesis. Photosynthetic proteins represent a large proportion to total leaf N (Evans, 1989; Field and Mooney, 1986). Leaf chlorophyll content is approximately proportional to leaf N content (Evans, 1983). Many studies showed that N application in cereals increased the above-ground biomass and LAI (Leaf Area Index) at anthesis increasing grains m^{-2} leading to higher grain yield (Cossani et al. 2009; Fois et al. 2009). However, N fertilisation probably has either no or a small effect on grain weight and HI (Barraclough et al. 1989, Cossani et al. 2009; Fois et al. 2009)

2.5 Nitrogen use efficiency and components

2.5.1 Nitrogen consumption in some countries

Wheat grain yield increased in UK by approximately 69 kg $ha^{-1}year^{-1}$ between 1948 and 2007, a value that translates into a relative rate of 0.76% $year^{-1}$ in 2007 (Mackay et al. (2011). Since 1982 at least 88% of improvement in yields across a range of species could be attributed to genetic improvement (Hall and Richards 2013; Mackay et al. 2011). In the UK HI was increased from 0.34 to 0.51 from 1900 to 1986 (Austin et al. 1989; Austin, 1999). The genetic yield increase registered in this period is also supplemented by progressively higher nitrogen (N) inputs (Austin, 1999) making new cultivars more tolerant to lodging compared with the old ones (Foulkes et al. 1998; Reynolds et al. 1999).

Over the last 40 years, the doubling of agricultural food production was associated with a sevenfold increase of N fertilisers (FAO 2001; Tilman et al. 2001) (see Figure 1.2 in the Chapter I). The use of N fertilisers in agriculture is therefore one of the key elements for producing sufficient food to meet demand of the increasing human population and global food security (Angus, 2001; Eikhout et al. 2006). The use of large amounts of N in intensive agricultural systems, however, has led to important environmental impacts such as gaseous emission of nitrous oxides and ammonia into the atmosphere (Galloway and Cowling, 2002) and through problems associated with eutrophication of marine ecosystems (Beman et al. 2005) and fresh water (London, 2005), and leaching of nitrates in ground water Bio-fuel production to ameliorate the impacts of climate change also requires the efficient use of N fertiliser in biofuel crops.

A substantial increase in yield and in crop stress resistance is therefore required, along with better use of N fertiliser to ensure food security and environmental protection in future decades. Improved efficiency of N-use will be particularly crucial as part of the dynamic to allow greater biomass production to be sustained.

Data compiled by FAO state that annual use rate of Nitrogen fertiliser is increasing worldwide (http://faostat.fao.org/faostat). The N fertiliser use efficiency has decreased exponentially from 1961 to 2001 in all countries except Western Europe and the United States with increasing amount of N fertiliser applied (Figure 2.2).

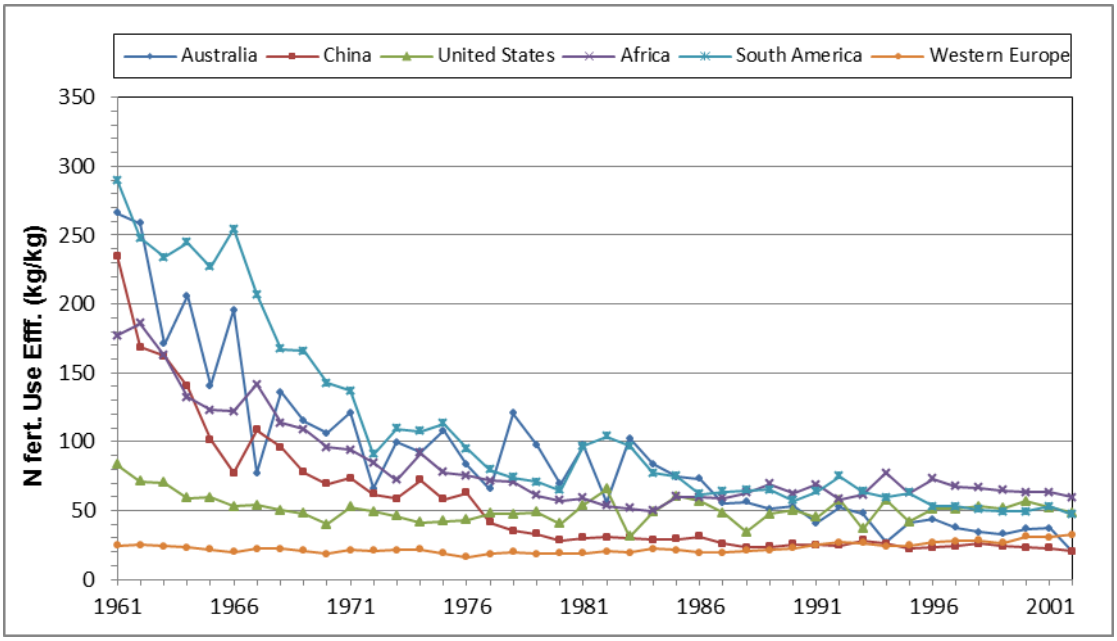


Figure 2. 1: Evolution of N fertiliser Use Efficiency (grain production / N consumption) in six regions in the world (data source: http://faostat.fao.org/faostat)

2.5.2 Nitrogen-Use Efficiency (NUE)

Nitrogen is the mineral nutrient required by plants in the highest concentration (Taiz and Zeiger, 2006). However, the N available to the plant is generally limited (Elser et al. 2007). Nitrogen is essential for reproduction (Sinclair and Jamieson, 2006), growth (Agren, 1985), and for physiological processes (Evans, 1989).

Nitrogen-use efficiency (NUE) is the grain dry matter per unit plant N availability (from the soil and/or fertiliser) and is divided it into two components: (i) N-uptake efficiency (crop N uptake/N available; NUPE) and (ii) N-utilization efficiency (grain dry matter yield/crop N uptake; NUTE) (Moll et al. 1982). This definition of NUE has since been found useful by crop physiologists. In the UK, NUE was estimated to be 25 kg DM kg^{-1} nitrogen available with an average fertiliser and soil nitrogen recovery of 65% and an average nitrogen utilization of 39 kg DM kg^{-1} nitrogen captured (Sylvester-Bradley and Kindred, 2009)

2.5.2.1 N-uptake efficiency

N-uptake efficiency (NUPE) is determined by the ability of the plant to remove N (as $NO3^-$ or $NH4^+$ ions) from the soil (soil N + N fertiliser). Full uptake of available N by fertilised crop is never completed and crops have a preference to recover soil mineral N compared to fertiliser N (Vaidyanathan, 1984). In wheat, in a review of

published literature data on fertiliser-N efficiency in cereal crops, the average N recovery efficiency in aboveground biomass (grain+straw) in research plots was 44% in rice, 54% in wheat and 63% in maize (Ladha et al. 2005). Similar results (50-60% of N fertiliser recovery) were found for winter wheat in Europe (Blankenau et al. 2002; MacDonald et al. 2002; Bloom et al. 1988).

In addition to nitrate (NO_3^-) and ammonium (NH_4^+) forms, plants roots take also organic N in the form of amino acids (Maathuis, 2009). The N status in the plant could be affected by root morphology since the roots are responsible for N-uptake. The root system plays a determinant role in supplying the plant with nutrients such as N and water (King et al. 2003; Wasson et al. 2012). Total N-uptake may also be affected by the capacity for N accumulation and storage in the stem (Foulkes et al. 2009; Hawkesford 2012).

Accumulating higher quantities of N in plant tissue is a desirable trait in wheat. NUPE is a trait under genetic control (LeGouis et al. 2000), but is also affected by the environment and by management strategies (Sylvester- Bradley et al. 1998). Several studies have reported high genetic variation of N-uptake in wheat (Van Sanford and MacKown, 1986; Ortiz-Monasterio et al. 1997; Le Gouis et al. 2000).

2.5.2.2 N-utilisation efficiency

N-utilisation efficiency (NUTE) is the product of the biomass per unit of crop N uptake and the partitioning of this biomass to the grain (harvest index, HI) at harvest (Ortiz-Monasterio et al. 1997). Therefore NUTE concerns grain storage capacity, grain filling through carbohydrates and N remobilisation, canopy formation, and the maintenance of photosynthetic apparatus (Pask, 2009). Many studies reported genetic variation in NUTE in wheat (Cox et al. 1985b; Dhugga and Waines, 1989; May et al. 1991). Austin et al. (1989) suggested that the genetic gain in NUTE from 1830 to 1985 was mainly associated with the increase in HI while biomass production was broadly maintained. Habash et al. (2001) illustrated that yield and protein concentration in wheat were improved by the manipulation of glutamine synthetase, the important enzyme for ammonia assimilation and recycling, therefore N remobilisation.

To improve NUTE, the improvement of nitrate assimilation could be a potential avenue (Foulkes et al. 2009). In the plant tissue, nitrate is reduced to nitrite (nitrate

reductase) then ammonium (nitrate reductase) after that to amino compound and other proteins in the plastid/chloroplast (synthetase/glutamate synthase GS/GOGAT) (Good et al. 2004; Hirel et al. 2007; Lea and Azevedo, 2007; Lea and Ireland, 1999). While nitrate reductase was considered to play the major role in this process, Crawford (1995) showed that this enzyme did not have any effect on plant growth. Furthermore, Kelly et al. (1995) found no correlation between nitrate reductase activity and grain yield in wheat.

At the leaf level, the photosynthetic N-use efficiency (PNUE) is the rate of C assimilation per unit leaf N (Field and Mooney, 1986). The relationship between NUE and PNUE is complex, not least because it depends on the extent to which PNUE measurements, performed at a given point in time, can be related to growth of the whole plant integrated over a much longer period of time.

2.6 Physiological traits related to NUE

To attain their maximum yields, modern cereal cultivars in developed countries require large amounts of fertilisers because they were selected under non-limiting fertilisation conditions (Bänziger et al. 1997 and Presterl et al. 2003; Foulkes 2009). However, fertiliser N uptake efficiency (crop N uptake/N available; NUPE) was estimated globally to be about 33% (Raun and Johnson, 1999).

2.6.1 Nitrogen root N capture

The possible agrotechnical measures for increasing nitrogen uptake from subsoil are of practical importance for farmers (Haberle et al. 2004, Hoad et al. 2004). The availability of mineral N in the soil and its distribution are affected by plant uptake, fertilisation, mineralization, denitrification, leaching and immobilization (Haberle et al. 2006). Crops absorb N in the form of nitrate and ammonium ions from different soil profiles (top and subsoil layers) (Miller and Cramer 2004). The above-ground plant growth and the water and nutrients balance in the soil are affected by the ability of plant to grow its root system as well as its capability to extract water and nutrients in different soil environments. There is evidence that N deficiency has an effect on the equilibrium between root and shoot growth (Novoa and Loomis, 1981; Brouwer, 1983). In fact, the ratio R/S (root weight to total plant weight ratio) is increased under N deficit (Dreccer et al. 2000; Mengel and Kirkby, 2001; Reich, 2002).

Furthermore, hydroponic pot and field experiments, increasing nitrogen supply enhanced both shoot and root growth, but usually shoot growth more than root growth, leading to decrease R/S dry weight ratio with increase in N supply (Robinson 1994, Marschner 1995, Lucas et al. 2000).

The horizontal spread of roots is usually 30-60 cm, while the roots grow in depth up to 200 cm (Barraclough and Weir, 1988; Gregory et al. 1978). However, Nagesh (2006) reported that about 70% of total root length is found within the top 30 cm of soil.

It is believed that the ability of root system to respond to special and temporal N supply affect root N capture (Fitter et al. 1991). In addition, a positive relationship was found between root length density (root length cm/ soil volume cm^3; RLD) and N capture in maize (Wiesler and Horst, 1993; Wiesler and Horst, 1994) and in wheat and barley (Ebrahim, 2008). N supply generally increases RLD increasing, therefore, the N uptake by the crop; however a decrease in NUPE and NUE was observed (Cabrera-Bosquet et al. 2007; Pask, 2009).

2.6.2 Nitrogen remobilisation efficiency

In wheat, N taken up after anthesis and N remobilised from pre-anthesis N uptake are the two sources of N used for grain protein synthesis. Grain N content is more influenced by the amount of N taken up than yield (Dupont and Altenbach, 2003). After anthesis, leaves provide N which is recycled and exported to the grains in the form of amino acids (Feller and Fischer, 1994 and Masclaux et al. 2000). Before anthesis N stored in roots and shoots provides 60 to 95% of N of the grain by remobilisation (Van Sanford and MacKown, 1986, Palta and Fillery, 1995a and Palta and Fillery, 1995b). After flowering and under N deficient conditions both the size and the N content of the grain can be significantly reduced by N deficit (Dupont and Altenbach, 2003). Yet, determination of genetic variation in grain yield and grain protein under N limitation is still unclear between N availability (N taken up after anthesis and N remobilised) and or storage protein synthesis.

Cox et al. (1985) reported in wheat grown in the field that post-anthesis N remobilisation efficiency N (NRE, the fraction of N in the whole plant or organ at anthesis which is not recovered in the straw at harvest) before anthesis depends on the genotype. However, in the absence of environmental factors limiting grain

filling, the capacity of the plant to store N pre-anthesis explained the differences observed in NRE (Barbottin et al. 2005). Remobilisation of the N stored before anthesis and N take up after anthesis can be estimated by the difference between the amount of above-ground N present at harvest and the amount of above-ground N present at anthesis in the different components of the plant. However, the necessity to combine data from the two sampling dates can lead to large experimental errors. One good alternative, less biased and precise method is the use of ^{15}N stable isotope labelling which allows better estimation of N uptake after anthesis and N stored and remobilised from source organs pre-anthesis. This technique was used by Cliquet et al. (1990) who applied ^{15}N nitrate to maize plants grown in hydroponics and by Palta and Fillery (1995a; 1995b) by infiltrating $_{15}N$-urea to the leaves of wheat plants grown in the field. In order to characterize genotypic differences in remobilisation of the N stored before anthesis and post-anthesis N uptake in wheat, Le Gouis et al. (2000) conducted field experiments during two years using post-flowering $_{15}N$ labelling at high and low levels of N fertilisation on five cultivars of winter wheat selected for their different sensitivity to N stress. They showed that both N uptake after flowering and N remobilisation of pre-anthesis stored N (once $_{15}N$ is applied just after flowering) can be estimated.

Many studies reported in wheat that NRE was higher for lamina than for stem and chaff (Gaju et al. 2014; Pask et al. 2012). N uptake post-anthesis can contribute between 5 and 50% of grain N (Van Sanford and MacKown, 1986; de Ruiter and Brooking, 1994) depending on N level and crop growth conditions. The genetic variation in NRE of the vegetative tissues has been reported in wheat from 0.52 to 0.92 (e.g. Gaju et al. 2014; Cox et al. 1986; Van Sanford and Mackown, 1987; Barbottin et al. 2005; Kichey et al. 2007; Pask, 2009,).

2.6.3 Nitrogen utilization and plant senescence

Senescence kinetics takes place after anthesis, allowing recycling of nutrients (N in particular) from vegetative tissues towards reproductive organs (Lim et al. 2007; 2008; Guiboileau et al. 2010), a processes called translocation. In wheat, leaf senescence is associated with the translocation of leaf N to the grains, which accounts for 40–90% of final grain N (Sanford and MacKown, 1986; Kichey et al. 2007).

Senescence is a normal phenomenon in the life cycle of the plants (Borrás et al. 2003). Lim et al. (2007a) defined the senescence as the age-dependent complex degenerative and degradation processes at full maturity which may ultimately lead to leaf death (Camp et al. 1982; Thomas et al. 2003; Tang et al. 2005). The most important event in leaf senescence is the loss of chlorophyll and the disturbance of photosynthetic apparatus, decreasing the leaf photosynthetic efficiency. In addition, leaf senescence leads to the decrease in the electron transport for the remaining components in the leaf (Weng et al. 2005; Zhang et al. 2006). The chlorophyll a/b ratio is also increased because chlorophyll "a" is degraded more rapidly than chlorophyll "b" during senescence process (Hotta et al. 1987; Humbeck and Krupinska, 2003; Fang et al. 1998). Lim et al. (2007) reported that this chlorophyll degradation is accompanied with structural changes in the chloroplasts of mesophyll cells. This can be easily assessed because plant leaves start to turn yellow.The balance between N supply and demand had a large effect on leaf senescence during grain filling in sorghum (Oosterom et al. 2010) and wheat (Triboi and Triboi-Blondel, 2002). The disturbance in this balance usually causes accelerated or delayed leaf senescence. If N demand is higher than N supply, an acceleration of the translocation N from leaves and stems occurs (Triboi and Triboi-Blondel, 2002). On the other hand, late N application led to higher N availability to plants in the soil leading to retention of leaf green (Bancal et al. 2008). According to Howarth et al. (2008), N is transported into the grain through the amino acid "acid glutamine" used to synthesise other amino acids and proteins.

As senescence is usually accelerated by abiotic stresses, varities with stay-green traits give higher yield in sorghum (Borrell et al. 2000) and in wheat under these conditions (Borrell and Hammer, 2000; Gaju et al. 2011; Bogard et al. 2011). A positive relationships between stay-green phenotypes and yield amongst wheat genotypes has been demonstrated also by Verma et al. (2004); Christopher et al. (2008); Lopes and Reynolds (2012); Bogard et al. (2011) and Pask and Pietragalla (2012). However, there may be a trade-off between keeping N in the leaves to produce biomass that might be translocated to the grains, and transporting N to the grain earlier and possibly more efficiently. Thus negative effects on grain N% have sometimes been reported (Jiang et al. 2004; Kichey et al. 2007; Derkx et al. 2012).

N remobilisation (NR) is driven by the source supply of the vegetative organs rather than by the demand for N by the grain (Martre et al. 2003). As leaf senescence regulated NR (Masclaux et al. 2001; Uauy et al. 2006), in bread-making wheat cultivars, delaying senescence will decrease grain quality (lower protein content); however, in feed wheat cultivars, delaying senescence (increasing the photosynthetic period during grain filling) will enhance grain yield (Foulkes et al. 2009).

2.6.4 Harvest Index, biomass and partitioning

In small-grain cereals yield potential has increased during the Green revolution by the introduction of semi-dwarf cultivars. This was due mainly to improvement in HI (Reynolds et al. 1999; Abdeledo et al. 2002). These increases were associated with reduction in stature, reduction in lodging, and also reduced competition resulting in greater spike, more grains per unit area, and then higher harvest index (Fischer and Stockman, 1986).

However, no significant genetic progress in HI is reported since 1990 with current values of ca. 0.50 in spring wheat (Reynolds et al. 1999) and ca. 0.55 in winter wheat (Shearman et al. 2005). In winter wheat the theoretical maximum of HI is 0.62, calculated based on an extrapolation from the mean value (0.49) observed in four UK modern cultivars (Austin et al. 1980). In the UK. Austin *et al.* (1980) reported decreased stem dry weight was the main reason for the increase of HI in UK cultivars increased from 1908 to 1978 and he argued that a further increase in HI (to 60%) could be achieved by decreasing stem-and-leaf sheath partitioning at harvest to 20% whilst maintaining the above-ground biomass. Further improvement in HI could be achieved by improving grain number by increasing partitioning to spike during stem elongation and decreasing partitioning to the true stem while maintaining lodging resistance through increase true stem material strength (Foulkes et al. , 2011).

2.7 Breeding and NUE

2.7.1 Genetic variation in N-use efficiency

Genetic as well as agricultural practices have both contributed to the increase in in NUE worldwide. In the UK, NUE at the optimum N amount increased by +1.2 kg DM kg N between 1994 and 2008 in wheat (Sylvester-Bradly and Kindred, 2009)

reaching 24.4 kg DM kg N. This increase was mainly due to the increase in NUPE rather than to NUTE. Fisher and Edmeades (2010) reported similar conclusion for Maize in USA. However, Foulkes et al. (1998) reported that genetic gains in NUE with breeding under low N supply have been related mainly to NUTE in winter wheat in UK.

Many reports stated that genetic progress in NUE was higher under high N than under low N conditions. Ortiz-Monasterio et al. (1997) studied 10 genotypes of spring wheat released between 1950 and 1985 and reported that the genetic progress in NUE was nearly tripled at high N supply (300 kg N ha^{-1}) compared to the nil N supply treatment. This could be normal, because new released cultivars were selected under non-limiting N conditions (Brancourt-Hulmel et al. 2005; Foulkes et al. 1998), thus modern genotypes were adapted to these conditions. While few studies examined cultivars under optimum N levels, Sylvester-Bradley and Kindred (2009) showed that the optimum nitrogen level increased by 1.24 kg N ha^{-1} $year^{-1}$, reaching 174 kg N ha^{-1} for cultivars released from 2001 to 2007. Similarly Foulkes et al. (1998) showed an increase of optimal N level in modern cultivars. Under Australian conditions for cultivars released between 1958 and 2007 there was a trend of increasing yield; yield per unit N remained largely unchanged due to increased N uptake. In spite of an increased NHI there was still a trend of decreasing grain N concentration (Sadras and Lawson, 2013).

2.7.2 Quantitative Trait Locus (QTL) for NUE

QTL analysis is usually carried out using Kruskal-Wallis (KW) or interval mapping (IM) (Lander and Botstein, 1989). Linkage maps are constructed from the analysis of many segregating markers. 'QTL mapping' is based on the principle that genes and markers segregate via chromosome recombination (crossing-over) during meiosis (i. e. sexual reproduction), thus allowing their analysis in the progeny (Paterson, 1996). There are three main steps affecting construction of a linkage map (Collard et al. 2005): (1) Size of the population (from 50-250 individuals or even larger). (2) Identification of polymorphism between parents in order to construct a linkage map, and (3) Linkage analysis of markers: This involves coding of data for each marker on each individual of a population and conducting linkage analysis using computer software programs. Linkage between markers is calculated as LOD score (logarithm

of odds; likelihood ratio statistic). The density of markers depends to some extent on the genome size and a higher density would give more precisely positioned markers on a linkage map.

Regions of the genome that show significant LOD values are assumed to contain a QTL (van Ooijen, 1999). After construction of a linkage map QTL detection is done by QTL analysis using single marker analysis, simple interval mapping and composite interval mapping (Liu, 1998; Tanksley, 1993). Phenotyping the population for the target trait is important and the laboratory marker data have to be correlated with the field data for QTL identification. Therefore, developing high-throughput precise phenotyping for the target traits is essential. Phenotypic correlations are usually used to link markers with traits and to genetically dissect complex traits into simple and Mendelian factors. After markers that are tightly linked to genes or QTLs of interest are found, they can be used by breeders as diagnostic tools to identify lines carrying the genes or QTLs in a marker-assisted selection (MAS) programme. The selected QTLs should account for a large proportion of the phenotypic variance for the target trait using a large population size and a high number of markers ares important to build high resolution mapping for precise and reliable markers for MAS. Multi-environmental trials can also help in achieving the goal of reliable markers and can also help to discriminate between a single gene and several linked genes (Mohan et al. 1997 and Michelmore, 1995).

Using a bread wheat mapping population exhibiting a contrasting response to N deficiency and three years of field experiment, Le Gouis et al. (2008) identified several chromosomal regions that explained variations for glutamate dehydrogenase (GDH) and glutamine synthetase (GS), two enzymes involved in N assimilation and recycling. A QTL for GS and GDH activities overlapped in four regions located on chromosomes 2A, 2D, 5A and 7A. Le Gouis et al. (2008) compared the QTL regions previously detected for yield, physiological and agronomical trait QTL. Three of the detected QTL were known to carry major genes acting on photoperiod sensitivity (Ppd-D1 on 2D), plant height (Rht-B1 on 4B) and awnedness (B1 on 5A).

Bogard et al. (2011) studied the genetic factors contributing to correlations observed between leaf senescence during grain filling, grain protein concentration, and grain yield in winter wheat. Using a DH CF9107 x Toisondor mapping population, QTLs affecting leaf senescence, protein concentration and grain yield were identified on

chromosomes 2D, 2A and 7D. The study showed that delaying leaf senescence was associated with high grain yield and protein content. This association was mainly due to the genetic variation in nitrogen availability during grain filling.

An et al. (2006) in a field experiment with high N and low N treatment detected few QTL for NUP on the chromosomes: 2B, 5B, 6A and 6B under low N and on the chromosomes 2D, 4B, and 5 A under high N conditions. An et al. (2006) reported also 2 more QTL on chromosome 1B and 5D in a hydroponics experiment.

However, the genetic control of whole-season N efficiency has rarely been examined in wheat. Some extensive molecular studies (Charmet et al. 2005; Quarrie et al. 2005; Habash et al. 2007; Laperche et al. 2008; Fontaine et al. 2009) identified numerous genome regions (QTLs) responsible for grain yield components and yield under N limitation, grain protein content and N metabolism in the uppermost foliage as well as for the activity of glutamine synthetase and glutamate dehydrogenase, the key enzymes involved in N assimilation. On root study, two QTLs for grain yield and N uptake co-localized with root QTLs on chromosomes 2B and 7D, respectively (Atkinson et al. 2015).

2.8 Physiological techniques for phenotyping NUE traits

2.8.1 Canopy temperature

As the plants need to fix carbon they transpire water. Canopy Temperature Depression (CTD) has had widespread application in plant breeding programs since it was shown that genetic variation in this trait is well associated with yield in irrigated conditions (Fisher et al. 1998) as well as moisture-limited conditions (Blum et al. 1982). Vapour pressure deficit has a large effect on CTD, while net radiation, air temperature, and wind speed have smaller effects (Smith et al. 1986). Water status of the soil and atmosphere, wind, evapotranspiration, air temperature, relative humidity, and radiation can affect CTD (Reynolds et al. 2001). CIMMYT began CTD measurements on different irrigated experiments in Northwest Mexico and it was found that phenotypic correlations of CTD with grain yield were positive (Reynolds et al. 1994; Fischer et al. 1998).

Canopy temperature should be measured with an infrared thermometer at midday (10:00h to 14:00h) throughout the growing season. Infrared temperature should be

measured under conditions of clear skies with moderately strong winds weekly when it is possible (Zhang et al. 2012). Canopy Temperature Depression (CTD) is a widely used method for estimating canopy photosynthesis which is well correlated with stomatal conductance of wheat genotypes.

2.8.2 Gas exchange and photosynthesis

Photosynthesis and transpiration are the two processes through which leaf stomata control plant CO_2 absorption and water loss. Water-use efficiency of crop and the partitioning of light energy into sensible and latent heat are regulated by the stomata. By regulating intercellular CO_2 concentration stomatal opening affects photosynthesis and the biochemical processes in chloroplasts (Yu et al. 2001). Light intensity and water balance of cells are responsible for the extent of stomatal opening. Stomatal conductance is relevant to drought tolerance. The correlations among grain yield, photosynthetic rate, and stomatal conductance were positive in irrigated dwarf spring wheat (Shimshi and Ephrat, 1975). Stomatal conductance depends on the environmental changes like photosynthetically active radiation (PAR), and temperature (Squire and Black, 1981).

Positive relations between gs and grain yield in 16 genotypes of CIMMYT bread wheat with high variation for yield were shown by Delgado et al. (1994). These relations change with the nitrogen amount in bread wheat under Mediterranean conditions (Koc et al. 2001). Provided that other constraints do not become limiting, increasing photosynthesis will increase crop yields, as demonstrated by the effects on yield of CO2-enrichment experiments (Ainsworth and Long, 2005; Parry 2010). Also, experiments on historic genotypes of CIMMYT spring wheat suggest that improvements in photosynthesis per unit leaf area may already have occurred, in concert with improvements in harvest index and grain number, although not apparently in greater biomass (Fischer et al. 1998).

Photosynthesis is strongly influenced by leaf N content despite it varies widely between species (Sinclair and Horie, 1989; Arora et al. 2001). Leaves with low N content have lower maximum rate of photosynthesis and lower carboxylation efficiency than those with high N content (Lawlor et al. 1987). However, Evans and Seeman (1984) showed that the rate of CO_2 assimilation per unit of N is lower in leaves with high N content than with low N content. Nitrogen metabolism affects

wheat photosynthesis and controls plant development, possibly through carbohydrate metabolism which is involved in other important plant signalling and regulatory pathways (Mcintyre, 2001).

2.8.2.1. Limitations to photosynthetic rate

The main factors affecting net photosynthesis at the leaf level are: (i) light limitation; (ii) CO_2 limitation; (iii) nitrogen limitation and photosynthetic capacity; (iv) water limitation; (v) temperature effects; and (vi) pollutants. (Chapin et al. 2002).

i- Light limitation

The linear increase in photosynthesis in response to increased light (in the range of light limitation) indicates relatively constant light-use efficiency. The light compensation point is the minimum irradiance at which the leaf shows a net gain of carbon (Chapin, 2011).

At low to moderate light levels, leaves have a near linear response to light intensity. The rate of change of net photosynthesis in this region to irradiance is the quantum yield of photosynthesis. This is similar for all C3 plants (in the absence of environmental stresses) (Chapin et al. 2002). At higher light levels, saturation occurs as the efficiency of the photosynthetic mechanism is reduced. At higher levels still, net photosynthesis can decline as a result of photorespiration as described above (Figure 2.3).

Plants have some capacity to respond to changes in light conditions over time scales of days to months, resulting in leaves in more direct sunlight having more cell layers and higher photosynthetic capacity than shade leaves by acclimation or adaptation over longer time periods (Chapin et al. 2002). Respiration rate depends on tissue protein content so shade leaves with low photosynthetic capacity generally have a lower protein content to minimise respiration losses. When upscaling light limitations to the canopy or ecosystem scale, the leaf area index (LAI) may be a major constraint.

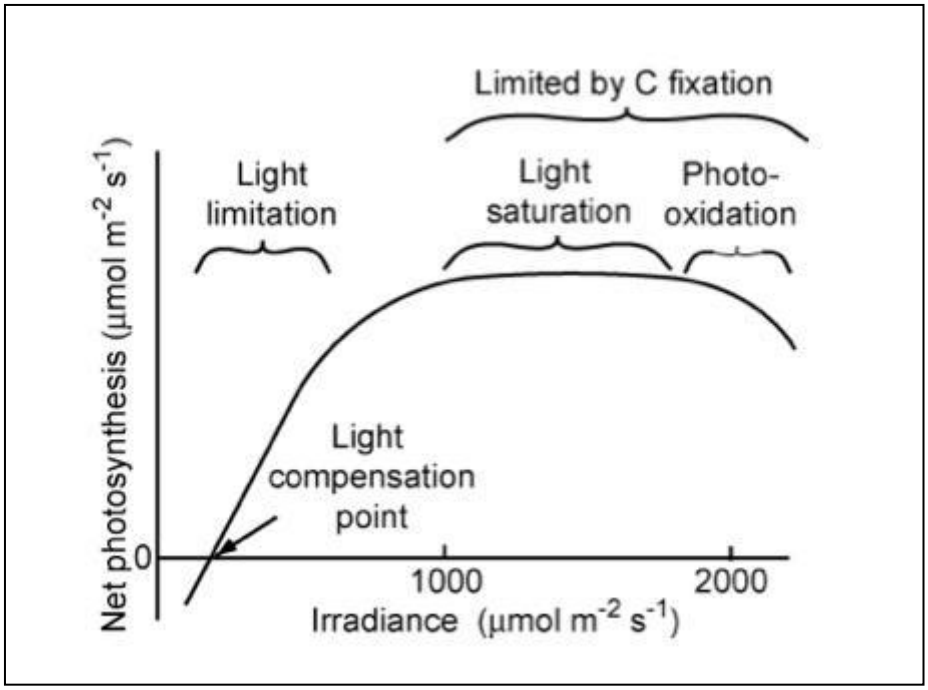


Figure2. 2: Relationship of the net photosynthesis rate with Photosynthetically Active Radiation (PAR)

ii- CO_2 limitation

Photosynthesis rate is limited by the rate of CO_2 diffusion into the chloroplast in the initial linear portion of the CO_2 response curve and by biochemical processes at higher CO_2 concentrations.

The response curve of net photosynthesis to CO_2 concentration inside the leaf of a C3 plant is shown above. At low levels, CO_2 diffusion limits photosynthesis. With current atmospheric CO_2 levels of around 390 ppm most C3 plants would show an increase in photosynthesis rate with further increases in concentration. The magnitude of this is however uncertain due to plant acclimation and other factors. Over the long term, it is likely that (*indirect*) effects of elevated CO_2 concentrations may be more important than increased net photosynthetic rates (Chapin et al. 2002), such as those arising from changes to the water cycle (Figure 2.4).

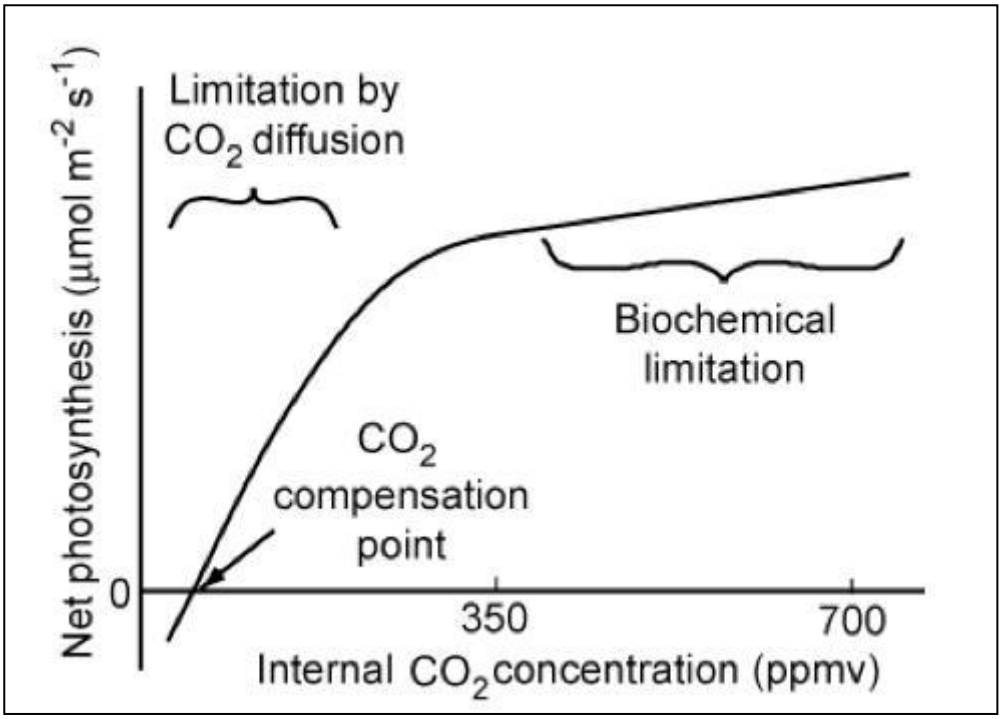


Figure2. 3: Relationship of internal CO2 and net photosynthesis

iii- Nitrogen limitation and photosynthetic capacity

In both natural and agricultural environments, nitrogen nutrition plays a crucial role in determining photosynthetic capacity (Abrol et al. 1999). Leaf growth is a key determinant of plant N demand because photosynthetic function accounts for a high proportion of the plant's reduced N requirements (Novoa and Loomis, 1981). In C3 plants, approximately 60-80% of leaf N is invested in the chloroplasts (Evans and Seemann, 1989; Makino and Osmond, 1991).

The rate of photosynthesis depends on i) the capacity of light harvesting, ii) the regeneration rate of NADPH, and ATP, iii) the capacity of carboxylation of ribulose-1,5-biphosphate (RuBP) by ribulose-1,5-biphosphate (Rubisco), and iv) the rate of sugar phosphate utilization. The influence of each of these processes on the photosynthesis rate varies depending on external conditions and internal regulation and resource availability. The photosynthetic apparatus accounts for a large proportion of plant N, therefore N availability is a key factor influencing photosynthetic capacity and plant growth. Many components containing N are responsible for photosynthesis including: i) the enzyme Rubisco, ii) the chlorophyll

complex; iii) Electron transport complex; and iiv) the enzyme of the reductive pentose phosphate pathway (Kumar et al. 2002).

N could be associated with non-soluble proteins (thylakoid membrane and electron transport complex), or with soluble proteins (phosphorylation, CO_2 assimilation, RuBP regeneration, starch and sucrose synthesis. N accounts for 25% of the leaf N in the thylakoid alone (Sivasankar et al. 1993) consisting of light harvesting chlorophyll "a" and "b" complex, photosystem I and II, the cytochrome b/f complex and the coupling factor (CF0-CF1).

The breakpoint for the relationship between specific leaf lamina N (N per unit green area of leaf lamina; SLN) and radiation use efficiency is estimated to be 2 g N m^{-2} (Pask et al. 2012; Lawlor et al. 1987a; Sinclair and Horie, 1989; Critchley, 2001). Furthermore, Araus and Tapia (1987) reported in spring wheat grown under Mediterranean conditions that the asymptote of the relationship between leaf N content and net CO_2 assimilation was similar for the flag leaf lamina and the leaf sheath at ca. 190 mmol N m^{-2} (Araus and Tapia, 1987).

iv- Water limitation

A very strong relationship between dry matter gain and water use is usually observed (Boyer, 1982) since at the leaf level there is CO_2 exchange for water (Cowan, 1982). Potentially useful variability in water-use efficiency (WUE, defined as the AGDW / total water use) among wheat cultivars has been demonstrated (Condon et al. 1990, Foulkes et al. 2007). Drought effects on photosynthesis rate and on WUE can be either stomatal or nonstomatal factors (Bunce 1988). However, it is clear that stomatal behaviour is important because variation in stomatal conductance (gs) affects transpiration rate (E) proportionally more than photosynthesis rate (A) (E increases linearly with gs, whereas A levels off at high gs values). Greater Calvin cycle capacity is associated with greater carboxylation efficiency, whereas greater steady-state capacity for photosynthetic electron transport and/or photo-phosphorylation is associated with greater A (Bjorn 1992).

2.8.2.2. Stomatal conductance and Rubisco content

Diffusion or CO_2 input through the stomata and carboxylation are two separate steps involves in the photosynthesis process. The CO_2 gradient is mainly a function of plant biological process affecting *Ci* (partial pressure of CO_2 inside the sub-stomtal

cavity), since *Ca* (partial pressure of CO_2 in the air surrounding the leaf) is relatively stable. Whereas, the water gradient is mainly a function of weather variables and leaf surface temperature. Content and activity of the Rubisco enzyme and the stomatal conductance (regulated by stomata) have an influence on carboxylation process which is involved in determining *Ci*.

For wheat, several strategies have been proposed for increasing photosynthesis rate per unit leaf area. These include improvement of Rubisco activity, and the introduction of carbon-concentrating mechanisms. These strategies all require modification of the photosynthetic components, which can only be achieved through genetic manipulation (Parry et al. 2011).

A large scale study by Wullschleger (1993) showed significant variation in photosynthetic capacity in a range of species. Analysis of CO_2 assimilation and intercellular CO_2 concentration of 109 species revealed that species-specific differences in CO_2 assimilation rate were due to differences in underlying biochemistry including carboxylation capacity and RuBP regeneration (via electron transport). Parry et al. (2014) reported significant variations in photosynthetic capacity in a panel of 16 elite wheat cultivars. They showed this variation was associated with components related to maximum capacity and operational rates of CO_2 assimilation, and to CO_2 diffusion.

2.8.2.3 Spectral reflectance indices

Canopy variables related to physiological characteristics can be estimated by different applications of remote sensing (Thenkabail, Smith and de Pauw, 2001). Imaging spectroscopy consists of acquiring images in many narrow wavebands providing a continuous spectrum for each pixel. Spectral reflectance indices have been developed such as difference or ratio between the reflectance at given wavelengths based on simple mathematical formula (Araus et al. 2001). Simple ratio (SR = NIR/VIS) and the normalized difference vegetation (NDVI = Rred - R_{nir}/ R_{red} + R_{nir}, where (R_{red}) is red reflectance and (R_{nir}) is infra-red reflectance (R_{nir}) were the first indices to be developed. This NDVI has been related to crop variables such as biomass, leaf area, plant cover, leaf gap fraction, nitrogen and chlorophyll in cereals (Aparicio, Villegas, Casadesus, Araus and Royo, 2000).

Spectral reflectance is a non-destructive and rapid technique that could estimate biomass at the genotypic level (Peňuelas et al. 1997). The measurement should be taken; on clear sunny days with negligible wind on leaf surfaces without dew or moisture. The time of the day is very important, so mesurements should be taken between 11:00h am and 14:00h to avoid diurnal variation.

To overcome the constraints in field phenotyping capability limiting the ability to dissect the genetics of quantitative traits, recent progress in high-throughput phenotyping that has been made by the development of new phenotyping technologies and highly automated platforms (Fiorani and Schurr, 2013; Araus and Cairns, 2014; Cooper et al. 2014a; Leitner et al. 2014).

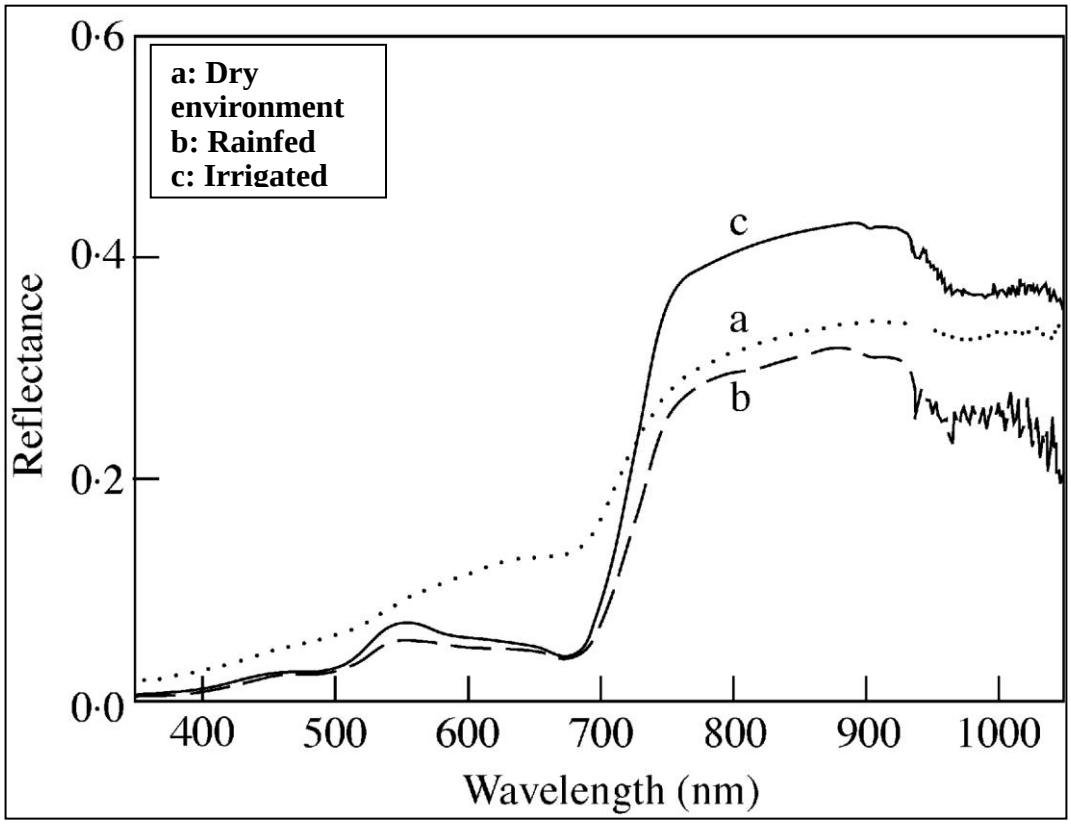


Figure2. 4: Typical variation in the spectra around mid-grain filling in three water regimes (adapted from Araus et al. 2002).

2.8.3 Chlorophyll content

Chlorophyll (Chl) is a green pigment found in almost all plants, algae, and cyanobacteria. Its name is derived from the Greek words chloros ("green") and phyllon ("leaf"). Chlorophyll is an extremely important biomolecule, critical in

photosynthesis, which allows plants to obtain energy from light. Chlorophyll absorbs light most strongly in the blue portion (at wavelength 665 nm for Chl. "a" and 640 for Chl. "b") of the electromagnetic spectrum, followed by the red portion (465 nm for chl. "a" and 450 for Chl. "b"). However, it is a poor absorber of green and near-green portions of the spectrum, hence the green colour of chlorophyll-containing tissues. Chlorophyll was first isolated by Joseph Bienaimé Caventou and Pierre Joseph Pelletier in 1817.

In the leaf chloroplast, chlorophyll "a" and "b" are the main pigments of photosynthesis. They absorb and exploit the light energy, thereby influencing photosynthetic efficiency (Pan and Dong 1995). Over the last century little change has been noticed with breeding in chlorophyll a/b ratio and is typically in the range 3.1 to 3.2 (Manderscheid and Weigel, 1997).

Chlorophyll content of spring CiMMYT wheat genotypes in a warm, irrigated environment was linked to increased net carbon dioxide fixation rate and positively correlated with grain yield and biomass (Gutierrez-Rodriguez et al. 2000; Gutierrez-Rodriguez et al. 2004 Araus et al. 1997). Biomass production and grain yield can be increased by increasing the chlorophyll content in crops (Wang et al. 2008). Therefore, for wheat yield improvement, it is important to understand the genetic mechanisms regulating chlorophyll content.

2.8.4 Chlorophyll fluorescence

The ability of the plants to absorb photosynthetically active radiation is the most important factor limiting winter wheat productivity. The maximum observed radiation-use efficiency for wheat is about 4 g DM/Mj^{-1} PAR. To improve wheat yield more efforts should focus on increasing photosynthesis efficiency, extending photosynthesis duration and improving the partitioning of assimilate to grain (Loomis and Amthor, 1996).

In photosynthesis, molecules of antenna absorb light photons; the excitation energy is transferred to reaction centres of the photosystems I and II. Photochemical reactions are driven primary by high energy initiating the conversion of photosynthetic energy in photochemical and biochemical pathways. Fluorescence is then the energy emitted by chlorophyll complex of PSII (Govindjee, 1995).

Useful information can be provided leaf photosynthetic performance can be provided by chlorophyll fluorescence, nevertheless, many papers of on agricultural and horticultural plants stated the complicated relationship between yield and fluorescence in wheat, therefore there are relatively few examples to date of its application in any crop breeding programs (Foulkes et al. 2009; Baker and Rosenqvist, 2004; Yan et al. 2011). The fluorescence of shaded (not dark adapted) leaves, subjected to double light pulses, have also been used as a fingerprint to distinguish maize and barley from six different weed species (Tyystjärvi et al. 2011).

2.9 Objectives of the study

2.9.1 General objectives

The present study aims to quantify genetic variation in NUE and NUE components and related physiological traits and to identify QTL linked to these traits in a panel of UK cultivars and AE Watkins landraces and two novel Paragon x Watkins SSD mapping populations. Therefore physiological traits linked to NUE, biomass and grain yield were investigated in a panel of elite UK winter wheat varieties and AE Watkins landraces. In addition a physiological and genetic analysis on two mapping populations from the cross between Paragon and two different AE Watkins lines was carried out. The overall objectives of the present study were to:

- Quantify the variation for grain yield, above-ground biomass, NUE, NUE components and related physiological traits amongst elite UK winter wheat varieties and AE Watkins landraces.

- Identify novel physiological traits determining genetic variation in biomass and NUE and NUE components under HN and LN conditions in two wheat SSD mapping populations and QTL on co-location with these traits.

- Identify variation in response to N supply for NUE, biomass and yield and underlying traits and mechanisms and QTLs associated these traits.

2.9.2 Specific objectives

The specific hypotheses tested in this study are:

1) There is variation between elite UK winter wheat varieties and AE Watkins landraces for grain yield, biomass, NUE, NUE components and related physiological traits in glasshouse and the field experiments under high and low N conditions.

2) Genotype performance in the glasshouse is correlated with performance in the field in regard to grain yield, biomass, NUE and components and their physiological determinants at the crop, plant and leaf scale.

3) Novel variation can be identified in grain yield, above-ground biomass and NUE components among lines of two SSD mapping populations (Paragon x landrace W481 and Paragon x landrace W141) under low N and high N conditions and the response to N availability.

4) Landraces can be identified with improved performance compared to UK check cultivars for grain yield, AGDM, NUE under LN conditions.

5) Genetic variation for physiological traits, canopy size and senescence traits is linked to grain yield biomass, NUE and NUE components.

6) The genetic maps for PxW481 and PxW141 populations can be constructed.

7) QTL collocations for yield and yield components, biomass, NUE can be found in the two SSD populations PxW481 and PxW141, under high N and low N conditions.

8) Stable QTLs for PxW481 mapping population can be identified under HN and LN conditions.

9) QTLs can be identified in two populations PxW481 and PxW141 that co-locate with each other and with QTLs in the literature.

Chapter 3: Materials and methods

This chapter describes the materials and methodologies used in the glasshouse and field experiments. In 2011/12, one glasshouse experiment under high N conditions (17 genotypes) and one field experiment under high N and low N conditions (10 genotypes) was carried out examining panels of Watkins landraces and UK check cultivars for grain yield, biomass, physiological traits and N-use efficiency (NUE) and NUE components. In addition, a second field experiment under HN and LN conditions examining 79 lines of a single seed descent (SSD) mapping population from the cross between UK spring wheat cultivar Paragon and Watkins landrace line 481 (PxW481) was carried out.

In 2012/13, one glasshouse experiment was carried out under HN conditions examining a panel of 17 genotypes (Watkins landraces and UK check cultivars) for grain yield, biomass, physiological traits, NUE and NUE components. In addition, two field experiments were carried out under HN and LN conditions; one experiment examined 79 lines of P x W481 SSD population and the second experiment examined 97 lines of a SSD mapping population from the cross between Paragon and Watkins landrace line 141 (PxW141).

Hereafter 2011/12 and 2012/13 are referred to as the years 2012 and 2013, respectively.

3.1 Glasshouse experiments

In each of 2012 and 2013, one glasshouse experiment was conducted at the University of Nottingham, School of Biosciences, Sutton Bonington Campus, UK ($52^{o}5^{'}$ N, $1^{o}3$' W). In each year a panel of 17 genotypes comprising five UK cultivars (spring wheat Paragon, and winter wheats Oakley, Rialto, Savannah and Xi19) and 12 Watkins landraces (W034, W040, W141, W209, W223, W292, W352, W355, W468, W481, W579 and W729) was assessed under controlled conditions in the glasshouse. The experimental design was a completely randomized block design with three replications. Each pot (25.8 cm diameter x 20.4 cm depth) contained 6 plants of each genotype. In the 1930s A. E. Watkins acquired landrace cultivars of bread wheat (*Triticum aestivum* L.) of which originated from local markets in 32

countries. The geographic distribution of the 826 landrace cultivars of the current collection, here called the Watkins collection, covers many Asian and European countries and some from Africa. The Watkins collection captures a snap shot of genetic diversity present before the start of modern breeding (Wingen et al. 2014)



Figure 3. 1: Glasshouse experiment in 2013 at about GS37 and GS61.

3.1.1 Experimental growing conditions

The glasshouse experiment was conducted in two years 2012 and 2013 at the University of Nottingham, School of Biosciences, Sutton Bonington Campus, UK (52.5^{o} N, 1.3^{o} W). The two experiments were sown on 10 February 2012 and on 13 February 2013 and harvested, depending on the genotype, from 1-15 August 2012 and from 25 July-8 August 2013, respectively. In each experiment, seeds of genotypes were germinated in modules of plastic seed trays using John Innes no. 2 compost (John Innes, Norfolk, UK), then grown in the growth room for vernalisation for eight weeks at 6°C (night and day) with a 12 hour photoperiod and 80-130 µmol $m^{-2}s^{-2}$ radiation intensity. This compost has been specially formulated with a mix of sterilised loam, peat and sand with essential plant nutrients. The plants were watered as required with water and with a complete nutrient solution (Vitax, 1:20, Leicestershire, UK) once during stem-extension.

After vernalisation, six plants of each genotype were transplanted into the 7.5 litre pots (25.8 cm diameter x 20.4 cm depth) filled with John Innes No. 2 compost on 6 April 2012 and 11 April 2013. The glasshouse was equipped with a vent allowing the automatic opening beyond the temperature of 25 °C. Minimum and maximum temperature was measured using a digital thermometer. Plants were watered as required to maintain them from water stress (three times aweek before anthesis then five times after that). To avoid lodging, plants were supported using a cane and two

plastic support rings in each pot. Plants were regularly sprayed with fungicides and pesticides to minimise effects of disease and pests. Fungicides were applied during the plant cycle as followed:

In 2012/12 experiment:

- 05/03/2012 : SB invigorator 2 ml l^{-1}
- 27/04/2012 : Cerbel 2.2 ml l^{-1}
- 27/04/2012 : Amistar 1ml l^{-1}
- 10/05/2012 : Apres 0.75 ml l^{-1}
- 01/06/2012 : Folicur 3.3 ml l^{-1}

In 2012/12 experiment:

- 13/04/2013 : Aphox 0.5 g l^{-1}
- 08/05/2013 : Apres 0.75 ml l^{-1}
- 08/05/2013 : Amistar 1ml l^{-1}
- 14/05/2013 : SB invigorator 2 ml l^{-1}
- 28/05/2013 : Folicur 3.3 ml l^{-1}
- 05/06/2013 : Cerbel 2.2 ml l^{-1}

3.1.2 Developmental stages

The main shoot of each plant was tagged using plastic wires. The developmental stages were assessed on the main shoot every 4-5 days according to the Zadoks' decimal scale (Zadoks et al. 1974; Tottman, 1987). The date when each main shoot reached GS31 and GS61 was recorded. At GS61, one plant amongst six was sampled for growth analysis. The date of physiological maturity was assumed to be reached when 100% of green leaf lamina area of the main shoot had senesced.

3.1.2.1 Growth analysis at anthesis

At GS61 in each experiment, one randomly selected plant per pot was sampled (different genotypes were sampled on different calendar dates according to the date of reaching the stage) for growth analysis in three replicates. Plants were cut at the soil surface and stored in the cold room at 4 °C for a maximum two days before

processing for growth analysis. After sampling, the plant height of the main shoot to the tip of the ear was measured using a meter rule, after which the shoots were separated into three categories: main shoot (MS), fertile tillers (those with an ear, FT) and infertile tillers (those with no ears apparent, IT) and counted. Each of the MS and FT groups were then separated into: (i) ear, (ii) green flag-leaf lamina, (iii) non-green flag-leaf lamina, (iv) remaining green leaf lamina, (v) non-green remaining leaf lamina, and (vi) stem with attached leaf sheath. The area of the green components was measured using a leaf area meter (Licor 3100, Lexicon instruments, Lincoln, Nebraska USA) was used. After drying all components in the oven at 80 °C for 48 hours, their weight was recorded separately. The weight of the infertile tillers was also recorded after drying to 80^{o}C for 48 h.

3.1.2.2 Growth analysis at harvest

At maturity samples were taken on the same calendar date for all genotypes. Three plants were selected randomly from each pot. Samples were taken by cutting the shoots as close to the soil surface as possible. The shoots were divided into three categories: main shoot (MS), fertile tillers (FT) infertile tillers (IT) and the following measurements were taken:

For MS, dry weight of: (i) spike, (ii) flag leaf, (iii) stem with attached leaf sheath and (iv) remaining lamina after drying for 48 h at 80^{o}C.

. For FS, dry weight of spike and straw after drying for 48 h at 80^{o}C.

. For IFS, above-ground dry weight after drying for 48 h at 80^{o}C.

The ears of each shoot category were hand-threshed separately and the dry weight of grain, chaff and all other parts were recorded separately after oven drying at 80 °C for 48 hours. The grain number was measured using a seed counter (CONTADOR, Hoffman Manufacturing, Inc. Jefferson, USA).

3.1.3 Nitrogen analysis

At maturity, nitrogen analysis was carried out on the following aboveground oven-dried plant material for each of main stem and fertile shoot groups:

i) Grain

ii) Flag leaf

iii) Leaf lamina

iii) Stem and sheath

All samples were milled to a fine powder (particle size of < 200 μm) using a Retsch, ZM 200 ultra-centrifugal mill, (Retsch Haan, Germany) and placed in re-sealable air-tight plastic bags to avoid moisture absorption (a sub-sample was taken where necessary). In order to avoid contamination, the mill was thoroughly cleaned between samples. Two different N analysis analyzers were used:

In 2011/12: 50 mg of samples were encapsulated in aluminium capsules, and put into the Fisons NA-2000 elemental analyzer (Fisons, Ipswich, UK) calibrated against a methionine standard (N content = 9.39%).

In 2012/13: 10 mg of samples were encapsulated in aluminium capsules and put into the Thermo-Scientific Flash 2000 CHNS-O analyzer calibrated against a sulphanilamide standard (N content = 16.24%).

For analyzers, the method described below for calculating NUE and components at the plant level was followed.

The total N content of plant components was calculated by multiplying the N% by dry weight of the plant component (Eqn. 3.1). The total N uptake per plant (NUP_{plant}) was calculated by summing the amounts of N in all aboveground plant components (Eqn. 3.2). Plant N-Uptake efficiency ($NUPE_{plant}$), plant N-utilization efficiency ($NUTE_{plant}$), plant N-use efficiency (NUE_{plant}) and plant N harvest index NHI_{plant} were calculated using the equations (Eqn. 3.3, 3.4, 3.5 and 3.6 respectively).

N plant component (g) = (N% / 100) * DW (g) Eqn. 3.1

NUP_{plant} = grain N_{plant} (g) + total straw N_{plant} (g) Eqn.3.2

$NUPE_{plant}$ = NUP_{plant} (g) / N available in the soil (g) Eqn. 3.3

$NUTE_{plant}$ = grain N_{plant} (g) / NUP_{plant} (g) Eqn. 3.4

NUE_{plant} = $NUPE_{plant}$ x $NUTE_{plant}$ Eqn. 3.5

NHI_{plant} = grain N_{plant} (g) / NUP_{plant} (g) Eqn. 3.6

In February in each year prior to the first fertilizer N application, a soil analysis was carried outfor soil N amount. Six soil cores were taken to 90 cm soil depth in two discard plots per replicate in the low N treatment; the soil cores were separated into 0-30 cm, 30-60 cm and 60-90 cm horizons for soil mineral nitrogen analysis. The 6

replicates per discard plot were bulked for each soil horizon to give three samples per plot. The samples were immediately stored in a chilled cool box, prior to storing in a freezer. The samples were sent to the laboratory for analysis (NO_3^- and NH_4^+), in a cool box to avoid defrosting.

The amount of soil N measured was 6.37, 4.90, 9.97 kg N ha^{-1} for the 0-30 cm, 30-60 cm and 60 -90 cm horizons in 2011/12; and 25.70, 17.50, and 14.17 kg N ha^{-1}, respectively in 2012/13. The total amounts of soil N to 90 cm was 21.23 and 57.37 kg ha^{-1} respectively in 2011/12 and 2012/13

3.1.4 Leaf activity measurements

3.1.4.1 Leaf chlorophyll fluorescence



FluorPen
FP 100 (PSI)

Using a portable fluorometer (FluorPen PF 100, PSI Ltd, Brno, Czech Republic) the photosystem II Quantum Yield (QY) was measured on three main-shoot flag leaves per pot. Measurements were carried out between 11.00 and 15.00 under bright conditions. In 2012, the measurements were carried out pre-anthesis on: 10 May, 21 May 09 June 2012 and post-anthesis on: 21 June, 30 June and 11 July 2012. In 2013, pre-athesis measurements were carried out on: 10 May, 19 May, 24 May and 03 June 2013 and post-anthesis measurements on: 23 June, 30 June and 10 July 2013 in centre of leaf abaxial surface.

3.1.4.2 Leaf chlorophyll Content (SPAD)



SPAD-502Plus
(Minolta)

Leaf chlorophyll content was measured using SPAD chlorophyll meter (SPAD-502 Plus Minolta, Japan). Three measurements per leaf (basal, central and distal) of five main-shoot flag leaves were measured per pot weekly for three replicates from 08 June to 12 July in 2012 and from 10 June to 31 July in 2013 in centre of leaf abaxial, surface.

3.1.4.3 Leaf gas exchange measurments

Gas-exchange measurements were carried out using a Li-Cor LI-6400XT portable potosynthesis system (Li-Cor Biosciences, Nebraska, USA). Light-saturated photosynthesis rate (A_{max}), stomatal conductance (gs) and transpiration rate (mmol) with the Licor light source set at PAR 2000 μmol m^{-2} s^{-1}were recorded on a weekly basis over the whole season from the onset of tillering until mid to late grain filling. Before anthesis, all measurements were taken on the middle of the newest fully emerged leaf, and after GS39 on the flag leaf. The protocol set radiation intensity to 1500 μmol m^{-2} s^{-1} for 90 seconds, then raising it to 2000 μmol m^{-2} s^{-1} for 90 seconds, and recording A_{max} (defined as the maximal rate of photosynthesis under light saturated conditions) at 2000 μmol m^{-2} s^{-1} once it had stabilised. The other instrument specifications were 400 ppm CO_2, 500 μmol s^{-1} flow rate, fan set to fast, block temperature set to 22°C, humidity maintained in the range 30 to 70%, stomatal ratio 1.4 (after Estiarte et al. 1994; Lu, 1989), measuring light off and radiation set to 10% blue (induces stomatal opening) (the protocol was adapted from instructions in Li-Cor (2008)). All measurements were made between 10.00 and 16.00, on the adaxial surface of three randomly selected flag leaves per pot avoiding outer rows in three replications on the 10 genotypes.

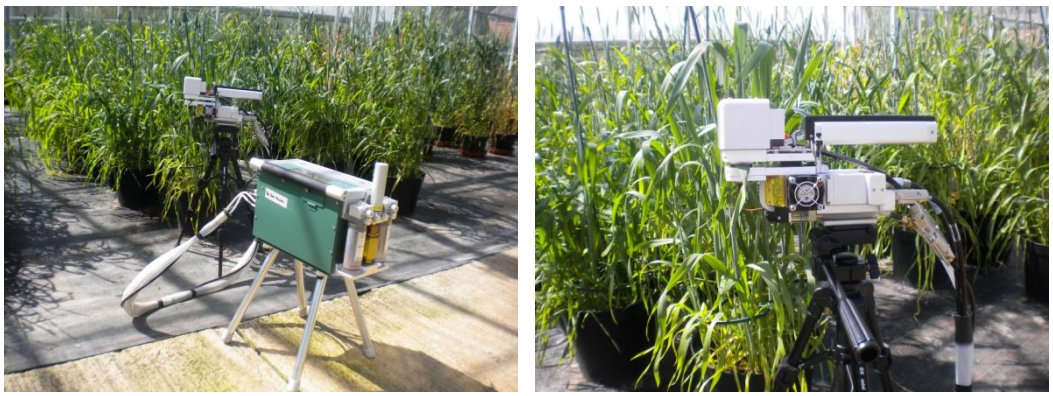


Figure 3. 2: LiCor 6400-XT Photosynthesis System in the glasshouse

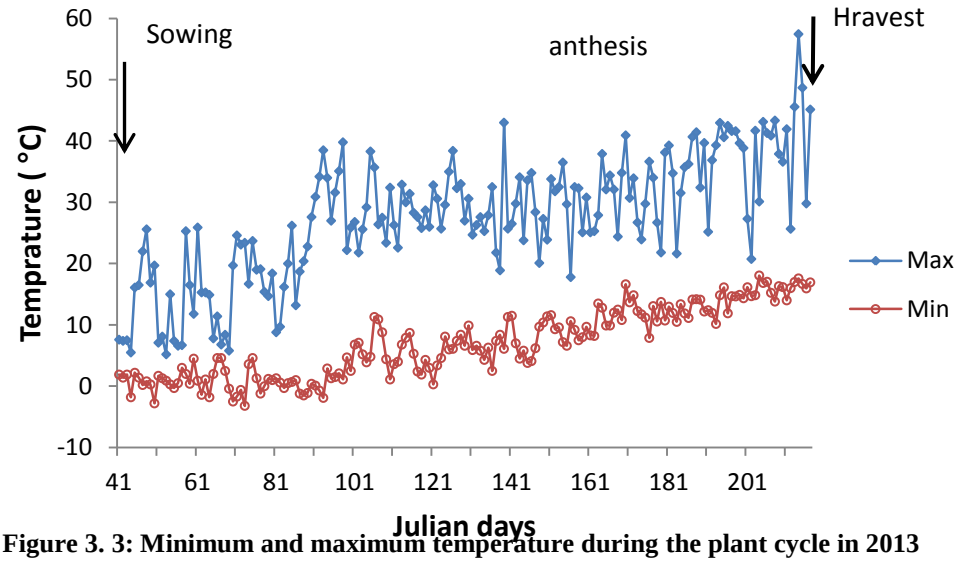


Figure 3. 3: Minimum and maximum temperature during the plant cycle in 2013

3.2 Field experiments

3.2.1 Experimental site and plot management

All field experiments were located at the University of Nottingham experimental station at Bunny Park, Nottinghamshire, UK latitude: 52° 51' 45" N longitude: 1° 7' 30.6" W. Experiments were sown on 28 October 2011 and 19 October 2012, respectively, for 2011/12 and 2012/13 experiments. For all experiments, the target spring plant population was 200 plants per m^2. Seed weights to achieve this plant population were based on thousand grain weight (TGW) and the seed rate was 350 seeds per m^2 in each experiment. All experiments were sprayed prophylactically with herbicides, fungicides and pesticides to minimize effects of weeds, diseases and pests. The previous cropping was oilseed rape for all experiments and the soil type was a sandy loam in both 2011/12 and 2012/13.

In 2011/12, the field was ploughed on 19 September, harrowed on 20 September and drilled and rolled on 31 October 2011. Manganese Jett (2.0 l ha^{-1}) was applied twice during early tillering (on 29 February and 20 March 2012). Herbicides were applied as: Lierator (0.6 l ha^{-1}) on 09 September 2011, Spitfire (1.0 l ha^{-1}) on 20 March and 23 May 2012, Foxtrot (1.0 l ha^{-1}) and Toil (1.0 l ha^{-1}) on 20 March 2012. Fungicides were applied as: Opus (0.75 l ha^{-1}), Bravo (1.0 kg ha^{-1}) and Justice (0.15 l ha^{-1}) on 20 March 2012, Tracker (1.5 l ha^{-1}) on 24 May 2012, Opus (0.75 l ha^{-1}) and Phoenix (1.3 l ha^{-1}) on 23 May 2012 and Orius (0.85 l ha^{-1}) and Vegas (0.15 l ha^{-1}) on 26 June

2012. In addition the insecticide Aphos (0.28 kg ha^{-1}) was applied on 26 June 2012, Plant growth regulators (PGR) were applied as: Moddus (0.2 l ha^{-1}) on 20 March 2012 and Chloromequat (2.0 l ha^{-1}) and Moddus (0.2 l ha^{-1}) on 24May 2012.

In 2012/13, the field was ploughed on 21 September and drilled and rolled on 19 October 2012. Manganese Jett (2.0 l ha^{-1}) was applied twice during early tillering (on 27 February and 21 March 2013) and twice during stem extension (on 03 May and 13 May 2013). Herbicides were applied as: Harta (l.0 l ha^{-1}), harge (2.0 l ha^{-1}) Lierator (0.3 l ha^{-1}) and Zeal (0.3 l ha^{-1}) on 11 April 2013 and Lorgate (25.0 g ha^{-1}) on 03 June 2013. Fungicides were applied as: Kestrel (1.0 l ha^{-1}), Phoenix (1.0 l ha^{-1}) and Flexity (0.14 l ha^{-1}) on 03 May 2013; Brutus (1.25 l ha^{-1}), Amistar Opti (1.0 l ha^{-1}) and Vegas (0.15 l ha^{-1}) on 03 June 2013, and finally Brutus (1.0 l ha^{-1}), Bravo (1.0 kg ha^{-1}) and Instinct (0.5 l ha^{-1}) on 26 June 2013. In addition, three applications of plant growth regulators (PGR) were applied, Chlormequat (1.4 l ha^{-1}) on 03 May 2013, Moddus (0.2 l ha^{-1}) and 13 May 2013 and Terpal (0.5 l ha^{-1}) on 03 June 2013.



Figure 3. 4: **Field experiment at stem elongation a) high N treatment, b) low N treatment and at grain filling c) high N treatment and d) low N treatment**

3.2.2 Experimental design and treatments

3.2.2.1 Experimental design

For all field experiments, a split-plot design was used with two nitrogen (N) treatments, Low N (LN) and High N (HN) and 3 replicates, randomised on main plots and genotypes randomised on sub-plots in three replicates. Each sub-plot was formed of 6 rows (1 m length and 20 cm row spacing) and the plot area was 1.2 m^2.

3.2.2.2 N treatments

In each of the two experiments in 2011/12 the N treatments were as follows:

Under LN, one application of 30 kg N ha^{-1} was applied on 10 May 2012 as Nitram (34.5% N).

Under HN, 200 kg N ha^{-1} was applied in three splits. Each N application was made to all genotypes on the same calendar date. Nitrogen fertiliser was applied as ammonium nitrate prill Nitram (34.5% N) in three doses: 40 kg N ha^{-1} (08 March 2012); 80 kg N ha^{-1} (11 April) and 80 kg N ha^{-1} (10 May 2012) in HN treatment.

In each of the two experiments in 2012/13 the N treatments were as follows:

Under LN, no N application was applied.

Under HN, 200 kg N ha^{-1} was applied in three splits. Each N application was made to all genotypes on the same calendar date. Nitrogen fertiliser was applied as ammonium nitrate prill Nitram (34.5% N) in three doses: 40 kg N ha^{-1} (06 March 2012); 80 kg N ha^{-1} (17 April) and 80 kg N ha^{-1} (21 May 2012) in HN treatment.

3.2.2.3 Genotype treatments

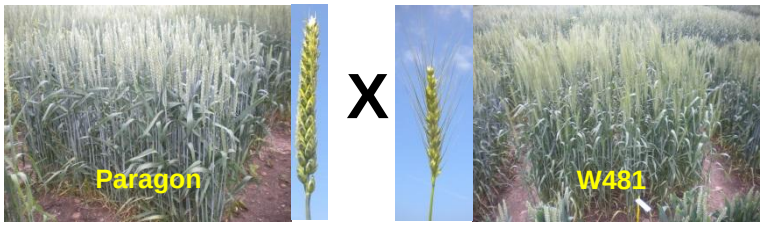
a Genotype panel 2011/12

In 20111/12, a panel of 10 genotypes comprising three UK cultivars (spring wheat Pargon and winter wheats Rialto and Savannah) and seven AE Watkins landraces namely (W034, W040, W141, W209, W223, W352 and W729) were assessed under field conditions.

b Single Seed Descent populations 2012 and 2013

Two populations of recombinant inbred lines derived from the crosses of Paragon and Watkins line 481 (PxW481) and Paragon and Watkins 141 (PxW141) were used

in this study. The lines were at F4 generation in 2011/12, they were developed by single seed descent (SSD) by John Innes Centre. Paragon is a spring bread wheat bred by RAGT Seeds Ltd (CSW 1742/19/6/68 x (Axona x Tonic)) first listed on the UK Recommended List in 1999. It is widely grown in the UK and is known for its bread-making quality. It has good all round disease resistance and good straw characteristics. Watkins lines were selected lines from an international collection of landraces collection from 32 countries established in 1920s and 1930s and curated at the John Innes Centre, UK. A total of 79 SSD lines of PxW481 and 97 SSD lines of PxW141 mapping populations were developed and used in the present study.



a) 79 SSD Lines (F5)



b) 97 SSD Lines (F5)

Figure 3. 5: **Field plots and single ears of parental lines of a) PxW481 b) PxW141 mapping populations**

These two mapping populations were selected based on the yield and lodging results of 2011/12 field experiment. The SSD populations were developed by Dr Simon Griffiths and Mr Simon Orford at John Innes Centre,Norwich,Norfolk, UK.

In 2011/12, the field experiments carried out were as follows:

- ✦ Experiment 1: 2 N treatments x 10 genotypes (namely: Paragon, Rialto, Savannah, and Watkins landraces W034, W040, W141, W209, W223, W352, and W729).
- ✦ Experiment 2: 2 N treatments x 79 lines of PxW481 SSD mapping population and parents

In 2012/13, the field experiments carried out were as follows:

- ✦ Experiment 3: 2 N treatments x 79 lines of PxW481 SSD mapping population
- ✦ Experiment 4: 2 N treatments x 97 lines of PxW141 SSD mapping population and parents

3.2.3 Physiological measurements

The following measurements were taken at different growth stages.

3.2.3.1 Plant development

In each experiment, developmental stages were recorded in two replicates of the HN and LN treatments on a weekly basis, and every 3-4 days during the anthesis window. This was done by visually observing each plot and examining the individual plants when necessary at GS30 and GS31. Growth stages were based on the decimal codes of the growth stages (GS) devised by Zadoks et al. (1974) as revised by Tottman and Broad (1987). For GS30 and GS31, three plants per plot were pulled up, and the stage of the main shoot recorded. The plot was considered to have reached GS30 or GS31 when more than 50% of main shoots were at the stage; it was considered to have reached GS39 (flag leaf emergence) and GS61 (anthesis) from a visual assessment of all shoots in the plot, when more than 50% of all shoots were at the particular stage. Physiological maturity was taken as the date when for more than 50% of the shoots all green lamina had senesced, and there was less than 10% of stem area remaining green.

3.2.3.2 Leaf chlorophyll fluorescence

a Genotype panel

Leaf chlorophyll fluorescence was measured on three flag leaves per sub-plot following the protocol described in paragraph 3.1.4.1 at anthesis (GS61); different genotypes were measured on different calendar dates according to the date of reaching the stage.

b SSD mapping populations

The measurements were taken as described in 3.2.2.4.a. The measurements were taken in the two experiments in 2013 under HN and LN treatments on two replicates. Two measurements were taken: one pre-anthesis (11 June) and one post-anthesis (5 July). Readings were taken during 11.00 - 13.00 under bright conditions. No flag-leaf QY assessments were taken in the experiments in 2012.

