IDENTIFYING GENETIC DIVERSITY FOR IMPROVED NITROGEN-USE EFFICIENCY AND ASSOCIATED PHYSIOLOGICAL TRAITS IN WHEAT (*Triticum aestivum* L.) AND WHEAT AMPHIDIPLOIDS.

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ABSTRACT

Wheat is one of the most widely consumed staple crops in the world including India and its demand is increasing with increasing population. Increased grain yield (GY) has been associated with increased use of nitrogen (N) fertilizers which represent a significant environmental and production cost. Developing cultivars which have higher grain yield but use N efficiently may allow reduced fertilizer N inputs. The objectives of this study were to: (i) quantify the genetic variability in N use-efficiency (grain dry matter (DM) yield per unit N available from soil and fertilizer, NUE) in a panel of modern Indian wheat cultivars and find new genetic variation in a panel of amphidiploids produced by crossing hexaploid bread wheat with wild wheat relatives and (ii) identify traits and understand physiological mechanisms determining improved NUE to exploit for development of new N efficient cultivars.

Thirty Indian elite bread wheat cultivars and 18 amphidiploid lines along with their five respective bread wheat parents were tested under high N (HN) and low N (LN) conditions in two years in field experiments at Agharkar Research Institute, Pune, India (2013 and 2014) and in glasshouse experiments at Nottingham University, UK, (2015 and 2016), respectively. Detailed growth analysis was conducted including GY, above-ground dry matter (AGDM), DM and N partitioning at anthesis and at harvest along with N remobilization efficiency (NRE) in the field experiment. Senescence kinetics of the flag-leaf were assessed from a visual score weekly from anthesis to complete canopy senescence in both sets of experiments. Physiological traits were assessed including flag-leaf light-saturated photosynthetic rate (A_{max}) under HN conditions in the field experiment and under both HN and LN conditions in glasshouse experiment. Flag-leaf relative chlorophyll content (SPAD) under HN and LN conditions was measured in both experiments; and Normalized Difference Vegetative Index (NDVI) under HN and LN conditions in field experiment. 2D seedling root phenotyping was carried out on subset of 12 genotypes selected based on contrasting performance under LN conditions from both the field and glasshouse experiments.

In field experiments, GY was reduced under low N (LN) conditions on average by 1.46 t ha^{-1} (28%). Crop above-ground N-uptake at harvest on average was reduced from 16.2 kg N ha^{-1} under HN to 8.5 kg N ha^{-1} under LN conditions while N-utilization efficiency (grain DM yield per unit above-ground N uptake at harvest; NUtE) increased from 32.7 to 44.6 g DM g^{-1} N. Significant N × genotype level interaction was observed for GY, N uptake at harvest and NUtE. Overall genetic variability in GY and NUE (which ranged from 15.6 - 23.7 g DM g^{-1} N under LN; $P<0.001$) related mainly to differences in N uptake rather than NUtE.

Overall, cultivars ranged significantly at anthesis in N accumulation in the flag-leaf N (1.1 -2.2 g N m^{-2} at HN and 0.5-1.0 g N m^{-2} at LN), the stem and remaining leaf with sheath (5.78-11.97 at HN and 3.61- 6.33 g N m^{-2} at LN) (P=0.01), and the ear (2.91-6.13 at HN and 2.06-4.23 g N m^{-2} at LN) (P<0.001). Cultivars ranged in N partitioning index (proportion of above-ground N in the crop component, NPI) at anthesis for the flag-leaf from 0.08 to 0.16 at HN and 0.07 to 0.13 at LN (P< 0.001); and for the stem-and remaining leaf with sheath from 0.54 to 0.68 at HN and from 0.52 to 0.65 at LN (P<0.001) and for ear from 0.21 to 0.34 at HN and from 0.28 to 0.41 at LN (P<0.001).

The post-anthesis NRE was positively associated with the duration of flag-leaf senescence amongst cultivars under LN. Genetic variation in grain yield and grain N% (through N dilution effects) appeared to be mainly influenced by pre-anthesis N accumulation rather than post-anthesis N remobilization under LN conditions. Under N stress conditions, there was evidence that NRE was a determinant of genetic variation in grain N%. Flag-leaf A_{max} was positively associated with AGDM (P=0.02), GY (P=0.14), and specific leaf N at anthesis (P=0.046). Flag-leaf onset (VS.OnsetRP) and end (VS.EndRP) of senescence was positively associated with GY, AGDM and NRE in both N treatments.

In the glasshouse experiments, out of 18 amphidiploid lines, two lines under HN and three lines under LN conditions showed transgressive segregation (TS) above the bread wheat parent for pre-anthesis A_{max} and 12 lines under HN and 7 lines under LN conditions showed TS for post-anthesis A_{max}. In addition, higher expression than the bread wheat parent was observed for *Thinopyrum turcicum* P208/201 x Chinese Spring Eup 94 under HN conditions for GY and for *Thinopyrum turcicum* P208/201 x Chinese Spring Eup 94, *Secale anatolicum* P208/142 x Highbury and *Secale anatolicum* P208/141 x Chinese Spring Eup 94 under HN conditions for AGDM showing potential to exploit these genotypes for wide crossing for NUE wheat breeding.

Seedling root architectural traits including seminal root number per plant showed association with field and glasshouse GY and NUE related traits in HN and LN conditions. Overall amphidiploids showed evidence for increased root depth than bread wheat cultivars in the hydroponics seedling platform under both HN and LN conditions.

In summary:

• N-use efficiency in thirty Indian wheat cultivars in the field was correlated with onset of flag-leaf senescence under high N and low N conditions and senescence timing was correlated with N accumulation at anthesis.

• Yield response to N limitation of 30 wheat cultivars was associated with responses in N uptake at anthesis under both N conditions.

• The grain yield in N stressed crops for the 30 cultivars was limited by post-anthesis source capacity.

• Three amphidiploids lines (*Thinopyrum turcicum* P208/201 x Chinese Spring Eup 94, *Secale anatolicum* P208/142 x Highbury and *Secale anatolicum* P208/141 x Chinese Spring Eup 94) in glasshouse conditions showed higher flag-leaf photosynthesis rate and prolonged flag leaf green area than their recurrent parents.

• The 2D seedling RSAT study showed seminal root number was correlated amongst 12 Indian wheat cultivars and amphidiploids with grain yield per shoot under high and low N conditions.

This thesis dedicated To

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LIST OF ABBREVIATIONS

AD	Anthesis date
AGDM	Above ground dry matter
AGN	Above-ground N
AGN An or NUpAn	above-ground N at anthesis
AGN H or NUpH	above-ground N at harvest
Amax	Maximum assimilation rate
ANOVA	Analysis of Variance
BPE	Biomass production efficiency
°C	Degree Celsius
C	Carbon
Ca	Calcium
CaCo3	Calcium carbonate
Ci	intracelluer CO2 concentration
CIMMYT	The International Maize and Wheat Improvement Centre
Cl	Chlorine
cm	Centimeter
CO2	Carbon dioxide
Cu	Copper
DAS	Days after sowing
df	Degrees of freedom
DM	Dry matter
DWR	Directorate of wheat research
E	Environment
Ear NA	Ear N at anthesis
Ear NPI	Ear N partitioning index
EC	Emulsifiable concentrate
eps	Earliness per se gene
FAO	Food and Agriculture Organisation of the UN
FE	Fruiting efficiency
Fe	Iron
FL	Flag-leaf
FL NA	Flag-leaf N at anthesis
FL NPI	Flag-leaf N partitioning index
Fv'/Fm'	Maximum efficiency of PSII
g	Gram
G	Genotype
G x N	Genotype x N treatment
GPC	Grain protein concentration
GS	Growth stage
gs	stomatal conductance
GY	Grain yield
H2	Hydrogen
ha-1	Per hectare
HI	Harvest index

HN	High N
h	hours
HY	High yielding
IFA	International Fertiliser Industry Association
IST	International standard time
K	Light extinction coefficient
K	Potassium
K	Maximum SPAD
K	Potassium
kg	Kilogram
LAI	Leaf area index
LN	Low N
LSD	List significant difference
LY	Low yielding
m	Meter
Mg	Magnesium
MMT	Million metric tons
Mn	Manganese
Mo	Molybdenum
MOP	Muriate of potash
N	Nitrogen
N2O	Nitrous oxide
NDIV	Normalized difference vegetation index
NDVI per-tt400	NDVI average over measurement from 0 to 400 °C post-anthesis
NDVI post-tt400	NDVI average over measurement from 400 to 800 °C post-anthesis
NH4NO3	Ammonium nitrate
NHI	N harvest index
NIR	Near infrared
NNI	N nutrition index NNI
NPI	N partitioning index
NR	N remobilisation
NRE	N remobilisation efficiency
NUE	N-use efficiency
NUpE	N-uptake efficiency
NUtE	N-utilisation efficiency
O2	Oxygen
P	Phosphorus
PANU	Post-anthesis N uptake
PAR	Photosynthetically active radiation
PCA	Principal component analysis
PCs	Principal components
PH	Plant height
PMD	Physiological maturity date
Post-An	Post anthesis
Ppd	Photoperiod insensitive gene
PPFD	Photosynthetic photon flux density

Pre-An	Pre anthesis
Pro	Probability
PSII	Photosystem II
QTL	Quantitative trait loci
RSA	Root system architecture
RSAT	Root system architectural traits
RUBISCO	Ribulose 1,5-bisphosphate carboxylase/oxygenase
RUE	Radiation-use efficiency
SED	SE of differences of means
Sh	shoot
SLN	Specific leaf N
SMN	Soil mineral N
SO4	Sulphate
SPAD	Single Photon Avalanche Diodes
SPADA	SPAD at anthesis
SS.Dur or D	SPAD senescence duration
SS.End	SPAD senescence end
SS.Mid or T	SPAD senescence mid-point
SS.Onset	SPAD senescence onset
Stem NPI	Stem N partitioning index
StL	Stem and remaining leaf
StL NA	Stem and remaining leaf N at anthesis
TGW	Thousand grain weight
TotLegAllRo	Total length of all roots
TotLegLatRo	Total length of lateral toots
TotLegSemRO	Total length of seminal roots
TS	Transgressive segregation
tt	Thermal time ^{o}Cd
UK	United Kingdom
Vrn	Vernalization-insensitive gene
VS.EndRP	Visual senescence end of rapid phase
VS.Mid	Visual senescence mid-point of rapid phase
VS.OnsetRP	Visual senescence onset of rapid phase
W/D ratio	Width-depth ration
Y x G x N	Year x Genotype x N treatment
yr	Year
Zn	Zinc
µm	Micrometre

CHAPTER 1

Introduction

1.1 The Context for enhancing the N-use efficiency

Wheat (*Triticum aestivum* L.) is the most widely grown staple cereal crops in the world including India after rice. World population is projected to be 9.7 billion whereas Indian population is projected to be 1.7 billion by 2050. To feed the increasing world population food crop production needs to be increased including wheat grain yield. In the past half-century, despite a doubling of the world population, plant scientists were able to produce new higher yielding cultivars of wheat to keep up with food requirement of increasing world population (Evenson and Gollin, 2003). Global production of the wheat crop has increased threefold since 1960 (Fig1.1 a)(FAO, 2016). This increase in yield hasn't come due to an increase in the area under cultivation but because of an increase in productivity per unit area associated with improved cultivars and agronomy (Fig 1.1 b). In future, yield will need to keep track with demand in a sustainable manner as the world is facing increasing demand for crop production due to the increasing human population, meat and dairy consumption from growing affluence, and biofuel consumption (Tilman *et al.*, 2011). The higher yield that has been achieved in the last few decades is due to of advances in technological knowledge of intensive cropping system which include high yielding wheat cultivars, new irrigation system, and higher fertilizer input.

The most important mineral nutrient in determining the grain yield of the crop is Nitrogen (N). The success of intensive cereal systems has been associated with technological improvement associated with the high input of N fertilizer along with semi-dwarf wheat cultivars that respond to the high levels of N. This phenomenon called the 'Green Revolution' was developed and adopted all over the world including India (Evenson and Gollin, 2003). As a result, there has been a dramatic increase in the amount of N fertilizer used in modern intensive agriculture in last 50 years. However, the wheat crop has a limitation for N uptake and after a certain limit, there is no further increase in grain yield even with increased in N application. This N-uptake of applied N fertilizer is reported to average 33% N uptake efficiency worldwide (Raun and Johnson, 1999) and 55% for European winter wheat (Bloom

et al., 1988). So, a significant amount of applied N is lost to soil representing a cost to the grower and also creating environmental problems due to N leaching, eutrophication of freshwater and marine ecosystems and N_2O (a greenhouse gas) emission associated with denitrification by soil bacteria (Foulkes *et al.*, 2009).

In India, the production of N fertilizer for the year 2015-16 was a record high of 13.48 MT and wheat after rice is the most N consuming crop affecting the Indian agriculture economy significantly (FAI, 2016). India is the second largest consumer of N fertilizers in the world after China (FAI, 2010) and therefore a reduction in fertilizer dependency in agriculture is one of the biggest challenge for policy makers, farmers, and plant breeders. In future, increasing yield without increase in land under cultivation is a priority for investigation for plant scientists all over the world including India.

Therefore, there is an increasing need to reduce fertilizer N inputs in present cereal systems in both developed and developing countries while maintaining acceptable grain yield along with adequate grain protein contents. Breeding to develop new cultivars that can use N more efficiently through improved uptake and utilization would be one of the ways to reduce N application in agriculture without compromising yield. Therefore, optimization of fertilizer N use through the identification, characterization, and selection of physiological traits that play a role in efficient use of N is now in a high priority in wheat improvement projects worldwide.

This thesis chapter starts with, introducing the context of this study. Then, this chapter discusses the global importance of wheat and explains how modern intensive agriculture systems rely on N fertilizers and discusses briefly how this is threatening the environment. The concept of N-use efficiency (NUE; grain dry matter yield / N available) is discussed in the context of improving NUE through breeding strategies along with NUE traits and genetic diversity for these traits in wheat to maintain or improve yield with minimum application of N fertilizers. Finally, the aims of this thesis are presented.

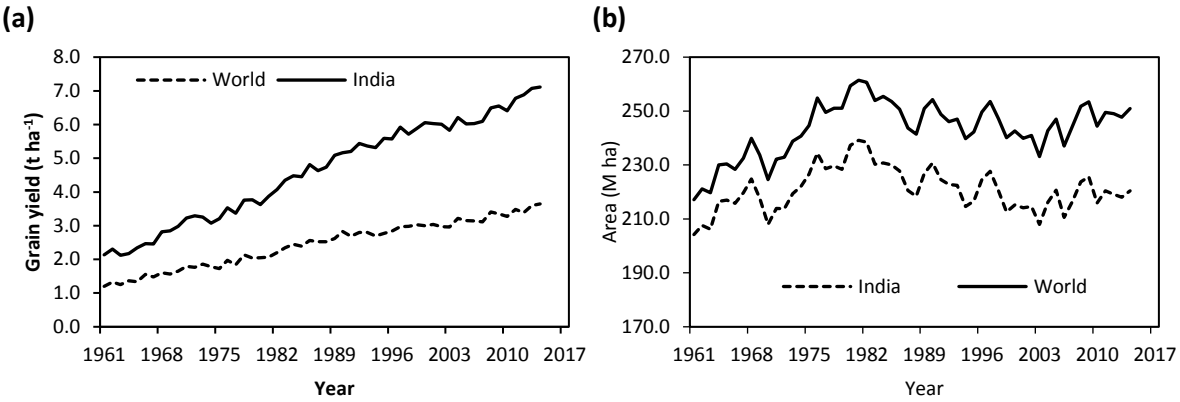


Figure 1. 1 World and Indian wheat (a) grain yield (t ha^{-1}) and (b) area under cultivation (Million ha) between 1961 to 2014 (FAO, 2016).

1.2 Wheat as a global crop and its status

Wheat was domesticated by humans in Southwest Asia about ten thousand years ago and since then it has been spread all over the world from hot climates of Asia and North Africa to colder climates of Europe (Curtis, 2002). Today, wheat is grown on more land area than any other commercial crop and continues to be the most important food grain source for humans. With the success of modern high yielding wheat cultivars, the consumption of wheat has been increased and wheat is consumed by a third of the world's population, providing the main source of calories for more than 1.5 billion people, and on average providing one-third of the total human calorific input (FAO, 2016). According to recent data available from the Food and Agriculture Organization (FAO) of United Nations, world wheat production in 2014 was approximately 729 M t from 220 M ha land and worth 46.8 billion $US (FAO, 2016). In India, it was 96 M t from 30.5 M ha of land (FAO, 2016). India stand 2nd in world wheat production after China (~13% of global share)(FAO, 2016). In the world, wheat is consumed by 2.5 billion consumers accounting for 20% of the calories of their diet (FAO, 2016) whereas in India it contributes 50% of the calories consumed, contributing substantially to the national food security (DWR, 2016). As mentioned earlier, by 2050 world population is expected to be increased to 9.7 billion and to produce food for this population wheat yield needs to be increased by 60% (FAO, 2016).

Since wheat domestication, wheat has been adopted almost from 60°N in North Europe (Norway) to 40°S in South America (Chile). It has adapted to a range of temperature conditions from below -35°C in the vegetative phase in Ontario (Haji and Hunt, 1999) to over

40°C during grain filling in the Sudan (El-Ahmadi, 1994). The wheat yield can range from 1 t ha^{-1} under severe drought conditions to over 15 t ha^{-1} where agronomical and environmental conditions are suitable for winter wheat crops e.g. the UK and New Zealand.

Bread wheat (*T. aestivum* L) originated around 10,000 BC and since then, during domestication, wheat has been selected by humans for improved grain yield, adapted to diverse environmental conditions and agriculture practices. Bread wheat is a hexaploid wheat which comes from natural outcrossing of wild progenitors (Fig. 1.2) (Morris and Sears, 1967). Hexaploid bread wheat has three sets of homeologous chromosomes, designated as AABBDD, which come from three different wild wheat species. The D chromosome may have come from wild diploid *Aegilops tauschii* (Kihara, 1944) through alloploidization with tetraploid *Triticum dicoccoides* which contributed the AABB genome. It seems that the A and B genome of that tetraploid came from an earlier hybridization between the wild AA genome donor diploid *Triticum urartu* (Dvořák *et al.*, 1993) and the wild B genome donor of which the source is still not certain. Traditionally some researchers thought that B genome donor may be from *Sitopsis* section of the genus *Aegilops* (Sarkar and Stebbins, 1956; Kerby and Kuspira, 1988).

Hexaploid wheat can be further classified by breeders or growers as i) winter wheat (which requires a cool climate for vernalization before flowering) and spring wheat (which doesn't require vernalization) depending on climate of adaptation; ii) high or low gluten content and/or hard vs. soft wheat depending on grain quality or grain texture respectively; and iii) white or red wheat depending on color of the grains. According to the quality of wheat grains, they are used in making different food products. Wheat cultivars which have high gluten content are mainly used for bread making because of their dough elasticity. Similarly, hard wheat is mostly used in bread or pasta (durum) making, while soft wheat is generally used for biscuits and breakfast foods. Soft low-quality wheat can also be used in making whiskey, beer, bio-ethanol or feed for livestock. In India, traditionally bread making wheat is used for making *Chapati* and its variants such as *roti*, *phulka* and *parotha* which form the staple food products of the major population of India and its subcontinent. The major proportion of wheat produced in these countries is utilized for this purpose after processing into whole wheat flour in plate mills (Rao *et al.*, 1986).

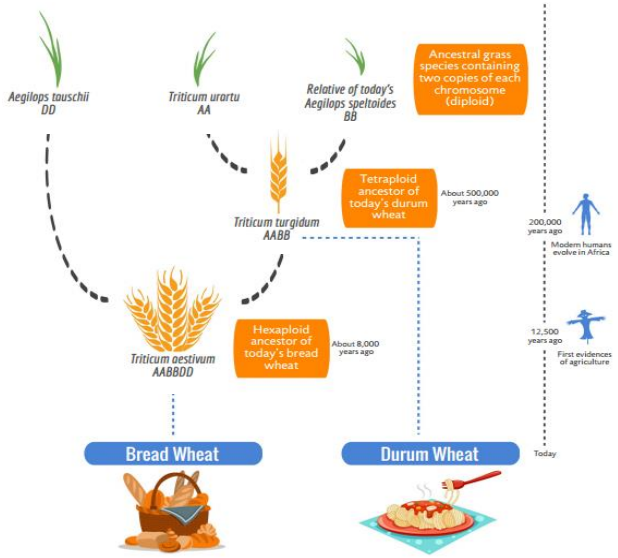


Figure 1. 2 The origin of hexaploid bread wheat from natural hybridization between three different species or genera of wild grasses (www.wheatinitiative.org).



Figure 1. 3 Wheat growing zones of India (Jaiswal, 2009)

In India, climate varies from North to South, therefore, developing wheat cultivars adaptable to specific climate zones are necessary. Overall, India is divided into six climatic wheat growing zones as 1. Northern Hills Zone (NHZ) 2. North Western Plain Zone (NWPZ) 3. North Eastern Plain Zone (NEPZ) 4. Central Zone (CZ) 5. Peninsular Zone (PZ) 6. Southern Hills Zone (SHZ) (Fig.1.3). In India, the Green Revolution started in the 1970s with the introduction of 'semi-dwarf' and 'photoperiod-insensitivite' wheat cultivars like Sonara 63, Sonara 64, Mayo 64 and Lerma Rojo 64-A having deep red color which were the common donors of reduced height (*Rht*) and photoperiod-insensitivity (*Ppd*) genes along with other segregating lines from International Maize and Wheat Improvement Center (CIMMYT), Mexico (Swaminathan, 2013). Using these cultivars as donor parents in selective breeding programs new wheat cultivars like Kalyan Sona, Sonalika, Choti Lerma and Safed Lerma having amber or white more acceptable grain color were developed which then became common parents for successive breeding programs in different breeding centers in India during the Green Revolution (Swaminathan, 2013; Ram, 2014).