3.2.3.3 Leaf chlorophyll content (SPAD)

a Genotype panel

The measurements were taken as described in 3.1.4.2 on five flag leaves of primary shoots (those within top 10 cm of tallest shoot per plot) for each sub-plot at anthesis (GS61) (different genotypes were measured in different dates according to the date of the satge flowering.

b SSD mapping populations

Three measurements per leaf (basal, middle and distal) of five randomly selected flag leaves were taken per plot on reaching anthesis (GS61) in two replicates at HN for the PxW481 and PxW141 experiments in 2013. The protocol described in 3.1.4.2 was used for measurements. No leaf SPAD measurements were carried out for the PxW481 experiment in 2012.

3.2.3.4 Crop spectral reflectance indices

a Genotype panel

In 2012, five spectral reflectance measurements were taken during the season, on: 16 May, 22 May, 20 June, 12 July and on 23 July. All measurements were taken using a remote-sensing multispectral radiometer (CROPSCAN: MSRSYS16R, CropScan Inc, USA). It measured reflectance at 16 wavelengths from 500 to 970 nm. The Normalized Difference Vegetation Index (NDVI) was calculated as:



Figure 3. 6: **CROPSCAN: MSRSYS-16R multispectral radiometer**

$$NDVI = (NIR-VIS) / (NIR+VIS) \quad \text{Eqn 3.7}$$

Where NIR is the reflectance at near infra-red wavelength 900 nm and VIS is the reflectance at visual wavelength 680 nm. Readings were taken between 11:00 and 15:00 on days with clear skies or lightly cloudy days with incident radiation readings above 300 W m^2. If there were cumulus type clouds, then readings were not taken until after the cloud had passed (when the incident radiation was at least 300 W m^{-2}). The radiometer was positioned toward the sun when taking readings to avoid the shadow of the operator from falling across the sample area

b SSD mapping populations

In 2012, at around anthesis (19 June) under HN conditions all sub-plots of the PxW481 experiment in one replicate were scanned; and post anthesis on 24 July under HN and LN conditions all sub-plots of two replicates were scanned. In 2013, in the PxW481 and PxW141 experiments, one assessment pre-anthesis was carried out on 6 June and three post-anthesis on 5, 17, 27 July 2013 on all sub-plots in two replicates under HN and LN conditions.

3.2.3.5 Gas exchange measurements

In 2012, in field experiment 1 examining the panel of 10 genotypes, flag leaf activity traits (light-saturated net rate of CO_2 exchange (A_{max}), intracellular carbon concentration (Ci) and stomatal conductance (g_s), were measured using the Li-Cor 6400 (Li-Cor Biosciences, Nebraska, USA), on 23 May, 14, 27 June and 11 July 2012, on the middle of the flag leaves post-anthesis, avoiding plot outer rows. The protocol used for glasshouse experiment (paragraph 3.1.4.3) was used for this field experiment. Three measurements were taken on the adaxial surface of three different well developed leaves for each sub-plot. The measurements were taken between 11:00 and 15:00 on clear sunny days.

3.2.3.6 Flag leaf senescence scoring

In 2013 in both mapping populations (PxW481 and PxW141), senescence kinetics were assessed visually by recording the percentage green area of the flag leaf senesced in the whole sub-plot using a standard diagnostic key based on a scale from 0 (no senescence) to 10 (100% senesced) (Gaju et al. 2011). For the scoring, the same operator assessed senescence in all sub-plots during the season. Assessment was carried out weekly after anthesis (GS61) until full canopy senescence. Data were fitted against thermal time from anthesis (GS61; base temperature of 0º C) using a monomolecular-logistic equation describing leaf senescence kinetics during grain filling as a two phase process with a slow and a rapid phases (Génard et al. 1999; Figure 3.8).

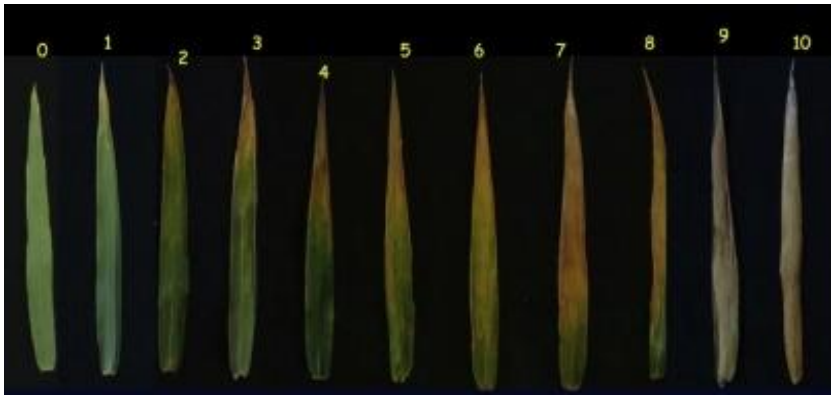
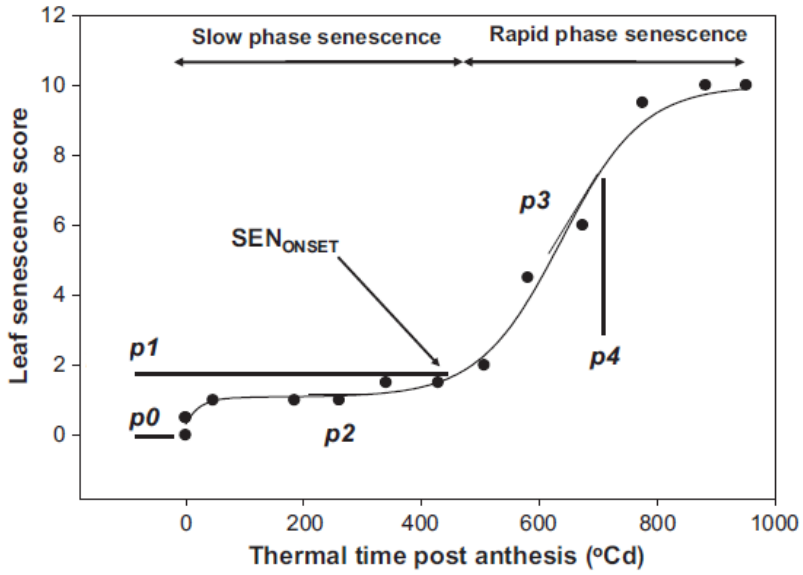


Figure 3. 7: Scale used for flag leaf senescence scoring

3.2.3.7 Plant height

In all experiments, plant height was assessed before harvest on two randomly selected fertile shoots (those with an ear) shoots per sub-plot to the tip of the ear in three replicates.



$$score = p0 + p1 \times \left(1 - e^{(-p2 \times TTA/p1)}\right) + \frac{10 - p1 - p0}{1 + e^{\left(-4 \times p3 \times ((TTA - p4)/(10 - p1 - p0))\right)}} \quad \text{Eqn 3.8}$$

Figure 3. 8: **Definitions of the parameters derived from the observed senescence scores during the post-anthesis period where p0 is the initial senescence score at GS61; p1 is the senescence score of first slow senescence phase, p2 is the maximum rate of first phase; p3 is the maximum rate of second rapid senescence phase; p4 is the time when rate is maximum in second phase; TTA is the thermal time post-anthesis (From Gaju et al. 2011).**

3.2.3.8 Biomass, grain yield and yield components

a Anthesis measurements

In 2013, at GS61 five fertile shoots in each sub-plot were sampled at random in the HN treatments in two replicates. Sampling was carried out on the actual date of reaching the stage GS61; therefore different lines were sampled on different calendar dates. Samples were processed on the day of field sampling.

b Harvest measurements

In each experiment, at maturity just before combining, a row-length sample was taken (1 m row-length for the two field experiments in 2012, PxW481 MP and the panel of 10 genotypes) and 0.5 m row length in 2013 long of one row for both PxW481 and PxW141 MPs in 2013, corresponding respectively to 0.2 m^2 and 0.1 m^2). In order to avoid a border effect, the sample was selected from the four middle rows avoiding the outer rows on each side of the plot. Each sample was put in a paper sack and brought to the laboratory for processing. At harvest, all genotypes were sampled within two days in each experiment.

In the laboratory, roots were removed at ground level and the number of fertile shoots (those with an ear) and infertile shoots were counted. The fertile shoots were separated into ears, leaf lamina and stem-and-leaf-sheath. The total fresh weight of the stem-and-leaf-sheath was recorded. The ears were threshed using a Walter and Wintersteiger LD180 thresher (Wintersteiger, Upper Austria) and the grain counted using a seed counter (Pfeuffer Contador, Bavaria, Germany) before grain and chaff were dried and weighed separately at 80 °C for 48 h. Above-ground dry matter (AGDM) was then calculated by dividing the combine grain yield expressed at 100% DM (Eqn 3.9) by the harvest index (proportion of AGDM in the grain; HI).

$$AGDM = (1 / HI) \times \text{combined yield (100\% DM)} \qquad \text{Eqn 3.9}$$

Combine yield was adjusted to account for the pre-harvest sampling at physiological maturity by adding the relevant hand-threshed grain weight to the weight of the machine-harvested grain per plot.

Combine grain yield of each plot (1.2 m^2) was measured through machine harvesting and the moisture content of the grain was recorded. Thousand grain weight (TGW) was calculated by counting out > 200 grains using the seed-counter, drying at 80°C

for 48h and weighing. Grains m^{-2} was calculated by dividing combine grain yield by TGW; grains ear^{-1} was calculated by dividing the total number of grains in the rowlength sample by the ear count for the row-sample at PM.

3.2.4 Plant nitrogen analysis

In experiments, at maturity, N analysis was carried out on the aboveground oven-dried plant material for:

(i) Leaf lamina, stems plus leaf sheaths, grain and chaff for PxW481 mapping population in 2012 and 2013 and

(ii) Grain and straw for subset of 10 genotypes in 2012 and PxW481 and PxW141 mapping populations in 2013.

All samples (except grain) were milled to a particle size of <200 μm using a Fritsch cutting mill "Pulverisette 15" (Fritsch Idar-Oberstein, Germany). However, nitrogen content for whole grains (without milling) was obtained by scanning using near-infrared reflectance spectroscopy (NIRs).

Grain and milled leaf lamina, stem plus leaf sheath and straw samples were scanned in a small circular cup cell (part number IH-0307, NIR Systems, USA). Samples were scanned in the visible and NIR regions of the electromagnetic spectrum (400–2500 nm) at 2 nm intervals using a scanning Foss-NIRSystems 6511 scanning monochromator (Silver Springs, Maryland, USA). Samples were scanned once and not rotated during spectra collection and spectral data were stored as a logarithm of the reciprocal of reflectance [log (1/R)]. The spectrum of each sample was the average of 32 successive scans. Data collection and instruments were operated with Infra-soft International (ISI) software (version 2.0).

The NIR data sets were calibrated by selecting approximately 10% of samples for each component (grain, leaf lamina, stem plus leaf sheath and straw) and analysing these samples for N% according to the Dumas method using the Fisson NA-2000 elemental analyzer (Fisson, Ipswich, UK) calibrated against a methionine standard (N content = 9.39%). About 10 mg of sample was weighed and encapsulated in an aluminium capsule. The Dumas N method consists of firstly converting all N forms in the sample into gaseous nitrogen oxides by complete combustion in pure oxygen at T>1000ºC; secondly, the gaseous products of the combustion are passed over a hot

copper catalyst to convert the oxides of N to gaseous N, after which CO_2 and water are removed, and the amount of N in the sample is determined by the change in thermal conductivity in the sample stream (N_2 gas in helium carrier gas) compared to a reference cell. The results are expressed as N% of the sample dry weight (Simonne et al. 1997; Pask, 2009).

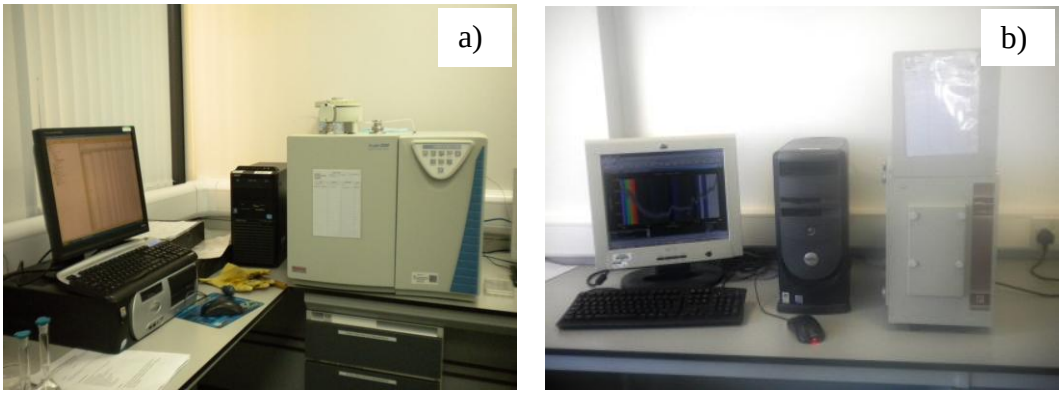


Figure 3. 9: **Machine used for N analysis in the lab: a) Flash 2000 Combustion CHNS/O Analyser and b) NIR System 6500 Spectrophoto-meter**

The total N content was calculated by multiplying the N% by dry weight of the plant component (Eqn. 3.10). The total N uptake (NUP) was calculated by summing the amounts of N in all aboveground parts (Eqn. 3.11). N-Uptake efficiency (NUPE), N-utilization efficiency (NUTE), plant N-use efficiency (NUE) and NHI were calculated using the equations (Eqn. 3.12, 3134, 314 and 3.15 respectively).

$N\ (g\ m^2) = (N\% / 100) * DW\ (g\ m^2)$ Eqn. 3.10

$NUP = \text{grain N}\ (kg\ ha^{-1}) + \text{total straw N}\ (kg\ ha^{-1})$ Eqn.3.11

$NUPE = NUP\ (kg\ ha^{-1}) / \text{N available in the soil}\ (kg\ ha^{-1})$ Eqn. 3.12

$NUTE = \text{grain N}\ (kg\ ha^{-1}) / NUP\ (kg\ ha^{-1})$ Eqn. 3.13

$NUE = NUPE \times NUTE$ Eqn. 3.14

$NHI = \text{grain N}\ (kg\ ha^{-1}) / \text{AGN (at harvest)}(kg\ ha^{-1})$ Eqn. 3.15

3.3 Statistical analysis

3.3.1 ANOVA, correlation and regression analysis

All data were entered into Microsoft EXCEL 2007 spreadsheets and the statistical analysis of data was carried out using version 15 of the GENSTAT package (VSN International (2011). GenStat *for Windows* 15th Edition. VSN International, Hemel

Hempstead, UK). For field data, analysis of variance (ANOVA) procedures for a split-plot design were used to analyse N treatments and cultivar effects and test their interaction in individual seasons where replicates were regarded as random effects and genotypes as fixed effects. A cross-site-season ANOVA was applied to analyse N treatments and cultivar effects across experiments and the interaction with site-season, assuming N treatments and cultivars were fixed effects and replicates and site-seasons were random effects. For glasshouse data ANOVA was carried out using procedures for a randomised block design, where replicates were regarded as random effects and genotypes as fixed effects. ANOVA was applied to analyse genotype effects across experiments and the experiment x genotype interaction, assuming replicates and experiments were random effects and genotypes were fixed effects. Treatment means were compared using the LSD of the means of Fisher, calculated from standard errors of the difference of the means using appropriate degrees of freedom, when the ANOVA indicated significant differences.

Regression analysis was performed using the regression procedure in Genstat 15.1 (www.genstat.com) using the genotype means across replicates. Pearson's correlation coefficient between traits amongst genotypes was calculated using the correlation procedure in GenStat 15.1 to quantify relationships between traits using the genotype means across replicates. For senescence data fitting data against thermal time, R package 'stats' (R Foundation for Statistical Computing, Vienna, Austria: http://www.R-project.org/) was used to calculate different senescence parameters of the monomolecularr logistic equation (Génard et al. 1999).

3.3.2 Map construction and QTL analysis

3.3.2.1 Data file for map construction

Linkage analysis was performed using JoinMap v 4 (Van Ooijen, 2006). Data files containing marker loci scores (Locus genotype file ".loc") were prepared for each mapping population by transferring data from MS-excel spreadsheets to text editor program (WordPad) and saved under extension ".loc". Each locus genotype file (loc-files, one for each mapping population), containing the marker segregation patterns within the population, was then loaded to two different JoinMap projects created for each mapping population. The loc-file has a sequential structure, containing four line header instructions with the following syntax:

name = NAME

popt = POPT

nloc = NLOC

nind = NIND

Where NAME was the name given to the population, POPT was the code for the population type (RI5: Recombinant Inbred lines at 5^{th} generation in our case), NLOC was the number of loci and NIND was the number of individuals. The data body contains the actual genotype information for all individuals grouped according to locus as defined in the manual.

It is important that the order of the individuals is identical over all loci in the file. The genotype code for population types RIL5 was as follows:

Table 3.1: Classification codes of mapping population scores; the ratios of expected Mendelian segregation.

Code	Description
a	homozygote as the one parent
b	homozygote as the other parent
h	Heterozygote
-	unknown genotype

3.3.2.2 Construction of linkage groups of markers

The genotyping data of Paragon x W481 and Paragon x W141 was developed using 201 Markers (27 SSRs and 174 SNPs) and 491 markers (23 SSRs and 468 SNPs) which are segregating markers, respectively for PxW481 and PxW141. and developed by John Innes Centre, Norfolk, UK and University of Bristol, UK as part of the BBSRC Wheat Improvement Strategic Programme. A total of 79 and 94 SSD lines, respectively, were screened by the SSR markers. Linkage analysis was performed using JoinMap v 4 (Van Ooijen, 2006).

Using the JoinMap package, the linkage groups of each mapping population were separately assembled by applying default calculation options starting with calculating the LOD scores and pairwise recombination frequencies between markers. The loci of all individuals in each population were grouped according to "independent LOD". The groups were manually selected using thresholds from LOD

3-5 and regression mapping was carried out using Haldane's mapping function to convert recombination frequencies into map distances (centiMorgan: cM). The third mapping round forces markers to be grouped which have been grouped, but rejected in the first and second rounds of mapping into the genetic map. The markers forced to be grouped after first round but which showed negative map distances and large *jumps* (threshold value >5) in mean chi-square values were removed from the map.

3.3.2.3 QTL analysis (data file preparation)

For QTL analysis, MapQTL 6.0 software (Van Ooijen, 2009) was used based on the mean values of phenotypic data of traits over replicates. MapQTL uses plain text files to load the data for analysis. Plain text files were made with editor text program "WordPad". For the QTL analysis three types of data files were required:

1- ***Loc-file***: the locus genotype file is the same file used in JoinMap to construct the linkage groups. This file contains the genotype codes (a, b, h and -) for the loci of segregating lines.

2- ***Map-file***: the map file contains the map positions of all loci in different linkage groups. It is the result of the grouping of the markers using the JoinMap package (see before). The nonparametric mapping of MapQTL (i.e. Kruskal Wallis, KW) analyses the loci one by one; the position of the loci is not important, it is used only to sort the loci. However, for Interval Mapping (IM) and MQM mapping, the positions were used to calculate the recombination frequencies necessary for likelihood calculation. The map file has no headers and starts with group numbers.

3- ***qua-file***: the quantitative file contains the data of quantitative traits of all individuals. Three instructions, as headers, should precede the data followed by the names of the traits. The syntax of the three instructions is:

ntrt = the numbers of traits
nind = the numbers of individuals
miss = missing value indicator usually "*"

3.3.2.4 Kruskal-Wallis, nonparametric mapping

The Kruskal-Wallis (KW) analysis is regarded as the nonparametric test equivalent of the one-way analysis of variance. It is suitable for normally as well as non-normally distributed traits. Unlike interval mapping (IM), the KW test does not rely on the assumption of probability distribution. The test is performed on each locus separately, regardless of its position in the linkage map. The test ranks all individuals according to the quantitative trait, while it classifies them according to their marker genotype. A segregating QTL (with large effect) linked closely to the tested marker will result in large differences in average rank of the marker genotype classes. A test statistic based on the ranks in the genotype classes is calculated. For individuals in ties, i.e. several individuals have equal values of the quantitative trait, the average rank (mid rank) is used, while for the test the statistic adjusted for ties is used (indicated by K*) (Lehmann, 1975).

The Kruskal-Wallis statistic K* is a Chi-square like distribution and the significance level is indicated in asterisks. (Significance level: *:0. 1, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001, ******: 0.0005, *******: 0.0001)

3.3.2.5 Interval mapping

The interval mapping method was developed by Lander and Botstein (1989). In interval mapping, the likelihood of presence of a segregating QTL under the null hypotheses, *H0*, is calculated for each position in the genome (every centiMorgan), as well the likelihood under the alternative hypothesis, *H1*, and the genotypic effects of the QTL and the residual variance are calculated. This likelihood under *H1* is compared to the likelihood for the situation when a locus with zero genetic effects (no segregation) (the likelihood under the null-hypothesis *H0*). This comparison is done with LOD score (likelihood ratio statistic), which is the 10-base logarithm of the quotient of the two respective likelihoods. The maximal LOD position is determined as the most likely position of the QTL and a one LOD decrease on either side of LOD peak is considered as a confidence interval for the effect. A genome-wide significance threshold with a p-value of 0.05 was calculated by a permutation test based on 1000 iterations. A QTL was considered significant if its LOD score was higher than the threshold value.

Chapter 4: Grain yield, nitrogen-use efficiency and yield components in Watkins parental lines and UK check cultivars

4.1 Introduction

Intensive agriculture raises costs for growers, through the use of fertilisers and chemical inputs (fungicide, insecticide and herbicides), and also exhausts energy resources. Modern wheat cultivars in developed countries which are selected under non-limiting fertilisation conditions require large amounts of N fertilisers (Foulkes et al. 2009), and environmental pollution by nitrates in water and gaseous emissions in the atmosphere is associated with the inefficient use of N. N-use efficiency (NUE) is defined as the grain dry matter (DM) yield (kg DM ha^{-1}) per unit N supply from soil or fertiliser (kg N ha^{-1}; Moll et al. 1982). NUE can be subdivided into two components that identify soil and above-ground plant processes that contribute to the overall use of N: (1) N-uptake efficiency (above-ground N uptake per unit N available from the soil and fertiliser; NUPE) and (2) N-utilization efficiency (grain yield DM per unit crop N uptake at harvest; NUTE) which is a function of the utilization or physiological efficiency. Genetic variation is reported in wheat for nitrogen-use efficiency (Van Sanford and Mackown 1986, Dhugga and Waines 1989 and Ortiz-Monasterio et al. 1997).

The ability to capture N depends mainly on the amount of nitrate present in the soil relative to the morphology of the root system (Foulkes et al. 2009). N-utilisation efficiency, by contrast, is dependent on the efficiency of nitrate reduction, biomass accumulation, N storage and N remobilisation from senesced tissues to grain (Lea and Azevedo, 2007, Good et al. 2004 and Foulkes et al. 2009). Developing cultivars with lower fertiliser N requirements will require improved efficiency of N absorption efficiency and N utilization processes (Le Gouis et al. 2000).

When growing wheat, grain quality along with grain yield must be considered, thus N fertilisation must be economically feasible and environment-friendly. To obtain higher protein content in wheat grain, presently, a high amount of nitrogen fertiliser must be applied (Ortiz-Monasterio et al. 1997, Strong and Holford, 1997). An inverse relationship exists between the grain protein concentration and yield (Triboi

et al. 2006), making the simultaneous genetic improvement of yield quantity and bread-making quality a difficult task (Cox et al. 1985). However, it is possible to identify bread-making genotypes having higher grain N% than would be expected from their grain yield in turn associated high post-anthesis N uptake (Kramer, 1979; Bogard et al. 2010, 2011). Ortiz Monasterio et al. (1997) reported that at low N input, NUPE is dominant in explaining genetic variation in NUE compared to NUTE, whereas under high N inputs, NUTE is relatively more important.

Ensminger et al. (2006) stated that photosynthetic capacity is tightly related with biomass accumulation through photosynthesis process. Indeed in the Calvin cycle by the Rubisco enzyme, plants are able to convert sunlight into usable chemical energy by a direct reduction of CO_2 into sugar (triose) phosphates. All metabolic processes of the plant, including growth and development use sucrose synthesised from CO_2 assimilated during photosynthetic processes. Canopy and leaf processes affecting photosynthesis per unit N uptake include: (i) leaf photosynthesis per unit leaf N and (ii) post-anthesis canopy N remobilization and senescence. The photosynthesis per unit area of flag leaf and carboxylation efficiency in both winter and spring wheat were proportional to the amount of total soluble protein up to 7.0 g m^{-2} and to the amount of RuBP protein up to 4.0 g m^{-2} (Lawlor et al. 1989). The amount of N remobilized to the grain is highly dependent on N stored at anthesis (Gooding and Davies, 1992; Palta and Fillery, 1993; Bly and Woodward, 2003). N remobilization is linked to the regulation of leaf senescence (Sinclair and De Wit, 1975; Masclaux et al. 2001; Uauy et al. 2006; Gaju et al. 2011). Modifying NRE to delay senescence could be advantageous to favour a longer period of active photosynthesis during grain filling and higher grain yield in feed, distilling, and biofuel cultivars (Gaju et al. 2013).

This chapter describes a panel of 17 genotypes (12 Watkins landraces and 5 UK check cultivars) examined in two glasshouse experiments conducted during 2011/12 and 2012/13 under high nitrogen conditions and in one field experiment in 2011/12 under high N (HN) and low nitrogen (LN) conditions. The results for above-ground dry matter (AGDM), yield and its components, growth and partitioning, leaf gas exchange, flag leaf senescence scores, and nitrogen-use efficiency (NUE) and its

components are presented and discussed. The comparison between field and glasshouse results is also discussed.

The specific hypotheses tested in this chapter are:

1- Genetic variation can be identified amongst AE Watkins landraces for grain yield, biomass, NUE, NUE components and related physiological traits.
2- Higher expression of biomass, NUE components and associated traits can be identified in AE Watkins landraces compared with elite UK wheat varieties under high N conditions in the glasshouse and under low and high N in the field
3- The genetic variation in grain yield, biomass, NUE components and related physiological traits in the glasshouse experiment is correlated with field expression of these traits.
4- The physiological processes determining genetic variation in NUE can be identified in relation to N accumulation, N partitioning and N remobilization within the plant

4.2 Materials and methods

Two glasshouse experiments in 2011/12 and 2012/13 and one field experiment in 2011/12 were carried out. The glasshouse experiments examined 17 genotypes: 5 UK RL cultivars (spring cultivar, Paragon and winter cultivars, Oakley, Rialto, Savannah, Xi19) and 12 A. E. Watkins landraces namely: W034, W040, W141, W209, W292, W352, W355, W468 and W729.

A completely randomized block design with three replications was used in both years. Six plants were grown in each pot. Full irrigation and fungicides were applied during the whole plant cycle to keep the plants healthy.

In the glasshouse the following measurements were taken for each genotype in three replicates:

- Leaf chlorophyll content (SPAD 502 meter, 4 and 7 measurement dates, respectively, in 2012 and 2013).
- Leaf chlorophyll fluorescence (Fluorpen FP10 meter: 6 measurement dates in 2012 and 7 in 2013).

- Leaf photosynthesis rate (LICOR 6400 : 4 measurement dates in 2012 and 4 in 2013).
- Growth analysis at GS61 (one plant per genotype at GS61 was harvested for biomass and N partitioning).
- Flowering date (GS61).
- Plant height at maturity.
- Yield and biomass at harvest (three plants were selected randomly per pot).
- N analysis for grain, lamina and stem at GS61 and harvest.

In the field, 10 genotypes (three UK cultivars, Rialto, Pargon and Savannah) and seven Watkins landraces namely, W034, W040, W141, W209, W223, W352 and W729) in both HN and LN in three replicates, the following measurements were taken for each genotype.

- Leaf chlorophyll content (SPAD, one measurement at anthesis, 5 shoots per plot, 2 replicates) under HN
- Leaf chlorophyll fluorescence (Fluorpen: one measurement date at around anthesis on one calendar day for all genotypes, 3 shoots per plot, 2 replicates).
- Photosynthesis rate (LICOR: 4 measurement dates, 3 shoots per plot, 3 replicates).
- Sampling and growth analysis GS61 (5 shoots per genotype reaching GS61 was harvested for biomass partitioning).
- Flowering date (for each genotype in both HN and LN, in three replicates)
- Plant height at maturity.
- Biomass at harvest (one row of 1 m length harvested and processed by separating grain from straw).
- N analysis for grain and straw for each genotype at both GS61 and maturity.

At maturity for glasshouse experiments, the aboveground oven-dried plant material for each of the main-shoot and fertile tiller groups, was separated into: i) true stem plus leaf sheath, ii) flag leaf, iii) remaining lamina. In the field experiment, the aboveground oven-dried plant material was separated into grain and straw. All samples were then milled to a particle size of < 200 μm. About 10 mg of milled sample was then weighed and encapsulated in aluminium capsules and analysed using the same Dumas protocol described for field experiment (see section 3.2.4).

All data were entered to Microsoft EXCEL 2007 and statistical analysis of data was carried out using version 15 of GENSTAT package. Analysis of variance (ANOVA) was used to test the significance of the treatment, and to calculate means, LSDs and standard errors. Linear regression analysis was used to establish causal relationships between different traits.

The genotype W481 (yield = 1.9 g and AGDM = 13.72 g plant^{-1}) was excluded from the analysis during 2012. This genotype, in 2012, was severely infected by powdery mildew which reduced its yield and AGDM.

The N remobilisation efficiency (NRE) was calculated as the fraction of N in the whole plant or organ at anthesis which was not recovered in the straw at harvest (Cox et al. 1986). Full details of the methodologies are presented in Chapter 3.

4.3 Results

4.3.1 Glasshouse experiments

4.3.1.1 Anthesis date (AD) and plant height (PH)

In 2012, anthesis date (GS61) of the genotypes ranged from 24 May (W034) to 22 June (W040) ($P<0.001$). Six genotypes (W034, W468, W292, W209, W223 and W729) had early flowering in the range 27 May - 2 June, whereas six (W040, W481, W352, W355, W141 and W579) along with UK cultivars (Savannah, Oakley and Paragon) were later in flowering in the range 10 - 22 June. Variation in plant height was observed in the range 66 (Savannah) to 122 cm (W355) ($P<0.001$). All UK varieties were shorter than Watkins lines except Paragon (86 cm), which was taller than W034 (79 cm).

In 2013, the earliest genotypes were W034, W209, W468, W729, W223, W292 and W579 (5 - 6 June). All other genotypes including UK varieties had anthesis dates after 15 June, with the latest genotype, Savannah, flowering on 20 June (P< 0.001). The plant height ranged from 67 (Savannah) to 122 cm (W355) (P<0.001). As in 2012, all UK cultivars were shorter than Watkins lines except Paragon (87 cm), which was taller than W034 (81 cm).

Year had a significant effect on anthesis date (P<0.001), but not on PH (P=0.895). The year x genotype interaction was also significant for anthesis date (P<0.001), but not for PH (P>0.05). Averaging over years, no correlation between either PH or AD and AGDM was observed, however, a trend of a negative correlation was found between each of PH and AD and grain yield (r = 0.35 and r = 0.40, respectively).

4.3.1.2. Grain yield and components and aboveground dry matter

In 2012, grain yield ranged amongst genotypes from 0.77 g (W468) to 2.66 g (Xi19) per main shoot (MS) (P<0.001) and from 0.56 g (W209) to 1.86 g (Paragon) per fertile tiller (FT) (P<0.001; Table 4.1). Overall grain yield per plant ranged from 5.02 g (W355) to 12.01 g (Xi19) (P<0.001). In 2013, MS grain yield ranged from 0.74 g (W468) to 2.73 g (Paragon) (P<0.001), and FT grain yield from 0.54 g (W209) to 1.77 g $tiller^{-1}$ (Paragon). Paragon produced the highest grain yield per plant (11.61 g) and W209 (5.57 g) the lowest (P<0.001).

Cross-year analysis showed that year did not have an effect on grain yield per plant (P=0.963) and overall grain yield ranged from 5.36 (W355) to 11.76 g $plant^{-1}$ (Xi19) (P<0.001). The year x genotype interaction was significant (P=0.006). Averaging over years two UK varieties, Xi19 (11.76 g $plant^{-1}$) and Paragon (11.72 g $plant^{-1}$), had higher yield than all other genotypes (P<0.05) and Oakley (10.33 g $plant^{-1}$), Rialto (10.16 g $plant^{-1}$) and W141 (9.51 g $plant^{-1}$) had similar yield (P=0.180).

In 2012, harvest AGDM per MS ranged from 1.84 (W468) to 5.53 g (Paragon) (P<0.001) and from 1.59 (W209) to 3.83 g (W040) per FT (P<0.001; Table 4.1). AGDM ranged from 15.33 (W468) to 25.64 g $plant^{-1}$ (Rialto) (P<0.001). In 2013, AGDM per MS ranged from 1.73 (W209) g to 3.93 g $plant^{-1}$ (Paragon) (P<0.001) and per FT AGDM ranged from 1.78 (W468) to 6.05 g $plant^{-1}$ (Paragon) (P<0.001).

The AGDM per plant ranged from 16.45 (W468) to 25.77 g plant^{-1} (Paragon) (P<0.001).

Across years ANOVA revealed no year effect on AGDM (P=0.725). However, genotype had an effect on AGDM in the range 15.87 (Savannah) - 25.07 g plant^{-1} (Paragon). The year x genotype interaction was significant (P=0.006). This interaction could be explained by Rialto, W352 and W579 which had much higher AGDM in 2012 than in 2013 while all other genotypes had similar or higher AGDM in 2013. Averaging over years, the highest biomass was recorded for Paragon (25.07 g plant^{-1}), Rialto (24.08 g plant^{-1}), Xi19 (23.63 g plant^{-1}) and W040 (23.19 g plant^{-1}).

In 2012, Xi19 had the highest harvest index (HI) for MS and FT groups (0.55 and 0.50, respectively) and W355 had the lowest values (0.33 and 0.29, respectively) (P<0.001). For the whole plant, HI ranged from 0.30 (W355) to 0.51 (Xi19) (P<0.001). In 2013, HI ranged from 0.36 (W040) to 0.58 (W729) (P<0.001) for the MS and from 0.27 (W481) to 0.49 (Oakley) (P<0.001) for FT. Harvest index per plant varied from 0.31 (W355) to 0.50 (Xi19) (P<0.001). No significant interaction between genotype and shoot category was observed (P=0.228 and P=0.421, respectively, in 2012 and 2013).

Averaging across two years, HI ranged from 0.23 (W481) to 0.50 (Xi19) (P<0.001). Amongst Watkins lines the range was 0.30 - 0.45; W034 had HI (0.45) which was not significantly lower than Xi19 (0.50) (Table 4.1). Three Watkins lines: W729 (0.44), W223 (0.43) and W141 (0.42) had HI which was not significantly lower than Paragon (0.47).

Cross-year analysis indicated that there was no year effect on yield, AGDM or HI (P=0.963, P=0.725 and P=0.669). The linear relationship amongst genotypes across years was significant for grain yield, HI and AGDM for values in 2012 versus 2013 (R^2= 0.70, R^2=0.65 and R^2= 0.81, respectively, for yield, AGDM and HI) (Figure 4.2). Nevertheless, the year x genotype interaction was significant for these variables (P=0.006, P=0.006 and P<0.001, respectively; Table 4.2).

Table 4.1: **Grain yield, aboveground biomass (AGDM) per plant and harvest index (HI) of 17 genotypes in 2012 and 2013**

Genotype (G)	Yield plant^{-1} (g)			AGDM plant^{-1} (g)			HI		
	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean
Oakley	9.76	10.91	10.33	19.74	22.17	20.96	0.49	0.49	0.49
Paragon	11.83	11.61	11.72	24.37	25.77	25.07	0.49	0.45	0.47
Rialto	11.41	8.91	10.16	25.64	22.51	24.08	0.44	0.40	0.42
Savannah	6.63	6.11	6.37	15.76	15.98	15.87	0.42	0.38	0.40
Xi19	12.01	11.52	11.76	23.63	23.64	23.63	0.51	0.49	0.50
W034	8.62	8.49	8.55	18.97	18.81	18.89	0.45	0.45	0.45
W040	7.66	6.23	6.94	22.31	19.76	21.04	0.34	0.31	0.33
W141	9.30	9.71	9.51	22.56	22.94	22.75	0.41	0.42	0.42
W209	6.47	5.57	6.02	17.90	17.34	17.62	0.36	0.32	0.34
W223	9.69	8.51	9.10	22.12	20.57	21.35	0.44	0.41	0.43
W292	6.43	7.01	6.72	16.78	18.14	17.46	0.38	0.39	0.38
W352	6.87	6.48	6.68	21.93	19.34	20.64	0.31	0.34	0.32
W355	5.02	5.69	5.36	16.78	18.36	17.57	0.30	0.31	0.30
W468	5.71	6.20	5.95	15.33	16.45	15.89	0.37	0.38	0.37
W481	1.90	6.14	4.02	13.72	19.07	16.40	0.14	0.32	0.23
W579	9.56	8.50	9.03	23.51	20.91	22.21	0.41	0.41	0.41
W729	6.68	8.13	7.40	16.11	17.43	16.77	0.41	0.46	0.44
Mean	7.97	7.98	7.98	19.83	19.95	19.89	0.39	0.40	0.39
S.E.D (df)									
G (32)	1.01***	0.76***	0.67***	1.71***	0.94***	0.99***	0.03***	0.03***	0.02***
Year (66)		0.229 NS			0.340 NS			0.007 NS	
GXY (66)		0.944 **			1.401 **			0.029 ***	

Probability significance: ***: P<0.001, **: P<0.01 and *: P<0.05

Overall grain yield was more closely associated amongst genotypes with HI per plant (R^2 = 0.76 P<0.001) than AGDM per plant (R^2 = 0.26 P<0.05) (Fig, 4.1).

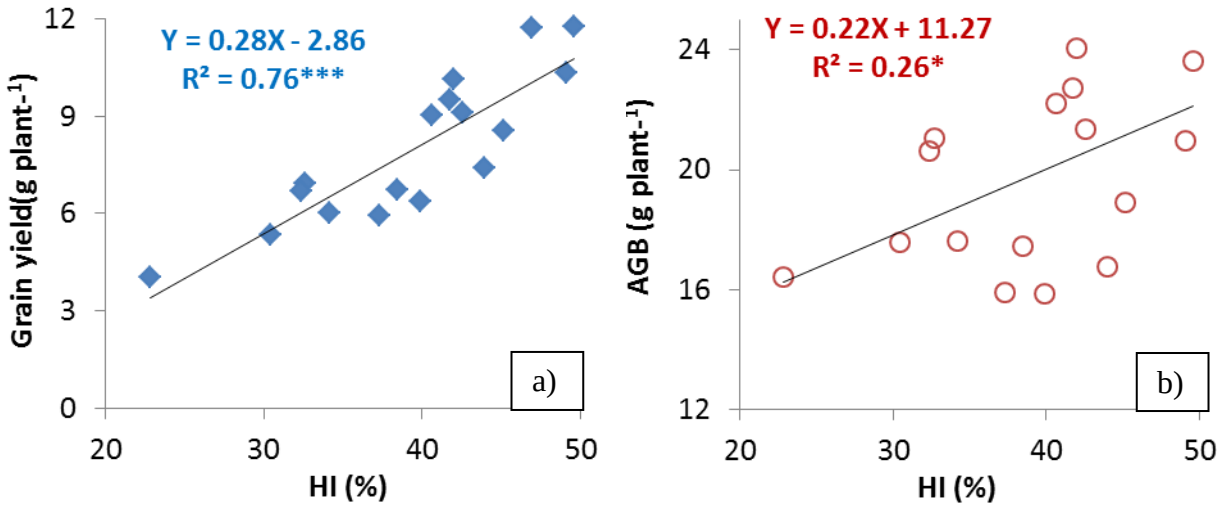


Figure 4.1: Linear relationship amongst 17 genotypes between harvest index (HI) and a) grain yield b) AGDM. Values represent mean across two years 2012 and 2013.

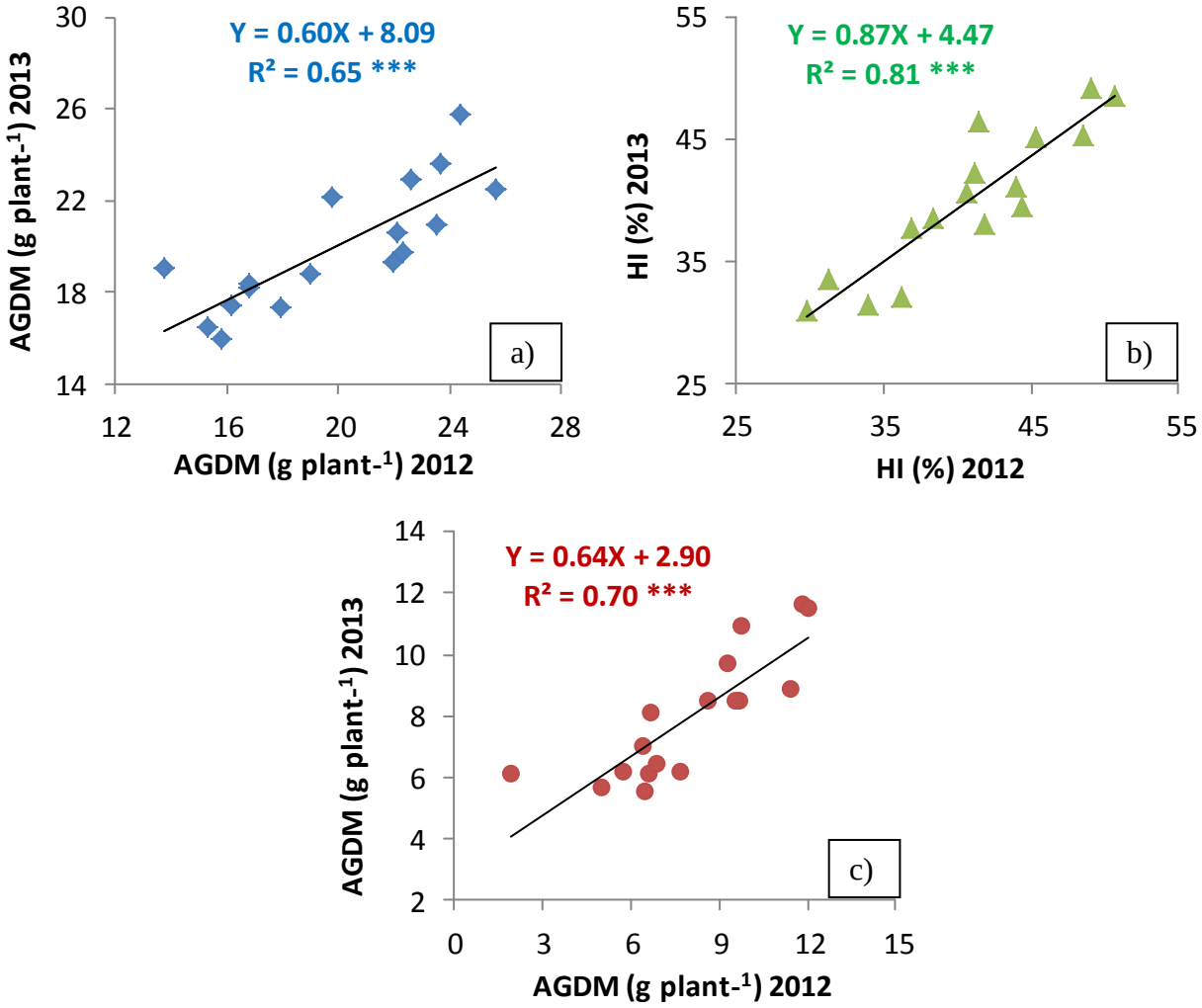


Figure 4.2: Linear relationship amongst 17 genotypes between 2012 and 2013 data for a) grain yield b) AGDM and c) Harvest Index (HI).

4.3.1.3 Yield components

In 2012, grain number per shoot ranged from 20.1 (W468) to 70.1 (Paragon) for the MS and from 8.8 (W468) to 44.6 (Paragon) for fertile tillers (P< 0.001; Table 4.2). Grains per plant ranged from 68.4 (W481) to 368.9 (Xi19) (P<0.001). Except for W579, which had more grains plant^{-1} than Savannah, other Watkins lines had fewer grains plant^{-1} than UK varieties, TGW ranged from 31.8 (Savannah) to 59.5 g (W141) for MS, and from 29.2 (Savannah) to 58.1 g (W141) for FT. For the whole plant, TGW ranged from 29.9 (Savannah) to 58.6 g (W141) (P<0.001). Ears per plant ranged from 5.5 for Savannah to 11.4 for W209 (P<0.001) (Table 4.2). In 2013, grains per MS and per FT differed between genotypes in the ranges 22.7 (W468) to 68.8 (Paragon) and 15.4 (W468) to 45.2 (Xi19), respectively (P<0.001).

Cross-year analysis (on whole plant data) showed that genotype and year had an effect on grains ear^{-1} (P<0.001 and P=0.019, respectively) and TGW (P<0.001 and P<0.001, respectively). The genotype x year interaction was also significant for both grains ear^{-1} (P=0.009) and TGW (P=0.006). The interaction for grains ear^{-1} could be explained by the genotype W481 (much lower grains per ear in 2012), however two genotypes explained the interaction for TGW W141 and W481 which had much lower TGW in 2013. For ears plant^{-1}, the effect of the genotype was significant (P<0.001) with differences (between 2012 and 2013) in the range -1.78 to +0.44; however, year did not affect ears plant^{-1} (P=0.416) and the year x genotype interaction was not significant (P=0.902).

Averaging over years, grains per ear varied amongst genotypes from 16.0 (W468) to 47.5 (Paragon) (P<0.001). TGW ranged from 28.3 g (Savannah) to 60.4 g (W141) (P<0.001). Overall ears plant^{-1} ranged from 5.6 (W481) to 10.6 (W209) (P<0.001).

Overall, grain yield in the glasshouse was highest for UK cultivars Xi19 (11.76 g plant^{-1}) and Paragon (11.72 g plant^{-1}). However, W141 (9.51 g plant^{-1}) had similar yield to UK cultivars: Oakley (10.33 g plant^{-1}) and Rialto (10.16 g plant^{-1}). on the other hand, W141 had the highest TGW (60.4 g) followed by W355 (45.7 g), W034 (42.6 g) and Paragon (40.8). Rialto (30.25 g) and Savannah (28.3g) had lower than the TGW majority of Watkins lines.

Table 4.2: Ear plant^{-1}, grain number ear^{-1} and Thousand Grain Weight (TGW) for 17 genotypes in 2012 and 2013.

Genotype (G)	Ears plant^{-1}			Grain ear^{-1}			TGW (g)		
	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean
Oakley	6.83	6.78	6.81	41.7	41.5	41.6	34.3	38.9	36.6
Paragon	6.00	6.11	6.06	48.9	46.2	47.5	40.4	41.2	40.8
Rialto	7.78	8.11	7.94	42.7	41.9	42.3	34.3	26.2	30.3
Savannah	5.50	5.78	5.64	40.3	39.4	39.9	29.9	26.7	28.3
Xi19	7.00	6.78	6.89	46.5	47.9	47.2	36.9	35.6	36.2
W034	9.56	8.56	9.06	21.1	23.6	22.3	42.8	42.4	42.6
W040	5.56	5.78	5.67	37.8	39.9	38.9	36.5	27.0	31.7
W141	6.33	5.89	6.11	25.1	26.7	25.9	58.6	62.2	60.4
W209	11.44	9.67	10.56	17.0	20.9	18.9	33.3	27.8	30.5
W223	8.67	9.00	8.83	25.5	25.5	25.5	43.9	37.1	40.5
W292	8.44	8.56	8.50	19.6	19.3	19.5	38.9	42.4	40.6
W352	8.11	8.56	8.33	22.6	20.0	21.3	37.6	37.9	37.8
W355	6.00	6.33	6.17	19.2	19.0	19.1	43.7	47.7	45.7
W468	9.22	9.22	9.22	15.8	16.2	16.0	39.3	41.8	40.6
W481	5.56	5.56	5.56	10.7	29.0	10.7	31.8	37.8	34.8
W579	6.78	6.22	6.50	34.8	39.5	37.1	40.6	34.7	37.7
W729	9.22	8.44	8.83	19.8	22.7	21.3	36.5	42.2	39.3
Mean	7.53	7.37	7.45	28.76	30.53	29.64	38.77	38.21	38.49
SED (DF)									
G (66)		0.67 ***			2.69 ***			2.45 ***	
Year (66)		0.23 NS			0.92 *			0.842 ***	
GXY (66)		0.94 **			3.81 **			3.470 **	

Probability significance: ***: P<0.001, **: P<0.01 and *: P<0.05

Grain yield per shoot (MS and FT included) was positively associated with grains per ear (R^2 = 0.75, P<0.001) and negatively with ears per plant (R^2 = 0.32, P<0.05) (Figure 4.3). There was also a negative relationship between grains per ear and ears per plant (R^2 = 0.28, P<0.05). There was no association between grains per ear and TGW (R^2 = 0.13, P=0.161) (Figure 4.4).

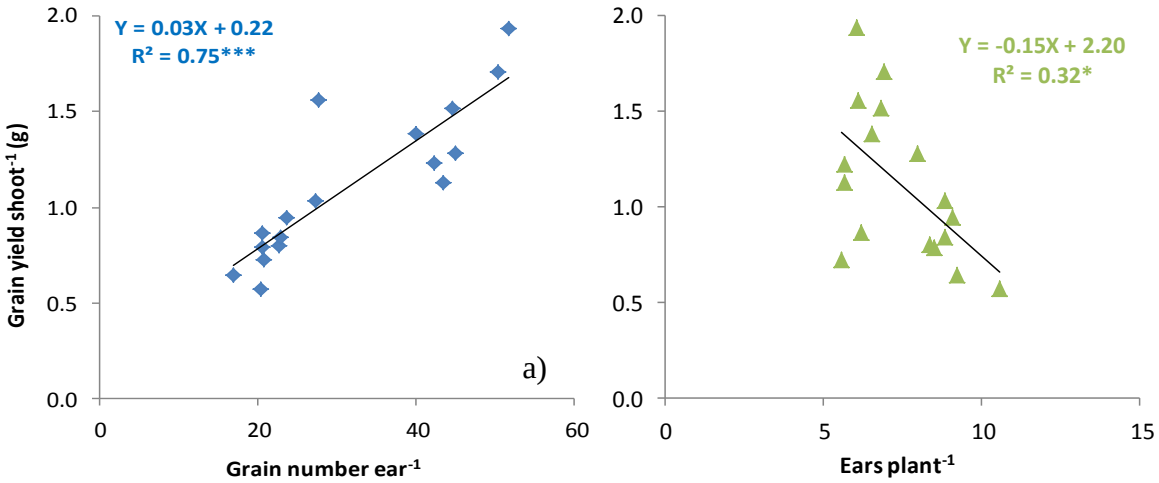


Figure 4.3: Linear relationship amongst 17 genotypes a) between grains ear^{-1} and grain yield shoot^{-1} b) between ears plant^{-1}, grains ear^{-1} and grain yield shoot^{-1}. Values represent mean across two years 2012 and 2013.

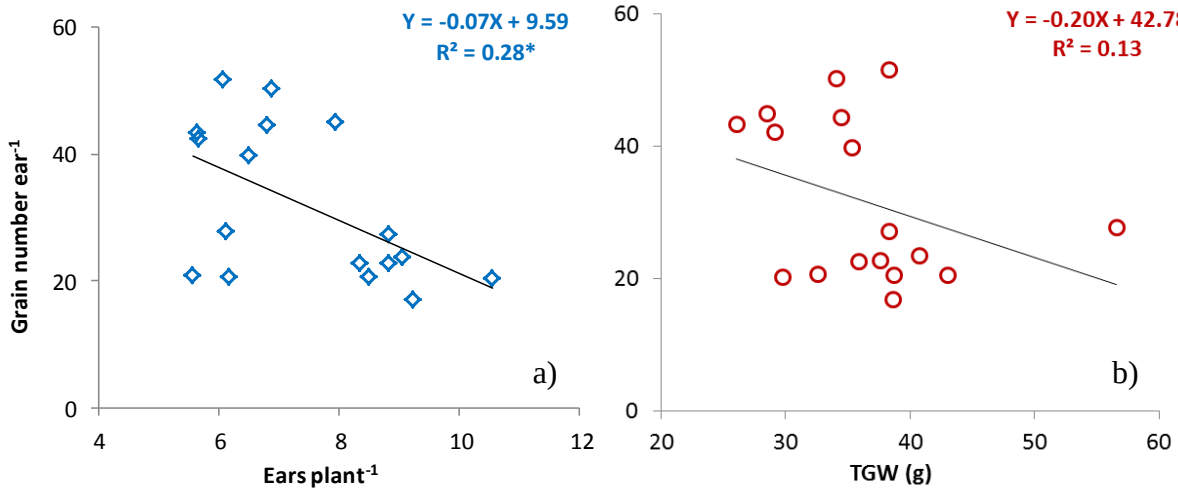


Figure 4.4: Linear relationship amongst 17 genotypes a) between ears plant^{-1} and Grain ear^{-1}, b) between TGW and grains ear^{-1}

4.3.1.4. Shoot number and biomass at anthesis

In 2012, genotype had an effect on the number of shoots per plant at all stages ($P<0.001$). W481 and Savannah, the latest genotypes to reach GS31, produced more shoots per plant (19 and 16 shoots, respectively) at this stage than other genotypes in the range 8 to 14. ($P<0.001$) (Table 4.3). The two genotypes W481 and Savannah lost respectively 70% and 66% of their shoots between GS31 and maturity, whereas shoots per plant was increased for W141 and stable for W468.

In 2013, genotype differences in shoots per plant were significant at all stages (P<0.001). W481 had the most shoots per plant at GS61 (16). Other genotypes ranged from 6 (W034) to 11 (Xi19). At maturity, shoots per plant varied from 5 (W481) to 9 (W209). Savannah and W034 continued to produce shoots after GS31 (+59% and +37%, respectively); whereas W481 showed high shoot death between GS31 and maturity (-71%, similar to 2012 experiment).

Cross-year analysis showed that genotype, year and genotype x year interaction had a significant effect on shoots per plant at GS61 (P<0.001, P=0.028, and P=0.028 respectively. However, at maturity, only genotype had a significant effect on shoots plant^{-1} (P<0.001) (Table 4.3).

Table 4.3: **Fertile shoots m^{-2} at GS61 and ears plant^{-1} at maturity for 17 genotypes in 2012 and 2013.**

Genotype (G)	Shoot number at anthesis			shoot number at maturity		
	2012	2013	Mean	2012	2013	Mean
Oakley	13.39	12.42	12.90	6.83	6.78	6.81
Paragon	9.11	10.83	9.97	6.00	6.11	6.06
Rialto	14.17	12.08	13.13	7.78	8.11	7.94
Savannah	16.17	10.33	13.25	5.50	5.78	5.64
Xi19	9.78	11.08	10.43	7.00	6.78	6.89
W034	8.28	7.33	7.81	9.56	8.56	9.06
W040	12.22	12.17	12.19	5.56	5.78	5.67
W141	12.33	10.25	11.29	6.33	5.89	6.11
W209	11.83	11.92	11.88	11.44	9.67	10.56
W223	10.67	10.50	10.58	8.67	9.00	8.83
W292	8.94	9.75	9.35	8.44	8.56	8.50
W352	14.39	14.83	14.61	8.11	8.56	8.33
W355	13.11	12.25	12.68	6.00	6.33	6.17
W468	9.22	8.92	9.07	9.22	9.22	9.22
W481	18.72	18.83	18.78	5.56	5.56	5.56
W579	9.72	10.83	10.28	6.78	6.22	6.50
W729	10.11	9.33	9.72	9.22	8.44	8.83
Mean	11.89	11.39	11.64	7.53	7.37	7.45
S.E.D (df)						
G (66)	1.33***	0.95***	0.85***	0.72***	0.56***	0.56***
Year (66)		0.29 *			0.19 NS	
GXY (66)		2.41 *			0.79 NS	

Probability significance: ***: P<0.001, **: P<0.01 and *: P<0.05

Cross-year ANOVA showed genotype affected AGDM per plant and per MS, and DM of plant components (ear, stem, lamina) ($P<0.001$) and main shoot flag leaf area (FLA) ($P=0.027$). Year affected all variables ($P<0.001$) except lamina DM per plant ($P=0.807$) and FLA ($P=0.141$). There was a year x genotype interaction for AGDM and stem DM per plant ($P<0.001$), but not for ear ($P=0.012$) or lamina DM per plant ($P=0.123$) and FLA (0.226).

Averaged over years, at anthesis Oakley had the highest AGDM per plant (14.04 g) and W468 the lowest (5.97 g). Overall, stem represent 55%, lamina 25% and ear 20% of the total AGDM at anthesis (Figure 4.5 and Table 4.4). No significant relationship was observed between plant height and AGDM per plant.

Table 4.4: Ear dry matter (DM), Lamina DM (Lam), stem DM, above-ground DM per plant at anthesis (GS61) for 17 genotypes in 2012 and 2013.

Genotype (G)	Ear DM (g)			Lam DM (g)			Stem DM (g)			AGDM (g)		
	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean
Oakley	3.96	2.66	3.31	3.27	3.17	3.02	7.59	7.43	7.51	14.82	13.26	14.04
Paragon	2.25	1.89	2.07	2.93	2.47	2.41	6.05	4.46	5.26	11.23	8.83	10.03
Rialto	2.70	2.91	2.81	2.72	2.94	2.63	6.66	5.58	6.12	12.08	11.43	11.75
Savannah	3.49	2.18	2.83	3.52	3.37	3.25	7.51	5.72	6.61	14.52	11.26	12.89
Xi19	3.11	2.49	2.80	2.86	2.72	2.56	5.95	5.89	5.92	11.92	11.11	11.51
W034	1.32	1.58	1.45	1.69	2.48	1.92	3.55	4.58	4.06	6.55	8.63	7.59
W040	1.90	1.73	1.82	3.66	3.22	3.23	9.18	5.21	7.19	14.73	10.16	12.44
W141	2.39	1.83	2.11	3.41	2.70	2.82	7.85	6.66	7.26	13.65	11.19	12.42
W209	1.83	1.67	1.75	2.08	2.39	2.07	4.76	5.60	5.18	8.67	9.65	9.16
W223	1.27	1.45	1.36	1.69	2.24	1.81	4.50	4.98	4.74	7.46	8.68	8.07
W292	1.48	1.68	1.58	1.54	2.28	1.69	3.52	3.79	3.66	6.54	7.76	7.15
W352	2.26	1.14	1.70	2.76	2.08	2.26	9.52	3.60	6.56	14.54	6.81	10.68
W355	2.62	2.14	2.38	3.52	2.65	2.93	10.55	5.13	7.84	16.68	9.92	13.30
W468	1.42	1.55	1.49	1.36	2.02	1.53	2.62	2.96	2.79	5.40	6.53	5.97
W481	1.31	1.97	1.64	2.63	2.80	2.55	6.75	5.88	6.32	10.69	10.65	10.67
W579	2.87	2.09	2.48	2.55	2.77	2.46	7.08	5.63	6.36	12.51	10.49	11.50
W729	1.14	1.48	1.31	1.68	2.05	1.72	3.48	3.75	3.62	6.31	7.28	6.79
Mean	2.20	1.91	2.05	2.58	2.61	2.40	6.30	5.11	5.70	11.08	9.63	10.35
SED (df)												
G (66)	0.284***			0.303 ***			0.724 ***			1.103 ***		
Year (Y) (66)	0.097**			0.104 NS			0.248 ***			0.378 ***		
GXY (66)	0.401*			0.428 NS			1.024 ***			1.560 ***		

Probability significance: ***: P<0.001, **: P<0.01 and *: P<0.05

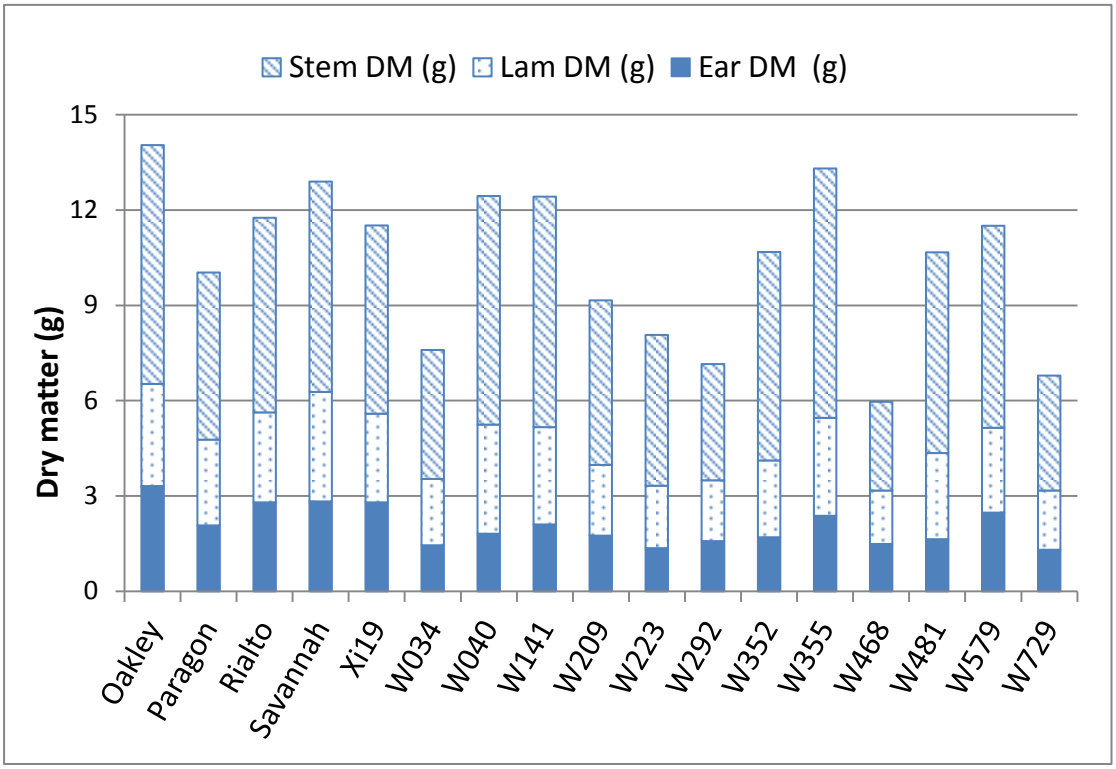


Figure 4.5: Dry matter partitioning at anthesis amongst 17 genotypes. Values represent mean across two years 2012 and 2013.

4.3.1.5 Nitrogen-use efficiency (NUE) and NUE components

In both years, genotype had an effect on N uptake at anthesis (GS61) (NUP_A), N uptake at maturity (NUP_M) and NUTE ($P<0.001$; Table 4.5). Year did not have any effect on these traits ($P=0.971$, $P=0.662$ and $P=0.686$, respectively). The genotype x year interaction was not significant for NUP_A ($P=0.726$) and for NUTE ($P=0.253$). However, it was significant for NUP_M ($P=0.020$). The interaction was explained by W481 (0.17 g/plant higher in 2013).

At anthesis, averaging across years, Savannah accumulated the most N amongst the genotypes (0.32 g N plant^{-1}) and W468 the lowest (0.15 g N plant^{-1}) ($P<0.001$). UK varieties Savannah and Oakley had higher NUP_A than all other genotypes. Amongst Watkins lines, W355, W141 and W040 had the highest values. These three lines had a trend to have higher values than Xi19, Rialto and Paragon. At maturity, Rialto accumulated the highest amount of N (0.43 g N plant^{-1}) and W292 the lowest (0.30 g N plant^{-1}) ($P<0.001$). For post-anthesis N uptake (NUP_{A-M}), genotypes ranged from 0.01 g N plant^{-1} (Savannah) to 0.18 g N plant^{-1} (W729) ($P<0.001$). No significant

relationship was found between NUP_{A-M} and anthesis date. Except for Rialto and Xi19, which had similar post-anthesis NUP to W729, W352, W223 and W468, all other UK varieties tended to have lower post-anthesis NUP than Watkins lines.

In 2012, post-anthesis nitrogen remobilisation (NR) for all genotypes ranged from 25 (W468) to 155 mg $plant^{-1}$ (Paragon) ($P<0.001$) and in 2013 from 44 (W352) to 200 mg $plant^{-1}$ for (Oakley) ($P<0.001$). The effects of year and year x genotype interaction were not significant ($P=0.132$ and $P=0.774$, respectively). Averaged across years, genotypes ranged from 0.05 mg $plant^{-1}$ (W468) to 0.18 mg $plant^{-1}$ ($P< 0.001$) NR was negatively associated with NUP_{A-M} ($R^2 = 0.34$, $P<0.001$).

Regarding N-remobilisation efficiency (NRE), four genotypes remobilised more than 50% of N accumulated at anthesis: Oakley (58.5%), Paragon (57.8%), W141 (55.0%) and W579 (54.2%). The lowest NRE was observed for W292 (24.8%). Averaging across years, a positive linear association amongst genotypes was found between NUP_A and N remobilisation (NR) ($R^2=0.81$, $P<0.001$).

Genotype had an effect on grain N concentration ($P<0.001$), but effects of year and the interaction Y x G were not significant ($P=0.391$ and $P>0.05$, respectively). Averaging over years, for all shoots per plant genotypes differed in grain N concentration in the range 2.22 (Oakley) - 3.66 (W481)% ($P<0.001$), whereas the year and Y x G interaction were not significant ($P= 0.391$ and $P>0.05$). Averaging across years, a negative relationship was found amongst the genotypes between grain N concentration and grain yield ($R^2= 0.42$, $P=0.005$), and NRE was positively associated with grain yield ($R^2= 0.46$, $P=0.002$) and AGDM ($R^2= 0.40$, $P=0.006$) (Figure 4.6). HI was also related to NUTE ($R^2= 0.40$, $P=0.006$).

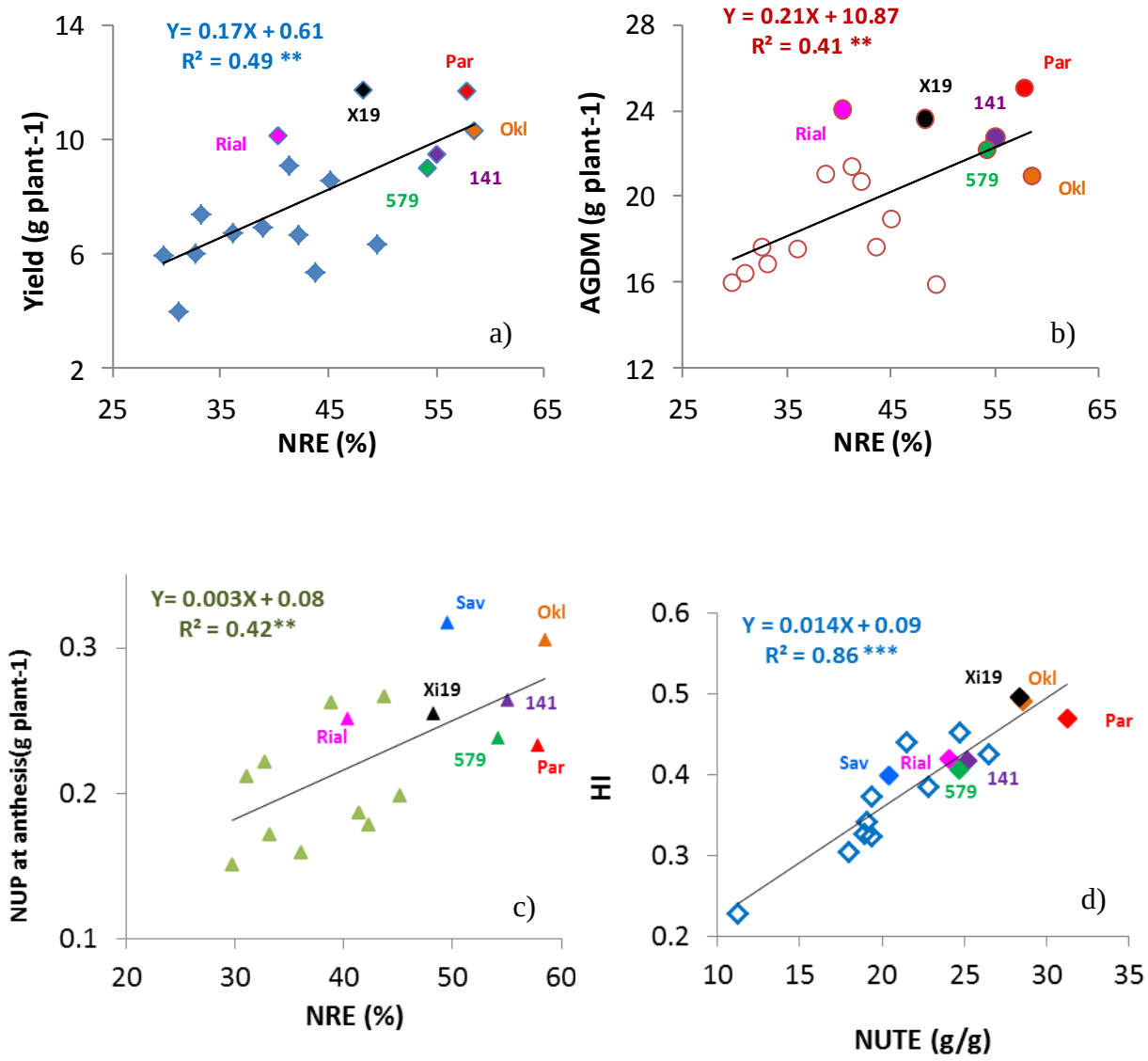


Figure 4.6: Linear relationship between a) NRE and grain yield b) NRE and AGDM c) NRE and NUP at anthesis and d) NUTE and Harvest index (HI) amongst 17 genotypes. Values represent means across 2012 and 2013.

Averaging across years, genotypes W468, W729, W352, W223 and W292 (range 46% - 51%) seem to have higher proportion of N uptake post-anthesis compared with Rialto (cultivar accumulating 41% of its N uptake at pre-anthesis) ($P<0.05$). All other UK varieties uptake less than 40% of total NUP at post-anthesis, and Savannah did not uptake any N post-anthesis (Figure 4.7). In addition no correlation was found between pre or post-anthesis NUP and anthesis date amongst the genotypes.