Since then wheat yield in India more has quadrupled from 52 million MT in 1951-52 to above 212.1 million MT in 2003-04 (FAI, 2016). In future, this increase in yield will be more important to avoid hunger of ever increasing population. The latest estimated demand for wheat production in India for the year 2020 is approximately about 13 million tonnes more than the record production of 75 million tonnes harvested in 1999-2000. Since 2000, India is facing a critical challenge in maintaining food security in the face of its growing population (Joshi *et al.*, 2007b). There are many examples which show the rates of yield increase in wheat is slower or has stopped in different parts of the world in recent years. For example, in India, despite the success of the Green Revolution, growth rates in wheat yield fell over the past decades. During the 1980s and 1990s yield increased at ca. 1% per year under optimum conditions. This increase was partly associated with breeding and selection of new cultivars by introducing yield-enhancing alien chromatin into wheat germplasm like 1BL/1RS chromosome translocation from rye (*Secale cereale* L.) (Foulkes *et al.*, 2007) and LR19 segment from tall wheat grass [*Agropyron elongatum* (Host) P.Beauv.] (Reynolds *et al.*, 1999). But recently the relative rate of global yield increase in wheat is declining in some countries and regions and is presently about 1.1% per year (Hall and Richards, 2013) and grain yields have stalled in 37% of the global wheat cropping areas (Ray *et al.*, 2012). Thus, a yield plateau

or a decrease in rate of genetic gains in grain yield potential is reported in the last decades, e.g. in spring wheat in Mexico (Fischer and Edmeades, 2010), Chile (Matus *et al.*, 2012) and Spain (Acreche *et al.*, 2008). Grassini *et al.* (2013) reported that some of the world's most intensive cropping systems (including UK, Netherlands, France, and India) have reached a long-lasting plateau. Hochman *et al.* (2017) have attributed the cause of slowing yield gains to climate change and some others have emphasized societal restrictions on the use of inputs and economic disincentives to intensify crop production (Peltonen-Sainio *et al.*, 2009; Lin and Huybers, 2012). The yield plateau may also be in part due to the fact that harvest index is approaching a theoretic maximum value estimated to be approximately 0.62 (Austin, 1980) in some countries. Apart from the above-mentioned challenges for increase wheat yield, there are other challenges which include increasing cost and scarcity of resources involved in intensive farming systems particularly fossil fuels for agro-chemical manufacture.

The use of wheat is also changing in the twenty-first century and the wheat market for non-food uses is increasing. The new emerging 'bio-fuels' market in Europe and North America is creating a demand for low protein grain (8-10%) to produce 'bio-ethanol' as a petrol additive or substitute. This demand in the future is most likely going to increase as new policies in some countries are formulated to promote the uses of bio-fuels. For example, EU countries have targeted 10% of road transport fuels to come from bio-fuels by 2020. Currently, it is difficult to know exactly how this change will influence wheat and fertilizer markets as government and industrial policies are evolving but it is estimated that bio-energy crops will account for 2-16% of fertilizer consumption by 2030 (Smeets and Faaij, 2006).

N is the most important crop nutrient and the major element that affects final crop economic yield. Management of N along with other essential nutrients is important in terms of a balanced fertilizer program and avoiding adverse effects on the environment. The knowledge and understanding of different forms of N in soil and how the plant uptakes these different forms are important in N management to increase N-use efficiency (NUE). This could also help to the producer to obtain higher yield with minimum additional N inputs and increased profit (King *et al.*, 2003).

1.3 Different forms of N:

In the atmosphere or in the soil, N is present in pure or in combined form with other elements which may be available to the plant directly or indirectly. In this section, some important inorganic chemical forms of N used by the plant as a source of N are discussed. Nitrogen gas (N_2) constitutes 78 percent of the atmosphere. Plants cannot use it directly but leguminous plants and free-living N_2-fixing bacteria can fix it from the atmosphere. Industrial N fertilizer production process fixes N from the atmosphere to produces fertilizers.

Ammonia (NH_3) is a gas which contains N. This form of N is not available to plants directly but involved in the N cycle in the soil and can be converted to nitrite (NO_2^-) and nitrate (NO_3^-) by nitrifying bacteria and then used by plants. NO_3^- is the most common form of N that can be used by plants in ionic form. This form of N is mobile and can leach through the soil. It is also the end-product of mineralization. Ammonium (NH_4^+) is another ionic from of N that is available easily to the plant as its need less energy for uptake compared to NO_3^-. Also in this form N is less likely to be lost from the soil than other forms (Habte and Krawinkel, 2015). NO_2^- is not available to plants and is more prone to be lost in soil by leaching than either NH_4^+ and NO_3^-. Nitrous Oxide (N_2O) is a gas which plays an important role in the greenhouse effect. In this form, N can be lost from the soil through the process of denitrification. Nitric Oxide (NO) is also a gas which can be lost through denitrification. It is harmful to the ozone layer (Schulten and Schnitzer, 1997). Generally, the denitrification process takes place in the soil when the soil is saturated with water and along with warm soil temperature. In situations of depleted oxygen, soil denitrifying microorganisms use NO_3^- as an oxygen source releasing N in the air as N_2O (Habte and Krawinkel, 2015)

1.4 N in soil and N cycle

In soil, most of the N is present in compound form (97 to 98%) tied with organic matter and unavailable for direct crop root uptake (Barbarick, 2013). Available forms of inorganic N are NO_3^- and NH_4^+, are present in the soil in lower amounts (2 to 3%) as compared to organic. N in organic matter is not available to the plant directly but can continuously be broken down by soil microorganisms at specific moisture, temperature and oxygen levels releasing N that can be used by plants. This process is called mineralization. Another process opposite to

mineralization is immobilization where microorganisms feed on inorganic N and incorporate N (Fig. 1.4) (Schulten and Schnitzer, 1997). In normal soil conditions, the processes of mineralization and mobilization occur at same time. After the crop harvest, large amounts of straw which contain high carbon and low N are incorporated into the soil, and the process of immobilization can take place depleting most of the available N from the soil. Later these materials can be broken down in the process of mineralization to add back N to soil making N available for plants. NO_3^- is highly mobile in the soil and can be lost from the soil in many ways like crop uptake, assimilation by microorganisms, leaching out with drainage water and loss to the atmosphere by denitrification.

N applied as fertilizer can be lost from the soil by different ways: the most commonly applied fertilizer worldwide urea can be converted to NH_3 and released as ammonium gas into the air if sufficient moisture, temperature, and the necessary enzyme urease are present in the soil. In field conditions, this can be avoided by incorporating urea below the surface of the soil or irrigating immediately after its application. Another common practice to avoid the N loss is to split the N application into different doses of application. In addition, some inhibitors can inhibit urease activities and reduce N loss (Peoples *et al.*, 1995).

Once plants uptake N, it also goes through conversion into different forms inside the plant cell before use for different purposes. The most common forms of N used by plants is NO_3^- and to a lesser extent the NH_4^+. In general, it has been found that plant growth is better if plants uptake both forms of N (Cramer and Lewis, 1993). After uptake of NO_3^-, it is converted into NH_4^+ by using energy produced through photosynthesis. NO_3^- reduction occurs in root and shoots but is separated between the cytoplasm where the reduction takes place and the plastids/chloroplasts where NO_2^- reduction take place. Reduction of NO_3^- to NO_2^- occurs in the cytosol and is catalyzed by the enzyme nitrate reductase (Meyer and Stitt, 2001). It has been found that there is a change in root: shoot ratio if wheat plants uptake more of one of these forms of N than other (Cramer and Lewis, 1993).

In leguminous plants like alfalfa and soybean, most of the necessary N is available from N fixation by symbiotic bacteria and rhizobia that are present in the root nodules of these plants. Not all nodules are able to fix N but healthy ones usually found on primary roots and larger in size and present in clusters with pink to red in centers generally fix N. The presence

of the red color is due to the leghaemoglobin associated with N fixation in rhizobia. In nature plants and fungi are the only eukaryotic organisms that actually assimilate the inorganic N. Apart from legumes, which fix N from the atmosphere, plants predominantly use NO_3^- under aerobic soil conditions and NH_4^+ in flooded wetland or acidic soils.

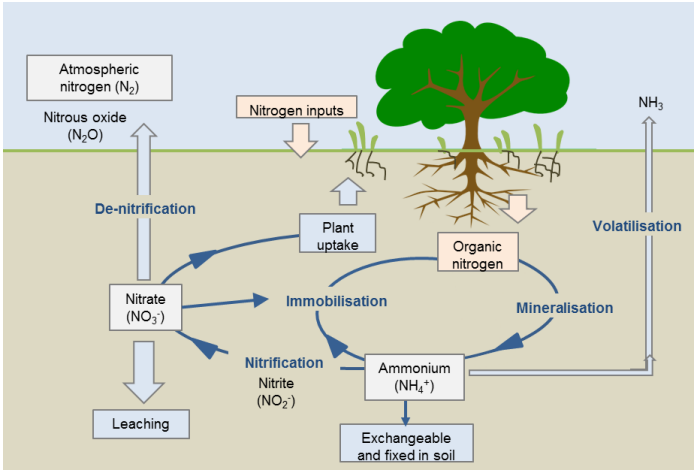


Figure 1. 4 Soil nitrogen (N) cycle. The cycling of N through different forms in the soil determines the quantity of N that is made available for plant uptake (Hoyle *et al.*, 2017).

1.5 Role of N in wheat plants

N is an essential component found in many secondary and signaling compounds like proteins, cell wall components, hormones, and vitamins. It is also a basic element of animal and plant proteins including genetic material DNA and RNA. In the wheat plant, tillering and stem elongation are the critical growth stages as N limitation at this stage affects ear growth and grains per unit area and final yield. For higher yield, wheat plants need an optimum green canopy for photosynthesis which depends on healthy chlorophyll in plant cells. The key photosynthetic enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) typically constitutes up to 30% of the total N in wheat leaves (Lawlor, 2002) and under field conditions, leaf photosynthetic rate is highly correlated with RuBisCo content.

Inside the plant, N assimilation occurs to form amino acid components which are the building blocks of proteins. These amino acids are used to form protoplasm that helps in cell division. They are also utilized in the production of enzymes and different structural parts of the plant including storage proteins in the grain. N in plants is mainly used as a component of chlorophyll and are constituent of the main photosynthetic enzyme Rubisco. This

combination of chlorophyll and Rubisco is necessary to utilize the sunlight in photosynthesis as an energy source and to conduct different functions at the cellular level including nutrient uptake. The function of chlorophyll is in harvesting the light energy in photosynthesis and of Rubisco is in catalyzing the conversion of CO_2 and H_2O to produce simple sugars. From these sugars, different products produced in the plant cell are used in plant growth along with the production of grain protein.

Therefore, N plays an important role in final biomass and grain yield production in the wheat plant and its application at particular growth stages is important to minimize the loss of N by leaching in soil and its efficient use without causing environmental problems.

1.6 Agronomy of N management

Modern wheat cultivars depend on higher N fertilizer amounts than older cultivars for their better agronomic performance. Of the total fertilizer N that is applied, only 33% globally is taken up by the crop and the rest is lost as surface runoff, leached nitrate (NO_3) in groundwater, volatilization to the atmosphere or by microbial denitrification, all of which pose environmental concerns (Raun and Johnson, 1999). In the UK, 60 to 70% of fertilizer N reported to be taken by winter wheat plant and the rest is loss in the soil. These losses could be reduced by using on-farm crop management techniques (Raun and Johnson, 1999; DEFRA, 2011) including:

(i) considering growing environment (i.e. soil type and climate)

(ii) fine tuning regimes for N fertilization (quantity, timing, and rate of applications) taking into account sources of N (soil, previous crops, animal manures)

(iii) optimizing soil management practices (tillage and organic matter) and irrigation practices.

Other ways to increase NUE include (a) application of the total N application split into two or three doses (Addiscott *et al.*, 1991), and placing N fertilizer below the soil surface layer to reduce the immobilization and increase plant N uptake (Sharpe *et al.*, 1988); (b) applying the N at particular times synchronizing with demand of the plant (Shanahan *et al.*, 2008) for

example the requirement of crop for N will be higher at the canopy expansion stage as major photosynthetic activities are taking place (Sylvester-Bradley *et al.*, 1998) (c) appropriate crop rotation for example wheat-soybean. These management strategies mainly improve N-uptake efficiency (NUpE).

During the Green Revolution, semi-dwarf and photoperiod-insensitive wheat cultivars were developed by using common wheat donors for *Rht* and *Ppd* genes, respectively. This led to a narrowing of the genetic base which is now affecting seriously the selection procedure for new wheat cultivars for N-use efficiency. Moreover, wheat is a naturally self-pollinated crop with less heterozygosity and heterosis than outcrossing crops (Longin *et al.*, 2012). Therefore, the option of hybrid seed production to break the yield plateau seems difficult.

However, genetic diversity can be created by incorporating genetic diversity into cultivated wheat from wild wheat relatives (Hawkesford, 2014; Nemeth *et al.*, 2015; King *et al.*, 2017). Genetic diversity can be created by introgression including production of interspecific hybrids by crossing ancestral or wild relatives of the wheat to cultivated wheat and then repeated backcrossing to one of the parents (Tiwari *et al.*, 2014) or by producing amphidiploids which involves production of an interspecific hybrids and subsequent chromosome doubling to complete the diploid chromosome set from each parent (Reynolds *et al.*, 2012; Jiang *et al.*, 2014; Nemeth *et al.*, 2015).

The use of ancestral-derived germplasm, introgressing genes from the ancestral wheat species and landraces has been suggested as a source of variation for biomass and NUE in wheat breeding programs (Moore, 2015). Wild relatives of bread wheat have been reported to have higher leaf photosynthetic rates than modern cultivars (Evans and Dunstone, 1970; Johnson *et al.*, 1987; Carver *et al.*, 1989; Gaju *et al.*, 2016) suggesting that wheat breeding may have resulted in a lower photosynthetic rate. Austin *et al.* (1982) reported that rate of net photosynthesis was in general highest in the diploid wheat species, intermediate for the tetraploid species and lowest for hexaploid *T. aestivum* L. with means of 38, 32 and 28 mg CO_2 $dm^{-2}h^{-1}$, respectively. However, higher photosynthesis may have related to smaller leaf size. There is also evidence that root size of landraces is larger compared with that of modern cultivars (Ehdaie and Waines, 1997) and could be useful for efficient N-uptake from the soil.

1.7 Aim of the thesis

The challenge to sustainable agriculture is to find the balance between acceptable grain yield and negative impact on the environment associated with the use of N fertilizers in the modern intensive farming systems. Breeding new N efficient wheat cultivars will be critical in achieving these challenges whilst maintaining higher grain yield to ensure food security.

This thesis aimed to quantify novel genetic variation in 30 elite Indian hexaploid bread wheat cultivars from six breeding zones for grain yield, above-ground biomass and NUE traits and responses to N availability and also quantify variation in 18 amphidiploid lines under HN and LN conditions in field and glasshouse conditions, respectively.

Therefore, the overall objectives of this thesis are to:

● Quantify genetic variation for N-use efficiency and its associated physiological traits in a panel of 30 modern Indian wheat cultivars in field experiments at Agharkar Research Institute, Pune, India under high and low N conditions and in a controlled-environment 2-D seedling root screen.

● Identify physiological traits and quantify genetic variation in comparison with bread wheat parental genotypes for N-use efficiency and associated traits in 18 novel ancestral amphidiploids lines (crosses between bread wheat and wild wheat relatives) under high and low N conditions in a glasshouse experiment and in a controlled-environment 2-D seedling root screens.

● Increase the physiological understanding of traits related to NUE and its components in the modern bread wheat cultivars and novel ancestral amphidiploid lines.

CHAPTER 2

Literature Review

2.1 Introduction

Nitrogen (N) is most important crop nutrient and the major element that affects final crop economical yield. N plays an important role from the plant's physiological activities to the structural roles. The crop requires sufficient N to produce and maintain a functional green canopy that can intercept and utilize solar radiation in photosynthesis and produce biomass, grain yield along with quality. Modern wheat cultivars need a large quantity of N to produce high grain yield and quality (Kaur *et al.*, 2016). Excessive N fertilizer application if not managed properly can cause loss by leaching which causes environmental problems along with an economic loss to producers (Foulkes *et al.*, 2009). In the UK, average N recovery is about 65% and the rest is lost in the environment (Sylvester-Bradley and Kindred, 2009). One of the ways to overcome such problem is to develop wheat cultivars which use N more efficiently. However, breeding N efficient cultivars requires an understanding of traits responsible for N-use efficiency along with the accessible genetic diversity of such traits in cultivated wheat and/or in wild wheats. The objective of identifying potential traits for NUE and the necessary genetic diversity requires a fundamental understanding of the complex inter-relationship between N-uptake, N-utilization, and N remobilization during crop growth in cultivated and wild wheat relatives.

This study examines the genetic diversity for NUE present in modern Indian wheat cultivars and in amphidiploid lines derived from crosses with wild relatives of wheat, and its physiological basis and compares these NUE traits using different techniques. NUE traits were studied using high-throughput phenotyping techniques under High and Low N levels in field and glasshouse conditions. A particular focus was given to studying senescence-related traits using different techniques. Two types of genetic material were selected, 30 elite Indian cultivars and 18 amphidiploids which were developed by crossing modern wheat cultivars with wild wheat relatives.

This chapter reviews the literature regarding the understanding of the physiological basis of the N-use efficiency in the wheat plant. It starts by briefly explaining the current wheat yield trends in relation to N fertilizer consumption and by introducing the definition of NUE and its components that are NUpE and NUtE. Then examining the previous studies done and reported genetic variation in NUE and its two components, NUpE and NUtE. Finally, different potential traits that could be useful in improving NUE along with and improvement strategies of these traits are discussed. The chapter concludes with the study objectives and hypotheses to be tested in this thesis.

2.2 Grain yield trends in relation to NUE

In general, NUE is depended on the amount of N applied to crop. Over time, if the amount of N application is constant and yield is increasing then the yield is the result of an increase in NUE. However, understanding the relative effect of high versus low N input systems of cropping is difficult with such a basic definition which may give misleading information about higher efficiency when the input is either low or high. In cereals, NUE is reported to be 33% estimated based on recovery of N in grain from applied fertilizer along with corrections for N acquired from the soil or atmosphere (Raun and Johnson, 1999). This amount represents a huge inefficiency.

Under conditions where N inputs are higher there is typically an increase in yield but this relationship is not linear showing that there is optimum threshold above which yield does not increase (Fig. 2.1). On the other hand, additional N increases the cost of production; therefore knowledge of exact input requirements for individual cultivars is necessary to obtain the economic optimum yield (Foulkes *et al.*, 1998b). Through the growing period of the wheat crop, N is essential in affecting seedling establishment, numbers of tillers, canopy size and function as well as grain filling and protein content. All of these crop processes influence the final grain yield and together determine the crop N requirement. Increasing grain yield through genetic improvement while increasing NUE is a complex issue and will need a complex set of solutions to fulfill the objectives of sustainable crop yield (Hawkesford, 2017).

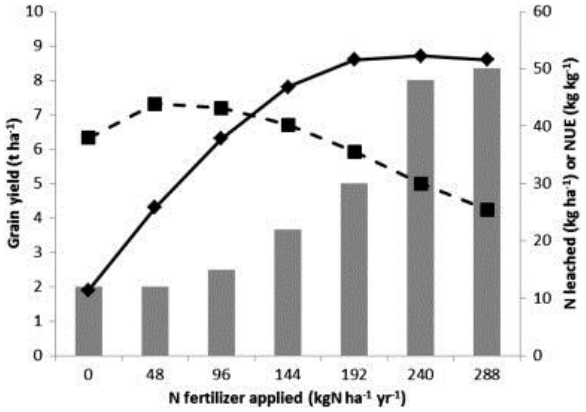


Figure 2. 1 Effect of N application on winter wheat yield (solid line, diamonds), loss of N through leaching (bar chart) and estimated grain NUE (dashed line, squares) (Hawkesford, 2012).

Kaur *et al.* (2016) reported that grain yield increase with an increase in N application up to 120 kg N ha^{-1} and agronomic N use efficiency was similar at 120 and 150 kg N ha^{-1} in Indian wheat. Hawkesford (2014) reviewed on-farm yield increased in the UK between 1970 to 1990 and reported a yield increase with N application from ca. 70 to 192 kg ha^{-1}. With the additional application of N up to 350 kg ha^{-1}, there was no increase in yield; the added N increase grain N content but here also only up to a certain level. From the study of Hawkesford (2014), it was concluded that the inability of the crop to increase the yield when applied N more than 200 kg ha^{-1} may not be because of the limitation of N but because of a physiological limitation of photosynthesis to produce more yield. This limitation may be because of photosynthetic efficiency and/or water limitation (Hawkesford, 2014). Consequently, there is likely a limitation to grain sink size.

2.3 Definitions of N-use efficiency and its components

For reduced N fertilizer inputs whilst maintaining acceptable grain production, plants should use N in an efficient manner. However, there are several obstacles to achieving such a goal through genetic improvement and improved understanding of traits determining grain yield and components of physiological traits determining NUE is an important objective. Achieving higher yield without increasing N fertilizer application will by definition give greater N use efficiency, but that may come with a compromise for grain quality traits. In the

literature, there are many definitions of N-Use Efficiency (Good *et al.*, 2004; Fageria *et al.*, 2008). In this thesis, the following definitions will be used for NUE and its components.

Moll *et al.* (1982) defined Nitrogen-use efficiency (NUE) as the grain dry matter (DM) yield (kg DM ha^{-1}) per unit of available N (kg N ha^{-1}) and represented by the following equation:

$$\text{NUE} = \text{Grain Yield } (kg\ ha^{-1}) \text{ / available N } (kg\ ha^{-1}) \qquad \text{Equation 2.1}$$

Available N includes N from fertilizer inputs, N deposition from the atmosphere and from N from soil mineralization which depends on soil organic matter content and the previous crop in the rotation.

NUE is a combination of the different process associated with whole plant N uptake and the utilization of N to produce grain and which includes absorption, translocation, assimilation, and redistribution of N (Moll *et al.*, 1982). Therefore, NUE can be further divided into two components:

(i) N-uptake efficiency (NUpE) which is crop N uptake, i.e. above-ground N at harvest (AGN_H), per unit N available from the soil and fertilizer represented by the following equation:

$$\text{NUpE} = \text{AGN}_\text{H}\ (kg\ ha^{-1}) \text{ / available N } (kg\ ha^{-1}) \qquad \text{Equation 2.2}$$

(ii) N-utilization efficiency (NUtE) which is grain dry matter yield per unit crop N uptake at harvest, i.e. above-ground N at harvest (AGN_H) represented by the following equation:

$$\text{NUtE} = \text{Grain Yield } (kg\ ha^{-1}) \text{ / AGN}_\text{H}\ (kg\ ha^{-1}) \qquad \text{Equation 2.3}$$

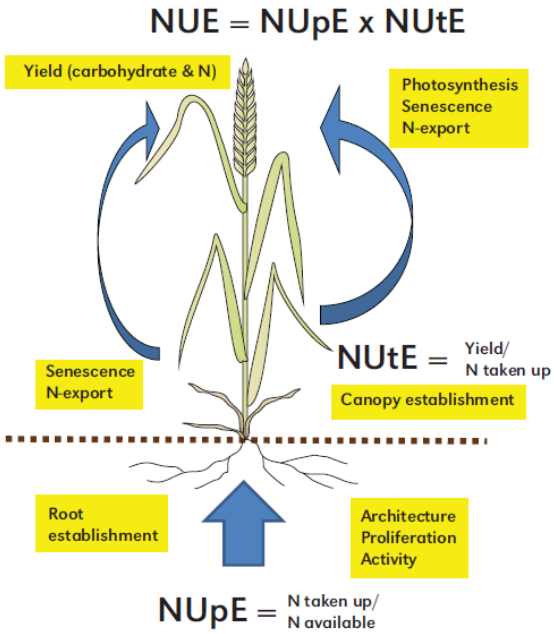


Figure 2. 2 Diagrammatic representation of the key terms used to describe wheat N-use efficiency, focusing on N. Underlying physiological processes are indicated (Hawkesford, 2012).

2.4 Traits relevant to high-throughput phenotyping for NUE

Some of the physiological parameters that could be measured to understand NUE are leaf chlorophyll and N content, biomass, N/P accumulation, N partitioning indices, leaf area index, grain N and P, grain yield and its components among others. In additions to the above traits root architecture traits e.g. length and density, number, angle, and distribution of roots could be studied as the root plays a vital role of acquiring N from the soil. Similar root architectural traits could measure at seedling stage in controlled environmental conditions (Atkinson *et al.*, 2015). Green area and N content could be measured at individual leaf level or canopy level using spectral reflectance techniques like Normalized difference vegetative index (NDVI) (Balyan *et al.*, 2016). At leaf level, traits like maximum photosynthesis rate (A_{max}) at saturated light conditions and the chlorophyll fluoresce quantum yield which indicates the efficiency of PSII could be measured (Gaju *et al.*, 2016). Further genetic studies applying molecular marker mapping approaches using appropriate segregating populations could identify Quantitative Trait Loci (QTL) for complex traits such as NUE (Hirel *et al.*, 2007) and underlying traits. The QTL can then be used to develop molecular markers with which to screen breeding lines for traits underlying NUE.

2.5 Traits and strategies to increase N-uptake efficiency (NUpE)

N-uptake efficiency reflects the ability of the plant to acquire N (as ammonium or nitrate ions) from the soil in relation to that available (soil mineral N and applied N). It is normally assumed that soil mineral N is recovered by the crop in preference to applied N, although full recovery of available N by fertilized cereal crops is never achieved (Vaidyanathan, 1984). From a summary of published data on N efficiencies in cereal crops, Ladha *et al.* (2005) found that the average recovery efficiency of fertilizer N was 54% in wheat. Some cultivars are genetically better able take up N more efficiently, indicating the influence of genetic control for N uptake (Le Gouis *et al.*, 2000). Differences in uptake may result from variation in the ability to absorb N at various soil depths which affect the quantity, rate, and duration of N-uptake associated with variation in root system architecture.

2.5.1. Increasing NUpE by manipulating root system architecture traits

Root system architecture traits (RSAT) are the most important traits associated with NUpE. But the role of root traits in NUE is not well understood due to difficulties in studying root morphology and functionality in field studies, although new methods are under development (Whalley *et al.*, 2017). Also in the field RSAT phenotyping of full-grown plant is labor-intensive, especially when experiment need to be conducted under uniform soil conditions and involve a large group of genotypes for study (Mace *et al.*, 2012). However, some studies on NUE QTL show positive coincidences between QTL for N-uptake and QTL for seedling RSA traits (Bai *et al.*, 2013; Atkinson *et al.*, 2015; Xie *et al.*, 2017). These studies suggest that RSAT like root length, rooting depth and root length density at depth will be key traits to enhance and apply in breeding strategies to increase NUE (Garnett *et al.*, 2009). However, traits to improve NUE also depend on whether plants are grown under high N or low N (Foulkes *et al.*, 2009). For example in maize, under high N conditions genetic variation in NUE appears to be related to N-uptake efficiency, whilst at low N it appears to relate to N-utilization efficiency (Moll *et al.*, 1982). In wheat, under low N conditions, genetic variation in NUE appeared to be mainly related to NUpE (Le Gouis *et al.*, 2000; Gaju *et al.*, 2009; Gaju *et al.*, 2016).

In recent years, major advances have been made in the study of root architecture, which has been often described as another important "hidden half" trait (Lynch, 2007; Lammerts van Bueren *et al.*, 2014). The wheat root system has two types of roots per plant i) six seminal roots and ii) 10-15 crown roots. Density and length of these roots increase continuously from germination till the flowering and after flowering, there is usually no significant change in root size and morphology (Barraclough and Leigh, 1984). The ratio of root dry weight to total dry weight of the plant changes from ca. 0.3 in early growth stage to ca. 0.1 at harvesting (Gregory *et al.*, 1978).

To maximize the N-uptake these root-related traits need to be optimized for N-uptake efficiency. For example, to extract N effectively from the deep soil, deep root systems play an important role (Liao *et al.*, 2006). The ability of a root system to acquire water and nutrients from a defined volume of soil is principally related to its Root Length Density [RLD; root length (cm) per unit soil volume (cm^{-3})] (Van Noordwijk, 1983) and to its maximum rooting depth and distribution of RLD with soil depth (Foulkes *et al.*, 2009). Barraclough *et al.* (1989) in field experiments in the UK showed that RLD of 1 cm cm^{-3} is necessary to abstract all available water by winter wheat. King *et al.* (2003) in a modeling study showed that relatively deeper root distribution in the soil profile combined with decreasing specific root length increased N capture and yield under low N availability. Cultivars that grow for a longer duration, and have an extended phase to anthesis, generally show deeper root systems and therefore winter wheat that grows for a long duration compared to spring wheat shows a deeper root system than spring wheat and could have higher NUE (Thorup-Kristensen *et al.*, 2009). In winter wheat the maximum rooting depth is typically 140-200 cm (Barraclough and Weir, 1988) whereas in spring wheat it is typically 80-120 cm (Siddique *et al.*, 1990). Wild wheats may have deeper root systems and this trait could be introgressed into cultivated wheat by breeding (Reynolds *et al.*, 2012). However, Aziz *et al.* (2017) studied wheat cultivars released in Australia between 1958 and 2007 and found that selection for yield, had reduced total RLD and total root length, and increased N uptake per unit root length that compensated for the reduction in root system size, effectively explaining the increase in N-uptake. Traits like root axis number and RLD at depth can be useful to absorb available nitrogen at the maximum level and rate. Root longevity may also be useful for post-anthesis N-uptake particularly in longer growing seasons (Bengough *et al.*, 2006; Lynch, 2013). Root growth angle (RGA) could

also play role in N uptake, for example, narrow-angle could reach a greater depth or a wider angle could cover the maximum area at shallow depth. In rice, by studying root distribution in the field (Uga *et al.*, 2013) and RGA in rhizotrons (Kitomi *et al.*, 2015), a narrow and deep root ideotype for improved drought resistance has been successfully developed. RGA is easily measured on seminal roots of seedlings (Richard *et al.*, 2015) or on the adventitious roots at the adult stage (Kato *et al.*, 2006).

The association between plant height and root length is not very clear. In some studies, no direct correlation found between plant height and root: shoot ratio (Cholick *et al.*, 1977; Siddique *et al.*, 1990) but root: shoot ratios tended to be negatively correlated with plant height in study of tall (no *Rht* alleles), semi-dwarf (*Rht1* or *Rht2*), dwarf (*Rht1* and *Rht2*) Canadian wheat cultivars (McCaig and Morgan, 1993) and showed positive association between plant height and root: shoot ratio in Indian wheat study (Gupta and Virmani, 1974).

While advances in genomic approaches to tackle complex traits have been made, the lack of high-throughput and large-scale phenotyping methods for root traits remains a bottleneck to gene discovery and selection for such traits in breeding programs. Recent progress in root measurement techniques has enhanced our ability to visualize, quantify and conceptualize root system architecture traits (RSAT) and their relationship to plant productivity (Lynch, 1995). In controlled environment conditions, the use of soil-filled root observation chambers (Rhizotrons) and non-destructive digital imaging techniques offer some promise (Manschadi *et al.*, 2010), but may be less suitable for screening of root traits that are expressed at later stages of crop development. Laboratory screens have focused mainly on seedlings, with seedlings growing on germination paper (Hund *et al.*, 2009; Bai *et al.*, 2013; Atkinson *et al.*, 2015; Xie *et al.*, 2017) or in growth pouches (Liao *et al.*, 2004a; Hund *et al.*, 2009). Several non-soil filled methods for evaluating roots are available, including hydroponic, aeroponic and agar-plate systems (Gregory *et al.*, 2009). Root measurements in laboratory screens tend to be more precise and more reproducible because the plants are grown in a more homogeneous environment compared to the field. Although several screening tests have been designed to generate accurate and robust data from seedlings grown under artificial conditions, these phenotypes have only rarely been extrapolated to field conditions partly because of the pronounced plasticity of root growth and development processes. Laboratory-based methods can be limited in their ability to reproduce field-like

conditions (Poorter *et al.*, 2012). For example, soil-environment × genotype interactions significantly affect the root length of wheat cultivars grown in sandy soil compared to agar plates (Gregory *et al.*, 2009). Encouragingly, as mentioned above, seedling root traits based on paper-based germination screens were shown to be linked to mature plant traits such as height and yield in a Savannah x Rialto DH population (Atkinson *et al.*, 2015) and plant height in an Avalon x Cadenza DH population (Bai *et al.*, 2013). At an intermediate scale, X-ray computed tomography (Mooney *et al.*, 2012; Mairhofer *et al.*, 2013) magnetic resonance imaging (Metzner *et al.*, 2015), mini-rhizotrons (Poorter *et al.*, 2012), and rhizotron (Nagel *et al.*, 2009) methods have been developed and offer promise.

In the field, most of the root phenotyping methods approaches are low-throughput. There are two relatively high-throughput field phenotyping techniques: the soil core break where a root auger is used to take soil-root cores from the field, the cores are then broken transversely and the roots on the exposed cross sections counted (Bennie *et al.*, 1987; Wasson *et al.*, 2012) and shovelomics which involves the excavation and visual scoring of crown roots extracted from the field, and results in maize have been shown to be well correlated with total plant depth and root system total length (Trachsel *et al.*, 2011).

2.6 Traits and strategies to increase N-utilization efficiency (NUtE)

NUtE is the ability to use the N taken up by the plant to produce grain yield, and is the product of: (i) the amount of above-ground biomass produced per unit of crop N uptake (Ortiz-Monasterio *et al.*, 1997) and (ii) the partitioning of this biomass to the grain (harvest index) at harvest. N-uptake and its partitioning to grain are important variables in determining the final grain yield in wheat crop. Plants need N for the production of proteins and nucleotides for developing grain in specific concentrations and therefore final grain quantity and quality depends on N supply during the grain filling stage. N for the development of grain can be obtained from N that is accumulated during grain filling or by N remobilization to grain from vegetative parts which is accumulated before anthesis. The efficiency of AGDM production per unit of N-uptake by a plant depends on principally four factors: the role of N in photosynthesis, nitrate assimilation, N partitioning amongst plant components and remobilization of N between stem, leaf lamina, leaf sheath and grain (Hirel *et al.*, 2007).

2.6.1 Increasing NUtE by manipulating N partitioning, storage, and remobilization

It is reported that in wheat, of total above-ground N, 35-42% is in leaf lamina, 14-20% in the leaf sheath, 20-31% in the true stem and 16-23% in the ear under optimal N application at anthesis (Pask *et al.*, 2012; Barraclough *et al.*, 2014; Gaju *et al.*, 2014). Under N limited conditions, the proportion of N in the ear increases compared to other plant components at anthesis (Gaju *et al.*, 2014; Gaju *et al.*, 2016). Foulkes *et al.* (2009) suggested that a higher capacity for storing accumulated N in the true stem before anthesis could theoretically favor a higher maximum rate of N uptake and hence higher NUpE. In addition, favoring a greater capacity to store N in non-photosynthetic organs (i.e. stem internodes) may enable the translocation of a larger amount of N to grains without reducing plant photosynthetic capacity (Bertheloot *et al.*, 2008), although the respiratory cost of maintaining a large non-photosynthetic pool of storage N is unclear. In wheat, genetic variation in stem N content at anthesis is reported (Pask, 2009; Barraclough *et al.*, 2014; Gaju *et al.*, 2014), as well as in post anthesis N remobilization efficiency from the stem (Pask, 2009; Gaju *et al.*, 2014). The importance of N content in the grain depends on the end-use market. Low grain N concentration is required for animal feed, distillation, and biofuel markets whereas high grain N concentration (> 13%) is required for the bread-making market.

N present in the true stem plays a role in the structure, transport, reserve/storage and metabolic functions (Critchley, 2001). Most of the reserve N in the plant (about 60%) is in the true stem N (Pask, 2009). Lemaire and Gastal (1997) defined N pools depending on their roles during growth of the wheat plant from emergence to anthesis as: i) the structural N – providing physiological support for photosynthetic surfaces and supplying them with vascular connections, ii) the photosynthetic N - maintaining proteins needed for photosynthetic machinery, and iii) the reserve N - acting as a 'reservoir' of accumulated excess N after satisfying the demands of i) and ii). Staswick (1994) sub-divided the reserve N pool as 'storage' that is remobilized post-anthesis and 'accumulation' N that is not remobilized post-anthesis. As previously discussed, the maximum rate of accumulation of N in true stem before flowering may be a good indicator of N-uptake efficiency (Foulkes *et al.*, 2009) and the storage reserve N in the stem may have a functional role and can be translocated to grain at maturity. Therefore, the strategy to select cultivars that have a greater capacity to store N in non-

photosynthetic organs like the true stem and higher N remobilization efficiency possibly extending the duration of post-anthesis photosynthetic may be one way to increase NUE (Martre *et al.*, 2007). Thus, N retranslocation is a trait that has been associated with "stay green" behavior showing delayed leaf senescence and improved grain yield (Borrell *et al.*, 2001b; Foulkes *et al.*, 2009; Gaju *et al.*, 2011).

The ear contains around 20 per cent of total above-ground N at anthesis and approximately quarter to half of this is translocated to grain N at harvest (Foulkes *et al.*, 2009). Furthermore, the glumes are also a major source of N for grain development. Compared to the other vegetative parts of the wheat plant, flag leaves and glumes are metabolically active for a longer period and their contribution to final grain N is also higher (Simpson *et al.*, 1983). In the period just prior to rapid grain filling, when there are no alternative sinks, the glumes act as a temporary sink for the N before remobilization to the grains (Waters *et al.*, 1980). Lopes *et al.* (2006) found that glumes lost twice as much of their total N content than the flag-leaf between the milk and early dough stages. Glumes, therefore, may act as a buffer similar to the true stem N that remobilizes N to grain at maturity (Foulkes *et al.*, 2009).

2.6.2 Increasing NUtE by manipulating leaf and canopy photosynthesis per unit N

In the wheat plant, up to 70% of N is located in mesophyll cells and its major function is photosynthesis as a constituent of chloroplastidic enzyme Rubisco (Evans, 1989). Thus, under conditions where N availability is low, there is an adverse effect on this enzyme which further could reduce, leaf expansion and duration, leaf N and chlorophyll content, leaf stomatal conductance and photosynthesis per unit of leaf area (Sylvester-Bradley *et al.*, 1990). Such changes reduce radiation interception and radiation-use efficiency (above-ground biomass per unit radiation interception; RUE) and hence biomass and yield (Gaju *et al.*, 2016). More specifically canopy and leaf processes affecting photosynthesis per unit of N uptake include: (i) radiation interception per unit of N uptake, (ii) optimizing vertical N distribution in relation to light in the canopy and (iii) leaf photosynthesis per unit of leaf N.

For a radiation interception of 95%, assuming a light extinction coefficient (*K*) value of 0.5, a green area index (green canopy area per unit of ground area; GAI) of 6 is required. Indeed,

$$K = -\ln(I/Io)/L \qquad \text{Equation 2.4}$$

where *Io* is the incident radiation and *I* is the amount of radiation not intercepted by a canopy having a green area index (GAI) = L.

At anthesis, modern wheat cultivars produce canopies with GAI (green canopy per unit grown area) values around 6 and hence achieve full interception at this stage (Moreau *et al.*, 2012; Gaju *et al.*, 2014). The only realistic way to increase fractional interception in the pre-anthesis phase is to increase fractional interception at the start of the stem elongation phase. However, in wheat, it is already around 60–70% at this stage (Moreau *et al.*, 2012). Thus, only marginal improvement seems possible.

An effective way to increase NUtE may be to increase the rate of photosynthesis per unit N concentration of leaf. In C3 plants under favorable conditions, maximum leaf photosynthesis rate under light-saturated conditions (A_{max}) can be up to 20 µmol CO_2 m^{-2} s^{-1} at leaf N concentration of 2 g m^{-2} (Evans, 1983). There is debate whether A_{max} increases linearly or exponentially with increase in leaf N concentration. If the maximum photosynthesis increases up to a certain limit with increasing leaf N concentration, then a strategy to increase N-use efficiency could be the reduction of leaf N content values to the break-point whilst maintaining the maximum assimilation rate (Foulkes *et al.*, 2009). At light-saturated conditions, for potential photosynthesis, the specific leaf N (leaf lamina N per unit leaf area, SLN) required is ca. 1.5 g N m^{-2} (Jamieson and Semenov, 2000). In general, cultivated wheat cultivars accumulated more than 2 g N m^{-2} under favorable conditions (Critchley, 2001; Pask *et al.*, 2012). So NUtE could be potentially increased by adopting selection strategy for lower SLN.

The optimization theory describes the N distribution among leaf lamina which optimizes whole canopy photosynthesis (Hirose and Werger, 1987). This theory explains that, within a dense canopy, SLN content depends on the amount of light received, and extinction coefficient for N (K_N) decreases as the rate of light interception with cumulative green area deeper in the canopy decreases. K_N (m^2 ground m^{-2} green laminae) is the nitrogen extinction coefficient which describes the vertical gradient in SLN from the top of the canopy downwards. At the canopy scale, matching the optimum level of SLN and its vertical distribution in the leaf layers of the canopy, especially in low N conditions, may be an avenue

to increase RUE through reduced light-saturation of photosynthesis and NUtE (Foulkes *et al.*, 2009). The interaction between light interception and leaf N content plays an important role in carbon assimilation at the crop canopy level (Evans, 1989). N distribution in canopies in relation to light attenuation also affects photosynthesis per unit of N uptake. Considering that the leaf N gradient is 'optimal' in accordance with the 'optimization theory' (Moreau *et al.*, 2012), theoretical studies indicate that leaf N maximizes canopy photosynthesis when it parallels the light gradient, that is, when the light (K_L) and N (K_N) extinction coefficients are equal. In wheat, observed N gradients are generally less steep than predicted with the 'optimization theory'; however, they do demonstrate that SLN follows an exponential gradient with vertical depth in the canopy (Pask, 2009; Moreau *et al.*, 2012). Therefore, vertical distribution of N in crop canopy may play a role in determining genetic variation in N-use efficiency. In wheat K_N reported is around 0·464 $m^2\ m^{-2}$ (Yin *et al.*, 2003). Genetic variability in SLN was observed from 1.4 to 2.6 g m^{-2} for 144 durum wheat genotypes (Araus *et al.*, 1997) and 2.1–2.4 g m^{-2} for 17 durum wheat cultivars (Giunta *et al.*, 2002); and in leaf N concentration from 43.7 to 47.6 mg g^{-1} for eight bread wheat cultivars (Fischer *et al.*, 1998).

In *Triticum* species, there is relatively little information available on genetic diversity on the vertical distribution of N in the canopy. Critchley (2001) studied two winter wheat cultivars, Soissons and Spark, for vertical N distribution and found that their distribution follows the optimization theory and there was not a large difference in the two cultivars for N distribution in top four leaves at anthesis. Bertheloot *et al.* (2008) also reported similar results for two French winter wheat cultivars, Apache and Isengrain. Moreau *et al.* (2012) studied the leaf N and vertical light gradient where it was found that the whole plant canopy and its size is determining the genetic variation in the ratio of the vertical gradients of leaf N and light in the canopy amongst 16 wheat cultivars grown in field experiments in France and the UK. It has also been observed that leaves which are more active photosynthetically, for example, the flag-leaf, have higher optimum SLN than other leaves in the canopy (Grindlay, 1997).

In this study, the introgression amphidiploid lines, representing novel genetic variation created by crossing wild wheat species to cultivated wheat, are examined as a source of improved photosynthesis as wild species are mostly diploids and have been

reported as having higher photosynthetic rate than tetraploid species or *Triticum aestivum* (Austin *et al.*, 1982; Sybesma, 2013).