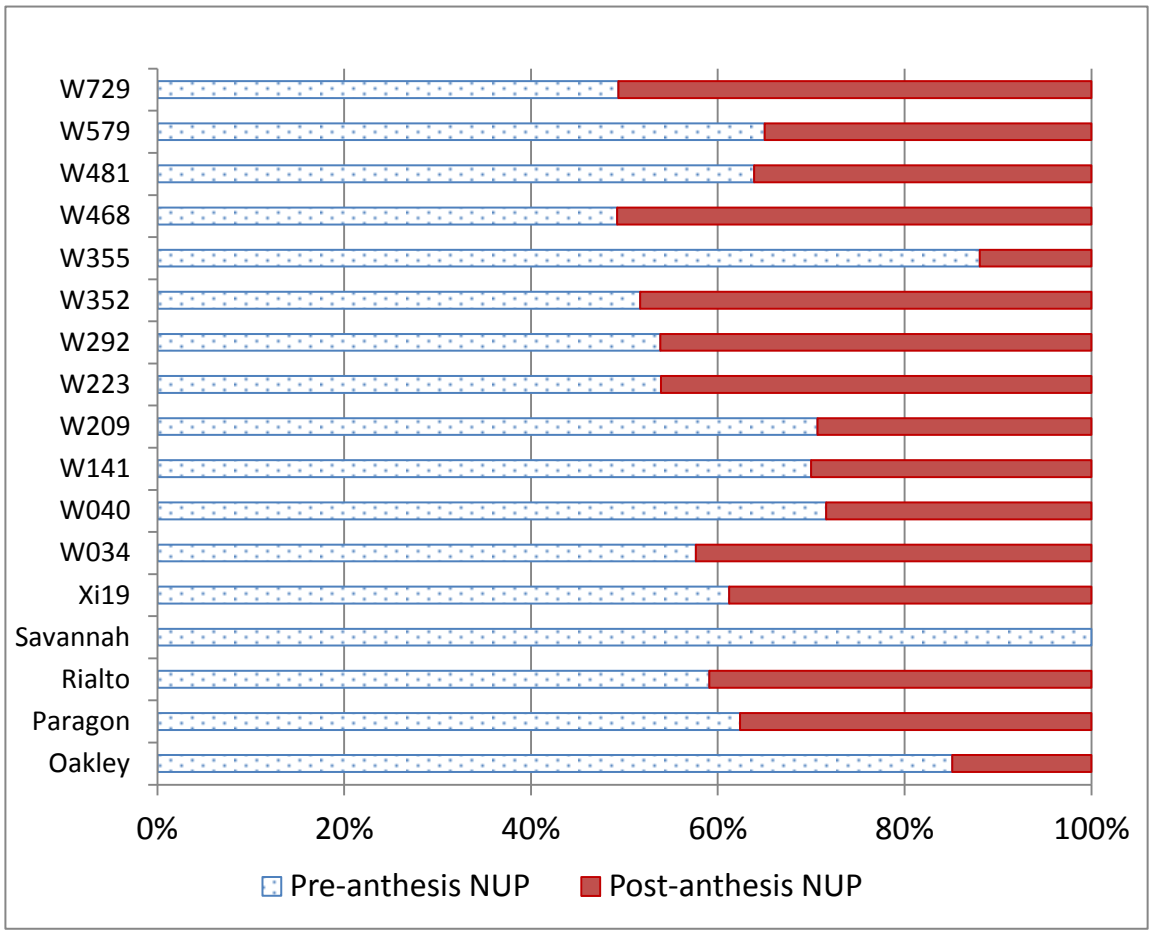


Figure 4.7: Percentage of N uptake at maturity in the pre- and post-anthesis period for 17 genotypes. Values represent means across 2012 and 2013

Table 4.5: **Nitrogen uptake at anthesis (NUP_A), at maturity (NUP_M), nitrogen utilization efficiency (NUTE), nitrogen remobilisation (NR), nitrogen remobilisation efficiency (NRE) and grain nitrogen concentration (grain N) in 2012, 2013 and the average across two years.**

Genotype	NUP Anthesis (g plant^{-1})			NUP Maturity (g plant^{-1})			NUTE (g g^{-1})			NR (g plant^{-1})			NRE (%)			Grain N (%)		
	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean
Oakley	0.28	0.34	0.31	0.34	0.38	0.36	28.28	28.85	28.57	0.16	0.2	0.18	57.35	59.63	58.49	2.23	2.21	2.22
Paragon	0.25	0.21	0.23	0.37	0.38	0.37	31.77	30.77	31.27	0.16	0.11	0.14	64.85	50.77	57.81	2.33	2.33	2.33
Rialto	0.23	0.27	0.25	0.46	0.39	0.43	25.28	22.89	24.08	0.09	0.11	0.1	41.24	39.44	40.34	2.42	2.5	2.46
Savannah	0.31	0.33	0.32	0.31	0.31	0.31	21.45	19.4	20.42	0.16	0.16	0.16	50.99	48.03	49.51	2.31	2.32	2.32
Xi19	0.24	0.27	0.26	0.42	0.41	0.42	28.62	28.11	28.36	0.11	0.14	0.12	46.17	50.28	48.22	2.38	2.4	2.39
W034	0.19	0.21	0.2	0.35	0.34	0.34	24.26	25.19	24.72	0.07	0.11	0.09	40.12	50.18	45.15	2.72	2.69	2.71
W040	0.26	0.27	0.26	0.39	0.34	0.37	19.61	18.24	18.93	0.1	0.11	0.1	37.29	40.53	38.91	2.9	2.93	2.91
W141	0.27	0.26	0.26	0.37	0.39	0.38	25.21	25.17	25.19	0.15	0.14	0.15	55.67	54.39	55.03	2.73	2.87	2.8
W209	0.22	0.22	0.22	0.32	0.31	0.31	20.01	18.19	19.1	0.08	0.07	0.07	34.44	31.03	32.73	2.76	2.78	2.77
W223	0.18	0.19	0.19	0.36	0.33	0.35	26.92	26.01	26.47	0.07	0.08	0.08	38.52	44.25	41.38	2.57	2.62	2.59
W292	0.16	0.16	0.16	0.28	0.31	0.3	23.06	22.58	22.82	0.05	0.06	0.06	31.91	40.36	36.14	2.49	2.51	2.5
W352	0.22	0.14	0.18	0.36	0.33	0.34	18.91	19.83	19.37	0.1	0.05	0.08	45.62	38.9	42.26	3.14	3.22	3.18
W355	0.27	0.26	0.27	0.29	0.32	0.3	17.7	18.26	17.98	0.13	0.11	0.12	46.62	40.9	43.76	2.77	2.84	2.81
W468	0.14	0.16	0.15	0.3	0.31	0.31	18.78	19.93	19.36	0.03	0.06	0.05	22.9	36.7	29.8	2.84	2.9	2.87
W481	0.18	0.24	0.21	0.25	0.42	0.33	7.79	14.7	11.25	0.05	0.09	0.07	25.13	37.1	31.11	3.61	3.71	3.66
W579	0.25	0.23	0.24	0.39	0.34	0.37	24.45	24.93	24.69	0.13	0.13	0.13	50.83	57.5	54.17	2.81	2.92	2.86
W729	0.18	0.17	0.17	0.33	0.36	0.35	20.39	22.67	21.53	0.06	0.05	0.06	34.2	32.15	33.17	3.03	2.98	3
Mean	0.22	0.23	0.23	0.35	0.35	0.35	22.50	22.69	22.59	0.10	0.10	0.10	42.58	44.24	43.41	2.75	2.71	2.73
S.E.D																		
Genotype	0.04*	0.04***	0.027***	0.03***	0.04*	0.03***	2.19***	1.44***	1.36***	0.33**	0.03*	0.02***	9.72***	8.05**	6.24***	0.18***	0.19***	0.13***
Year		0.09 NS			0.09 NS			0.465 NS			0.008 NS			2.120 NS			0.044 NS	
GXY		0.038 NS			0.037 *			1.916 NS			0.033 NS			8.830 NS			0.183 NS	

Probability significance: ***: P<0.001, **: P<0.01 and *: P<0.05

4.3.1.6 Relationship between NUE components, yield and AGDM

Regression analysis showed that overall grain yield per plant was more positively associated with NUTE (R^2=0.89, P<0.001) than with NUP at harvest (R^2=0.61, P<0.001). In contrast, AGDM per plant was more positively associated with NUP (R^2=0.73, P<0.001) than with NUTE at harvest (R^2=0.52 P<0.001) (Figure 4.8).

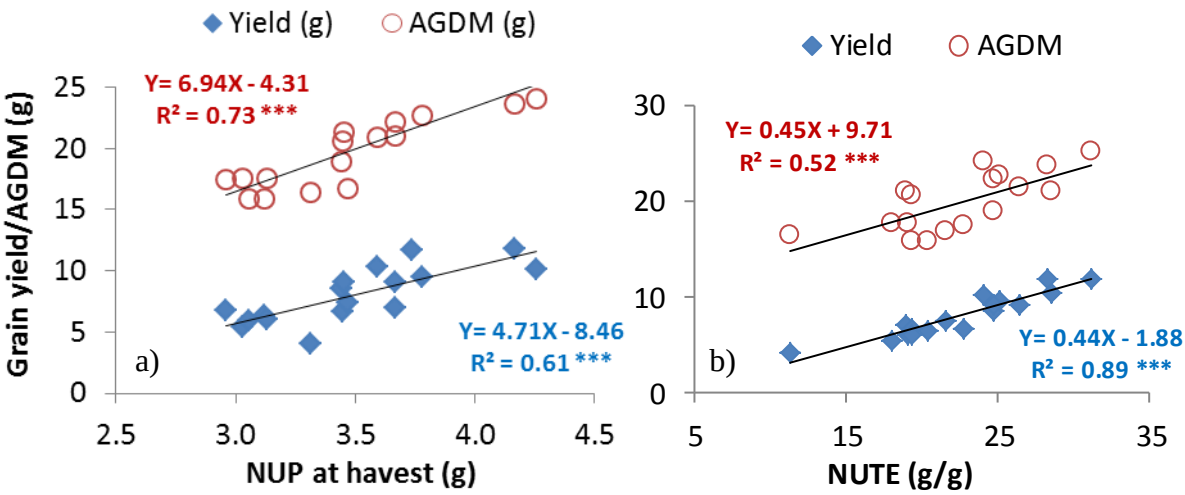


Figure 4.8: Linear relationship between a) grain yield and AGDM per plant vs NUP and b) grain yield and AGDM per plant vs NUTE amongst 17 genotypes. Values represent mean across two years 2012 and 2013.

4.3.1.7 Photosynthesis, chlorophyll fluorescence and content (SPAD)

In both years, leaf A_{max} and stomatal conductance were measured on three pre-anthesis dates and two post-anthesis dates.

a- Leaf photosynthetic rate

Averaging over genotypes and years, flag-leaf light-saturated photosynthesis rate (A_{max}) was higher pre-anthesis (20.91 µmol m^{-2} s^{-1}) than post-anthesis (13.78 µmol m^{-2} s^{-1}) (P<0.001) without significant interaction with genotype. For individual years, the A_{max} was higher in 2012 (22.76 µmol m^{-2} s^{-1}) than in 2013 (19.05 µmol m^{-2} s^{-1}) (P<0.05; Table 4.6).

Overall the 5 UK RL varieties (18.00 - 21.50 µmol m^{-2} s^{-1}), W141 (20.66 µmol m^{-2} s^{-1}), W352 (18.35 µmol m^{-2} s^{-1}) and W040 (18.07 µmol m^{-2} s^{-1}) had higher A_{max} (P<0.05) than other genotypes in the range 13.55 - 17.23 µmol m^{-2} s^{-1}. Amongst UK

varieties, Savannah had the highest A_{max} (21.50 µmol m^{-2} s^{-1}) ($P<0.05$) post-anthesis and Oakley, Rialto, and Xi19 had similar A_{max}. Amongst Watkins lines, W141 (20.66 µmol m^{-2} s^{-1}) had higher A_{max} than other lines ($P<0.005$). Watkins lines reduced from 27% (W141) to 56% (W579) pre-antthesis leaf A_{max} compared to post-anthesis; however UK varieties lost only between 2% (Savannah) and 36% (Rialto).

A positive linear relationship was found between pre-anthesis A_{max} and grain yield per plant (R^2=0.25, $P<0.01$), between pre-anthesis A_{max} and AGDM per plant (R^2=0.36, $P<0.05$) and between pre-anthesis A_{max} and grain number per ear (R2=0.35, P=0.002) (Figure 4.9).

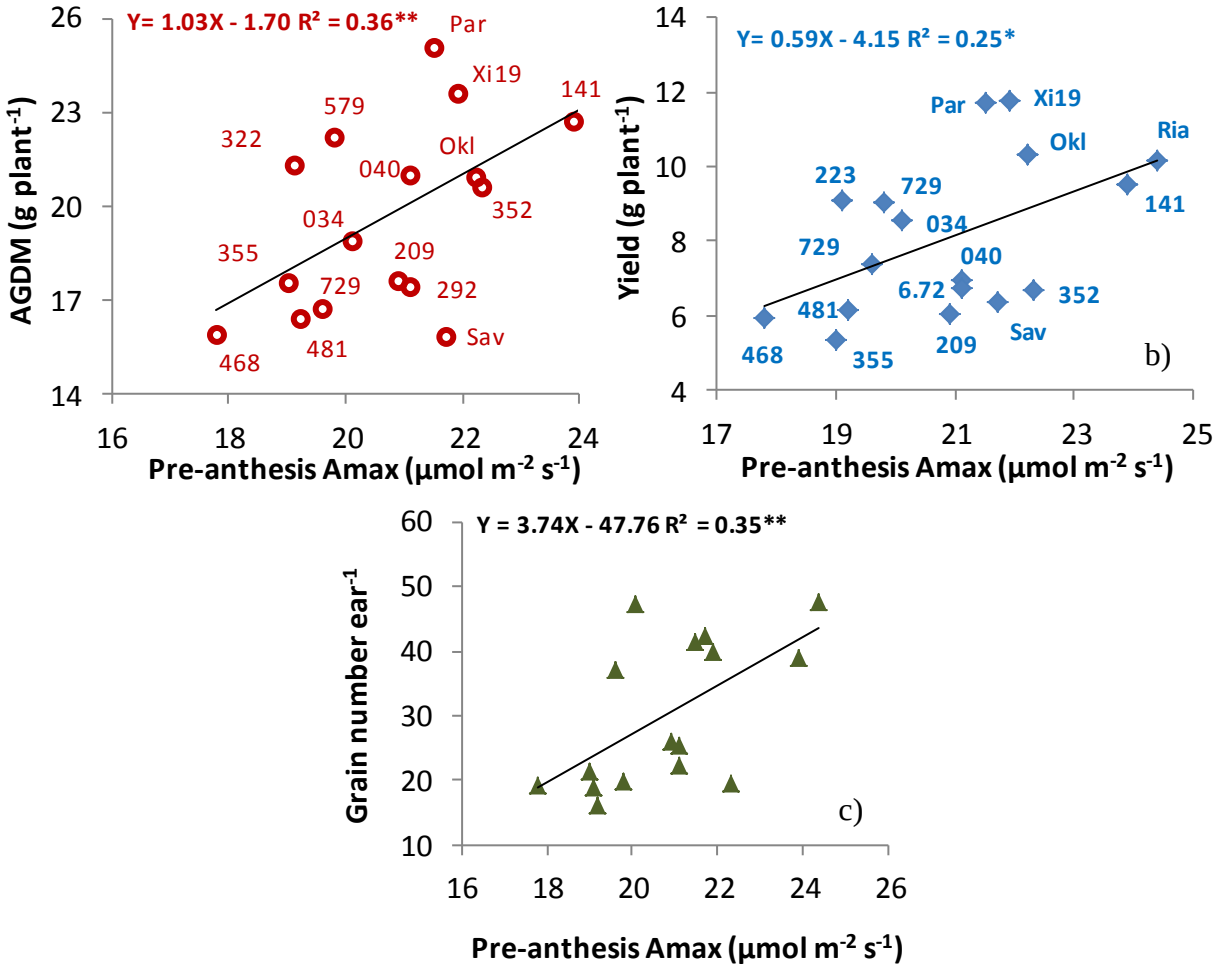


Figure 4.9: Linear relationship amongst 17 genotypes between a) pre-anthesis photosynthesis rate (A_{max}) and AGDM; b) pre-anthesis A_{max} and grain yield; c) pre-anthesis A_{max} and grain number per ear. Values represent mean across two years 2012 and 2013.

b- Flag leaf chlorophyll fluorescence (Quantum Yield: QY)

In 2012, pre-anthesis QY ranged amongst genotypes from 0.39 (W223) to 0.61 (Paragon and W729) ($P<0.001$), and post-anthesis in the range 0.35 (W468 and W481) to 0.70 (Savannah) ($P<0.001$). Measured at anthesis QY ranged from 0.35 (W292) to 0.70 (Savannah) ($P<0.01$).

In 2013, pre-anthesis QY ranged from 0.36 (W355) to 0.64 (W729) and post-anthesis from 0.34 (W579 and W223) to 0.72 (Savannah) ($P<0.001$). At anthesis QY ranged from 0.34 (W292) to 0.70 (savannah) ($P<0.01$). Averaging over all genotypes, QY decreased from 0.53 pre-anthesis to 0.47 at post-anthesis ($P<0.005$).

c- Chlorophyll content (SPAD) and leaf senescence

In 2012 genotype had an effect on flag leaf chlorophyll content (SPAD value) at anthesis and at all dates of measurements (9, 16 June as pre-anthesis; and 21, 30 June, 11, 21 and 31 July post-anthesis) ($P<0.01$). At anthesis, SPAD values were the highest and ranged from 41.7 (W352) to 56.7 (Oakley) ($P<0.001$). Just after anthesis, SPAD values started to decrease for all genotypes. Overall genotypes ranged from 41 (W141) to 51 (W040) ($P<0.001$) pre-anthesis and from 30 (W223) to 35 (Savannah and Xi19) ($P<0.001$) post anthesis.

In 2013 genotype differed in flag leaf chlorophyll content (SPAD value) at anthesis and at all dates of measurements (10, 16 June as pre-anthesis and 23, 30 June, 10, 17 and 27 July as post-anthesis) ($P<0.01$). At anthesis, SPAD values were the highest and ranged from 36.2 (W223) to 57.2 (Xi19) ($P<0.001$). Just after anthesis, SPAD values started to decrease for the majority of the genotypes. Overall genotypes ranged from 39 (W223) to 53 (Xi19) ($P<0.001$) pre-anthesis and from 27 (Oakley and Paragon) to 37 (W481 and W729) post-anthesis ($P<0.001$).

In 2013, leaf senescence duration in thermal time (SEN_{TOT}), leaf senescence duration in calendar days (SEN_{DUR}) and senescence rate (SEN_{RATE}) in the post-anthesis period were measured. SEN_{TOT} ranged from 739 °Cd (W481) to 1021 °Cd (Oakley) ($P=0.006$). A positive relationship amongst genotypes was found between SEN_{TOT} and grain yield per plant ($R^2=0.51$, $P<0.001$) and with the last leaf SPAD measurement (27 July 2013) ($R^2=0.34$, $P<0.013$). SEN_{DUR} ranged from 19 (W468)

days to 37 days (Paragon) ($P<0.001$). Finally SEN_{RATE} varied from 26 °Cd^{-1} (Paragon and W352) to 42 °Cd^{-1} (W468).

Cross-year ANOVA analysis showed no year effect on flag-leaf SPAD ($P=0.16$), though the year x genotype interaction was significant ($P=0.036$). Indeed, SPAD values decreased significantly for W223 (-27%) and for W141 (-15%) in 2012 compared to 2013, however, no significant differences between years for all other genotypes. Overall Xi19 had the highest chlorophyll content at anthesis (55.74) and W355 the lowest (38.36) ($P<0.001$).

Averaging over years regression analysis showed amongst genotypes: 1) a positive linear relationship between SPAD and NUP at anthesis ($R^2=0.37$, $P=0.030$); 2) a negative linear relationship between leaf SPAD and NRE ($R^2=0.46$, $P<0.001$) and 3) a positive relationship between leaf SPAD at anthesis and leaf N concentration (%) ($R^2=0.35$, $P<0.013$) (Figure 4.10).

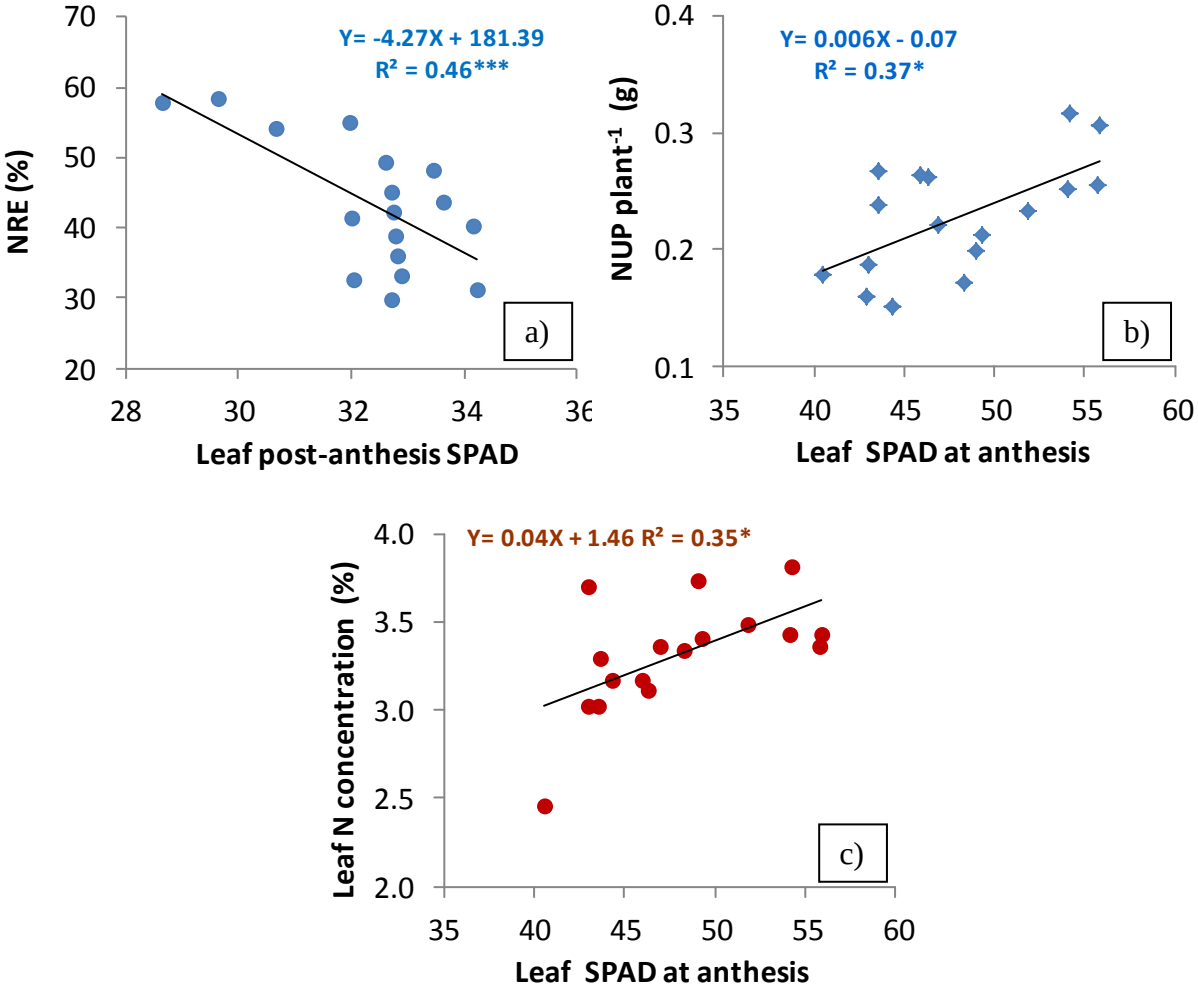


Figure 4.10: Linear relationship between a) leaf post-anthesis chlorophyll content (SPAD) and nitrogen remobilisation efficiency (NRE,%) amongst 17 genotypes, b) N uptake and SPAD at anthesis, c) SPAD at anthesis and leaf N content (%) Values represent mean across two years 2012 and 2013.

Grain yield was positively associated amongst genotypes with AGDM (R = 0.86, P < 0.001), NUP (R = 0.78, P < 0.001), NUTE (R = 0.94, P < 0.001), NRE (R = 0.70, P < 0.01), flag leaf green area (FLA) (R = 0.59, P < 0.05) and with pre-anthesis photosynthesis rate (PrA_A_{max}) (R = 0.52, P < 0.05). While AGDM was positively associated with FLA (R = 0.57, P < 0.01), NRE (R = 0.64, P < 0.01), NUP_M (R = 0.85, P < 0.001), NUTE (R = 0.72, P < 0.001) and PrA_A_{max} (R = 0.60, P < 0.05) (Table 4.17).

Table 4.6: Flag leaf photosynthesis rate (A_{max}), relative chlorophyll content (SPAD) and chlorophyll fluorescence for 17 genotypes.

Genotype	A_{max} (µmol m^{-2} s^{-1})						SPAD						Chl. Fluorescence					
	Pre-anthesis			Post-anthesis			Pre-anthesis			Post-anthesis			Pre-anthesis			Post-anthesis		
	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean	2012	2013	Mean
Oakley	24.8	19.7	22.2	19.9	15.6	17.7	45.3	50.6	48.0	32.4	26.9	29.7	0.57	0.60	0.59	0.60	0.57	0.58
Paragon	21.8	21.2	21.5	16.8	12.2	14.5	43.9	42.1	43.0	30.2	27.1	28.7	0.61	0.63	0.62	0.43	0.46	0.45
Rialto	25.7	23.0	24.4	15.6	15.4	15.5	46.6	46.9	46.8	34.2	34.1	34.1	0.54	0.55	0.54	0.62	0.59	0.61
Savannah	22.3	21.1	21.7	22.1	20.5	21.3	47.4	44.4	45.9	35.2	30.0	32.6	0.58	0.65	0.63	0.70	0.71	0.71
Xi19	22.6	21.3	21.9	17.4	15.0	16.2	46.7	53.4	50.1	35.2	31.8	33.5	0.58	0.60	0.59	0.60	0.60	0.60
W034	22.5	17.7	20.1	16.9	11.9	14.4	43.9	43.9	43.9	31.4	34.0	32.7	0.51	0.54	0.52	0.38	0.44	0.41
W040	23.8	18.3	21.1	16.9	13.2	15.1	51.0	49.4	50.2	34.7	30.8	32.8	0.43	0.44	0.44	0.40	0.44	0.42
W141	26.2	21.6	23.9	19.5	15.4	17.4	41.4	42.4	41.9	31.4	32.6	32.0	0.52	0.47	0.47	0.41	0.37	0.39
W209	22.9	18.8	20.9	13.9	10.8	12.3	45.2	45.3	45.3	31.5	32.6	32.0	0.58	0.61	0.59	0.48	0.52	0.50
W223	22.5	15.7	19.1	14.5	10.4	12.5	41.7	38.7	40.2	29.9	34.1	32.0	0.39	0.42	0.41	0.46	0.43	0.44
W292	23.9	18.3	21.1	11.0	9.5	10.2	47.4	44.7	46.0	34.1	31.6	32.8	0.44	0.44	0.44	0.37	0.34	0.36
W352	24.8	19.7	22.3	13.9	15.0	14.4	44.5	43.9	44.2	30.7	34.8	32.7	0.48	0.52	0.50	0.37	0.42	0.40
W355	21.5	16.5	19.0	16.0	10.2	13.1	46.1	44.9	45.5	32.7	34.6	33.6	0.43	0.36	0.36	0.38	0.36	0.37
W468	19.9	15.8	17.8	10.2	8.40	9.30	44.2	50.6	47.4	33.8	31.6	32.7	0.53	0.53	0.53	0.35	0.43	0.39
W481	19.9	18.5	19.2	11.4	8.50	9.90	47.2	44.3	45.8	31.4	37.1	34.2	0.56	0.60	0.58	0.35	0.39	0.37
W579	20.9	18.6	19.8	11.4	6.10	8.70	42.3	41.2	41.8	32.6	28.8	30.7	0.52	0.47	0.44	0.38	0.29	0.34
W729	21.1	18.1	19.6	13.0	10.1	11.6	42.4	45.3	43.9	29.0	36.8	32.9	0.61	0.64	0.63	0.41	0.46	0.44
Mean	22.8	19.1	20.9	15.3	12.2	13.8	45.1	45.4	45.3	32.4	32.3	32.3	0.52	0.53	0.52	0.45	0.46	0.46
S.E.D (df)																		
Genotype (66)	1.4***	1.0***	2.3***	1.4***	2.1***	1.7***	1.6***	3.8***	4.1***	1.8***	3.9***	1.5***	0.04***	0.02***	2.02***	1.04***	0.02***	0.03***
Year (66)		0.4*			0.009 NS			0.7 NS			0.5 NS			0.009***			0.01***	
GXY (66)		3.6**			0.037 *			5.8 NS			4.2***			0.035 ***			0.08***	

Probability significance: ***: P<0.001, **: P<0.01 and *: P<0.05

Table 4.7: Pearson's correlation coefficient between different traits (AGDM: above-ground dry matter, AD: anthesis date, PH: plant height, Pr_A_{max}: pre-anthesis photosynthetic rate, Po_A_{max}: post anthesis photosynthetic rate, Pr_SPAD: pre-anthesis Chlorophyll content, Po_SPAD: post-anthesis Chlorophyll content, Pr_QY: Pre-anthesis chlorophyll fluorescence, Po_QY: Post-anthesis chlorophyll fluorescence, FLA: flag leaf area, SFLA: specific flag leaf area, NUPA: N uptake at anthesis, NUPM: N uptake at maturity, NUTE: N utilisation efficiency, NRE: N remobilisation efficiency and GNC: grain N concentration (%). The correlations are based on means of 2012 and 2013.

Traits	Yield	AGDM	AD	PH	PrA_A_{max}	PoA_A_{max}	PrA_SPAD	PoA_SPAD	PrA_QY	PoA_QY	FLA	SFLA	NUPA	NUPM	NUTE	NRE
AGDM	0.86***															
AD	-0.35	0.08														
PH	-0.40	-0.04	0.90***													
Pr_A_{max}	0.51*	0.60**	-0.03	-0.26												
Po_A_{max}	0.37	0.27	-0.21	-0.49*	0.67**											
Pr_SPAD	-0.51*	-0.41	0.15	0.14	-0.09	-0.11										
Po_SPAD	-0.08	-0.07	-0.07	-0.28	0.25	0.37	0.36									
Pr_QY	0.18	-0.07	-0.64*	-0.73***	0.22	0.32	-0.17	-0.03								
Po_QY	0.35	0.14	-0.58*	-0.83***	0.49*	0.75***	0.00	0.43	0.59*							
FLA	0.59*	0.66**	-0.05	-0.27	0.71**	0.39	-0.47*	0.11	0.39	0.33						
SFLA	0.10	0.06	0.14	0.18	-0.16	0.07	0.01	0.35	-0.47	-0.19	-0.21					
NUPA	0.28	0.29	0.06	-0.28	0.48*	0.75***	-0.20	0.43	0.14	0.61**	0.33	0.23				
NUPM	0.78***	0.85***	-0.06	-0.24	0.62**	0.32	-0.08	0.08	0.18	0.33	0.55*	-0.05	0.32			
NUTE	0.94***	0.72***	-0.43	-0.40	0.39	0.33	-0.65**	-0.14	0.11	0.29	0.49*	0.17	0.20	0.54*		
NRE	0.70**	0.64**	0.01	-0.16	0.47	0.56**	-0.67**	-0.08	0.03	0.26	0.55*	0.17	0.64**	0.43	0.72***	
GNC	-0.65**	-0.37	0.58*	0.64**	-0.37	-0.52*	0.46	-0.29	-0.15	-0.61**	-0.36	-0.26	-0.42	-0.21	-0.78***	-0.56*

Probability significance: ***: P<0.001, **: P<0.01 and *: P<0.05

4.3.2 Field experiment (10 genotypes) 2011/12

4.3.2.1 Biomass, grain yield and components

AGDM increased from 11.1 t ha^{-1} under LN to 15.60 t ha^{-1} under HN ($P< 0.05$). Genotypes ranged from 9.5 t ha^{-1} (W209) to 21.2 t ha^{-1} (Paragon) under HN and from 6.8 t ha^{-1} (W209) to 14.6 t ha^{-1} (Paragon) under LN ($P<0.001$). The N x genotype interaction was not significant ($P=0.806$).

Grain yield increased from 4.61 t ha^{-1} under low N to 5.47 t ha^{-1} under high N conditions ($P=0.005$). Genotypes ranged from 3.21 t ha^{-1} (W729) to 9.05 t ha^{-1} (Paragon) under HN and from 2.30 t ha^{-1} (W209) to 6.37 t ha^{-1} (Paragon) ($P< 0.001$) under LN conditions. There was a trend for a N treatment x genotype interaction ($P=0.096$). Under high N, UK varieties generally had higher grain yield than Watkins lines. Furthermore, Rialto, Paragon, and Savannah lost relatively more yield under low N (-30%, -30% and -24% respectively) than W040 (-9.8%) and W141 (6%).

HI increased from 0.35 under HN to 0.42 under LN ($P=0.005$). Amongst genotypes, HI ranged from 0.27 (W141 and W352) to 0.43 (Rialto) under HN and from 0.33 (W352) to 0.50 (Savannah) under LN ($P<0.001$). There was a trend for a N x genotype interaction ($P=0.096$); the lines W141, W034 and W729 decreased HI relatively more under HN conditions (-32%, -29% and -24% respectively) than UK varieties Rialto, Savannah and Paragon (-2.4%, -7.82% and -15%, respectively).

A positive correlation was found amongst genotypes between AGDM and grain yield ($R^2=0.79$, $P<0.001$ under HN and $R^2=0.85$, $P<0.001$ under LN) and between HI and grain yield ($R^2=0.36$, $P<0.05$ under HN and $R^2=0.17$, $P=0.188$) (Figure 4.11).

Table 4.8: Grain yield, above-ground biomass (AGDM) and Harvest Index (HI) amongst 10 genotypes

Genotype (G)	Grain Yield (t 100% DM ha^{-1})		AGDM (t 100% DM ha^{-1})		HI	
	HN	LN	HN	LN	HN	LN
Paragon	9.05	6.37	21.2	14.6	0.42	0.44
Rialto	8.86	6.24	21.0	13.8	0.43	0.46
Savannah	6.01	4.56	14.3	9.2	0.42	0.50
W034	4.34	4.79	13.5	10.5	0.32	0.46
W040	6.27	5.65	18.3	14.2	0.36	0.41
W141	4.98	5.29	18.7	13.3	0.27	0.40
W209	3.18	2.30	9.5	6.8	0.35	0.34
W223	4.37	4.51	12.8	10.9	0.34	0.41
W352	4.47	3.84	16.7	11.5	0.27	0.33
W729	3.21	2.55	9.9	5.9	0.33	0.44
Mean	**5.47**	**4.61**	**15.60**	**11.1**	**0.35**	**0.42**
SED (df)						
Genotype (36)	0.38***		1.78***		0.026***	
Nitrogen(2)	0.25*		0.85*		0.005**	
GXN (36)	1.29***		2.54NS		0.035NS	

Probability significance: ***: P<0.001, **: P<0.01 and *: P<0.05

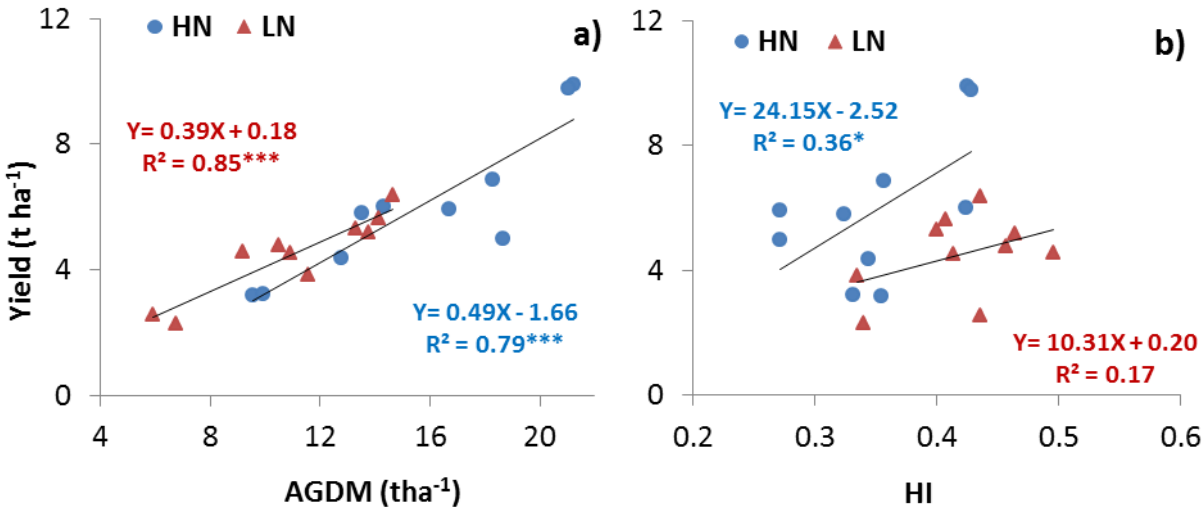


Figure 4.11: Linear relationship amongst 10 genotypes between grain yield (100% DM) and a) AGDM, b) harvest index (HI).

Ears per m^2 increased from 408 under LN to 535 under HN (P<0.032). Genotypes ranged from 412 (W040) to 863 (W223) m^{-2} under HN and from 252 (Savannah) to 490 (W352) m^{-2} under LN (P<0.001). The N x genotype interaction was not significant (P=0.232). Amongst UK cultivars, Rialto had the most ears m^{-2} under HN (523), and Paragon (450) had the most under LN. However, amongst Watkins lines, the highest ear density was recorded in W223 (92% significantly higher than Rialto) under HN in the range 412 - 863 and in W352 (10% higher than Paragon) under LN in the range 327 - 490.

Grains per m^2 increased from 12679 under LN to 15539 under HN (P=0.046). Genotypes ranged from 10,018 (W209) to 24,941 (Rialto) under HN and from 7,051 (W209) to 17,778 (Paragon) undr LN (P<0.001). The N x genotype interaction was significant (P=<0.001). W209, Rialto, Paragon and Savannah lost relatively more grains per m^2 under LN (-30%, -29% -26% and -22% respectively) than W034, W223 and W141 (+3%, -7% and -7%).

Regarding TGW, the effects of genotype and the genotype x N treatment interaction were highly significant (P<0.001, P<0.001). However, N treatment did not have any effect (P=0.309). UK cultivars lost less TGW under LN (-5%, -3% and -1% for Paragon, Savannah and Rialto respectively) than W141 (+13%), W223 (+9%) and W209 (+95%).

Table 4.9: Ears per m^2, grains per m^2 and thousand grain weight (TGW) amongst 10 genotypes

Genotype	Ears m^{-2}		Grains m^{-2}		TGW (g)	
	HN	LN	HN	LN	HN	LN
Paragon	450	444	24 117	17 778	37.7	35.8
Rialto	523	331	24 941	17 719	35.9	35.4
Savannah	416	252	15 658	12 168	38.6	37.4
W034	583	446	12 734	13 106	34.3	36.5
W040	412	401	16 015	14 018	39.5	40.3
W141	545	327	13 094	12 166	37.9	43.5
W209	649	456	10 018	7 051	31.6	34.5
W223	863	488	13 262	12 383	33.2	36.4
W352	441	490	15 105	12 328	29.1	31.2
W729	463	432	10 444	8 074	30.7	31.6
Mean	**535**	**417**	**15 539**	**12 679**	**34.8**	**36.3**
SED (df)						
Genotype (36)	57.8***		1148***		0.72 ***	
Nitrogen (2)	23.7*		793*		1.05 NS	
GXN (36)	81.1NS		1733***		1.43 ***	

Probability significance: ***: P<0.001, **: P<0.01 and *: P<0.05

4.3.2.2 N-use efficiency components and grain N content (NG)

a- NUE and NUE components

NUP ranged from 164 kg N ha^{-1} (W209) to 414 kg N ha^{-1} (Rialto) under HN and from 108.4 kg N/ha (W209) to 214.6 kg N ha^{-1} (W040) under LN (P<0.001). The N treatment x genotype interaction was significant (P<0.001). A larger decrease in NUP under HN was recorded in W355 (-66%) and in Paragon (-55%) than for W040 (-23%) and W223 (-37%).

There was a positive linear relationship between NUP and grain yield (R^2=0.69, P<0.05) under HN and (R^2=0.81, P<0.001) under LN; and also between NUP and AGDM (R^2=0.73, P<0.001) under HN and (R^2=0.65, P<0.01) under LN conditions (Figure 4.12).

Both N treatment (P=0.002) and genotype (P<0.001) had an effect on NUTE. The N treatment x genotype interaction was not significant (P=0.158). NUTE ranged from

17.7 kg kg^{-1} (W040) to 26.7 kg kg^{-1} (Paragon) under HN and from 24.0 kg kg^{-1} (W729) to 38.2 kg kg^{-1} (Paragon) under LN.

A positive linear association was found between NUTE and grain yield under HN (R^2=0.39, P<0.05) and LN (R^2=0.64, P<0.01). Also a positive relationship between NUTE and AGDM under LN was found (R^2=0.56, P<0.05), However under LN there was no significant relationship between AGDM and NUTE (Figure 4.13).

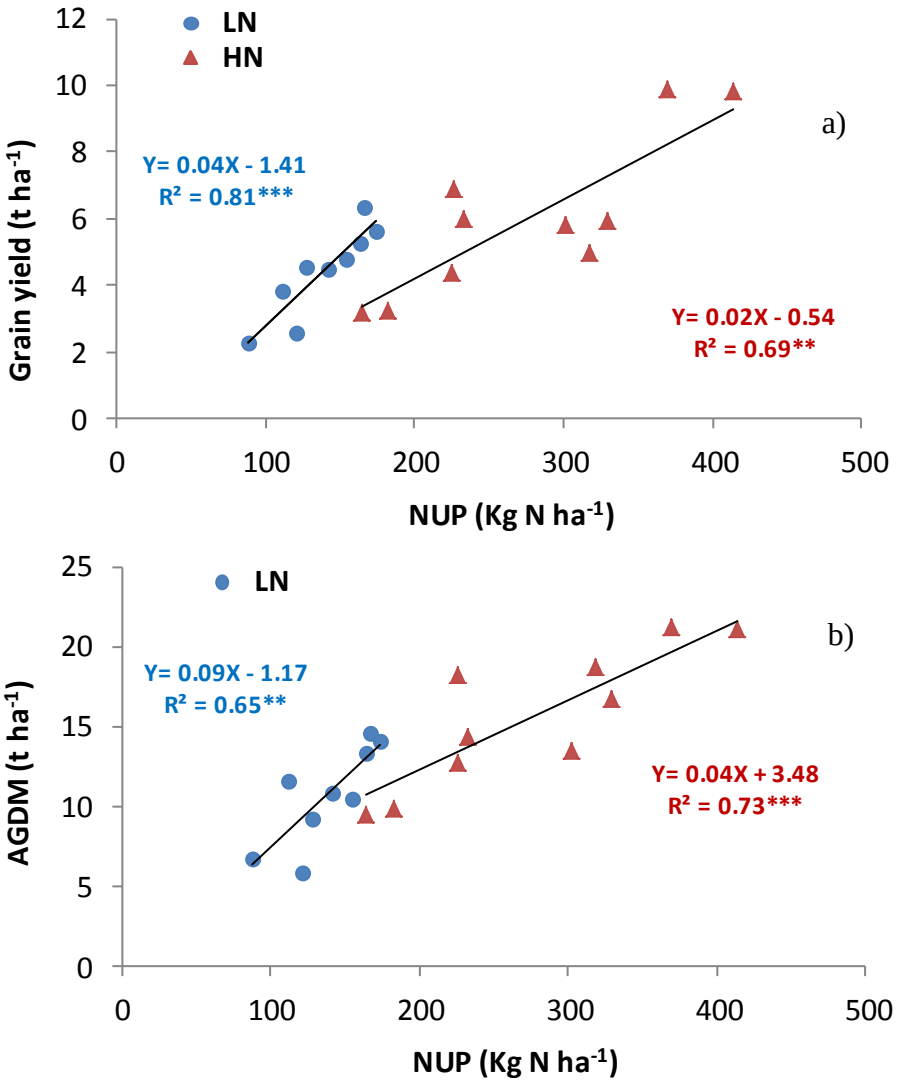


Figure 4.12: Linear relationship amongst 10 genotype under HN and LN between: a) grain yield and NUP and b) AGDM and NUP

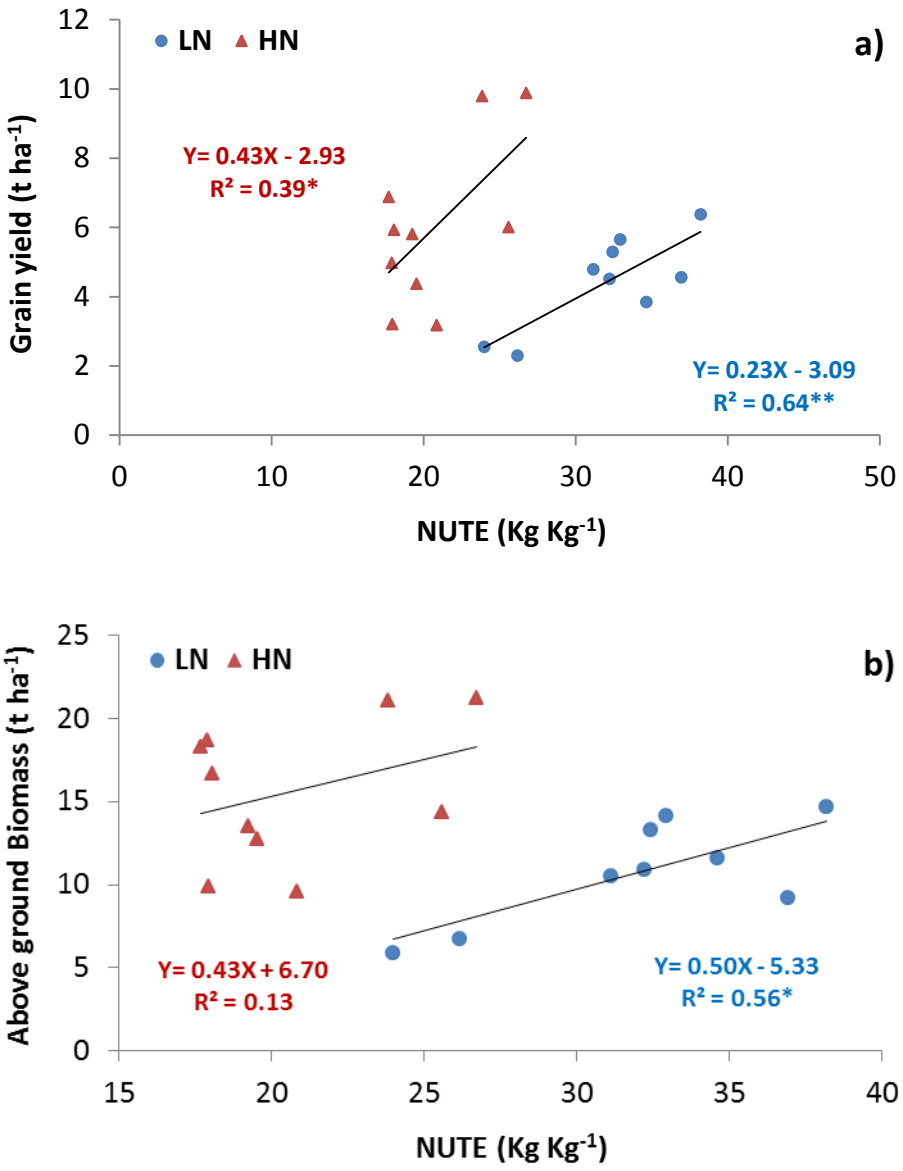


Figure 4.13: Linear relationship amongst 10 genotypes under HN and LN a) between NUTE and grain yield, and b) between NUTE and above-ground biomass (AGDM)

A positive linear association was found between NUTE and NUE (R^2=0.31, P=0.093 under HN and R^2=0.66, P<0.001 under LN) and between NUPE and NUE (R^2=0.75, P<0.001 under HN and R^2=0.80, P<0.001 under LN) (Figure 4.14).

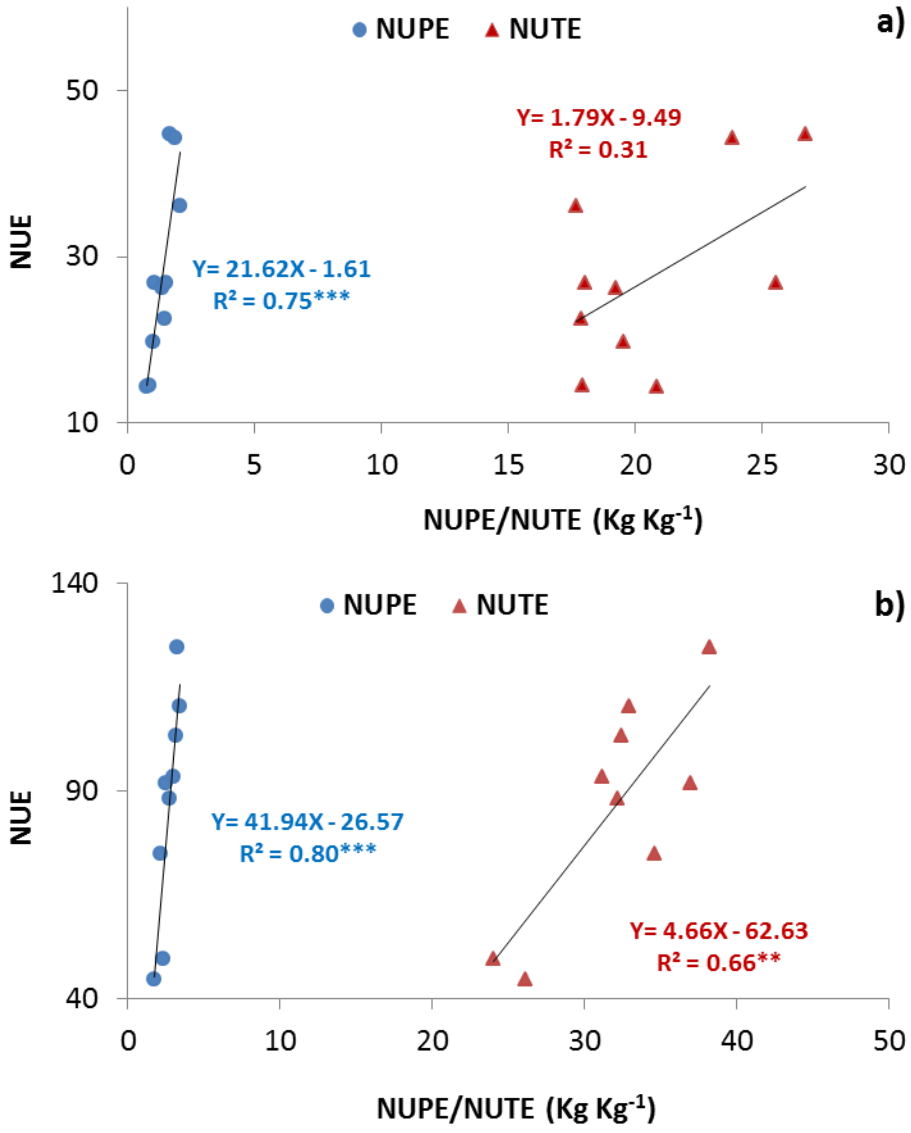


Figure 4.14: Linear relationship amongst 10 genotypes between NUPE and NUTE a) under HN, b) under LN conditions

b- Grain nitrogen concentration (GNC)

Both N treatment and genotype had an effect on grain N concentration (GNC) ($P=0.008$ and $P<0.001$ respectively). However, the N treatment x genotype interaction was not significant ($P=0.052$). GNC ranged from 2.44 (Savannah) to 3.37 (W352) under HN and from 1.95 (Paragon) to 2.75 (W729) under LN. A negative linear relationship was found between grain N content and grain yield ($R^2=0.41$, $P<0.05$ and $R^2=0.62$, $P<0.01$, respectively, for HN and LN) (Figure 4.15).

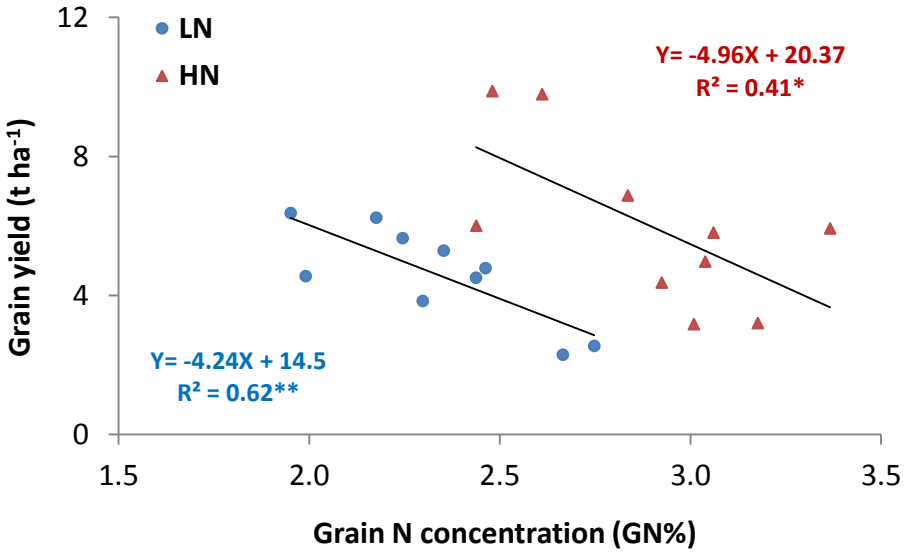


Figure 4.15: Linear relationship between grain yield (t ha^{-1} 100% DM) and grain N content (GNC) under HN and LN amongst 10 genotypes

Table 4.10: N-uptake (NUP, kg N ha^{-1}), N-utilisation Efficiency (NUTE, kg DM kg^{-1} N) and grain N concentration (%) amongst 10 genotypes under high (HN) and low (LN) N treatments.

Genotype	NUP (kg N t ha^{-1})		NUTE (Kg Kg^{-1})		GN (%)	
	HN	LN	HN	LN	HN	LN
Paragon	370	166	26.7	38.2	2.48	1.95
Rialto	414	-	23.8	-	2.61	2.18
Savannah	233	127	25.6	36.9	2.44	1.99
W034	302	154	19.2	31.1	3.06	2.46
W040	226	174	17.7	32.9	2.84	2.24
W141	318	163	17.9	32.4	3.04	2.35
W209	164	88	20.8	26.2	3.01	2.67
W223	226	142	19.5	32.2	2.92	2.44
W352	329	111	18.0	34.6	3.37	2.30
W729	183	121	17.9	24.0	3.18	2.75
Mean	**276**	**138**	**20.53**	**32.1**	**2.9**	**2.3**
SED (df)						
Genotype (36)	14.7***		1.93***		0.10***	
Nitrogen (2)	12.4*		0.52**		0.05**	
GXN (36)	23.3***		2.65NS		0.14*	

Probability significance: ***: P<0.001, **: P<0.01 and *: P<0.05

4.3.2.3 Photosynthesis rate and canopy size

a- Flag-leaf Photosynthesis (A_{max})

Overall, leaf A_{max} increased from 22.6 µmol m^{-2} s^{-1} under HN to 24.80 µmol m^{-2} s^{-1} under LN pre-anthesis (P<0.001) and from 20.95 µmol m^{-2} s^{-1} to 22.60 µmol m^{-2} s^{-1} post-anthesis (P=0.002).

Leaf photosynthesis rate differed between genotypes in the range 19.64 - 24.14 µmol m^{-2} s^{-1} under HN and 19.31 - 24.71 µmol m^{-2} s^{-1} under LN pre-anthesis (P=0.002) and in the range 16.81 - 23.62 µmol m^{-2} s^{-1} under HN and 19.31 - 24.71 µmol m^{-2} s^{-1} under LN post-anthesis (P<0.001). The N treatment x genotype interaction was not significant pre-anthesis (P=0.216). However, it was significant post-anthesis (P=0.003): Rialto, W729, W034, W209 and W352 had relatively higher A_{max} under LN conditions (+29%, +26%, +17%, +17% and +12% respectively), than W141 and Savannah (-9% and -6% respectively) compared to HN conditions.

Photosynthesis rate decreased between pre-anthesis and post-anthesis from 24.80 to 25.60 µmol m^{-2} s^{-1} under LN (P<0.001), and from 22.56 to 20.96 µmol m^{-2} s^{-1} under HN. (Figure 4.16).

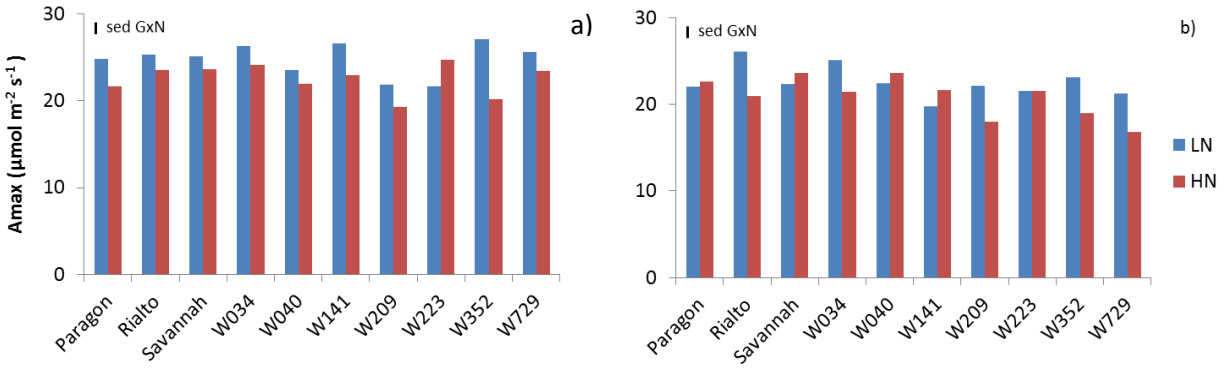


Figure 4.16: Leaf photosynthesis rate (A_{max}, µmol m^{-2} s^{-1}): a) pre-anthesis and b) post-anthesis.

There was a linear relationship between post-anthesis A_{max} and grain yield under HN (R^2=0.37, <0.05) than under (R^2=0.09, NS). Similarly, the linear regression of AGDM on post-anthesis A_{max} was significant (R^2=0.35, P<0.05) under HN, but not under LN conditions. Post-anthesis A_{max} had a trend of positively associated with NUP at maturity under HN (R^2=0.11, P<0.001) but not under LN (Figure 4.17).

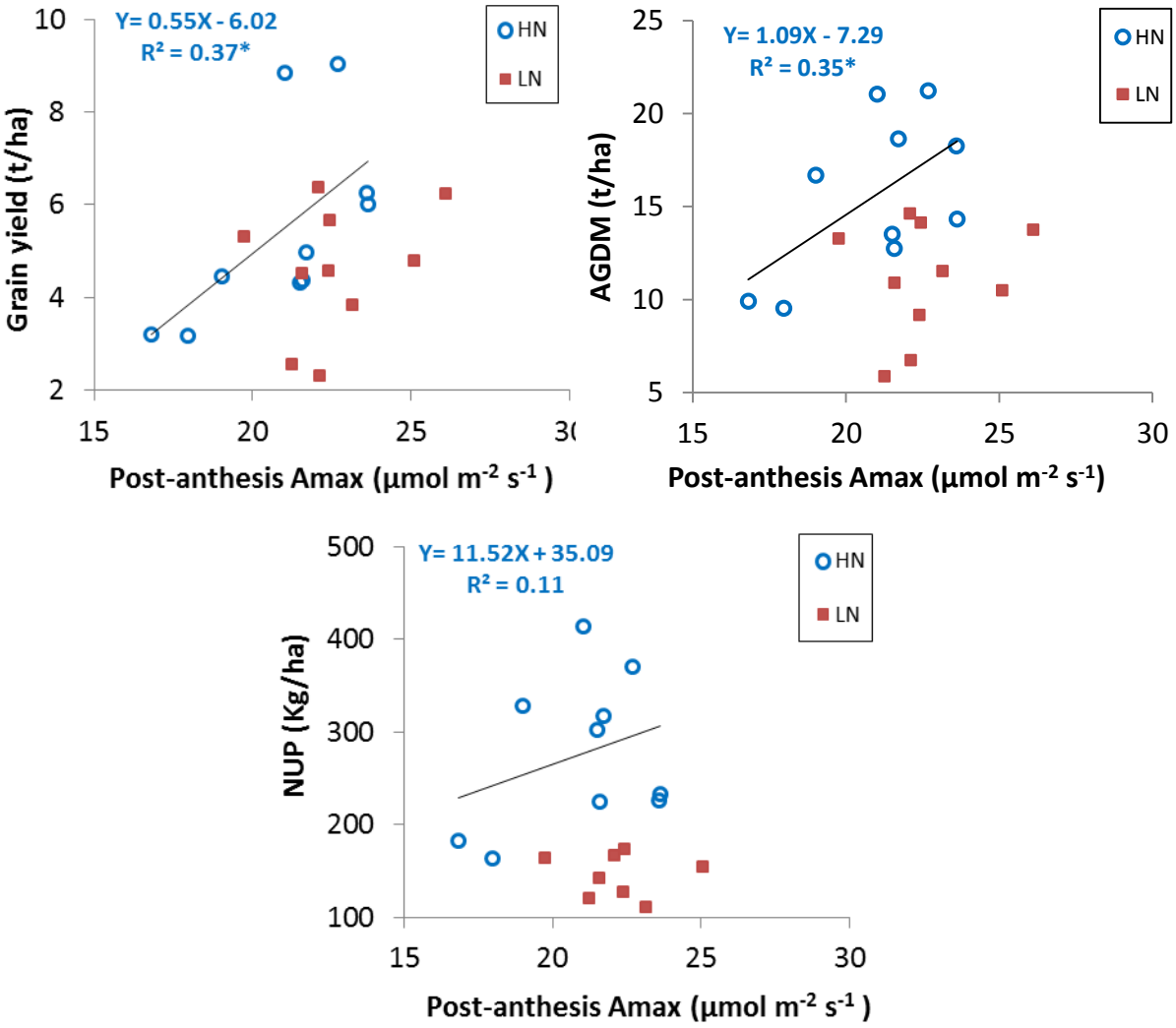


Figure 4.17: Linear regression of a) grain yield on post-anthesis flag-leaf A_{max} b) above-ground DM on post-anthesis flag-leaf A_{max} and c) N uptake on post-anthesis flag-leaf A_{max}.

b- Normalized difference vegetation Index (NDVI)

Five NDVI measurements were taken using the CropScan MSR16 radiometer. Differences between genotypes and between N treatments were observed for all measurements ($P<0.01$; Figure 18). The measurements were grouped into pre-anthesis NDVI (PrA_NDVI: average of two measurements on 16 and 22 May) and post-anthesis NDVI (PoA_NDVI: average of three measurements on 20 June, 12 and 23 July).

Under HN, there were two groups of genotypes having similar NDVI in all dates of measurements. The first group was formed by UK cultivars (Paragon, Rialto and Savannah) in addition to W040 and the second group by all other Watkins lines

(W034, W141, W209, W223, W352 and W729). For the first group, NDVI was higher pre-anthesis (0.90), and the decrease slower after anthesis (0.85) and finally this group decreased to about 0.70. The second group had also high NDVI pre-anthesis (0.90) but decreased more rapidly after anthesis to lower NDVI (0.50) at the last measurement (Figure 14.19).

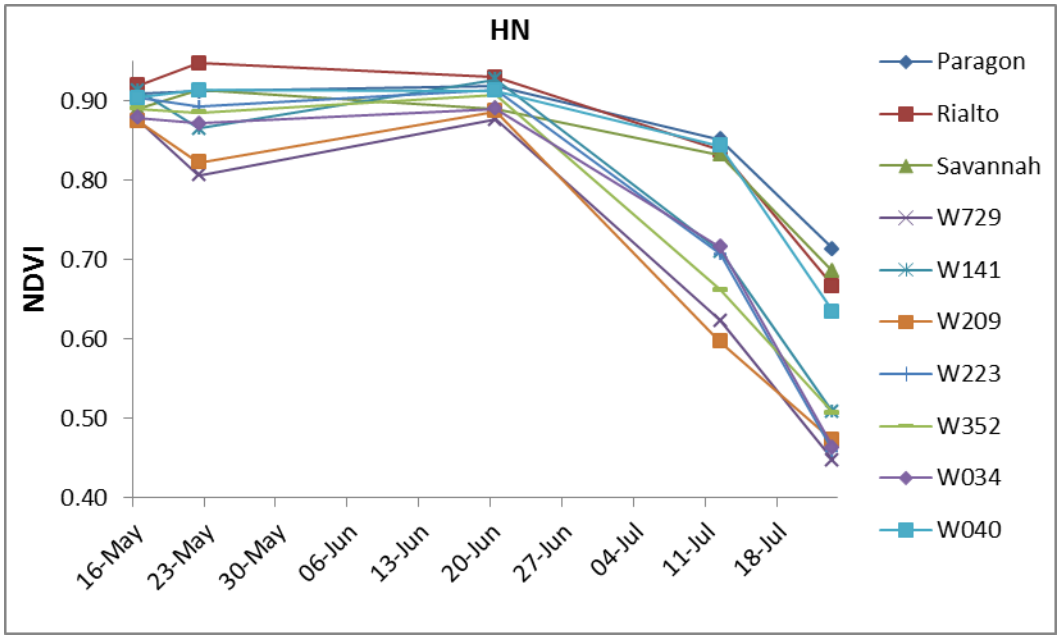


Figure 4.18: NDVI on four dates under HN and LN conditions

Under HN, overall NDVI decreased from 0.90 pre-anthesis to 0.65 post-anthesis ($P<0.001$). Pre-anthesis NDVI ranged from 0.88 (W729, W209 and W034), to 0.92 (W141 and Rialto). However, post-anthesis, NDVI ranged from 0.53 for W729 to 0.78 for Paragon. Paragon, W141 and W040 had generally higher NDVI than most other genotypes on all assessment dates, whereas Savannah had the lowest NDVI on the first assessment date (0.65) but did not decrease as quickly as other genotypes (Figure 4.19).

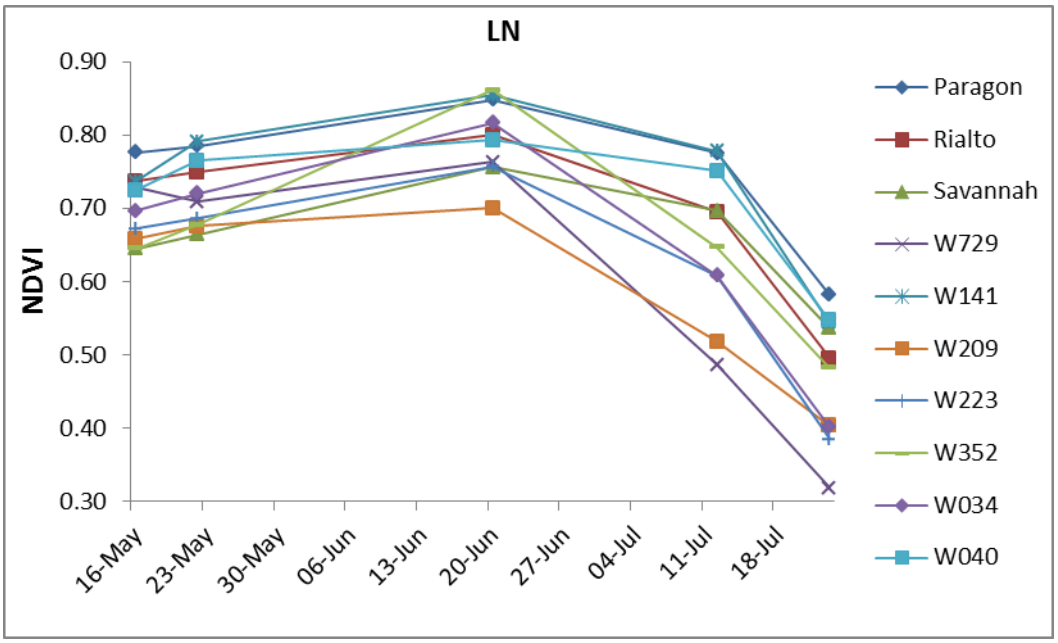


Figure 4.19: NDVI measurements in 4 different dates under HN and LN conditions

N treatment had an effect on PrA_NDVI (P=0.003) and PoA_NDVI (P=0.006). NDVI both pre- and post-anthesis was higher in HN for all genotypes; there was no significant genotype x N treatment interaction for either prA or PoA NDVI, P=0.143 and P=0.067, respectively).

Under LN, overall NDVI decreased from 0.70 pre-anthesis to 0.56 post-anthesis (P<0.001). The highest NDVI pre-anthesis was for Paragon (0.77) and the lowest for Rialto (0.63). Post-anthesis the highest NDVI was for Paragon (0.67) and the lowest for W729 (0.42).

Regression analysis showed that:

- Under HN, grain yield was more strongly associated with Pre-anthesis NDVI (R^2=0.71, P<0.01) than with post-anthesis (R^2=0.53, NS) (Figure 4.20 a).
- Under LN, the linear relationship between grain yield and NDVI was stronger post-anthesis (R^2=0.67, P<0.05) than pre-anthesis (R^2=0.51, P<0.05) (Figure 4.20 b).

- Under HN, the linear relationship between NDVI and AGDM was stronger pre-anthesis (R^2=0.68, P<0.01) than post-anthesis (R^2=0.27, NS) (Figure 4.20 c).
- Under LN, the relationship between NDVI and AGDM was similarly associated with pre-anthesis NDVI (R^2=0.59, P<0.05) and post-anthesis (R^2=0.53, P<0.05).

Table 4.11: Regression coefficient (R^2) between harvest AGDM and NDVI assessments on four dates in both HN and LN treatments.

NDVI assessments		16-May	22-May	11-Jul	23-Jul
AGDM	HN	0.68**	0.58**	0.25	0.42*
	LN	0.52*	0.64**	0.01	0.31

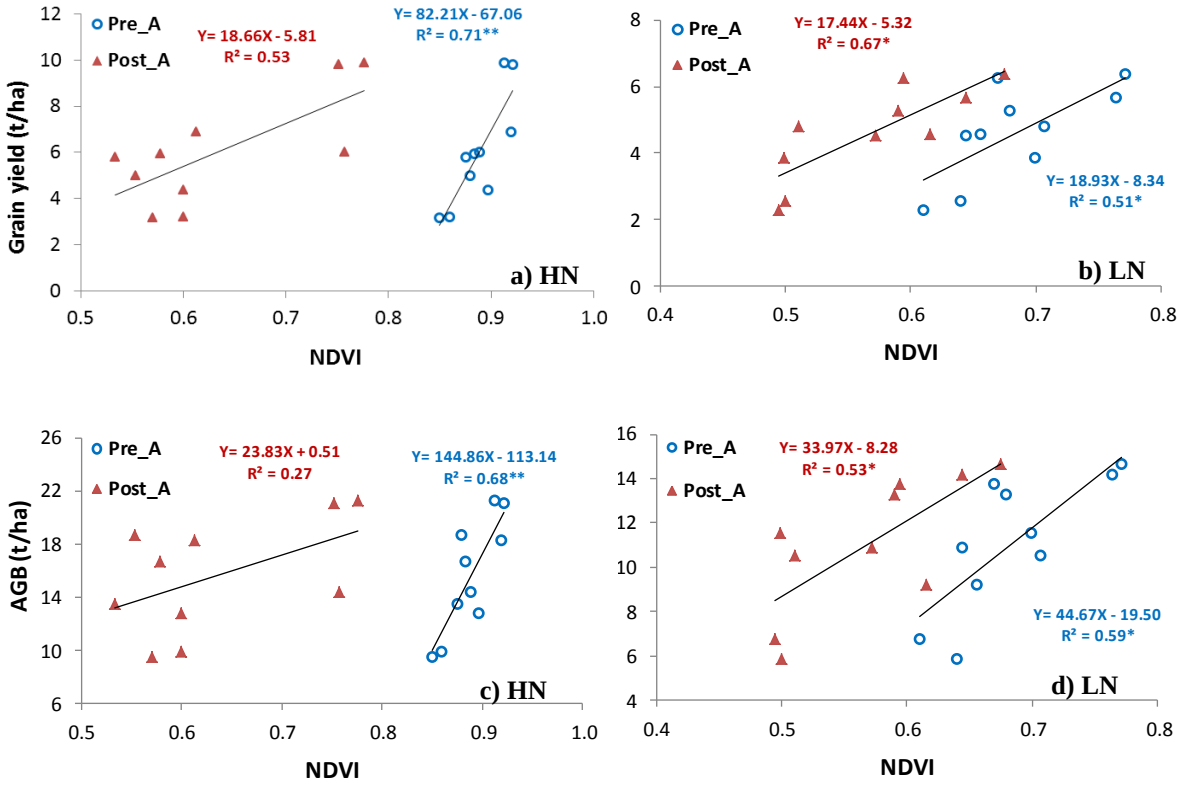


Figure 4.20: Relationship between NDVI and a) grain yield (100% DM) under HN b) grain yield under LN c) AGDM under HN d) AGDM under LN.

4.3.3 Relationship between results in glasshouse and field experiments

For the same set of 10 genotypes, linear regression analysis of glasshouse data and field data under HN conditions showed a positive association between (Figure 4.21):

- Grain yield in the field (t ha^{-1}) and in the glasshouse (g $plant^{-1}$) (R^2 = 0.45, P<0.05).
- AGDM in the field (t ha^{-1}) and in the glasshouse (g $plant^{-1}$) (R^2 = 0.71, P<0.01).
- NUP in the field (t ha^{-1}) and NUP in the glasshouse (g $plant^{-1}$) (R^2 = 0.58, P<0.01).
- Post-anthesis flag-leaf photosynthesis rate in the field and in the glasshouse (R^2 = 0.45, P<0.05).
- Grain N concentration in the field and in the glasshouse (R^2 = 0.82, P<0.001).

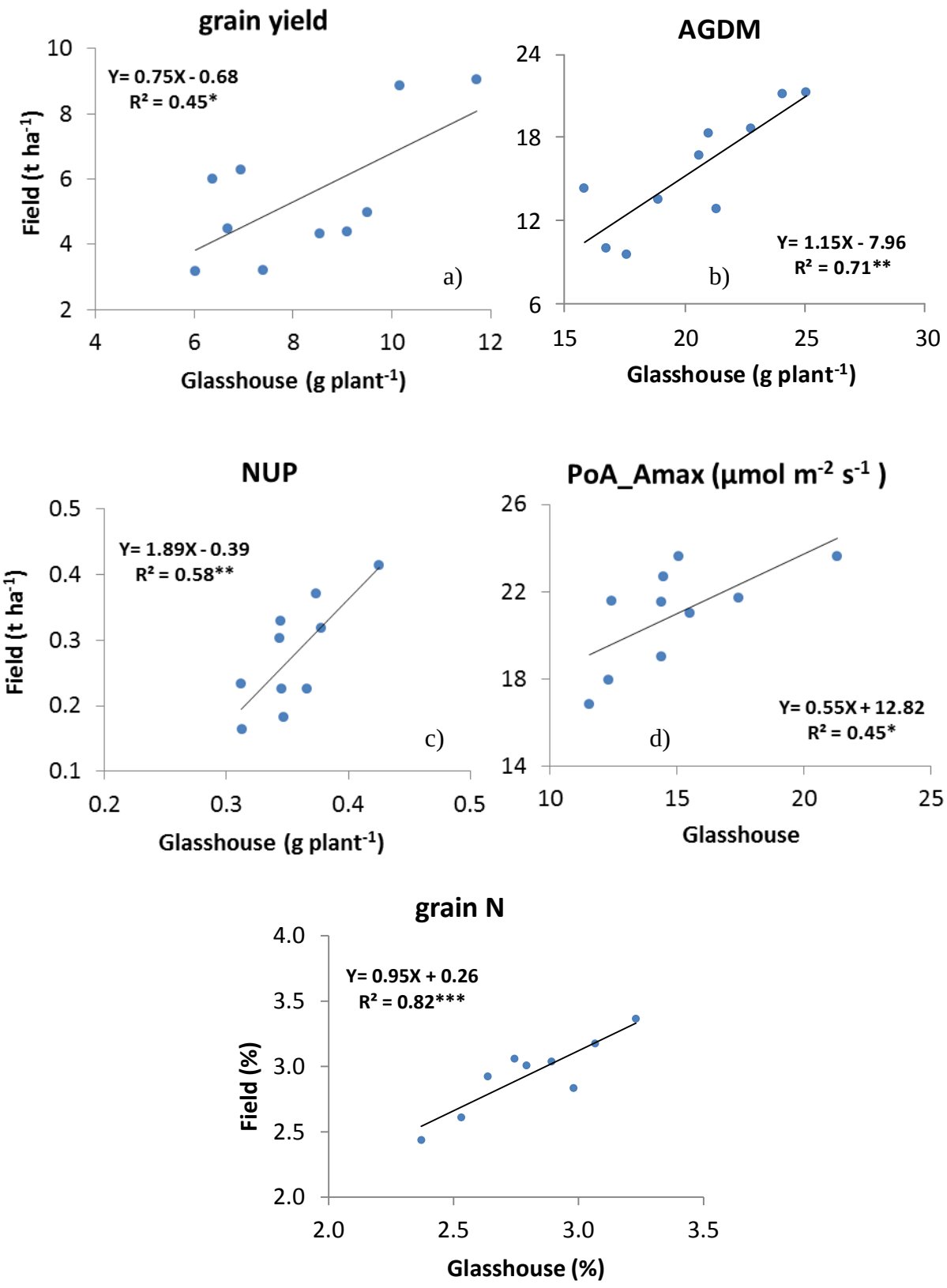


Figure 4.21: Linear relationship between glasshouse and field data for a) grain yield; b) AGDM; c) NUP; d) PoA_A_{max} and e) grain N concentration.