In wheat plants, other organs than the leaf lamina also contain chlorophyll, for example, the leaf sheath, true stem, and ear and can participate in photosynthesis. As mentioned earlier at anthesis with regard to N partitioning, about 35% of the total N in a wheat canopy is in the leaf lamina. The remaining approximately 65% of the crop N is partitioned between the leaf sheath, true stem (i.e., stem with the leaf sheath removed) and the ear (Pask *et al.*, 2012; Barraclough *et al.*, 2014; Gaju *et al.*, 2014) and could play an important role in photosynthesis. In wheat, the ear including awn, glume, lemma, and palea are capable of photosynthetic CO_2 fixation and contribute considerable assimilate to final grain mass (Araus and Tapia, 1987). Furthermore, photosynthesis in the leaf sheath and true stem also contributes to final grain biomass (Araus and Tapia, 1987; Wang *et al.*, 2001).

2.6.3 Increasing NUtE by manipulating the stay-green trait

During the grain filling stage, N is translocated to growing grains from the vegetative parts. This decreases the N in the canopy resulting in a reduction in photosynthetic capacity and indirectly hampers the ability of roots to further take up N. Stay-green is the trait which allows the plant to prolong the green canopy duration and final death of vegetative parts during senescence (Thomas and Howarth, 2000). Jiang *et al.* (2004) studied the stay-green trait in rice and classified it into two types, a 'functional stay green' where leaves photosynthesize longer and might, therefore, contribute to higher yield and 'non-functional stay green' where plants remain green as a result of lesions in chlorophyll catabolism, but lack in photosynthetic competence. Leaf senescence is the effect of numerous environmental and developmental factors and also the genetic background play an important role in it (Wingler *et al.*, 1998). The stay-green trait is also associated with improved yield through enhanced heat tolerance (Kumari *et al.*, 2007). In this study, the stay-green trait is one of the traits that will be examined in detail at the flag-leaf level. Generally, under the conditions where available N in the soil is not limiting then yield may be limited by the sink size rather than source size and in that case, the stay green trait may not show any correlation with GY. In contrast, GY shows a correlation with the stay-green trait under low N availability conditions

(Gaju *et al.*, 2011). In wheat, it has been observed from source-sink manipulation experiment that the remobilization of N from source to sink does not depend on the 'demand' of the sink (grain), but it depends on the supply from the source (vegetative tissues) (Martre *et al.*, 2003).

Genetic variation for functional stay-green (delayed senescence associated with extended photosynthesis) has been reported in bread wheat (Silva *et al.*, 2001; Verma *et al.*, 2004; Foulkes *et al.*, 2007; Gaju *et al.*, 2011). Recently stay-green traits were studied in synthetic derived wheat lines (Gaju *et al.*, 2016). However, at the physiological level, this trait still needs further study. Christopher *et al.* (2008) reported that stay-green trait (maintaining green leaf area longer during the grain-filling period) in spring wheat SeriM82 was responsible for the extraction of water from the deep soil in Australia. Genetic variation in this trait also in durum wheat showing correlation with higher rate and duration of grain filling (Spano *et al.*, 2003). Kumari *et al.* (2007) have reported a significant association between tolerance of abiotic stresses like high temperature with stay-green in 936 wheat lines. Kumar *et al.* (2010) suggested that delay in leaf senescence near physiological maturity stage may promote higher translocation and grain filling rates with direct effects on yield components. Kumar *et al.* (2016) reported an association between QTL of a stay-green trait with heat tolerance in Indian wheat lines. Bogard *et al.* (2011) reported that stay-green trait in winter wheat in filed experiments in France and the UK was associated with QTL for a flowering date.

2.7 High-throughput phenotyping

In recent years, a number of new high-throughput phenotyping techniques (indices) have been developed which are low cost, allowing rapid measurements with high precision. These techniques are reproducible and can measure a range of traits like chlorophyll content, photosynthetic activities, leaf area, and N content in crop plants. Many different types of indices have been developed to measure biomass, chlorophyll content, carotenoid and N/P status of plant e.g. Normalized Difference Vegetation Index (NDVI), (Balyan *et al.*, 2016). These indices are collectively described as vegetation/spectral reflectance indices (VI), where spectral reflectance is determined as the inverse of spectral absorbance. These indices make use of electromagnetic (EM) energy reflected by vegetation in the visible region of the EM spectrum. The indices are influenced by the presence of chlorophyll/carotenoid pigments in

the leaf tissues that have been related to the concentration of leaf N/P (Haboudane *et al.*, 2002). Therefore, these spectral features can provide a measure of plant N and P content. Normalized difference vegetation index (NDVI), a type of VI has recently become popular as an indirect measure of N and P contents of the canopy. This index has been shown to have a significant correlation with genetic variation in grain yield in wheat (Babar *et al.*, 2006; Gaju *et al.*, 2016). As an alternative to NDVI, canopy chlorophyll content index (CCCI) and near infrared spectroscopy (NIRS) have also been proposed for more accurate measurement of N (Haboudane *et al.*, 2002). More recently, a remote-sensing-based non-destructive method for crop N status assessment known as NDDAig has also been reported specifically for wheat (Feng *et al.*, 2014). With the availability of these indices, measurements of crop N and P status could be made more rapidly allowing fast screening of large segregating populations, while breeding for improved NUE in wheat. Recently unmanned aerial vehicles (UAVs) to take NDVI images in field conditions is becoming possible (Kyratzis *et al.*, 2017) and could take field rapid screening to next level.

Plants with greater leaf N concentrations typically have more chlorophyll. Chlorophyll in leaves absorbs most strongly in the blue (around 450 nm) and red (around 670 nm) light, and reflects in the green (around 550 nm) region of the light spectrum. The Minolta SPAD 502 CM measures light transmission in the red (650 nm) and near-infrared (940 nm) parts of the spectrum and has been used to measure leaf chlorophyll content in wheat (Uddling *et al.*, 2007), to predict grain N requirements in wheat (Lopez-Bellido *et al.*, 2004) and to monitor nitrogen status and schedule fertigation in wheat (Prost and Jeuffroy, 2007).

Chlorophyll fluorescence is a popular technique in plant physiology because of the ease with which the user can gain detailed information on the state of photosystem II (PSII) at a relatively low cost. Chlorophyll fluorescence is light re-emitted by chlorophyll molecules during return from excited to non-excited states and used as indicator of photosynthetic energy conversion in plants and give information about efficiency of PSII. It has had a major role in understanding the fundamental mechanisms of photosynthesis, the responses of plants to environmental change, genetic variation, and ecological diversity. In wheat, chlorophyll fluorescence has successfully used to see the effect of N nutrition on net photosynthesis (Shangguan *et al.*, 2000) and also for early detection of fusarium infection (Bauriegel *et al.*, 2011). Gaju *et al.* (2016) found a positive association of flag-leaf chlorophyll

fluorescence with pre-anthesis A_{max} which shows that higher efficiency of PSII may also related to higher photosynthesis in 15 winter wheat genotypes.

2.8 Objectives and Hypothesis

A major gap identified in the literature review was that there is a lack of information on genetic variation NUE and its physiological basis particular in wider wheat germplasm. Also to date, there a few studies on genetic variation in NUE in Indian wheat germplasm. Therefore, the present study aimed to identify genetic diversity in an elite panel of 30 Indian spring wheat cultivars which are representative cultivars from a range of Indian breeding zones, in field experiment carried out at ARI, Pune in two years 2013-4 and 2015-6. The literature review also showed there is relatively little previous information on genetic diversity in NUE and related traits in wild wheat relatives. Therefore, in the present study, amphidiploids which are produced by crossing hexaploid bread wheat with diploid wild wheat relatives were used to study and identify novel NUE traits in ancestral backgrounds.

Therefore, specific hypotheses of this thesis were:

1. There is genetic variation for N-use efficiency and its components traits that are related to NUpE and NUtE in the panel of 30 elite Indian spring wheat cultivars.

2. There is genetic variation for N-use efficiency and its components traits that are related to NUpE and NUtE in the panel of 18 amphidiploid lines of wheat.

3. There is a N x genotype interaction for grain yield in Indian cultivars which can be explained by physiological traits.

4. Under LN conditions NUpE plays a more important role in determining genetic variation in NUE than NUtE.

5. Genetic variation in NUpE is linked to root system architecture traits, post-anthesis N-uptake, source-sink strength, and stay-green traits under LN conditions.

6. Genetic variation in NUtE is linked to source-sink strength, stay-green traits, and N remobilization efficiency under LN condition.

7. Stay-green trait explain variation in NUE under low N but not under high N.

8. Genetic variation in stay-green trait is related to N remobilization efficiency and post-anthesis N-uptake.

9. There is a novel genetic variation for increased photosynthesis rate in amphidiploid lines under low N conditions compared to bread wheat parental lines.

10. Genetic variation in flag-leaf A_{max} is related to genetic variation in specific leaf N (SLN).

11. Early seedling root vigor is responsible for uptake of N from soil and increases yield under N limiting conditions.

12. Genetic variation in 2D seedling root system architectural traits in controlled environment conditions related to N-uptake in field conditions.

2.9 Thesis structure

This thesis presents work of experimental investigation of N-use efficiency conducted in field conditions at ARI near Pune, India, and glasshouse conditions in Nottingham, the UK using different genetic materials. Chapter 3 describes materials and methodologies used in conducting two field and two glasshouse experiments and one controlled environment condition experiment under two levels of N applications. The results of these experiments are presented in Chapters 4, 5, 6 and 7. Chapter 4 and 5 is based on Indian field experimental results where Chapter 4 presents the results of harvest data which include grain yield, biomass accumulation, N-uptake and yield and NUE components and Chapter 5 presents the results of crop phenological data which include plant phenology, N remobilization, N partitioning, leaf and canopy senescence and photosynthesis traits. Chapter 6 presents results from glasshouse experiment which include harvest (grain yield, biomass, and N-uptake) and physiological data (plant phenology, leaf senescence, and leaf photosynthesis). Chapter 7

includes 2D seedling root architecture phenotyping data of selected genotypes from the field and glasshouse experiments. Chapter 8 discusses the experimental results in the context of the objectives and hypotheses being tested and concludes the thesis by relating the findings to wider perspectives for the application of physiology for the genetic improvement of NUE in future years.

CHAPTER 3

Materials and Methods

This chapter describes the plant genetic materials and experimental methodology used in five experiments conducted in the field, the glasshouse and controlled environment (hydroponic) conditions during 2013 to 2016 in India and the UK to test the hypotheses set out in the previous chapter of this thesis.

3.1 INDIAN FIELD EXPERIMENTS

Three field experiments were conducted at Agharkar Research Institute, Pune, India during the wheat growing seasons of 2013-4, 2014-5 and 2015-6. In this location spring wheat is generally grown from mid-November to mid-March (about 16 weeks) representing the Indian winter growing season. The experiment of 2014-5 was severely affected by lodging due to heavy rain and therefore data of this season were not considered for this thesis.

3.1.1 Experimental conditions

3.1.1.1 Experimental site

The field experiments were conducted at Agharkar Research Institute, Pune, India at Hol farm (LA:18.1171893, LO:74.31072280000001) located 60 km from Pune in the wheat climatic Peninsular Zone (PZ) (latitude; 16^{o} 30’ to 22^{o} 03’ M, longitude: 73 ^{o} 47’ to 74 ^{o} 40’ N, altitude: 557.5) in 2013-4 and 2015-6 (Fig. 3.1). The previous crop was soybean in 2013-4 and sorghum in 2015-6 (Table 3.1). This site has a temperate climate with mean annual rainfall fall of 457 mm and mean annual maximum temperature of 36^{o}C and minimum temperature of 9^{o}C. Average intensity of incoming solar radiation during the daytime hours (0800 hr to 1600 hr IST) is about 609 Wm^{-2}. Details of weather at Hol farm of daily minimum and maximum temperature, relative humidity, and rain-fall for the two experimental years are shown in Table 3.1.

Table 3. 1 Weather data of Hol farm, Pune, India for two field experiments 2013-4 and 2015-6.

2013-4		Nov-13	Dec-13	Jan-14	Feb-14	Mar-14
Temperature (0C)	Max	30.28	28.99	29.56	32.65	34.90
	Min	15.21	11.13	12.55	12.1	16.56
Relative humidity (%)	Max	88.43	88.63	91.87	88.86	86.33
	Min	49.73	42.47	43.97	35.23	35.6
Rainfall (mm)		00.00	00.00	00.00	0.80	8.00

2015-6		Nov-13	Dec-13	Jan-14	Feb-14	Mar-14
Temperature (0C)	Max	31.52	31.2	30.22	33.9	36.43
	Min	17.31	14.03	11.03	13.62	17.36
Relative humidity (%)	Max	86.87	91.74	90.48	87.02	79.00
	Min	43.77	38.1	33.58	29.08	26.69
Rainfall (mm)		22.20	00.00	00.00	25.00	00.00

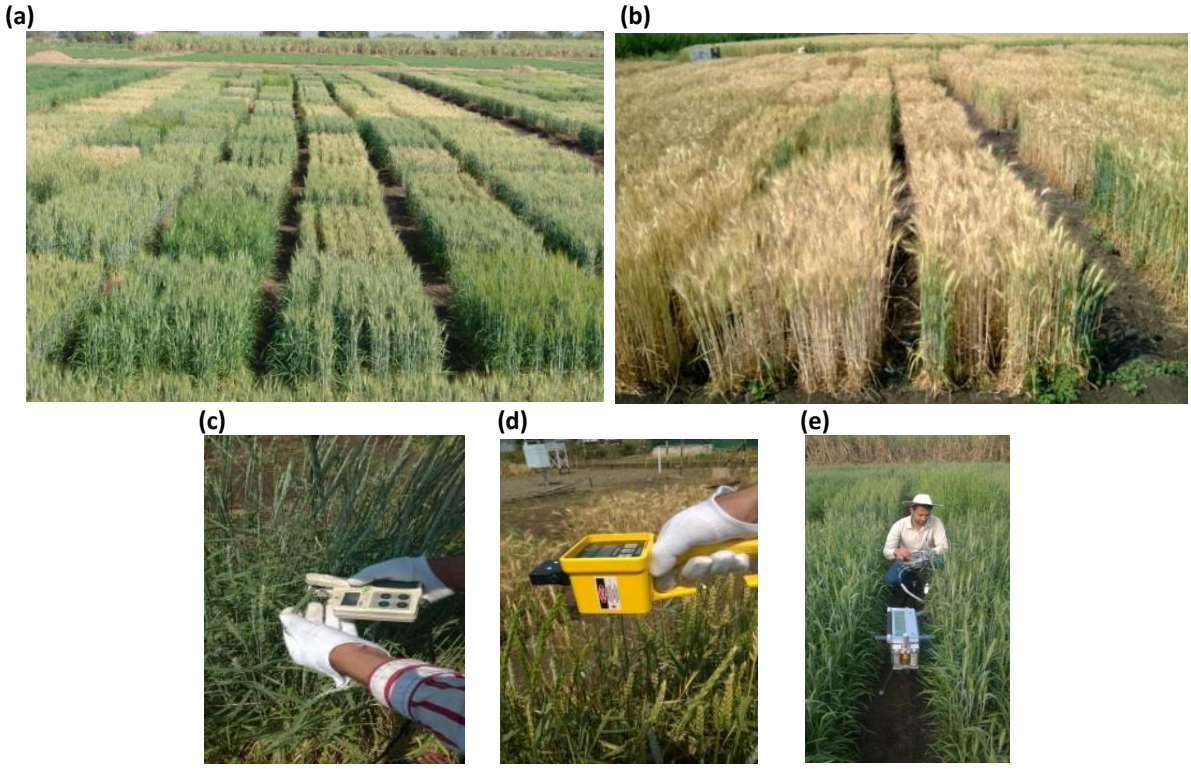


Figure 3. 1 The experimental site at Hol farm, Pune, India in 2015-6 (a) around anthesis (b) at maturity (c) Minolta SPAD-502 leaf chlorophyll meter (d) NDVI (e) LI-6400XT portable photosynthesis infrared gas analyzer.

In each year, soil samples were taken after land cultivation which included ploughing, disking, leveling and furrowing and before sowing. Soil samples were taken at depth of 0-90 cm in 2013-4 and 0-60 cm in 2015-6 due to a rocky soil below 60 cm depth which made soil coring difficult. Six soil cores were taken in a 'W' shape in each replication and soil from these samples was bulked and mixed thoroughly. Around 1 kg representative soil samples per

replication were sent from this bulk for analysis of macro (N, P and K) and micro (Fe, Mn, Zn and Cu) nutrients at Agriculture University, Rahuri, India in 2013-4 and Agriculture College Pune, India in 2015-6. Soil analysis was done to obtain information on ammonium N and nitrate N and total available N, as well as pH, electrical conductivity, $CaCO_3$ and organic C. The soil type was a Black Cotton soil or Black Vertisol with good drainage and water holding capacity in both years.

Table 3. 2 Experimental conditions and soil properties in 2013-4 and 2015-16

Soil properties	2013-4	2015-6
Sowing date	26 Nov 2013	2 Nov 2015
Fertilizer N2 under HN (kg ha-1)	120	120
Fertilizer N2 under LN (kg ha-1)	0	40
Fertilizer P2O5 (kg ha-1)	60	60
Fertilizer K2O (kg ha-1)	40	40
Soil sampling depth	0-90 cm	0-60 cm
Available N (kg ha-1)	196.9	141.4
Available P (kg ha-1)	25	15.7
Available K (kg ha-1)	578.1	229.9
NH4-N (kg ha-1)	107.4	102.1
NO3-N (kg ha-1)	69.1	39.4
PH (1: 2.5)	7.9	8.1
E.C. (ds m-1)	0.2	0.1
Calcium Carbonate (%)	13.5	8.1
Organic Carbon (%)	0.8	0.7
Fe (ppm)	5.5	-
Mn (ppm)	11.2	-
Zn (ppm)	3.9	-
Cu (ppm)	4.9	-
Crop rotation before 1yr	Soybean	Sorghum
Crop rotation before 2yr	Wheat	Wheat
Soil type	Black cotton/ Vertisol	Black cotton / Vertisol

3.1.1.2 Experimental design

The experiment was conducted in split-plot design with two replications in 2013-4 and three replications in 2015-6; the main plot treatment was two levels of nitrogen fertilizer application and the subplot treatment was 30 elite Indian spring wheat cultivars. Each genotype was sown in four rows 25 centimeters apart and with 2.5 m length (plot size 1.0 x 2.5 m) in 2013-4 whereas there was 6 rows and row width was 25 cm and row length was 4.0 m (plot size 1.5 x 4 m) in 2015-6. In each year, seeds were sown at 240 seeds m^{-2}. Buffer plots

were planted between the two N treatments to avoid inter plot interaction for the high and low N treatment.

3.1.1.3 Nitrogen treatments

Two levels of N treatment, optimum level of N or high N (HN) and low N (LN) were applied (Table 3.2). High N was given as per the recommendation of the Directorate of Wheat Research (DWR) for PZ wheat cultivars. The N rate was estimated according to previous knowledge of the Hol site taking into account soil N availability and yield potential using the recommendations by DWR (DWR, 2016). The HN rate in both years was 120 kg N ha^{-1} and was split into two doses, of 60 kg N ha^{-1} applied after final land preparation and before sowing and 60 kg N ha^{-1} at onset of stem extension approximately 20 days after sowing (GS31, Zadoks *et al.* (1974)). In the LN level, there was no application of N fertilizer in 2013-4 and 40 kg N ha^{-1} in 2015-6. All N fertilizer was applied as granules of urea (34.5% N) and each split was applied on the same calendar date for the 30 cultivars by hand.

3.1.1.4 Cultivars treatments

Thirty elite Indian spring wheat cultivars were selected, representative of six wheat growing zones of India. Mostly these cultivars were used as checks in the breeding programs in these breeding zones. All the cultivars were semi-dwarfs, except Kharchia-65 and BH-1146. There were two durum wheat (*Triticum turgidum ssp. durum*, 2 n= 4 x= 28, genomes AB) cultivars (PDW- 314 and HI- 8498); the remaining 28 cultivars were bread wheat cultivars. More information about pedigree, year of release, zone of adaptation and optimum growing conditions of these cultivars is given in Table 3.3.

Table 3. 3 The 30 Indian cultivars studied in field experiments in 2013-4 and 2015-6.