4.4 Discussion

The discussion will start with grain yield and biomass results. Secondly, it will consider N-use efficiency and components and finally it will finish with physiological measurements in both glasshouse and field trials. The discussion will focus on the comparison between UK cultivars and Watkins landraces, followed by a comparison between field and glasshouse results for the 10 genotypes sown in both environments.

4.4.1 Grain yield and biomass

For glasshouse experiments, all genotypes were pre-vernalized for 8 weeks and grown in the same conditions in both years. The year effect was not significant for grain yield, AGDM and HI.

Grain yield in the glasshouse was highest for UK cultivars Xi19 and Paragon. However, other UK cultivars: Oakley and Rialto had similar yield to W141, with the highest grain yield amongst Watkins lines. In the field, grain yield increased under HN. Under HN, Paragon and Rialto had the highest yield amongst genotypes with Savannah, W034 and W141 also showing high yields. Under LN Paragon, Rialto, W040, W141 had the highest and significantly similar yields.

The genetic gain in wheat grain yield in the last decades was mainly associated with improvement in HI (Foulkes, 2009). Grain yield improvement was also associated with improvement in biomass in some countries since ca. 1985 (Austin, 1982, Shearman et al. 1990; Foulkes et al. 2009). Consistent with theoretical predictions for C3 species (Zhu et al. 2010), increases in above-ground biomass of wheat have been reported in recent years (Shearman et al. 2005; Bustos et al. 2013 Sadras et al. 2013) and some physiological and genetic bases identified (Reynolds et al. 2009).

In the current study, grain yield in glasshouse and field experiment under HN conditions was more associated with AGDM. Breeders should exploit the positive association between yield and biomass for increased yield in wheat varieties. Under HN, the two Watkins lines, W040 and W141 had significantly AGDM to the three UK cultivars, while under LN all Watkins genotypes had similar AGDM to UK

cultivars except W209, and W729. However, five lines (W034, W040, W209, W223 and W729) maintained AGDM better under LN than all UK cultivars.

In the glasshouse, grain yield was stongly associated with grains per ear, however no association was found with ears plant^{-1} or with TGW. In the field experiment, the increase in grain yield was mainly associated with ears m^{-2} and grain m^{-2} rather than with TGW. Among the yield components, the grains m^{-2} was observed to be the best indicator of grain yield response to N application. However, yield components frequently mutually compensate and high yields are often attainable by diametrically opposite routes (Pask, 2009).

The grain "sink size" is determined by the grains m^{-2}, which is typically more closely associated with grain yield under optimal conditions than under abiotic stress (Satorre and Slafer, 1999; Shearman et al. 2005; Gaju et al. 2011) than grain weight (Fischer, 1985; Savin and Slafer, 1991). During development ears m^{-2} is determined first, then grains ear^{-1}, and finally grain weight. Grain number per m^2 in wheat is largely determined by the number of florets that survive to anthesis (Gonzalez et al. 2012). Wheat plants generally produce a large number of floret primordia but only a small proportion survive and complete their development to produce a fertile floret (Kirby, 1988; Sibony and Pinthus, 1988). Miralles et al. (2000) suggested that ear growth during the stem elongation phase, in which the spike and stem competing for assimilates, was associated with the number of fertile florets per spike. Thus the extending of the stem-elongation period could be a way to reduce assimilate competition and thereby increase the number of fertile florets and grain yield.

In the glasshouse experiments, W141 had the highest TGW followed by W355, W034 and Paragon. Rialto and Savannah had lower TGW than the majority of Watkins lines.

The yield response to the N availability of UK cultivars is different from Watkins lines. Indeed, the UK cultivars Paragon, Rialto, W209 and Savannah lost more yield under LN. In contrast, the grain yield did not change for W034, W141 and W223. This confirms previous findings that to attain their maximum yield, modern varieties in developed countries require more N inputs because they were selected under non-limiting fertization conditions (Brancourt-Hulmel et al. 2005; Bänziger et al. 1997

and Presterl et al. 2003; Foulkes, 2009; 1998). On the other hand, wheat landraces could have a deeper rooting system. Studies on landraces noted that root size of landraces is bigger than that of modern cultivars. The root system of which may be too small to uptake more water and nutrients from deeper layers resulting in reduced biomass and reduced grain yield (Waines, Ehdaie 2007; Grando and Checcarelli, 1995).

Regarding AGDM in the glasshouse, Paragon, Rialto, Xi19 and W141 produced more biomass per plant than all other genotypes, however; some Watkins lines produced similar AGDM to Xi19 and more than Oakley (W579 and W040). Miralles and Slafer, (1997) found that AGDM was positively associated with plant height in wheat, however, in the present experiment, no association was found between AGDM and plant height either in the glasshouse or in the field.

In the field averaging overall genotypes, AGDM was increased under HN. UK cultivars lost more biomass under LN than some Watkins lines. Biomass was associated with post-anthesis photosynthesis rate under HN conditions in the field. Extended duration of photosynthesis increases total photosynthesis, biomass and yield (Richards, 2000). Chlorophyll content, total photosynthesis, the onset of senescence and green leaf duration were positively associated with biomass and grain yield (Wand et al. 2008 and Kichey et al. 2007). A positive relationship was found between post-anthesis senescence duration (SEN_{TOT}) and grain yield in the glasshouse experiment (2013). Genetic variation for AGDM response to N was found, and, W729,Savannah, Rialto, W352 and Paragon lost significantly more biomass under LN than W040, W034 and W223. UK cultivars seemed to be more adapted to the high N environment, and landraces may have deeper root allowing keeping higher AGDM under LN conditions.

4.4.2 N use efficiency and components

Overall NUE and NUE components differed between genotypes in the glasshouse, and field experiment, which confirms that there is a genetic component of NUE that potentially allows for breeding.. High correlation was found between genetic variation in the field (under HN conditions) and glasshouse experiments for NUP.

Some Watkins landraces had high NUP in both glasshouse and field experiments (W040, W141 and W579), with similar NUP to Paragon, and better than Oakley.

In the field, there was high genetic variation in NUE response to increasing N supply. This variation was more associated with NUTE than with NUPE.. A negative relationship was also found between NUE response to N and grain N concentration response to N.

The genetic variation in NUTE was positively associated with NHI in the glasshouse and in the field experiment under HN, however, no association was found under LN in the field. The association between NUTE and grain N concentration was negative in the glasshouse, and in the field under HN and under LN . These results are consistent with previous findings (Ortiz-Monasterio et al. 1997; Brancourt-Hulmel et al. 2003; Foulkes et al. 1998). Increasing NUTE is associated with decrease in GNC, which is advantageous for feed, distilling and biofuel wheats (high ratio of starch to protein required); however, for bread wheat cultivars for which protein concentration is required, increasing NUTE should be associated with the increase in NHI. The minimum amount of grain protein concentration (GPC) reported in wheat is in the range 6.8-7.2% (Martre et al. 2006; Bogard et al. 2011; Kindred et al. 2006), equivalent (assuming conversion ratio of 5.7) to 1.2% of grain N. It is uncertain whether is is possible to decrease below this value since N is required for the cells structure and other metabolisms. This gives grain a minimum N concentration of approximately 1.5% (Sinclair and Amir, 1992), after which, the synthesis of grain storage proteins typically increases the grain N concentration to 2.1-2.3% (about 12-13% protein, typical of milling wheat).

Previous studies reported genotypic variation for NUTE in wheat and barley (Cox et al. 1986; May et al. 1991; Gaju et al. 2011; Barraclough et al. 2011). In addition, Slafer et al. (1996) and Ortiz-Monasterio et al. (1997) stated that NUTE could be the trait the most affected by breeding. Increased HI by breeding (Austin et al. 1980) has a substantial effect on the improvement of NUTE (Calderini et al. 1999). In fact, examination of the NUTE in this study showed that the effect of genotype on HI was the main cause of the variety effect on NUTE in the glasshouse and in the field.

4.4.3 Physiological traits

Significant genetic variation in leaf photosynthesis rate was identified both pre- and post-anthesis. In the field, all genotypes had higher A_{max} under LN both pre- and post-anthesis except for W223. In the field, significant variation in A_{max} exceeding UK cultivars was found pre-anthesis in W352 and W141 under LN. However, under HN, W223 and W034 had the highest values of A_{max} pre-anthesis. In the glasshouse, UK cultivars generally had higher pre-anthesis. However, post-anthesis W141 and W040 had higher A_{max} than all UK cultivars. Since A_{max} was partially associated with yield and biomass, it could be a useful breeding trait to improve wheat biomass and yield. Pre-anthesis A_{max} was also associated with grains per ear. This is in agreement with findings by Slafer and Andrade, (1993), which demonstrated that grain number per m^2 was related to floret survival determined just before anthesis.

Some recent evidence has suggested that an association between breeding progress for yield and leaf activity traits, in particular increased photosynthetic may exist for modern semi-dwarf wheats. Positive correlation between grain yield, photosynthetic rate, and stomatal conductance in irrigated short spring wheats was observed by Shimshi and Ephrat (1975). Fischer et al. (1998) reported higher stomatal conductance pre-anthesis with year of release and found a positive association between grains m^{-2} and grain yield and crop growth rate and RUE in the three week period before flowering in one year out of the three in CIMMYT experiment in Mexico. Greater light-saturated photosynthesis rate (A_{max}) was found in the more recent cultivars. Changes in the flag leaf with breeding progress at CIMMYT (e.g., smaller, more erect flag leaves, higher N and chlorophyll per unit area) were similar to those reported for winter wheat progress in the United Kingdom (Shearman et al. 2005).

However, Fischer et al. (1981) were unable to confirm fully this result in northwest Mexico, possibly because photosynthesis measurements were indirect and imprecise, relying upon carbon-14 uptake by the adaxial leaf surface. On the other hand, Ensminger et al. (2006) demonstrated that photosynthesis rate and biomass accumulation were tightly related. This was consistent with our finding since, genetic variation pre-anthesis photosynthesis rate was better associated with biomass than with yield.

Genetic variation was also observed for leaf chlorophyll content (SPAD), pre-anthesis, post-anthesis and at anthesis. A negative relationship was found between leaf SPAD and NRE. Previous investigations have reported that genetic variation in leaf senescence was associated with post-anthesis N remobilization in maize (Masclaux et al. 2001) and sorghum (Van Oosterom et al. 2010a and b).

A high correlation between post-anthesis photosynthesis rate (A_{max}) and chlorophyll fluorescence (QY) indicates scope for phenotyping for photosynthesis rate by chlorophyll fluorescence measurement, since photosynthesis measurement is laborious and time consuming. Also, since the most important traits (yield, AGDM, NUP and A_{max}) in the field and in the glasshouse (all per plant) were correlated, high-throughput phenotyping may be carried out in the glasshouse and extended to the field measurements, mainly the photosynthesis, since measurements are laborious in the field associated with heavy equipment and also with variables affected by weather conditions.

Chapter 5: Grain yield, yield components, above-ground biomass and NUE components in Paragon x W481 and Paragon x W141 SSD mapping populations

5.1 Introduction

Since the current rate of genetic gain of grain yield in wheat is about 0.5-1.0% per year (Miralles and Slafer, 2007; Foulkes al. 2010; Fischer and Edmeades, 2010) which is lower than the predicted rate of increase of demand for wheat of 1.2% year^{-1} (lower biofuel requirement estimate) or 1.3% year^{-1} (upper biofuel requirement estimate) from now until 2050 (Rosegrant and Cline, 2003; Singh and Trethowan, 2007; Foulkes et al. 2010; Hall and Richards, 2013), an acceleration in genetic yield potential improvement is required. This must be achieved whilst at the same time meeting the challenge of climate change which makes grain production more unpredictable.

Future gains in grain yield by increasing HI may be difficult since HI in some countries is already approaching its theoretical limit of *ca*. 0.64 (Mann, 1999, Evans and Fischer 1999, Foulkes, 2009). Therefore, the most promising option for yield potential increase in the future years will be increasing aboveground biomass. To improve biomass productivity genetically, wheat scientists have focused on a number of physiological traits in the last two decades, including increased leaf carbon fixation rate, leaf stay-green and spike fertility (Reynolds et al. 2012).

Modern wheat cultivars in developed countries require large amounts of N fertilisers to produce high yields and are selected under non-limiting N fertilisation conditions (Foulkes et al. 2009). Consumption rates of nitrogen in the world for cereals increased from 13.5 million tons in 1962 to ~ 120 million tons in 2012 (FAO 2012). Nevertheless, due to economical and ecological factors, agricultural practices have tended towards extensive systems with lower inputs of nitrogen (N) fertilisers. Therefore, wheat breeders must aim to release cultivars with a lower requirement for N fertiliser not only to decrease the pollution risk to ecosystems (Le Gouis et al. 2000), but also to lower production cost.

Since breeding is more focused and effective when limiting processes are known, the improvement of breeding programs through selection for physiological traits needs

better understanding of the traits limiting the performance of cultivars across different target environments (e.g. according to N availability). More effective approaches to wheat improvement require knowledge of the important physiological limits to yield potential.

New genetic resources should be tested for NUE to enhance the selection base of wheat germplasm and pre-breeding investigations on wider germplasm are required Crossing more adapted cultivars with landrace accessions is one avenue to increase allelic diversity for abiotic stress tolerance (Reynolds et al. 2006).

The objective of this chapter was to quantify genetic variation in grain yield, N-use efficiency and NUE components and associated traits at harvest in two SSD mapping populations under contrasting N supply. The specific aims were to:

1) Quantify novel variation in grain yield, above-ground biomass, NUE and NUE components among lines of two SSD mapping populations (Paragon x landrace W481 and Paragon x landrace W141) under low N and high N conditions and the response to N availability.

2) Increase the understanding of the physiological processes determining genetic variation in grain yield, biomass production, NUE, N-Uptake Efficiency, N-Utilization Efficiency and N partitioning within the crop at harvest.

3) Identify novel genetic variation in the two SSD populations for physiological determinants of crop growth at harvest linked to grain yield, above-ground biomass, NUE and NUE components.

4) Determine the contribution of each NUE component in the genetic variation in NUE in the two SSD populations under high N and low N conditions.

5) Determine the relationships amongst genotypes in the two SSD populations between anthesis date and plant height NUE, NUE components, grain yield and above-ground biomass.

5.2 Materials and methods

5.2.1 Plant material and experimental design

In 2011/12, one field experiment examining 79 SSD lines of a mapping population from the cross between the UK spring wheat cultivar Paragon and the AE Watkins landrace line W481 (P x W481) and the two parents was carried out. Two N treatments were applied, high N (HN = 200 kg N ha^{-1}) and low N (LN = 30 kg N ha^{-1}). In 2012/13, two field experiments, one examining 79 SSD lines of P x W481 and the two parents and another examining 97 SSD lines of a mapping population from the cross between Paragon and AE Watkins landrace line W141 (P x W141) and the tow parental lines, were conducted under two N treatments (HN =200 kg N ha^{-1} and LN = 0 kg N ha^{-1}). In each experiment, a split-plot design with three replicates was used with N treatments randomised on main plots and genotypes randomised on sub-plots. Sub-Plots (1 x 1.2 m) comprised 6 rows of 1 m length with 20 cm between rows.

5.2.2 Grain yield and biomass

At physiological maturity, a sample of above-ground material of one row of 1 m length in 2011/12 and 0.5 m row length in 2012/13 was harvested (avoiding the outer rows on either side of the plot) and analysed for above-ground biomass (for more details see chapter 3.1.6).

Grain yield, HI (Harvest Index) and above-biomass were measured on the sample at harvest and the remaining plot area was machine-harvested to obtain grain yield. The total grain yield per plot was calculated by adding the grain from the 1.0/0.5 m row length and machine harvested grain together and biomass was calculated by dividing total grain yield by HI (from the 1.0/0.5 m row length sample).

5.2.3 Crop N content, NUE and NUE components

To estimate N content of plant material (grains and straw) at harvest, all samples were scanned (milled samples for straw and whole grain without milling) using Near Infra-Red Spectroscopy (monochromator NIRS 6511 analyzer systems).

This technique needed to be calibrated by chemical analysis on a subset of samples using the combustion method (Dumas) for the straw and grain separately. NIRS

calibration was carried out for each mapping population and each year. The Dumas method using the Fisson NA-2000 elemental analyzer (Fisson, Ipswich, UK) was calibrated itself against Sulfonamide (N content = 16.24%).

N-use efficiency (NUE) was calculated using the equation 4.1:

$$NUE = NUPE \times NUTE: \qquad \text{Equ 4.1}$$

NUPE (N-uptake efficiency) = above-ground N uptake per unit N available from the soil and fertiliser.

NUTE (N-utilisation efficiency) = grain DM yield per unit above-ground N uptake at harvest.

Genotype, N treatment, year and interaction effects were analysed using GENSTAT version 15 software. Full details are given in the Material and Methods section (Chapter 3).

5.3 Results

5.3.1 Paragon x W481 mapping population

5.3.1.1 Anthesis date (AD) and plant height (PH)

a- 2011/12

There was a trend for increased N supply to slightly delay anthesis date (AD) ($P=0.055$), with SSD lines ranging from 17 June (L481-19) to 20 June (L481-22) under HN and from 16 June (L481-50) to 21 June (L481-69) under LN ($P<0.001$). There was no N treatment x genotype interaction ($P = 0.119$). There was no significant difference between Paragon (19 June under HN and 18 June under LN) and W481 (19 June under both HN and LN) ($P=0.768$). Transgressive segregation was observed; under HN five SSD lines flowered before either Paragon (19 June) or W481 (19 June) and under LN one line: L481-50 (16 June) flowered before Paragon (18 June) and 12 lines (16 - 17 June) were earlier than W481 (19 June). Under high N six lines (20 June) flowered later than W141 (19 June) however, no line flowered later than Paragon (19 June). Under LN, 29 lines had later flowering than Paragon (18 June) and one line: L481-69 (21 June) than W481 (19 June).

Plant height (PH) was increased from 98 cm under LN to 116 cm (+18%) under HN conditions (P=0.007). Amongst SSD lines plant height ranged from 93 to 133 cm under HN and from 72 to 120 cm under LN (P<0.001). The N treatment x genotype interaction was significant (P<0.001), with response to increase N supply ranging from -2 cm (L481-49) to +43 cm (L481-28). Plant height increasing under HN by 23% (from 81 to 100 cm) for Paragon and by 34% (from 100 to 134 cm) for W481. Under HN, no line was taller than W481 (133.5 cm), however, under LN seven lines, in the range 109.3 (L481-59) - 119.8 cm (L481-27) were taller than W481 (100.2 cm).

b- 2012/13

Increased N supply did not have any effect on anthesis date (P=0.212). All SSD lines ranged from 14 June (L481-28) to 24 June (L481-19) under HN and from 16 June (L481-30) to 24 June (L481-13) under LN (P<0.001). There was a N treatment x genotype interaction (P = 0.728) with response to increased N supply ranging from -5 days (L481-02) to +3 days (L481-61) days. There was no significant difference between Paragon (21 June under both HN and LN) and W481 (21 June under both HN and LN). Transgressive segregation was observed with, 26 SSD lines flowering before either Paragon (21 June) or W481 (21 June) under HN and 17 SSD lines flowering before either Paragon (21 June) or W481 (21 June) under LN.

There is a trend for increase plant height from 89 under LN to 99 cm under HN (P=0.055). However, SSD lines ranged in height from 86 (L481-09) to 112 (L481-19) cm under HN and from 72 (L481-28) - 106 (L481-19) cm under LN (P<0.001). The N treatment x genotype interaction was significant (P=0.008). Under HN, no line was taller than W481 (111.5 cm), however, all SSD lines were taller than Paragon (82.0 cm) except L481-09 (86.67 cm). Under LN two lines, L481-27 (105.8) and L481-19 (106.0 cm) were significantly taller than W481 (98.3 cm), while 54 SSD lines were taller than Paragon (80.3 cm) in the range of 86.2 cm (L481-71) to 106.0 cm (L481-19) (Table 5.1).

Table 5.1: Plant height (PH) days to anthesis (DA, days after sowing) for parental lines and mean, maximum and minimum of 79 lines of Paragon x W481 SSD population under HN and LN treatments and in 2011/12 and 2012/13 experiments

	PH (cm)				DA (days)			
	2012		2013		2012		2013	
	HN	LN	HN	LN	HN	LN	HN	LN
Paragon	100	81	82	80	236	234	245	245
W481	134	100	112	98	235	235	245	245
Min	93	72	87	72	233	233	238	240
Max	133	120	112	106	236	238	248	248
Mean	116	98	99	89	117	99	243	244
N-trt.	0.007		0.055		0.007		0.212	
Genot.	<0.001		<0.001		<0.001		<0.001	
N X G	<0.001		0.119		<0.001		0.728	

5.3.1.2 Grain yield and yield components

a- 2011/12

N treatment increased grain yield from 7.25 under LN to 8.63 t ha^{-1} under HN (P=0.06; Table 5.1). Amongst SSD lines, grain yield ranged from 5.24 (L481-14) to 11.02 (L481-64) t ha^{-1} under HN and from 5.29 (L481-74) to 9.81 t ha^{-1} (L481-76) under LN (P<0.01). The N treatment x genotype interaction was significant (P<0.001) with responses to reduced N ranging from -2.89 (L481-14) to -4.75 t ha^{-1} (L481-74).

Overall there was a significant difference in grain yield between the parental genotypes, Paragon (10.45 under HN and 8.08 t ha^{-1}under LN) and W481 (6.80 and 6.81 t ha^{-1}, respectively). Grain yield of Paragon was amongst the highest genotypes under HN; however, 10 SSD lines significantly exceeded its yield under LN. Frequency distributions for grain yield under HN and LN are shown in Figure 5.1. Grain yield under both N levels showed an approximately normal distribution, suggesting polygenic inheritance.

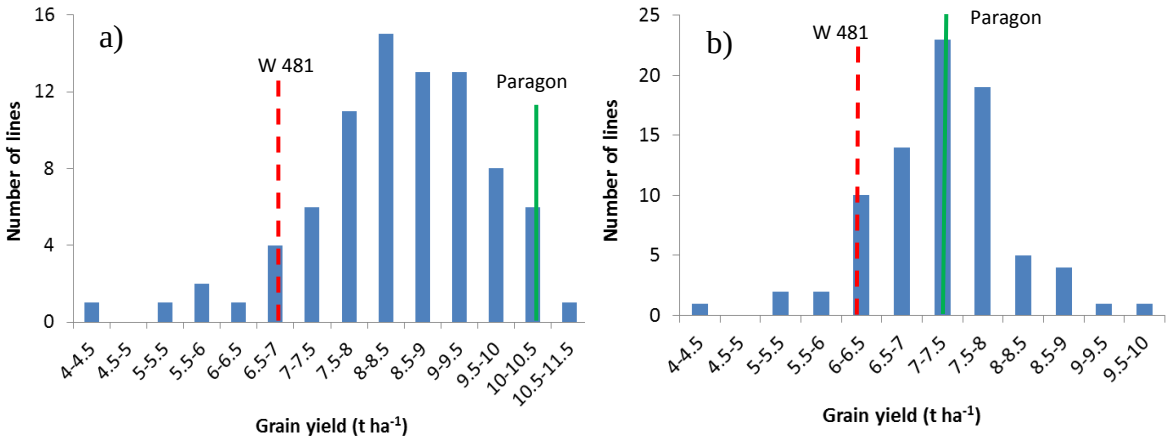


Figure 5.1: Frequency distribution of 79 Paragon x W481 SSD lines for grain yield (t ha^{-1}) in 2012 **a) under HN and b) under LN.**

Overall, ears m^{-2} increased from 389 under LN to 582 under HN conditions (P=0.032) (Table 5.2). Amongst SSD lines, ears m^{-2} ranged from 378 to 838 under HN and from 282 to 557 under LN (P<0.001). The N x genotype interaction was significant (P=0.025). There was no significant difference in ears m^{-2} between the two parental lines (625 under HN and 343 under LN for Paragon vs 653 under HN and 323 under LN for W481) (P=0.929), and there was no interaction N treatment x parental genotype (P=0.613).

There was a trend for increasing N supply to decrease TGW (P = 0.083) from 38.1 (LN) to 33.74 (HN) g (Table 5.2). Thousand grain weight of SSD lines ranged from 24.1 to 42.1 g under HN and from 30.0 to 44.3 g under LN (P<0.001). The N treatment x genotype interaction was significant (P<0.001) with increases under LN ranging from -2.9 g (L481-69) to +11.0 g (L481-57). Parental line W481 (38.1 g) had higher TGW than Paragon (35.4 g).

In 2012, grain number per ear (GE) increased from 44.7 under HN to 50.7 under LN (P<0.001). SSD lines ranged from 31.3 (L481-47) to 61.0 grains ear^{-1} (L481-29) under HN and from 33.7 (L481-33) to 75.2 grains ear^{-1} (L481-64) under LN (P<0.001). The N treatment x genotype interaction was significant (P=0.021), with response to decreasing N supply ranging from -14.9 (L481-14) to +34.1 grains ear^{-1} (L481-68). Under HN no SSD lines had more grains ear^{-1} than Paragon (55.7) but 68 SSD lines had significantly higher GE than W481 (26.3). Under LN, no SSD line

had higher GE than Paragon (66.3); however, three lines had significantly higher values than W481 (55.8). The grains ear^{-1} response to decreasing N supply was higher for six SSD lines than Paragon (+10.6), whilst no SSD line had significantly higher values than W481 (+29.4).

Increased N fertiliser overall increased grains m^{-2} in SSD lines by 32% (from 19,26 to 26,34) (P=0.027; Table 5.3). Differences were observed between genotypes (P<0.001) in the range 17,56 (L481-60) to 36,45 (L481-64) under HN and 13,26 (L481-74) to 28,10 (L481-64) under LN. The N treatment x genotype interaction was significant (P=0.023) with decreases under low N ranging from 599 (L481-14) to 14,748 (L481-74). Under HN no line had higher grains m^{-2} than Paragon (34.703), however under LN; three lines: L481-80 (26,480), L481-44 (27,731) L481-64 (27,102) had significantly higher grains m^{-2} than Paragon (22,813).

Regression analysis showed that grain yield was more strongly associated amongst SSD lines with grains m^{-2} under both HN an LN (R^2= 0.68, P<0.001 under both HN and LN) (Figure 5.2) than with TGW (P=0.088 and P=0.804, respectively).Grains m^{-2} was negatively associated amongst SSD lines with TGW under both HN (R^2= 0.26, P<0.001) and LN (R^2= 0.34, P<0.001) (Figure 5.3).

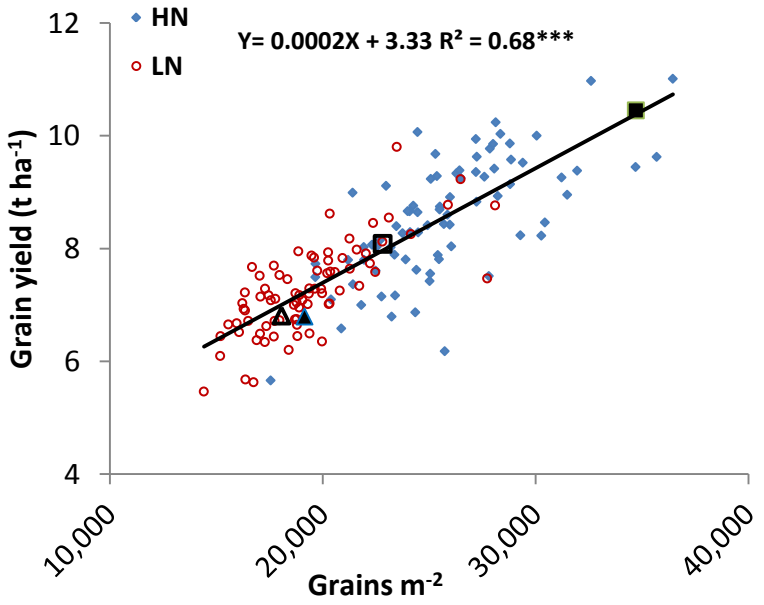


Figure 5.2: Linear regression of grain yield (100% DM) on grains m^{-2} amongst 79 Paragon x W481 mapping population lines in HN (◆) and LN (○).The parental lines are indicated by: ■ Paragon under HN, ▲ W481 under HN, □ Paragon under LN and △ W481 under LN.

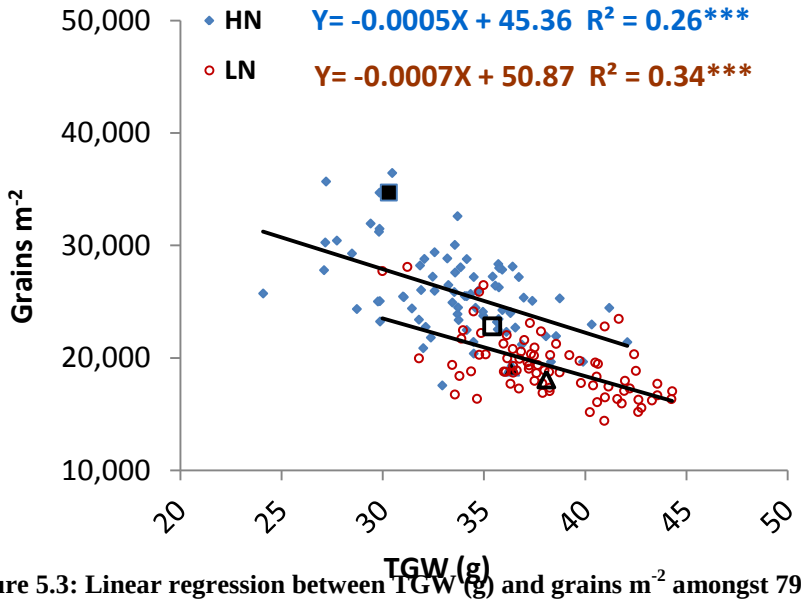


Figure 5.3: Linear regression between TGW (g) and grains m^{-2} amongst 79 mapping population lines in HN (◆) and LN (O).The parental lines are indicated by: ■ Paragon under HN, ▲ W481 under HN, □ Paragon under LN and △ W481 under LN.

Table 5.2: Grain yield and thousand grain weight (TGW) for parental lines and mean, maximum and minimum of 79 lines of Paragon x W481 SSD population under HN and LN treatments and in 2011/12 and 2012/13 experiments

	Grain Yield (t ha^{-1}) 100% DM				TGW (g)			
	2012		2013		2012		2013	
	HN	LN	HN	LN	HN	LN	HN	LN
Paragon	10.45	8.08	11.51	8.7	30.3	35.4	36.4	37.7
W481	6.8	6.81	9.23	8.02	36.3	38.1	37.9	39.4
Min	5.24	5.29	7.23	5.97	24.1	30.0	27.3	31.0
Max	11.02	9.81	11.81	10.68	42.1	44.3	51.4	45.9
Mean	8.63	7.25	9.74	8.29	33.7	38.1	37.0	38.0
N-trt.	0.006		0.017		0.083		0.267	
Genot.	<0.001		<0.001		<0.001		<0.001	
N X G	<0.001		<0.001		<0.001		<0.001	

Table 5.3: Ears per m^2, grains per m^2 and grain per ear for parental lines and mean, maximum and minimum of 79 lines of Paragon x W481 SSD population in 2011/12**.**

	Ears m^{-2}				Grains m^{-2}				Grains ear^{-1}			
	2012		2013		2012		2013		2012		2013	
	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN
Paragon	625	343	580	413	34,703	22,813	31,935	23,138	55.69	66.34	57.99	57.37
W481	653	323	607	427	19,145	18,065	24,327	20,534	26.34	55.75	40.96	48.02
Min	378	282	390	280	15,502	13,262	17,037	15,420	31.27	33.71	29.05	36.13
Max	838	557	820	640	36,453	28,102	38,984	29,390	60.95	75.19	71.91	65.41
Mean	582	389	554	447	26,341	19,263	26,670	21,959	44.71	50.73	49.43	50.26
N-trt.	0.032		0.003		0.027		0.042		<0.001		0.674	
Genot.	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
N X G	0.025		0.01		0.023		<0.001		0.021		0.024	

b- 2012/13

Grain yield overall decreased from 9.73 t ha^{-1} under HN to 8.29 t ha^{-1} under LN (-15%) (P< 0.05) (Table 5.2). Amongst SSD lines, grain yield varied from 7.23 (L481-73) to 11.81 t ha^{-1} (L481-36) under HN and from 5.97 (L481-96) to 10.68 t ha^{-1} (L481-82) under LN (P<0.001). The N treatment x genotype interaction was significant (P<0.001), with decrease under LN ranging from -2.32 (L481-37) to 4.91 (L481-39). Paragon (11.51 t ha^{-1} under HN and 8.70 t ha^{-1} under LN) tended to have higher yield than W481 (9.23 t ha^{-1} under HN and 8.02 t ha^{-1} under LN) (P=0.111). Under HN, no line had higher yield than Paragon (11.51 t ha^{-1}); however, under LN, two lines: L481-37 (10.66 t ha^{-1}) and L481-82 (10.68 t ha^{-1}) had significantly higher yield than Paragon (8.70 t ha^{-1}).

The frequency distribution of grain yield amongst the 79 lines (Figure 5.4) shows that yield was approximately normally distributed, suggesting polygenic inheritance.

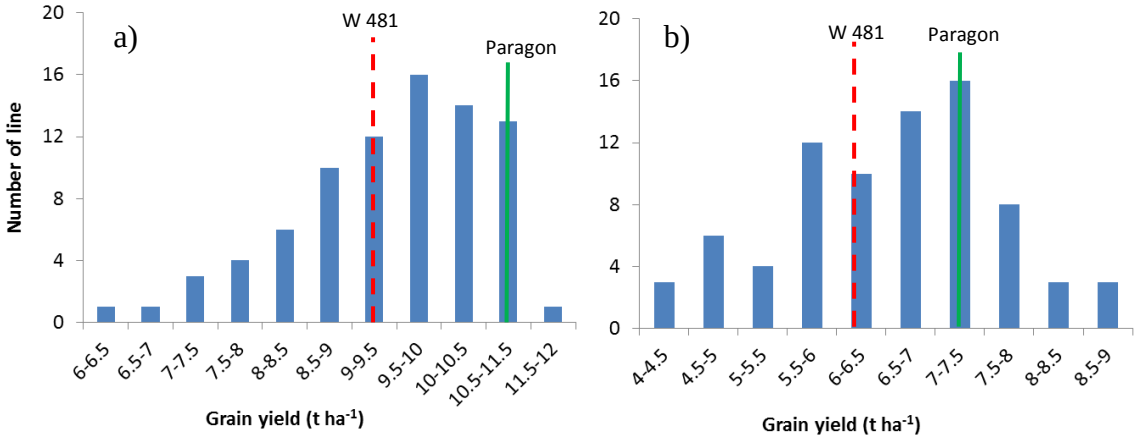


Figure 5.4: Frequency distribution of 79 Paragon x W481 lines for grain yield (t ha^{-1} at 100% DM) in 2013 **a) under HN and b) under LN.**

Averaging over all SSD lines, ears m^{-2} increased from 447 under LN to 554 under HN conditions (Table 5.2). Genotype ranged from 390 (L481-41) to 820 ears m^{-2} (L481-69) under HN and from 280 (L481-58) to 640 ears m^{-2} (L481-67) under LN. The N treatment x genotype interaction was significant (P=0.010), with decreases under LN supply ranging from +55 (L481-67) to -290 (L481-69). For parental lines no significant effect of the genotype or N treatment x genotype interaction was observed (P=0.130, and P=0.910 respectively). Under HN three lines had

significantly higher ears m^{-2} than two parental lines (580 and 607 ear m^{-2} for Paragon and W481, respectively). However, under LN six lines had significantly higher values than both Paragon (413) and W481 (427) in the range 530 - 640 ear m^{-2}.

N treatment did not affect TGW (P=0.399) (Table 5.3). SSD lines ranged from 27.3 (L481-05) to 51.4 g (L481-77) under HN and from 31.0 (L481-64) to 45.9 g (L481-57) under LN (P< 0.01). A N treatment x genotype was found (P<0.001), with responses to increasing N supply ranging from -8.5 (L481-05) to +6.0 g (L481-14). Under HN, ten lines had significantly higher TGW than the higher parent W481 (37.8 g) in the range 41.3 (L481-08) - 51.4 g (L481-77). Under LN only seven lines had higher TGW than the higher parent W481 (39.4 g), in the range 43.01 (L481-17) – 45.9 g (L481-57).

In 2013, grains ear^{-1} was not affected by N treatment (P=0.671). Grains ear^{-1} ranged amongst SSD lines from 29.1 (L481-35) to 71.9 grains (L481-67) under HN and from 36.1 (L481-70) to 65.4 grains (L481-58) under LN (P<0.001). Response to increasing N supply ranged amongst SSD lines from -28.5 (L481-58) to 32.1 (L481-67) (P = 0.024). Under HN one SSD line, L481-67 (71.9), had higher grains per ear than the higher parent Paragon (58.0); however, under LN, no line had higher values than the higher parent Paragon (57.4).

Grain m^{-2} increased from 21,959 under LN to 26,670 under HN (P=0.042) (Table 5.3). SSD lines overall varied from 17,037 to 38,984 under HN and from 15,420 to 29,390 under LN (P<0.001). The interaction was also significant (P<0.001), with responses to increasing N supply ranging from -3,772 (L481-19) to +15 491 (L481-39).

As in 2012, regression analysis showed that variation in grain yield amongst DH lines was more strongly associated with grains m^{-2} (R^2= 0.43, P<0.001) under both HN (R^2= 0.70, P<0.001) and LN than with grain weight (TGW) (Figure 5.5).

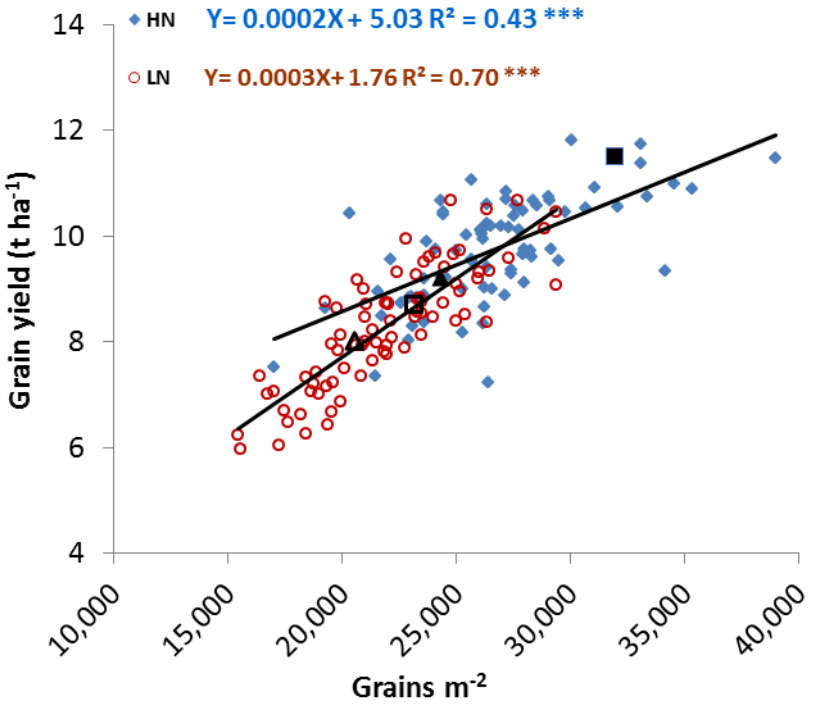


Figure 5.5: Linear regression of grain yield (100% DM) on grains m^{-2} amongst 79 SSD lines of Paragon x W481 population for HN (◆) and LN (○).The parental lines are indicated by: ■ Paragon under HN, ▲ W481 under HN, □ Paragon under LN and △ W481 under LN.

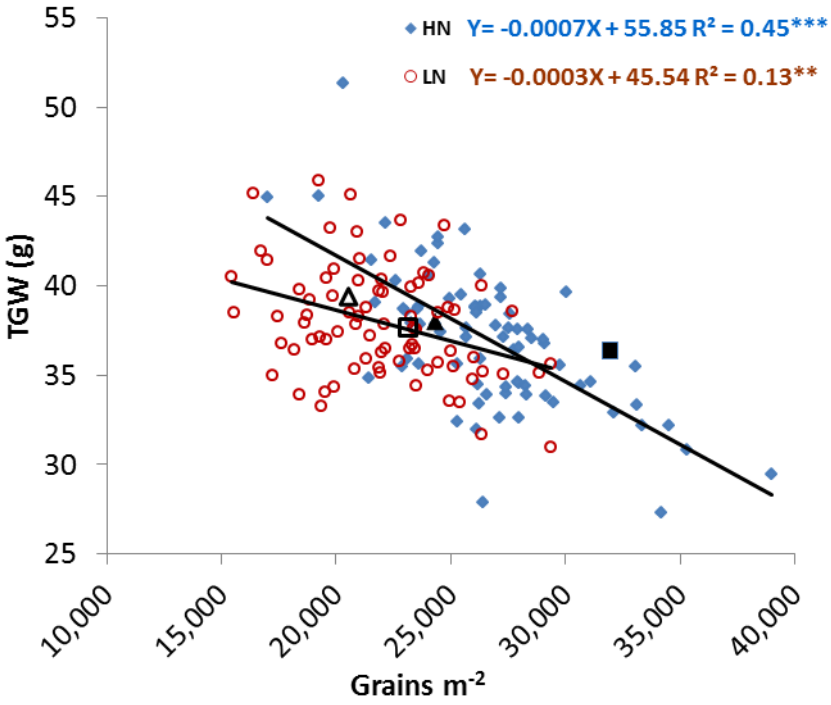


Figure 5.6: Linear regression of TGW (g) on grain number par m^{-2} amongst 79 SSD lines of Paragon x W481 for HN (◆) and LN (○).The parental lines are indicated by: ■ Paragon under HN, ▲ W481 under HN, □ Paragon under LN and △ W481 under LN.

Cross-year ANOVA showed that grain yield was lower in 2012 (8.63 t ha^{-1}) than in 2013 (9.74 t ha^{-1}) under HN conditions (-15%), and also lower in 2012 (7.25 t ha^{-1}) than in 2013 (8.29 t ha^{-1}) under LN conditions (-15%) ($P<0.001$). The year x N treatment interaction was not significant ($P=0.296$), however the year x N treatment x genotype interaction was significant ($P=0.011$).

From the cross year ANOVA, the N treatment x genotype interaction was significant for grains m^{-2} ($P=0.006$), ears m^{-2} ($P< 0.001$) and TGW ($P = 0.002$); and the year x N treatment x genotype interaction for grains ear^{-1} (P =m0.002) and TGW ($P = 0.001$).

5.3.1.3 Above-ground dry matter and HI

a- 2011/12

Averaging over SSD lines, AGDM increased from 17.38 t ha^{-1} under LN to 25.29 t ha^{-1} under HN (+42%) ($P=0.004$) (Table 5.4). Biomass ranged amongst SSD lines from 15.89 to 20.36 t ha^{-1} under HN and from 11.98 t ha^{-1} to 22.93 t ha^{-1} under LN ($P<0.001$; Table 5.4). The responses of SSD lines to increasing N supply ranged from -2.23 t ha^{-1} (L481-14) to +15.08 t ha^{-1} (L481-74) ($P = 0.024$). Under HN amongst all genotypes, Paragon (30.85 t ha^{-1}) had highest AGDM, whereas under LN two lines: L481-76 (22.19 t ha^{-1}) and L481-48 (22.93 t ha^{-1}) lines had significantly higher AGDM than the higher parent Paragon (18.0 t ha^{-1}).

The frequency distribution of biomass amongst SSD lines followed an approximately normal distribution (Figure 5.7).

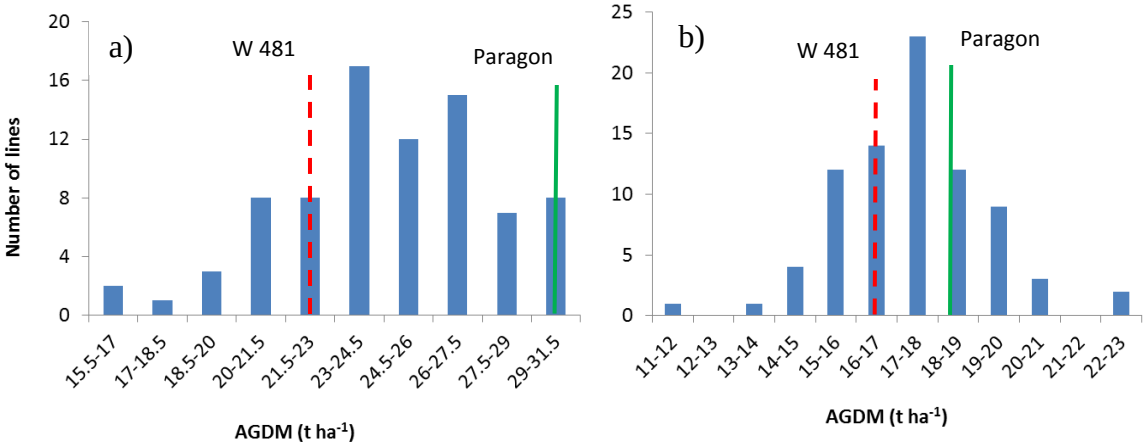


Figure 5.7: Frequency distribution of 79 Paragon x W481 SSD lines for biomass (t ha^{-1}) in 2012 **a) under HN and b) under LN.**

Grain yield was highly associated with AGDM amongst the genotypes in both HN (R^2=072, P<0.001) and LN (R^2=084, P<0.001) conditions (Figure 5.8).

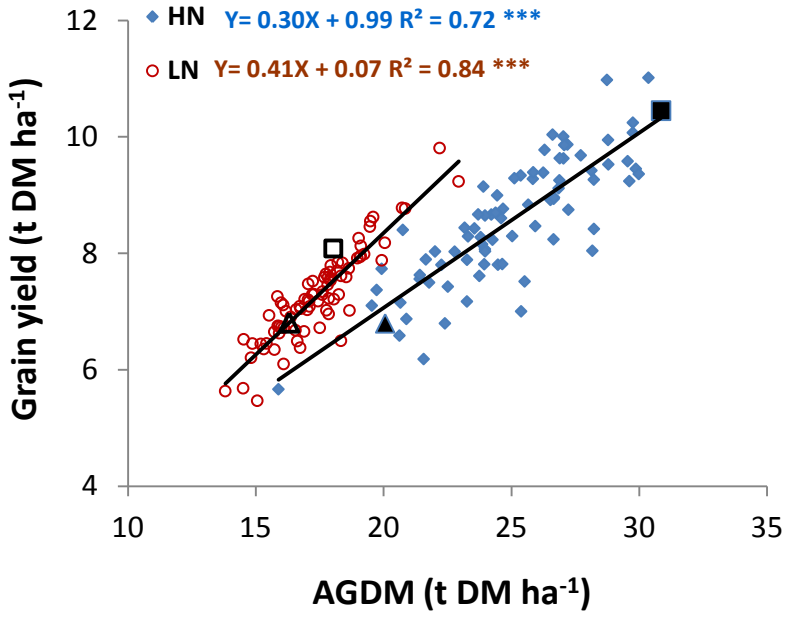


Figure 5.8: Linear regression of grain yield (100% DM) on above-ground dry matter (AGDM t ha^{-1}) amongst 79 SSD lines of Paragon x W481 population for HN (◆) and LN (○).The parental lines are indicated by: ■ Paragon under HN, ▲ W481 under HN, □ Paragon under LN and △ W481 under LN.

Averaging over SSD lines, HI increased from 0.34 under HN to 0.42 under LN (+22%) (P<0.01) (Table 5.4). Amongst SSD lines, HI ranged from 0.24 to 0.42 under HN and from 0.30 to 0.44 under LN conditions (P<0.001). The N treatment x genotype interaction was significant (P=0.007), with responses to increasing N supply ranging from -0.14 (L481-73) to -0.02 (L481-69). Under HN, four lines had significantly higher HI than either Paragon (0.31) or W481 (0.30) in the range 0.38

(L481-28) to 0.41 (L481-49). However under LN no line had higher HI than the higher parent Paragon (0.45).

Table 5.4: Above-ground dry matter (AGDM) and harvest index (HI) for parental lines and mean, maximum and minimum of 79 lines of Paragon x W481 SSD population under HN and LN treatments and in 2011/12 and 2012/13 experiments

	AGDM (t ha^{-1})				HI			
	2012		2013		2012		2013	
	HN	LN	HN	LN	HN	LN	HN	LN
Paragon	30.85	18.03	21.99	17.45	0.35	0.45	0.52	0.50
W481	22.40	16.30	21.30	18.69	0.30	0.42	0.43	0.43
Min	15.89	11.98	15.35	13.08	0.28	0.38	0.41	0.43
Max	30.36	22.93	25.05	23.54	0.41	0.46	0.51	0.49
Mean	25.29	17.38	20.91	18.03	0.34	0.42	0.47	0.46
N-trt.	0.004		0.027		0.007		0.140	
Genot.	<0.001		<0.001		<0.001		<0.001	
N X G	0.041		<0.001		0.007		0.008	

b- 2012/13

Averaging over SSD lines AGDM increased from 18.03 t ha^{-1} under LN to 20.91 t ha^{-1} under HN ($P< 0.05$) (Table 5.4). SSD lines ranged from 15.35 to 25.05 t ha^{-1} under HN and from 13.08 t ha^{-1} to 23.54 t ha^{-1} under LN ($P<0.001$). Responses of SSD lines to increasing N supply ranged from -8.22 (L481-48) to +12.41 t ha^{-1} (L481-67) ($P< 0.001$). Two lines: L481-81 (24.88 t ha^{-1}) and L481-39 (25.05 t ha^{-1}) had significantly higher AGDM than Paragon (21.99 t ha^{-1}) or W481 (21.30) under HN, whereas under LN, four lines had significantly higher values than W481 (18.69 t ha^{-1}) or Paragon (17.45 t ha^{-1}), in the range 21.82 (L481-82) - 23.54 t ha^{-1} (L481-37).

N treatment did not have any significant effect on HI ($P=0.267$). SSD lines ranged from 0.41 to 0.51 under HN and from 0.43 to 0.49 under LN ($P<0.001$) (Table 5.4). The N treatment x genotype interaction was significant ($P<0.001$), with responses to increasing N supply ranging from -0.06 (L481-24) to +0.04 (L481-35). Under HN and LN Paragon (0.52 and 0.50, respectively) had higher HI than all other genotypes.

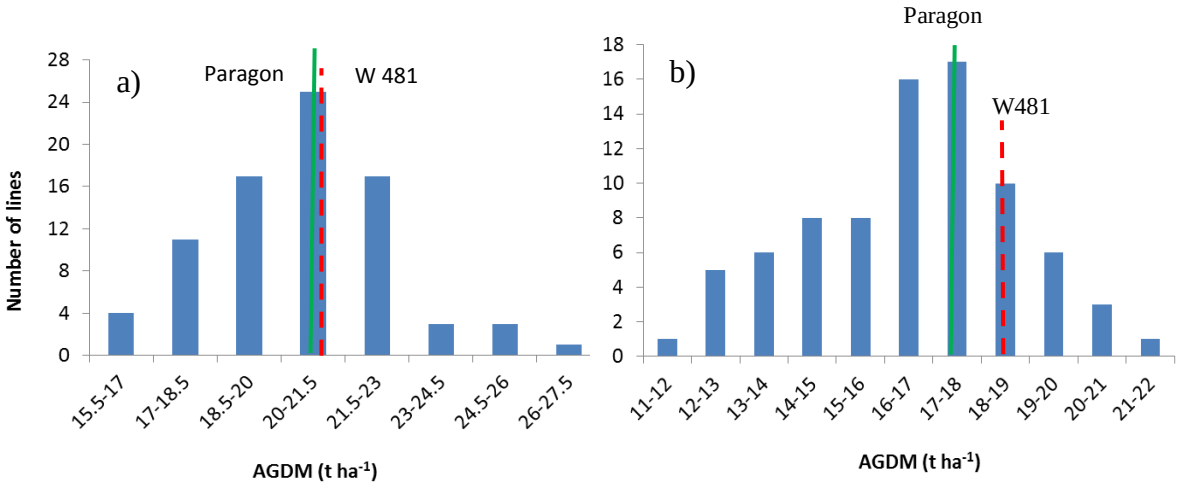


Figure 5.9: Frequency distribution amongst 79 Paragon x W481 SSD lines for grain above-ground dry matter (AGDM t ha^{-1}) in 2013 **a) under HN and b) under LN conditions.**

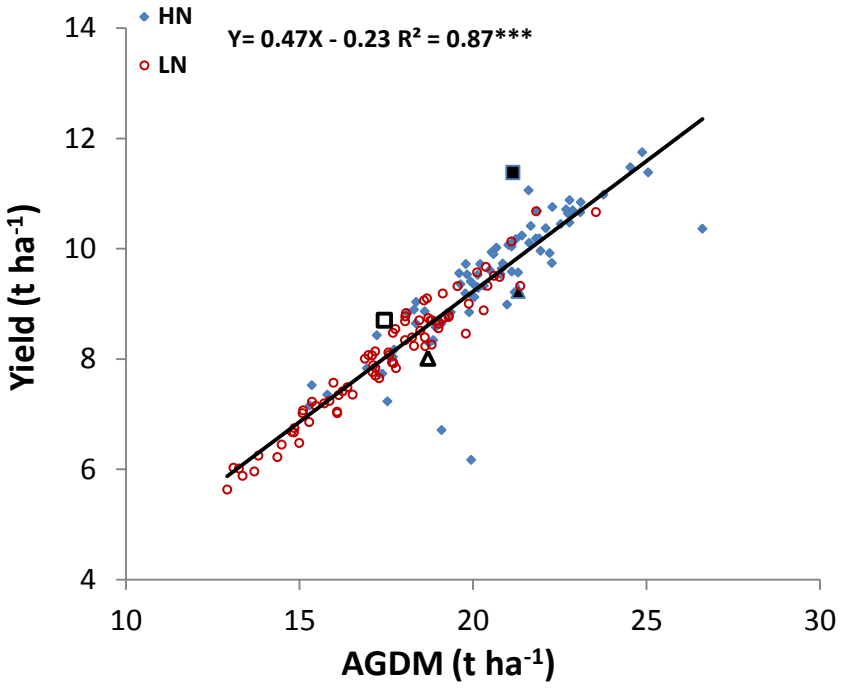


Figure 5.10: Linear regression of grain yield (100% DM) on on above-ground dry matter (AGDM t ha^{-1}) amongst 79 SSD lines of Paragon x W481 population for HN (◆) and LN (○).The parental lines are indicated by: ■ Paragon under HN, ▲ W481 under HN, □ Paragon under LN and △ W481 under LN.

Cross-year ANOVA showed that for AGDM the N treatment x genotype and year x N treatment x genotype interactions were significant (P = 0.005 and P = 0.027, respectively). For HI there was a N treatment x genotype interaction (P = 0.02) and year x N treatment x genotype interaction (P = 0.004).

5.3.1.4 N-use efficiency (NUE) and NUE components

a- 2011/12

N-use efficiency increased from 39.2 kg kg^{-1} under HN to 140.5 kg kg^{-1} under LN (P=0.002, Table 5.5). Within SSD lines NUE varied from 25.2 kg kg^{-1} (L481-60) to 49.8 kg kg^{-1} (L481-64) under HN and from 110.0 kg kg^{-1} (L18468) to 191.5 kg kg^{-1} (L481-76) under LN (P<0.001). The N treatment x genotype interaction was significant (P=0.003), with responses to increasing N supply amongst SSD lines ranging from -150.7 kg kg^{-1} (L481-01) to -67.5 kg kg^{-1} (L481-68). Under HN no line had higher NUE than the higher parent Paragon (47.2 kg kg^{-1}), whereas under LN, three lines: L481-48 (180.3 kg kg^{-1}), L481-01 (184.9 kg kg^{-1}) and L481-76 (191.5 kg kg^{-1}) had higher NUE than the higher parent Paragon (157.8 kg kg^{-1}).

NUP overall increased from 310 kg N ha^{-1} under LN to 503 kg N ha^{-1} under HN (P=0.005, Table 5.5). SSD lines ranged in NUP from 254 to 503 kg ha^{-1} under HN and from 174 kg ha^{-1} to 310 kg ha^{-1} under LN (P<0.001). A N treatment x genotype interaction was also found (P<0.001), with responses to increasing N supply ranging amongst genotypes from 41.8 kg ha^{-1} (L481-60) to 269.1 kg ha^{-1} (Paragon). Under HN Paragon (497.7 kg N ha^{-1}) had higher NUP than all genotypes; however, under LN, two lines: L481-01 (304.4 kg N ha^{-1}) and L481-76 (310.5 kg N ha^{-1}) had higher NUP than the higher parent Paragon (253.4 kg ha^{-1}). Finally, amongst all genotypes, Paragon (269.1 kg N ha^{-1}) had the highest NUP response to increasing N supply.

N-uptake efficiency (NUPE) overall increased from 1.84 kg kg^{-1} under HN to 4.53 kg DM kg^{-1} N under LN (P=0.009, Table 5.6). SSD lines ranged from 1.15 (48160) to 2.28 (L481-64) kg kg^{-1} under HN and 3.39 (L481-68) to 6.06 kg kg^{-1}(L481-76) under LN (P<0.001); however, the N treatment x genotype interaction was not significant (P=0.102). Amongst SSD lines under HN, no line had higher NUPE than the higher parent Paragon (2.36 kg kg^{-1}); however, under LN two lines, L481-01

(5.94 kg kg^{-1}) and L481-76 (6.06 kg kg^{-1}), had higher NUPE than the higher parent Paragon (4.95 kg kg^{-1}).

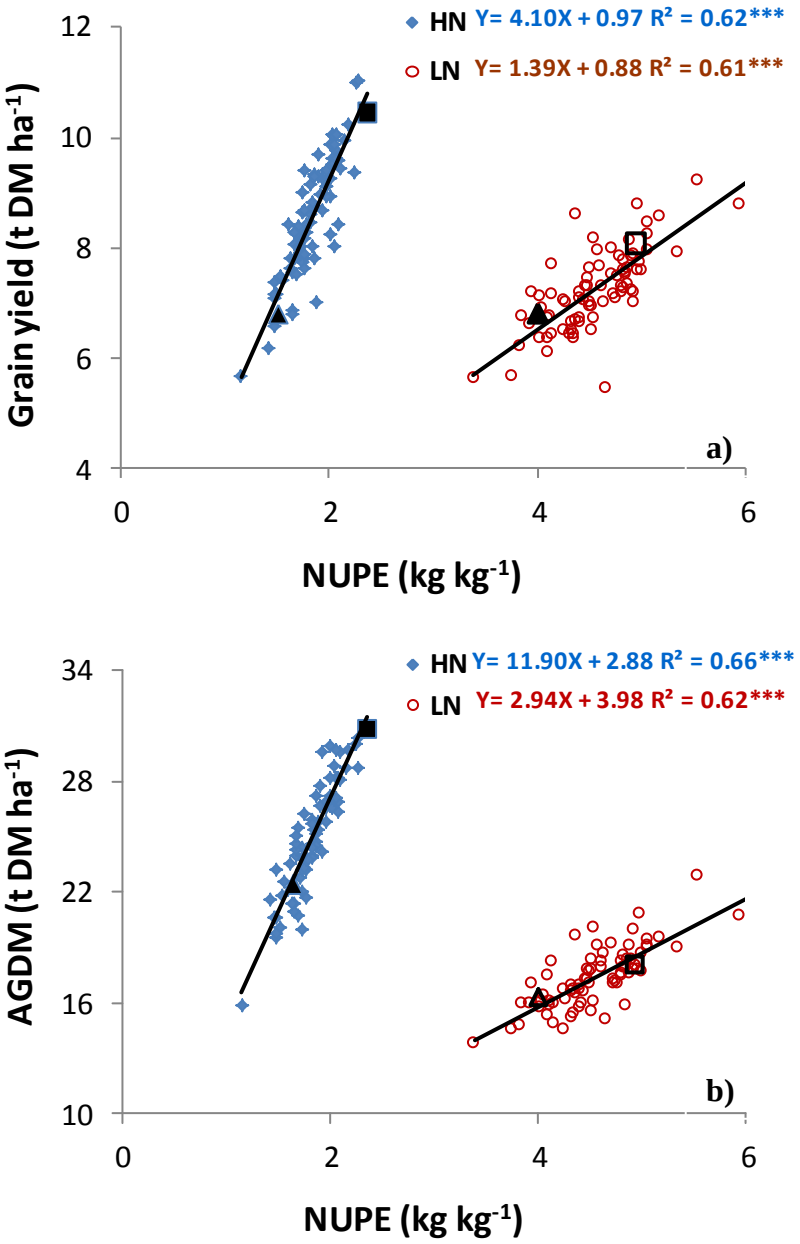


Figure 5.11: Linear regression amongst 79 SSD Paragon x W481 mapping population lines of a) grain yield (DM t ha^{-1} 100% DM) on N uptake efficiency (NUPE kg kg^{-1}) under HN (◆) and LN (○) and b) above-ground dry matter (AGDM t ha^{-1}) on N uptake efficiency (NUPE kg kg^{-1}) under HN (◆) and LN (○).The parental lines are indicated by: ■ Paragon under HN, ▲ W481 under HN, □ Paragon under LN and △ W481 under LN.

N-utilisation efficiency (NUTE) overall was higher under LN (31.2 kg DM kg^{-1} N) than HN (17.1 kg DM kg^{-1} N) (P=0.011; Table 5.6). N-utilization efficiency ranged

amongst SSD lines from 17.1 (L481-33) to 24.2 kg DM kg^{-1} N (L481-54) under HN and from 24.7 (L481-33) to 38.5 kg DM kg^{-1} N (L481-24) under LN (P<0.001). The N treatment x genotype interaction was significant (P<0.001) with responses to increasing N supply amongst SSD lines ranging from -17.3 (L481-24) to -5.9 kg DM kg^{-1} N (L481-34). Under HN 25 lines had higher NUTE than Paragon (20.1 kg DM kg^{-1} N) in the range 22.0 (L481-10) - 24.2 kg DM kg^{-1} N (L481-06) and four lines had significantly higher values than W481 (18.8 kg DM kg^{-1} N) in the range 23.1 (L481-26) - 24.2 kg DM kg^{-1} N (L481-06). However, under LN only 30 lines had significantly higher NUTE than Paragon (29.9 kg DM kg^{-1} N) in the range 31.9 (L481-29) - 38.5 kg DM kg^{-1} N (L481-67); while only seven lines had higher values than W481 (32.0 kg DM kg^{-1} N) in the range 34.1 (L481-30) - 38.5 kg DM kg^{-1} N (L481-67).

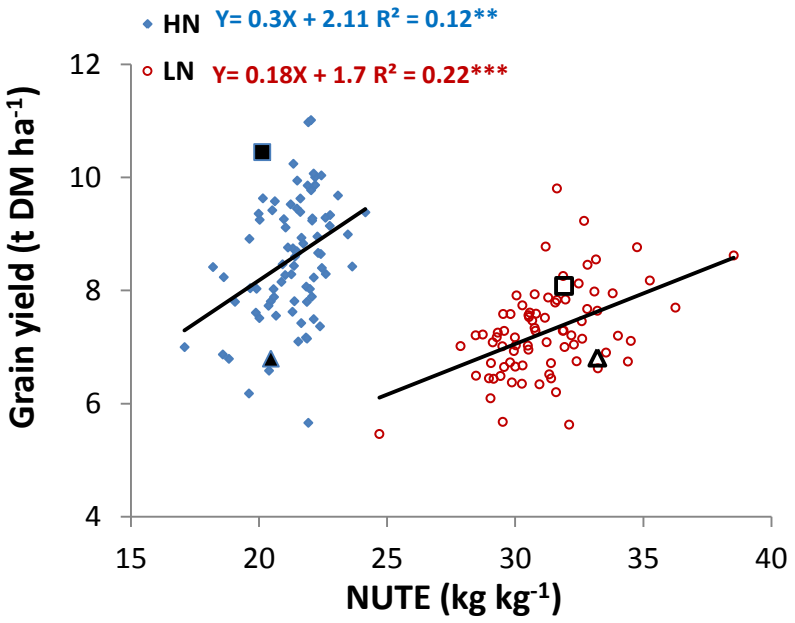


Figure 5.12: Linear regression of grain yield (t DM ha^{-1}) on N-utilisation efficiency (NUTE kg kg^{-1}) amongst 79 mapping population lines of PxW481 in HN (◆) and LN (O).The parental lines are indicated by: ■ Paragon under HN, ▲ W481 under HN, □ Paragon under LN and △ W481 under LN.

Table 5.5: N-use efficiency (NUE, kg kg^{-1} N) and N uptake (NUP kg N ha^{-1}) for parental lines and mean, maximum and minimum of 79 lines of Paragon x W481 SSD population in 2011/12 and 2012/13.

	NUE (kg DM kg^{-1} N)				NUP (kg N ha^{-1})			
	2012		2013		2012		2013	
	HN	LN	HN	LN	HN	LN	HN	LN
Paragon	47.2	158	42.7	152	523	254	367	230
W481	30.7	133	35.9	140	364	205	323	204
Min	25.2	110	27.8	98	255	174	230	127
Max	49.8	192	45.6	186	504	311	384	273
Mean	39.2	141	36.9	140	406	232	317	202
N-trt.	0.002		<0.001		0.005		0.025	
Genot.	<0.001		<0.001		<0.001		<0.001	
N X G	0.003		<0.001		<0.001		0.041	

Table 5.6: N-uptake efficiency (NUPE kg N kg N^{-1}) and N-utilisation efficiency (NUTE in kg DM kg^{-1} N) for parental lines and mean, maximum and minimum of 79 lines of Paragon x W481 SSD population in 2011/12 and 2012/13

	NUPE (kg kg^{-1})				NUTE (kg kg^{-1})			
	2012		2013		2012		2013	
	HN	LN	HN	LN	HN	LN	HN	LN
Paragon	2.36	4.95	1.42	4.01	20.1	29.9	30.0	37.9
W481	1.64	4.01	1.26	3.55	18.8	32.0	28.5	39.3
Min	1.15	3.39	0.89	2.21	17.1	24.7	27.8	35.5
Max	2.28	6.06	1.49	4.76	24.2	38.5	32.1	47.7
Mean	1.84	4.53	1.23	3.52	21.4	31.2	30	40.2
N-trt.	0.009		<0.001		0.011		0.003	
Genot.	<0.001		<0.001		<0.001		<0.001	
N X G	NS		0.001		<0.001		0.009	

Table 5.7: N harvest index (NHI kg N kg N^{-1}) and biomass production efficiency (BPE in kg DM kg^{-1} N) for parental lines and mean, maximum and minimum of 79 lines of Paragon x W481 SSD population in 2011/12 and 2012/13

	NHI (kg kg^{-1})				BPE (kg kg^{-1})			
	2012		2013		2012		2013	
	HN	LN	HN	LN	HN	LN	HN	LN
Paragon	0.55	0.71	0.83	0.86	58.6	71.1	58.88	75.93
W481	0.55	0.77	0.78	0.84	62.1	79.5	66.01	91.43
Min	0.50	0.67	0.75	0.80	51.7	63.0	59.21	74.33
Max	0.67	0.79	0.85	0.88	70.8	87.5	72.01	105.10
Mean	0.60	0.74	0.81	0.84	62.0	74.5	64.72	88.09
N-trt.	0.01		0.079		0.016		0.003	
Genot.	<0.001		<0.001		<0.001		<0.001	
N X G	0.431		<0.001		<0.001		0.126	

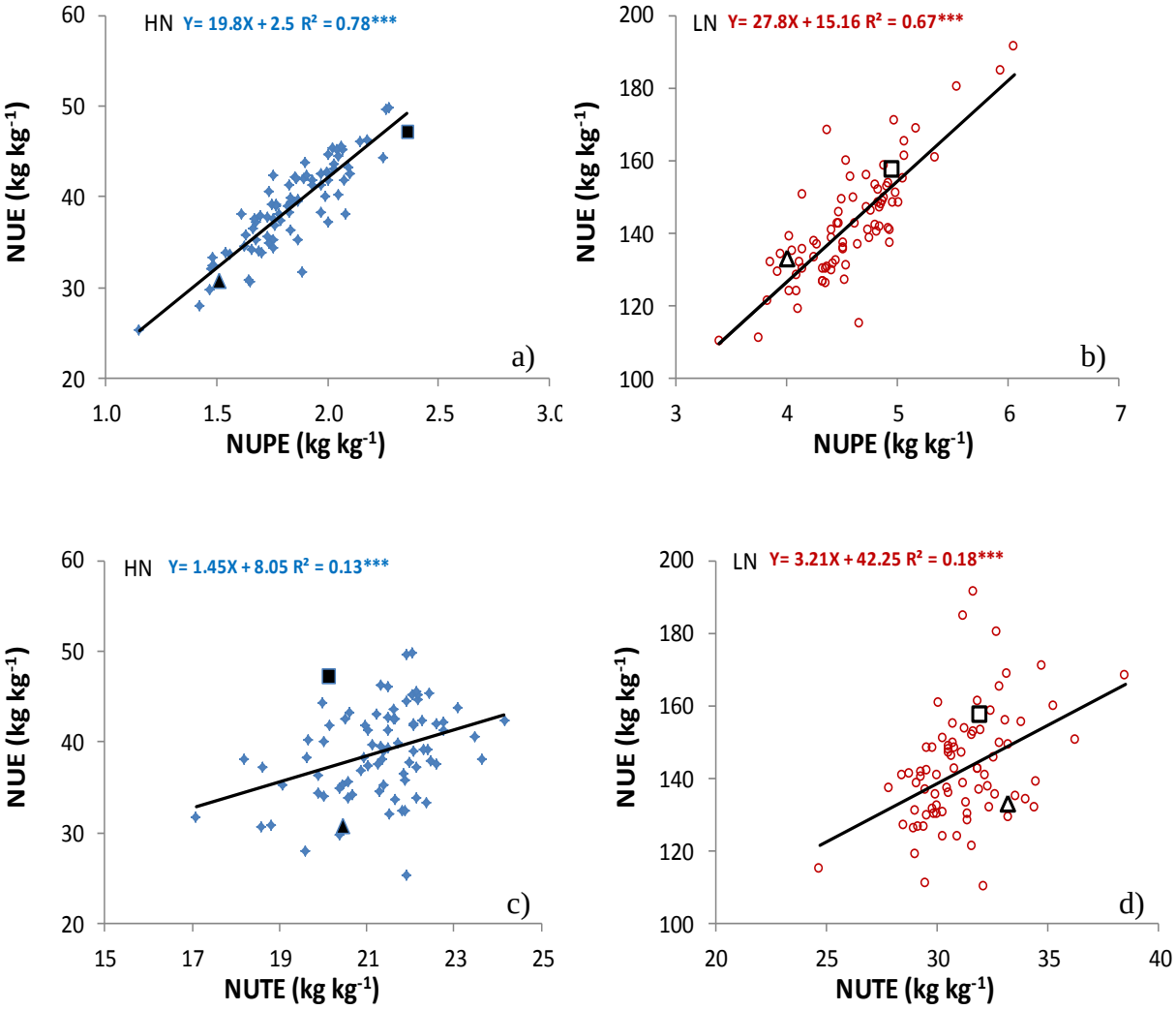


Figure 5.13: Linear regression amongst 79 SSD lines of Paragon x W481 mapping population lines of N use efficiency (NUE kg kg^{-1}) on N uptake efficiency (NUPE kg kg^{-1}) a) under HN and b) under LN, of NUE (kg kg^{-1}) on N utilisation efficiency (NUTE kg kg^{-1}) c) under HN and d) under LN. The parental lines are indicated by: ■ Paragon under HN, ▲ W481 under HN, □ Paragon under LN and △ W481 under LN.