Cultivar	Pedigree	Date of release	Zone of adaptation	Production condition
BH- 1146	Eonta ponta grossa 1//Fretes/Martin	Stock (1987)		
CBW- 38	Cndo/r143//Ente/Mexi-2/3/Ae.squarrosa(taus)/4/Weaver/5/2*Pastor	2008	NEPZ	TS, IR
DBW- 14	Raj 3765/PBW 343	2002	NEPZ	LS, IR
DBW- 16	Raj 3765/WR 484//HUW 468	2006	NEPZ	TS, IR
DBW- 17	Cmh79a.95/3*Cno79//Raj 3777	2007	NWPZ	TS, IR
DBW- 39	Attila/Hui	2010	NEPZ	TS, IR
DBW- 46	PBW 343/Inq21	Stock (2011)		
DBW- 51	Site/Milan	2010	NEPZ	LS, IR
DBW- 71	Prinia/UP 2425	2013	NWPZ	LS, IR
DPW- 621-50	Kauz//Altar84/aOS/3/Milan/Kauz/4/Huites	2011	NWPZ	TS, IR
GW- 322	PBW 173/GW 196	2002	CZ PZ	TS, IR
HD- 2733	Attila/3/Tui/Carc//Chen/Chto/4/Atila	2001	NEPZ	TS, IR
HD- 2967	Ald/Coc//User/HD 2160m/HD22778	2011	NWPZ	TS, IR
HD-2932	Kauz/Star//HD 2643	2008	CZ	TS, IR
HI- 8498 †	CR 'S'-GS'S'//A-9-30-1/Raj 911	1999	CZ	TS, IR
HW- 2044	HD 226*5/Sunstar*6/C-80-1	1999	SHZ	TS, IR
K- 0307	K 8321/UP 2003	2007	NEPZ	TS, IR
KHARCHIA- 65	Kharchia Local/EG 953	1970	All zones	TS, IR
KRL- 19	PBW 255/KRL 1–4	2000	All zones	TS, IR
KRL- 210	PBW 65/2*Pastor	2010	NWPZ/NEPZ	TS, IR
KRL-1-4	Kharchia 65/WL 711	1990	All zones	TS, IR
KRL-213	Cndo/r143//Ente/Mexi-2/3Aegilopssquarrosa(taus)/4/Weaver/5/2*Kauz	2010	NWPZ	TS, IR
MACS- 6222	HD 2189*2//MACS 2496	2011	PZ	TS, IR
MACS-2496	CM 33027-F-15M-500Y-OM	1991	PZ	TS, IR
MACS-6478	CS/TH.SC//3*PVN/3/MIRLO/BUC/4/MILAN/5/TILHI	2014	PZ	TS, IR
NW-1067	TR 380-16-3-614/chat'S'	2004	UP	TS, IR
PDW- 314 †	Ajaia12/F3Local(SEL.Ethio.135.85)//Platai 13/3/Somat3/4/Sooty/Rascon37	2010	NWPZ	TS, IR
RAJ- 4229	HW 2048/Raj 4000	2012	NEPZ	TS, IR
RAJ- 4238	HW 2021/Raj 3765	2012	CZ	LS, IR
WH- 1021	Nyot 95/Sonak	2007	NWPZ	LS, IR

Northern Hills Zone (NHZ), North Western Plain Zone (NWPZ), North Eastern Plain Zone (NEPZ), Central Zone (CZ), Peninsular Zone (PZ), Southern Hills Zone (SHZ), Irrigated (IR), Timely sown (TS), Late sown (LS). Stock refers to advanced breeding line used in breeding program. (†) are durum wheat cultivars.

3.1.1.5 Crop management

In both years pesticides to control pests were applied to avoid limiting yield and there was no significant incidence of diseases. To control the pests dimethoate 30 % EC, 2.5ml/l (brand name: TAFGOR; company: Rallis India TATA Ltd.) was applied in each year. Weed control was done manually when necessary. Phosphate and potassium fertilizers were applied at levels to prevent non-N nutrients limiting yield (Table 3.2). P_2O_5 and K_2O fertilizer were given in the form of triple super phosphate (TSP) and muriate of potash (MOP), respectively. In 2014, four (on 26/11/13, 21/11/13, 18/1/14, 12/2/14) and in 2016 seven (on

2/11/15, 22/11/15, 9/12/15, 28/12/15, 16/1/16, 3/2/16) ground-based flood irrigations were given the amounts of which were adjusted according to visual inspection of the field for water stress using local experience of the site. In both years, first irrigation was applied immediately after the N fertilizer application and seed sowing as to wash fertilizer into the soil.

3.1.2 Crop measurements

3.1.2.1 Crop development

Regular monitoring of crop growth stages was done following the decimal code of Zadoks growth stages (Zadoks *et al.*, 1974). The growth stages were assigned when 50% of the plants (before onset of stem extension, GS31) or fertile shoots (after GS31) were at the specific growth stage. In both years anthesis date (AD) was recorded when 50% of the fertile shoots carried visible stamens (GS61). Dates of heading (GS55, 50% ear visible) and physiological maturity (GS89, 50% Yellow peduncle) were also recorded when 50% of the fertile shoots were at the stage. Depending of crop growth stage of the crop, data was recorded once or twice a week.

3.1.2.2 Sampling for growth analysis (DM and N partitioning)

2013-4: For dry mater and N partitioning analysis, five primary shoots at anthesis (GS65) and 10 primary shoots at maturity (GS93) were randomly selected and labelled in the field. Samples of each sub-plot were harvested by cutting shoots at ground level. While harvesting samples, care was taken to avoid 'edge' effects by taking samples from inner rows and avoiding the ends of the plot. These samples were then immediately transferred to laboratory to record fresh weight using a balance to three decimal places (g). Shoot samples harvested at both stages then were divided into four components: (i) ear, (ii) flag leaf-lamina, (iii) remaining leaf lamina with leaf sheath and (iv) true stem.

2015-6: In 2015-6 samples were taken by cutting shoots at ground level in a 25 cm row-length from four rows giving a sampled area of 1 m^2 per plot at anthesis (GS65) and at maturity (GS93). To avoid the 'edge' effects samples were taken from inner rows and avoiding the ends of the plot. A buffer gap of 25 cm was left between sequential sample areas. Harvested

samples were immediately put in plastic bags to avoid moisture loss and transferred to the laboratory to record fresh weight. From these samples, for final growth analysis, 15 primary shoots at anthesis and 12 primary shoots at maturity were randomly selected and the fresh weight was again recorded. These samples were then divided in to (i) ear, (ii) flag leaf-lamina, (iii) remaining leaf lamina and (iv) stem and leaf sheath.

In both years, all samples were analyzed within 24 hours of collection. All samples were oven dried at 80^{o} C for 48 h and the dry weight recorded.

3.1.2.3 Plant population and fertile and infertile shoot number

2013-4: Plant population density was calculated by counting the number of plants in a tagged one-meter row length between GS10 and GS15. There was no fertile and infertile shoot count at anthesis taken in this year. At maturity (GS93) from same tagged one-meter row, the number of fertile and infertile shoots was counted. A shoot was considered fertile if had a healthy ear with grains inside and infertile if there was no ear or ear with no grains. Ear number per m^2 at harvest was calculated by dividing total grain yield per unit area by grain weight per shoot measured from 10 primary shoots.

2015-6: Plant population was counted one-meter row length at GS10 to GS15. A shoot count of fertile and infertile was carried out at anthesis (GS61) and at maturity (GS93) in a quadrat sample (section 3.1.2.2). Ears per m^2 at harvest was calculated as described for 2013-4.

3.1.2.4 Crop grain yield, biomass and yield components

Grain yield was calculated from the bulk harvest of the sub-plot in both years. Before harvesting the sub-plot by hand in bulk, a one-meter row length was harvested separately in 2013-4 and the four 0.25 m row lengths in 2015-6 (as described above) for DM and N partitioning analysis. The ends of the plots (up to 0.25 m) were removed as discard. The rest of the plot (2 x 1 m in 2013-4 and 4 x 1.5 m 2015-6) was harvested as a bulk by cutting at ground level. Ears were threshed with use of a threshing machine for the bulk harvested plot.

Harvest index (grain DM / aboveground DM) was calculated from the randomly selected 10 fertile shoots harvested at physiological maturity in 2013-4 and from 12 shoots selected randomly from quadrat sample at harvest in 2015-6. Dried ears were threshed by hand and grain dry weight was measured. Chaff DM was calculated by subtracting the DM of grain from the DM of the ear. AGDM was calculated from the grain yield of the bulk harvested sample (without one-meter row in 2013-4 and two quadrat samples in 2015-6) and converted to t ha^{-1}. Grains from the harvested samples were counted manually by removing very small shriveled or broken grains and thousand grain weight calculated (TGW, g). From these measurements, above-ground DM per m^2, grains per ear and ears per m^2 were also calculated.

3.1.2.5 Plant height

In 2013-4 plant height was measured from ground level to the tip of ear using a ruler at five random positions per plot in the field at anthesis (GS 61) and in 2015-6 from three shoots randomly selected in laboratory from the quadrat sample harvested at anthesis.

3.1.2.6 Determination of crop N content

In both years, N content of the plant components were analyzed by the Dumas method. The dried samples were firstly ground into fine powder by using a mixer-grinder machine with mechanical modification of a *chattani* pot for small quantity samples. Milled samples were immediately put in an air-tight plastic bag to avoid moisture re-absorption. Between each sample, the pot was cleaned thoroughly to avoid cross contamination. For N analysis milled samples were re-dried at 80^oC for 48 h and weighed to 0.00001 g decimal precision before analysis.

Samples at anthesis were separated into: i) flag-leaf lamina ii) rest of the leaf lamina with stem and leaf sheath and iii) ears; and at physiological maturity separated into i) flag-leaf lamina ii) rest of the leaf lamina and stem with leaf sheath and chaff and iii) grains. These samples were milled to a particle size of <200 μm and encapsulated in tin aluminum capsules. The encapsulated sample was then analyzed for N% using the Dumas method using a Fisons

NA-2000 elemental analyzer calibrated against an atropine standard (N content = 4.8%). The Dumas N method functions in three steps: (i) converting all N forms in the sample into gaseous nitrogen oxides by complete combustion in pure oxygen, (ii) further reducing to N^2 (in a hot copper catalyst) and (iii) N^2 quantification by comparing the change in thermal conductivity with a reference cell (Simonne *et al.*, 1997).

The results are expressed as N% of the sample dry weight from which the total amount of N in the plant component was calculated by following equation:

$$\mathrm{N}\ (\mathrm{g\,m^{-2}}) = (\mathrm{N\%}/100) * \mathrm{DW}\ (\mathrm{g\,m^{-2}}) \qquad \text{Equation 3.1}$$

The **N harvest index** (NHI; the proportion of N in the above-ground plant which is in the grain at harvest) was calculated as:

$$\mathrm{NHI} = \text{grain N}\ (\mathrm{kg\,ha^{-1}}) \,/\, \mathrm{AGN_H}\ (\mathrm{kg\,ha^{-1}}) \qquad \text{Equation 3.2}$$

where AGN_H is the above-ground N at harvest.

3.1.2.7 Determination of N-use efficiency and components

N-use efficiency (NUE) was calculated as (Moll *et al.*, 1982):

$$\mathrm{NUE} = \text{Grain Yield DM}\ (kg\ ha^{-1}) \,/\, \text{available N}\ (kg\ ha^{-1}) \qquad \text{Equation 3.3}$$

Available N includes N from the N fertilizer and the soil N.

(i) N-uptake efficiency (NUpE) was calculated as:

$$\mathrm{NUpE} = \mathrm{AGN_H}\ (kg\ ha^{-1}) \,/\, \text{available N}\ (kg\ ha^{-1}) \qquad \text{Equation 3.4}$$

(ii) N-utilization efficiency (NUtE) was calculated as:

$$\mathrm{NUtE} = \text{Grain Yield DM}\ (kg\ ha^{-1}) \,/\, \mathrm{AGN_H}\ (kg\ ha^{-1}) \qquad \text{Equation 3.5}$$

3.1.2.8 Post-anthesis N-uptake

Post-anthesis N-uptake (PANU; kg N ha^{-1}) was calculated as the difference between AGN_H and AGN_A. A small amount (<10%) of this N is likely to be the result of remobilization from the roots (Dalling, 1985), which was not measured in the present study.

N Nutrition Index (NNI) was estimated according to the ratio of the actual above-ground crop N% at anthesis (GS61) and the critical N% estimated according to the 'critical dilution curve' described for wheat by Justes *et al.* (1994).

3.1.2.9 Post-anthesis N remobilization components

Comparison of the anthesis and harvest data sets allowed estimation of the amount and efficiency of N remobilized to the grain from the above-ground crop and also from the individual crop components during the post-anthesis period, as follows:

i. Post-anthesis N remobilization (NR; kg N ha^{-1}) is the amount of N in the crop or crop component at anthesis which is not recovered in the straw or straw component at harvest.

ii. Post-anthesis N remobilization efficiency (NRE; %) is the percentage of N in the crop or crop component at anthesis which is not present in the straw or straw component at harvest.

$$\mathrm{NRE} = (\mathrm{AGN_A} - (\mathrm{AGN_H} - \mathrm{GN}))/\mathrm{AGN_A} \qquad \text{Equation 3.6}$$

where AGN_A is above-ground N at anthesis, AGN_H is the above-ground N at harvest and GN is the grain N per unit area at harvest.

N partitioning index is the proportion of above-ground N in the crop component.

3.1.2.10 Fruiting efficiency, ear partitioning index and biomass production efficiency

The fruiting efficiency (grains per unit ear DM at anthesis, GS61) was calculated by dividing the grains per ear by the ear DM per fertile shoot at anthesis. The ear partitioning index was calculated as the ratio of ear DM to above-ground DM at anthesis and biomass

production efficiency (BPE; above-ground DM / above-ground N) was calculated by dividing above-ground dry matter by above-ground N at harvest.

3.1.2.11 Flag-leaf senescence parameters

Senescence kinetics were assessed visually every 7 days in each sub-plot by recording the percentage of flag-leaf area senesced after anthesis (GS61) of main shoots on five tagged plants in 2013-4 and for the whole flag-leaf layer in 2015-6 using a standard diagnostic key based on a scale of 0 to 10 (0 to 100% senesced, Fig. 3.2 a). The same senescence scoring key was used in each year with same operator. For each sub-plot, the regression analysis was done by fitting senescence score against thermal time (base temperature of 0°C) by using a modified version of an equation with five parameters consisting of a monomolecular and a logistic function (Eq. 3.7) (Génard *et al.*, 1999), as described by Gaju *et al.* (2011).

$$\text{Score} = \text{p0} + \text{p1x}(1 - \text{e}^{(-\text{p2xTTA/p1})} + \frac{10 - \text{p1} - \text{p0}}{1 + \text{e}^{(-4\text{xp3x}((\text{TTA/p4})/(10-\text{p1}-\text{p0})))}} \qquad \text{Equation 3.7}$$

where the senescence score of *p*0 is at GS61; *p*1 is the senescence score at the end of the first slow phase of senescence; *p*2 is of maximum rate of first phase; *p*3 is the maximum rate of second rapid phase; *p*4 is the time of when rate is maximum in second phase; TTA is the post-anthesis thermal time.

This function gives information about the flag-leaf senescence duration and rate through two phases of post-anthesis senescence, a slow phase (SP) and rapid phase (RP) (Fig. 3.2 b). The duration of each phase and the total duration of the senescence period were derived after the inflection point separating the two phases had been determined as the thermal time when the second derivative of the equation reached its maximum. The duration of the first phase of senescence, in which green area loss is slow, corresponds to the onset of senescence of the rapid phase (VS.OnsetRP; °C days); the duration of the second phase, in which most of the green area loss occurs, is the duration of the rapid senescence phase (VS.DurRP; °C days) and the inflection point of this phase is defined as the mid-point of the rapid phase (p4, VS.MidRP). In the present study, the onset of post-anthesis senescence is defined as the end of the rapid post-anthesis senescence (VS.OnsetRP or SEN_{ONSET}) and the rate of post-anthesis senescence is defined as the rate of the rapid phase of senescence

(SEN_{RATE}), i.e. *p*3 in Eq. (3.7). The end of post-anthesis senescence is taken as when senescence of the flag leaf reaches 90% flag-leaf senesced area (VS.EndRP). The senescence parameters were estimated in each sub-plot and then subjected to ANOVA.

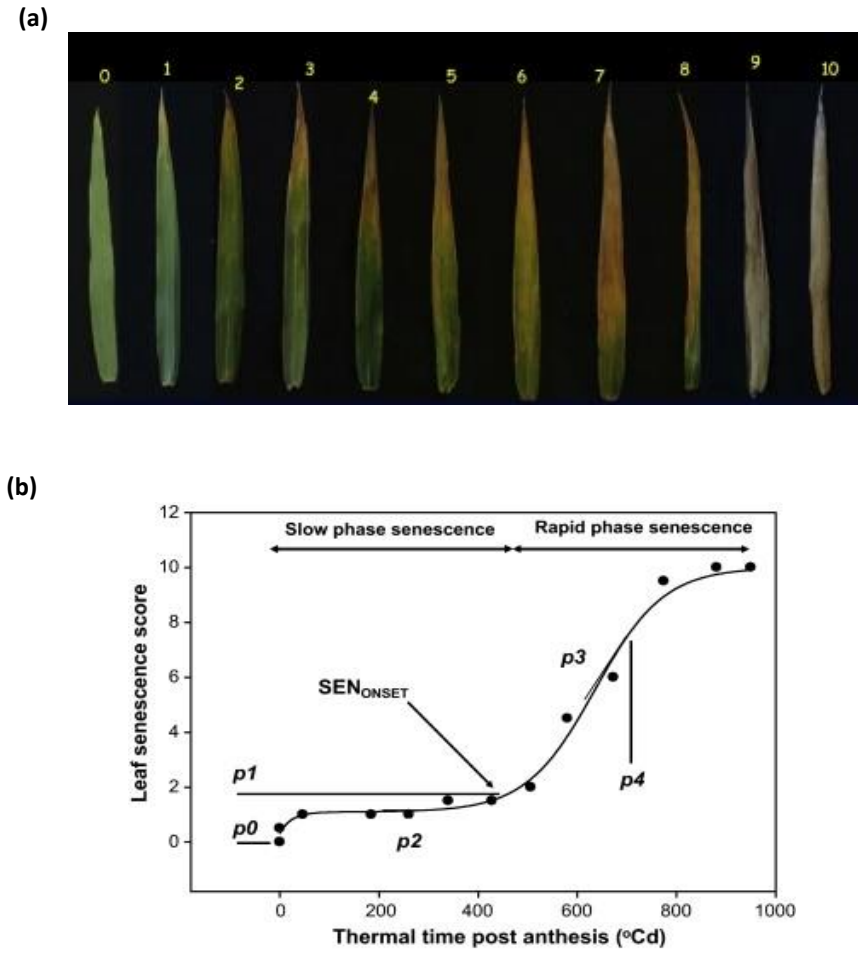


Figure 3. 2 (a) Flag-leaf senescence scoring key from 0 to 100% senescence and (b) diagram shows the visual-senescence kinetics for the monomolecular and a logistic function of Génard *et al.* (1999). Curve was fitted using senescence score against the thermal time (base temp. 0°C) and demonstrates the slow and rapid phase of post-anthesis senescence. Scoring for senescence was from zero (leaves fully green) to 10 (leaves fully senesced). Visually observed scores were fitted against accumulated thermal time after anthesis (GS61). Parameters calculated are: *p*0, senescence score at anthesis; *p*1, senescence score at the end of the slow phase; *p*2, maximal rate of senescence during the slow phase; *p*3 maximum rate of senescence during the rapid phase; and *p*4, thermal time when *p*3 is reached (VS.MidRP). The onset of the rapid senescence phase (VS.OnsetRP or SEN_{onset}) is taken as the end of the slow phase, and the rate of the senescence phase (SEN_{RATE}) is taken as *p*3.

3.1.2.12 Flag-leaf chlorophyll content

The relative chlorophyll content of the flag-leaf was measured from anthesis (GS61, Fig. 3.1 c) to flag-leaf 100% senesced using a hand-held SPAD meter (SPAD 502, Minolta, Japan) as an indicator of the relative chlorophyll content (Miller *et al.*, 1999; Lopez-Bellido *et al.*, 2004). In 2013-4 five fertile shoots were tagged whereas in 2015-6 three fertile shoots per sub-plot were randomly selected. The measurements were made every 7 days for each flag leaf at different positions (basal, middle and apical). Modified Gompertz curves (Eq 3.8) were fitted to the flag-leaf SPAD values against thermal time (t) (base temp. 0^{o}C after anthesis, GS61; Fig. 3.3). The Gompertz time (T) parameter was fitted as the thermal time for the flag leaf to senesce to 37% flag-leaf SPAD value at GS61. The thermal time (t) when SPAD values were 90% and 10% of the value were taken as the onset of SPAD-senescence (SS.Onset) and end of SPAD-senescence (SS.End), respectively, whereas the duration from 90% flag-leaf SPAD to 37% flag-leaf SPAD remaining was considered as the SPAD-senescence duration (SS.Dur).

$$y = K * \exp\left\{-\exp\left(\frac{(t - T) * 2}{D}\right)\right\} \qquad \text{Equation 3.8}$$

where *t* is thermal time (base temp. 0°C), *D* is duration of senescence (SS.Dur), *T* is inflection point at 37% SPAD value remaining in flag leaf from initial point at GS61, *K* is the y-axis value which shows maximum SPAD at GS61. The senescence parameters were estimated for each sub-plot and then subjected to ANOVA.

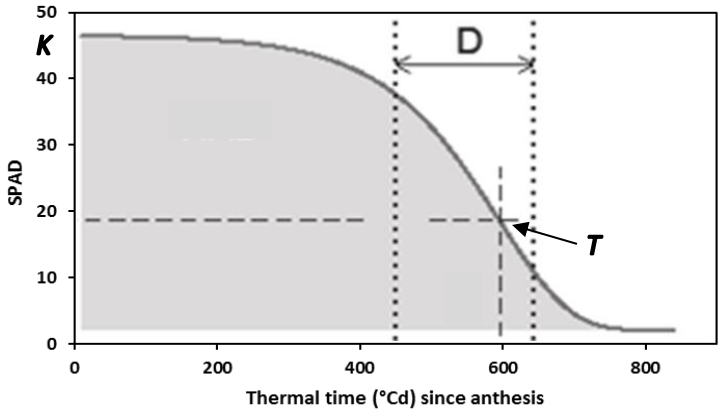


Figure 3. 3 Diagram shows the SPAD-senescence kinetics for the modified Gompertz curve fit (Gooding *et al.*, 2000) which fitted using SPAD-senescence against the post-anthesis thermal time (°Cd, base temp. 0°C).

3.1.2.13 Normalised difference vegetation index (NDVI)

The NDVI was measured in each experiment in each sub-plot approximately every 7 days from booting (GS41) until late grain-filling using a FieldScout CM 1000 NDVI Chlorophyll Meter (Spectrum Technologies, USA) (Fig. 3.1 d). The measurements were taken between 10.00 and 15.00 on clear sunny days. The NDVI chlorophyll meter sensor was held 50 cm above the crop. NDVI was calculated from measurements of light reflectance in the red and near infra-red (NIR) regions of the spectrum as:

$$\mathrm{NDVI} = (\mathrm{R}_{900} - \mathrm{R}_{680})/(\mathrm{R}_{900} + \mathrm{R}_{680}) \qquad \text{Equation 3.9}$$

where R_{680} and R_{900} are the reflectance at 680 and 900 nm, respectively. Similar to the SPAD analysis, modified Gompertz curves (Eq 3.8) were fitted to NDVI values over thermal time (t) post-anthesis (GS61) for each replication in each treatment combination. NDVI average values from 0 to 400 °Cd (NDVI pre-tt400) and after 400 °Cd (NDVI post-tt400) post-anthesis (GS61) were used as indicators of stay-green.