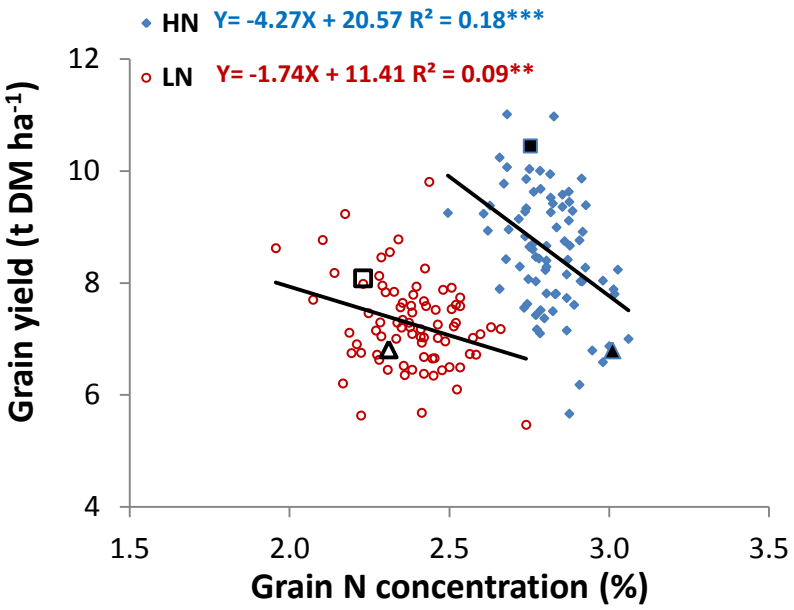


Figure 5.14: Linear regression of grain yield (t DM ha^{-1}) on grain N concentration amongst 79 mapping population lines of PxW481 (211/12 data). The parental lines are indicated by: ■ Paragon under HN, ▲ W481 under HN, □ Paragon under LN and △ W481 under LN.

N harvest index (NHI) increased from 0.60 under HN to 0.74 under LN (P=0.010). Within genotypes, NHI varied from 0.50 kg kg^{-1} (L481-421) to 0.67 kg kg^{-1} (L481-26) under HN and from 0.67 kg kg^{-1} (L18433) to 0.79 kg kg^{-1} (L481-41) under LN (P<0.001). The N treatment x genotype interaction was not significant (P=0.431). The responses to N supply amongst SSD lines ranged from -0.21 kg kg^{-1} (L481-45) to -0.07 kg kg^{-1} (L481-69). NHI in Paragon decreased from 0.71 kg kg^{-1} under LN to 0.55 kg kg^{-1} under HN (-23%) and W481 from 0.77 kg kg^{-1} under LN to 0.55 kg kg^{-1} under HN (-29%).

Biomass production efficiency (BPE; AGDN/Above-ground N uptake) increased from 61.9 kg kg^{-1} under HN to 74.5 kg kg^{-1} under LN (P=0.016). Amongst SSD lines, BPE ranged from 51.7 kg kg^{-1} (L481-417) to 70.8 kg kg^{-1} (L481-14) under HN and from 63.0 kg kg^{-1} (L481-33) to 87.5 kg kg^{-1} (L481-24) under LN (P<0.001). The N treatment x genotype interaction was significant (P<0.001) with responses to increasing N supply amongst SSD lines ranging from -23.2 kg kg^{-1} (L481-24) to +0.06 kg kg^{-1} (L481-73). Under HN, 29 SSD lines had higher BPE than Paragon (58.6 kg kg^{-1}) or W481 (62.1 kg kg^{-1}). However, under LN, only 11 SSD lines had higher values than Paragon (71.1 kg kg^{-1}) or W481 (79.6 kg kg^{-1}).

b- 2012/13

NUE overall increased from 36.9 kg kg^{-1} under HN to 140 kg kg^{-1} under LN (P<0.001, Table 5.5). The SSD lines ranged from 27.8 to 45.6 kg kg^{-1} under HN and from 98.2 kg kg^{-1} to 186 kg kg^{-1} under LN (P<0.001). The responses to decreasing N supply ranged amongst SSD lines from 67.6 kg kg^{-1} (L481-26) to 153 kg kg^{-1} (L481-37) (P< 0.001). Under HN, 5 SSD lines had higher NUE than the higher parent Paragon (42.7 kg kg^{-1}), whereas under LN 22 lines had higher values than higher parent Paragon (152 kg kg^{-1}).

NUP increased from 201.9 kg ha^{-1} under LN to 317.37 kg ha^{-1} under HN (P< 0.05, Table 5.5). SSD lines ranged from 229.7 to 383.9 kg ha^{-1} under HN and from 126.7 to 273.2 kg ha^{-1} under LN (P<0.001). The N treatment x genotype interaction was significant (P=0.041), with responses to increasing N supply ranging amongst genotypes from 8.5 kg ha^{-1} (L481-37) to 222 kg ha^{-1} (L481-39). Under HN, 6 lines had higher NUP than the higher parent Paragon (366.7 kg ha^{-1}), whereas under, LN 16 lines had higher NUP then the higher parent Paragon (230.2 kg ha^{-1}).

NUPE overall increased from 1.23 kg kg^{-1} under HN to 3.52 kg kg^{-1} under LN (P=0.003, Table 5.6). Amongst SSD lines NUPE ranged from 0.89 to 1.49 kg kg^{-1} under HN and 2.21 to 4.76 kg kg^{-1} under LN (P<0.001). The N treatment x genotype interaction was significant (P=0.005), with responses to increasing N supply ranging from -0.98 kg ha^{-1} (L481-26) to -3.67 kg ha^{-1} (L481-37). Under HN, four SSD lines had higher NUPE than the higher parent Paragon (1.42 kg ha^{-1}), whereas under LN 16 lines had higher NUPE than the higher parent Paragon (4.01 kg ha^{-1}).

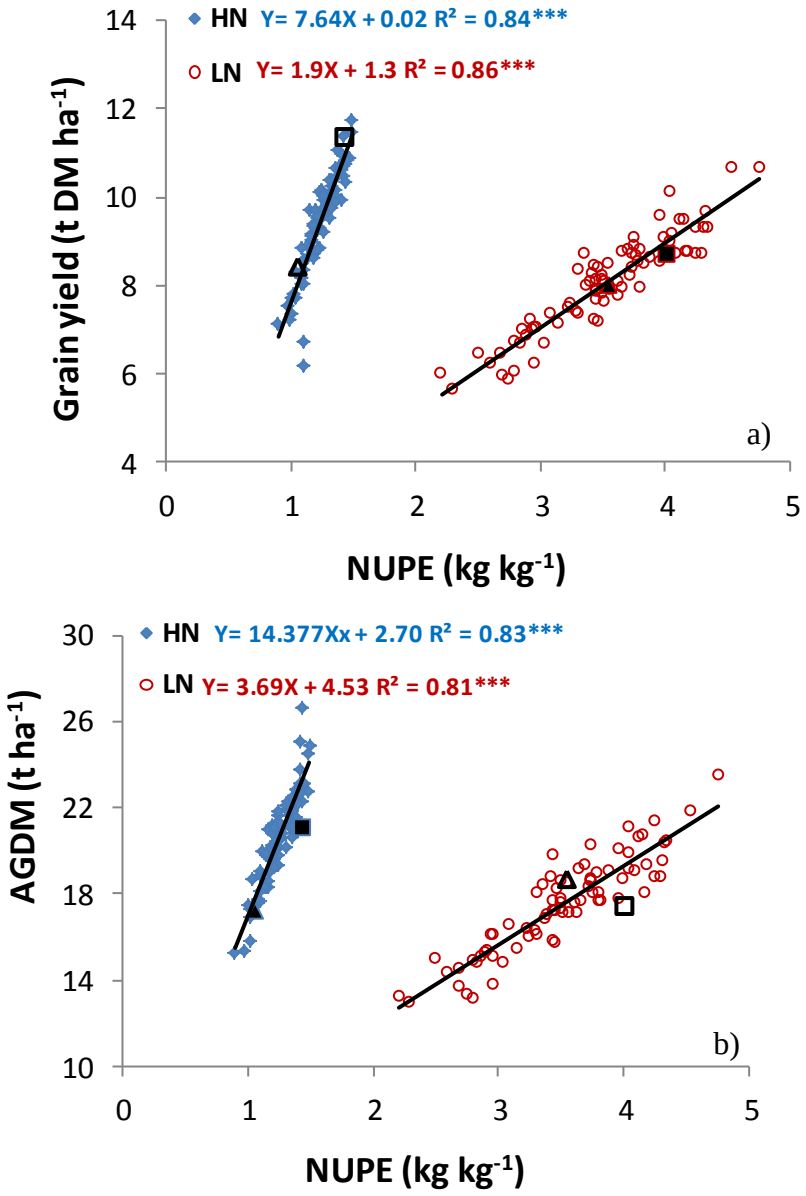


Figure 5.15: Linear regression amongst 79 SSD lines of Paragon x W481 mapping population lines of a) grain yield (DM t ha^{-1}) on N uptake efficiency (NUPE kg kg^{-1}) under HN (◆) and LN (O), b) bove ground dry matter (AGDM t ha^{-1}) on N uptake efficiency (NUPE kg kg^{-1}) under HN (◆) and LN (O).The parental lines are indicated by: ■ Paragon under HN, ▲ W481 under HN, □ Paragon under LN and △ W481 under LN.

N-utilisation efficiency (NUTE) increased from 30.0 kg kg^{-1} under HN to 40.23 kg kg^{-1} under LN (P=0.003, Table 5.6). The SSD lines ranged from 27.8 (L481-83) to 32.1 kg DM kg^{-1} N (L481-07) under HN and from 35.5(L481-23) to 47.7 kg DM kg^{-1} N (L481-26) under LN (P<0.001). The N treatment x genotype interaction was significant (P=0.009), with responses to increasing N supply amongst SSD lines ranging from -17.8 (L481-24) to -6.2 kg kg^{-1} (L481-34). Under HN, 11 SSD lines

had significantly higher NUTE than the higher parent Paragon (30 kg kg^{-1}), whereas under LN 18 SSD lines had higher NUTE than the higher parent W481 (39.3 kg kg^{-1}).

There was a trend for a small increase of N harvest index from 0.81 under HN to 0.84 kg kg^{-1} under LN (P=0.079) (Table 5.7). Within SSD lines, NHI varied from 0.75 kg kg^{-1} (L481-424) to 0.85 kg kg^{-1} (L481-76) under HN and from 0.80 kg kg^{-1} (L18433) to 0.88 kg kg^{-1} (L481-57) under LN (P<0.001). The N treatment x genotype interaction was significant (P<0.001) with responses to increasing N supply amongst SSD lines ranging from -0.11 kg kg^{-1} (L481-24) to -0.01 kg kg^{-1} (L481-35). Under HN, 12 SSD lines had higher NHI than higher parent Paragon (0.83), whereas under LN, only 13 SSD lines had higher NHI than the higher parent Paragon (0.86).

Biomass production efficiency increased from 64.8 kg DM kg^{-1} N under HN to 88.0 kg kg^{-1} under LN (P=0.003; Table 5.7). Amongst SSD lines, BPE ranged from 58.9 kg kg^{-1} (L481-475) to 72.0 kg kg^{-1} (L481-24) under HN and from 74.3 kg kg^{-1} (L18449) to 105.1 kg kg^{-1} (L481-26) under LN (P<0.001). The N treatment x genotype interaction was not significant (P0.126). Under HN, 26 SSD lines had higher BPE than the higher parent W481 (66.0 kg kg^{-1}), whereas under LN 24 SSD lines had higher values than the higher parent W481 (91.4 kg kg^{-1}).

Cross-year ANOVA (Table 5.5 and 5.6) revealed the main effect of genotype was significant for all traits (P< 0.001). For NUP there was a trend for a N treatment x genotype interaction (P=0.083) and a year x N treatment x genotype interaction (P=0.039) For NUPE the N treatment x year interaction (P<0.001) was also significant but the year x N treatment x genotype interaction was not significant (P=0.703). For NUTE and NUE the N treatment x genotype interaction was significant (P<0.001), but the year x N treatment x year, genotype interaction was not significant (P=0.450 and P=0.253, respectively).

Table 5.8: Pearson's correlation coefficient in 79 SSD lines of Paragon xW481 SSD population between Grain Yield (GY kg DM ha^{-1}), Harvest Index (HI), Above-ground Dry Matter (AGDM, kg DM ha^{-1}), N-Use Efficiency (NUE, kg DM kg^{-1} N), N-Uptake Efficiency (NUPE, kg kg^{-1}), N-Utilisation Efficiency (NUTE, kg kg^{-1}), Anthesis Date (AD), Plant Height (PH), Grains per m^2 (GM) and Thousand Grain Weight (TGW, g). Values based on means in 2012 and in 2013. The values are for HN (unshaded) and under LN (shaded).

	GY	HI	AGDM	NUE	NUPE	NUTE	AD	PH	GM	TGW
2012 experiment										
GY		0.44***	0.91***	0.99***	0.77***	0.47***	-0.25*	0.23*	0.75***	0.04
HI	0.37***		0.05	0.46***	0.26*	0.37***	-0.18	-0.14	0.36***	0.01
AGDM	0.83***	-0.19		0.90***	0.79***	0.30**	-0.14	0.28*	0.69***	0.01
NUE	0.98***	0.35*	0.84***		0.82***	0.42***	-0.20	0.19	0.75***	0.03
NUPE	0.87***	0.05	0.91***	0.88***		-0.18	-0.08	0.05	0.65***	-0.03
NUTE	0.35*	0.65***	-0.02	0.36***	-0.11		-0.20	0.24*	0.26*	0.11
AD	-0.12	-0.10	-0.06	-0.10	-0.02	-0.17		-0.31**	-0.09	-0.22
PH	-0.08	-0.27*	0.03	-0.08	-0.16	0.13	-0.23*		-0.22	0.60***
GM	0.72***	0.05	0.72***	0.71***	0.67***	0.15	0.03	-0.10		-0.59***
TGW	0.22	0.38***	0.02	0.21	0.13	0.22*	-0.17	0.05	-0.51***	
2013 experiment										
GY		0.43***	0.96***	1.00***	0.93***	-0.20	0.12	0.30**	0.75***	0.18
HI	0.42***		0.19	0.43***	0.37**	0.00	-0.07	0.08	0.39***	0.17
AGDM	0.85***	0.01		0.96***	0.90***	-0.20	0.17	0.32**	0.70***	0.16
NUE	1.00***	0.39***	0.91**		0.93***	0.05	0.13	0.30*	0.74***	0.18
NUPE	0.91***	0.29**	0.91**	0.96***		-0.53***	0.10	0.23	0.66***	0.18
NUTE	0.16	0.35**	0.02	0.18	-0.11		0.00	0.09	-0.07	-0.06
AD	0.05	-0.17	0.19	0.10	0.12	-0.09		0.30*	0.05	-0.06
PH	0.13	-0.27*	0.30**	0.18	0.13	0.15	0.12		0.22	0.16
GM	0.48***	0.10	0.49***	0.50***	0.48***	0.15	0.15	-0.11		-0.35**
TGW	0.10	0.29**	0.00	0.09	0.08	0.02	-0.19	0.20	-0.67***	

Significance: *: P<0.005, **: P<0.001 and ***: P<0.001

5.3.2 Paragon xW141 Mapping population

5.3.2.1 Anthesis date (AD) and plant height (PH)

For the PxW141 mapping population, anthesis date was only recorded in HN conditions on two replicates. SSD lines ranged from 20 June (L481-01) to 27 June (L481-68) (P=0.032). Paragon flowered on 26 June and W141 on 21 June. Regarding transgressive segregation, no lines flowered earlier than W141 (21 June). However, one line L141-67 (24 June) flowered later than Paragon.

Anthesis date was positively correlated amongst SSD lines with grain yield (r=0.30, P=0.003 under HN and r=0.30, P=0.031 under LN), harvest index (r=0.30, P=0.003 under HN and R=0.34, P<0.001 under LN), and grains per m^2 (r=0.49, P<0.001 under HN and r=0.40, P<0.001 under LN). However, no significant relationship amongst SSD lines was found between AD and AGDM, and the relationship was negative with TGW (r=-0.49, P<0.001 under HN and r=-0.40, P<0.001 under LN).

Averaging over SSD lines, plant height increased from 87 cm under LN to 96 cm under HN (P=0.047). Amongst SSD lines PH varied from 72 to 112 cm under HN and from 62 to 107 cm under LN (P<0.001). There was a trend for a N treatment x genotype interaction (P=0.089). The parent W141 (115 cm under HN and 106 cm under LN) was overall 34% taller than Paragon (86 cm under HN and 78 cm under LN) (P<0.001). Under HN, W141 was the tallest genotype, whereas under LN, two SSD lines L141-22 (107 cm) and L141-20 (107 cm) were taller than W141 (106 cm). In total, 11 genotypes were shorter than Paragon (86 cm) under HN and, 18 genotypes were shorter than Paragon (78 cm) under LN.

Plant height was negatively correlated amongst SSD lines with grain yield under HN (r=-0.29, P=0.005) and LN and (r=-0.18, P=0.079), harvest index (r=-0.75, P<0.001 under HN and r=-0.78, P<0.001 under LN), grains m^{-2} (r=-0.67, P<0.001 under HN and r=-0.57, P<0.001 under LN). However, a positive correlation was found between PH and AGDM (r=0.30, P=0.003 under HN and r=0.40, P<0.001 under LN) and TGW (r=0.64, P<0.001 under HN and r=0.76, P<0.001 under LN).

5.3.2.2 Grain yield and yield components

Averaging over 79 lines of the PxW141 mapping population, grain yield increased from 8.47 t ha^{-1} under LN to 9.72 t ha^{-1} under HN (P=0.001; Table 5.9). SSD lines ranged from 7.55 (L141-41) to 11.94 t ha^{-1} (L141-83) under HN and 5.96 t ha^{-1} (L141-83) to 10.92 (L141-46) under LN (P<0.001). Responses to increasing N supply ranged amongst SSD lines from -2.93 t ha^{-1} (L141-46) to 5.98 t ha^{-1} (L141-83) (P< 0.001). Under HN conditions, three lines, L141-52 (11.80 t ha^{-1}), L141-04 (11.91 t ha^{-1}) and L141-83 (11.94 t ha^{-1}), had significantly higher yield than the higher parent Paragon (10.33 t ha^{-1}), whereas under LN, one SSD line: L141-46 (10.9 t ha^{-1}), had higher yield than Paragon (9.62 t ha^{-1}).

Table 5.9: Values of parental lines and mean, maximum, minimum and ANOVA significance for treatments of the 97 SSD lines of Paragon x W141 mapping population for grain yield (t ha^{-1}) Ears per m^2, thousand grain weight (TGW), grains m^{-2} and grains ear^{-1}.

	Grain yield (t ha^{-1}) 100% DM		Ears m^{-2}		TGW (g)		Grains m^{-2}		Grains ear^{-1}	
	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN
Paragon	10.33	9.62	527	370	34.47	36	30066	26806	57.29	68.00
W141	8.39	6.68	450	337	47.94	46.45	17544	14393	38.99	43.64
Min	7.55	5.96	333	280	28.46	31.08	18132	13731	32.48	31.88
Max	11.94	10.92	637	570	48.8	48.36	39733	30865	89.32	85.08
Mean	9.72	8.47	500	425	38.26	40.31	26019	21311	53.79	51.66
N-trt.	0.001		0.055		0.015		0.003		0.213	
Genot.	<0.001		<0.001		<0.001		<0.001		<0.001	
N X G	0.004		0.062		<0.001		<0.001		0.003	

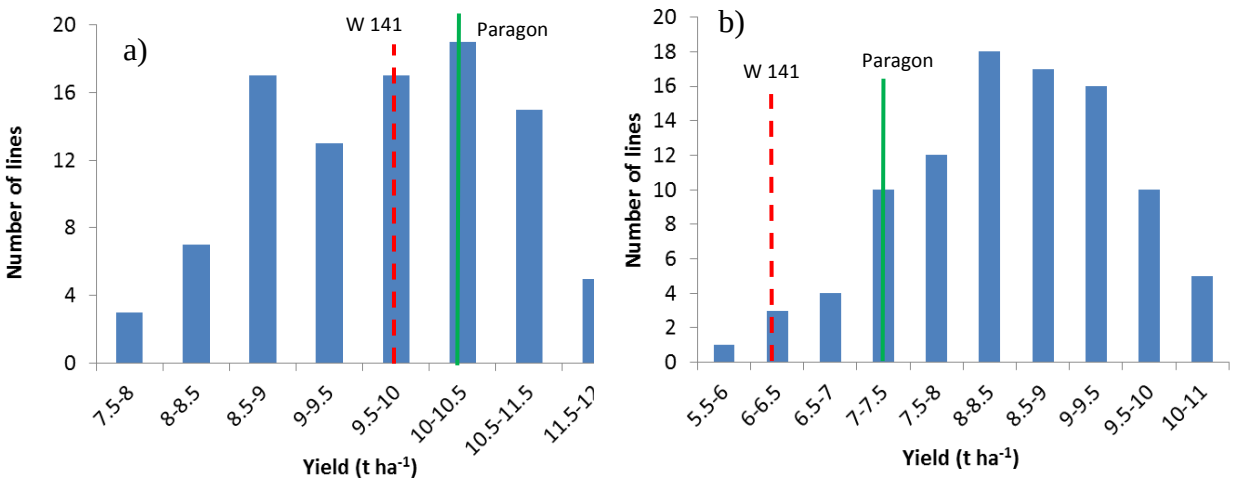


Figure 5.16: Frequency distribution of 97 SSD Paragon x W141 lines for grain yield (t ha^{-1} 0% moisture content a) under HN and b) under LN.

There was a trend for ears m^{-2} to increase from 425 under LN to 500 under HN conditions (P=0.055). There was also a trend for N treatment x genotype interaction (P=0.062), with responses to increasing N supply ranging from -107 to +278 ears m^{-2}. SSD lines under HN ranged from 333 to 637 and under LN from 280 to 570 ears m^{-2} (P< 0.001). Under HN, six lines had more ears m^{-2} than the higher parent Paragon (527) in the rage 620 (L141-21) - 637 (L141-57), whereas under LN 22 lines had more ear m^{-2} than the higher parent Paragon (370) in the range 463 (L141-11) - 570 (L141-90).

N treatment did not have any effect on grains ear^{-1} (GE) (P=0.372). Amongst SSD lines, GE varied from 32 (L141-21) to 89 (L141-40) and under HN and from 32 (L141-06) to 85 (L141-61) under LN (P<0.001). Response to N supply amongst lines ranged from -35 (L481-61) to 38 (L481-83) GE (P = 0.004). Paragon (57 under HN and 68 under LN) had higher GE than W141 (39 and 44, respectively). Under HN, seven SSD lines had higher GE than Paragon (58) in the range 71 (L141-76) - 89 (L141-40) and under LN, no line had higher GE than Paragon (73).

Thousand grain weight increased from 38.3 g under HN to 40.3 under LN (P=0.015). SSD lines ranged from 28.5 to 48.8 g under HN and from 31.1 to 48.4 g under LN (P<0.01). The N treatment x genotype interaction was significant (P<0.001), with responses to increasing N supply ranging from -9 g to +4 g. W141 (47.9 g under HN and 46.5 g under LN) had higher TGW than Paragon (34.5 g and 36.0 g,

respectively). Under HN, no line had higher TGW than the higher parent W141 (47.9 g), and under LN also no line had higher TGW than the higher parent W141 (46.5).

Grains m^{-2} overall increased from 21,311 under LN to 26,019 under HN ($P< 0.01$; Table 5.9). SSD lines ranged from 18,132 (L141-61) to 39,733 (L141-83) grains m^{-2} under HN and from 13,731 (L141-02) to 30,865 (L141-92) grains m^{-2} under LN ($P< 0.001$). There was a trend for an N treatment x genotype interaction ($P=0.062$), with responses to increasing N supply ranging from 3,874 (L141-46) to 22,211 (L141-83) Paragon had more grains m^{-2} (30,066 under HN and 26,806 under LN) than W141 (17,544 under HN and 14,393 under LN). Under HN, 11 lines had more grains m^{-2} than Paragon, whereas under LN only two lines: L141-86 (30,737) and L141-92 (30,865) had higher values than Paragon (26,806).

Similar to P x W481 in both 2012 and 2013, grain yield was more strongly associated with grains m^{-2} than with grain weight amongst SSD lines under both HN and LNconditions ($R^2=0.49$, $P<0.001$ and $R^2=070$, $P<0.001$ respe6ctively) (Figure 5.17).

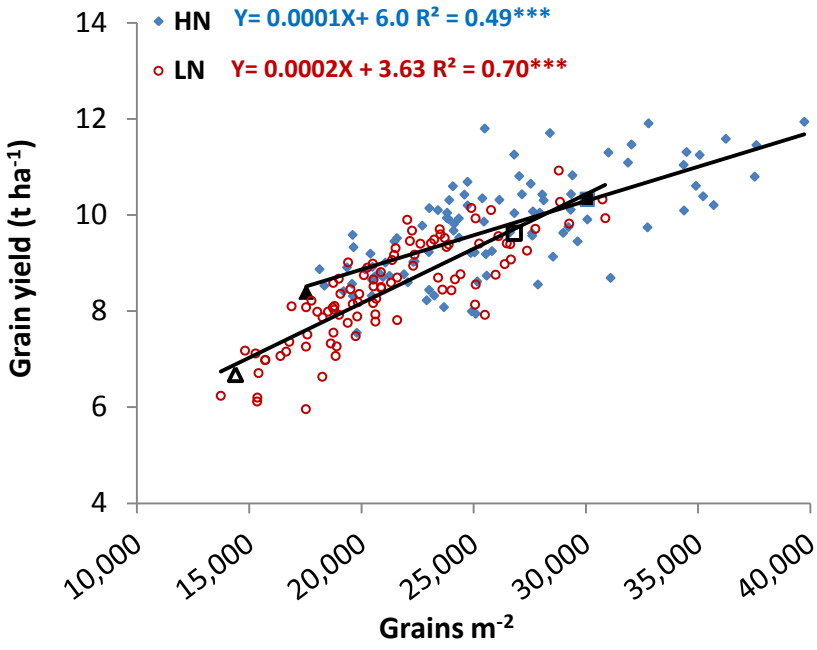


Figure 5.17: Linear regression of grain yield (100% DM) on grain number per m^{-2} amongst 97 Paragon x W141 mapping population SSD lines in HN (◆) and LN (O).The parental lines are indicated by: ■ Paragon under HN, ▲ W141 under HN, □ Paragon under LN and △ W141 under LN.

5.3.2.3- Aboveground dry matter and HI

Averaging over SSD lines, AGDM increased from 18.3 t ha^{-1} under LN to 20.7 t ha^{-1} under HN (P=0.013, Table 5.10). SSD lines varied in the range 15.9 t ha^{-1} to 26.6 t ha^{-1} under HN and from 10.9 t ha^{-1} to 24.4 t ha^{-1} under LN (P<0.001). Responses of SSD lines to increasing N supply ranged from -5.48 t ha^{-1} (L141-46) to 11.83 t ha^{-1} (L141-83) (P= 0.015). Under HN, six SSD lines had higher AGDM than the higher parent Paragon (21.0 t ha^{-1}), and under LN four lines had higher AGDM than the higher parent Paragon (18.9 t ha^{-1}).

The N treatment did not affect HI (P=0.43). SSD lines overall, ranged from 0.36 to 0.57 (P<0.001, Table 5.10). There was a trend for an N treatment x genotype interaction (P=0.06), with responses to increasing N supply ranging from -0.05 to +0.16. Under HN, ten SSD lines had higher HI than the higher parent Paragon (0.49), and under LN four lines had higher HI than the higher parent Paragon (0.51).

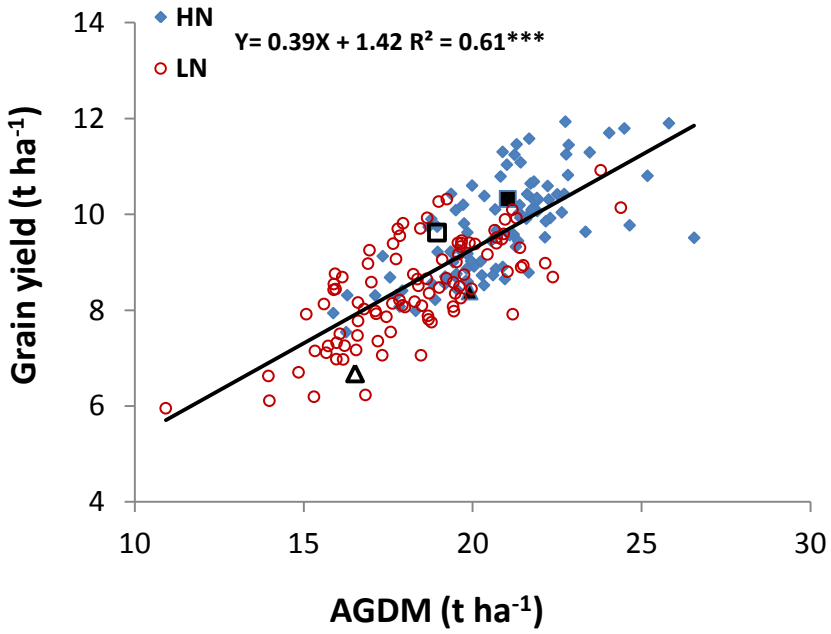


Figure 5.18: Linear regression of AGDM (t ha^{-1}) on grain yield (t ha^{-1}) amongst 97 SSD lines of PxW141 mapping population in HN (◆) and LN (○).The parental lines are indicated by: ■ Paragon under HN, ▲ W141 under HN, □ Paragon under LN and △ W141 under LN.

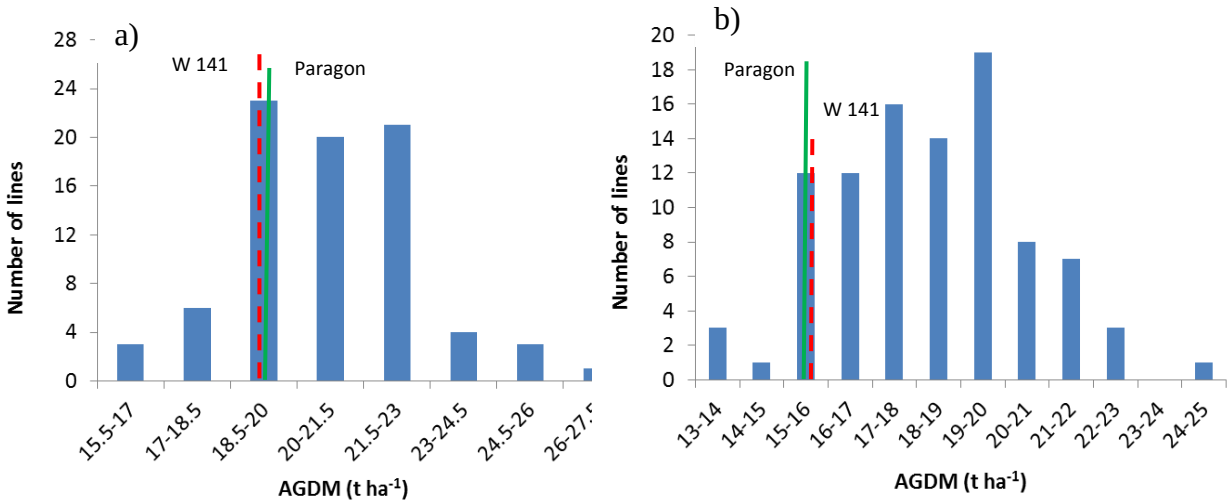


Figure 5.19: Frequency distribution of 97 Paragon x W141 lines for biomass (t ha^{-1}) a) under HN and b) under LN.

Table 5.10: Values of parental lines and mean, maximum, minimum and ANOVA significance results of the 97 SSD lines of Paragon x W141 mapping population, for above-ground dry matter (AGDM) harvest index (HI) and plant height (PH).

	AGDM (t ha^{-1})		HI		PH (cm)	
	HN	LN	HN	LN	HN	LN
Paragon	21.04	18.94	0.49	0.51	86	78
W141	19.91	16.51	0.42	0.41	115	106
Min	15.87	10.91	0.36	0.36	72	62
Max	26.56	24.39	0.57	0.55	112	107
Mean	20.74	18.33	0.47	0.46	96	87
N-trt.	0.013		0.426		0.047	
Genot.	<0.001		<0.001		<0.001	
N x G	0.015		0.061		0.089	

5.3.2.4- N-use efficiency and NUE components

Averaging over SSD lines, NUE decreased from 148 kg kg^{-1} under LN to 37.6 kg kg^{-1} under HN (-75%) (P<0.001, Table 5.11). Amongst SSD lines, NUE ranged from 29.3 (L141-41) to 46.4 kg kg^{-1} (L141-83) under HN and from 104 kg kg^{-1} (L141-83) to 190 kg kg^{-1} (L141-46) under LN (P< 0.001). Responses of SSD lines to N supply ranged from -57 (L141-83) to 159 kg kg^{-1} (L141-46). Under HN, no line had higher NUTE than the higher parent Paragon (40.3 kg kg^{-1}), while one SSD line, L141-46 (190 kg kg^{-1}), had greater NUE than the higher parent Paragon (168 kg kg^{-1}).

N-uptake overall increased from 238 kg ha^{-1} under LN to 349 kg ha^{-1} under HN (P< 0.01). SSD lines ranged from 260 kg ha^{-1} to 425 kg ha^{-1} under HN and from 149 kg ha^{-1} to 309 kg ha^{-1} under LN (P< 0.01). The N treatment x genotype interaction was significant (P<0.001), with response to N supply ranging from -12.1 kg ha^{-1} (L141-46) to 263.1 kg ha^{-1} (L141-83). Under HN, one line, L141-04, had higher NUP than the higher parent Paragon (363 kg ha^{-1}), and under LN no SSD lines had higher NUP than the higher parent Paragon (271 kg ha^{-1}).

N-uptake efficiency overall decreased from 4.15 kg kg^{-1} under LN to 1.35 kg kg^{-1} under HN (P< 0.01). SSD lines ranged from 1.01 kg kg^{-1} to 1.65 under HN and from 2.59 kg kg^{-1} to 5.39 kg kg^{-1} under LN (P<0.01). Responses of SSD lines to increasing N supply ranged from -4 kg kg^{-1} (L141-46) to -0.99 (L141-83) (P = 0.002; Table 5.11). Amongst SSD lines, no lines had higher NUPE than Paragon either under HN (1.41 kg kg^{-1}) or LN (4.72 kg kg^{-1}).

Table 5.11: Mean values of parental lines and mean, maximum, minimum and ANOVA significance results of the 97 SSD lines of Paragon x W141 mapping population, for N use efficiency (NUE, kg DM ha^{-1} N) N uptake (NUP, kg N ha^{-1}) and N uptake efficiency (NUPE, kg N kg^{-1} N).

	NUE (kg DM ha^{-1} N)		NUP (kg N ha^{-1})		NUPE (kg N kg^{-1} N)	
	HN	LN	HN	LN	HN	LN
Paragon	40.3	168	363	271	1.41	4.72
W141	32.6	118	308	197	1.19	3.44
Min	29.3	104	260	149	1.01	2.59
Max	46.4	190	425	309	1.65	5.39
Mean	37.6	148	349	238	1.35	4.15
N-trt.	<0.001		0.006		<0.001	
Genot.	<0.001		0.006		0.002	
N x G	<0.001		0.006		0.002	

Overall N-utilisation efficiency increased from 27.8 kg kg^{-1} under HN to 36.0 kg kg^{-1} under LN (P<0.01) and SSD lines ranged from 23.5 kg kg^{-1} to 30.6 kg kg^{-1} under HN and from 29.98 kg kg^{-1} to 42.13 under LN (P<0.001) (Table 5.12). Response to N supply ranged amongst SSD lines from -13.2 kg kg^{-1} (L141-80) to -4.17 (L141-33). Under HN, no line lines had higher NUTE than the higher parent Paragon (28.6 kg kg^{-1}), whereas under LN 28 lines had higher NUTE than the higher parent Paragon (35.6 kg kg^{-1}).

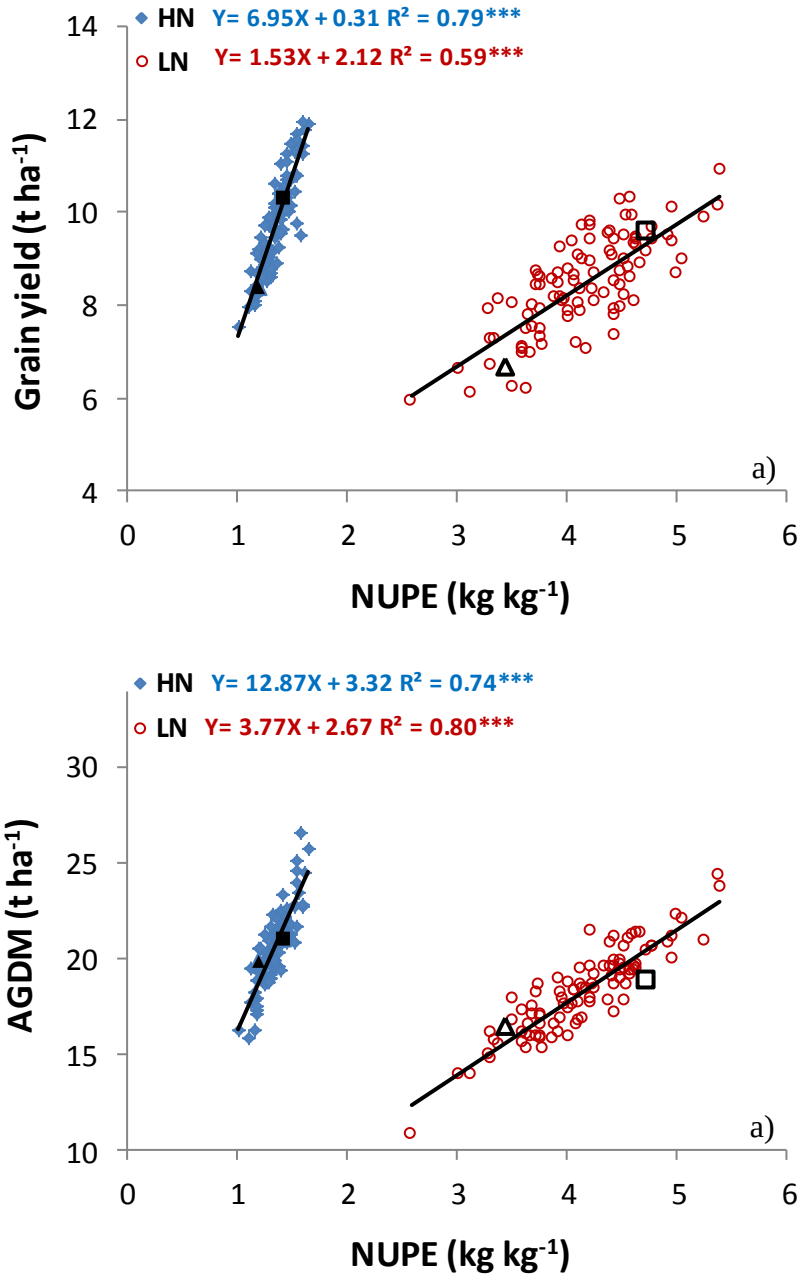


Figure 5.20: Linear regression amongst 97 Paragon x W141 mapping population SSD lines of a) grain yield (DM t ha^{-1}) on N uptake efficiency (NUPE kg kg^{-1}) under HN (◆) and LN (○) and b) above-ground dry matter (AGDM t ha^{-1}) on N uptake efficiency (NUPE kg kg^{-1}) under HN (◆) and LN (○).The parental lines are indicated by: ■ Paragon under HN, ▲ W141 under HN, □ Paragon under LN and △ W141 under LN.

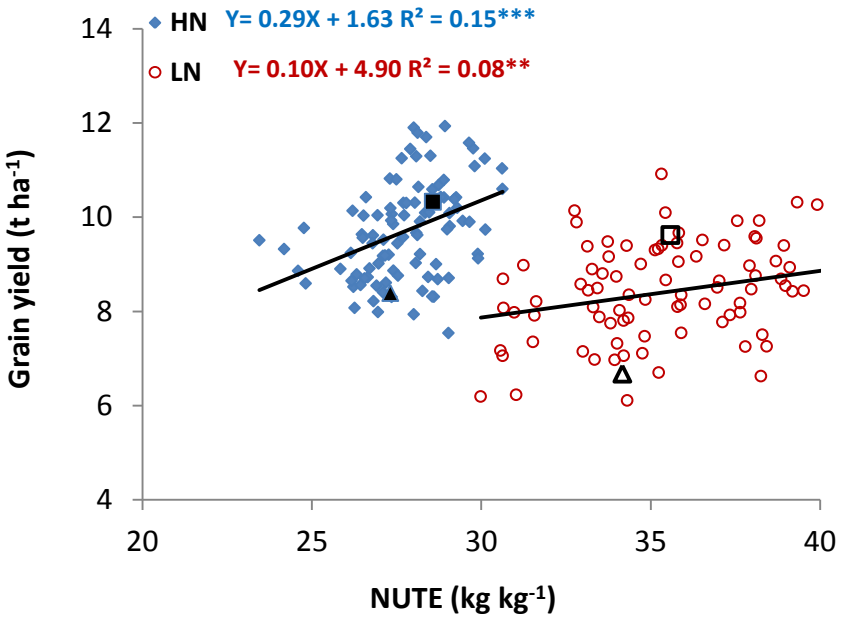


Figure 5.21: Linear regression of grain yield (t DM ha^{-1}) on N utilisation efficiency (NUTE kg kg^{-1}) amongst 97 mapping population lines of PxW141 in HN (◆) and LN (O).The parental lines are indicated by: ■ Paragon under HN, ▲ W141 under HN, □ Paragon under LN and △ W141 under LN.

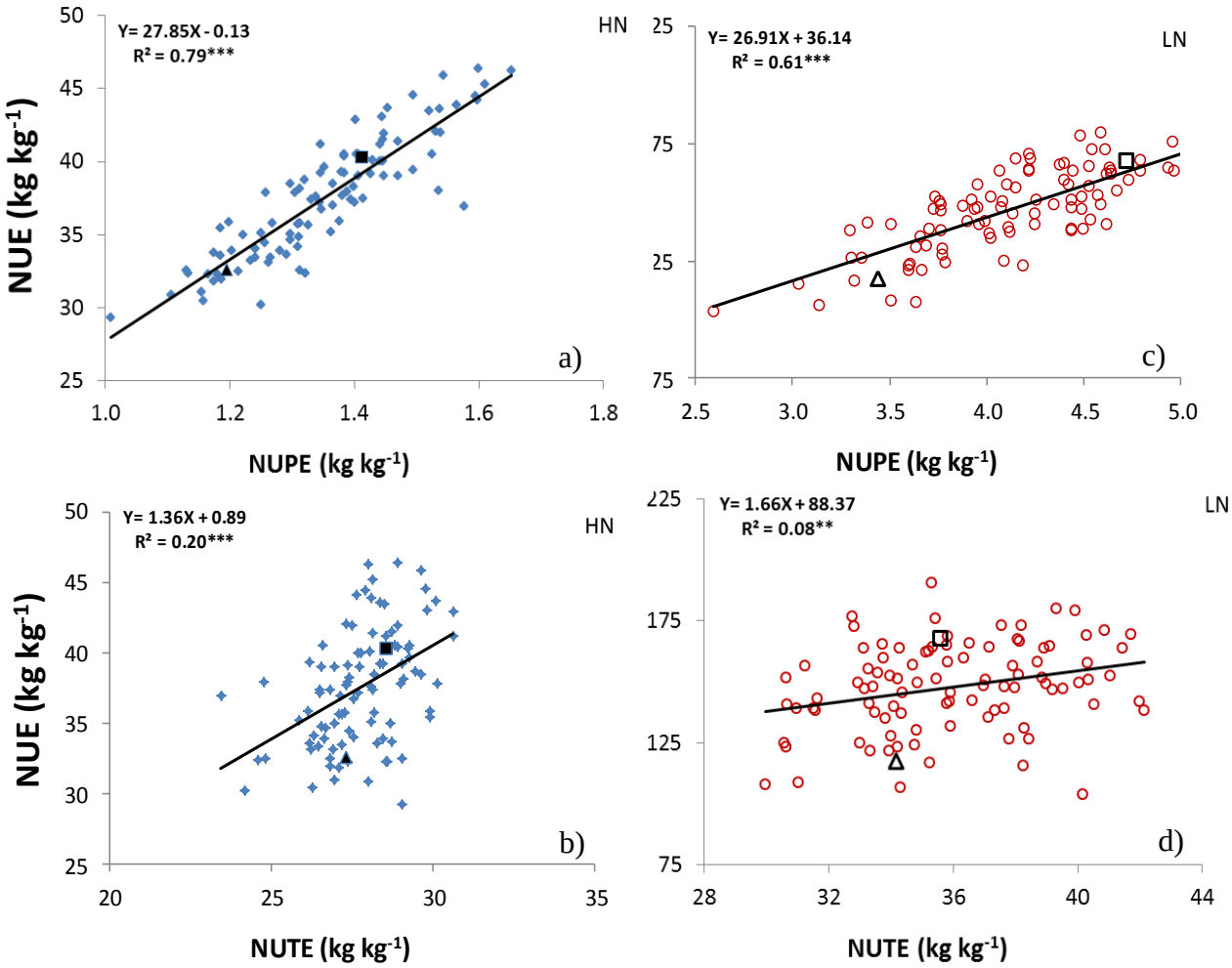


Figure 5.22: Linear regression amongst 97 mapping population lines of N use efficiency (NUE kg kg^{-1}) on N-uptake efficiency (NUPE kg kg^{-1}) a) under HN (◆) and b) under LN (O) and NUE (kg kg^{-1}) on N-utilisation efficiency (NUTE kg kg^{-1}) c) under HN (◆) and d) under LN (O).The parental lines are indicated by: ■ Paragon under HN, ▲ W141 under HN, □ Paragon under LN and △ W141 under LN.

Averaging all SSD lines, grain N concentration increased significantly from 2.31% under LN to 2.74% under HN (P=0.006). SSD lines ranged from 2.58 (L141-94) to 2.94 (L141-39) under HN and from 1.90 (L141-53) to 2.63% (L141-23) under LN. The responses of SSD lines to N increasing supply ranged from +0.7% (L141-22) to +0.8% (L141-80). Under HN, one SSD line, L141-39 (2.94%), had higher GNC than W141 (2.78%), and six lines had higher values than Paragon (2.72%). However under LN, 10 lines had higher GNC than the higher parent W141 (2.38%). N harvest index increased from 0.76 under HN to 0.82 under LN (P = 0.03), and amongst SSD lines varied from 0.67 (L141-435) to 0.81 kg kg^{-1} (L141-58) under HN and from 0.75 (L141-35) to 0.87 kg kg^{-1} (L141-58) under LN (P<0.001) (Table 5.12). The N treatment x genotype interaction was not significant (P<0.001) Under HN, one SSD line: L141-58 (0.81) had higher NHI than the higher parent Paragon (0.78); under LN three SSD lines: L141-71 (0.87), L141-68 (0.87) and L141-58 (0.87) had higher NHI than the higher parent Paragon (0.84).

A significant negative linear relationship was found amongst SSD lines between grain yield and grain N concentration (R^2=0.09, P=0.002 under HN and R^2=0.07, P=0.007 under LN) (Figure 5.23).

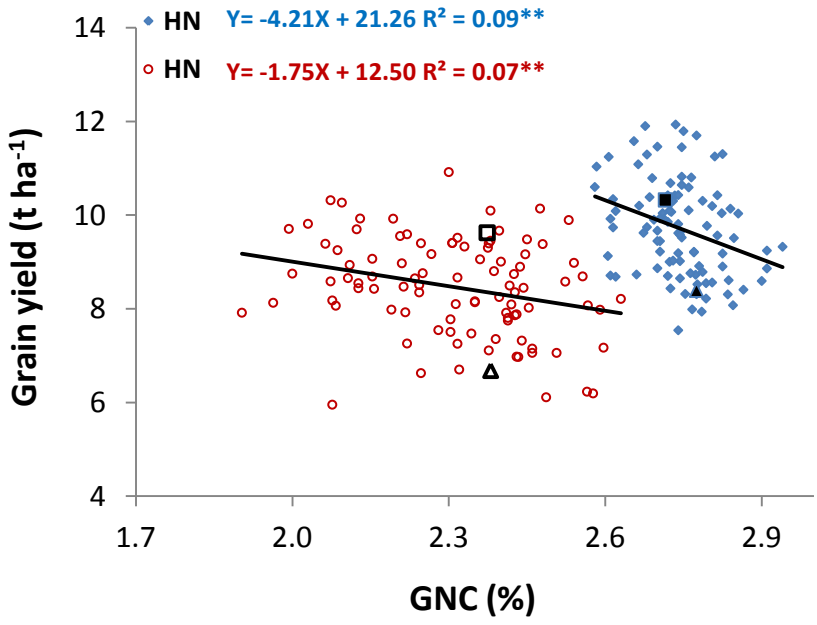


Figure 5.23: Linear regression of grain yield (t 100% DM ha^{-1}) on N grain concentration (GNC) amongst 97 line of PxW141 mapping population in HN (◆) and LN (○).The parental lines are indicated by: ■ Paragon under HN, ▲ W141 under HN, □ Paragon under LN and △ W141 under LN.

Biomass production efficiency increased from 59.7 kg kg^{-1} under HN to 77.5 kg kg^{-1} under LN (P=0.004) (Table 5.12). Amongst SSD lines, BPE ranged from 52.5 kg kg^{-1} (L141-12) to 67.9 kg kg^{-1} (L481-22) under HN and from 68.2 kg kg^{-1} (L114-33) to 90.3 kg kg^{-1} (L141-42) under LN (P<0.001). The N treatment x genotype interaction was not significant (P=0.249). BPE in Paragon decreased from 70.1 kg kg^{-1} under LN to 57.3 kg kg^{-1} under HN (-18%) and in W141 from 83.5 kg kg^{-1} under LN to 65.1 kg kg^{-1} under HN (-22%). Under HN, there was no SSD lines with higher BPE than the W141 (65.1 kg kg^{-1}) and under LN, one SSD line, L141-42 (90.3 kg kg^{-1}), had higher BPE than W141 (83.5 kg kg^{-1}).

Table 5.12: Values of parental lines and mean, maximum, minimum and ANOVA significance results of the 97 SSD lines of Paragon x W141 mapping population, for N utilisation efficiency (NUTE, kg DM ha^{-1} N) N harvest index (NHI, kg kg^{-1}) and biomass production efficiency (BPE, kg DM kg^{-1} N).

	NUTE (kg DM kg^{-1} N)		NHI (kg kg^{-1})		BPE (kg DM kg^{-1} N)	
	HN	LN	HN	LN	HN	LN
Paragon	28.6	35.6	0.78	0.84	57.3	70.1
W141	27.3	34.2	0.76	0.81	65.1	83.5
Min	23.5	30.0	0.67	0.75	52.5	55.6
Max	30.6	42.1	0.81	0.87	67.8	90.3
Mean	27.8	36.0	0.76	0.82	59.5	77.1
N-trt.	0.007		0.03		0.005	
Genot.	<0.001		<0.001		<0.001	
N x G	<0.001		0.459		0.774	

Across years for the PxW481 population, the increase in grain yield with N supply (+18%) resulted from increased AGDM (+30%) whilst HI was almost stable. For PxW141, however, the increase in yield with N supply (+15%) resulted also from AGDM (+13%) whereas HI did not change under HN. Under HN, lines of PxW481 produced slightly more AGDM (22.9 t ha^{-1}) than PxW141 mapping population (20.7 t ha^{-1}); however, PxW141 seemed to produce more yield (9.71 t ha^{-1}) than PxW481 (9.15 t ha^{-1}). Under LN, PxW141 seemed to produce more AGDM and More yield (18.31 t ha^{-1} and 8.46 t ha^{-1} respectively) than PxW481 (17.73 t ha^{-1} and 7.78 t ha^{-1} respectively for AGDM and grain yield).

Table 5.13: Pearson's correlation coefficient in 97 SSD lines of PxW141 between Grain Yield (GY kg DM ha^{-1}), Harvest Index (HI), Above-ground Dry Matter (AGDM, kg DM ha^{-1}), N-Use Efficiency (NUE, kg DM kg^{-1} N), N-Uptake Efficiency (NUPE, kg kg^{-1}), N-Utilisation Efficiency (NUTE, kg kg^{-1}), Anthesis Date (AD), Plant Height (PH), Grains per m^2 (GM) and Thousand Grain Weight (TGW, g). Values based in 2013. The data are under HN (unshaded) and under LN (shaded). 2012 experiment

	GY	HI	AGDM	NUE	NUPE	NUTE	AD	PH	GM	TGW
GY		0.40***	0.71***	0.99***	0.76***	0.26*	0.18	-0.17	0.83***	-0.25*
HI	0.47***		-0.35***	0.39***	-0.14	0.77***	0.34**	-0.78***	0.66***	-0.67***
AGDM	0.69***	-0.29**		0.72***	0.89***	-0.32**	-0.05	0.42***	0.34**	0.24*
NUE	0.98***	0.48***	0.66***		0.77***	0.25*	0.19	-0.16	0.83***	-0.25*
NUPE	0.88***	0.14	0.86***	0.88***		-0.40***	0.00	0.28**	0.41***	0.21*
NUTE	0.39***	0.75***	-0.22*	0.44***	-0.03		0.27**	-0.65***	0.56***	-0.69***
AD	0.29**	0.30**	0.02	0.32**	0.14	0.39***		-0.26*	0.37***	-0.39***
PH	-0.29**	-0.75***	0.31**	-0.28**	-0.07	-0.45***	-0.21*		-0.57***	0.76***
GM	0.70***	0.65***	0.20	0.72***	0.50***	0.57***	0.47***	-0.67***		-0.74***
TGW	-0.21	-0.52***	0.23*	-0.26*	-0.03	-0.49***	-0.46***	0.64***	-0.83***	

Significance: *: P<0.005, **: P<0.001 and ***: P<0.001

5.4 Discussion

The genetic variation in grain yield, yield components, above-ground biomass and NUE components and responses to N availability are presently discussed. The traits determining genetic variation in grain yield and NUE in two mapping populations are considered in relation to the N response and the transgressive segregation of the traits.

5.4.1 Grain yield and yield components

In the present study, grain yield increased with N supply in all three experiments. It increased for PxW481 mapping population in both 2012 and 2013 and and for PxW141 mapping population. Large genetic variation in yield was found in each mapping population. Averaging across years for PxW481 MP, no SSD line had significantly higher yield than Paragon under HN; however, 13 SSD lines had similar grain yield. Under LN, two SSD lines, L481-48 and L481-76 , had higher grain yield than Paragon. The two lines maintained yield better under LN conditions because L481-48 maintained higher AGDM and higher grains per m^2 (, while L481-76 maintained higher AGDM and TGW than Paragon.

Regarding the PxW141 mapping population, three SSD lines, L141-83, L141-04 and L141-52, had higher grain yield than Paragon under HN. The three lines had higher AGDM than Paragon. In addition, L141-83 and L141-04 had higher grains m^{-2} than Paragon, while L141-52 had much higher TGW than Paragon. Under LN one SSD line, L141-46, had higher yield than Paragon associated with higher TGW.

For PxW481, yield was more positively correlated with grains m^{-2} under HN (and under LN than with grain weight. Regarding PxW141, grain yield was associated with grains m^{-2} under HN and under LN.

Grains m^{-2} (GM) is an indicator of grain sink size of the crop and is historically better associated with genetic progress in grain yield under optimal conditions than grain weight (GW) (Sayre et al. 1997; Shearman et al. 2005; Peltonen-Sainio et al. 2007; Savin and Slafer, 1991). In this study, the increase in grain yield under HN conditions for PxW481 was associated with the increase in GM. For the PxW141

mapping population, the increase in yield under HN was again associated with GM rather than GW.

5.4.1.1 Grain number per m^2 and grain weight

Grain number can be expressed as a product of ears m^{-2} (EM) and grain per ear (GE). During the wheat development cycle, EM is the first yield component to be determined, followed by GE and finally GW determined during grain filling period. However, yield components are not independent (Slafer, 2003); For example, GM is negatively correlated with GW and also GE is negatively correlated with EM.

The anthesis stage is considered as the transition from vegetative to reproductive growth, at which point grain filling starts. Before anthesis, the N supply has an effect on the grain sink capacity, mainly through increased GM determined by GE (Fisher, 2008) and EM (fertile shoot). Post-anthesis photosynthesis and pre-anthesis storage provide assimilates to the grain. For PxW481, anthesis date did not have any effect on grain yield under HN in both 2012 and 2013 and under LN in 2013, however, a negative relationship was found under LN in 2012.

In contrast, plant height (PH) did not have any effect on grain yield under HN and had a positive effect under LN conditions. For PxW141, PH had a positive effect on grain yield under HN, while no effect on grain yield under LN conditions. On the other hand, PH had negative effect on grain yield under HN, while no effect was observed under LN conditions. Plant height reduction leads to an increase in DM partitioning to the grain by reducing the competition from the stem resulting in greater ear and more grain per ear and higher harvest index (HI) (Reynolds et al. 1999; Peng et al. 2000; Riggs et al. 1981; Abeledo et al. 2002).

Genetic variation in grains m^{-2} was found to be strongly associated with grain yield (Austin et al. 1980; Slafer et al. 1990; Slafer et al. 1996) and determined in the period between 20-30 days before flowering and 10 days after anthesis. This period coincides with active stem elongation and rapid spike growth. Grain number, however, is not translated directly to the yield because of the grain weight compensation (Acevedo et al. 2002). In the present study, grains m^{-2} and TGW increased under HN in both 2012 and 2013 for PxW481 and for PxW141 in 2013.

Under HN conditions GM was better associated amongst SSD lines with GE than with EM. Under LN, GM was again more associated with GE than EM for PxW141; but for Px481 the relationship between GM and GE was similar to that between GM and EM for PxW481. Geleto et al. (1995) stated that N level and application time affect the ear population density through influencing tillering ability of the plant (Ayoub et al. 1994; Verma et al. 1993). Wheat plants require N for growth and development, allowing them to produce more grains per ear (Geleto et al. 1995; Ayoub et al. 1994; Slafer et al. 2003; Jan et al. 2000).

Grain weight is determined during the grain filling period. Amongst all yield components grain weight is the most stable component (Gallagher et al. 1975) and less affected by N supply (decrease with N supply) than grains m^{-2} (Siddique et al. 1989; Waddington et al. 1986). In 2012 for PxW481 there was a trend for a decrease of TGW under HN supply; however N did not have any significant effect on TGW in 2013. For the PxW141, GW decreased under HN for all SSD lines (N treatment x genotype interaction). Under optimal conditions at grain filling, once grain number is established, competition between grains for assimilates increased within the spike, which results in reduction in grain weight (source limitation). Slafer et al. (1996) argued that the lower grain weight observed with increased grain number is also the result of an increased number of grains with a lower weight potential coming from more distal florets.

5.4.1.2- Above-ground biomass and HI

Yield is the product of above-ground biomass and HI. In PxW481 averaging over years, grain yield was more strongly associated amongst SSD lines with AGDM than with HI. In PxW141 mapping population, genetic variation in grain yield was again more strongly associated with AGDM than HI. These results are generally consistent with findings by Donmez et al. (2001) who examined 13 winter wheat cultivars and found significant correlation between grain yield and both biomass and HI. Shearman et al. (2005) attributed yield increase in eight winter wheat varieties introduced in the UK between 1972 and 1995 to increased HI in those varieties introduced before 1980 and increased biomass in varieties introduced after 1983. It has been reported also that variation in grain yield was more strongly associated with variation in biomass than in HI by White at al. (2006) in 10 UK cultivars.

Averaging two years for PxW481, no SSD line had significantly higher AGDM than Paragon under HN. However under LN, two SSD lines had higher AGDM than Paragon. In addition, L481-76 had higher HI than Paragon while L481-48 and Paragon had similar HI.

For PxW141, five SSD lines had higher AGDM than Paragon under HN; while 10 lines had higher HI. Under LN, four SSD lineshad higher AGDM than Paragon, while four lineshad higher HI than Paragon.

Since harvest index for winter wheat is approaching its theoretical maximum value, in some countries and regions, which is 62% (Austin at al., 1982), above-ground biomass has been reported as the main target to improve grain yield. There is recent evidence of genetic gains in biomass in UK winter wheat (Shearman et al. 2005; Clarke et al. 2012).

Increasing HI can be achieved mainly by reducing the partitioning to the stem. Further small increases can be achieved, although HI is approaching its maximum (Foulkes et al. 2009). Another option for increasing HI is by stay-green trait. Stay-green in genotypes was associated with higher grain filling rate and duration increasing HI (Spano et al.2003). More discussion on senescence and a sty green will be presented in the next Chapter 6.

For both PxW481 and PxW141 mapping populations, no effect of anthesis date was observed either under HN or under LN. However, a positive relationship between plant height and AGDM was observed for PxW481 under LN in 2012, and under HN and LN in 2013. Similar results were found for PxW141 under HN and under LN.

5.4.2 N-use efficiency (NUE) and components

N uptake and utilisation can be analysed by two physiological models, the first concerns N uptake efficiency (NUPE) and N utilisation efficiency (NUTE), whereas the second includes grain yield (GY), N uptake, N harvest index (HNI) and grain N concentration (GNC):

$$NUE = NUPE \times NUTE$$

$$GY = \frac{NUP \times NHI}{GNC}$$

NUE is a complex trait because its two components (NUPE and NUTE) affect each other, and the relationship between them is variable and mainly affected by the environmental factors and N supply (Ortiz-Monasterio et al. 1997; Le Gouis et al. 2000). An ideal N-efficient wheat variety would acquire N from soil efficiently, and use the absorbed N efficiently to produce biomass and grain yield.

NUE decreased significantly under HN in all experiments (In 2012, NUE decreased for PxW481 in 2012 and in 2013 and for PxW141 in 2013). The NUE was similar in the two mapping populations under both HN and LN. The values of NUE in the present experiments are higher than the average NUE of 25 kg DM kg^{-1} N reported in UK by Sylvester-Bradley and Kindred, (2009). These differences are due first to the big gap usually found between experiment trials and farm fields, and secondly the present experiment was based on small plots (1.2 m^2) giving the ability to over-estimate yield, biomass and therefore NUE parameters due to egde effects.

For PxW481 the decrease in NUE between HN and LN was associated with NUTE, but not with the decrease in NUPE. For PxW141, the NUE response to N supply was associated with a decrease in both NUTE and NUPE.

Furthermore, for the three experiments, the genetic variation in NUE was more closely associated with NUPE than with NUTE under both LN and HN conditions. The relative contributions of NUPE and NUTE to NUE have been compared in previous studies (Moll et al. 1982; Kessel et al. 2012; Berry et al. 2010; Ortiz-Monasterio et al. 1997; LeGouis et al. 2000; Gaju et al. 2011; Liang et al. 2014). In maize, it has been reported that the genetic variation of NUE was due largely to variation in NUTE with low N supply and NUPE with high N supply (Moll et al. 1982). Hirel et al. (2007) confirmed the relationship by genetic studies on quantitative traits loci associated with NUE in maize. Gaju et al. 2011 found stronger association between NUE and NUTE than NUPE in winter wheat.

Present results showed that there was an association between NUPE and NUE at all three experiments almost equally under HN and LN. In contrast, the association between NUTE and NUE was significant at PxW481-2012 and PxW141-2013 under

both HN and LN, whereas no association was found for PxW481-2013 either under HN or under LN conditions. This result is consistent with NUE studies on durum wheat, bread wheat and rice (Ehdaie et al. 2001; Liang et al. 2014; Le Gouis et al. 2000; Singh et al. 1998; Ortiz-Monasterio et al. 1997; Dhugga and Waines, 1989).

Chapter 6: Physiological traits and associations with grain yield, biomass and NUE in Paragon x W481 and Paragon x W141 SSD mapping populations

6.1 Introduction

Above-ground dry matter is associated with genetic variation in grain yield in wheat (Reynolds et al. 2005) as found in both the PxW481 and PxW141 mapping populations (Chapter 5). In this chapter, results for traits underlying biomass including canopy size and flag-leaf senescence for PxW481 mapping population in 2011/12 and 2012/13 and for PxW141 mapping population in 2012/13 are presented and discussed. The relationship between the Normalized Deference Vegetation Index (NDVI) and and biomass is investigated.

Grain yield is limited by either the grain number per unit area or the potential weight per grain (sink) or the assimilate availability to fill these grains (source), whichever is the lower. The balance between grain sink (storage capacity of the grains to accumulate available carbohydrates) and grain source (supply of carbohydrates to grains) determines the final grain yield. Carbohydrates for grain filling are supplied by current photosynthetic activity post-anthesis and from carbohydrates accumulated in the stem and leaf sheath pre-anthesis and remobilized during grain filling. In wheat, grain growth under optimal conditions is generally shown to be sink-limited. Borras et al. (2004) in a review of responses to source sink manipulation treatments concluded that the yield of wheat is mainly limited by the size of the sink during grain filling. However, some studies on modern germplasm have reported that grain yield of wheat is co-limited by both the source and the sink (Beed et al. 2007; Kruk et al. 1997; Achreche et al. 2008. With regard to genetic variation in grain yield under low N, there is evidence for a strong correlation between onset of flag-leaf senescence and grain yield (Gaju et al. 2011).

With regard to N accumulation in the grain, a study by Ma et al. (1995) suggested that the accumulation of N in the grain may be largely source limited, i.e limited by N supply to grains during grain filling. The grain normally has an ability to store the entire translocatable N accumulated before anthesis (Borghi et al. 1986). However, under optimal N conditions, N in the grain may become co-limited by both supply and demand (Martre et al. 2006). Bogard et al. (2011) reported that post-anthesis flag

leaf senescence was associated with genetic variation in grain yield and grain N concentration in a doubled-haploid population of French winter wheat from the cross Toisondor X CF9107. Post-antheis senescence is a process allowing the translocation of N from vegetative tissues to grains. This translocation accounts for 40-90% of all grain N (Kichey et al. 2007). Leaf senescence is reported to be affected by the balance between N supply and N demand in sorghum (Triboi and Triboi-Blondel, 2002; van Oosterom et al. 2010). Accelerated senescence process could result from an inadequate balance between N demand and N availability: if N demand from grain is higher than source N from the soil, the N translocation occurs earlier leading to early senescence. Stay-green phenotypes and broader genetic variation in senescence have been reported in hexaploid wheat (Silva et al. 2000; Verma et al. 2004; Joshi et al. 2007; Christopher et al. 2008; Chen et al. 2010; Bogard et al. 2011; Chen et al. 2011; Gaju et al. 2011; Naruoka et al. 2012; Derkx et al. 2013). A better understanding of the mechanisms determining post-anthesis N remobilization and senescence and their genetic regulation in wheat offers scope to raise grain yield in feed wheats and to increase the stability of high GPC in bread-making cultivars, particularly under low N conditions.

The aims of the experiments reported in this chapter were:

1) To quantify novel genetic variation for physiological traits (flag leaf chlorophyll content (SPAD), flag leaf chlorophyll fluorescence (as an indicator of maximum rate of photosystem II), NDVI and flag leaf senescence parameters) and associations with grain yield, biomass, NUE and NUE components amongst SSD lines from the two crosses Paragon x W481 and Paragon xW141 under HN and LN conditions.

2) To increase the understanding of the physiological processes determining genetic variation in grain yield biomass, NUE and NUE components and associations with the NDVI and senescence parameters amongst SSD lines SSD lines of P x W481 and PxW141 populations under LN and HN conditions.

6.2 Methodology

6.2.1 Materials and experimental design

The results of three field experiments are included in this chapter: Seventy nine lines (and parents) of the Paragon x W481 SSD population in 2012 and in 2013 and 97 lines (and parents) of the Paragon x W141 SSD population in 2013. The experiments were carried out at Bunny Park, University of Nottingham Farm, Nottinghamshire. In each case, the experimental design was a split plot with two N treatments (HN = 200 kg N ha^{-1} and LN = 30 and 0 kg N ha^{-1} respectively in 2012 and 2013) and three replicates. The N treatment was randomised on main plots and genotypes on sub-plots. Each sub-plot comprised 1.2 m^2 (6 rows of 1 m of length with 20 cm between rows).

6.2.2 Crop measurements

6.2.2.1 Canopy size

In each experiment, Normalised Difference Vegetation Index (NDVI) was determined using a spectral radiometer (CROPSCAN: MSRSYS16R multispectral radiometer, Cropscan Inc, Rochester, MN, USA). The spectroradiometer measured reflectance at 16 wavelengths from 500 to 970 nm. The NDVI is calculated as: (NIR-VIS) / (NIR+VIS), where NIR is the reflectance at near infra-red wavelength (900 nm) and VIS is the reflectance at visual wavelength (680 nm). During 2011/12, two measurements were carried out, one at around anthesis (19 June) in one replicate and one post-anthesis (24 July) in two replicates of the PxW481 population in HN and Ln treatments. In 2013, four measurements in two replicates in both HN and LN treatments were carried out in each sub-plot on two populations PxW481 and PxW141. One measurement was pre-anthesis (06 June) and three post-anthesis (05, 17 and 27 July). Each measurement was taken on the same calendar day for the two mapping populations in 2013.

6.2.2.2 Flag leaf senescence

Senescence kinetics of the flag leaf were assessed visually by recording the percentage green area senesced using a standard diagnostic key based on a scale from 0 (no senescence) to 10 (full senescence) (Gaju et al. 2011). The assessment was carried out approximately weekly from anthesis until physiological maturity.

The data were then fitted against thermal time from anthesis (GS61; base temperature of 0 ◦C) using a modified version of an equation with five parameters consisting of a monomolecular and a logistic function (Génard et al. 1999 see Chapter 3, section 3.2.3.6). The fitted curves were discibed two phases of senescence: a slow phase and a rapid phase. In this chapter, data are presented for the duration of the first phase of senescence (i.e. the onset of the rapid phase of senescence; ONSEN), the duration of the rapid phase (SEN) and the total duration of the post-anthesis senescence (DURTOT).

6.2.2.3 Leaf photosynthetic activity

In 2013, two flag-leaf chlorophyll fluorescence measurements, one pre-anthesis and one post-anthesis, were carried out in both experiments (PxW481 and PxW141). The measurements were taken on 11 June and 5 July 2013 in each sub-plot in two replicates in HN and LN treatments. In addition, 5flag-leaf chlorophyll content (SPAD) measurements were taken in each sub-plot in two replicates in the HN treatment. The measurement was taken for each genotype on the date of reaching anthesis. For each measurement, assessments were carried out on the randomly selected fertile shoots (those with an ear) per sub-plot at GS61.

6.2.2.4 Dry matter per shoot and DM partitioning at anthesis

In each experiment in 2013, five fertile shoots (those with an ear) were sampled randomly at GS61 random per sub-plot by cutting at ground level in the HN treatment in each sub-plot in two replicates. The shoots were separated into three components: leaf lamina, stem (with attached leaf sheath) and ear. After drying for 48 hours at 80 °C, the weight of each component was recorded.

6.3 Results

6.3.1 Crop NDVI

6.3.1.1 PxW481 population 2012

At anthesis (19 June), NDVI increased from 0.80 under LN to 0.93 under HN; and SSD lines ranged from 0.88 (L481-58) to 0.95 (L481-55) under HN and from 0.74 (L481-32) to 0.85 (L481-55) under LN. The response to increasing N supply ranged

from 0.07 (L481-58) to 0.20 (L481-10). Under HN 34 SSD lines had higher NDVI than parental lines (Paragon 0.93 and W481 0.92), and under LN 47 lines had higher NDVI than Paragon (Paragon 0.80 and W481 0.76).

Post-anthesis (24 July), NDVI increased from 0.43 under LN to 0.60 under HN (P=0.014, Table 6.1). SSD lines ranged from 0.46 (L481-52) to 0.77 (L481-26) under HN and from 0.34 (L481-55) to 0.51 (L481-67) under LN (P<0.001); and responses to increasing N supply from 0.02 (L481-01) to 0.32 (L481-54) (P< 0.001). Under HN, two SSD lines, L481-09 (0.76) and L481-26 (0.77), had higher NDVI than the higher parent Paragon (0.70) (P<0.05). Under LN conditions, no SSD line had higher NDVI than Paragon (0.49) but 19 SSD lines had higher values than W481 (0.41) (P<0.05).

Table 6.1: NDVI at anthesis (GS61) on 19 June and post anthesis (Post_GS61) on 24 July .of parental lines and mean, maximum, minimum for HN and LN treatments of the 79 SSD lines of Paragon x W481 mapping population in 2011/12 and ANOVA significance. N/A = not/applicable, measurement in one replicate.

	NDVI GS61		NDVI Post GS61	
	HN	LN	HN	LN
Paragon	0.93	0.80	0.70	0.49
W481	0.92	0.76	0.55	0.41
Min	0.88	0.74	0.46	0.34
Max	0.95	0.85	0.77	0.51
Mean	0.93	0.80	0.60	0.43
Sig.				
N-trt. (N)	N/A		0.014	
Genot. (G)	N/A		<0.001	
N X G	N/A		<0.001	

Averaging over SSD lines, NDVI decreased between anthesis (19 June) and late grain filling (24 July 2012) by 0.33 under HN and by 0.37 under LN (Figure 6.1).

6.3.1.2- PxW481 mapping population 2013

N limitation in the low N treatment decreased NDVI at all measurement dates (P=0.028, P=0.042, P=0.044 and P=0.038 on 06 June, 05 July, 17 July, and 27 July respectively). Pre-anthesis, NDVI decreased from 0.89 under HN to 0.82 under LN (P<0.05) and lines ranged from 0.79 (W481-35) to 0.92 (W481-09, W481-20, W481-62 and W481-64) under HN and from 0.72 (W481-72 and W481-73) to 0.89

(W481-50) under LN (P<0.001, Table 6.2). Responses to increasing N supply ranged from -0.03 (L481-35) to +0.17 (L481-14) (P< 0.001). No SSD line had higher NDVI than the higher parent W481 (0.90), under HN or Paragon (0.87) under LN. However, three SSD lines: L481-35 (+0.03), L481-34 (-0.00) and L481-82 (-0.00) maintained NDVI better under LN than both Paragon (-0.03) and W484 (-0.04).