3.1.2.14 Canopy temperature

Canopy temperature was recorded in each experiment on a per sub-plot basis using a hand-held infrared thermometer (sixth Sense LT300 Infrared Thermometer) on bright sunny days between 1100 and 1400 hours, at seven days intervals, starting from anthesis (GS61) until the late milk stage (GS79).

3.1.2.15 Leaf photosynthesis (A_{max}), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and maximum efficiency of PSII (Fv'/Fm') in HN treatment

Post-anthesis flag-leaf photosynthesis measurements on two assessment dates (07/01/2016 and 23/01/206) were taken only in 2015-6 under HN conditions. Light-saturated photosynthetic rate (A_{max}), stomatal conductance (g_s), intercellular carbon dioxide concentration (C_i) and maximum efficiency of PSII (Fv'/Fm') of the flag-leaf were measured using a Li-Cor LI-6400XT Portable Photosynthesis System (Licoln, NE, USA) (Fig. 3.1 e). In each sub-plot, single reading on a single flag-leaf of two randomly selected fertile primary shoot were taken. The instrument was calibrated for humidity at 50–60% relative humidity and the

settings for the A_{max} readings were: photosynthetically active radiation (PAR) 1500 µ mol $m^{-2}s^{-1}$, sample chamber CO_2 concentration 360 µl l^{-1} and flow rate 400 µ mol s^{-1}. The measurements were taken between 10.00 and 15.00 around 1-2 weeks after anthesis. Chlorophyll fluorescence of the flag-leaf was also measured at the same time of A_{max} measurements. This gives the information about the efficiency of excitation capture by open PSII reaction centre (Fv'/Fm') which is a quantitative indicator of reductions in the photon yield of O_2 evolution from intact leaves when exposure to high light levels under favorable temperatures and water status results in photoinhibition (Murchie and Lawson, 2013).

3.1.2.16 Problems with plant establishment and lodging

Overall there was no serious problem of plant establishment and lodging in both years. Regarding lodging, two tall cultivars in the experiment (Kharchia-65 and BH-1146) showed lodging at maturity but crop yield was not affected significantly. Also because of manual harvesting in both years, care was taken during harvesting these two cultivars to collect all lodged shoots.

3.1.3 Environmental measurements

In each year, daily meteorological data were manually collected from a weather station for rainfall, humidity, minimum and maximum air temperature from a meteorological station located 100 m from the experimental site. Average intensity of incoming solar radiation during the daytime hours (0800 hr to 1600 hr IST) during wheat growing season (November to March) was about 17.54 J m^{-2} s^{-1}. Solare radiation data was obtained from National Institute of Abiotic Stress Management, Baramati, Indian which is 20 km away from experimental site. Details of weather at Hol farm of daily minimum and maximum temperature, relative humidity, and rain-fall for two experimental years are shown in Table 3.1.

3.1.4 Statistical analysis

Data were entered in to excel spreadsheets (Microsoft Corporation 2013). Statistical analysis was done using Genstat 17th edition (Lawes Agricultural Trust, Rothamsted

Experimental Station; 2006). Analysis of variance (ANOVA) procedures for a split-plot design were used to analyze N treatments and cultivar effects and test their interaction in individual years where replicates were regarded as random effects and cultivar as fixed effects. A cross-year ANOVA was applied to analyze N treatments and cultivar effects across year and the interaction with year, assuming N treatments and cultivars were fixed effects and replicates and year were random effects.

ANOVA was used to test the significance of differences between years (Y), N treatments (N), genotypes (G) and their interactions using the standard error of the difference of the means. A probability values of less than or equal to 0.05 was considered as significant, although values between 0.05 and 0.10 may be included in comments. In chapter 4 ANOVA was done to see the effect of breeding zone. In this chapter ANOVA for split-plot design was use in same way as described above, only the treatment structure was changed to Year*N T treatment*(Breeding Zone/Genotype).

Associations between crop variables were calculated by linear regression analysis or simple correlation analysis using the mean values across replicates. Pearson's correlation coefficient and linear regressions were calculated using Genstat 17th edition.

Principal component analysis (PCA) was used to examine associations between the cross-year mean of different traits. PCA uses a multi-dimensional dataset to explain the most variation among the variables (Jolliffe, 2002). The PCA were done in R (https://www.r-project.org/) using the package FactoMineR (Lê *et al.*, 2008) (http://factominer.free.fr/).

Broad-sense heritability (H^2) of 30 cultivars of a given trait across years (2013-4 and 2015-6) was calculated as described in equation 3.10 (Cooper and DeLacy, 1994) using META-R, an R-program for multi-environment analysis (Lopez-Cruz *et al.*, 2015):

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{e} + \frac{\sigma_e^2}{re}} \qquad \text{Equation 3.10}$$

where σ_g^2 and σ_e^2 are the genotypic and error variance. σ_{ge}^2 is the genotype x environment interaction. The number of environments and number of replications are represented by *e* and *r*, respectively.

3.2 UK GLASSHOUSE EXPERIMENTS EXAMING AMPHIDIPLOID LINES

This section explains the methodology used in two glasshouse experiments conducted in 2014 and 2015 at Sutton Bonington, University of Nottingham, UK campus (52° 5 ′ N, 1° 3′ W).

3.2.1 Experimental conditions

3.2.1.1 Experimental design

For the two glasshouse experiments, the seed sowing dates were 23 June 2014 and 6 July 2015. The experimental procedures and measurements were the same in both years (Fig. 3.4 a). Seeds were sown in plastic modules filled with F2 + S compost (Levington Advance Seed & Modular F2S). After seed germination (~6 days after sowing) seedling of 2 to 4 cm length were transfer to a cold room for vernalization. Seeds were put for vernalization for 6 weeks at 6 °C temperature with a 12 hour of photoperiod and then transplanted into 2L pots in the glasshouse. Twenty amphidiploid lines along with five bread wheat parental cultivars were grown in the experiment (Table 3.6). Among bread wheat parent cultivars, Paragon (PAV) and Highbury (HB) were the UK parent spring check cultivars whereas Pavon was a CIMMYT and Chinese Spring (CS) or CS Euploid was a Chinese spring wheat parental cultivar. CS mutant was having mutant *ph1* locus which facilitates gene transfer between the chromosomes of wheat and its wild relatives. The experimental design use was a split-plot where two levels of N treatment (high N and low N) were randomized on the main-treatment and 25 genotypes were randomized on the sub-plot treatment. A single seedling of each line was transplanted into each pot and represented one replication. For each genotype, there was four replications. Pot size was 2 L with 16.8 cm diameter and a F block low N peat-compost (Klasmann Medium Peat 818) was used as the soil medium and supplemented with nutrients. Details of the supplemented nutrients are give in Table 3.3.

3.2.1.2 Genotype treatment

Twenty amphidiploid lines (Table 3.6) derived from crosses between ancestral wheat wild grass accessions and bread wheat cultivars along with five bread wheat parents were planted.

Table 3. 4 Amphidiploid lines with bread wheat parent and wild parent used to produce amphidiploid lines

Bread wheat parent 2n = 6x =42 (AABBDD)	Wild grass parent	Grass species genome	Abbreviations
CS Eup 94	Aegilops mutica 2130004	2n = 2x = 14 (TT)	Ae. mut4 x CSE
CS Eup 94	Aegilops mutica 2130008	2n = 2x = 14 (TT)	Ae. mut8 x CSE
Paragon	Aegilops mutica 2130012	2n = 2x = 14 (TT)	Ae. mut12 x Par
Highbury	Aegilops mutica 2130012	2n = 2x = 14 (TT)	Ae. mut12 x HB
CS Eup 94	Aegilops mutica 2130012	2n = 2x = 14 (TT)	Ae. mut12 x CSE
CS Eup 94	Aegilops speltoides 2140008	2n = 2x = 14 (SS)	Ae. spe8 x CSE
Pavon 76	Aegilops speltoides 2140008	2n = 2x = 14 (SS)	Ae. spe8 x Pav
Pavon 76	Aegilops speltoides 449340	2n = 2x = 14 (SS)	Ae. spelt40 x Pav
Pavon 76	Aegilops umbellulata 542377	2n = 2x = 14 (UU)	Ae. umb77 x Pav
CS Eup 94	Aegilops umbellulata 554410	2n = 2x = 14 (UU)	*Ae. umb*10/0 x CSE
CS Eup 94	Aegilops umbellulata 554410	2n = 2x = 14 (UU)	*Ae. umb*10/3 x CSE
Paragon	Aegilops comosa var. comosa 276970	2n = 2x = 14 (MM)	Ae. com70 x Par
CS Eup 94	Thynopyrum turcicum P208/201	2n=10x=70 (EE)	*Th. tur*201 x CSE
Highbury	Secale anatolicum P208/142	2n = 2x = 14 (RR)	Se. ana142 x HB
CS Eup 94	Secale anatolicum P208/142	2n = 2x = 14 (RR)	*Se. ana*142 x CSE
CS Eup 94	Secale anatolicum P208/141	2n = 2x = 14 (RR)	*Se. ana*141 x CSE
CS Eup 94	Secale anatolicum P208/142	2n = 2x = 14 (RR)	*Se. ana*142 x CSE
CS Eup 94	Secale anatolicum P208/142	2n = 2x = 14 (RR)	NA
CS Mut P208/535	Thinopyrum bessarabicum 531712	2n = 2x = 14 (JJ)	NA
Chinese Spring	Thinopyrum bessarabicum Abers.	2n = 2x = 14 (JJ)	Th. bes x CS
CS Euploid 94	Parent	2n = 6x =42 (AABBDD)	CSE
Paragon	Parent	2n = 6x =42 (AABBDD)	PAR
Highbury	Parent	2n = 6x =42 (AABBDD)	HB
Pavon 76	Parent	2n = 6x =42 (AABBDD)	PAV
CS Mutant P208/535	Parent	2n = 6x =42 (AABBDD)	CSM

3.2.1.3 Glasshouse environmental conditions

Plants were grown in a glasshouse with a light cycle of 16 h at 20 °C and dark cycle of 8 h at 15 °C. Plants were irrigated with a complete nutrient solution (minus N) regularly with an automatic irrigation system to maintain plants free from water stress. The composition of complete nutrient solution is described in Table 3.3. Daily minimum and maximum air temperature was measured using a tiny-tag temperature data logger (Tinytag Ultra 2 - TGU-4500, Gemini data loggers). On average, during the wheat growing season in the glasshouse from June to December, PAR was 194 µ mol $m^{-2}s^{-1}$ between 10.00 to 13.00 and 153 µ mol $m^{-2}s^{-1}$ between 13.00 to 16.00. Overall ambient solar radiation during experiment was 11501 KJ m^{-2} between June to December in 2014 and 11743 KJ m^{-2} between July to December 2015, respectively. During cloudy whether extra light was provided by zinc lamps.

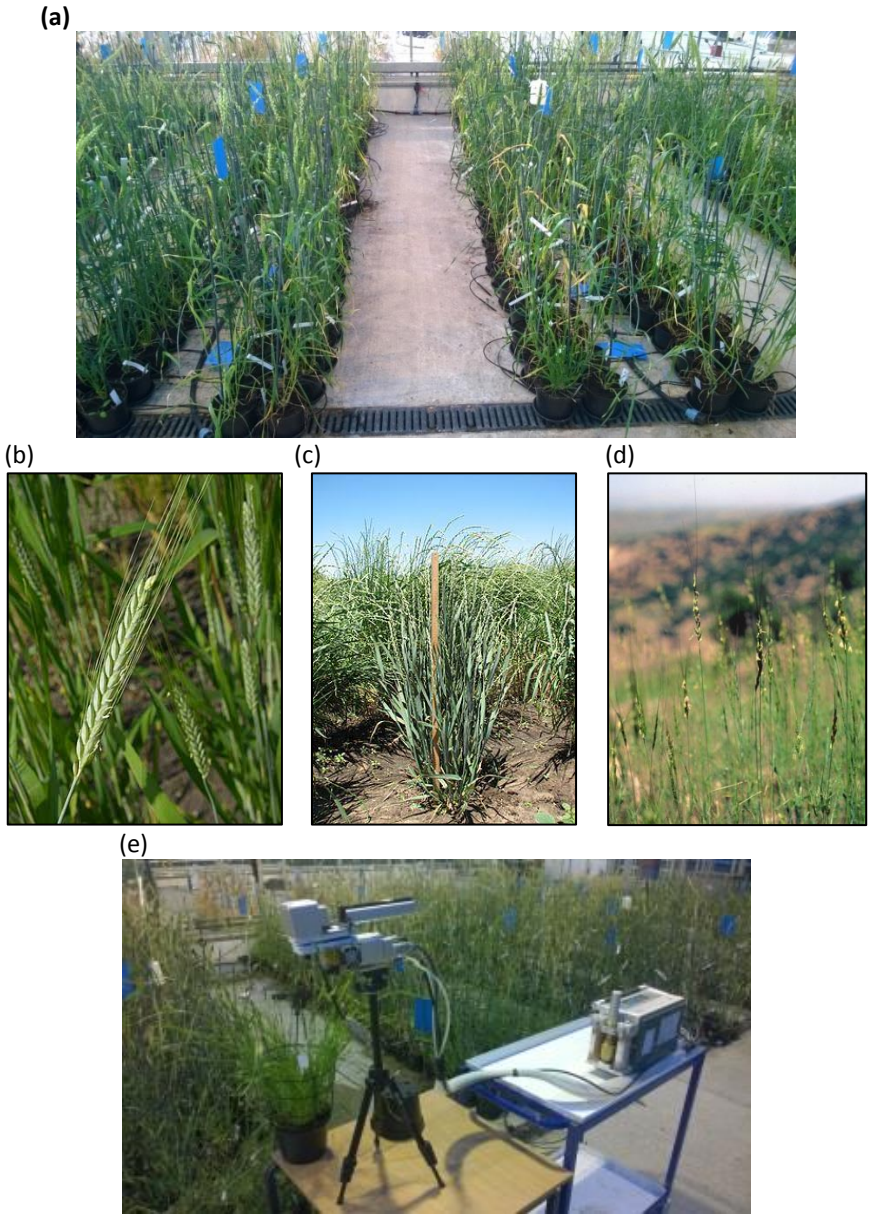


Figure 3. 4 (a) Glasshouse experiment of amphidiploids 2015-6 (b) *Triticum urartu* (c) *Thinopyrum bessarabicum* (d) *Aegilops speltoides* (e) Photosynthesis measurements in glasshouse.

Table 3. 5 Composition of low N peat-compost and nutrient solution used in glasshouse experiment in 2014 and 2015.

Peat-composts F blocks low N (winter)	Amount	Units
Peat: Sand	100:0	% volume
Single super phosphate	1.5	Kg m^{-3}
Potassium sulphate	0.75	Kg m^{-3}
Ground lime chalk	2.25	Kg m^{-3}
Ground magnesian limestone	2.25	Kg m^{-3}
Fritted trace elements WN225	0.4	Kg m^{-3}
P_2O_5	125	g m^{-3} = mg $litre^{-1}$
K_2O	300	g m^{-3} = mg $litre^{-1}$
Nutrient sol. NPK:0,36,36 (for watering)	100	g per 2L diluted for a stock solution 1 FEED/100 water (1 g/L)

3.2.1.4 Nitrogen treatment

Nitrogen was applied manually by dissolving ammonium nitrate (NH_4NO_3) in water (34% N). Two levels of N were applied, low N 60 kg ha^{-1} equivalent and high N 200 kg ha^{-1} equivalent. For low N, N application was split into two doses each of 30 kg N ha^{-1} and for high N into three doses of 50 kg N ha^{-1}, 50 kg ha^{-1} and 100 kg ha^{-1}. Total actual amount of N fertilizer applied was 0.25 g and 1.27 g of NH_4NO_3 per pot under LN and HN conditions, respectively. The first application was applied immediately after transplanting of seedlings in pots and the second at GS31 for both treatments. The last application for the high N treatment was at flag-leaf emergence (GS39).

3.2.2 Plant measurements

3.2.2.1 Plant development

Regular monitoring of plant growth stages was done by following the decimal code of Zadoks growth stages (Zadoks *et al.*, 1974). During the plant growing period, the growth stages were assigned when the main shoots were at specific growth stage. In both years, anthesis date (AD) was recorded when the ears on the main shoot carried visible stamens (GS61). Date of heading (GS55) was taken when half of ear was visible on the main shoot and physiological maturity (GS89) when peduncle of main shoot was 100% senesced. Depending on growth stage of the plant, data were recorded once or twice a week. Plant height to the tip of the ear was also measured on the main shoot at harvest.

3.2.2.2 Leaf photosynthesis (A_{max}), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and maximum efficiency of PSII (Fv'/Fm') in HN and LN conditions.

Gas-exchange photosynthesis measurements were taken on the flag-leaf of each plant on the main shoot using a Li-Cor 6400 XT Portable Photosynthesis System with chlorophyll fluorescence attachment (Li-Cor Biosciences, NE, USA) under HN and LN conditions. Light-saturated photosynthetic rate (A_{max}), stomatal conductance (g_s), intercellular carbon dioxide concentration (C_i) and maximum efficiency of PSII (Fv'/Fm') of the flag leaf were measured. Measurements were taken twice a week in between 10.00 and 15.00 h from flag-leaf

emergence (GS37) to mid-grain filling (GS85). Li-cor settings were: flow rate 400 µmol s^{-1} and block temperature 20°C with ambient relative humidity. The sample (cuvette) CO_2 concentration was set to 400 µmol mol^{-1} and PAR was 1500 µmol m^{-2} s^{-1} (10% blue). All parameters were analyzed by taking average values per plant during each of the pre-anthesis and post-anthesis periods.

3.2.2.3 Flag-leaf senescence measurements

Senescence kinetics were assessed visually every 7 days in each plant by recording the percentage of green area senesced of the flag-leaf after anthesis (GS61) of main stems in both years using a standard diagnostic key based on a scale of 0 to 10 (0 to 100% senesced). All other methodology used and senescence parameter calculations were as described in section 3.1.2.12 of this chapter to measured VS.OnsetRP, VS.Dur, VSMidRP, and VS.EndRP.

3.2.2.4 Harvesting and sampling

Harvesting was done at physiological maturity by cutting the whole plant at ground level from each pot separately. Harvested plants were weighed for fresh weight and divided into the main shoot, remaining fertile shoots (those with ear) and infertile shoots (those without an ear), for growth analysis. Plant components from each fertile shoot category were divided into: i) ear, ii) leaf lamina and iii) stem and leaf sheath and each component weighed after oven drying at 80°C for 48 h. After drying at 80°C for 48 h, thousand grain weight (TGW) and N% of: i) grain ii) leaf lamina and iii) stem and leaf sheath of main shoot and the rest of the shoots was carried out as described for the field experiments. The dry weight of the infertile shoots was weighed after oven drying at 80°C for 48 h.

Grain yield and yield components and NUE and NUE components were calculated as described in section 3.1 but for glasshouse data were calculated on a per plant basis. Flag-leaf chlorophyll content measurement was done using the SPAD meter as described in section 3.1.2.13. Anthesis date, physiological maturity date and plant height were measured on the main shoot of each plant.

3.2.3 Statistical analysis

ANOVA for a split-plot experimental design, correlation, and regression analysis of data was carried out as described in section 3.1.4 using GenStat 17th Edition. PCA carried out using R program as described in 3.1.4. Analysis of variance was carried out for a split-plot design for individual years if there was a significant effect for year otherwise cross-year ANOVA are presented. Analysis of variance (ANOVA) procedures for a split-plot design were used to analyze N treatments and cultivar effects and test their interaction in individual years assuming replicates were random effects and genotypes were fixed effects. A cross-year ANOVA was applied to analyze N treatment and genotype effects across year and the interaction with year, assuming N treatments and genotypes were fixed effects and replicates and year were random effects. ANOVA was used to test the significance of differences between years (Y), N treatments (N), genotypes (G) and their interactions using the standard error of the difference of the means. A probability value of less than or equal to 0.05 was considered as significant, although values between 0.05 and 0.10 may be included in comments. Skewed data was transformed and probability values were used from transformed data for significance.

3.3 2D SEEDLING ROOT PHENOTYPING EXPERIMENT

3.3.1 Plant material

The genotypes used in this experiment were selected based on their contrasting performance for grain yield (GY) and N uptake in the two field experiments (2013 and 2015) and two glasshouse experiments (2014 and 2015) under LN conditions (Table 3.6). Out of the 12 genotypes, four were amphidiploids and eight were bread wheat cultivars. One of the bread wheat parental cultivars (Chinese Spring Euploid) used in this experiment was the parent of four selected amphidiploid lines whereas the second was a UK spring wheat parental cultivar (Paragon).

Table 3. 6 List of genotypes studied for 2D seedling RSA under HN and LN conditions. (HY=high yielding, LY=low yielding under LN conditions in field or in glasshouse experiment).

No.	Genotypes	Rationale for selection (HY/LY = high/low yield)	Seed size (mm)
1	GW- 322	HY, Field expt.	3.35
2	HD-2932	HY, Field expt.	2.8
3	DBW- 39	HY, Field expt.	3.35
4	DBW- 14	LY, Field expt.	3.35
5	KHARCHIA- 65	LY, Field expt.	2.8
6	HW- 2044	LY, Field expt.	2.8
7	*Th. turcicum* P208/201 x CS Eup 94	HY, Glasshouse expt.	2.8
8	*Se. anatolicum* P208/141 x CS Eup 94	HY, Glasshouse expt.	2.8
9	*Ae. umbellulata* 554410 x CS Eup 94	LY, Glasshouse expt.	2
10	*Se. anatolicum* P208/142 x CS Eup 94	LY, Glasshouse expt.	2
11	CS Euploid 94	Parent, Glasshouse expt.	2.8
12	Paragon	Parent, Glasshouse expt.	2.8

3.3.2 Root system architecture phenotyping

Seeds were sieved through a set of calibrated graduated sieves (Scientific Laboratory Supplies Ltd, Hessle, UK). All seeds used in the experiment were taken from the fraction collected between 2 mm and 3.35 mm mesh sizes (Table 3.6). This size was selected as it represented the average seed size of all the genotypes. Seeds were surface sterilized by rinsing in 70% (v/v) ethanol for 30 s, followed by transfer to 5% (v/v) sodium hypochlorite solution for 10 min, and finally washed three times with sterile water. Sterilized seeds were placed onto moistened germination paper crease-side down and incubated at 4°C for 5 days

to synchronize germination on 22 March 2017. Following cold treatment, seeds were transferred to a light-impermeable box for 48 h to complete germination. The box was placed inside the controlled environment room where phenotyping was conducted (12 h photoperiod: 20°C day, 15°C night, with a light intensity of 400 µmol m^{-2} s^{-1} PAR). Uniformly germinated seeds with roots ~5 mm in length were transferred to individual growth pouches on 27 March 2017. The growth pouch system was based on the design developed for maize (Hund *et al.*, 2009). Each pouch consisted of a sheet of germination paper (24 × 30 cm; Anchor Paper Company, St Paul, MN, USA), covered with a black polythene film of equal area (75 µm thick; Cransford Polythene Ltd, Suffolk, UK). The germination paper and film were fixed to an acrylic rod (316 × 15 × 5 mm; Acrylic Online, Hull, UK) using two 18 mm fold back clips. A matrix barcode label affixed to the rod allowed identification of each seedling. A single seedling was placed in each pouch centred 2 cm from the top edge and held in place by the adhesion of the polythene sheet to the wet germination paper. Growth pouches were fitted into four aluminium and polypropylene frame assemblies (Fig. 3.5 A) in the controlled environment chamber. Each assembly consisted of an aluminum profile frame (104 × 62 × 102 cm; KJN Ltd, Leicester, UK) supporting toothed acrylic holders to suspend each pouch in a set position. Black polypropylene side panels (101 × 31 × 0.3 cm and 63 × 31 × 0.3 cm; Cut Plastic Sheeting, Devon, UK) maintained the pouches in darkness.

The base of each frame held a black polypropylene tray (99 × 61 × 10 cm; Stansa Plastic Fabrication Ltd, Suffolk, UK) containing 18 L modified one-quarter Hoagland's solution (Hoagland and Arnon, 1950) with HEDTA as the iron chelator (Piñeros *et al.*, 2005). The composition (mg L^{-1}) of the nutrient solution for HN and LN was as follows:

HN: $(NH_4)_3PO_4$, 29 mg; $Ca(NO_3)_2$, 165 mg; $MgSO_4$, 251.87 mg; KNO_3, 151.99 mg; H_3BO_3, 28.44 mg; Cu_2SO_4, 75 mg; $MnCl_2(H_2O)_4$, 10.1 mg; MoO_3, 0.2 mg; $ZnSO_4$, 2.29 mg; FeHEDTA, 25.49 mg.

LN: $(NH_4)_3PO_4$, 29 mg; $CaSO_4{\cdot}½H_2O$, 101.15 mg; KCL, 112.09 mg); H_3BO_3, 28.44 mg; Cu_2SO_4, 75 mg; $MnCl_2(H_2O)_4$, 10.1 mg; MoO_3, 0.2 mg; $ZnSO_4$, 2.29 mg; FeHEDTA, 25.49 mg.

The solutions were adjusted to pH 6 using KOH. The volume of nutrient solution in each tray was maintained automatically via a float valve system and header tank containing deionized water. Each frame assembly consisted of three rows of 30 pouches allowing 90

plants per frame. Pouches were suspended so that the bottom 3 cm of the pouch was submerged in the nutrient solution.

A split-plot design was used with two levels of N (HN and LN) as the main treatment and 12 genotypes randomized as the sub-plot treatment. There were 15 replicates. Phenotyping of all genotypes was done in one run of the experiment. Nine days after emergence (leaf 2 stage), individual pouches were transferred to a copy stand (model number SGCS-920; Speed Graphic, Hampshire, UK) for imaging using a Nikon D600 DSLR camera (Fig. 3.3.1B) controlled using NKRemote software (Breeze Systems Ltd, Camberley, UK). The copy stand was modified with two draw slides (RS UK, Northants, UK), a Nylatron block (600 × 260 × 22 mm), and white acrylic sheets (330 × 290 × 9 mm and 290 × 290 × 9 mm; Cut Plastic Sheeting, Devon, UK) to form a template to ensure consistent placement of each pouch. The polythene film covering each pouch was carefully peeled back leaving the roots fixed to the blotting paper for imaging. The draw slides then enabled the template block to be repositioned allowing shoots to be imaged without moving the pouch. Images were taken of all 15 replicate seedlings of the 12 genotypes in two N treatments, resulting in 360 RSA images.

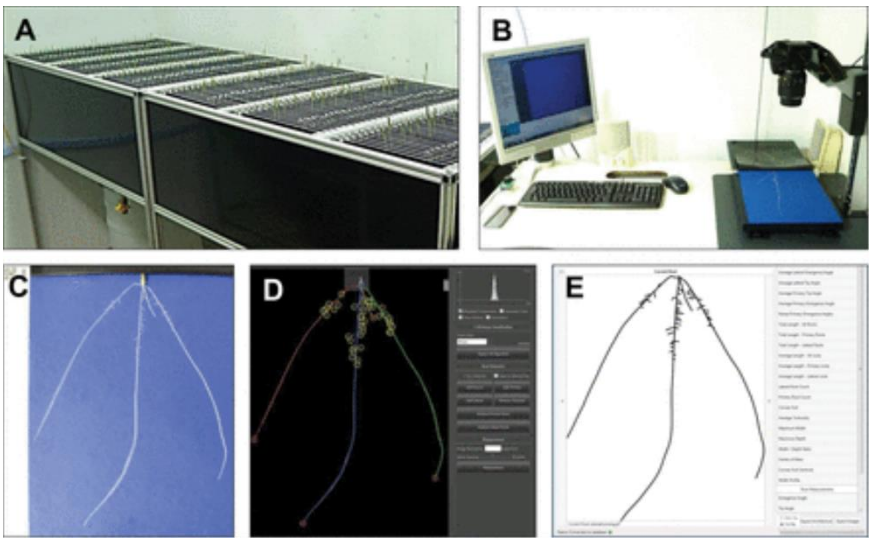


Figure 3. 5 Root phenotyping pipeline. (A) Growth assembly. (B) Image acquisition. (C) Example root image. (D) Root system extraction and quantification using RootNav software. (E) Reconstruction of root system in silico and trait measurement (Atkinson *et al.*, 2015).

3.3.3 Image analysis

Root system images were processed using RootNav software (Pound *et al.*, 2013). Traits were quantified from extracted RSA using RootNav's standard functions (Table 3.7) as detailed by Pound *et al.* (2013). For this study, RootNav functionality was extended to measure additional traits describing the distribution of the root system (the co-ordinates of the centre of mass of the root system and the coordinates of the centroid of the convex hull), determined as follows.

Centroid of convex hull: The convex hull of the root system is calculated using the standard definition, that of the smallest convex set of X points containing the entire root system. This results in a set of points $(x_1, y_1), \ldots, (x_n, y_n)$, the centroid (geometric centre) of which is calculated as:

$$C_x = \frac{1}{6A}\sum_{i=0}^{n}(x_i + x_{i+1})(x_i y_{i+1} - x_{i+1} y_i) \qquad \text{Equation 3.11}$$

$$C_y = \frac{1}{6A}\sum_{i=0}^{n}(y_i + y_{i+1})(x_i y_{i+1} - x_{i+1} y_i) \qquad \text{Equation 3.12}$$

where A is the signed area of the convex hull,

$$A = \frac{1}{2}\sum_{i=0}^{n}(x_i y_{i+1} - x_{i+1} y_i) \qquad \text{Equation 3.13}$$

It should be noted that the point (x_{n+1}, y_{n+1}) is equal to (x_1, y_1) in the above formulae.

As root angle varies with position along the root and the angle between pairs of seminal roots has been shown to be significant (Christopher *et al.*, 2013), additional angle measurements were calculated at a fixed point 15 mm along each root, and at 50, 75, and 100% of root length (Fig. 3.5 C). The RSAT quantified by the RootNav functions are listed in Table 3.7.

Table 3. 7 Root system architecture traits measured in seedling platform.

Acronym	Description (units)
TotLegAllRo	Total length of all roots (mm)
TotLegSemRo	Total length of seminal roots (mm)
TotLegLatRo	Total length of lateral roots (mm)
	Number of seminal roots
	Number of lateral roots
	Maximum width of the root system (mm)
	Maximum depth of the root system (mm)
W/D ratio	Width-depth ratio (RMW/RMD)
	Angle of emergence between the outermost seminal roots measured at the first quartile of the total length (degrees)
	Angle of emergence between the second pair of seminal roots measured at the first quartile of the total length (degrees)
	Angle of emergence between the outermost seminal roots measured at the second quartile of the total length (degrees)
	Angle of emergence between the second pair of seminal roots measured at the first quartile of the total length (degrees)
	Angle of emergence between the outermost seminal roots measured at the third quartile of the total length (degrees)
	Angle of emergence between the second pair of seminal roots measured at the third quartile of the total length (degrees)
	Angle of emergence between the outermost seminal roots measured at the root tip (degrees)
	Angle of emergence between the second pair of seminal roots measured at root tip (degrees)

3.3.4 Statistical analysis

ANOVA for split-plot experimental design, correlation, and regression of data was carried out using Genstat version 17th as described in section 3.1.4. PCA analysis was carried out using R program as described in section 3.1.4. ANOVA was carried out for a split-plot design, where two N treatments were the main treatment level and genotypes were the split-plot treatment level. Additional unbalanced ANOVA to see amphidiploid (AMP) and cultivars (CV) group effect were carried out using GenStat version 17th.

CHAPTER 4

Field Experiment, Harvest Results

4.1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most widely grown cereal crop in the world (for global human consumption 2nd after rice) including India. In 2014, world wheat production was approximately 729 M t from 220 M ha land (FAO, 2016). In India, it was 96 M t from 30.5 M ha of land (FAO, 2016). India stood 2nd in world wheat production after China (FAO, 2016). In the world, wheat accounts for 20% of the calories consumed by the global population (FAO, 2016) and in India it contributes 50% of the calories consumed, contributing substantially to the national food security (DWR, 2016). The world population is predicted to reach 9 billion by 2050 and global demand for food to increase at an annual rate of ca. 1.6% yr^{-1} (Godfray *et al.*, 2010; Hall and Richards, 2013). The relative rate of global yield increase in wheat is declining and is currently about 1.1% yr^{-1} (Hall and Richards, 2013). Furthermore, in some countries and regions, a yield plateau or a decrease in rate of genetic gains in grain yield potential is reported in the last decades, e.g. in spring wheat in Mexico (Fischer and Edmeades, 2010), Chile (Matus *et al.*, 2012) and Spain (Acreche *et al.*, 2008). Although there is scope for raising harvest index (HI) in the short to medium term from current values of ca. 0.50–0.55 in winter wheat and ca. 0.45–0.50 in spring wheat (Foulkes *et al.*, 2011) towards the theoretical maximum of ca. 0.64 (Austin *et al.*, 1982; Foulkes *et al.*, 2011), future increases in grain yield will increasingly depend on raising above-ground biomass production. There is some evidence that above-ground biomass has started to increase in recent decades in wheat breeding programs worldwide (Lopes *et al.*, 2012; Aisawi *et al.*, 2015) but there is a need to accelerate the rate of genetic gains.

Increasing biomass of the wheat crop implies an additional requirement for N capture to support photosynthesis. Up to 75% of the reduced N in cereal leaves is located in the chloroplasts of mesophyll cells, mainly as Rubisco, and is involved in photosynthetic processes (Evans, 1989). Increased N fertilizer inputs, however, will have economic implications as well as environmental impacts. Nitrogen (N) fertilizer represents a significant cost for the grower and also has environmental impacts through N leaching causing eutrophication of freshwater

and marine ecosystems and N_2O (a greenhouse gas) emission associated with denitrification by soil bacteria. India is the second largest consumer of nitrogen fertilizers in the world after China (FAI, 2010). Production of N fertilizer for the year 2015-16 was a record high (13.476 MMT) in India and wheat after rice is most N consuming crop affecting the Indian agriculture economy significantly (FAI, 2016). Therefore, developing new cultivars that have a higher yield and more efficient in N uptake and utilize to produce grain will be of economic benefit to farmers in the world and will help to reduce environmental impacts associated with fertilizers N application (Foulkes *et al.*, 2009; Hawkesford, 2014).

Several field studies have been carried out on historic sets of wheat cultivars to quantify genetic gains in NUE under contrasting N inputs between 1997 to 2016 as summarized below. Under low N supply, genetic gains in NUE were mainly related to improvement in NUpE in Mexico (Ortiz-Monasterio *et al.*, 1997), in France (Le Gouis *et al.*, 2000), in Finland (Muurinen *et al.*, 2006), in Spain (Cossani *et al.*, 2012) and in Australia (Sadras and Lawson, 2013), but to NUtE in France (Brancourt-Hulmel *et al.*, 2003) and in the UK (Foulkes *et al.*, 1998a; Barraclough *et al.*, 2010; Gaju *et al.*, 2011). Under high N supply, NUpE and NUtE explained genetic gains in NUE approximately equally in Mexico (Ortiz-Monasterio *et al.*, 1997), the UK (Foulkes *et al.*, 1998a; Gaju *et al.*, 2016) and Finland (Muurinen *et al.*, 2006). From these studies, it is apparent that effects of N supply and genotype on yield often interact. It also shows that, at low N supply, the genetic variation in NUE was more usually associated with NUpE, whereas at high N supply it was usually associated with NUtE. There are relatively few studies on Indian wheat cultivars where NUE and its components traits were studied in detail. In recent experiments conducted on Indian wheat cultivars to estimate optimum N application for higher grain quality with maximum N efficiency it was reported to be 120 kg ha^{-1} (Kaur *et al.*, 2016). Sathisha and Desai (2016) compared 84 released cultivars, local germplasm of India and CIMMYT advanced breeding lines from different nurseries (SATYN, CISA-HTEM, WYCYT and SAWSN) for yield and NUE related traits under two N conditions (T1-100 kg ha^{-1} and T0- 0 kg ha^{-1}) and found that N-use efficient traits like grain N, straw N, N-uptake efficiency were higher under the treatment T1 than under T0.

Traditionally wheat breeding is carried out under non-limiting N supply, and plant breeders select cultivars that perform well under optimum N supply conditions. From the above studies, since genotype and N supply do interact, cultivars selected for high yield under

high N conditions may not be the highest yielding under lower N conditions. Therefore, to obtain reliable information about which genotypes will yield well under low N supply may require both breeding and testing at low levels of N input (Brancourt-Hulmel *et al.*, 2005) along with improved understanding of the physiological traits that determine higher yield.

These experiments aimed to quantify genetic variation in 30 Indian hexaploid wheat cultivars for grain yield, above-ground biomass and NUE traits under HN and LN conditions and responses to N availability by screening elite cultivars representing six breeding or climatic zones of India. The overall objective was to quantify genetic variation in elite Indian cultivars in N-uptake and N-utilization efficiency and in grain yield response to N limitation in modern Indian cultivars.

Therefore, the objectives addressed in this chapter are to:

(i) quantify genetic variation in grain yield, yield components, above-ground DM, NUE, NUpE, and NUtE and their responses to N in 30 modern Indian wheat cultivars.

(ii) understand the effects of anthesis date and plant height on harvest traits underlying the genetic variation N-use efficiency in the Indian wheat cultivars and associated traits.

(iii) understand the physiological difference between the cultivars and their responses to N on the basis of their origin of climatic or breeding zone.

4.2. MATERIAL AND METHODS

In this section, briefly, materials and methods used in the field experiments are described investigating the 30 cultivars panel (Table 3.3) in 2013-4 and 2015-6.

4.2.1 Experimental conditions

4.2.1.1 Experimental site

The field experiments were conducted at Agharkar Research Institute, Pune, India at Hol farm (LA:18.1171893, LO:74.31072280000001) located 60 km from Pune in the wheat climatic Peninsular Zone (PZ) (latitude; 16^{o} 30' to 22^{o} 03' M, longitude: 73 ^{o} 47' to 74 ^{o} 40' N, altitude: 557.5) in 2013-4 and 2015-6 (Fig. 3.1). The previous crop was soybean in 2013-4 and sorghum in 2015-6 (Table 3.1). This site has a temperate climate with mean annual rain fall of 457 mm and mean annual maximum temperature of 36^{o}C and a minimum temperature of 9^{o}C. The average intensity of incoming solar radiation during the daytime hours (0800 hr to 1600 hr IST) during wheat growing season (November to March) is about 17.53 MJ m^{-2}. Details of weather at Hol farm of daily minimum and maximum temperature, relative humidity, and rainfall for two experimental years are shown in Table 4.1.

In each year, soil samples were taken after land cultivation which included ploughing, disking, leveling and furrowing and before sowing. Soil samples were taken at depth of 0-90 cm in 2013-4 and 0-60 cm in 2015-6 due to a rocky soil below 60 cm depth which made soil coring difficult. Six soil cores were taken in a 'W' shape in each replication and soil from these samples was bulked and mixed thoroughly. Around 1 kg representative soil samples per replication were sent for analysis of macro (N, P and K) and micro (Fe, Mn, Zn and Cu) nutrients at Agriculture University, Rahuri, India in 2013-4 and Agriculture college Pune, India in 2015-6. The soil type was a Black Cotton soil or Black Vertisol with good drainage and water holding capacity.

4.2.1.2 Experimental design

The experiment was conducted in a split-plot design with two replications in 2013-4 and three replications in 2015-6; the main plot treatment was two levels of nitrogen fertilizer

application and the subplot treatment was 30 elite Indian spring bread wheat cultivars. Plot size was 1.0 x 2.5 m in 2013-4 and 1.5 x 4 m in 2015-6.

4.2.1.3 Nitrogen treatments

Two levels of N treatment, optimum level of N or high N (HN) and low N (LN) were applied (Table 3.2). The HN rate in both years was 120 kg N ha^{-1}. In the LN level, there was no application of N fertilizer in 2013-4 and 40 kg N ha^{-1} in 2015-6.

4.2.1.4 Cultivar treatments

Thirty elite Indian spring wheat cultivars were selected, representative of six wheat growing zones of India. Mostly these cultivars were used as controls in the breeding programs in these zones. All the cultivars were semi-dwarfs, except Kharchia-65 and BH-1146. There were two durum wheat cultivars (PDW- 314 and HI- 8498); the remaining 28 cultivars were bread wheats. More information about pedigree, year of release, the zone of adaptation and optimum growing conditions of these cultivars is given in Table 3.3.

4.2.1.5 Crop management

In both years pesticides to control pest were applied to avoid limiting yield and there was no significant incidence of diseases. Phosphate and potassium fertilizers were applied at levels to prevent non-N nutrients limiting yield and plots were irrigated with ground-based flood irrigations as described in chapter 3.

Table 4. 1 Weather data of Hol farm, Pune, India for two field experiments 2013-4 and 2015-6.

2013-4		Nov-13	Dec-13	Jan-14	Feb-14	Mar-14
Temperature (^{0}C)	Max	30.28	28.99	29.56	32.65	34.90
	Min	15.21	11.13	12.55	12.1	16.56
Relative humidity (%)	Max	88.43	88.63	91.87	88.86	86.33
	Min	49.73	42.47	43.97	35.23	35.6
Rainfall (mm)		00.00	00.00	00.00	0.80	8.00
2015-6		Nov-13	Dec-13	Jan-14	Feb-14	Mar-14
Temperature (^{0}C)	Max	31.52	31.2	30.22	33.9	36.43
	Min	17.31	14.03	11.03	13.62	17.36
Relative humidity (%)	Max	86.87	91.74	90.48	87.02	79.00
	Min	43.77	38.1	33.58	29.08	26.69
Rainfall (mm)		22.20	00.00	00.00	25.00	00.00

4.2.2 Crop measurements

4.2.2.1 Crop development

Regular monitoring of crop growth stages was done following the decimal code of Zadoks growth stages (Zadoks *et al.*, 1974). The growth stages were assigned when 50% of the plants (before the onset of stem extension, GS31) or fertile shoots (after GS31) were at the specific growth stage. In both years anthesis date (AD) was recorded when 50% of the fertile shoots carried visible stamens (GS61). Dates of heading (GS55, 50% ear visible) and physiological maturity (GS89, 50% yellow peduncle) were also recorded when 50% of the fertile shoots were at the stage. Depending on crop growth stage, data was recorded once or twice a week.

4.2.2.2 Growth analysis (DM and N partitioning)

Growth analysis for dry matter and N partitioning analysis was carried out as desctibed in Chapter 3. Shoot samples at anthesis and harvested were divided into four components: (i) ear, (ii) flag leaf-lamina, (iii) remaining leaf lamina with leaf sheath and (iv) true stem. In both years, all samples were analyzed within 24 hours of collection. All samples were oven drying at 80^{o} C for 48 h and the dry weight recorded.

4.2.2.3 Plant population and fertile and infertile shoot number

Plant population density between GS10 to GS15 and the number of fertile (those with an ear) and infertile shoots at anthesis and at maturity was measured as described in section 3.1.2.3.

4.2.2.4 Crop grain yield, biomass and yield components

Grain yield was calculated from the bulk harvest of the sub-plot in both years as described in Chapter 3 (section 3.1.2.4). Harvest index (grain DM / aboveground DM) and TGW was calculated from measurements on the randomly selected 10 fertile shoots harvested at physiological maturity in 2013-4 and from 12 shoots selected randomly from

quadrat sample harvest in 2015-6 as described in Chapter 3 (section 3.1.2.4). From these measurements, grains per ear and ears per m^2 were also calculated.