Post-anthesis (average for three measurements) NDVI decreased overall from 0.66 under HN to 0.58 under LN (P<0.05), and SSD lines ranged from 0.50 (W481-01 and W481-50) to 0.80 (W48-106) under HN and from 0.46 (W481-44, W481-46 and W481-72) to 0.70 (W481-67) under LN conditions (P<0.001, Table 6.2). Response to decreasing N supply ranged from -0.26 (L481-46) to +0.17 (L481-48) to (P< 0.001). Under HN, no SSD line had higher NDVI than the higher parent Paragon (0.76) under HN or the higher parent Paragon (0.65) under LN. Finally, two SSD lines, L481-21 (+0.24) and L481-46 (+0.26), maintained better NDVI under LN than Paragon (-0.11) and 10 lines better than W481 (-0.07) (P<0.05).

Table 6.2: NDVI of parental lines of the 79 SSD lines of Paragon x W481 mapping population and mean, maximum, minimum and ANOVA significance for HN and LN treatments in 2012/13 for NDVI pre-anthesis (Pre_A, 6 June) and post anthesis (Post_A, 5, 17, 27 July).

	Pre_A NDVI		**Post_A NDVI**	
	HN	LN	HN	LN
Paragon	0.89	0.87	0.76	0.65
W481	0.90	0.86	0.70	0.62
Min	0.79	0.72	0.50	0.46
Max	0.92	0.89	0.80	0.70
Mean	0.89	0.82	0.66	0.58
Sign.				
N-trt. (N)	0.028		0.035	
Genot. (G)	<0.001		<0.001	
N X G	<0.001		<0.001	

Averaging over PxW141 SSD lines, NDVI increased from 0.84 at around flag-leaf emergence (22 May) to 0.90 shortly before anthesis (06 June) under HN and from 084 to 0.87 under LN, respectively. After anthesis, NDVI decreased to 0.88 (5 July) then to 0.75 (15 July) and 0.54 (24 July) under HN and decreased to 0.84, 0.67 and 0.44, respectively under LN (Figure 6.1).

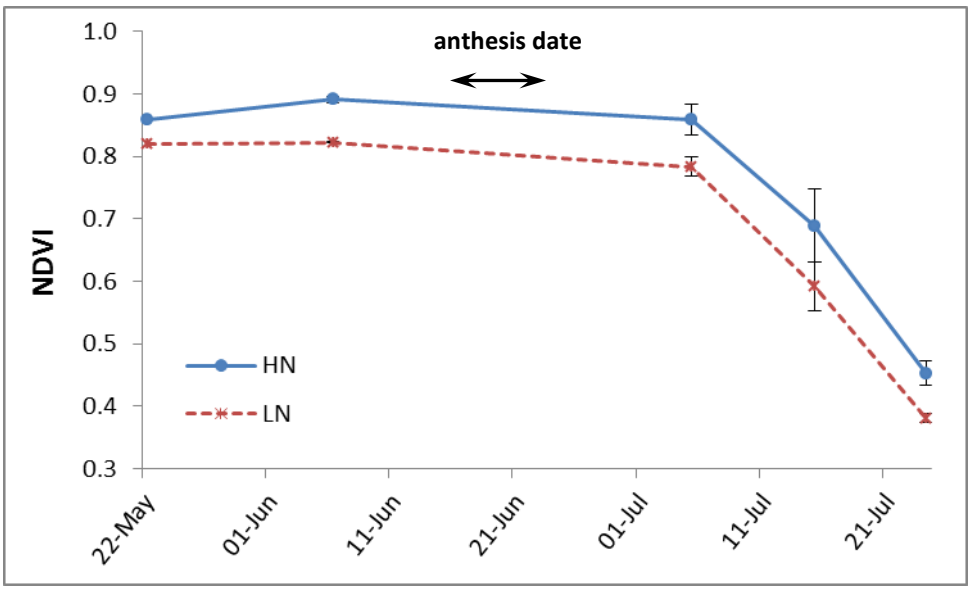


Figure 6.1: NDVI averaging over 79 SSD lines of PxW481, between 22 May and 24 July 2013 under HN (●) and LN (×).

Cross-year ANOVA showed that post-anthesis NDVI was higher in 2013 (0.66) than in 2012 (0.60) under HN conditions (+10%), and also under LN conditions (0.43 vs 0.58, respectively, +35%) (P<0.001). All the interactions (N treatment x genotype, N treatment x year, genotype x year and N treatment x year x genotype) were significant (P<0.001). Overall 21 lines maintained significantly higher post-anthesis NDVI under LN than both the parental lines. The NDVI response of these lines to N decrease ranged from -0.00 (L481-43) to - 0.17 (L481-48).

6.3.1.3 PxW141 mapping population 2013

N treatment did not have any effect on NDVI at the first two measurements; however, the N effect at the last two measurements was significant (P<0.05 and P<0.01, respectively). Pre-anthesis, SSD lines ranged from 0.87 (W141-33 and W141-36) to 0.93 (W141-81 and W141-96) under HN and from 0.84 (W141-36) to 0.86 (W141-01) under LN (P< 0.001).

Post-anthesis (average for three measurements) NDVI increased from 0.52 under LN to 0.64 under HN (P=0.031). SSD lines ranged from 0.45 (W141-39) to 0.77 (W141-84 and W141-93) under HN and from 0.39 (W141-60) to 0.64 (W141-49 and W14167) under LN (P<0.001, Table 6.3). Responses to increasing N supply ranged

from -0.10 (L141-82) to +0.32 (L141-93) (P = 0.06). Under HN, three SSD lines, L481-44 (0.76), L141-93 (0.77) and L141-84 (0.77), had significantly higher NDVI than the higher parent Paragon (0.67) (P<0.05). Under LN no lines showed higher NDVI than Paragon (0.58), whereas 22 SSD lines were significantly higher than W141 (0.48) (P<0.05).

Table 6.3: NDVI pre-anthesis (Pre_A) and post anthesis (Post_A).of parental lines and mean, maximum, minimum for HN and LN treatments of the 97 SSD lines of Paragon x W141 mapping population in 2012/13 and ANOVA significance.

	Pre_A NDVI		**Post_A NDVI**	
	HN	LN	HN	LN
Paragon	0.90	0.86	0.67	0.58
W141	0.90	0.88	0.58	0.48
Min	0.87	0.79	0.45	0.39
Max	0.93	0.90	0.77	0.64
Mean	0.90	0.86	0.65	0.52
P values				
N-trt.	0.206		0.031	
Genot.	0.435		<0.001	
N X G	0.351		0.006	

Averaging over all SSD lines, NDVI increased slightly from 0.86 pre-anthesis (22 May) to 0.89 (06 June) under HN whilst it was stable between the two measurements under LN (0.82). NDVI was then stable up to 5 July (mid grain filling) in both N treatments, After mid grain filling, NDVI decreased to 0.86 (5 July) then to 0.69 (15 July) and finally to 0.45 (24 July) under HN. Under LN, overall NDVI decreased to 0.78 (5 July) then to 0.59 (15 July) and finally to 0.38 (24 July (Figure 6.3).

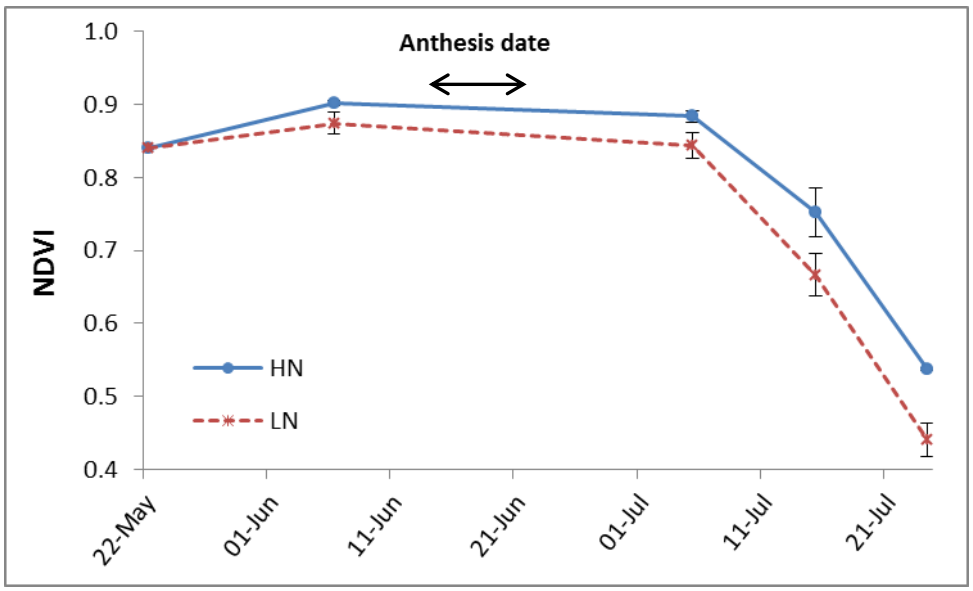


Figure 6.2: NDVI averaged over 97 SSD lines of PxW481, between 22 May and 24 July 2013 under HN (●) and LN (×).

6.3.2 Relationships between NDVI and GY, AGDM and NUP

6.3.2.1 PxW481 mapping population 2012

In the present study, post-anthesis NDVI was more strongly associated amongst SSD lines with grain yield under HN (R^2=0.21, P<0.001) than under LN (R^2=0.07, P<0.001, Figure 6.3a). Similarly, post-anthesis NDVI was more strongly associated with AGDM under LN (R^2=0.12, P<0.001,) than under HN (R^2=0.09, P<0.01 Figure 6.3b). The relationship between post-anthesis NDVI and NUP amongst SSD lines was also stronger under LN (R^2=0.16, P<0.001) than under HN conditions (R^2=0.05, P<0.05, Figure 6.3 c). However, post-anthesis NDVI was not associated with NUTE either under HN or under LN conditions. No relationship was found between pre-anthesis NDVI and NUTE, while pre-anthesis NDVI was associated with NUP only under HN conditions (Figure 6.3 d).

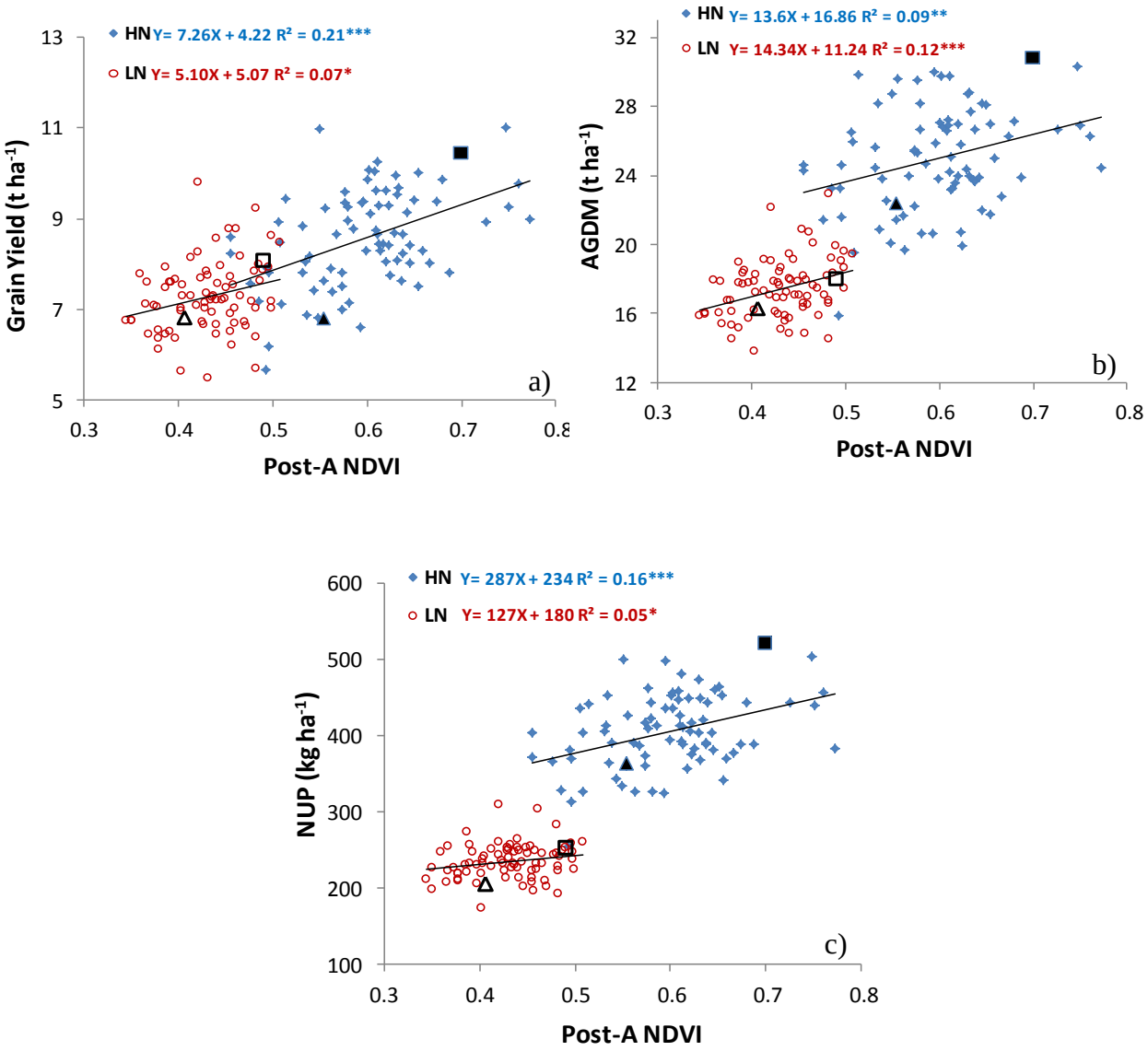


Figure 6.3: Linear regression between post-anthesis NDVI (post-A NDVI) and a) grain yield (100% DM), b) above-ground biomass (AGDM, 100% DM) and c) Above-ground N uptake (kg ha^{-1}) amongst 79 Paragon x W481 mapping population lines in HN (◆) and LN (○) treatments in 2011/12. The parental lines are indicated by: ■ Paragon under HN, ▲ W481 under HN, □ Paragon under LN and △ W481 under LN.

6.3.2.2 PxW481 mapping population 2013

For the PxW481 population in 2013, post-anthesis NDVI was more strongly associated amongst SSD lines with grain yield under LN (R^2=0.32, P<0.001) than under HN (R^2=0.20, P<0.001, Figure 6.4a). Similarly, post-anthesis NDVI was more strongly associated with AGDM under LN (R^2=0.28, P<0.001, Figure 6.4 b) than under HN (R^2=0.20, P<0.001). Similar effects were observed for the relationship between NDVI and NUP under LN (R^2=0.33, P<0.001) and under HN (R^2=0.25, P<0.001, Figure 6.4c). Post-anthesis NDVI was negatively correlated with NUTE under LN (R^2 =0.05, P< 0.05); however no significant association was found under HN.

A correlation matrix showing the associations amongst the 79 SSD lines between NDVI measurements, NUE components, grain yield and AGDM is shown in Table 6.4 for both LN and HN conditions. Under LN conditions, grain yield was positively correlated with the four NDVI measurements (P<0.001, and r = 0.50, 0.56, 0.59, and 0.46 respectively for measurements on 04 June, 05, 15 and 24 July). Grain yield was also correlated with NUPE and AGDM (P<0.001,. r=0.93 and 0.96 respectively) and correlated with NUE (P<0.05, r=0.27).

Similarly, AGDM was positively correlated with the four NDVI measurements (P<0.001, and r = 0.50, 0.53, 0.56, and 0.43 respectively for measurements in 04 June, 05, 15 and 24 July). AGDM was also correlated with NUPE (P<0.001. r=0.90).

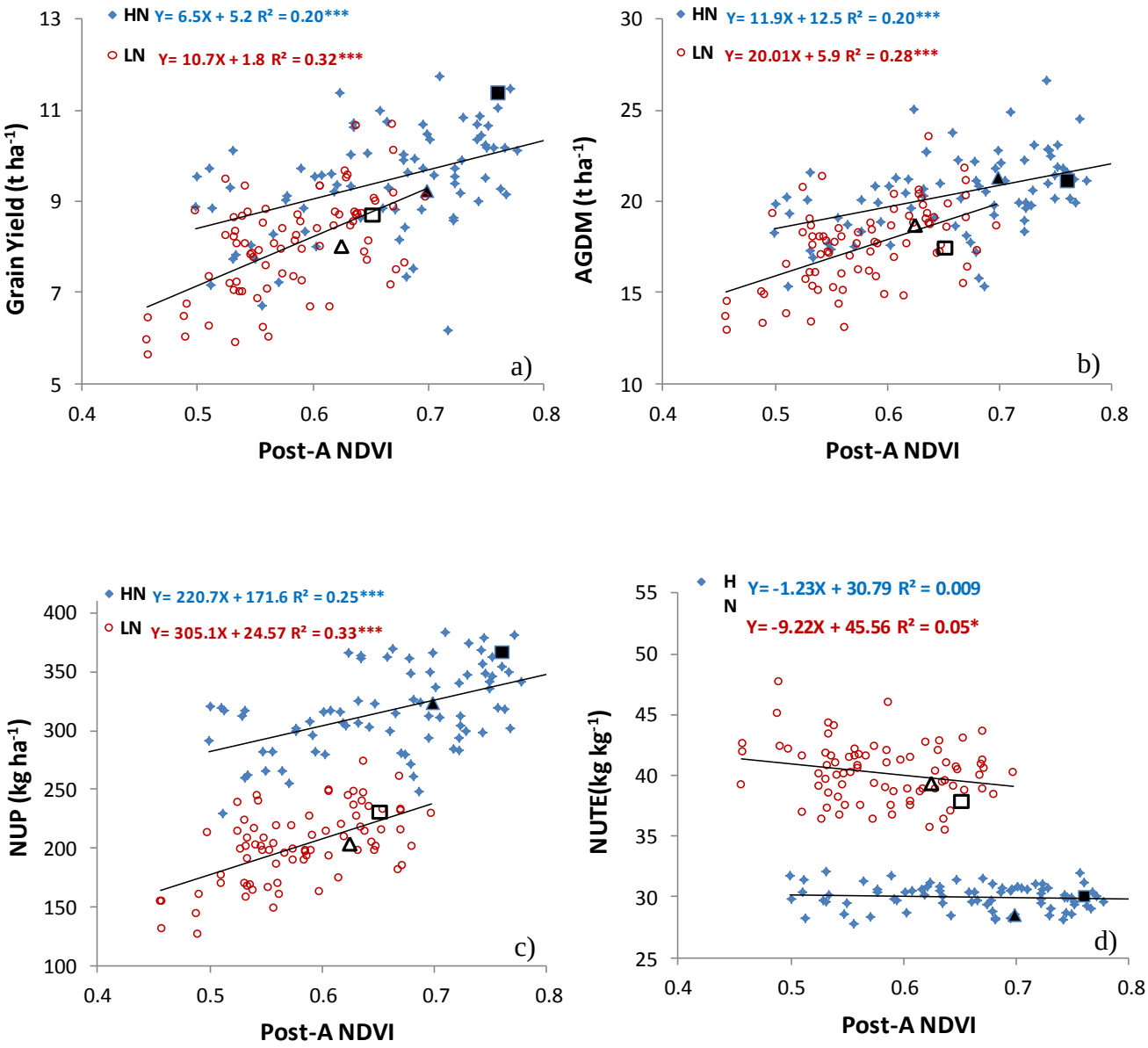


Figure 6.4: Linear regression between post-anthesis NDVI (post-A NDVI) and a) grain yield (100% DM), b) above-ground biomass (AGDM, 100% DM) and c) NUP (kg ha^{-1}) and d) NUTE amongst 79 Paragon x W481 mapping population lines in HN (◆) and LN (○) treatments in 2012/13. The parental lines are indicated by: ■ Paragon under HN, ▲ W481 under HN, □ Paragon under LN and △ W481 under LN.

Table 6.4: Pearson's correlation coefficient for 79 SSD lines of the PxW481 population under HN (Unshaded) and LN (shaded) treatments in 2013 for grain yield (100% DM), AGDM: above-ground dry matter, NUPE: N-uptake efficiency, NUTE: N-utilisation efficiency, and NDVI on 04 June, 05 July, 15 July and 24 July 2013.

	NDVI 04 Jun	NDVI 05 Jul	NDVI 15 Jul	NDVI 24 Jul	NUE	NUPE	NUTE	Yield	AGDM
NDVI 04 Jun		0.82***	0.68***	0.52***	0.24*	0.47***	-0.09	0.50***	0.50***
NDVI 05 Jul	0.58***		0.89***	0.73***	0.26*	0.56***	-0.19	0.56***	0.53***
NDVI 15 Jul	0.54***	0.92***		0.82***	0.19	0.62***	-0.27*	0.59***	0.56***
NDVI 24 Jul	0.29*	0.68***	0.75***		0.11	0.46***	-0.16	0.46***	0.43***
NUE	0.29**	0.24*	0.17	0.01		0.24*	0.06	0.27*	0.19
NUPE	0.53***	0.62***	0.57***	0.30**	0.20		-0.47***	0.93***	0.90***
NUTE	-0.08	-0.07	-0.08	-0.13	0.32**	-0.10		-0.12	-0.13
Yield	0.45***	0.55***	0.52***	0.26*	0.21	0.91***	0.16		0.96***
AGDM	0.55***	0.59***	0.54***	0.20	0.23*	0.91***	0.03	0.85***	

Significance: *: P<0.005, **: P<0.001 and ***: P<0.001

6.3.2.3 PxW141 mapping population

For the PxW141 mapping population, post-anthesis NDVI was more strongly associated amongst SSD lines with grain yield under LN (R^2=0.21, P<0.001) than under HN (R^2=0.12, P<0.001, Figure 6.5 a). Post-anthesis NDVI showed positive associations with AGDM under LN (R^2=0.07, P<0.01) and under HN (R^2=0.05, P<0.05, Figure 6.5 b), but associations were less strong than with grain yield. Post-anthesis NDVI was associated with NUP under both HN (R^2=0.14, P<0.001) and LN conditions (R^2=0.13, P<0.001, Figure 6.5 c) conditions. However, post-anthesis NDVI was not associated with NUTE either under HN or LN conditions.

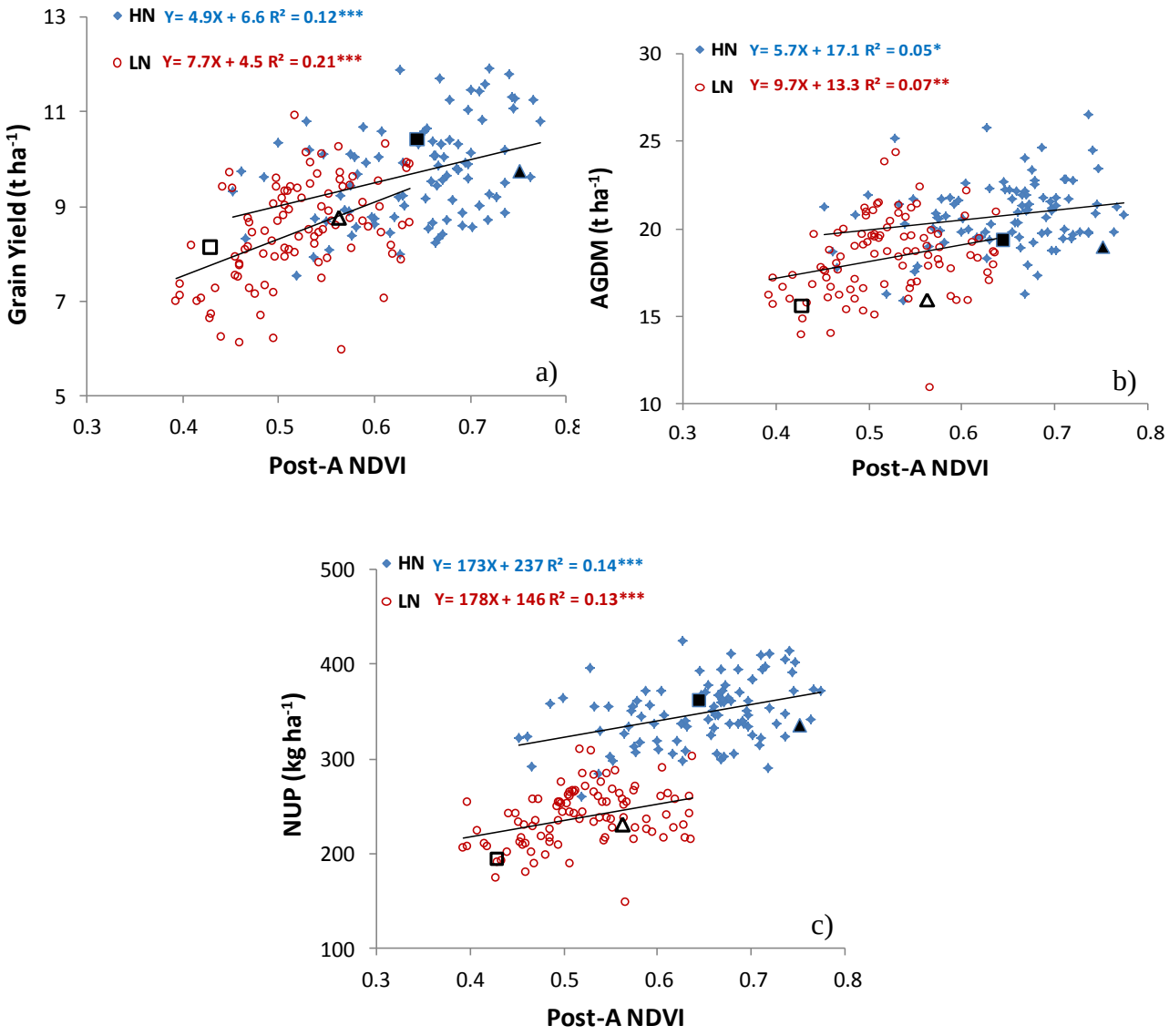


Figure 6.5: Linear regression between post-anthesis NDVI (post-A NDVI) and a) grain yield (100% DM), b) above-ground biomass (AGDM, 100% DM) and c) N uptake (kg ha^{-1}) amongst 97 Paragon x W141 SSD lines in HN (◆) and LN (○) conditions in 2012/13. The parental lines are indicated by: ■ Paragon under HN, ▲ W141 under HN, □ Paragon under LN and △ W141 under LN.

Table 6.5: Pearson's correlation coefficient in 97 SSD lines of PxW141 population between grain yield (100% DM), AGDM: above-ground dry matter, NUPE: N uptake efficiency, NUTE: N utilisation efficiency, and NDVI in high N (HN unshaded) and low N (LN shaded) conditions in 2013 in four different dates: 04 Jun, 05 July, 15 July and 24 July 2013.

	NDVI 04 Jun	NDVI 05 Jul	NDVI 15 Jul	NDVI 24 Jul	NUE	NUPT	NUTE	Yield	AGDM
NDVI 04 Jun		0.62***	0.31**	0.14	0.30**	0.46***	-0.27**	0.30**	0.49***
NDVI 05 Jul	0.58***		0.78***	0.61***	0.41***	0.47***	-0.13	0.41***	0.50***
NDVI 15 Jul	0.41***	0.76***		0.86***	0.51***	0.39***	0.16	0.51***	0.36**
NDVI 24 Jul	0.31**	0.48***	0.67***		0.34***	0.27**	0.11	0.34***	0.20
NUE	0.44***	0.48***	0.42***	0.30**		0.78***	0.29**	0.99***	0.73***
NUPE	0.50***	0.47***	0.41***	0.28**	0.89***		-0.36***	0.77***	0.89***
NUTE	-0.03	0.13	0.12	0.08	0.44***	-0.03		0.30**	-0.27**
Yield	0.45***	0.47***	0.39***	0.27**	0.98***	0.89***	0.39***		0.72***
AGDM	0.41***	0.45***	0.30**	0.15	0.68***	0.86***	-0.22*	0.69***	

Significance: *: P<0.005, **: P<0.001 and ***: P<0.001

6.3.3 Flag leaf senescence

6.3.3.1 PxW481 mapping population 2012/13

The onset of the rapid senescence phase (ONSEN) was advanced from 392°Cd under HN conditions to 308 (°Cd after GS61) under LN conditions (P<0.05; Table 6.6). Amongst SSD lines, ONSEN ranged from 161°Cd°Cd (L481-25) to 854°Cd (L481-36) under HN and from 161°Cd (L481-77) to 854°Cd (L481-56) under LN (P<0.001) conditions. Responses to decreased N availability ranged from -502°Cd (L481-43) to +216°Cd (L481-25) (P< 0.001). There were 16 SSD lines with higher ONSEN than W481 (377°Cd) and Paragon (377°Cd) (P<0.05). However, under LN, only two SSD lines L481-32 (754°Cd) and L481-56 (854°Cd) had significantly higher ONSEN than the higher parent Paragon (377°Cd)= (P<0.05). Eleven SSD lines maintained ONSEN better under LN than the better stay-green parent Paragon (+0°Cd) (P<0.05).

The total duration of post-anthesis flag-leaf senescence (DURTOT) increased from 615°Cd under LN to 708°Cd under HN (P = 0.020; Table 6.6). SSD lines ranged from 399°Cd (L481-49) to 1376°Cd (L481-05) under HN and from 395°Cd to 794°Cd under LN (P<0.001). Responses to increased N ranged from -239°Cd (L481-49) to +453°Cd (L481-26)°Cd (L481-56) (P< 0.001). Under HN, six SSD lines had significantly higher DURTOT than the higher parent W481 (735 ^{o}Cd (P<0.05). Under LN, no SSD line had higher DURTOT than the higher parent Paragon (709°Cd).

N treatment did not have any effect on the duration of the rapid phase of senescence (SEN) (P=0.482). Averaging over the HN and LN treatments, SSD lines ranged from 164°Cd (L481-26) to 468°Cd (L481-46) (P<0.001). Responses to increasing N availability ranged from -297°Cd (L481-43) to +323°Cd (L481-44) (P = 0.002). One SSD lines: L481-46 (468°Cd) had significantly higher SEN than the higher parent Paragon (341°Cd) (P<0.05).

Table 6.6: Flag-leaf senescence parameters of parental lines and mean, maximum, and minimum of 79 SSD lines of Paragon x W481 for HN and LN treatments in 2012/13 mapping population and ANOVA significance: onset of senescence (ONSEN,°Cd) senescence duration of the second phase (SEN,°Cd) and total duration of the senescence (DURTOT,°Cd).

	ONSEN °Cd		SEN °Cd		DURTOT °Cd	
	HN	LN	HN	LN	HN	LN
Paragon	377.0	377.0	351.0	332.0	728	709.0
W481	377.0	252.0	357.5	242.0	734.5	624.0
Min	161.0	161.0	144.0	120.0	399.0	395.0
Max	854.0	854.0	583.0	490.0	1376	794.0
Mean	392.4	307.7	296.8	324.3	707.7	614.8
N trt Prob.	0.038		0.482		0.020	
Gen. Prob.	<0.001		<0.001		<0.001	
N X GProb.	<0.001		0.002		<0.001	

6.3.3.2 PxW141 mapping population

The onset of the rapid senescence phase (ONSEN) was advanced from 431°Cd under HN to 366°Cd under LN (P<0.05; Table 6.7). Amongst SSD lines, ONSEN ranged from 252°Cd (L141-39 and L481-58) to 687°Cd (L141-94) under HN and from 252°Cd (L141-58) to 854°Cd (L141-47) under LN (P<0.01). Responses to reduced N ranged from +167°Cd (L141-04) to -373°Cd (L141-49) (P< 0.001). Under HN, one SSD line (L141-49) had higher ONSEN than either W141 (448.5°Cd) or Paragon (448.5°Cd) (P<0.05). Under LN also one line had higher ONSEN than the higher parent Paragon (520°Cd) (P<0.05). No SSD line maintained higher ONSEN under LN than W141 (+196.5°Cd) whilst 13 maintained higher ONSEN than Paragon (-71.5°Cd) (P<0.05).

For PxW141, N treatment did not have any effect on the total duration of leaf senescence (DURTOT); 686°Cd under LN vs 696°Cd under HN (P=0.671; Table 6.7). Averaging over HN and LN treatments, SSD lines ranged from 500°Cd (L141-56) to 818°Cd (L141-49) (P<0.05). Two SSD lines, L141-56 (+390°Cd), L141-60 (+386°Cd) maintained higher DURTOT than both W141 (+133°Cd) and Paragon (+57°Cd).

N treatment did not affect the duration of the rapid phase of senescence (SEN) (P=0.208), although there was decrease from 324 under HN to 266 under LN°Cd. Averaging over HN and LN treatments, all SSD lines ranged from 198°Cd (L141-

06) to 637°Cd (L141-51) (P<0.001). The ability to maintain SEN values under LN ranged from -126 (L141-21) to +308 (L141-11) (P=0.003).

Table 6.7: Flag-leaf senescence parameters of parental lines and mean, maximum, and minimum of 97 SSD lines of Paragon x W141 mapping population for HN and LN treatments and ANOVA significance: onset of senescence (ONSEN,°Cd) senescence duration of the second phase (SEN,°Cd) and total duration of the senescence (DURTOT,°Cd).

	ONSEN °Cd		SEN °Cd		DURTOT °Cd	
	HN	LN	HN	LN	HN	LN
Paragon	448.5	520.0	298.5	170.0	747.0	690.0
W141	448.5	252.0	287.0	351.0	735.5	603.0
Min	252.0	252.0	136.0	152.0	486.0	297.0
Max	687.0	854.0	697.0	545.5	826.0	964.0
Mean	430.6	365.6	268.7	325.6	695.5	685.8
N-trt.	0.024		0.208		0.591	
Genot.	<0.001		0.029		<0.001	
N X G	<0.001		0.004		<0.001	

6.3.4 Relationships between harvest traits and leaf senescence

6.3.4.1 PxW481 mapping population

For 2013, Pearson's correlation coefficient amongst SSD lines between senescence-related traits in the PxW481 population (ONSEN, SEN, and DURTOT) and grain yield, NUPE and NUTE are shown in Table 6.8. Under HN, no significant correlations were found between any of the senescence-related traits and grain yield and NUE traits (Figue 6.6). However, under LN a positive correlation between SEN and grain yield (r = 0.35, P=0.001) and between DURTOT and grain yield (r = 0.46, P<0.001) was found. In addition, a positive correlation was found between SEN and NUP (r = 0.40, P<0.001) and between DURTOT and NUP (r = 0.49, P<0.001).

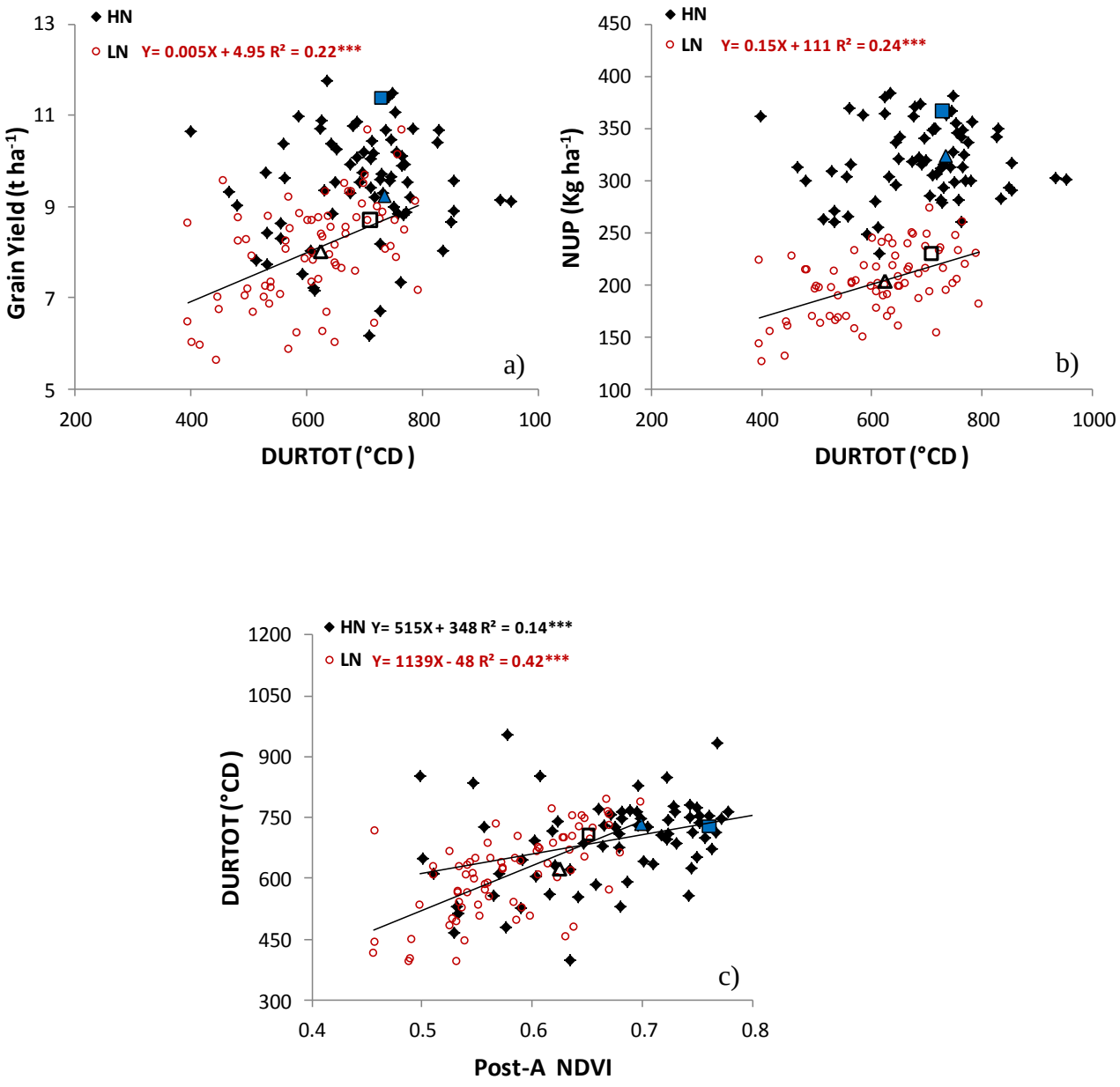


Figure 6.6: Linear regression between a) grain yield (100% DM) and total duration of senescence (DURTOT), b) N uptake (NUP, kg ha^{-1}) and DURTOT and c) N uptake (kg ha^{-1}) and post-anthesis NDVI (post-A NDVI) amongst 79 Paragon x W481 SSD lines in HN (◆) and LN (O) treatments in 2013.The parental lines are indicated by: ■ Paragon under HN, ▲ W481 under HN, □ Paragon under LN and △ W481 under LN.

For the PxW481 population, post-anthesis NDVI was more strongly associated with grain yield under LN (r=0.57, P<0.001) than under HN (r=0.47, P<0.001). Similarly, post-anthesis NDVI was better associated with AGDM under LN (r=0.53, P<0.001) than under HN (r=0.46, P<0.001). Post-anthesis NDVI was also better associated amongst SSD lines with ONSEN, SEN and DURTOT under LN (r=0.38, P<0.001; r=0.42, P<0.001 and r=0.65, P<0.001 respectively) than under HN (r=0.32, P<0.001; r=0.01, NS and r=0.38, P<0.001, respectively) (Table 6.8).

Table 6.8: Pearson's correlation coefficient amongst 79 SSD lines of Paragon x W481 for grain yield (Yield, 100% DM), AGDM: above-ground dry matter, NUPE: N-uptake efficiency, NUTE: N-utilisation efficiency, ONSEN: onset of post-anthesis rapid phase of senescence, SEN: duration of the post-anthesis rapid phase of senescence, DURTOT: total duration of post-anthesis senescence, PH: plant height, AD: date of anthesis (GS61) and GNC: grain N concentration (GNC). The values are based on means in 2013 with HN (Unshaded) and LN (Shaed)

	Yield	AGDM	NUE	NUPE	NUTE	ONSEN	SEN	DURTOT	NDVI	PH	AD	GNC
Yield		0.97***	1.00***	0.93***	-0.12	0.12	0.35**	0.45***	0.57***	0.31**	0.15	0.13
AGDM	0.85***		0.97***	0.90***	-0.13	0.10	0.33**	0.40***	0.53***	0.34**	0.17	0.10
NUE	1.00***	0.91***		0.93***	-0.12	0.13	0.35**	0.45***	0.57***	0.31**	0.15	0.13
NUPE	0.91***	0.91***	0.96***		-0.47***	0.11	0.40***	0.49***	0.58***	0.27**	0.16	0.46***
NUTE	0.16	0.02	0.18	-0.11		-0.01	-0.27*	-0.31**	-0.22*	0.02	-0.07	-0.94***
ONSEN	0.14	0.11	0.15	0.14	0.07		-0.17	0.43***	0.20	0.12	0.14	0.02
SEN	-0.11	-0.12	-0.12	-0.1	-0.13	-0.23*		0.80***	0.42***	0.18	0.19	0.24*
DURTOT	0.08	0.06	0.09	0.09	-0.01	0.70***	0.21		0.62***	0.15	0.27*	0.27*
NDVI	0.49***	0.47***	0.52***	0.53***	-0.11	0.32**	-0.03	0.36**		0.33**	0.50***	0.17
PH	0.13	0.30**	0.18	0.13	0.15	0.09	-0.08	-0.01	0.12		0.24*	0.03
AD	0.05	0.19	0.1	0.12	-0.09	-0.02	0.19	0.09	0.17	0.12		0.06
GNC	-0.07	-0.14	-0.12	0.1	-0.77***	-0.06	0.06	-0.03	-0.05	-0.24*	-0.16	

Significance: *: P<0.005, **: P<0.001 and ***: P<0.001

6.3.4.2 PxW141 mapping population 2013

The correlations amongst SSD lines between senescence-related traits in the PxW141 population (ONSEN, SEN, DURTOT, GY, NUPE and NUTE) are shown in Table 6.9. Under HN, ONSEN was positively correlated with grain yield (r = 0.25, P=0.012) and with NUPE (r = 0.30, P=0.002) and DURTOT was positively correlated with grain yield (r = 0.23, P=0.021) and with NUPE (r = 0.23, P=0.023). However, no significant correlation was found between SEN and grain yield or NUPE or between the three senescence parameters and NUTE.

Under LN, ONSEN was positively correlated with grain yield (r = 0.29, P = 0.004) and NUPE (r = 0.25, P = 0.013). The correlation between DURTOT and each of yield and NUPE was significant (r = 0.29, P = 0.003 and r = 0.22, P = 0.03, respectively). The correlation was not significant between SEN and yield and NUPE or between senescence traits and NUTE.

Under HN, post-anthesis NDVI (PoA_NDVI) was more strongly correlated with DURTOT (r = 0.59, P<0.001) than ONSEN (r = 0.48, P=0.001); the correlation with SEN was not significant. Under LN, PoA_NDVI was again highly positively correlated with DURTOT (r = 0.60, P<0.001) and ONSEN (r = 0.59, P<0.001); no significant relationships were found with SEN.

Under HN, similar to the PxW481 mapping population, no correlation was found between senescence traits (ONSEN, SEN and DURTOT) and TGW. However under LN, TGW was negatively correlated with DURTOT (r = -0.28, P<0.01) and with ONSEN (r = -.021, P<0.005). Therefore, overall as for PxW481, senescence traits for the PxW141population were more strongly associated with post-anthesis NDVI under LN than under HN.

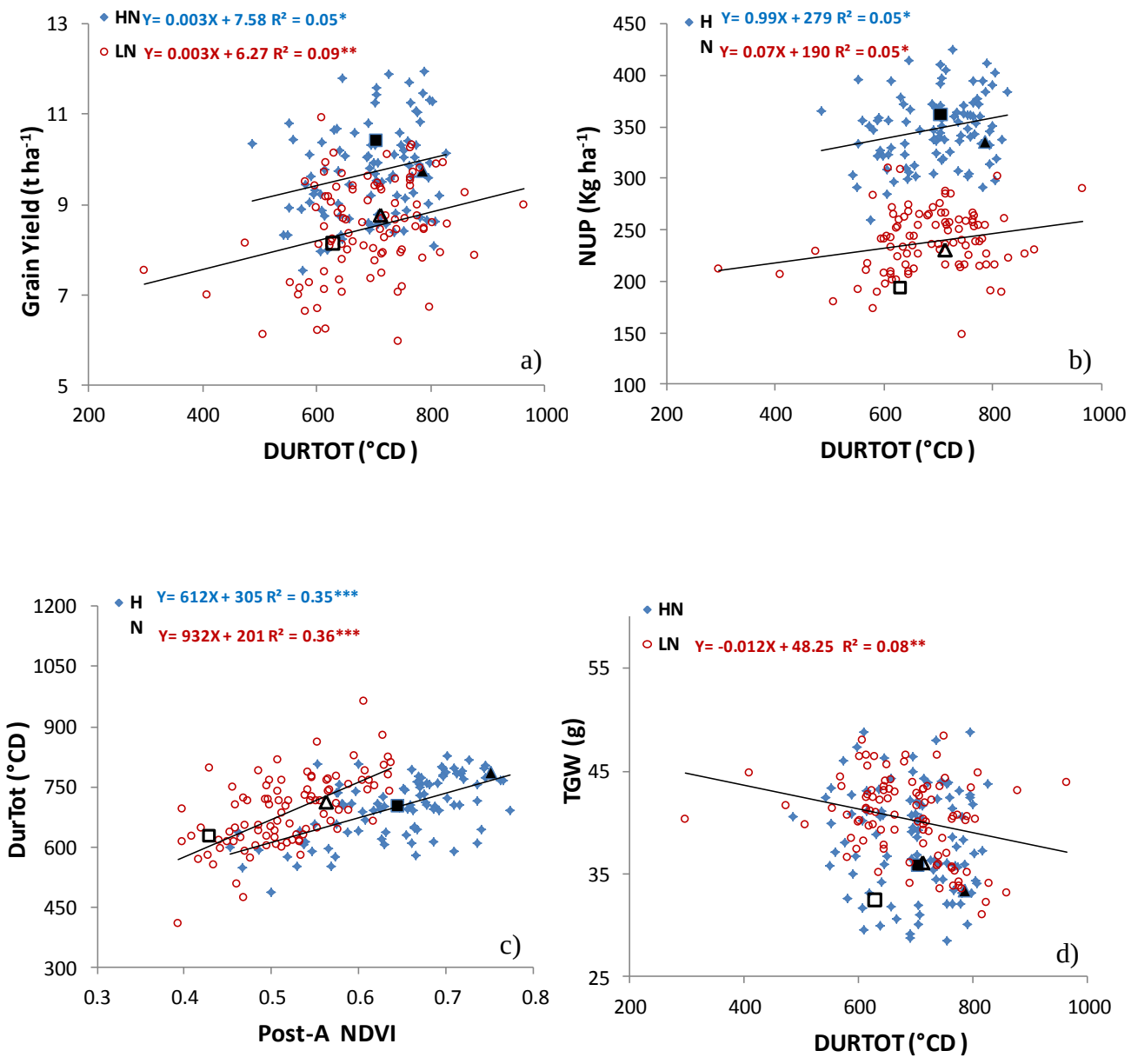


Figure 6.7: Linear regression between a) grain yield (100% DM), and total duration of senescence (DURTOT), b) N uptake (NUP, kg ha^{-1}) and DURTOT, c) DURTOT and post-anthesis NDVI (post-A NDVI) and d) 1000 grain weight and DURTOT amongst 97 Paragon x W141 SSD lines in 2013 lines in HN (◆) and LN (○) conditions. The parental lines are indicated by: ■ Paragon under HN, ▲ W141 under HN, □ Paragon under LN and △ W141 under LN.

Averaging over SSD lines, flag-leaf senescence score against thermal time is shown in Figure 6.8 for the PxW481 and PxW141 populations.

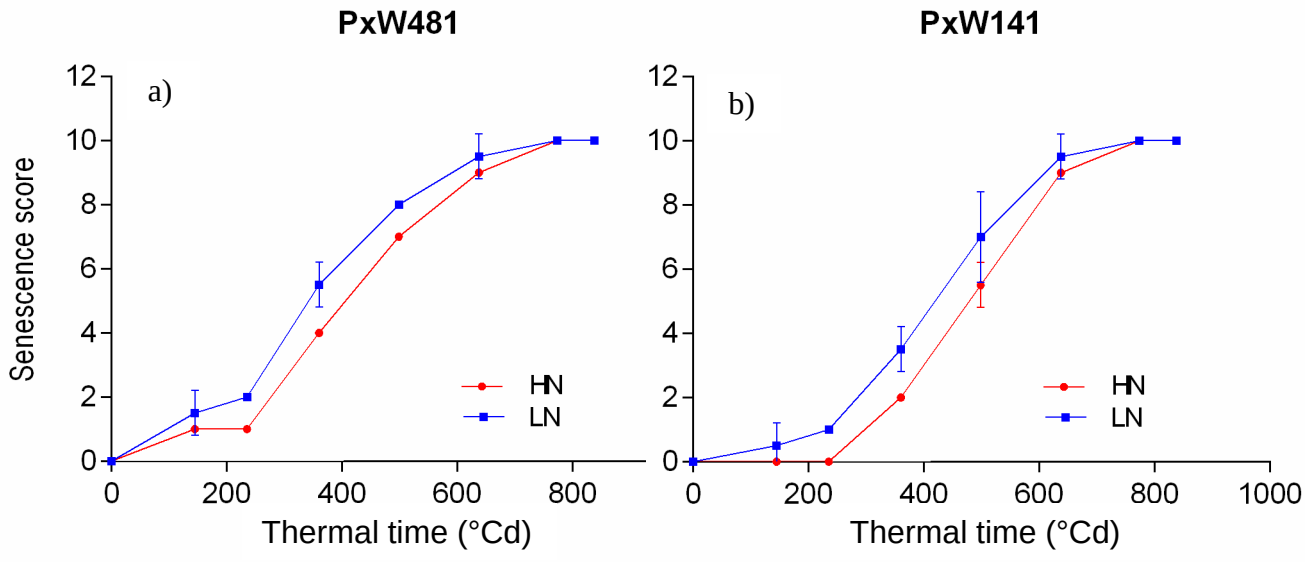


Figure 6.8: Post-anthesis flag-leaf senescence scores against thermal time post-anthesis (GS61)(°Cd) in in HN and LN for a) PxW481 PxW481 b) PxW141 mapping population in 2013

Table 6.9: Pearson's correlation coefficient for 97 SSD lines of PxW141 in 2013 between grain yield, AGDM: above-ground dry matter, NUPE: N-uptake efficiency, NUTE: N-utilisation efficiency, ONSEN: onset of the rapid flag-leaf post-anthesis senescence phase, SEN: duration of the rapid flag-leaf post-anthesis senescence phase DURTOT: total duration of flag-leaf senescence, PH: plant height and AD; date of anthesis (GS61). HN (Unshaded) and Ln (shaded)

	Yield	AGDM	NUPE	NUTE	ONSEN	SEN	DURTOT	NDVI	PH	AD	GNC
Yield		0.71***	0.77***	0.29**	0.29**	-0.01	0.29**	0.45***	-0.18	0.22*	-0.27**
AGDM	0.69***		0.89***	-0.29**	0.13	0.00	0.12	0.27**	0.40***	-0.01	0.25*
NUPE	0.88***	0.86***		-0.38***	0.25*	0.01	0.22*	0.36***	0.27**	0.02	0.36***
NUTE	0.38***	-0.23*	-0.04		0.06	0.00	0.13	0.12	-0.65***	0.30**	-0.96***
ONSEN	0.25*	0.19	0.31**	0.03		-0.23*	0.54***	0.59***	-0.18	0.31**	-0.10
SEN	0.02	-0.01	0.01	-0.07	-0.16		0.42***	0.01	-0.05	-0.05	-0.06
DURTOT	0.26*	0.16	0.26*	0.06	0.71***	0.36***		0.60***	-0.22*	0.34***	-0.18
NDVI	0.34***	0.20*	0.36***	0.10	0.48***	0.15	0.63***		-0.12	0.54***	-0.16
PH	-0.32**	0.27**	-0.11	-0.44***	-0.07	-0.01	-0.08	-0.15		-0.23*	0.65***
AD	0.31**	0.05	0.17	0.37***	0.23*	-0.16	0.19	0.33***	-0.18		-0.37***
GNC	-0.27**	0.07	0.04	0.04	0.06	0.07	0.02	-0.07	0.29**	-0.34***	

Significance: *: P<0.005, **: P<0.001 and ***: P<0.001

6.3.5 Leaf photosynthetic activity

6.3.5.1 Leaf chlorophyll fluorescence PxW481

Post-anthesis chlorophyll fluorescence was overall slightly higher than at anthesis (P<0.001), increasing from 0.64 pre-anthesis to 0.67 post-anthesis under HN and from 0.59 to 0.61, respectively, under LN.

For the PxW481 population in 2013, N treatment did not have a significant effect on flag-leaf chlorophyll fluorescence (Quantum yield: QY) at around anthesis (11 June 2013) (P=0.288). SSD lines ranged from 0.53 (L481-06) to 0.71 (L481-34) under HN and from 0.32 (L481-53) to 0.69 (L481-75) under LN (P<0.001). Response to increasing N supply ranged from -0.14 (L481-35) to +0.32 (L481-23) (P< 0.001).

Post-anthesis (5 July 2013), even though QY increased overall from 0.60 to 0.67, no significant effect of N treatment on QY was observed (P=0.219). SSD lines ranged from 0.59 (L481-59) to 0.76 (L481-54) under HN and from 0.44 (L481-44) to 0.73 (L481-76) under LN (P=0.03). The N treatment x genotype interaction was not significant (P= 0.114).

6.3.5.2 Chlorophyll fluorescence PxW141

At anthesis, flag-leaf QY increased overall from 0.59 under LN to 0.65 under HN (P=0.05). SSD lines ranged from 0.53 (L141-33) to 0.71 (L141-52) under HN and from 0.27 (L141-72) to 0.70 (L141-28) under LN (P<0.001). Response to increasing N supply ranged from -0.10 (L141-04) to +0.40 (L141-72) (P< 0.001).

Post-anthesis, neither N treatment nor genotype had an effect on flag-leaf chlorophyll fluorescence; however, there was a trend for a N treatment x genotype interaction (P= 0.085) with responses to increasing N supply ranging from -0.16 (L141-82) to +0.36 (L141-21).

Table 6.10: Pearson's correlation coefficient in 79 SSD lines of PxW481 between Chlorophyll fluorescence on 11 June and 5 July 2013 and SPAD at anthesis (GS61, 11 June), (only under HN) and Grain yield, AGDM: above-ground dry matter, NUPE: N uptake efficiency and NUTE: N-utilisation efficiency.

	Chlorophyll fluorescence (QY)				SPAD
	11 June		5 July		11 June
	HN	LN	HN	LN	HN
Yield	-0.17	0.21*	0.03	0.00	0.16
AGDM	-0.16	0.20	0.07	0.01	0.10
NUPE	-0.14	0.15	0.11	-0.07	0.21*
NUTE	0.14	0.09	-0.12	0.17	-0.07
QY1	-	-	0.05	-0.26	0.19
QY2	0.05	-0.26	-	-	-0.03

6.3.5.3 Flag leaf chlorophyll content (SPAD) under HN

Flag-leaf chlorophyll content was only measured under high N conditions in 2013. Flag-leaf chlorophyll content (SPAD) under HN ranged from 39.2 (L481-73) to 55.9 (L481-55) (P = 0.02). Twenty SSD lines had higher SPAD than both Paragon (53.45) and W481 (49.35).

For the PxW141 population, SPAD under HN (P=0.035) ranged from 48.0 (L141-86) to 57.0 (L141-40). Thirty seven SSD lines had higher SPAD than both parental lines Paragon (53.7) and W141 (51.9).

Table 6.11: Pearson's correlation coefficient for 97 SSD lines of PxW141 popuation in 2013 for correlations between flag-leaf chlorophyll fluorescence on 11 June and 5 July 2013 and SPAD at around anthesis (11 June) under HN each of grain yield,above-ground dry matter (AGDM), N-uptake efficiency (NUE) and N-utilisation efficiency (NUTE).

	Chlorophyll fluorescence (QY)				SPAD
	11 June		5 July		11 June
	HN	LN	HN	LN	HN
Yield	0.14	0.15	0.25*	0.15	0.22*
AGDM	0.06	0.09	0.22*	0.07	-0.10
NUPE	0.15	0.06	0.27**	0.07	0.07
NUTE	0.01	0.06	0.01	0.14	0.35***
QY1	-	-	0.22*	-0.09	0.28**
QY2	0.22*	-0.09	-	-	0.17

6.3.6 Dry matter per shoot and partitioning at anthesis under HN

6.3.6.1 PxW481 mapping population 2013

Ear dry weight of SSD lines at anthesis ranged from 232 (L481-72) to 551 g m^{-2} (L481-56) (P=0.025). Lamina dry weight of SSD lines ranged from 192 (L481-72) to 440 g m^{-2} (L481-75) (P<0.001). Stem dry weight ranged from 775 (L481-67) to 1729 g m^{-2} (L481-76) (P=0.032).

AGDM_A of SSD lines at anthesis ranged from 1307 g m^{-2} (L481-67) to 2700 g m^{-2} (L481-47) (P=0.003). Only one line, L481-47 (2700 g m^{-2}) had higher AGDM_A than Paragon (2690 g m^{-2}).

Table 6.12: Ear dry matter, (Ear DM), lamina dry matter (Lamina DM), stem dry matter (Stem DM) and total above-ground dry matter at anthesis (AGDM_A), parental lines and mean, maximum, minimum and ANOVA significance for HN treatment of the 79 SSD lines of Paragon x W481 mapping population in 2013

	Ear DM (g m^{-2})	Lamina DM (g m^{-2})	Stem DM (g m^{-2})	AGDM_A (g m^{-2})
Paragon	616	450	1625	2690
W481	442	294	1344	2081
Min	232	192	775	1307
Max	551	440	1729	2700
Mean	369	285	1224	1877
Gen. Prob	0.025	<0.001	0.032	0.032

6.3.6.2 PxW141 mapping population

Ear dry weight of SSD lines at anthesis ranged from 184 (L141-82) to 536 (L141-92) (P=0.008). Lamina dry weight of all SSD lines at anthesis ranged from 110 (L141-82) to 503 g m^{-2} (L141-37) (P = 0.039). Stem dry weight ranged from 567 g m^{-2} (L141-82) to 1911 g m^{-2} (L141-78) (P = 0.011).

At anthesis AGDM of SSD lines ranged from 861 (L141-82) to 2905 g m^{-2} (L141-47) (P=0.02). Three SSD lines, L141-63 (2714 g m^{-2}), L141-21 (2760 g m^{-2}) and L141-78 (2905 g m^{-2}) had higher AGDM_A than either W141 (1820 g m^{-2}) or Paragon (1700 g m^{-2}). The values of AGDM at anthesis were obviousely overestimated, because they are extrapolated from five shoots sample to the m^2.

Table 6.13: Mean, maximum, minimum and ANOVA significance for the HN treatment for 97 SSD lines of Paragon x W141 mapping population and parental lines for ear dry matter (DM), leaf lamina DM (Lamina DM), stem dry matter (Stem DM) and above-ground dry matter (AGDM) per m^2 at anthesis (GS61).

	Ear DM (g m^{-2})	Lamina DM (g m^{-2})	Stem DM (g m^{-2})	AGDM (g m^{-2})
Paragon	372	327	1001	1700
W141	347	242	1231	1820
Min	184	110	567	861
Max	536	503	1911	2905
Mean	362	298	1131	1791
Genot.	0.008	0.039	0.011	0.020

6.4 Discussion

This discussion considers the response of NDVI and senescence parameters to the N supply in two mapping populations (PxW481 and PxW141) and the relationship of these parameters with grain yield and biomass as well as NUE components.

6.4.1 Canopy size

In the present investigation, restricted N availability reduced NDVI on four assessments (pre- and post-anthesis) for PxW481 mapping population; and on two post-anthesis measurements for PxW141 mapping population. Pre-anthesis, N had an effect on NDVI of the PxW481 mapping population, but not on the PxW141 mapping population. However, N supply significantly affected post-anthesis NDVI in both mapping populations.

The canopy reflectance results confirmed that an increase in greenness results in lower reflectance at visual (VIS) wavelengths (400-700 nm), because of increased light absorption due to a higher density of pigments per unit soil surface area (Hinzman, Bauer, and Daughtry, 1986). However, the opposite effect was observed for near infr-red reflectance (NIR) (700-1300 nm), the relatively higher reflectance observed is due to the canopy and leaf internal scattering (Colwell, 1974).

All wheat genotypes in the populations had highest NDVI value at about flowering; NDVI then declined during the grain-filling period. Reflectance-based measurements of NDVI often become saturated at a high leaf area index near anthesis (Christopher et al. 2013). As plant tissues become older, the crop loses its greenness; it reflects more light and the spectral reflectance characteristics of the canopy change. Adamsen et al. (1999), using a digital camera on spring wheat, reported that NDVI and SPAD were associated with senescence in wheat. However, the difference between highest and lowest NDVI was more pronounced during grain-filling compared with pre-anthesis stage since certain genotypes had a sharper decline. Present results showed; averaging all SSD lines; that NDVI decreased in the end of grain filling to 0.45 under HN and to 0.38 under LN for PxW481 and to 0.54 under HN and 0.42 under LN for PxW141 mapping population.

a) 6.4.1.1 Relationship between NDVI and grain yield

Post-anthesis NDVI was positively and more strongly associated with grain yield amongst SSD lines under LN for both PxW481 and PxW141 than under HN. Indeed, under LN conditions, the crop has lower biomass but higher biomass production efficiency (BPE). Therefore, under LN present results indicated that there is more source limitation, while under HN the crop is more sink limited. In addition, when leaf area index (LAI) is approaching 4, NDVI becomes saturated and less sensitive to changes in vegetative development (Hatfield et al. 2010) which is the case under HN in our experiment. Although under optimal conditions wheat crops are in general little limited by the assimilate supply during grain filling (Dreccer et al. 1997; Borra´ s et al. 2004; Calderini et al. 2006), under low to moderate N fertiliser levels yields may be more limited by post-anthesis assimilate supply.

The post-anthesis NDVI response to N was associated amongst genotypes with grain yield response to N, therefore NDVI measurements could detect SSD lines maintaining high yield under LN conditions.

In the past, selection for yield *per se* has been effective in raising wheat yield (Ferrio et al. 2005). Nevertheless, the assessment of grain yield requires the harvesting of field plots, which is expensive and time consuming for large sets of genotypes in plant breeders's trials. A non-destructive way to estimate yield before harvesting would therefore be an important tool in breeding programs. Bauer (1975) showed that monitoring crop growth conditions by using spectral signature of crop canopies in visible and near-infrared regions was successful. Thus, spectral reflectance measurements have been successfully used to estimate biomass, leaf area index, photosynthesis and yield in several species like rice (Vaesen et al. 2001), barley (Bort et al. 2002), bread wheat (Filella et al. 1995 Babar et al. 2006b) and durum wheat (Aparicio et al. 2000; Royo et al. 2003). The NDVI and simple ratio (SR) are related to the photosynthetic active biomass (Penuelas et al. 1997).

NDVI was positively associated with yield under different environments (Lopes and Reynolds, 2012). Many studies reported that NDVI could be used to predict genetic variation in grain yield in cereals (Raun et al. 1999, Babar et al. 2006, Teal et al.

2006, Wang et al. 2010). However, other studies have reported that NDVI recorded at different growth stages of the wheat crop differed in its relationship with grain yield. Freeman et al. (2003) reported that NDVI was well correlated with wheat grain yield at growth stages when the flag leaf was fully emerged and heading was complete. Under HN, the correlation between NDVI two weeks after anthesis and grain yield was highest in both mapping populations (r=0.55 and r=0.47 respectively for PxW481 and PxW141). These correlations are similar to those reported by Aaparicio et al. (2000) (r=0.55) and lower than those reported by Ferrio et al. (2005) (r=0.64). Under LN condition, the best correlation between NDVI and grain yield was found at three weeks after flowering. The milky-ripe grain filling stage was also found to be the best developmental stage for recording genetic variation in NDVI as correlations with yield are stronger than for earlier measurements (Royo et al. 2003).

b) 6.4.1.2 Relationship between NDVI and biomass

The post-anthesis NDVI was positively correlated with biomass production under HN and LN in PxW481 and in PxW141. In both HN and LN, post-anthesis NDVI was better associated with biomass in PxW481 than in PxW141. Also in both mapping populations, post-anthesis NDVI was better associated with biomass under LN than under HN.

Similar to grain yield, NDVI measured two weeks after anthesis date was the better correlated with biomass under HN and LN for PxW141 mapping population, and under HN for PxW481 mapping population, and NDVI measured three weeks after flowering was the best correlated with biomass under LN conditions. These results are consistent with those reported by Babar et al. (2006a and b) who showed that the relationship between biomass and NDVI was strongest at about two weeks after flowering under normal N conditions.

PxW481 and PxW141 had similar post-anthesis NDVI under HN conditions; however, under LN conditions PxW481 had higher NDVI than PxW141. The difference between the two populations could be explained by the higher biomass for PxW481. Indeed, averaging two years, PxW481 produced much more biomass under HN and LN conditions than PxW141. The results on the two mapping populations

used in this study confirm the presence of genotypic variation for NDVI, which can be utilized to predict biomass in breeding programmes.

In order to produce high yielding, stress-tolerant plants rapidly and efficiently, connecting genotype to phenotype is required. Given the rapid development of plant genomic technologies (i.e. next generation DNA sequencing), a lack of access to high-throughput field phenotyping capabilities limits our ability to dissect the genetics of quantitative traits related to growth, yield and adaptation to stresses (Phillips et al. 2010). However, new high-throughput phenotyping platforms are now being developed functional including a variety of imaging spectroscopy (multispectral and hyperspectral remote sensing), thermal infrared imaging, fluorescence imaging, 3D imaging and tomographic imaging (MRI, Magnetic Resonance Imagers, PET Positron Emission Detectors and CT, X-ray Computed Tomography) (Li et al. 2014). New high throughput phenotyping platforms (HTPPs) were reported by Araus and Cairns (2014). A combination of advances in aeronautics, remote sensing and high performance computing leads to develop ground based platforms to aerial systems of HTPPs. Most of the ground-based HTPPs include modified vehicles equipped with GPS, navigation devices, cameras and sensors (Araus and Cairns 2014).

c) 6.4.1.3 Relationship between NDVI and N uptake

There were significant correlations between NUPE and NDVI at all measurement dates (before and after anthesis) in both PxW481 and PxW141 mapping populations. These results are consistent with findings by Stone et al. (1996). The correlations in the present study increased from the early stages of stem elongation to flowering, and then started to decrease up to maturity. However, the highest correlations were observed around anthesis. Indeed, under HN, the highest correlation was observed in the two weeks before flowering time for PxW481 and PxW141, whereas under LN conditions the correlation was highest three weeks after anthesis for PxW481 and two weeks after anthesis for PxW141. Dekker et al. (2003) emphasized that the early readings (around booting) were far superior for predicting total N uptake than the later readings. Poole and Craig (2010) using a panel of wheat cultivars under different N treatments showed that NDVI correlation with the nitrogen content of the

plant biomass, becomes weaker at NDVI levels approaching 0.75 (due to saturation of greenness).

Crop reflectance (light wavelengths reflected from the crop) can be measured by remote sensors from aircraft/satellite or proximal sensors mounted on a spray boom or tractor cab (Roberts al. 2009). New plant phenomics offer technologies to enable breeders to develop new wheat germplasm with improved NUE to support future sustainable wheat production (Furbank and Tester 2011).

6.4.2 Flag leaf senescence

In the present study, pre-anthesis NDVI was highly correlated with the total duration of post-anthesis senescence (DURTOT) for both the PxW481 and PxW141 mapping populations under HN and LN conditions. Indeed, high NDVI pre-anthesis relates to high N uptake pre-anthesis allowing delayed senescence by providing N for remobilisation during grain filling.

For wheat, there is evidence that the N sink demand for grain protein initiates senescence in order to remobilise nitrogen from vegetative organs to the grain (Gan and Amasino, 1997; Martre et al. 2006). This process is affected by the genetic variability of the N source-sink balance. Several investigations have concluded that the genetic control of N remobilization seems likely to be involved in the regulation of leaf senescence (Sinclair and De Wit, 1975; Masclaux et al. 2001), and an improved understanding of N remobilization could be important in developing cultivars with stay-green properties. The stay-green phenotype has been attributed to higher cytokinin levels and/or reduced ethylene production or perception (Thomas and Howarth, 2000). Christopher et al. (2008) postulated under drought in Australia that a stay-green genotype had a narrower root system which conferred a yield advantage in areas where moisture was available in deep non-constrained soils. This characteristic allows these genotypes to extract a small amount of extra soil moisture and nutrients (N) from deep in the profile late in the season (Lopez and Reynold, 2012).

Present results suggested no significant associations between ONSEN and either grain yield or NUTE or NUPE under both HN and LN conditions for the PxW481

mapping population. However, for PxW141 mapping population, ONSEN was slightly better associated with yield under HN and LN conditions, and ONSEN was slightly better associated with NUPE under HN than under LN. Previous studies in wheat have demonstrated that genetic variation in grain growth is not associated with post-anthesis senescence parameters under HN conditions whereas associations between grain yield and NUTE and post-anthesis senescence parameters were reported under LN conditions (Gaju et al. 2011). This is in general agreement with present results where under LN conditions grain yield and NUTE were more strongly correlated with the timing of the onset of the rapid phase of senescence hence potentially limited by assimilates supply during grain filling.

The results of this study show that the genetic variation in ONSEN was associated with anthesis date (AD). Indeed, under LN, later onset of the rapid phase of senescence was associated with later anthesis date for PxW481 and for PxW141; however under HN, ONSEN was correlated with AD only in the PxW141 mapping population. This difference between the two mapping populations under HN could be due to the difference in the range of AD amongst lines for the two populations. The findings of the current study are consistent with those of Gaju et al. (2011) and Bogard et al. (2011). Bogard et al. (2011) added that the relationship between AD and flag-leaf senescence depended on the environment. Another explanation of relationship between AD and ONSEN could be that increased rooting capacity may be associated with the genotype differences in N acquisition under restricted N supply. Later AD could be associated with deeper roots (Foulkes et al. 2004) and/or a relatively deeper distribution of roots or a lower specific root mass (King et al. 2003).

A positive correlation amongst SSD lines was found between grain N concentration (GNC) and both SEN and DURTOT for PXW481 under LN, however no correlation between these traits was found for PxW141 either under HN or under LN. The effect in PxW481 could be due to staygreen genotypes accumulating more N before anthesis while maintaining high N remobilization efficiency (Slafer et al. 1990). These two processes may increase GNC, SEN and DURTOT simultanously.

Delayed leaf senescence has been reported to increase grain yield in durum wheat (Spano et al. 2003) and bread wheat genotypes (Gong et al. 2005; Luo et al. 2006;

Christopher et al. 2008; Chen et al. 2010; Bogard et al. 2011), presumably because of prolonged photosynthetic duration. Bogard et al. (2011) argued that the use of the relationship between leaf senescence traits and grain N should be interpreted with caution. Indeed leaf senescence during grain filling was highly prone to the genotype x environment interaction (GxE) so leaf senescence, as a selection criterion to improve grain N, strongly depends on the targeted environments and presumably the N availability during the post-anthesis period.

A negative correlation was also found between grain N concentration (GNC) and AD for PxW141 mapping population under HN ($r=-0.34$, $P<0.001$) and under LN. For PxW481, there is a trend of negative relationship under HN and no relationship under LN. This may be associated with the positive correlation between GY and anthesis date leading to starch dilution of N in the grain.

NUTE was negatively associated with GNC in both mapping populations. This finding is consistent with findings by Gaju et al. (2011). In feed and biofuel wheat cultivars, increasing NUTE with lower GNC is feasible. However, in bread-making genotypes any trade-off with grain protein concentration (GPC) with regard to the ca. 13% threshold for bread-making must be considered carefully (Gaju et al. 2011). The minimum amount of GNC required for viable grain in wheat was reported to be 1.25% (Pask et al. 2012). One strategy to increase NUTE would be to develop grain with a lower minimum GNC in future phenomics studies linked to genomics studies to identify the genes regulating grain protein structural and functional requirements.

Chapter 7: Genetic analysis of the phenotypic data of Paragon x W481 and Paragon x W141 mapping populations

7.1 Introduction

This chapter describes the results of the genetic analysis carried out on the Paragon x W481 (2012 and 2013) and Paragon x W141 (2013) mapping populations for traits measured in the field experiments at University of Nottingham. The phenotypic evaluation was carried out for grain yield, above-ground biomass, NUE and NUE components and target physiological traits. The two maps (for PxW481 and PxW141) were constructed using JoinMap version 4 (Van Ooijen, 2006). The quantitative trait loci (QTL) analysis was performed using the software package MapQTL version 5 (van Ooijen, 2004). The nonparametric mapping or Kruskal Wallis (KW) analysis was performed first to classify the effect of the markers one by one, followed by Interval Mapping (IM) identifying loci between markers. The results are presented for significant QTL (LOD> significant threshold of each trait) and putative QTLs (LOD>2) and their location on the chromosomes of interest.

The objective of this chapter was to identify quantitative trait loci (QTL) for grain yield and yield components, above-ground biomass, NUE and NUE components and physiological traits under high N and low N conditions. The specific aims were to:

- Construct the genetic map for the PxW481 and PxW141 mapping populations.
- Identify quantitative trait loci (QTL) for grain yield and yield components, above-ground biomass, NUE, NUE components and underlying traits in the two SSD mapping populations, PxW481 and PxW141, under high N and low N conditions.
- Identify the stable QTLs for PxW481 and Px141 mapping populations under HN and LN and QTLs which interaction with N treatment.
- Compare the locations of the identified QTLs in two mapping populations PxW481 and PxW141.

- Compare the locations of the QTLs with previous published NUE QTLs in wheat.

7.2 Methodology

7.2.1 Phenotyping

The methodology for phenotyping grain yield and the target traits including yield components, biomass, NUE and NUE components and physiological traits for PxW481 in 2012 and 2013 and for PxW141 in 2013 for PxW141 SSD mapping populations was described in Chapter 3 and the results presented in Chapters 5 and 6. In each experiment for each trait mean values across replicates were used for QTL analysis.

7.2.2 Genotyping

The initial mapping work was carried out at John Innes Centre, Norwich, UK and University of Bristol, UK in the two mapping populations as part of the BBSRC-funded collaborative programme (Wheat Improvement Strategic Programme, WISP). This was achieved using Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphism (SNPs). From the characterized genotype data provided by John Innes Centre, one map for each of the mapping populations (PxW481 and PxW141) was presently constructed using JoinMap version 4.1 (Van Ooijen, 2006). For PxW481, a total of 201 Markers (27 SSRs and 174 SNPs) were used to build a genetic map; and for PxW141 mapping population a total of 491 markers (23 SSRs and 468 SNPs) were used. The Haldane mapping function was used to calculate the centiMorgan (cM) distances.

In the mapping of the 201/491 marker loci into linkage groups several LOD thresholds were tested. Usually, a LOD of 3.0 is widely applied for linkage analysis (Collard at al., 2005). Using this threshold (LOD of 3.0), one large linkage group containing almost all marker loci was obtained. Linkage analysis with a LOD of 5.0 and 8.0 still resulted in large linkage groups. The LOD threshold for significant linkage was gradually increased until obtaining individual loci in a group. Only at a LOD of 10.0 were linkage groups obtained which in all cases were in agreement with nulli-tetrasomic data. However, markers with a genetic distance of more than 20

cM were no longer significantly linked at this threshold. After finding the best order of markers within these linkage groups, they were assembled into larger units by lowering the LOD threshold.

After the maps were constructed, a QTL analysis was performed using MapQTL version 5 (van Ooijen, 2004). Two methods were used for QTL analysis of each trait: Kruskal Wallis (KW) for individual QTL identification and Interval Mapping (IM) to estimate the position of a QTL within two markers. After IM analysis to test the (LOD) significance, a Permutation Test (PT) was performed to estimate the logarithm of the odds (LOD) threshold for each trait. In this study, when LOD > threshold, the QTL is considered significant, but when the LOD > 2.00 and < threshold, the QTL is considered as putative, finally, when LOD > 1.00 and < 2.00 the QTL is considered as candidate.

In the results, the significant and putative effects identified by IM analysis are described and discussed. In addition, candidate QTL identified are referred in relation to colocation of QTLs where appropriate in the discussion.

7.3 Results

7.3.1 Genetic map construction

7.3.1.1 PxW481

For PxW481, the mapping of the 201 marker loci with a LOD of 10.0 resulted in 35 distinct linkage groups consisting of 172 loci and 29 unlinked loci. After flowering, the markers were assembled into larger groups. This resulted in a genetic map with 26 linkage groups comprising 173 loci and spanning 852.7 cM. These linkage groups could be assigned to the 21 wheat chromosomes based on published maps (Sommers et al. 2004, Sourdille et al. 2004) and the available nulli-tetrasomic data, except for one linkage group 26 containing two markers, which belongs to either 6D or 1B. There was relatively poor coverage of markers on chromosomes 1D, 4B, 3A and 7B, and no markers on chromosomes 3D, 4D, 6D and 7D.

The map lengths were 416, 330 and 108 cM for the A, B and D genomes, respectively (Table 7.1). The D genome had lower marker numbers (M) coverage

and lower density (13 M and 8.27 cM M^{-1}) than the B genome (82 M and 4.01 cM M^{-1}) and A genome (75 M and 5.54 cM M^{-1}) (Table 7.1).

The map lengths of individual chromosomes ranged from 5.0 (chr 1D) to 95.0 cM (chr 6A). The number of markers per chromosome ranged from 2 on 1D to 33 on 6A. The chr 6A was formed by 3 linkage groups and chr 1B, 3B, 5A, 7A and 7B each comprised of two linkage groups.

The marker data were used for QTL analysis of selected quantitative traits using 79 SSD lines of the population PxW481 in the present study. The most relevant QTLs are discussed in this chapter.

7.3.1.2 PxW141

For PxW141, the mapping of the 491 marker loci with a LOD of 10.0 resulted in 45 distinct linkage groups consisting of 475 loci and 16 unlinked loci. After lowering the LOD threshold, the markers were assembled into larger groups. This resulted in a genetic map with 28 linkage groups comprising 482 loci and the total genetic distance of the linkage maps was 1585 cM with an average density of one marker per 3.29 cM. These linkage groups could be assigned to the 21 wheat chromosomes based on published maps and the available nulli-tetrasomic data. The map lengths were 667, 650 and 268 cM for the A, B and D genomes, respectively (Table 7.2). Genome D had lower marker number (M) coverage but higher density (268 M and 2.79 cM M^{-1}) than genome B (650 M and 3.19 cM M^{-1}) and genome A (667 M and 3.67 cM M^{-1}) (Table 7.2).

The chr 4D did not contain any markers and map lengths of other individual chromosomes ranged from 18.8 (chr 6D) to 141.9 (chr 2B) cM. The number of markers per chromosome (excluding 4D) ranged from 8 on chr 6D to 60 on 6B. The chr 3B and 6D were formed by 3 linkage groups and the chromosomes 1B, 2B, 3B 5A and 6A each comprised of two linkage groups.

These marker data were used for QTL analysis of selected quantitative traits using 97 SSD lines of the population PxW481 in the present study. The most relevant QTLs are discussed in this chapter.

Table 7.1: Marker coverage for the Paragon x W481 genetic map by chromosome

Chromosome	1A	1B	1D	2A	2B	2D	3A	3B	3D	4A	4B	4D	5A	5B	5D	6A	6B	6D	7A	7B	7D
No. markers (M)	4	8	2	4	23	3	7	13	0	5	6	0	12	10	8	33	14	0	10	8	0
Length (cM)	47	46	5	31	67	24	19	57	0	66	13	0	89	89	78	95	30	0	68	28	0
Density (cM/M)	11.83	5.78	2.50	7.68	2.89	8.10	2.77	4.38	-	13.28	2.12	-	7.43	8.92	9.78	2.87	2.13	-	6.81	3.54	-

Genome	A	B	D	Total
No. marker (M)	75	82	13	**170**
Length (cM)	416	330	108	**853**
Density (cM/M)	5.54	4.02	8.27	**5.02**

Table 7.2: Marker coverage for the Paragon x W141 genetic map by chromosome

Chromosome	1A	1B	1D	2A	2B	2D	3A	3B	3D	4A	4B	4D	5A	5B	5D	6A	6B	6D	7A	7B	7D
No. marker (M)	15	19	29	26	37	23	21	33	9	15	13	0	21	60	18	57	17	8	27	25	9
Length (cM)	64	86	66	95	142	73	120	89	52	71	58	0	89	97	29	92	108	19	136	70	28
Density (cM/M)	4.27	4.53	2.30	3.64	3.83	3.20	5.73	2.70	6.00	4.73	4.44	-	4.24	1.62	1.59	1.62	6.33	2.00	5.03	2.81	3.00

Genome	A	B	D	Total
Marker (M)	182	204	96	**482**
Length (cM)	667	650	268	**1585**
Density (cM/M)	3.67	3.19	2.79	**3.29**

7.3.2 Genetic analysis of grain yield and components

In the text unless stated otherwise the QTLs described are those identified by interval mapping.

7.3.2.1 PxW481

a) 2012

Grain yield: Under HN conditions, one QTL (LOD = 3.91) was found at 23.9 cM on chr 6A (Table 7.3, Figure 7.1) close to marker BS0001037 (K*=14.4; P<0.001; LOD = 3.26) at 23.9 cM explaining 20.4% of the phenotypic variation. Yield was increased by the presence of the Paragon allele. In addition, two putative QTLs were identified, one on 3B (LOD = 2.71) and one on 4B (LOD = 2.18) explaining respectively 14.6 and 11.9% of the phenotypic variation.

Under LN conditions, one QTL was found at 17.05 cM on chr 2B which coincided with marker BS00003589 (K*=2.73; P=0.1; LOD = 3.09) explaining 16.5% of the phenotypic variation (Table 7.4, Figure 7.1). Yield was increased by the W481 allele. In addition, eight more putative QTLs on 2B (1), 2D (1), 3A (1), 3B (2), 4B (1), 5A (1), and 6A (1) were identified explaining between 11.8 and 14.3% of the total phenotypic variation.

Grains per m^2: Under HN, a major QTL was found on chr 7B coinciding exactly with marker barc32 at 20.3 cM (K*=12.33; P<0.001; LOD = 3.88) explaining 20.3% of the phenotypic variance (Table 7.3, Figure 7.1). Grains per m^2 was increased by the Paragon allele. In addition three putative QTLs were identified, one on chr 2D (LOD = 2.74), one on 5B (LOD = 2.30) and one on 5A (LOD = 2.16). These QTLs explained respectively 14.7, 12.5 and 11.8% of the phenotypic variation.

Under LN, four putative QTLs were found for GM using the IM method (Table 7.4, Figure 7.1): two on 3B (LOD = 2.51 and 2.06), one on 2D (LOD = 2.48) and one on 5A (LOD = 2.07). These QTLs explained between 11.3 and 13.6% of the phenotypic variation.

Grains per ear: Under HN no QTL was significant for GE (Table 7.3, Figure 7.1). The highest LOD (1.68) was recorded for a marker BS00009531 Chr 5A at 55.04

cM. Under LN conditions, one QTL was found at 2.00 cM on chr 2D close to marker gwm577 (K*=6.6; P<0.05; LOD = 2.90) (Table 7.4, Figure 7.1). The QTL explained 15.7% on the phenotypic variance and GE was increased by the W481 allele. In addition, two putative QTLs were found on 5A (LOD = 2.34) and on 1A (LOD = 2.18) explaining, respectively, 12.7 and 11.9% of the phenotypic variation.

Ears per m^2: Under HN, two putative QTLs were found on 7B (LOD = 2.10) and on 3B (LOD = 2.06) explaining respectively 11.3 and 11.5% of the phenotypic variation (Table 7.3, Figure 7.1).

Under LN, one QTL was found coinciding with the marker BS00021895 at 0.00 cM position on 1A (K*=3.01; P=0.1; LOD = 4.12) (Table 7.4, Figure 7.1). In addition, three putative QTLs were found on 3B (LOD = 2.48), 3A (LOD = 2.34) and 7A (LOD = 2.15). These QTLs explained respectively 13.4, 12.8 and 11.8% of the total phenotypic variation.

Thousand grain weight: Under HN, one QTL was found at 84.2 cM on chr 5B (LOD = 2.80) (Table 7.3, Figure 7.1) close to marker BS00016286 (K*=11.44; P<0.001; LOD = 2.67) at 87.4 cM position explaining 15.1% of the phenotypic variance. TGW was increased by the W481 allele. In addition, two putative QTLs were found, one on 5B (LOD = 2.25) and one on 2A (LOD = 2.08) explaining, respectively, 12.3 and 11.4% of the total variation. Under LN, four putative QTLs were found for TGW (Table 7.4, Figure 7.1): two on 7B (LOD = 2.59 and 2.00), one on 2D (LOD = 2.49) and one on 5B (LOD = 2.07). These QTLs explained between 11.0 and 14.0% of the phenotypic variation.

Table 7.3: QTLs identified using both Kruskal Wallis (KW) and Interval Mapping (IM) methods for grain yield and components under HN treatments for Paragon x W481 mapping population in 2011/12.

Trait	Chr.	Position (cM)	Locus	Kroskal Wallis		Interval mapping				
				K*	Signif.	LOD	PT	Var.	%Expl.	Add.
Grain yield	3B	34.6	BS00011570	7.76	***	2.71	2.9	0.95	14.6	0.39
	4B	12.7	BS00012107	8.11	****	2.18		0.98	11.9	0.36
	6A	23.9	BS00001037	14.40	******	3.91		0.88	20.4	0.45
1000 grain weight	2A	24.9	BS00004004	7.73	***	2.08	2.7	10.06	11.4	-1.15
	5B	84.2	BS00016286	11.44	*****	2.80		9.64	15.1	-1.39
	5B	43.2	BS00003637	0.47	-	2.25		9.96	12.3	-0.18
Grains per m^2	2D	5.0	gwm577	3.72	*	2.74	2.8	1.1E+07	14.7	-2067
	5A	63.0	BS00009531	0.52	-	2.16		1.1E+07	11.8	-972
	5B	31.4	BS00010573	1.91	-	2.30		1.1E+07	12.5	-658
	7B	20.3	barc32	12.53	******	3.88		1.0E+07	20.3	1762
Ears per m^2	3B	1.00	BS00020861	2.44	-	2.06	2.8	5223	11.3	8.90
	7B	17.49	Barc32	4.55	**	2.10		5211	11.5	29.24

Chr.: chromosome; significance level *: 0.1, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001; LOD: log likelihood score; PT: permutation test threshold; Var.: residual variance after fitting the QTL;%Expl.: the percentage of variance explained for by the QTL; Additive effect (μA-μB)/2

Table 7.4: Summary of QTLs identified using both Kruskal wallis (KW) and Interval Mapping (IM) methods for grain yield and components under LN treatments for PxW481 mapping population in 2011/12.

Trait	Chr.	Position (cM)	Locus	Kroskal Wallis		Interval mapping				
				K*	Sign.	LOD	PT	Var.	%Expl.	Add.
Grain yield	2B	17.1	BS00003589	2.73	*	3.09	2.8	0.51	16.5	-0.13
	2B	61.7	BS00009442	5.17	**	2.53		0.52	13.7	-0.15
	2D	24.3	BS00009575	6.61	**	2.65		0.52	14.3	-0.3
	3A	18.6	gwm2-2	7.07	***	2.46		0.53	13.4	0.23
	3B	31.1	BS00010881	9.13	****	2.43		0.53	13.2	0.28
	3B	2	BS00020861	2.01	-	2.27		0.53	12.4	0.21
	4B	12.6	BS00015504	0.05	-	2.5		0.52	13.5	0.03
	5A	65	BS00009531	8.31	****	2.16		0.53	11.8	-0.33
	6A	9.9	BS00003881	2.73	*	2.37		0.53	12.9	-0.17
1000 grain weight	2D	0	gwm577	4.81	**	2.49	2.8	8.87	13.5	1.48
	5B	86.2	BS00010679	4.08	**	2.07		9.09	11.4	-0.75
	7B	17.5	barc32	11.28	*****	2.59		8.81	14	-1.43
	7B	1	BS00011217	6.17	**	2		9.12	11	-1.03
Grains per m^2	2D	0	gwm577	8.04	****	2.48	2.7	6.50E+06	13.5	-1582
	3B	1	BS00020861	0.81	-	2.51		6.50E+06	13.6	521
	3B	31.1	BS00010881	6.67	***	2.06		6.70E+06	11.3	918
	5A	70	BS00000645	2.37	-	2.07		6.70E+06	11.4	-1131
Grains per ear	1A	19.45	BS00010416	8.74	****	2.18	2.8	43.7	11.9	3.51
	2D	2	gwm577	6.61	**	2.93		41.8	15.7	-4.46
	5A	40.36	BS00011890	13.01	******	2.34		43.3	12.7	-2.46
Ears per m^2	1A	0	BS00021895	6.77	***	4.12	2.7	1648	21.4	-9.55
	3A	19.36	gwm2-2	2.62	-	2.34		1828	12.8	-4.57
	3B	8.51	BS00009651	0.01	-	2.48		1814	13.4	10.23
	7A	1	BS00011724	5.11	**	2.15		1848	11.8	14.95

Chr.: chromosome; significance level *: 0.1, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001; LOD: log likelihood score; PT: permutation test threshold; Var.: residual variance after fitting the QTL;%Expl.: the percentage of variance explained for by the QTL; Additive effect (μA-μB)/2

b) 2013

Grain yield: Under HN conditions, one QTL was found at 32.8 cM on chr 5A (LOD = 3.06) close to the marker BS00011227 (K*=1.75; NS; LOD = 0.74) at 30.8 cM. This QTL explained 16.3% of the phenotypic variance and yield was increased by the W481 allele (Table 7.5, Figure 7.1). In addition, five putative QTLs were found, two on 6A (LOD = 2.44 and 2.29), one on 4A (LOD = 2.23) and one on 3B (LOD = 2.06). These QTLs explained between 11.3 and 13.3% of the phenotypic variation.

Under LN, five putative QTLs were found, two on 2B (LOD = 2.54, 2.29 and 2.21), explaining 12.1 and 13.8% of the phenotypic variation, respectively, one on 7A (LOD = 2.47) explaining 13.4% of the phenotypic variation and two on 5A (LOD = 2.37 and 2.11) explaining 12.9 and 11.6% of the phenotypic variation, respectively.

Thousand grain weight: No marker was significant under HN. The highest LOD was observed for BS0001405 in 6A (1.91) (Table 7.5, Figure 7.1). Under LN, one QTL was found at 29.9 cM on chr 5B (LOD = 2.86) close to the marker BS00010573 (K*=7.47; P<0.001; LOD = 2.67) at 31.4 cM explaining 15.4% of the phenotypic variance; yield was increased by the Paragon allele. In addition a putative QTL was found on 6A (LOD = 2.38) explaining 13.0% of the phenotypic variation.

Grain per m^2: Under HN conditions, one QTL was found at 6.0 cM on chr 2D (LOD = 3.53) close to marker gwm577 (K*=4.97; P<0.05; LOD = 3.09) at 0.00 cM (Table 7.5, Figure 7.1) explaining 18.6% of the phenotypic variance; yield was increased by the W481 allele.

Under LN conditions, three significant QTLs were found for GM:

- QTL at 32.4 cM on chr 5B (LOD = 4.29) close to the marker BS00010573 (K*=13.10; P<0.001; LOD = 3.97) explaining 20.6% of the phenotypic variance; GM was increased by the W481 allele.
- QTL at 65.0 cM on chr 5B (LOD = 2.81) close to the marker BS00009531 (K*=3.04; P<0.1; LOD = 2.80) explaining 15.1% of the phenotypic variance GM was increased by the W481 allele.

- QTL at 8.00 cM on chr 5A (LOD = 3.15) close to the marker BS00011846 (K*=7.02; P<0.01; LOD = 3.12) explaining 16.8% of the phenotypic variance; GM was increased by the Paragon allele.

Grains per ear: Under HN, one putative QTL was found on 2D (K*=4.34; P<0.01; LOD = 2.32) explaining 12.6% of the phenotypic variation (Table 7.5, Figure 7.1). Under LN conditions, one QTL on marker BS00011846 (K*=7.02; P<0.01; LOD = 3.78) was found at 8.20 cM on chr 5A explaining 19.8% of the phenotypic variance and GE was increased by the Paragon allele. In addition, two putative QTLs were found on 6A (LOD = 2.814) and on 5B (LOD = 2.27) explaining 15.1 and 12.4% of the phenotypic variation, respectively.

Ears per m^2: Under HN, one QTL: BS00011047 (K*=6.72; P<0.01; LOD = 2.95) on chr 2B at 16.8 cM was identified explaining 15.8% of the phenotypic variance (Table 7.5, Figure 7.1). Under LN, one putative QTL (K*=4.95; P<0.05; LOD = 2.28) was found on chr 2B explaining 12.4% of the phenotypic variation.

Table 7.5: Summary of QTLs identified using both Kruskal wallis (KW) and Interval Mapping (IM) methods for grain yield and components under HN and LN treatments for PxW481 mapping population in 2012/13.

N Treat	Trait	Chr.	Position (cM)	Locus	Kroskal Wallis		Interval mapping				
					K*	Sign.	LOD	PT	Var.	%Expl.	Add.
HN conditions	Grain yield	3B	7.5	BS00009651	0.065	-	2.06	2.8	1.13	11.3	0.07
		4A	20	BS00010455	0.32	*	2.23		1.12	12.2	-0.05
		5A	32.8	BS00011227	1.754	-	3.06		1.07	16.3	-0.28
		6A	12.3	BS00010576	3.654	*	2.44		1.11	13.3	-0.23
		6A	0.2	BS00010326	11.83	****	2.29		1.12	12.5	0.39
	Grains per m^2	2D	6	gwm577	4.973	**	3.53	2.7	1.1E+07	18.6	-2505
	Grains per ear	2D	0	gwm577	4.341	**	2.32	2.9	70	12.6	-4.83
	Ears per m^2	2B	16.8	BS00011047	6.718	***	2.95	2.8	4900	15.8	22.9
LN conditions	Grain yield	2B	46.3	BS00021713	10	****	2.54	2.9	315	13.8	-7.23
		2B	58.6	BS00003585	10.44	****	2.21		321	12.1	-6.66
		5A	8	BS00011846	5.872	**	2.37		318	12.9	4.57
		5A	66	BS00009531	3.986	**	2.11		323	11.6	-4.28
		7A	14	BS00001450	0.9	-	2.47		316	13.4	-1.44
	1000 grain weigh	5B	29.9	BS00010573	7.47	***	2.86	2.8	7.94	15.4	1.07
		6A	9.1	BS00011122	8.734	****	2.38		8.16	13	-1.12
	Grains per m^2	5A	8	BS00011846	7.016	****	3.15	2.8	8.4E+06	16.8	1107
		5A	65	BS00009531	4.879	**	2.81		8.6E+06	15.1	-894
		5B	32.4	BS00010573	13.1	******	4.29		7.9E+06	22.1	-1627
	Grains per ear	5A	8.2	BS00011846	13.26	******	3.78	2.8	39.19	19.8	3.26
		5B	33.4	BS00010573	3.525	*	2.27		42.8	12.4	-2.67
		6A	0	BS00003818	8.782	****	2.81		41.5	15.1	2.29
	Ear per m^2	2B	62.7	BS00011630	4.949	**	2.28	2.7	3300	12.4	-15.3

Chr.: chromosome; significance level *: 0.1, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001; LOD: log likelihood score; PT: permutation test threshold; Var.: residual variance after fitting the QTL;%Expl.: the percentage of variance explained for by the QTL; Additive effect (µA-µB)/2

7.3.2.1 Paragon x W141

Grain yield: Under HN, two putative QTLs were found on 6D (LOD = 2.37) and on 7B (LOD = 1.9) explaining 11.0 and 8.9% of the phenotypic variation (Table 7.6, Figure 7.2). Under LN, three putative QTLs were found on 4A (LOD = 2.06), 1B (LOD = 2.03) and 6B (LOD = 2.02) (Table 7.7, Figure 7.2) explaining 9.6, 9.5 and 9.4% of the total phenotypic variation, respectively.

Thousand grain weight: Under HN, two significant QTLs were found (Table 7.6, Figure 7.2):

- QTL at 43.6 cM on chr 1A (LOD = 4.93) close to marker BS00016654 (K*=0.25; NS; LOD = 4.51) at 41.56 cM.explaining 21.5% of the phenotypic variance. TGW was increased by the W141 allele.
- QTL at 10.00 cM on chr 3A (LOD = 3.39) close to marker BS00025739 (K*=9.46; P<0.001; LOD = 2.75) explaining 20.51% of the phenotypic variance. TGW was increased by the W141 allele.

In addition, three putative QTLs were found, one on chr 6B (LOD = 2.48), one on 6A (LOD = 2.16) and one on 2D (LOD = 2.01) explaining 21.5, 21.8 and 22.0% of the phenotypic variation, respectively. Under LN conditions, two QTLs were found (Table 7.7, Figure 7.2):

- QTL at 43.6 cM on chr 1A (LOD = 3.52) close to marker BS00016654 (K*=0.09; NS; LOD = 3.37) at 41.56 explaining 15.8% of the phenotypic variance. TGW was increased by the W141 allele.
- QTL at 41.10 cM on chr 7B (LOD = 3.30) close to marker BS00080621 (K*=15.37; P<0.001; LOD = 3.10) explaining 15.8% of the phenotypic variance. Thousand grin weight was increased by the W141 allele.

In addition, two putative QTLs were found on 7A (LOD = 3.08) and on 3A (LOD = 2.78) explaining 12.8 and 13.0% of the phenotypic variation, respectively.

Grains per m^2: Under HN, one QTL (LOD = 5.53) at 42.6 cM on chr 1A near marker BS00016654 (K*=1.24; NS; LOD = 5.35) at 41.6 cM was found explaining 23.7% of the phenotypic variance (Table 7.6, Figure 7.2). Grains per m^2 was

increased by the Paragon allele. In addition, three putative QTLs were found on 3A (LOD = 2.91), 7B (LOD = 2.35) and 6B (LOD = 2.01) explaining 13.3, 10.9 and 9.4% of the total phenotypic variation, respectively. Under LN conditions, four putative QTLs were found on 1A (LOD = 2.95), 7A (LOD = 2.88), 4A (LOD = 2.38) and 3A (LOD = 2.18) (Table 7.7, Figure 7.2) explaining 13.4, 13.2, 11.0 and 10.1% of the total phenotypic variation, respectively.

Grains per ear: Under HN, one QTL was found: BS00011036 (K*=4.84; P<0.01; LOD = 3.60) at 44.6 cM on chr 6B explaining 16.2% of the phenotypic variance (Table 7.6, Figure 7.2). Grains per ear was increased by the W141 allele. In addition, two putative QTLs were found, one on 1A (LOD = 2.54) and one on 3D (LOD = 2.10) explaining 11.7 and 9.80% of the total phenotypic variation, respectively.

Under LN conditions, one QTL at BS00011769 (K*=4.87; P<0.01; LOD = 3.72) was found at 21.82 cM on chromosome 5B explaining 16.7% of the phenotypic variation (Table 7.7, Figure 7.2). Grains per ear was increased by the W141 allele. In addition, two putative QTLs were found, one on 3D (LOD = 2.54) and one on 7B (LOD = 1.97) explaining 11.7 and 9.20% of the total variation, respectively.

Ears per m^2: Under HN, five putative QTLs were identified, two on 5A (LOD = 2.77 and 2.22), one on 3A (LOD = 2.22) and two on 7B (LOD = 2.01 and 1.97) (Table 7.6, Figure 7.2). These QTLs explained from 9.20 to 12.7% of the total phenotypic variation. Under LN, three putative QTLs were found on 5A (LOD = 2.62), 3A (LOD = 2.50) and 6A (LOD = 2.45) (Table 7.7, Figure 7.2) explaining 12.1, 11.5 and 11.3% of the total phenotypic variation, respectively.

Table 7.6: QTLs identified using both Kruskal Wallis (KW) and Interval Mapping (IM) methods for grain yield and yield components under the HN treatment for Paragon x W141 mapping population in 2012/13.

Trait	Chr.	Position (cM)	Locus	Kroskal Wallis		Interval mapping				
				K*	Sign.	LOD	PT	Var.	%Expl.	Add.
Grain yield	6D	1.32	BS00022094	4.355	**	2.37	3.2	0.91	11.0	-0.26
	7B	48.0	BS00023800	3.059	*	1.9		0.93	8.90	0.15
1000 grain weight	1A	43.6	BS00016654	0.253	-	4.93	3.2	19.0	21.5	-0.58
	2D	58.8	cfd36	5.114	**	2.01		22.0	9.40	-1.17
	3A	10.0	BS00025739	9.46	****	3.39		20.5	15.3	-2.30
	6A	41.6	psp3152	9.321	****	2.16		21.8	10.0	-1.67
	6B	39.1	BS00076465	5.061	**	2.48		21.5	11.5	1.55
Grains per m^2	1A	42.6	BS00016654	1.236	-	5.53	3.2	1.8E+07	23.7	677
	3A	8.00	BS00025739	7.076	***	2.91		2.1E+07	13.3	2059
	6B	39.1	BS00076465	4.284	**	2.01		2.2E+07	9.4	-1541
	7B	48.0	BS00010251	7.724	***	2.35		2.1E+07	10.9	1724
Grains per ear	1A	42.6	BS00016654	3.703	*	2.54	3.3	96.0	11.7	2.60
	3D	52.4	BS00098495	5.992	**	2.10		98.0	9.80	-2.51
	6B	44.6	BS00011036	4.843	**	3.60		91.1	16.2	-2.51
Ears per m^2	3A	14.0	BS00032524	6.785	***	2.22	3.3	4786	10.3	34.4
	5A	3.00	BS00031177	9.749	****	2.77		4658	12.7	-30.0
	5A	33.8	BS00010966	3.378	*	2.22		4786	10.3	-10.0
	7B	47.0	BS00009398	5.23	**	2.01		4835	9.40	25.2
	7B	22.7	BS00012117	4.68	**	1.97		4844	9.20	18.1

Chr.: chromosome; significance level *: 0.1, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001; LOD: log likelihood score; PT: permutation test threshold; Var.: residual variance after fitting the QTL;%Expl.: the percentage of variance explained for by the QTL; Additive effect (µA-µB)/2

Table 7.7: Summary of QTLs identified using both Kruskal Wallis (KW) and Interval Mapping (IM) methods for grain yield and yield components under the LN treatment for Paragon x W141 mapping population in 2012/13.

Trait	Chr.	Position (cM)	Locus	Kroskal Wallis		Interval mapping				
				K*	Sign.	LOD	PT	Var.	%Expl.	Add.
Grain yield	1B	35.0	BS00010392	6.385	**	2.03	3.2	0.98	9.5	0.34
	4A	56.7	BS00101512	0.319	-	2.06		0.98	9.6	-0.04
	6B	44.6	BS00011036	1.967	-	2.02		0.98	9.40	-0.17
1000 grain weight	1A	43.6	BS00016654	0.091	-	3.52	3.2	12.5	15.8	-0.26
	3A	8.0	BS00025739	7.155	***	2.78		13.0	12.7	-1.64
	7A	131.8	BS00089844	0.263	-	3.08		12.8	14.0	0.41
	7B	41.1	wmc517	13.81	******	3.30		12.7	14.9	-1.61
Grains per m^2	1A	44.6	BS00016654	0.01	-	2.95	3.3	1.2E+07	13.4	424
	3A	10.0	BS00025739	6.022	**	2.18		1.3E+07	10.1	1511
	4A	56.7	BS00101512	0.953	-	2.38		1.3E+07	11.0	213
	7A	131.8	BS00089844	0.946	-	2.88		1.2E+07	13.2	-506
Grains per ear	3D	55.7	BS00101512	0.641	-	2.54	3.2	87.6	11.7	-0.01
	5D	21.8	BS00011769	4.869	**	3.72		82.6	16.7	-1.84
	7B	45.0	BS00009398	1.583	-	1.97		90.1	9.20	1.23
Ears per m^2	3A	66.9	BS00039498	3.015	*	2.50	3.1	2677	11.5	10.4
	5A	11.28	BS00011278	10.38	****	2.62		2660	12.1	-20.1
	6A	38.9	BS00010749	8.339	****	2.45		2683	11.3	19.9

Chr.: chromosome; significance level *: 0.1, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001; LOD: log likelihood score; PT: permutation test threshold; Var.: residual variance after fitting the QTL;%Expl.: the percentage of variance explained for by the QTL; Additive effect (μA-μB)/2

7.3.3 Genetic analysis of AGDM, NUE and components

7.3.3.1 PxW481

a) 2012

Anthesis date: Under HN, three putative QTLs were identified, on chr. 7A (LOD = 2.53), on 5B (LOD = 2.37) and on 1A (LOD = 2.20) (Table 7.8, Figure 7.1). These QTLs explained 13.7, 12.9 and 12.0% of the phenotypic variation, respectively. Under HN, two putative QTLs were identified on chr 5B (LOD = 2.04) and 5A (LOD = 1.95) explaining 11.2 and 10.7% of the phenotypic variation, respectively.

Plant height: Using the IM method under HN, two significant QTLs were found (Table 7.8, Figure 7.1):

- QTL at 61.0 cM on chr 4B (LOD = 3.90) close to marker BS00012071 (K*=8.96; P<0.001; LOD = 3.55) explaining 20.4% of the phenotypic variance. Plant height was increased by the W481 allele.
- QTL at 6.00 cM on chr 5A (LOD = 3.22) close to marker BS00011846 (K*=12.7; P<0.001; LOD = 3.04) explaining 17.1% of the phenotypic variance. Plant height was increased by the Paragon allele.

Under LN conditions, three QTLs were found:

- QTL at BS00011789 (K*=8.06; P<0.005; LOD = 3.47) at 8.5 cM on chr 6A explaining 18.3% of the phenotypic variance. Plant height was increased by the presence of W481 allele.
- QTL (LOD = 2.87) at 17.5 cM on chr 7B close to marker barc32 at 17.5 cM position (K*=20.33; P<0.001; LOD = 2.75) explaining 15.4% of the phenotypic variance; plant height was increased by the W481 allele.
- QTL at gwm577 (K*=5.06; P<0.05; LOD = 2.89) at 0.00 cM on chr 2D explaining 15.5% of the phenotypic variance. Plant height was increased by the presence of the Paragon allele.

In addition, three putative QTLs were identified, one on chr. 7B (LOD = 2.62) and two on 3A (LOD = 2.16 and 2.02) explaining 14.1, 11.8 and 11.1% of the phenotypic variation, respectively.

Above-ground dry matter: Under HN conditions, one significant QTL was found on chr 4B with a marker BS00009426 at 12.6 cM (K*=8.56; P<0.005; LOD = 3.10) explaining 16.5% of the phenotypic variance (Table 7.8, Figure 7.1). AGDM was increased by the Paragon allele. In addition, two putative QTLs were found on 3B (LOD = 2.53 and 2.32) explaining 13.7 and 12.6% of the phenotypic variation, respectively. Under LN conditions, four putative QTLs were found for AGDM one each on 3A (LOD = 2.331), 2B (LOD = 2.15), 5D (LOD = 2.137) and 2D (LOD = 2.09). These QTLs explained between 11.5 and 12.7% of the phenotypic variation.

Harvest index: Under HN conditions, one QTL was found on chr 7A with marker BS00003894 at 55.6 cM position (K*=0.83; NS; LOD = 2.67) explaining 14.4% of the phenotypic variance (Table 7.8, Figure 7.1). HI was increased by the Paragon allele. In addition, two putative QTLs were found on 5B (LOD = 2.53 and 2.05) explaining 13.7 and 11.3% of the phenotypic variation, respectively.

Under LN conditions, four QTLs were found:

- QTL at 8.50 cM on chr 3B (LOD = 4.88) close to marker BS00009651 (K*=17.5; P<0.001; LOD = 3.45) explaining 24.8% of the phenotypic variance. HI was increased by the Paragon allele.
- QTL at 2.00 cM on chr 6A (LOD = 2.95) close to marker BS00014510 (K*=5.69; P<0.05; LOD = 2.00) explaining 15.8% of the phenotypic variance. HI was increased by the Paragon allele.
- QTL at BS00010881 (K*=6.83; P<0.01; LOD = 2.82) at 30.5 cM on chr 3B. This QTL explained 15.2% of the phenotypic variance. HI was increased by the W481 allele.
- QTL at BS00018510 (K*=5.69; P<0.01; LOD = 2.81) at 0.00 cM on chr 5A. This QTL explained 15.1% of the phenotypic variance. HI was increased by the Paragon allele.

In addition, two putative QTLs were found on 5A (LOD = 2.40) and 6A (LOD = 2.35) explaining 13.1 and 12.8% of the phenotypic variation, respectively.

Table 7.8: QTLs identified using both Kruskal wallis (KW) and Interval Mapping (IM) methods for biomass (AGDM), harvest index (HI), plant height (PH) and anthesis date (AD) under HN and LN treatments for Paragon x W481 mapping population in 2011/12.

	Trait	Chr.	Position (cM)	Locus	Kroskal Wallis		Interval mapping				
					K*	Sign.	LOD	PT	Var.	%Expl.	Add.
HN conditions	Anthesis Date	1A	10	BS00010416	5.4	**	2.2	2.8	0.53	12	0.29
		5B	47.9	BS00012038	1.185	-	2.37		0.52	12.9	0.13
		7A	51.73	BS00003894	5.7	**	2.53		0.52	13.7	0.24
	Plant Height	2B	61	BS00009442	6.868	***	3.9	2.8	34.5	20.4	-1.94
		2B	49.2	BS00011737	8.899	****	3.11		36.1	16.6	-2.72
		5A	6	BS00011846	12.67	******	3.22		35.9	17.1	2.8
	Above-ground dry matter	3B	25.1	BS00010060	6.022	**	2.53	2.7	7.49	13.7	1.02
		3B	34.6	BS00011570	5.448	**	2.32		7.59	12.6	1.08
		4B	12.6	BS00009426	8.564	****	3.1		7.25	16.5	1.09
	Harvest index	5B	42.4	BS00003637	0.008	-	2.53	2.6	4.96	13.7	0.08
		5B	78.6	BS00011465	2.82	*	2.05		5.1	11.3	-0.66
		7A	55.6	BS00003894	0.828	-	2.67		4.92	14.4	0.25
LN conditions	Anthesis Date	5A	2.46	BS00010702	1.5	-	1.95	2.6	0.6	10.7	-0.08
		5B	48.55	BS00012038	7.6	***	2.04		0.6	11.2	0.26
	Plant Height	2D	0	gwm577	5.059	**	2.89	2.8	67.7	15.5	3.61
		3A	16.6	wmc264	5.627	**	2.16		70.6	11.8	3.59
		3A	1	BS00010531	4.423	**	2.02		71.2	11.1	2.39
		6A	8.5	BS00011789	8.059	****	3.47		65.4	18.3	-3.55
		7B	17.5	barc32	8.73	****	2.87		67.7	15.4	-4.2
		7B	3.2	BS00003756	6.293	**	2.62		68.7	14.1	-2.92
	Above-ground dry matter	2B	61.7	BS00009442	8.616	****	2.15	2.7	2.54	11.8	-0.43
		2D	24.3	BS00009575	4.248	**	2.09		2.55	11.5	-0.56
		3A	18.6	gwm2-2	7.663	***	2.33		2.52	12.7	0.5
		5D	18.2	BS00004065	2.309	-	2.13		2.54	11.7	-0.03
	Harvest index	3B	8.5	BS00009651	17.466	*******	4.88	2.7	2.41	24.8	0.94
		3B	30.5	BS00010881	6.831	***	2.82		2.71	15.2	0.59
		5A	0	BS00018510	5.687	**	2.81		2.71	15.1	-0.51
		5A	10.9	BS00011360	5.658	**	2.4		2.78	13.1	-0.6
		6A	2	BS00014510	1.568	-	2.95		2.69	15.8	-0.24
		6A	19.1	psp3152	3.35	*	2.35		2.79	12.8	-0.41

Chr.: chromosome; significance level *: 0.1, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001; LOD: log likelihood score; PT: permutation test threshold; Var.: residual variance after fitting the QTL;%Expl.: the percentage of variance explained for by the QTL; Additive effect (µA-µB)/2

N-use efficiency: One significant QTL was found under HN conditions at 23.1 cM on chr 6A near marker BS00001037 (K*=13.96; P<0.001; LOD = 3.08) explaining 17.6% of the phenotypic variance (Table 7.9, Figure 7.1). NUE was increased by the Paragon allele. In addition three putative QTLs were identified, one on chr 3B (LOD = 2.84), one on 4B (LOD = 2.16) and one on 5A (LOD = 2.10) explaining 15.2, 11.8 and 11.5% of the phenotypic variation, respectively.

Under LN, one QTL was found at BS00003589 (K*=0.014; NS; LOD = 3.07) at 17.1 on chr 2B explaining 16.4% of the phenotypic variation. In addition, nine more putative QTLs on 2D (2), 3B (2), 3A (1), 2B (1), 4B (1), 6A (1) and 5A (1), were identified explaining between 11.0 and 13.9% of the total phenotypic variation.

N-uptake efficiency: Under HN conditions, two QTLs were found (Table 7.9, Figure 7.1):

- QTL (LOD = 2.97) at 23.1 cM on chr 3B close to marker BS00001037 at 23.94 cM position (K*=11.78; P<0.001; LOD = 2.38) explaining 15.9% of the phenotypic variance. NUPE was increased by the Paragon allele.
- QTL (LOD = 3.08) at 34.08 cM on chr 3B close to marker BS00011570 at 34.56 cM position (K*=7.46; P<0.01; LOD = 3.07) explaining 16.4% of the phenotypic variance. NUPE was increased by the Paragon allele.

In addition, one putative QTL was found on chr. 4B (LOD = 2.56) explaining 13.8% of the phenotypic variation. Under HN, two significant QTLs were found:

- QTL at marker BS00003589 (K*=2.52; NS; LOD = 3.02) at 17.10 cM on chr 4Bexplaining 16.2% of the phenotypic variance. NUPE was increased by the W481 allele.
- QTL at marker BS00021895 (K*=3.21; P<0.1; LOD = 2.77) at 0.00 cM on chr 1A explaining 14.9% of the phenotypic variance. NUPE was increased by the Paragon allele.

In addition, three putative QTLs were identified, one on each of chr 4B (LOD = 2.56), 6A (LOD = 2.34) and 5B (LOD = 2.08) explaining 13.8, 12.7 and 11.4% of the phenotypic variation respectively.

N-utilisation efficiency (NUTE): one putative QTL was found on chr. 7A (LOD = 2.16) explaining 11.8% of the phenotypic variation (Table 7.9, Figure 7.1).

Under LN conditions, one significant QTL was found at 7.00 cM on chr 3B near marker gwm261 at 20.01 cM position (K*=2.21; NS; LOD = 0.77) explaining 15.8% of the phenotypic variance. NUTE was increased by the Paragon allele. In addition, two putative QTLs were found, one on 3A (LOD = 2.49) and one on 6B (LOD = 2.37) explaining, respectively, 13.5 and 12.9% of the total variation.

Table 7.9: Summary of QTLs identified using both Kruskal wallis (KW) and Interval Mapping (IM) for N-uptake efficiency (NUPE), N-utilisation efficiency (NUTE) and N-use efficiency (NUE) under HN and LN treatments for PxW481 mapping population in 2011/12.

	Trait	Chr.	Position (cM)	Locus	Kroskal Wallis		Interval mapping				
					K*	Sign.	LOD	PT	Var.	%Expl.	Add.
HN conditions	N-use efficiency	3B	34.6	BS00011570	8.558	****	2.84	2.9	19.9	15.2	1.85
		4B	12.6	BS00009426	6.307	**	2.16		20.7	11.8	1.6
		5A	0	BS00001953	5.053	**	2.1		20.8	11.5	1.64
		6A	23.1	BS00001037	14	******	3.32		19.3	17.6	2
	N-uptake efficiency	3B	34.1	BS00011570	7.461	***	3.08	2.7	0.04	16.4	0.09
		4B	12.6	BS00009426	5.653	**	2.56		0.04	13.8	0.07
		6A	23.1	BS00001037	11.782	*****	2.97		0.04	15.9	0.08
	N-utilisation efficiency	7A	13	BS00001450	6.463	**	2.16	2.8	1.32	11.8	0.46
LN conditions	N-use efficiency	2B	17.1	BS00003589	3.533	*	3.07	2.7	199	16.4	-2.89
		2B	61.7	BS00009442	4.113	**	2.17		210	11.9	-2.41
		2D	24.3	BS00009575	6.137	**	2.56		205	13.9	-5.92
		2D	5	gwm577	3.156	*	2.1		210	11.5	-6.18
		3A	18.6	gwm2-2	6.354	**	2.44		206	13.3	4.29
		3B	31.1	BS00010881	9.531	****	2.52		205	13.6	5.53
		3B	1	BS00020861	1.897	-	2.41		207	13.1	3.57
		4B	12.6	BS00015504	0.034	-	2.35		207	12.8	0.19
		5A	64	BS00009531	7.4	***	2		212	11	-6.26
		6A	9.9	BS00003881	3.966	**	2.35		207	12.8	-3.67
	N-uptake efficiency	1A	0	BS00021895	3.211	*	2.77	2.7	0.17	14.9	0.09
		2B	17.1	BS00003589	2.517	-	3.02		0.17	16.2	-0.07
		4B	12.6	BS00009426	0	-	2.56		0.17	13.8	-0.02
		5B	57.5	BS00003879	1.727	-	2.08		0.18	11.4	-0.03
		6A	1.2	BS00010326	4.61	**	2.34		0.18	12.7	0.16
	N-utilisation efficiency	3A	12.6	wmc264	10.639	****	2.49	2.8	3.57	13.5	0.81
		3B	7	BS00009651	9.832	****	2.94		3.48	15.8	0.73
		6B	1	BS00003852	6.989	***	2.37		3.6	12.9	-0.59

Chr.: chromosome; significance level *: 0.1, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001; LOD: log likelihood score; PT: permutation test threshold; Var.: residual variance after fitting the QTL;%Expl.: the percentage of variance explained for by the QTL; Additive effect (µA-µB)/2

b) 2013

Anthesis date: Under HN, two putative QTLs were found, one on 3B (LOD = 2.67) and one on 6B (LOD = 1.94) explaining 14.4 and 11.2% of the phenotypic variation, respectively (Table 7.10, Figure 7.1). Under LN, two putative QTLs were found, one on 3A (LOD = 2.59) and one on 4A (LOD = 2.12) explaining14.2 and 11.7% of the phenotypic variation, respectively.

Plant height: Three putative QTLs were found on 4A (LOD = 2.04), 5A (LOD = 2.17) and 4A (LOD = 2.04) explaining 14.5, 11.9 and 11.2% of the total phenotypic variation, respectively (Table 7.10, Figure 7.1). Under LN conditions, one QTL at BS00010531 (K*=15.82; P<0.001; LOD = 3.55) at 0.00 cM on chr 3A was found explaining 18.7% of the phenotypic variance. Plant height was increased by the Paragon allele. In addition, a QTL on 2B (LOD = 2.48) explained 13.5% of the total variation.

Above-ground dry matter: Under HN two putative QTLs, one on 3A (LOD = 2.451) and one on 5A (LOD = 1.99) explaining 13.3 and 10.9% of the phenotypic variation, respectively, were found (Table 7.10, Figure 7.1Under LN conditions, four putative QTLs were found, one on 7A (LOD = 2.65) and one on 2B (LOD = 2.56) and two on 5A (LOD = 2.38 and 2.07) explaining between 11.4 and 14.3% of the phenotypic variation.

Harvest index: Three putative QTLs were found on 6A (LOD = 2.79), 2B (LOD = 2. 74) and 1B (LOD = 1.98) explaining 15, 13.4 and 10.9% of the total phenotypic variation, respectively (Table 7.10, Figure 7.1).

Under LN conditions, one QTL at psp3152 (K*=13.69; P<0.001; LOD = 4.07) was found at 19.1 cM position on chr 6A explaining 21.1% of the phenotypic variance. Harvest index was increased by the presence of W481 allele. In addition, a QTL was found on 2B (LOD = 2.52) explaining 13.7% of the phenotypic variation.

Table 7.10: QTLs identified using both Kruskal Wallis (KW) and Interval Mapping (IM) methods for biomass (AGDM), harvest index (HI), plant height (PH) and anthesis date (AD) under HN and LN treatments for PxW481 mapping population in 2012/13.

	Trait	Chr.	Position (cM)	Locus	Kroskal Wallis		Interval mapping				
					K*	Sign.	LOD	PT	Var.	%Expl.	Add.
HN conditions	Anthesis date	3B	2	BS00011277	7.615	***	2.7	3	2.2	14.4	-0.7
		6B	9	BS00010420	7.492	***	1.9		2.3	10.7	-0.5
	Plant height	3B	9.5	BS00009651	4.71	**	2.7	3	20	14.5	-0.8
		4A	54.8	BS00018651	4.71	****	2		21	11.2	-1.9
		5A	8.2	BS00011846	8.81	****	2.2		21	11.9	1.58
	Above-ground biomass	3A	9.6	BS00011660	9.86	****	2.5	3	4.1	13.3	0.75
		5A	31.8	BS00011227	2.27	-	2		4.2	10.9	-0.5
	Harvest index	1B	17.3	BS00004129	3.42	*	2	2.9	2.8	10.9	-0.5
		2B	61.7	BS00009442	0.94	-	2.5		2.7	13.4	0.2
		6A	0	BS00003818	10.01	****	2.8		2.6	15	0.63
LN conditions	Anthesis date	3A	19.4	gwm2	9.61	****	2.6	3	2	14.2	0.61
		4A	65.8	BS00018651	8.66	****	2.1		2.1	11.7	-0.6
	Plant height	2B	63.6	BS00011630	9.08	****	2.5	2.7	45	13.5	-2.3
		3A	0	BS00010531	15.82	*******	3.6		42	18.7	3.12
	Above-ground biomass	2B	46.3	BS00021713	9.96	****	2.6	2.8	1	13.9	-0.4
		5A	8	BS00011846	5.91	**	2.4		1.1	12.9	0.26
		5A	66	BS00009460	1.24	-	2.1		1.1	11.4	-0.2
		7A	15	BS00001450	0.85	-	2.7		1	14.3	-0.1
	Harvest index	2B	5	BS00009531	0.58	-	2.5	3.0	2	13.7	-0.8
		6A	19.1	psp3152	13.69	******	4.1		1.8	21.1	-0.7

Chr.: chromosome; significance level *: 0.1, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001; LOD: log likelihood score; PT: permutation test threshold; Var.: residual variance after fitting the QTL;%Expl.: the percentage of variance explained for by the QTL; Additive effect (µA-µB)/2

N-Use efficiency: Under HN, one putative QTL was found on 6A (LOD = 2.3) explaining 14.9% of the phenotypic variation (Table 7.11, Figure 7.1). Under LN, three putative QTLs were found on 5A (LOD = 2.39), 7A (LOD = 2.39) and 2B (LOD = 2.34) explained 13.0, 13.0 and 12.8% of the total phenotypic variation, respectively.

Grain N concentration: Under HN, two markers were found (Table 7.11, Figure 7.1)

- QTL on BS0001713 (K*=15.82; P<0.001; LOD = 2.92) at 47.7 cM on chr 2B explaining 15.6% of the phenotypic variance. Grain N% was increased by the Paragon allele.
- QTL at 10.00 cM on chr 1B (LOD = 2.97) close to marker BS00010392 (K*=8.32; P<0.001; LOD = 2.50) explaining 15.9% of the phenotypic variance. Grain N% was increased by the presence of W481 allele.

In addition, two putative QTLs were found on 2B (LOD = 2.72) and on 6B (LOD = 2.27) explaining 14.7 and 12.4% of the phenotypic variation, respectively. Under LN, two putative QTLs were found on 5A (LOD = 2.59) and on 2B (LOD = 2.23) explaining 14.0 and 12.2% of the phenotypic variation, respectively.

N-uptake efficiency: Under HN, one putative QTLs was found on chr 6A (LOD = 2.09) explaining 11.5% of the phenotypic variation (Table 7.11, Figure 7.1). Under LN, two putative QTLs were found one on 5A (LOD = 2.14) and one on 7A (LOD = 2.01) explaining 13.1 and 11.1% of the phenotypic variation, respectiverly.

N-utilisation efficiency: Under HN conditions, one QTL coinciding with BS00011670 (K*=3.85; P<0.05; LOD = 3.73) at 0.00 cM on chr 1B was found explaining 19.5% of the phenotypic variation (Table 7.11, Figure 7.1). NUTE was increased by the presence of Paragon allele. In addition, seven putative QTLs on 5B (1), 2B (2), 4A (1), 6B (1), 5D (2) and 3A (1), were identified explaining between 11.1 and 13.5% of the total phenotypic variation.

Under LN, three putative QTLs were identified, one on chr 3B (LOD = 2.47), one on 5A (LOD = 1.99) and one on 2B (LOD = 1.99) explaining 13.4, 11.0 and 10.2% of the phenotypic variation, respectively.

Table 7.11: QTLs identified using both Kruskal wallis (KW) and Interval Mapping (IM) for N-uptake efficiency (NUPE), N-utilisation efficiency (NUTE) and N-use efficiency (NUE) under HN and LN treatments for the Paragon x W481 mapping population in 2012/13.

	Trait	Chr.	Position (cM)	Locus	Kroskal Wallis		Interval mapping				
					K*	Sign.	LOD	PT	Var.	%Expl.	Add.
HN conditions	N-use efficiency	6A	0.2	BS00010326	11.53	*****	2.3	2.9	15	12.5	1.39
	N-uptake efficiency	6A	0	BS00003818	6.566	**	2.1	3	0	11.5	0.04
	N-utilisation efficiency	1B	0	BS00011670	3.846	**	3.7	3	0.8	19.5	0.19
		2B	47.7	BS00021713	13.26	******	2.5		0.9	13.4	-0.4
		2B	6	BS00010688	3.929	**	2.1		0.9	11.7	-0.5
		3A	10.6	BS00011660	4.229	**	2		0.9	11.1	0.29
		4A	45.8	BS00003674	3.066	*	2.3		0.9	12.3	0.25
		5B	82.6	BS00011465	6	**	2.5		0.9	13.5	-0.3
		5D	7	BS00021731	0.948	-	2.1		0.9	11.7	-0.2
		6B	3	BS00003893	0.4	-	2.2		0.9	11.9	-0.1
	Grain N concentration	1B	10	BS00010392	8.316	****	3	3	0	15.9	-0
		2B	47.7	BS00021713	14.96	******	2.9		0	15.6	0.03
		2B	64.6	BS00011630	9.939	****	2.7		0	14.7	0.03
		6B	2	BS00003852	6.745	***	2.3		0	12.4	0.02
LN conditions	N-use efficiency	2B	46.3	BS00021713	7.4	***	2.3	2.8	4.2	12.8	-0.8
		5A	8	BS00011846	4.47	**	2.4		4.2	13	0.44
		7A	14	BS00001450	0.83	-	2.4		4.2	13	-0.2
	N-uptake efficiency	5A	8	BS00011846	3.99	**	2.4	3	0.3	13.1	0.1
		7A	15	BS00001450	0.78	-	2		0.3	11.1	-0.1
	N-utilisation efficiency	2B	16.8	BS00011047	1.18	-	2	2.7	5.1	10.9	-0.3
		3B	2	BS00020861	4.72	**	2.5		4.9	13.4	0.67
		5A	27.9	BS00001735	10.25	****	2		5.1	11	0.84
	Grain N concentration (%)	2B	16.8	BS00011047	1.14	-	2.2	3	0	12.2	0.01
		5A	28.9	BS00001735	11.32	*****	2.6		0	14	-0.1

Chr.: chromosome; significance level *: 0.1, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001; LOD: log likelihood score; PT: permutation test threshold; Var.: residual variance after fitting the QTL;%Expl.: the percentage of variance explained for by the QTL; Additive effect (µA-µB)/2

7.3.3.2 Paragon x W141

Anthesis date: Under HN one QTL, BS00011036 (K*=4.89; P<0.05; LOD = 3.13) at 44.63 cM on chr 6B was found explaining 14.5% of the phenotypic variation (Table 7.12, Figure 7.2). Anthesis date was increased by the W141 allele. In addition, three putative QTLs were identified, two on chr. 7A (LOD = 2.31 and 2.27) and one on 2B (LOD = 2.31) explaining, 10.9, 10.7 and 10.9% of the phenotypic variation, respectively. Under LN, six putative QTLs on 1A (1), 1D (1), 2D (1), 5B (1), 6A (1) and 7B (1) were identified explaining between 10.8 and 14.1% of the total phenotypic variation.

Plant height: Under HN, one major QTL (LOD = 3.83) at 42.56 cM on chr 1A, close to marker BS00016654 (K*=4.66; P<0.05; LOD = 3.81) was identified explaining 17.3% of the phenotypic variance (Table 7.12, Figure 7.2). Plant height was increased by the W141 allele. In addition, three putative QTLs were found, two on 5B (LOD = 2.55 and 2.38) and one on 3A (LOD = 2.42), explaining 11.9, 11.1 and 11.3% of the total phenotypic variation, respectively. Under LN, six putative QTLs on 1A (1), 1D (1), 2D (1), 5B (1), 6A (1) and 7B (1) were identified explaining between 9.5 and 11.4% of the total phenotypic variation.

Above-ground dry matter: Under HN, five putative QTLs were found, two on 5B (LOD = 2.53 and 2.06), one on 7D (LOD = 2.47) and two on 6A (LOD = 2.20 and 2.13) explaining between 9.9 and 11.7% of the phenotypic variation (Table 7.12, Figure 7.2). Under LN conditions, three putative QTLs were found on chr 6A (LOD = 2.68), 4A (LOD = 2.65), 2D (LOD = 2.57) and 2A (LOD = 2.30).

Harvest index: four putative QTLs were found on ch 5B (LOD = 2.46), 6D (LOD = 2.27), 7A (LOD = 2.16) and on 1A (LOD = 2.02) (Table 7.12, Figure 7.2) explaining 11.7, 11.8, 11.9, and 11.9% of the total phenotypic variation, respectively.
Under LN conditions, one QTL, BS00016654 (K*=13.69; P<0.001; LOD = 3.28), was found at 41.56 cM on chr 1A explaining 14.9% of the phenotypic variance. Harvest index was increased by the presence of W481 allele. In addition, three putative QTLs were found on chr 2D (LOD = 2.95), 5B (LOD = 2.17) and 2D (LOD = 2.95) explaining 13.5, 10.1, and 9.1% of the total phenotypic variation, respectively.

	Trait	Chr.	Position (cM)	Locus	Kroskal Wallis		Interval mapping				
					K*	Sign.	LOD	PT	Var.	%Expl.	Add.
HN conditions	Anthesis date (AD)	2B	19.7	BS00022493	2.72	*	2.31	3.1	3.59	10.9	-0.17
		6B	44.6	BS00011036	4.89	**	3.13		3.44	14.5	-0.44
		7A	78.6	BS00088825	5.53	**	2.31		3.59	10.9	-0.45
		7A	42.1	BS00108786	4.64	**	2.27		3.6	10.7	0.59
	Plant height (PH)	1A	42.6	BS00016654	4.66	**	3.83	3.1	106	17.3	-2
		3A	0	BS00025739	5.91	**	2.42		113	11.3	-3
		5B	3.2	BS00003739	11.42	*****	2.55		113	11.9	-4
		5B	50.9	BS00000592	5.47	**	2.38		114	11.1	3
	Above-ground biomass (AGDM)	5B	0	BS00009264	4.35	**	2.53	3.1	3.46	11.7	-0.4
		5B	43.2	BS00010366	6.43	**	2.06		3.54	9.6	0.65
		6A	41.6	psp3152	6.91	***	2.20		3.52	10.2	-0.46
		6A	0	BS00003616	6.39	**	2.13		3.53	9.9	0.51
		7D	9.3	BS00018030	4.89	**	2.47		3.47	11.4	0.54
	Harvest index (HI)	1A	41.6	BS00016654	1.94	-	2.02	3.2	11.9	9.4	0.52
		5B	50.9	BS00000592	5.02	**	2.46		11.7	11.4	-1
		6D	0	BS00042153	13.08	******	2.27		11.8	10.5	-1.18
		7A	122.4	BS00001450	5.01	**	2.16		11.9	10	0.93
LN conditions	Anthesis date (AD)	1A	39.9	BS00011286	0.16	-	2.18	3.3	2.98	10.8	-0.08
		2D	48.6	BS00064538	0.79	-	2.91		2.87	14.1	-0.25
		5B	92.8	BS00011633	8.4	****	2.27		2.97	11.2	0.64
		5D	21.8	BS00021687	4.66	**	2.67		2.9	13.1	-0.48
		6A	67.4	BS00035914	2.45	-	2.40		2.95	11.8	0.32
		7B	12.2	BS00055861	3.83	*	2.32		2.96	11.4	-0.71
	Plant height (PH)	1A	42.6	BS00016654	0.99	-	2.46	3.3	113	11.4	-1.19
		1D	66.4	BS00010946	0.19	-	2.07		115	9.7	0.43
		2D	58.8	cfd36	11.9	*****	2.13		115	9.9	-3.12
		5B	50.9	BS00000592	4573	**	2.04		116	9.5	2.84
		6A	60.4	BS00035914	2.68	-	2.38		114	11	-3.17
		7B	25.7	BS00003350	5632	**	2.21		115	10.2	-3.45
	Above-ground biomass (AGDM)	2A	15.1	BS00011075	3.19	*	2.30	3	4.45	10.6	-0.52
		2D	24.6	BS00003804	10.5	****	2.57		4.39	11.8	-0.83
		4A	67	wmc262	13.7	******	2.65		4.38	12.2	-0.79
		6A	64.4	BS00021965	7.83	***	2.68		4.37	12.3	-0.95
	Harvest index (HI)	1A	41.6	BS00016654	0.05	-	3.28	3.2	15.8	14.9	-0.12
		2D	58.9	cfd36	9.68	****	2.95		16	13.5	1.42
		5B	50.9	BS00000592	7.41	***	2.17		16.7	10.1	-1.21
		6B	41.1	BS00076465	5.35	**	1.94		16.9	9.1	-1.16

Table 7.12: QTLs identified using both Kruskal Wallis (KW) and Interval Mapping (IM) methods for biomass (AGDM), harvest index (HI), plant height (PH) and anthesis date (AD) under HN and LN treatments for Paragon x W141 mapping population in 2012/13.

Chr.: chromosome; significance level *: 0.1, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001; LOD: log likelihood score; PT: permutation test threshold; Var.: residual variance after fitting the QTL;%Expl.: the percentage of variance explained for by the QTL; Additive effect (µA-µB)/2

N-use efficiency: Under HN, three putative QTLs were identified, one on chr. 6D (LOD = 2.23), one on 7B (LOD = 2.23) and one on 3A (LOD = 2.06) (Table 7.13, Figure 7.2) explaining14.7, 14.7 and 14.8% of the phenotypic variation, respectively. Under LN, two putative QTLs were found, one on 6B (LOD = 2.27) and one on 1B (LOD = 2.05) explaining10.5 and 9.6% of the total variation, respectively.

N-uptake efficiency: Under HN, two putative QTLs were found, one on 2A (LOD = 2.86) and one on 6D (LOD = 2.760) explaining 13.1 and 12.7% of the total phenotypic variation, respectively (Table 7.13, Figure 7.2).

Under LN, two putative QTLs were found, one on 7B (LOD = 2.23) and one on 4A (LOD = 2.06) explaining 10.3 and 9.6% of the total phenotypic variation, respectively.

N-utilisation efficiency: Under HN, one QTL at BS00011036 (K*=4.89; P<0.05; LOD = 5.09) was found at 44.6 cM on chr 6 explaining 22.1% of the phenotypic variation (Table 7.13, Figure 7.2). NUTE was increased by the W141 allele. In addition, two putative QTLs were found on 4A (LOD = 2.96) and on 7B (LOD = 2.24) explaining 13.5 and 10.4% of the phenotypic variation, respectively.

Under LN, two QTLs were found:

- QTL at 41.1 cM on chr 7B close marker BS00080621 (K*=12.91; P<0.001; LOD = 3.94) explaining 17.6% of the phenotypic variance. NUTE was increased by the Paragon allele.
- QTL at 14.00 cM on chr 3A close to marker BS00032524 (K*=8.72; P<0.001; LOD = 3.45) at 25.37 cM explaining 15.6% of the total phenotypic variation. NUTE was increased by the W141 allele.

In addition, two putative QTLs were found, one on 6D (LOD = 3.16) and one on 2D (LOD = 1.99) explaining respectively 14.3 and 9.3% of the total phenotypic variation.

Table 7.13: QTLs identified using both Kruskal Wallis (KW) and Interval Mapping (IM) for N-uptake efficiency (NUPE), N-utilisation efficiency (NUTE) and N-use efficiency (NUE) under HN and LN treatments for Paragon x W141 mapping population in 2012/13.

	Trait	Chr.	Position (cM)	Locus	Kroskal Wallis		Interval mapping				
					K*	Sign.	LOD	PT	Var.	%Expl.	Add.
HN conditions	N use efficiency (NUE)	3A	30.8	BS00010531	0.91	-	2.1	3	15	9.6	0.5
		6D	1.3	BS00022094	3.69	*	2.2		15	10.3	-1.1
		7B	48	BS00010251	5.19	**	2.2		15	10.3	0.77
	N uptake efficiency (NUPE)	2A	7.2	BS00004090	3.4	*	2.9	3	0	13.1	-0
		6D	1.3	BS00022094	4.74	**	2.8		0	12.7	-0
	N utilisation efficiency (NUTE)	4A	18.1	wmc491	7.41	***	3	3	1.6	13.5	0.59
		6B	44.6	BS00011036	3.46	*	5.1		1.4	22.1	-0.2
		7B	40.1	wmc517	8.13	****	2.2		1.6	10.4	0.33
LN conditions	N use efficiency (NUE)	1B	36	BS00021876	9.93	****	2.1	3	295	9.6	5.55
		6B	44.6	BS00011036	2.47	-	2.3		292	10.5	-3.3
	N uptake efficiency (NUPE)	4A	67	wmc262	9.51	****	2.1	3	0.3	9.6	-0.2
		7B	23.7	BS00012117	5.29	**	2.2		0.3	10.3	-0.1
	N utilisation efficiency (NUTE)	2D	58.9	cfd36	4.12	**	2	3	8.2	9.3	0.61
		3A	14	BS00032524	8.72	****	3.5		7.6	15.6	1.62
		6D	0	BS00042153	12.6	******	3.2		7.7	14.3	-1.2
		7B	41.1	wmc517	12.4	******	3.9		7.4	17.6	1.21

Chr.: chromosome; significance level *: 0.1, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001; LOD: log likelihood score; PT: permutation test threshold; Var.: residual variance after fitting the QTL;%Expl.: the percentage of variance explained for by the QTL; Additive effect (μA-μB)/2

7.3.4 Genetic analysis of physiological and senescence data

7.3.4.1 PxW481

a) 2012

Pre-anthesis NDVI: Under HN, one QTL (LOD = 2.91) was found at 6.84 cM on chr 3B. This QTL is close to the marker BS00011438 (K*=6.8; P<0.001; LOD = 2.88) at 5.84 cM and explained 15.8% of the phenotypic variation (Table 7.14, Figure 7.1). In addition, three putative QTLs were identified, one on chr. 3B (LOD = 2.49), one on 6A (LOD = 2.29) and one on 3A (LOD = 2.04) explaining 13.6, 12.6 and 11.3% of the phenotypic variation, respectively.

Under LN conditions, , two QTLs were found:

- QTL at 51.0 cM on chr 2B coinciding with the marker BS00003788 (K*=12.0; P<0.001; LOD = 3.4) explaining 18.0% of the phenotypic variance. NDVI was increased by the W481 allele.
- QTL at 61.7 cM on chr 2B coinciding with the marker BS00009442 (K*=12.0; P<0.001; LOD = 3.58) explaining 15.8% of the phenotypic variance. NDVI was increased by the W481 allele.

Post-anthesis NDVI: Under HN, two putative QTLs were found, one on 5A (LOD = 2.62) and one on 6A (LOD = 2.16), explaining 14.2 and 11.8% of the total phenotypic variation, respectively (Table 7.14, Figure 7.1). Under LN, no putative QTL was found. The highest LOD was for BS00003818 (LOD = 1.74) which explained 9.7% of the total phenotypic variation.

Table 7.14: QTLs identified using both Kruskal Wallis (KW) and Interval Mapping (IM) for Pre-anthesis NDVI, Per-anthesis NDVI under HN and LN treatments for PxW481 mapping population in 2011/12.

	Trait	Chr.	Position (cM)	Locus	Kruskal Wallis		Interval mapping				
					K*	Sign.	LOD	PT	Var.	%Expl.	Add.
HN	Pre-anthesis NDVI	3A	18.6	gwm2-2	5.1	**	2.04	2.9	7.4E-05	11.3	-0.003
		3B	6.84	BS00011438	6.8	***	2.91		7.0E-05	15.8	0.004
		3B	13.5	BS00009651	8.8	****	2.49		7.2E-05	13.6	0.004
		6A	20.1	psp3152	3.5	*	2.29		7.2E-05	12.6	0.002
	Post-anthesis NDVI	5A	0.00	BS00018510	6.2	**	2.62	2.7	0.004	14.2	0.02
		6A	2.00	BS00014510	4.2	**	2.16		0.004	11.8	0.02
LN	Pre-anthesis NDVI	2B	51.0	BS00003788	12.0	*****	3.58	2.8	6.0E-04	18.8	-0.01
		2B	61.7	BS00009442	12.0	*****	3.40		6.0E-04	18.0	-0.01

Chr.: chromosome; significance level *: 0.1, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001; LOD: log likelihood score; PT: permutation test threshold; Var.: residual variance after fitting the QTL;%Expl.: the percentage of variance explained for by the QTL; Additive effect (µA-µB)/2

a) 2013

Pre-anthesis NDVI: Under HN one putative QTL was found on 5A (K*=2.41; NS; LOD = 1.98) explaining 11% of the phenotypic variation (Table 7.15, Figure 7.1). NDVI was increased by the Paragon allele. Under LN, one putative QTL was found on 7A (K*=6.76; P<0.001; LOD = 227) explaining 12.4% of the phenotypic variation. NDVI was increased by the W481 allele.

Post-anthesis NDVI: Under HN, three putative QTLs were found two on 3A (LOD = 2.63 and 2.02) and one on 5B (LOD = 2.32) (Table 7.15, Figure 7.1) explaining 14.4, 11.3 and 12.8% of the total phenotypic variation, respectively. Under LN, one QTL (LOD = 3.31) at 64.6 cM on chr 2B near marker, BS00011630 (K*=2.38; NS; LOD = 5.35) at 63.55 cM, was found explaining 17.6% of the phenotypic variance. GM was increased by the W481 allele. In addition, two putative QTLs were found, one on 2B (LOD = 2.37) and one on 3A (LOD = 2.25) explaining 12.9 and 12.3% of the total phenotypic variation, respectively.

Onset of rapid phase of post-anthesis senescence: Under HN, two putative QTLs were found, one on 5A (LOD = 2.41) and one on 3B (LOD = 2.10) explaining 13.4 and 11.8% of the total phenotypic variation, respectively. Under LN, the highest LOD = 1.72 was recorded at 17.2 cM on chr 5D, close to marker BS00021742 (K*=5.62; P<0.05; LOD = 1.71) at 16.15 cM.

Total duration of post-anthesis senescence: Under HN one putative QTL was found on 5B (LOD = 2.24) explaining 13.2% of the phenotypic variation (Table 7.15, Figure 7.1). Under LN conditions, two putative QTLs were found on 2B (LOD = 2.31 and 2.13) explaining 12.9 and 11.9% of the total phenotypic variation, respectively.

Duration of rapid phase of post-anthesis senescence: Under HN, no maker was significant for SEN. The highest LOD = 1.72 (BS00011274) was observed at 0.00 cM on chr 1B (Table 7.15, Figure 7.1).

Under LN, one putative QTL was found on 2B (LOD = 1.99) explaining 11% of the phenotypic variation.

Table 7.15: QTLs identified using both Kruskal Wallis (KW) and Interval Mapping (IM) for Pre-anthesis NDVI, Per-anthesis NDVI, Onset of rapid phase of post-anthesis senescence, Total duration of post-anthesis senescence, Duration of rapid phase of post-anthesis senescence under HN and LN treatments for Paragon x W481 mapping population in 2012/13.

	Trait	Chr.	Position (cM)	Locus	Kruskal Wallis		Interval mapping				
					K*	Sign.	LOD	PT	Var.	%Expl.	Add.
HN conditions	Pr-anthesis NDVI	5A	0.0	BS00018510	2.41	-	1.98	2.6	0.00	11	0.01
	Post-anthesis NDVI	3A	18.6	gwm2	0.06	-	2.63	3	0.01	14.4	0.00
		3A	6.8	BS00011660	0.28	-	2.02		0.01	11.3	0.00
		5B	64.6	BS00003879	1.47	-	2.32		0.01	12.8	-0.01
	ONSEN	5A	3.0	BS00001953	2.52	-	2.41	2.7	14019	13.4	44.9
		3B	31.1	BS00010881	0.13	-	2.10		14279	11.8	-1.76
	DURTOT	5B	43.9	BS00000592	8.13	****	2.24	2.9	9869	13.2	-41.0
LN conditions	Pr-anthesis NDVI	7A	32.7	BS00000663	6.76	***	2.27	2.9	0.001	12.4	-0.01
	Post-anthesis NDVI	2B	64.6	BS00011630	12.2	******	3.31	2.9	0.003	17.6	-0.02
		2B	46.3	BS00021713	10.2	****	2.37		0.003	12.9	-0.02
		3A	0.0	BS00010531	8.66	****	2.25		0.003	12.3	0.02
	SEN	2B	61.0	BS00009442	5.00	**	1.99	2.8	6549	11.0	-21.2
	DURTOT	2B	45.3	BS00009247	7.64	***	2.31	2.9	9140	12.9	-41.7
		2B	66.5	BS00011275	5.02	**	2.13		9243	11.9	-31.2

Chr.: chromosome; significance level *: 0.1, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001; LOD: log likelihood score; PT: permutation test threshold; Var.: residual variance after fitting the QTL;%Expl.: the percentage of variance explained for by the QTL; Additive effect (μA-μB)/2

7.3.4.2 Paragon x W141

Pre-anthesis NDVI: Under HN, three putative QTLs were found on 6D (LOD = 2.57) and 5B (LOD = 2.14) and 7A (LOD = 1.9) (Table 7.16, Figure 7.2). These QTLs explained, respectively 12.0, 10.1 and 9% of the total phenotypic variation. Under LN, six putative QTL on 2D (1), 6A (1), 3D (1), 2B (1), 3B (1) and 7B (1) were identified explaining between 9.6 and 12% of the total phenotypic variation.

Post-anthesis NDVI: Under HN three putative QTLs were identified, two on chr. 1B (LOD = 2.39; LOD = 2.33) and one on 6D (LOD = 2.21) (Table 7.16, Figure 7.2) explaining 11.3, 10.9 and 10.5% of the phenotypic variation, respectively. Under LN conditions, three putative QTLs were identified, one on chr. 5A (LOD = 3.04), one on chr. 4A (LOD = 2.62) and one on 2B (LOD = 2.40). These QTLs explained 13.9, 12.0 and 11.1% of the phenotypic variation, respectively.

Onset of rapid phase of senescence: Under HN, one putative QTL was found on chr. 7B. Under LN, one QTL (LOD = 4.34) was found at 19.02 cM on chr 2D close to marker BS00021923 (K*=0.05; NS; LOD = 0.17) explaining 19.2% of the phenotypic variation. ONSEN was increased by the W141 allele.

Total duration of the senescence: Under HN, one QTL: BS00011539 (K*=6.28; P<0.05; LOD = 3.32) located at 2.90 cM on chr 2A explaining 15% of the total phenotypic variation (Table 7.16, Figure 7.2). DURTOT was increased by the W141 allele. In addition, four putative QTLs were found, two on 6A (LOD = 2.36 and 2.01), one on 1D (LOD = 2.19) and one on 2B (LOD = 2.14). Under LN conditions, five putative QTLs were found, one on 1A (LOD = 2.25), two on 2B (LOD = 2.17 and 2.16), one on 4A (LOD = 2.14) and one on 5B (LOD = 2.05) explaining between 9.6 and 10.5% of the phenotypic variation.

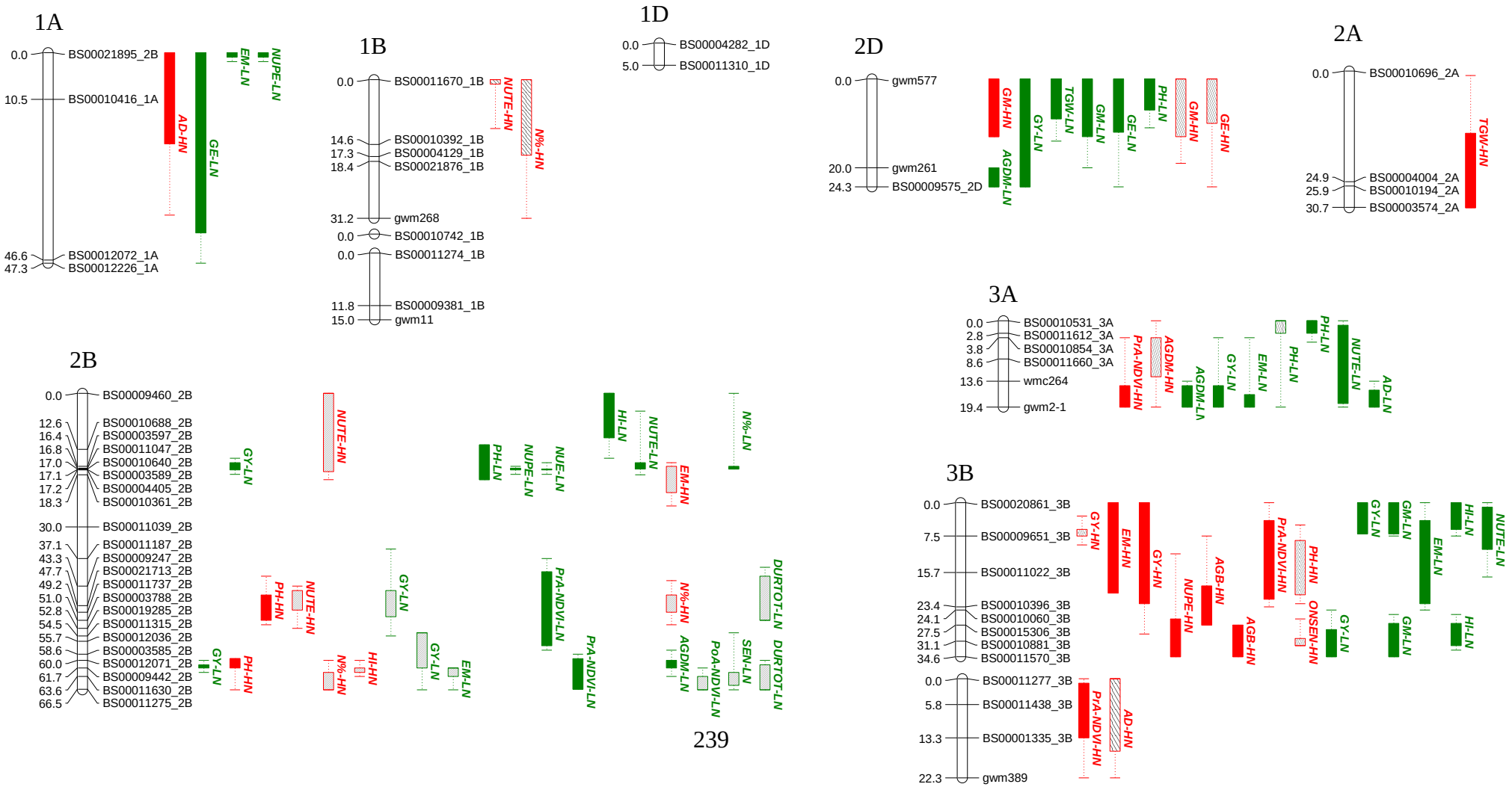
Duration of the senescence rapid phase: Under HN, two putative QTLs were found on 5A (LOD = 2.76) and on 7B (LOD = 2.24) explaining 12.6% of the total phenotypic variation (Table 7.16, Figure 7.2). Under LN conditions, one putative QTL (LOD = 2.21) was found on 6A explaining 10.3% of the phenotypic variation.

Table 7.16: QTLs identified using both Kruskal Wallis (KW) and Interval Mapping (IM) for Pre-anthesis NDVI , Per-anthesis NDVI , Onset of rapid phase of post-anthesis senescence , Total duration of post-anthesis senescence , Duration of rapid phase of post-anthesis senescence under HN and LN treatments for Paragon x W141 mapping population in 2012/13.

	Trait	Chr.	Pos. (cM)	Locus	Kruskal Wallis		Interval mapping				
					K*	Sign.	LOD	PT	Var.	%Expl.	Add.
HN conditions	Pr-anthesis NDV	5B	0	BS00009264	6.81	***	2.14	3.1	0.0002	10.1	-0.004
		6D	1.3	BS00022094	5.51	**	2.57		0.0001	12	-0.003
		7A	135.1	BS00009797	1.19	-	1.9		0.0002	9	0.002
	Post-anthesis NDVI	1B	27	BS00009709	6.23	**	2.39	3.3	0.005	11.3	-0.03
		3B	65.9	BS00009701	5.81	**	2.3		0.005	10.9	0.02
		6D	0	BS00009806	4.94	**	2.21		0.005	10.5	-0.01
	ONSEN	7B	23.7	BS00055861	0.06	-	2.7	3.2	6263	12.5	1.13
	SEN	5A	1	BS00031177	3.39	*	2.76	2.8	5029	12.6	-10.2
		7B	13.2	BS00055861	1.67	-	2.24		5159	10.4	8.58
	DURTOT	1D	12	IS00020531	0.59	-	2.19	3.2	5424	10.2	-6.6
		2A	2.9	BS00011539	6.28	**	3.32		5131	15	-21.7
		2B	64.6	BS00064156	1.97	-	2.14		5438	10	18.4
		6A	38.9	BS00009871	2.92	*	2.36		5380	10.9	12.4
		6A	6.9	BS00012414	0.45	-	2.01		5473	9.4	-5.03
LN conditions	Pr-anthesis NDVI	2B	24.76	BS00003585	0.06	-	2.18	3	0.0005	10.2	0
		2D	24.58	BS00003804	8.27	****	2.58		0.0005	12	-0.01
		3B	0	barc164	4.96	**	2.06		0.0005	9.7	0.01
		3D	30.74	gwm3	0.1	-	2.25		0.0005	10.5	0
		6A	61.39	BS00035914	1.93	-	2.27		0.0005	10.6	-0.01
		7B	30.09	BS00022053	1.68	-	2.04		0.0005	9.6	0
	Post-anthesis NDVI	2B	20.73	BS00022493	3.14	*	2.4	3.2	0.003	11.1	-0.01
		4A	54.73	BS00018651	2.36	-	2.62		0.003	12	-0.01
		5A	57.19	BS00011360	12.98	******	3.04		0.003	13.9	0.03
	ONSEN	2D	19	BS00021923	0.4	-	4.34	3	7751	19.2	-4.95
	SEN	6A	66.4	BS00021965	1.77	-	2.21	3.2	5367	10.3	18.2
	DURTOT	1A	41.56	BS00016654	4.43	**	2.25	3.1	8372	10.5	13.8
		2B	61.57	BS00009604	7.72	***	2.17		8408	10.1	-46.6
		2B	24.76	BS00003585	0.93	-	2.16		8409	10.1	11.9
		4A	52.73	BS00018651	6.79	***	2.14		8417	10	-28.3
		5B	26.4	BS00010679	0.42	-	2.05		8456	9.6	-1.42

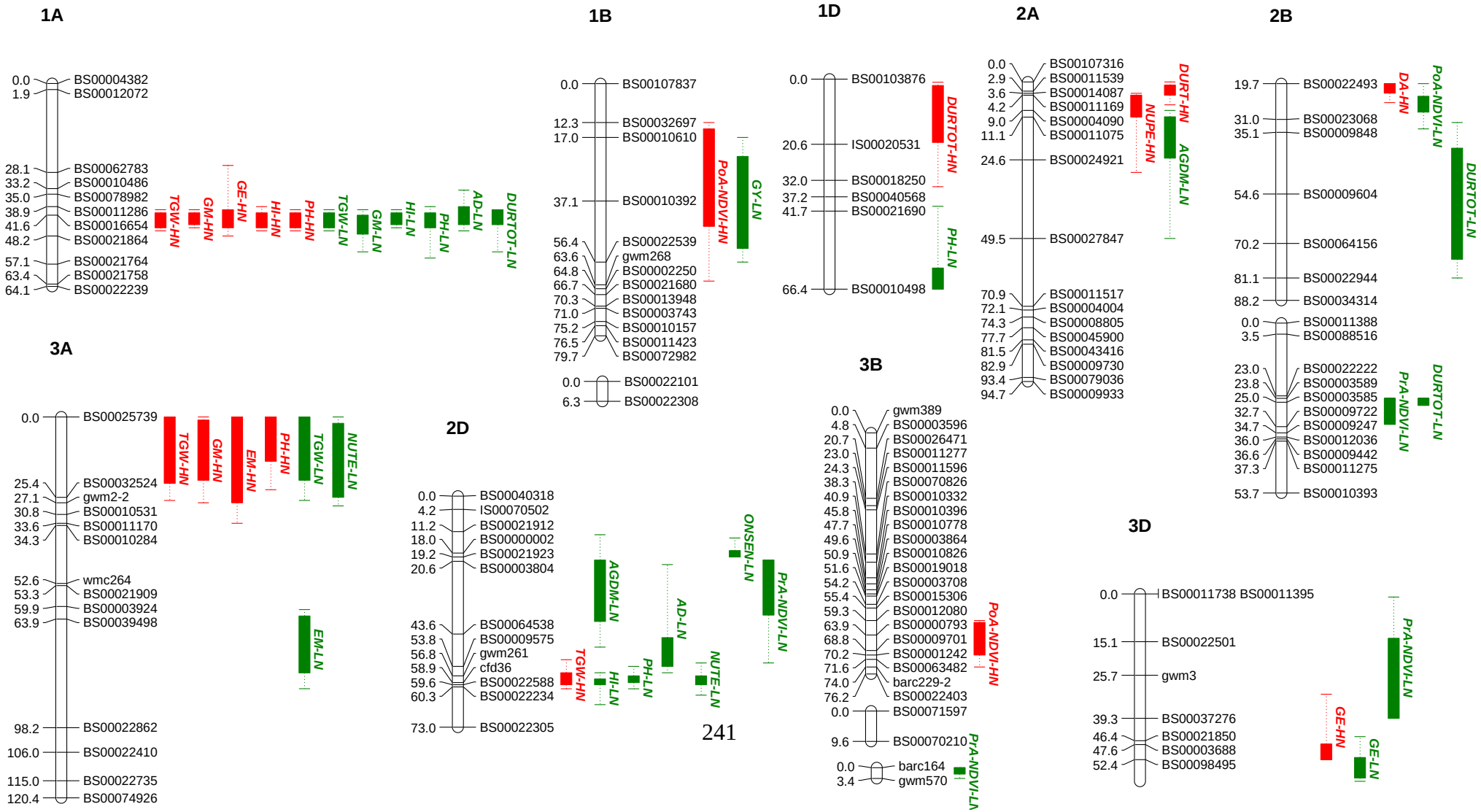
Chr.: chromosome; significance level *: 0.1, **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001; LOD: log likelihood score; PT: permutation test threshold; Var.: residual variance after fitting the QTL;%Expl.: the percentage of variance explained for by the QTL; Additive effect (µA-µB)/2

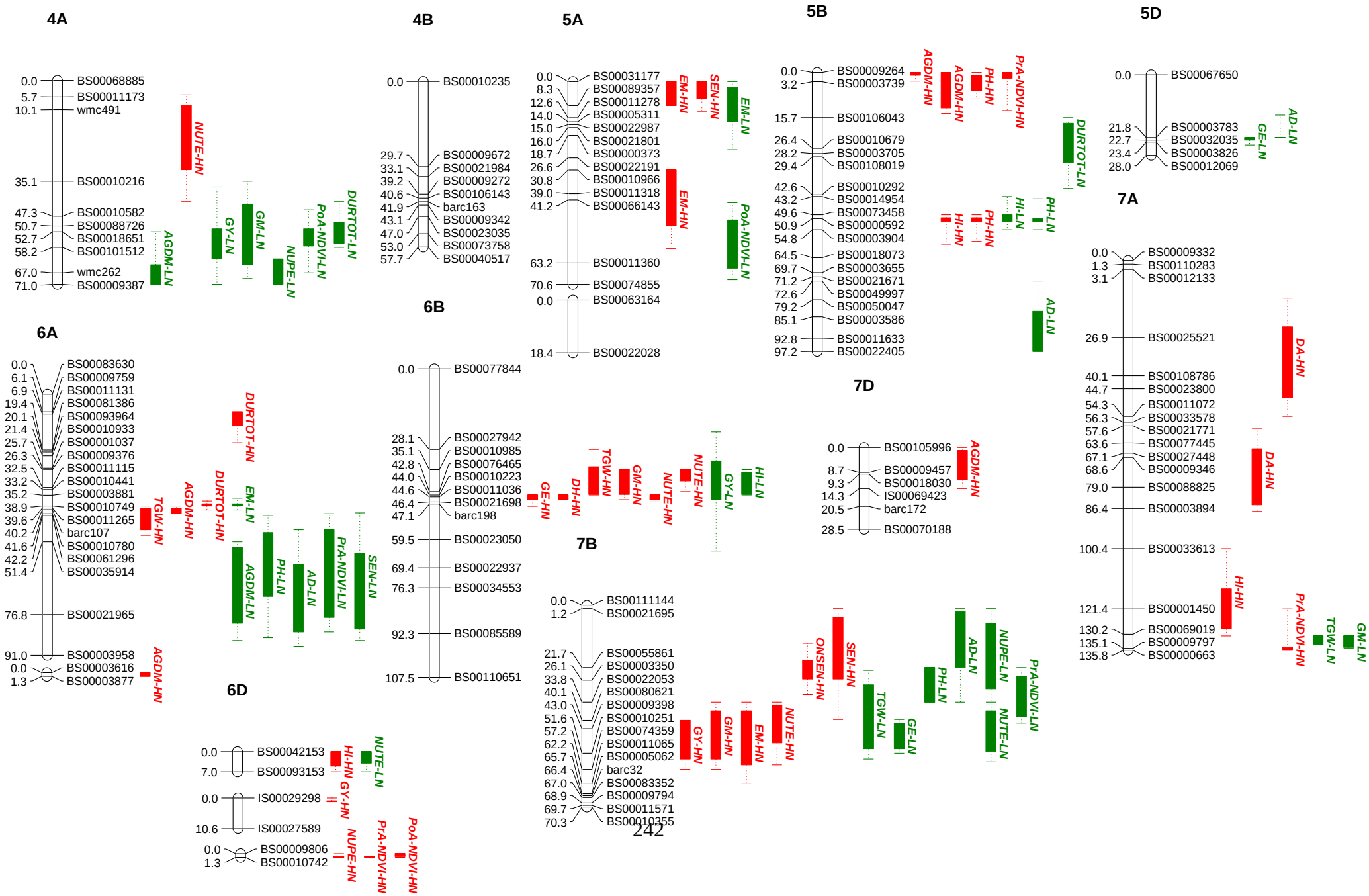
Figure 7.1: Summary of location of QTL using Interval Mapping (IM) method for Paragon xW481 MP for grain yield (GY), 1000 grain weight (TGW), grains per m^2 (GM), grains per ear (GE), ears per m^2 (EM), above-ground dry matter (AGDM), harvest index (HI), plant height (PH), days to anthesis (DA), N-uptake efficiency (NUPE), N-utilisation efficiency (NUTE), N-use efficiency (NUE), pre-anthesis NDVI (PrA-NDVI), post-anthesis NDVI (PoA-NDVI), Onset of rapid phase of senescence (ONSEN), Total duration of the senescence (DURTOT), and Duration of the rapid senescence phase (SEN). Red-filled rectangle: 2012-HN, green-filled rectangle: 2012-LN, red dashed rectangle: 2013-HN and green dashed rectangle: 2013-LN. Error bar = 2 x LOD of the QTL.




4A
0.0 wmc491
21.7 BS00010455_4A1
40.8 BS00010216_4A
41.8 BS00003674_4A1
66.4 BS00018651_4A
0.0 BS00011148_4B
3.0 BS00010235_4B
12.6 BS00009426_4B
12.7 BS00012107_4B
GY-HN
NUTE-HN
AD-LN
NUPE-HN
GY-LN
NUPE-LN
5A
0.0 BS00018510_5A
0.5 BS00011605_5A
2.5 BS00010702_5A2
4.9 BS00011360_5A2
29.4 BS00001735_5A2
30.8 BS00011227_5A
40.4 BS00011890_5A
55.0 BS00009531_5A1
79.2 BS00000645_5A1
80.9 BS00005311_5A1
0.0 BS00001953_5A3
8.2 BS00011846_5A
PoA-NDVI-HN
GY-HN
GM-HN
PH-HN
ONSEN-HN
HI-LN
AD-LN
AGDM-HN
N%-LN
GE-LN
GY-LN
GM-LN
AGDM-LN
5B
0.0 BS00021671_5B
4.9 BS00009378_5B
31.4 BS00010573_5B1
43.2 BS00003637_5B1
43.9 BS00000592_5B1
48.5 BS00012038_5B
58.6 BS00003879_5B1
84.2 BS00011465_28
87.4 BS00016286_5B
89.2 BS00010679
GM-HN
TGW-HN
HI-HN
AD-HN
ONSEN-HN
NUTE-HN
TGW-LN
GM-LN
GE-LN
AD-LN
NUPE-LN
6A
0.0 BS00014510_6A
5.7 BS00010497_6A
6.4 BS00009871_6A
7.7 BS00011265_6A
8.0 BS00014117_6A
8.5 BS00011789_6A
8.9 BS00013851_6A
9.1 BS00011122_6A
9.4 BS00003581_6A
9.9 BS00003881_6A
10.1 BS00014317_6A
10.2 BS00001132_6A
10.5 BS00010749_6A
11.2 BS00009319_6A
11.3 BS00010872_6A
11.4 BS00009783_6A
12.3 BS00010576_6A
13.6 BS00010730_6A
15.5 BS00012028_6A
16.8 barc107
18.3 BS00001405_6A
19.1 psp3152
23.9 BS00001037_6A
29.7 BS00009746_6A
31.7 BS00010933_6A
32.4 BS00009664_6A
51.5 BS00003861_6A
0.0 BS00003818_6A
0.2 BS00010326_6A
2.8 BS00010129_6A
7.1 BS00003723_6A
0.0 BS00010922_6A
36.0 BS00012414_6A
GY-HN
NUPE-HN
NUE-HN
PrA-NDVI-HN
PoA-NDVI-HN
GY-LN
HI-LN
PH-LN
HI-HN
GE-LN
NUPE-LN
5D
0.0 BS00003783_5D
7.0 BS00021731_5D
8.4 BS00021687_5D
10.8 BS00012069_5D
14.2 BS00000622_5D
16.2 BS00021742_5D
18.9 BS00004065_5D
78.2 barc172
NUTE-HN
AGDM-LN
7B
0.0 BS00010355_7B
1.1 BS00011217_7B
3.0 BS00004376_7B
3.2 BS00003756_7B
8.5 BS00021717_7B
20.3 barc32
0.0 BS00003350_7B
8.0 wmc517
EM-HN
GM-HN
TGW-LN
PH-LN
6B
0.0 BS00003852_6B
4.1 BS00003893_6B
4.6 BS00011665_6B
4.7 BS00010985_6B
6.0 BS00010420_6B
14.0 BS00021698_6B
15.8 BS00003562_6B
21.5 barc198
0.0 BS00010870_6B
2.5 BS00010627_6B
3.2 BS00011795_6B
5.1 BS00011603_6B
8.3 BS00010618_6B
BS00011365_6B
AD-HN
NUTE-HN
N%-HN
NUTE-LN
7A
0.0 BS00003621_7A2
16.3 BS00001450_7A2
26.3 BS00014246_7A
32.7 BS00000663_7A2
55.6 BS00003894_7A2
56.8 BS00011701_7A
59.8 BS00009398_7B
0.0 BS00011724_7A
8.3 BS00012133_7A
NUTE-HN
GY-LN
AGDM-LN
NUPE-LN
HI-HN
AD-HN
EM-LN

Figure 7.2: Summary of location of QTL using Interval Mapping (IM) method for Paragon x W141 MP for grain yield (GY), 1000 grain weight (TGW), grains per m^2 (GM), grains per ear (GE), ears per m^2 (EM), above-ground dry matter (AGDM), harvest index (HI), plant height (PH), days to anthesis (AD), N-uptake efficiency (NUPE), N-utilisation efficiency (NUTE), N-use efficiency (NUE), pre-anthesis NDVI (PrA-NDVI), post-anthesis NDVI (PoA-NDVI), Onset of rapid phase of senescence (ONSEN), Total duration of the senescence (DURTOT), and Duration of the rapid senescence phase (SEN). Red rectangle: HN and green rectangle: LN. The error bar = 2 x LOD of the QTL.




4A
0.0 BS00068885
5.7 BS00011173
10.1 wmc491
35.1 BS00010216
47.3 BS00010582
50.7 BS00088726
52.7 BS00018651
58.2 BS00101512
67.0 wmc262
71.0 BS00009387
AGDM-LN
NUTE-HN
GY-LN
GM-LN
NUPE-LN
PoA-NDVI-LN
DURTOT-LN
4B
0.0 BS00010235
29.7 BS00009672
33.1 BS00021984
39.2 BS00009272
40.6 BS00106143
41.9 barc163
43.1 BS00009342
47.0 BS00023035
53.0 BS00073758
57.7 BS00040517
5A
0.0 BS00031177
8.3 BS00089357
12.6 BS00011278
14.0 BS00005311
15.0 BS00022987
16.0 BS00021801
18.7 BS00000373
26.6 BS00022191
30.8 BS00010966
39.0 BS00011318
41.2 BS00066143
63.2 BS00011360
70.6 BS00074855
0.0 BS00063164
18.4 BS00022028
EM-HN
SEN-HN
EM-LN
EM-HN
PoA-NDVI-LN
5B
0.0 BS00009264
3.2 BS00003739
15.7 BS00106043
26.4 BS00010679
28.2 BS00003705
29.4 BS00108019
42.6 BS00010292
43.2 BS00014954
49.6 BS00073458
50.9 BS00000592
54.8 BS00003904
64.5 BS00018073
69.7 BS00003655
71.2 BS00021671
72.6 BS00049997
79.2 BS00050047
85.1 BS00003586
92.8 BS00011633
97.2 BS00022405
AGDM-HN
AGDM-HN
PH-HN
PrA-NDVI-HN
HI-HN
PH-HN
HI-LN
PH-LN
AD-LN
5D
0.0 BS00067650
21.8 BS00003783
22.7 BS00032035
23.4 BS00003826
28.0 BS00012069
DURTOT-LN
GE-LN
AD-LN
7A
0.0 BS00009332
1.3 BS00110283
3.1 BS00012133
26.9 BS00025521
40.1 BS00108786
44.7 BS00023800
54.3 BS00011072
56.3 BS00033578
57.6 BS00021771
63.6 BS00077445
67.1 BS00027448
68.6 BS00009346
79.0 BS00088825
86.4 BS00003894
100.4 BS00033613
121.4 BS00001450
130.2 BS00069019
135.1 BS00009797
135.8 BS00000663
DA-HN
DA-HN
HI-HN
PrA-NDVI-HN
TGW-LN
GM-LN
6A
0.0 BS00083630
6.1 BS00009759
6.9 BS00011131
19.4 BS00081386
20.1 BS00093964
21.4 BS00010933
25.7 BS00001037
26.3 BS00009376
32.5 BS00011115
33.2 BS00010441
35.2 BS00003881
38.9 BS00010749
39.6 BS00011265
40.2 barc107
41.6 BS00010780
42.2 BS00061296
51.4 BS00035914
76.8 BS00021965
91.0 BS00003958
0.0 BS00003616
1.3 BS00003877
TGW-HN
AGDM-HN
AGDM-HN
DURTOT-HN
DURTOT-HN
EM-LN
AGDM-LN
PH-LN
AD-LN
PrA-NDVI-LN
SEN-LN
6B
0.0 BS00077844
28.1 BS00027942
35.1 BS00010985
42.8 BS00076465
44.0 BS00010223
44.6 BS00011036
46.4 BS00021698
47.1 barc198
59.5 BS00023050
69.4 BS00022937
76.3 BS00034553
92.3 BS00085589
107.5 BS00110651
GE-HN
DH-HN
TGW-HN
GM-HN
NUTE-HN
NUTE-HN
GY-LN
HI-LN
6D
0.0 BS00042153
7.0 BS00093153
0.0 IS00029298
10.6 IS00027589
0.0 BS00009806
1.3 BS00010742
HI-HN
GY-HN
NUTE-LN
NUPE-HN
PrA-NDVI-HN
PoA-NDVI-HN
7B
0.0 BS00111144
1.2 BS00021695
21.7 BS00055861
26.1 BS00003350
33.8 BS00022053
40.1 BS00080621
43.0 BS00009398
51.6 BS00010251
57.2 BS00074359
62.2 BS00011065
65.7 BS00005062
66.4 barc32
67.0 BS00083352
68.9 BS00009794
69.7 BS00011571
70.3 BS00010355
GY-HN
GM-HN
EM-HN
NUTE-HN
7D
0.0 BS00105996
8.7 BS00009457
9.3 BS00018030
14.3 IS00069423
20.5 barc172
28.5 BS00070188
AGDM-HN
ONSEN-HN
SEN-HN
TGW-LN
GE-LN
PH-LN
AD-LN
NUPE-LN
NUTE-LN
PrA-NDVI-LN

7.4 Discussion

7.4.1 Population size

In this study, the map construction was based on 79 SSD lines from the cross between Paragon and Watkins line 481 (PxW481) and on 97 SSD lines from the cross between Paragon and Watkins line 141. While a large population size is required for a dense linkage map, generally the population size used for mapping studies ranges from 50 to 250 individuals (Young 1994; Mohan et al. 1997). Ericson et al. (2004) reported that the power of an experiment depends on the genetic population size. It has been demonstrated that when the population size is reduced from 100 to 50 individuals in a map, the confidence interval drops from 90% to 60% (Liu, 1998). However, many QTLs were identified using mapping population with approximately 80 - 100 lines (Freyre et al. 1998; Quarrie et al. 2006).

7.4.2 Mapping yield and components

Grain yield: Grain yield is the most commonly agronomic trait studied in QTL analysis. One hundred seventy-eight QTL controlling grain yield were identified in 23 investigations reviewed by Zhang et al. (2014). These QTL were unrandomly distributed around the genome, the most of QTL for grain yield were reported on chromosome 4AL (17), and followed by 3B and 2B, accounting, respectively, for 15 and 14 QTL For Paragon x W481 under HN, one major QTL (LOD = 3.91) was identified for grain yield on Chr. 6A under HN in 2012 and two putative ones on chr. 3B and 4B (LOD = 2.71 and 2.18, respectively). The two QTLs on chr. 6A and 3B were stable, because they appeared as putative QTLs in 2013 (LOD = 2.44 and 2.06, respectively). These two QTLs were also putative under LN conditions in 2012 (LOD = 2.37 and 2.43 respectively), whereas they did not have any effect on yield under LN on 2013.

The current marker on 6A explained 20.4, 13.3 and 12.9% of the phenotypic variation of grain yield, respectively, for PxW481 under HN in 2012, and in 2013 and under LN in 2012. This QTL (on 6A) was also major a QTL for HI (LOD = 2.81; 15.1%), and was putative for grains per ear (LOD = 2.81; 15.1% of variation) and for TGW (LOD = 2.38; 13.0% of variation) under LN conditions in 2013. The QTL on 3B explained 14.6, 11.3 and 13.2% of phenotypic variation in yield

respectively for PxW481 under HN in 2012 and in 2013 and under LN in 2012. This QTL was also putative for grains per m^2 under LN in 2012 and for ears per m^2 in 2012 under HN as well as under LN conditions, while it was not detected in the 2013 experiment either under HN or under LN.

For Paragon x W141 no major QTL was found, however, two putative QTLs were significant, (one on 6D, LOD = 2.37 and one on 7B, LOD = 2.00; 11 and 8.90 of variation, respectively) under HN and three under LN on 1B (LOD = 2.03), 4A (LOD = 2.06) and 6B (LOD = 2.02) explaining 9.5, 9.6 and 9.4% of the phenotypic variation, respectively.

Several studies in wheat also reported QTL for yield on chr 6A (Qin et al. 2014; Sukumaran et al. 2015), chr 3B (Graziani et al. 2014; Bonneau et al. 2013) and on 6D (Atkinson et al. 2015). Wu et al. (2012) reported that yield QTLs were more frequently observed on chromosomes 1B, 2B, 2D, 5A and 6B, and were concentrated in a few regions on individual chromosomes, exemplified by three striking yield-related QTL clusters on chromosomes 2B, 1B and 4B that explained the correlations between grain yield and other traits.

Grain yield components: For TGW, one major QTL (LOD = 2.8; 15.1%) on chr 5B and two putative QTLs on 5B (LOD = 2.25; 12.3%) and 2A (LOD = 2.08; 11.4%) were found in PxW481 under HN. The QTL on 5B was putative for TGW under LN in 2012 (LOD = 2.07; 11.4%). In addition, it explained 7.1% of the phenotypic variation in grain yield under LN. A putative QTL (LOD = 2.59; 14%) close to barc32 on chr 7B was also found under LN conditions near a significant QTL for grain per m^2 These QTLs were not co-located in the PxW141 mapping population. One major QTL on chr. 1A (LOD = 4.93; 21.5%) under HN was also found under LN conditions (LOD = 3.52; 15.8%). Another major QTL (LOD = 3.39; 20.5%) was found on chr. 3A. This QTL was also putative for this mapping population under LN conditions and was not significant for grain yield (LOD = 1.67 and 1.58) explaining 7.8 and 7.4% of the phenotypic variation respectively under HN and LN conditions.

Ramya et al. (2010) reported seven QTLs on chromosome 5B. Many other studies found a QTL for TGW in 5B (QH et al. 2015; Wu et al. 2015). Rustgi et al. 2013, by

dissection of yield components of wheat chromosome 3A report major QTL on this chromosome.

Turning to consider grains m^{-2}, for PxW481 in 2012 under HN, a major QTL for GM was found on 7B at 20.3 cM co-locating with a QTL for grain yield (LOD = 1.52 at 19.49 cM). A putative QTL on 7B at 17.49 CM under HN was also fond for ears per m^2. For PxW141 under HN, a putative QTL on 7B at 48.0 cM was found for GM (LOD = 2.35) explaining 10.9% of the phenotypic variation. The same QTL was putative for grain yield (LOD = 1.9) explaining 8.9% of the phenotypic variation in the same mapping population under the same conditions. A study on 95 bread wheat doubled-haploid lines revealed that three QTL were found for yield and yield components on the long and short arm of 7B (Quarrie et al. 2006). The QTL was found to be stable in different environments. However, under LN, the current QTL explained only 6% of the variation.

Another major QTL (LOD = 3.53; 18.6%) for GM was found on chr 2D at 5.0 cM, for PxW481 under HN in 2013. This co-located with a putative QTL in 2013 under HN (LOD = 2.74; 14.7%) at 6.0 cM and under LN (LOD = 2.48; 13.5%). The current QTL is close to the marker gwm261 associated with the dwarfing gene Rht8 reported by Worland et al. (1986).

A third major QTL (LOD = 4.29; 22.1%) for GM was found on chr 5B at 32.4 cM under LN in PxW481 in 2013 co-locating with a putative QTL for PxW481 at 31.4 cM under HN in 2012 (LOD = 2.30) and a putative QTL at 33.4 cM (LOD = 2.27; 12.4%) for grains per m^2.

For PxW141 under HN, one major QTL on 1A (LOD = 5.53; 23.7%) at 42.6 cM and one putative QTL on 3A (LOD = 2.91; 13.3%) at 8.0 were found. These two QTL also collated with putative QTL under LN conditions (LOD = 2.95 and 2.18 13.4 and 10.1%, respectively) at 44.6 cM and 10.0 cM. The QTL on 1A at 42.6 also co-located with a putative QTL for grain per ear in this population under HN.

Li et al. (2007) in chinese winter wheat RILs found major QTL on the chromosomes 1A 1D 2A 2D 3B 4B 7D with LOD ranging from 2.81 to 5.17 and a contribution to the phenotypic variation ranging from 5.57 to 52.25%. In addition a study by Ma et

al. (2015) on the Avalon x Cadenza mapping population, reported that 11 loci were detected on chromosomes 1A, 1D, 2A, 2D, 3A, 4A, 4D, 5A (two QTL), 6A and 7A, and accounted for phenotypic variation ranging from 2.47 to 11.24%. They added that the QTL detected on 2D contributed the largest additive effect and phenotypic variation.

For PxW481 in 2012, under LN, a major QTL was found on chr. 2D (LOD = 2.93; 15.7%) at 2.0 cM. It coinciding with a putative QTL (LOD = 2.32; 12.6%) at 0.0 cM under HN in 2013. The QTL was also co-located (LOD = 1.76 8.3%) in PxW141 under LN conditions. The QTL is close to the marker gwm261, which is linket to the photoperiod trait, controlled by the gene Ppd-D1A (Pestsova and Röder, 2002). This finding also is consistent with what have been found by Millet (1986, 1987) in common wheat showing that chromosome 2D carries a major recessive gene for the number of spikelets per spike (the major component of grain per ear). A Major QTL in 2D was also published by Echeverry-Solarte et al. (2014) in 163 RIL derived from a cross between the hexaploid hard winter wheat line a hexaploid hard red wheat line.

For PxW481 in 2012, a putative QTL on 3B was found under both HN (LOD = 2.06; 11.3%) at 1.0 cM and LN (LOD = 2.48; 13.4%) at 8.51 cM in 2012 and in 2013 (LOD = 1.82; 10.1%) in different position (27.5 cM) under HN. Another QTL was found on 7B under HN for PxW481 in 2012 (LOD = 2.10; 11.5%) and in PxW481 in 2013 under HN (LOD = 1.53; 8.6%) and in PxW14 under HN (LOD = 1.97; 9.20%). The QTL co-located with the QTL found in 7B for GM. Law (1967) discovered a genetic factor responsible for tiller number on chr 7B, which has an effect on ears per m^2. A significant QTL in the same chromosome was also mapped for tillering on chromosome arm 7B (Shah et al. 1999).

Under LN for PxW481 in 2012, major QTL (LOD = 4.12; 21.4%) was found on chr. 1A at 0.0 cM. This QTL explained 8.5% of the phenotypic variation of GM and 10.5% of EM under HN conditions (LOD = 1.89). This result is consistent with what have been found by Zhang et al. (2014), they reported one major QTL for EM on the chr 1A.

7.4.3 Mapping biomass, anthesis date and plant height

7.4.3.1. Anthesis date and plant height

For PxW481 under HN, a putative QTLs were found on 7A at 51.7 CM (LOD = 2.53), with QTL also collated with a putative QTL under HN in PxW141 at 42.1 cM (LOD = 2.31; 10.9%), and a QTL candidate in 2013 under HN (LOD = 1.16; 8.5%) at 47.7 cM. For PxW141 under HN, one major QTL was found on 6B (LOD = 3.13, 14.5%). The QTL was co-located under LN (LOD = 1.26 and explained 6.4% of the variation). These findings are consistent with have been found by Quarrie et al. (2006). They reported two QTLs on Chr 7A, one on long arm (7AL) and another on the short arm (7AS). In addition, Cuthbert et al. (2008) reported QTLs for AD on 5B, 6B and 7A.

For plant height, in PxW481 under HN, a major QTLs were found, on 5A (LOD = 3.22; 17.1%). Co-locating with a putative in for PxW481 in 2013 (LOD = 2.17; 11.9%) This finding is consistent with several studies reporting QTLs for PH on 5A in durum and bread wheat (Cadalen et al. 1997; Chu et al. 2008; Hai et al. 2008; Maccaferri et al. 2008; Marza et al. 2006; McIntyre et al. 2010).

Under LN for PxW481, three major QTLs were found on: 2D (LOD = 2.89; 15.5%), 7B (2.87; 15.4%) and 6A (LOD = 3.47; 18.3%). These QTL co-located with putative QTL for PxW141 under LN conditions. In previous studies a few QTLs were reported for PH in 2D and 6A (Marza et al. 2006; Borner et al. 2002). Another major QTL under LN for PxW481 was found in 2013 (LOD = 3.55 and explained 18.7% of the variation) on the chr 3A at 9.6 cM. Campbell et al. 2003 in Wichita x Cheyenne (RIL) found one QTL on the similar position of the chromosome 3A. A QTL in a similar position was reported by Maccaferri et al. (2008) on durum wheat mapping population reporting the QTL on length arm (3AL).

7.4.3.2. Above-ground biomass (AGDM)

For PxW481 under HN in 2012, one major QTL was found on 4B (LOD = 3.1; 16.5%). The QTL co-located with a QTL for grain yield under HN (LOD = 2.18; 11.9%). For PxW481 under LN in 2012, one stable QTL was found on 2B at 61.7 cM (11.8 and 13.9% in 2012 and 2013, respectively) which was also putative for

grain yield in the same conditions. For PxW141, one stable QTL was found putative on chr. 6A under both HN (LOD = 2.20; 10.2%) and under LN. Ayala et al. (2002) reported five QTLs for biomass on chromosomes 2B, 2D, 3B, 4D, and 6A, while a study by Li et al. (2014), reported also QTLs on 3A, 4B, 4D and 5A.

7.4.4 Mapping NUE components and senescence traits

7.4.4.1 N-uptake efficiency

For PxW481 in 2012 under HN, two major QTLs were found: one on 3B at 34.1 cM and one on 6A at 23.1 cM. The QTL in 6A was stable and co-located with a putative QTL under HN in 2013 (LOD = 2.09) and under LN in 2012 (LOD = 2.35). This QTL was also putative for grain yield under HN in 2013. The same QTL on 3B and 6A also co-located with putative QTLs for grain yield under HN in 2012.

Under LN in 2012, a major QTL was found on 2B was co-located with a major QTL for grain yield under LN, while the QTL on 4B and on 6A were co-located with putative QTLs for grain yield.

For PxW481 under LN in 2013, two putative QTLs were found on 5A (LOD = 2.41) and on 7A (LOD = 2.01), which were co-located for putative QTL for grain yield for the same mapping population and under the same conditions (LOD = 2.37 and 2.47; 12.9 and 13.4%, respectively).

In the literature, for NUE components relatively few previous investigations in wheat are available. In a field trial, An et al. (2006) reported several QTLs for NUP on chr 2B, 2D, 4A, 4B 5B, 6A, 6B and 7D under LN conditions, however, QTLs here on 1B, 2D, 3B, 4B, 5A and 7B under HN conditions. Several QTL in which some are major, were also reported on 1B, 1D, 2A, 4A, 5A, 5B, 6A and 7A in wheat for NUPE (Cormier et al. 2014).

In a study by Bogard et al. (2011), five QTLs for N-uptake on 2A, 2D, 3A and 5A were reported in 4 different environments. An et al. (2006) detected 21 QTLs for NUP of which 17 were detected in the two field trials, and four were detected in the hydroponic culture. The 21 NUP QTLs were distributed at 16 loci on 14 chromosomes. Of the 16 loci, five were detected consistantlyacross different

environments. Atkinson et al. (2015), on wheat population from Rialto x Savannah cross, suggested that QTLs for NUPE may relate to rooting traits.

Several genes from the N assimilation pathway have already been associated to NUE QTL including the genes coding for glutamate synthase (NADH-Gogat) located in QTL on chromosome 3A, and 3B (Quraishi et al. 2011; Habash et al. 2007; Quarrie et al. 2005; leGouis 2008).

7.4.4.2 N-utilisation efficiency (NUTE)

For PxW481 under HN 7 putative QTLs on 4A, 5B, and 6B were found in both 2012 and 2013. The QTL on 6B was also found in PxW141 as a major QTL (22.1%), while the one on 4A co-located with a putative QTL (13.5%) in this population and a QTL for grain yield under HN in 2013 in PxW481.

Under LN (PxW481), one major QTL was found on 3B at 7.0 CM (LOD = 2.94; 15.8%) and a putative QTL on 3A at 12.6 cM. The QTL on 3B was co-located with a putative QTL in 2013 (explained 13.4%) and the QTL on 3A was co-located with a major QTL in PxW141 (LOD = 3.45; 15.6%). The two QTLs (on 3A and 3B) were also co-located with putative QTL for grain yield under LN conditions (LOD = 2.46 and 2.43, respectively).

Using genome-wide association in a 214 variety panel experimented in eight environments, Cormier et al. (2014) found several QTLs for NUTE on the chromosomes: 2B, 2D, 3A, 4A, 5B and 6D.

7.4.5 NDVI and flag-leaf senescence traits

7.4.5.1. Canopy size (NDVI)

Pre-anthesis NDVI: In PxW481 in 2012 under HN, a major QTL (LOD = 2.91) was found on 3B at 6.84 cM. Under LN, two major QTL were found in PxW481 in 2012 (LOD = 3.58 and 3.40) on 2B at 51.0 CM and 61.7 cM, respectively. A putative QTL on chr 2B at 24.76 cM was also found in PxW141 (LOD = 2.18).

Post-anthesis NDVI: Under LN one major QTL (LOD = 3.31; 17.6%) was found on 2B. This QTL was more or less stable because it was candidate QTL in 2012 (LOD

= 1.56 explaining 8.7% of the variation). The QTL co-located with a putative QTL in PxW141 (LOD = 2.4).

Pinto et al. (2010) reported repeatable QTL on 2B and 5A for NDVI under different water stress regimes. In wheat, QTL have been detected for NDVI on chromosomes 2BL and 3BS based on the study conducted on a population of 249 RILs of durum wheat which were evaluated across a broad range of Mediterranean environments (Maccaferri et al. 2008). El-Feki (2010) identified three marginally significant QTL for NDVI on chromosomes 3A, 4B and 6A in a single environment for a winter wheat bi-parental doubled haploid population.

7.4.5.2. Flag-leaf senescence traits

The flag-leaf senescence scoring was carried out only in 2013, therefore the following discussion focuses on the comparison between PxW481 and PxW141 in terms of ONSEN (Onset of rapid phase of post-anthesis senescence), SEN (Duration of rapid phase of post-anthesis senescence) and DURTOT (Total duration of post-anthesis senescence).

Under LN, a major QTL found for ONSEN in PxW141 on 2D (LOD = 4.34) but it did not co-locate with QTL in PxW481.

DURTOT: Under HN a major QTL was found on 2A for DURTOT (LOD = 3.32) in PxW141 which was co-located with candidate GNC QTL (LOD = 1.75; 8.2%) for the same population, and with DURTOT QTL candidate in PxW481 (LOD = 1.34; 8.1%). For PxW141, four more putative were reported in 6A (2), 1D (1), and 2B (1). Amongst these QTL, the QTL on 6A co-located with a putative for GNC for the same population QTL for GNC, and candidate QTL in PxW481 (LOD = 1.40; 8.4%) under HN.

Several QTLs were previousely reported for leaf senescence in wheat. Indeed, Bogard et al. (2011) reported several QTLs for ONSEN on chromosomes 1B, 2A, 2D, 6B, 7B and 7B, for SEN 2D, 5A, 7B and 7D and for DURTOT. They reported 5 QTLs on 2A, 2D, 4D, 7B and 7D. However, Li et al. (2014) reported 18 QTL for leaf senescence rate on: 1A, 2A, 2D, 3B, 4D, 6A, and 6D and 8 QTLs for leaf

senescence score on: 1A, 2D, 3B, 4D, 5B, 2D, 7D, 2B and 3A. Verma et al. (2003) reported two major QTLs in 2B and 2D affecting grain yield in wheat.

A locus associated with early leaf senescence and increased grain protein content (GPC) was identified on chromosome 6B of wild emmer wheat (*Triticum turgidum subsp. dicoccoides*). Recombinant wheat with this Gpc-B1 locus displayed early leaf senescence and improved remobilisation of amino acids from the flag leaf, thereby increasing GPC without reducing grain yield (Kade et al. 2005; Uauy et al. 2006b). Members of the NAC transcription factor family have been shown to be involved in developmental and physiological processes, such as embryo and shoot meristem development, lateral root formation, auxin signalling, defence, abiotic stress responses and senescence (Olsen et al. 2005; Nakashima et al. 2012; Nuruzzaman et al. 2012).

Amongst the 39 major QTLs found for PxW481 under both HN and LN conditions, the traits were increased for 19 QTLs by W481 allele. They include QTL for grain yield, TGW, PH, GNC under HN and QTLs for grain yield, grains ear^{-1}, ear m^{-2}, grain m^{-2}, PH, HI, NUPE and pre-anthesis NDVI.

For PxW141, amongst the 19 Major QTLs found, the traits were increased for 14 QTLs by W141 allele, including QTL for TGW, GE, AD, PH, HI, NUPE, NUTE, DURTOT, SEN and ONSEN.

Chapter 8: General discussion

In the present study, landraces were used to identify novel genetic resources and physiological traits for improved N-use efficiency in wheat. In addition, the combination of physiological and quantitative genetic approaches using molecular markers allowed for the identification of key genetic loci involved in the expression of specific traits, to enable the selection of genotypes for increased N-Use efficiency in wheat.

A main objective was to find novel variation for NUE and related traits amongst a panel of wheat landraces and check UK cultivars and two SSD populations and to identify potential QTLs determining the variation in two mapping populations from the cross between Paragon (UK cultivars) and two AE Watkins lines, W141 and W481.

Improved NUE in wheat by introgressing novel traits and genes into adapted wheats would not only improve sustainability of agricultural systems, but also minimise the N fertiliser application and associated environmental impacts of wheat cultivation whilst maintaining acceptable yields. Genetic variation in NUE in wheat is due to variation in N-uptake efficiency and/or N-utilisation efficiency. Specific traits may be identified for each component for NUPE and NUTE and QTLs for each trait can be identified that are N treatment specific and combined in new genotypes contributing to additive effects.

8.1 Novel variation in panel of Watkins lines and UK check cultivars

In the field both main effect genotype and treatment had an effect on grain yield and on AGDM and the N treatment x genotype interaction was highly significant indicating some genotypes maintained higher yield and AGDM better under LN than others. All UK cultivars generally lost more yield and more biomass than the Watkins lines. Modern cultivars were selected under non-limiting conditions (high N input), so they may not be not well adapted to low N environments (Brancourt-Hulmel et al. 2005; Bänziger et al. 1997 and Presterl et al. 2003; Foulkes 2009, 1998). In barley (Hordeum vulgare L.) seedlings, Grando and Ceccarelli (1995) reported an initial increase in root size from ten wild barleys to ten landrace barleys,

then a marked decrease in root size from landraces to ten modern barley cultivars. Wild collections of bread wheat do not exist, only landraces. Landraces are generally cultivated in harsh environments to which they are well adapted since they are exposed to many years of selection and can survive the harsh climatic conditions of extreme years. Several studies reported that the root size of landraces is larger compared with that of modern cultivars. Differences were observed in root size between 'Chinese Spring' a tall landrace with large root biomass and cultivar Hope with small root biomass.. Three old tall landraces from China and Iran had 2- to 4-fold larger root biomass than four modern semi-dwarf cultivars descended from CIMMYT breeding material (Ehdaie et al. 1991; Ehdaie and Waines, 1993, 1997; Ehdaie, 1995). A study of the evolution of tall landraces to semi-dwarf and dwarf cultivars in the last 100 years, in large pots in sand culture, showed that four mid generation green revolution semi-dwarf cultivars (Anza, Chenob70, Sholeh and Yecora Roja) had lower root biomass per plant than three tall landraces (Iran 14, Iran 49 and Chinese spring) (Waines, Ehdaie 2007). They concluded root size of modern cultivars was smaller compared compared with that of landraces. The root system of modern UK cultivars may be too small for optimum uptake of water and nutrients and maximum biomass and grain yield. In summary, UK cultivars are likely better adapted to take up more N under HN conditions. However, under LN conditions, landraces may absorb more N from deeper layer as they may have deeper root system. However, grain growth of landraces may also be sink-limited. Indeed, either in the glasshouse or in the field, apart from W040 and W579 which had similar grains per m^2 to Paragon, all other Watkins lines had lower grains m^{-2} than all other UK cultivars.

In the glasshouse as well as in field under both HN and LN, genetic variation in grain yield was better associated with AGDM than with HI. Furthermore, the yield response to N supply was positively associated amongst genotypes with the responses of grains per m^2 (R^2=0.95, P<0.001), AGDM (R^2=0.54, P<0.05) and HI (R^2=0.44, P<0.05) to N supply. Several studies reported that genetic progression in grain yield of wheat was associated with increased HI and biomass (Austin et al. 1982; Foulkes et al. 2009). However, HI for winter wheat is approaching its theoretical maximum value, in some countries and regions, which is ca. 62-64% (Austin, 1980; Foulkes, 2009), so above-ground biomass has been reported as the

main target to improve grain yield. There is recent evidence of genetic gains in biomass in UK winter wheat (Shearman et al. 2005; Clarke et al. 2012).

For NUE traits, genetic variation was found in both glasshouse and field experiments. A positive relationship was found between different NUE parameters in the field and in the glasshouse. The UK cultivars tended to have lower post-anthesis NUP. Indeed, the Watkins lines W040, W141 and W579 had similar NUP than Paragon and better than Oakley. In the field, NUE response to increased N was similarly associated with NUTE than NUPE. The two Watkins lines W209 and W141 showed higher NRE and NUTE in both glasshouse and field experiments.

Genetic variation was found amongst the genotypes for NUTE. Variation in NUE was also reported by other investigations in barley and wheat (Barraclough et al. 2011; Cox et al. 1986; Gaju et al. 2011). In the present study, NUTE was positively associated with NHI in both glasshouse and in field experiments (under both HN and LN), while it was negatively associated with grain N concentration (GNC). Several studies reported similar negative relationship between NUTE and GNC (Ortiz-Monasterio et al. 1997; Brancourt-Hulmel et al. 2003; Foulkes et al. 1998; Slafer et al. 1990; Simmonds, 1995). This effect is advantageous in non-bread making cultivars (feed, biofuel and distilling industry), however, in bread making varieties, the increase in NUTE should be associated with an increase in NHI to maintain high GNC and grain protein content, the first grain quality trait.

Regarding photosynthesis, genetic variation was found in both glasshouse and field experiments for pre and post-anthesis measurements. Pre-anthesis, UK cultivars generally had higher A_{max} in both experiments; however, post anthesis, W141 and W040 had higher values than Paragon. In the field, post-anthesis A_{max} was higher for W352 and W034 under LN and for W141 and W040 under LN than Paragon.

Leaf photosynthesis rate was previousely shown to be tightly associated with biomass accumulation (Ensminger et al. 2006). In the present results, pre-anthesis A_{max} was positively associated with biomass and with grain yield in the glasshouse, while the correlation was stronger with post-anthesis A_{max} than pre-anthesis in the field uder both HN and LN. Indeed some previous evidence suggested a positive

relationship between grain yield and yield components with photosynthesis rate and stomatal conductance (Shimshi and Ephrat 1975; Fisher et al. 1998).

Some wheat landraces in both glasshouse and field conditions (W141, W040 and W352) had comparable pre-anthesis photosynthetic rates to and UK cultivars while other had lower values. The maximal rate of photosynthesis under light saturated conditions was significantly reduced with time (from pre to post-anthesis), without significant interaction with genotype.

It is possible that the variation in photosynthesis rate has beendriven by the intrinsic physiology of the leaf, which may be determined by Rubisco limitations, Rubisco concentrations or variation in leaf structure (Reich et al. 1997). In the current work, only leaf structure was investigated. Specific leaf area (leaf area per unit mass, SLA) indicates efficient light capture and photosynthetic capacity (Reich et al. 1997). Previous investigations revealed that modern wheat cultivars had significantly thicker leaves, compared to wheat landraces leading them to have reduced photosynthesis rate per unit nitrogen (N) (Reich et al. 1998). Furthermore, Watkins lines seemed to have higher post-anthesis N uptake and lower N remobilisation, indeed post-anthesis N uptake was negatively associated with N remobilisation. This result implies that Watkins lines could have a large root system.

8.2 Comparability of glasshouse and field experiments of the traits

The two-year means of the main traits in the glasshouse correlated with the field experiment (under HN, as glasshouse was considered HN) for the same genotypes (3 UK cultivars and 7 Watkins lines). The results in the glasshouse were measured on a per plant basis, while in the field it was on per ha basis. Indeed, grain yield in the field ($th^{-1}a$) and in the glasshouse (g $plant^{-1}$) ($R^2 = 0.45$, $P<0.05$), AGDM in the field (t ha^{-1}) and in the glasshouse (g $plant^{-1}$) ($R^2 = 0.71$, $P<0.01$) NUP in the field (t ha^{-1}) and NUP in the glasshouse (g $plant^{-1}$) ($R^2 = 0.58$, $P<0.01$), post-anthesis flag-leaf photosynthesis rate in the field and in the glasshouse ($R^2 = 0.45$, $P<0.05$) and grain N concentration in the field and in the glasshouse ($R^2 = 0.82$, $P<0.001$) were all significantly correlated. These correlations confirmed the scope for extrapolation of the genetic variation measured in glasshouse (per plant) to the field scale (area).

8.3 Variability in yield, AGDM and NUE components

8.3.1 Grain yield and above-ground biomass

The crop variables grain yield and components and, above-ground biomass were affected to a different extent by the experimental factors N-level, year and genotype. Large genetic variation in grain yield was found in each population. Indeed, under LN, two SSD lines (L481-48 and L481-76) had significantly higher grain yield than Paragon in the PxW481 population. For PxW141, the results showed that three lines (L141-52, L141-04 and L141-83) had higher grain yield under HN. The first two lines (L141-52 and L141-04) had significantly higher AGDM, while L141-83 had higher HI and higher grains per m^2 than Paragon. Under LN, only one line (L141-46) had higher grain yield than Paragon with significantly higher values of AGDM, grains per m^2 and TGW, but lower HI than Paragon.

Regarding yield response to N supply for PxW481, results showed also that 13 SSD lines maintained grain yield significantly better under LN than Paragon. Amongst the 13 Lines, nine lines maintained AGDM better, one line maintained HI better and TGW better and 11 lines maintained grains per m^2 better than Paragon. For PxW141, only one SSD line, L141-46, maintained grain yield better under LN conditions than Paragon. This line along with L141-41 maintained better AGDM, while it had similar HI response to N supply to Paragon.For AGDM, genetic variation was found in both the PxW481 and PxW141 populations. While no line had higher AGDM than Paragon under HN, L481-76 and L481-48 had higher values than Paragon under LN. Also as the first line had higher HI, and the second had similar HI to Paragon, the two lines had also higher yield than Paragon. For PxW141, five SSD lines had higher AGDM than Paragon under HN (L141-52, L141-47, L141-48, L141-04 and L141-35), while four SSD lines (L141-47, L141-50, L141-46, and L141-48) had higher AGDM under LN conditions. Regarding HI, four other lines also had higher HI than Paragon.

Averaging two years for the PxW481 population, no SSD line maintained better AGDM under LN than Paragon. However, for PxW141, two SSD lines, L141-46 and L141-41 maintained higher AGDM under LN than Paragon. This could be explained by the fact that the two lines had higher NUPE response to reduced N than Paragon;

they had similar ONSEN and DURTOT response to reduced N than Paragon. It can be speculated that these two wheat landraces had large root system allowing higher post-anthesis N-uptake under low N conditions (Waines, Ehdaie 2007).

8.3.2 N-uptake and N-utilisation efficiency

The present study demonstrated genetic variation in NUPE and NUTE amongst SSD lines in two mapping populations under both HN and LN treatments. N-uptake efficiencyand N-utilisation efficiency were all affected by N level in all experiments. Indeed NUPE increased by 59, 65 and 67% for experiments, PxW481 in2012 and in 2013 and PxW141 in 2013 respectively and NUTE decreased under HN by 31, 25 and 22% in PxW481 in 2012, and 2013 and PxW141 in 2013, respectively. The positive association between genetic variation in NUPE and NUE was observed under HN and LN conditions in all experiments, while the association between NUTE and NUE was observed only in two experiments, PxW481-2012 and PxW141-2013 under both HN and LN. It can be speculated that this transgressive segregation could be partly physiologically explained by the difference in post-anthesis N uptake.

For the PxW481 population, no transgressive segregation under HN was observed, however under LN two SSD lines: L481-76 and L481-48 had higher NUPE than Paragon. For NUTE, 17 lines had higher NUTE under HN and 10 lines had higher values than Paragon. For PxW141, while no SSD line had higher NUPE under HN, two SSD lines: L141-48 and L141-46 had higher values than Paragon.

Regarding NUTE, two lines, L141-95 and L141-94, had higher NUTE under HN and 39 lines had higher NUTE under LN than Paragon. NUTE is dependent on N efficiency of biomass formation, the effect of N on carbohydrate partitioning, nitrate reduction efficiency and remobilisation of N from senescence tissue as well as storage functions (Hirel et al. 2007; Azevedo, 2007; good et al. 2004).

Wheat senescence occurs earlier in crops with the least N reserve (Pask, 2009) and in the present study, the total duration of senescence was reduced under LN compared to HN. In addition, post-anthesis N uptake by roots can contribute between 5 to 50% of grain N in wheat (De Ruiter and Brooking, 1994; Kichey et al. 2007) depending

on the environmental conditions and soil N availability. Several studies reported that grain N accumulation in wheat is likely principally driven by N availability from the soil (Triboi and Triboi-Blondel, 2002; Martre et al. 2003).

In this study, genetic variation in NUE was much more associated with NUPE than with NUTE in both mapping populations. Ortiz- Monasterio et al. (1997) by studying old and new wheat cultivars, showed that the genetic gain in NUE under LN was mainly associated with gains in NUPE. Similar results were reported by Foulkes et al. (1998) in winter wheat. These studies agreed that under HN supply, the contribution of NUPE and NUTE to the genetic gain in NUE was approximately equal. Furthermore, LeGouis et al. (2000) added that NUPE accounts for more genetic variation in NUE than NUTE under LN conditions in wheat.

8.4 Genetic analysis of traits

For PxW481 under HN, one major QTL on chr 6AL at 10.0 - 30.1 cM, linked to the marker BS00010749, was stable for grain yield. It was major in 2012, but putative in 2013. The current QTL was also putative for HI in LN in 2012 and in 2013, but there was no association with AGDM. In this QTL cluster on chr 6AL also, a putative and major QTL for TGW and grain per ear in PxW481 in 2012 under LN were found. For PxW141, another group of QTLs was also found in the similar region of 6AL at 35 - 50 cM, linked to the same marker BS00010749 in PxW481. In this region, QTLs for AGDM, TGW and stay-green trait were identified in 2012 under HN. Forthermore, QTLs for AGDM, PH, anthesis date, pre-anthesis NDVI and stay-green traits were significant under LN conditions. This result is consistent with what have been reported by Simonds et al. (2014). They have identified a major stable QTL for grain yield and TGW on wheat chromosome 6AL of the Spark x Rialto population, across 12 North European environments in a similar position to that reported in this study (close to the marker SNP: BS00009871). In the same region, QTLs for NUPE and pre and post-anthesis NDVI were identified. El-Feki (2010) identified three marginally significant QTL for NDVI on 6A in a single environment for a winter wheat bi-parental doubled haploid population, while An et al. (2006) found a QTL for NUPE on the same chromosome 120 DH lines derived from winter wheat varieties Hanxuan 10 and Lumai 14.

A second QTL cluster was found on 2B at 61.7 - 64.6 cM, including a major QTL for grain yield for PxW481 under LN in 2012 and putative QTL in 2013 and a putative QTL for AGDM in 2012 under LN. QTLs for pre- and post-anthesis NDVI as well as the stay-green trait (DURTOT) under LN in 2013 were also found in this region. This finding is consistent with those of Borner et al. (2002); Marza et al. (2006); Sommer et al. (2004) reporting a major QTL on 3BS was also associated with yield components. For PxW141, just a few QTLs were found in this region mainly for anthesis date under HN and post-anthesis NDVI and stay-green trait under LN.

A third QTL cluster was found on 3BL at 0.0 - 15.0 cM. This region includes a stable QTL for grain yield in two years as well as for AGDM, NUPE and pre-anthesis NDVI under HN. In addition, major QTLs for HI and putative QTLs for grain yield, grains m^{-2} and NUTE under LN were found. Also for PxW141, just QTL for post-anthesis NDVI was found in this region. Several studies on both durum and bread wheat previousely reported major QTL on chr 3B (Campbell et al. 2003; Borner et al. 2002; Sourdille et al. 2004; Liu et al. 2006; Maccaferri et al. 2007).

On chr. 2DS at 0.0 to 15.0 cM, close to the centromere, several QTLs were found mainly under LN in PxW481. A QTL for grain yield co-located with a QTL for grains m^{-2}, TGW and grains ear^{-1} under LN. In addition, the QTL for grains m^{-2} under HN was stable in the same region since it appears in 2012 and in 2013. These QTLs were linked with the markers gwm577 and gwm261. For PxW141, a QTL cluster was found in the same region linked to the SSR marker gwm261. This fourth QTL cluster also contained a QTL for TGW under HN and QTLs for AGDM, HI, PH AD, NUTE, pre-anthesis NDVI and the stay-green traits under LN. Rht8 was found to be tightly linked to the microsatellite marker gwm261 on chromosome arm 2DS (Korzun et al. 1998). This was confirmed by Ellis et al. (2005) who identified an additional microsatellite marker, wmc503, linked to Rht8 on chromosome arm 2DS. The micosatellite marker gwm261 co-segregates with Rht8 and produced three different allelic variants of 192 bp, 174 bp and 165 bp (Korzun et al. 1998). It is possible that this QTL cluster was associated with allelic variation for Rht8 in the present study.

The fourth QTL cluster in was found in PxW481 on chr. 3AL at 8.0 - 18 cM close to the SSR markers gwm2 and wmc264. This region contained a QTL for grain yield, AGDM, PH, and NUTE under LN. In addition a QTL for AGDM under HN in 2013 was also found on this part of 3A chromosome. For PxW141, QTLs for TGW, grains per m^2, ear per m^2, PH under HN and TGW, NUTE under LN conditionswere also located in this region. Maccaferri et al. (2008) evaluated a RIL durum wheat population in 16 environments over two years in different Mediterranean locations (10 rainfed and 6 irrigated locations). They identified a major QTL for PH, on chromosomes 3AL in 10 environments. The QTL was inconsistent over environments, especially under different moisture levels (Maccaferri et al. 2008). In addition Rustgi et al. (2013) reported a major QTL for biomass on 3AL at 17.7 cM which is similar to the position of the reported QTL in this study.

The sixth cluster to describe for the PxW481 mapping population was located on 5A at 0.0 - 8.2 cM. A major and stable QTL in two years for PH under HN was found in this region as well as QTLs for grain yield, grains m^{-2} and grain per ear under LN and the stay-green trait under HN in 2013. In addition, a QTL for AGDM and for NUPE in 2012 under LN was co-located.

For PxW141, four more QTL clusters were found on 1A at 38 - 42 cM, 4AL at 50 - 60 cM, 6BS at 42 - 44 cM and 7BL at 40 - 60 cM.

The cluster in the Chr 1A included QTLs for grain per m^2, TGW, HI and PH for each of HN and LN conditions. In addition, QTLs for grains per ear under HN and anthesis date and stay-green trait under LN were also co-located in this region.

The second cluster for this mapping population was in 4AL. The region 50 - 60 cM on this chromosome contained QTLs for grain yield, AGDM, grain per m^2, NUPE and the stay-green trait under LN, in addition to a QTL for NUTE under HN. Kirigwi et al. (2007) mapped several QTL for biomass production and biomass production rate on the proximal region of chromosome 4AL in wheat under differing soil moisture regime treatments.

On chr 6BS, 6 QTL for grains per m^2, TGW, grains per ear, anthesis date NUTE under HN were co-located. These QTLs were also co-located with grain yield and HI

under LN conditions. Huang et al. (2003) reported that a significant QTL on chr 7B increased TGW by 11.7%. In the population ITMI (W7984×Opata85), QTL for TGW were previousely mapped on 3AS, 5AL and 6BS (Borner et al. 2002; Breseghello et al. 2007).

The last main QTL cluster for the PxW141 population was located on 7BL. Indeed, in addition to QTLs for each of grain yield, grain per m^2 ear per m^2, NUTE and stay-green under HN, QTLs for TGW, grains per ear, PH, anthesis date, NUPE and NUTE and pre-anthesis NDVI were co-located in this region. these findings were also consistent with several studies which reported major QTL on 7BL for grain yield and grain number (Quarrie et al. 2005 and 2007; McCartney et al. 2005; Huang et al. 2006).

8.5 Variation in canopy traits and high-throughput phenotyping

In the present study, post-anthesis NDVI (PoA-NDVI) was more closely associated with grain yield and above-ground biomass (AGDM) under LN than under HN in all experiments. These findings are consistent with other studies on wheat (Syeda Refat et al. 2014; Serrano et al. 2002, Gaju et al. 2011). In contrast to the LN treatment in which the grain growth is likely source limited, grain growth is sink limited leading to lower biomass production efficiency under HN conditions, partly because the efficiency of N remobilisation is reduced (Hatfield et al. 2010; Calderini et al. 2006).

With regard to N uptake, significant correlations amongst SSD lines were found with all NDVI measurements in all experiments. The strongst correlations were recorded between two weeks and three weeks after anthesis. When NDVI approaches 0.75, the light saturation of canopy photosynthesis occurs, leading to less correlation with NUP (Poole and Craig, 2010). In plant breeding, connecting genotype to phenotype is required. However, a lack of access to high-throughput plant phenotyping capabilities limits the ability to dissect the genetics of quantitative traits related to growth, yield and adaptation to abiotic stresses (Phillips et al. 2010). Therfore developing high-throughput phenotyping platforms is required particularly field based platforms. Indeed a variety of imaging methodologies, e.g. visible imaging (machine vision), imaging spectroscopy (multispectral and hyperspectral remote

sensing), thermal infrared imaging, fluorescence imaging, 3D imaging and tomographic imaging (magnetic resonance imagers, positron emission detectors and X-ray computed tomography) have been recently developped (Li et al. 2014). Araus and Cairns (2014) reviewed new high throughput phenotyping platforms (HTPPs). A combination of advances in aeronautics, remote sensing and high performance computing has allowed the application of the canopy sensors in aerial systems. In addition, ground based new HTPPs include modified vehicles equipped with GPS, navigation devices, cameras and sensors (Araus and Cairns 2014; Furbank and tester 2011). These new phenomics technologies will accelerate progress in understanding gene function and environmental responses to enable breeders to develop new wheat germplasm with enhanced biomass and NUE.

Chapter 9: Conclusions and future work

Based on the results presented in this thesis, the following conclusions can be drawn:

- Genetic variation was found in the glasshouse and in the field experiments for grain yield and above-ground biomass. The Watkins landrace lines did not exceed the grain yield of UK cultivars, but some genotypes had similar biomass mainly under LN conditions in the field. Indeed, the majority of Watkins lines maintained grain yield and biomass better under reduced N fertiliser compared to the UK check cultivars. Similar results were found for NUP and NUTE. The UK cultivar Rialto tended to have higher NUP in both glasshouse and field experiments under HN than Watkins lines. However, some Watkins lines showed similar NUP and NUT to Paragon and higher than other UK check cultivars under LN conditions. In addition, the Watkins lines generally maintained NDVI (canopy size) and green area duration better under LN than the check cultivars in the field experiments.

- The correlations amongst genotypes between field and glasshouse (plant grown in pots) expression of traits were significant for, GY, AGDM, NUP, NUTE, photosynthesis rate and grain N concentration. This supports the extrapolation of genetic variation results in the glasshouse to the field.

- For the PxW481 population under HN, no SSD line had significantly higher grain yield than Paragon, while 13 SSD lines had higher values under LN conditions. However, for the PxW141 population, three and one SSD lines under HN and LN, respectively, had higher grain yield than Paragon. The grain yield response to increasing N supply showed that one SSD line had a higher yield response than W481 for PxW481, while 37 lines had higher than W141 for PxW141 population.

  Similar to grain yield result, no SSD line had higher AGDM than Paragon for the PxW481 population under HN, while two SSD lines had higher grain yield under LN. For the PxW141 population, five and four SSD lines had higher AGDM than Paragon, respectively, under HN and LN conditions.

- In all three experiments (PxW481-12, PxW481-13 and PxW141) either under HN and LN, genetic variation in NUE was more closely associated with

NUPE than with NUTE. Averaging over years for PxW481 under HN, no SSD line had higher NUPE than Paragon, while under LN L481-76 had higher NUPE than Paragon. For PxW141, no SSD line had higher NUPE than Paragon either under HN or under LN. For NUTE, 17 SSD lines of the PxW481 population had significantly higher NUTE than Paragon under HN and 10 SSD lines higher under LN conditions. For PxW141 under HN, L141-95 and L141-94 had higher NUTE than Paragon under HN, while under LN 12 SSD lines had higher NUT. The genetic variation in the response of NUE to decrease in N supply, however, was more strongly associated with the response to NUTE than with the response to NUPE in both mapping populations.

- For both the PxW481 and PxW141 populations, genetic variation in NDVI (canopy size) at around anthesis was more strongly correlated with grain yield, biomass and NUE components compared to other assessment dates. The relationships were stronger under LN conditions implying under LN grain growth is more source-limited (or the extent of sink limitation is reduced) compared to HN conditions. For PxW141, genetic variation in grain yield was also positively associated with the flag-leaf senescence parameters ONSEN and DURTOT mainly under LN conditions.

- Using 201 (27 SSRs and 174 SNPs) and 491 (23 SSRs and 468 SNPs) Markers, respectively, for PxW481 and PxW141, two genetic maps were constructed with 35 and 28 linkage groups for PxW481 and PxW141 respectively.

- For grain yield in PxW481, two stable QTLs in 2012 and 2013 were found under HN on chr 3B and 6A. Under LN, two QTLs on chr 2B and 5A were found which were each stable across years. For AGDM, one QTL on 2B was found in both years under LN conditions. With regard to NUE traits, one QTL which was stable across years was found under HN for NUPE on chr 6A and one for NUTE under LN conditions on chr 3B.

- For the PxW481 population under HN, two major yield QTLs on chr 6A and 3B and the putative QTL on 4B were co-located with QTLs on the same

chromosomes for NUPE. Under LN, three QTLs on chr 2B, 4B, 6A and were also co-located for both grain yield and NUPE. For PxW141 one QTL on 6D under HN and one on 4A were co-located for grain yield and NUPE.

- For AGDM one QTL was found in both the PxW481 and PxW141 populations on chr 2D. For NUTE, three QTLs were found in both PxW481 and PxW141on 3A, 4A and 6B.

This study has identified significant novel variation for grain yield, biomass, NUE components and physiological related traits amongst AE Watkins lines and amongst SSD lines from the crosses between Paragon and both W481 and W141. Future research to enhance grain yield, biomass and NUE in wheat should focus on the following suggestions:

- Investigate a wider range of AE Watkins lines under contrasting nitrogen treatments to identify wider novel variation for these traits., also phenotype synthetic-derived wheat lines and introgression lines from crosses with ancestral wheat species for wider genetic diversity for these traits (and genotyping these resources for the markers discovered in this project for allelic diversity).

- The variation observed for NUPE either in AE Watkins lines or for SSD lines of the two populations associated with AGDM and grain yield under LN conditions in the present study implies improved NUE may be linked to root system architecture traits. So future work should focus on phenotyping these SSD populations for root system architecture traits and identifying QTL for the key rooting traits.

- The difference between the two SSD populations and the variation within the populations should be further investigated in relation to mechanisms underlying N-uptake efficiency, N-utilisation efficiency and senescence-related traits. Thus, multi-site-season experiments should be carried out on near isogenic lines developed for the novel QTLs for the key traits identified in this study quantifying N partitioning at anthesiss, post-anthesis N

remobilisation efficiency and post-anthesis N-uptake and its relationship with root system.

- High-throughput phenotyping systems should be further developed to facilitate genetic analysis for the complex traits of the canopy photosynthesis and its determinants, e.g. hyperspectral reflectance imaging including the use of canopy sensors mounted on aerial platforms.

- Further genetic analysis on the two mapping populations should be carried out including adding more markers to the two SSD populations. Some chromosomes contained only few markers (D genome mainly). This will allow identification of more QTL in the gaps in the map and, together with the development of backcross lines for target QTLs, fine mapping of QTLs to more precise locations on the chromosomes. These QTLs can then be developed as targets for marker-assisted selection, and candidate genes established. In addition, lines carrying suitable QTL can be crossed with the best-performing current cultivars to introgress the QTLs and traits into adapted UK wheat backgrounds for use in trait-based breeding for improved yield, biomass and NUE.

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