4.2.2.5 Plant height

In 2013-4, plant height was measured from the ground level to the tip of the ear using a ruler at five random positions per plot in the field at anthesis (GS 61) and in 2015-6 from three shoots randomly selected in the laboratory from the quadrat sample harvested at anthesis.

4.2.2.6 Determination of crop N content, N use efficiency and NUE components

In both years, N content of the plant components was analyzed by the Dumas method. N content was measured for i) flag-leaf lamina ii) rest of the leaf lamina with stem and leaf sheath and iii) ears at anthesis; and for i) flag-leaf lamina ii) rest of the leaf lamina and stem with leaf sheath and chaff and iii) grains at harvest. NUE, NUE components, NHI, N partitioning indices and post-anthesis N remobilization and post-anthesis N remobilization efficiency were measured as described in Chapter 3 (section 3.1.2.6/7/8/9). N Nutrition Index (NNI) was estimated according to the ratio of the actual above-ground crop N% at anthesis (GS61) and the critical N% estimated according to the 'critical dilution curve' described for wheat by (Justes *et al.*, 1994). Biomass production efficiency (BPE; above-ground DM / above-ground N) was calculated by dividing above-ground dry matter by above-ground N. Fruiting efficiency and ear partitioning index were calculated as described in Chapter 3 (section 3.1.2.10).

4.2.3 Environmental measurements

In each year, daily meteorological data were manually collected for rainfall, humidity, minimum and maximum air temperature from a meteorological station located 100 m from the experimental site. Average intensity of incoming solar radiation during the daytime hours (0800 hr to 1600 hr IST) during wheat growing season (November to March) was about 17.54 J m^{-2} s^{-1}. Details of weather at Hol farm including daily minimum and maximum temperature, relative humidity, and rainfall for two experimental years are shown in Table 3.1.

4.2.4 Statistical analysis

Data were entered into excel spreadsheets (Microsoft Corporation 2013). Statistical analysis was done using Genstat 17th edition (Lawes Agricultural Trust, Rothamsted Experimental Station; 2006). Analysis of variance (ANOVA) procedures for a split-plot design was used to analyze N treatments and cultivar effects and test their interaction in individual years where replicates were regarded as random effects and cultivar as fixed effects. A cross-year ANOVA was applied to analyze N treatments and cultivar and N as effects across years and the interaction with the year, assuming N treatments and cultivars were fixed effects and replicates and years were random effects.

ANOVA was used to test the significance of differences between years (Y), N treatments (N), genotypes (G) and their interactions using the standard error of the difference of the means. A probability values of less than or equal to 0.05 was considered as significant, although values between 0.05 and 0.10 may be included in comments. An ANOVA was also done to test the effect of the breeding zone. The split-plot design was used in the same way as described above, only the treatment structure was changed to Year*N treatment*(Breeding Zone/Genotype). Associations between crop variables were calculated by linear regression analysis or simple Pearson's coefficient correlation analysis using the mean values across replicates.

Principal component analysis (PCA) was used to examine associations between the cross-year mean of different traits. PCA uses a multidimensional data set to explain the most variation among the variables (Jolliffe, 2002). The PCA was done in R (https://www.r-project.org/) using the package FactoMineR (Lê *et al.*, 2008) (http://factominer.free.fr/).

Broad-sense heritability (H^2) of 30 cultivars of a given trait across years (2013-4 and 2015-6) was calculated as in equation 4.3 (Cooper and DeLacy, 1994) using META-R, an R-program for multi-environment analysis (Lopez-Cruz *et al.*, 2015):

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{e} + \frac{\sigma_e^2}{re}} \qquad \text{Equation 4.3}$$

where σ_g^2 and σ_e^2 are the genotypic and error variance. σ_{ge}^2 is the genotype x environment interaction. The numbers of environments and numbers of replications are represented by *e* and *r*, respectively.

4.3 RESULTS

4.3.1. Plant height, anthesis date and physiological maturity date

In 2014, overall plant height (PH) increased from 79.0 cm under LN to 84.1 cm under HN conditions (P = 0.023; Table 4.4). PH ranged amongst cultivars from 74.8 to 116.8 cm under HN and 68.17 to 105.80 cm under LN conditions (P<0.001). There was no N x G interaction. In 2016, there was a weak trend for PH to increase from 57.8 cm under LN to 60.1 cm under HN conditions (P=0.093; Table 4.4). PH ranged amongst cultivars from 41.5 to 101.7 cm under HN and 42.7 to 97.5 cm under LN conditions (P<0.001). There was no significant N x G interaction. Averaging across years and cultivars, PH increased from 68.4 cm under LN to 72.1 cm under HN conditions (P=0.006; Table 4.4). Cultivars ranged from 58.2 (KRL- 19) to 109.2 cm (KHARCHIA- 65) under HN and 58.7 (KRL- 19) to 98.4 cm (KHARCHIA- 65) under LN conditions (P<0.001). There was no N x G or Y x N x G interaction.

In 2014, days to anthesis (AD) advanced from 64 DAS under HN to 61 DAS under LN conditions (P=0.015; Table 4.4). AD ranged from 55 to 69 under HN and 55 to 68 DAS under LN conditions (P<0.001). There was an N x G interaction (P=0.005) with advancement in AD under N limitation ranging from -2 to 6 DAS. In 2016, there was no effect of N treatment on AD (Table 4.4). AD ranged amongst cultivars from 48 to 74 DAS under HN and 48 to 75 DAS under LN conditions (P<0.001). There was no N x G interaction. Averaging across years, there was no significant effect of N treatment on AD (62 DAS under LN and 63 DAS under HN) (Table 4.4). AD ranged from 53 to 71 DAS under both N treatments (P<0.001). There was no N x G interaction but there was a Y x N x G interaction (P=0.021).

In 2014, there was no significant effect of N treatment on the physiological maturity date (PMD) (Table 4.4). Overall PMD increased from 93 DAS under LN to 98 DAS under HN conditions (P=0.054). There was no significant N x G interaction. In 2016, there was also no significant effect of N treatment on PMD (Table 4.4). PMD ranged from 94 to 116 DAS under HN and 93 to 112 DAS under LN treatment (P<0.001). There was no N x G interaction. Averaging across years, PMD was delayed from 98 DAS under LN to 103 DAS under HN conditions (P = 0.023; Table 4.4). PMD ranged from 93 (DBW- 14) to 111 (DBW- 17) DAS under HN and 89 (DBW- 14) to 108 (HD- 2733) DAS under LN conditions (P<0.001). There was no N x G interaction but there was a Y x N x G interaction (P=0.015).

4.3.2 Grain yield

In 2014, grain yield (GY) was reduced by 30 % under LN (3.54 t ha^{-1}) compared to HN (5.04 t ha^{-1}) conditions (P=0.11; Table 4.5; Fig. 4.1. a). Genotypes ranged from 3.60 (KHARCHIA- 65) to 5.98 t ha^{-1} (PDW- 314, durum wheat) under HN and 2.38 (K- 0307) to 4.84 t ha^{-1} (PDW- 314, durum wheat) under LN conditions (P<0.001). The N x G interaction was not significant. In 2016, GY was reduced by 26% under LN (3.92 t ha^{-1}) compared to HN (5.35 t ha^{-1}) conditions (P= 0.02; Table 4.5; Fig. 4.1. b). Cultivars ranged from 3.00 (DBW- 14) to 7.25 (MACS-6478) t ha^{-1} under HN and 2.76 (DBW- 14) to 5.29 (DBW- 46) t ha^{-1} under LN (P<0.001). The N x G interaction was significant (P= 0.018) with reduction under LN ranging amongst cultivars from -4.42% (DBW- 46) to 44.31% (KRL- 210). Averaging across years, GY was reduced by 28% under LN (3.73 t ha^{-1}) compared to HN (5.19 t ha^{-1}) conditions (P=0.003; Table 4.5; Fig. 4.1. c). Cultivars ranged from 3.72 (DBW- 14) to 6.48 (MACS-6478) t ha^{-1} under HN and 2.95 (BH- 1146) to 4.51 (GW- 322) t ha^{-1} under LN conditions (P<0.001). The N x G interaction was significant (P=0.046) with reduction under LN ranging from 14 (DBW- 14) to 41.8% (MACS- 6222). There was no Y x N x G interaction.

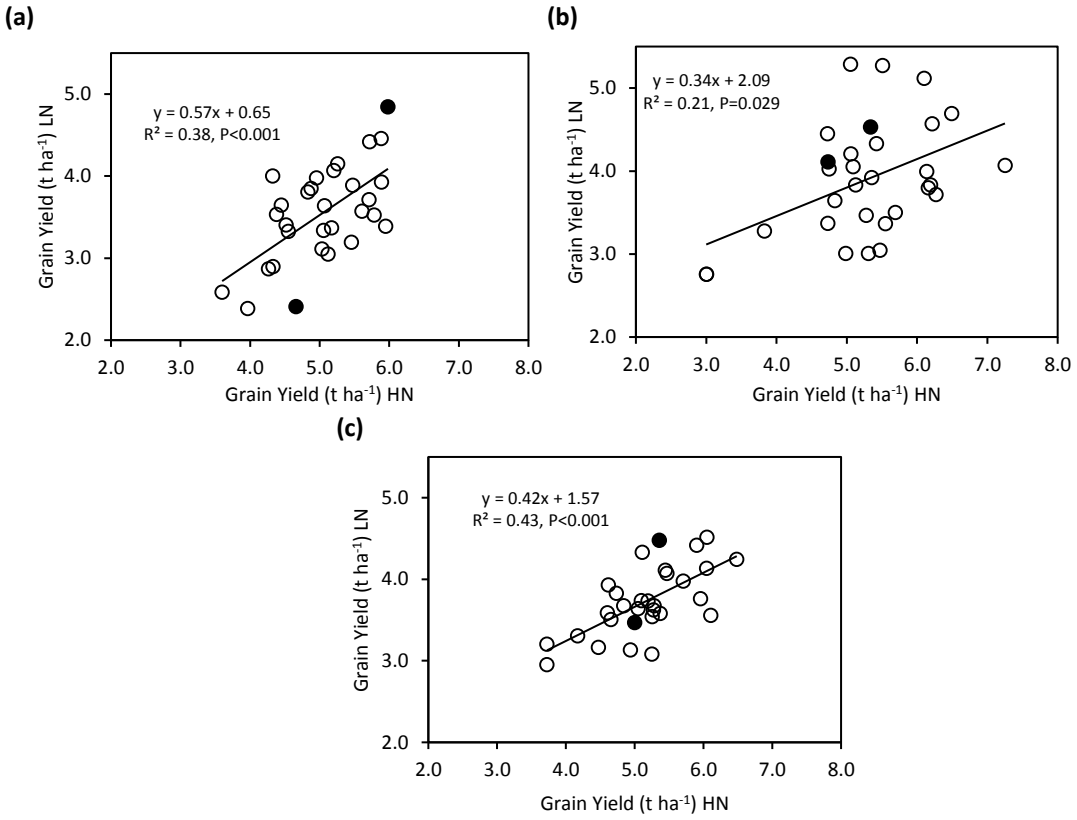


Figure 4. 1 Linear regression of grain yield (100% DM) in High N (HN) vs. Low N (LN) treatment for 30 wheat cultivars for (a) 2014 (b) 2016 and (c) mean 2014 and 2016. (●) durum wheat cultivars.

In 2014, there was no association between AD and GY under HN or LN amongst the cultivars (Fig. 4.2. a). However, there was a trend for a positive association amongst cultivars between PMD and GY under HN but not under LN conditions (Fig. 4.2. b). In 2016, there were positive associations between anthesis date and GY under HN (R^2= 0.14, P=0.043) and under LN (R^2= 0.41, P<0.001, Fig. 4.2. c.) conditions. Similarly, there are positive associations between PMD and GY under HN (R^2= 0.20, P=0.014) and under LN (R^2= 0.46, P<0.001) conditions (Fig. 4.2. d). Across years there was a significant association between AD and GY under HN (R^2= 0.18, P=0.02) and under LN (R^2= 0.30, P=0.002) conditions (Fig. 4.2. e.), and also between PMD and GY under HN (R^2= 0.22, P=0.009) and under LN (R^2=0.25, P<0.004) conditions (Fig. 4.2. f).

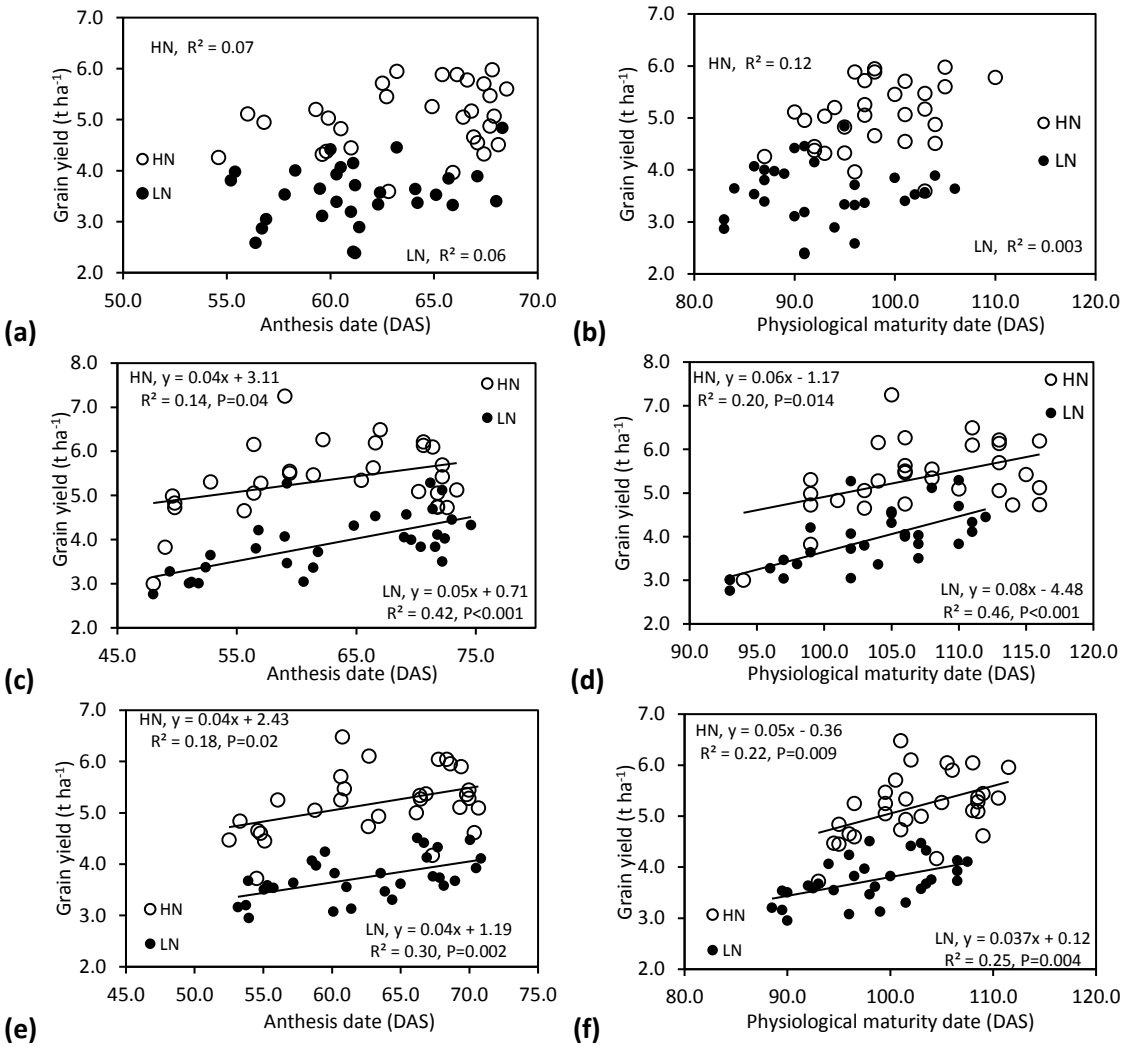


Figure 4. 2 Linear regression of anthesis date (DAS) and physiological maturity date (DAS) on grain yield (t ha^{-1}; 100% DM) for 30 cv. in year (a and b) 2014, (c and d) 2016 and (e and f) across years 2014-16.

4.3.3 Above-ground dry matter and Harvest Index

In 2014, AGDM was reduced by 27% under LN compared to HN conditions (P=0.11; Table 4.5; Fig. 4.3. a). Cultivars ranged from 8.10 (KHARCHIA- 65) to 11.60 (HD-2932) t ha^{-1} under HN and from 4.98 (HI- 8498, durum wheat) to 10.10 (WH- 1021) t ha^{-1} under LN conditions (P<0.001). The N x G interaction was not significant. In 2016, AGDM was reduced by 26% under LN compared to HN conditions (P=0.002; Table 4.5; Fig. 4.3 b) and cultivars ranged from 6.01 (DBW- 14) to 14.40 (DBW- 51) t ha^{-1} under HN and from 5.17 (DBW- 14) to 12.24 (DBW- 46) t ha^{-1} under LN conditions (P<0.001). Cultivar responses to N limitation ranged from -4.27 to 41.9% (P=0.038).

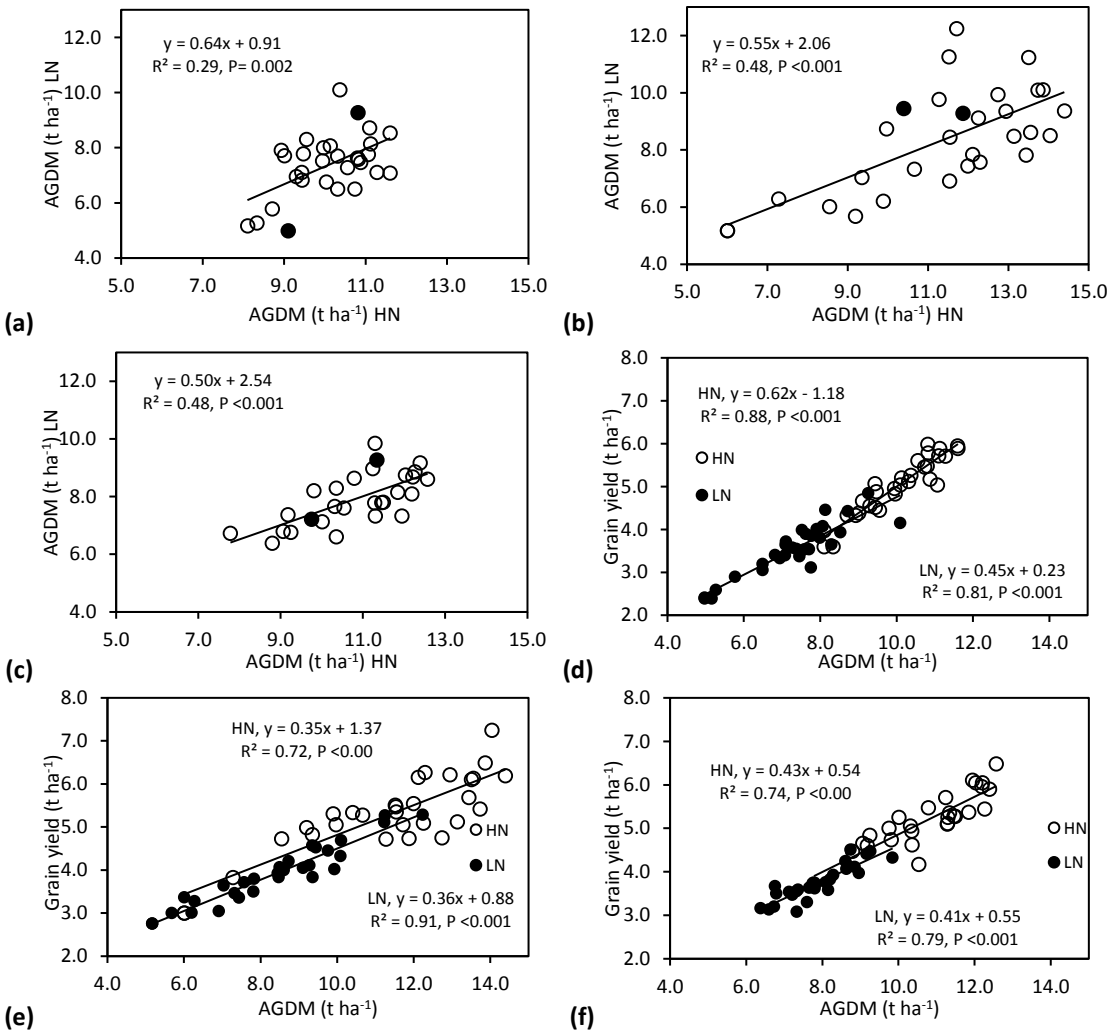


Figure 4. 3 Linear regression of above-ground dry matter (AGDM, 100% DM) in High N (HN) vs. Low N (LN) treatment (a) 2014-5 (b) 2015-6 and (c) across years; and association between grain yield (100% DM) and above-ground dry matter for 30 cv for (d) 2014 (e) 2016 and (f) across year 2015-6. (● showing durum wheat cultivars).

Averaging across years, AGDM was reduced by 27% under LN compared to HN conditions (P=0.002; Table 4.5; Fig. 4.3. c), with cultivars ranging from 7.78 (DBW- 14) to 12.57 (MACS-6478) t ha^{-1} under HN and from 6.38 (HW- 2044) to 9.85 (DBW- 46) t ha^{-1} under LN conditions (P<0.001). The N x G and Y x N x G interactions were not significant.

In 2014, there was no association between AD and AGDM (Fig. 4.4. a) or between PMD and AGDM under HN or LN conditions (Fig. 4.4. d). In 2016, there was a positive association between AD and AGDM under HN (R^2= 0.61, P<0.001) and under LN (R^2= 0.60, P<0.001) conditions (Fig. 4.4. b) and between PMD and AGDM under HN (R^2=65, P<0.001) and under LN (R^2= 0.64, P<0.001) conditions (Fig. 4.4. e).

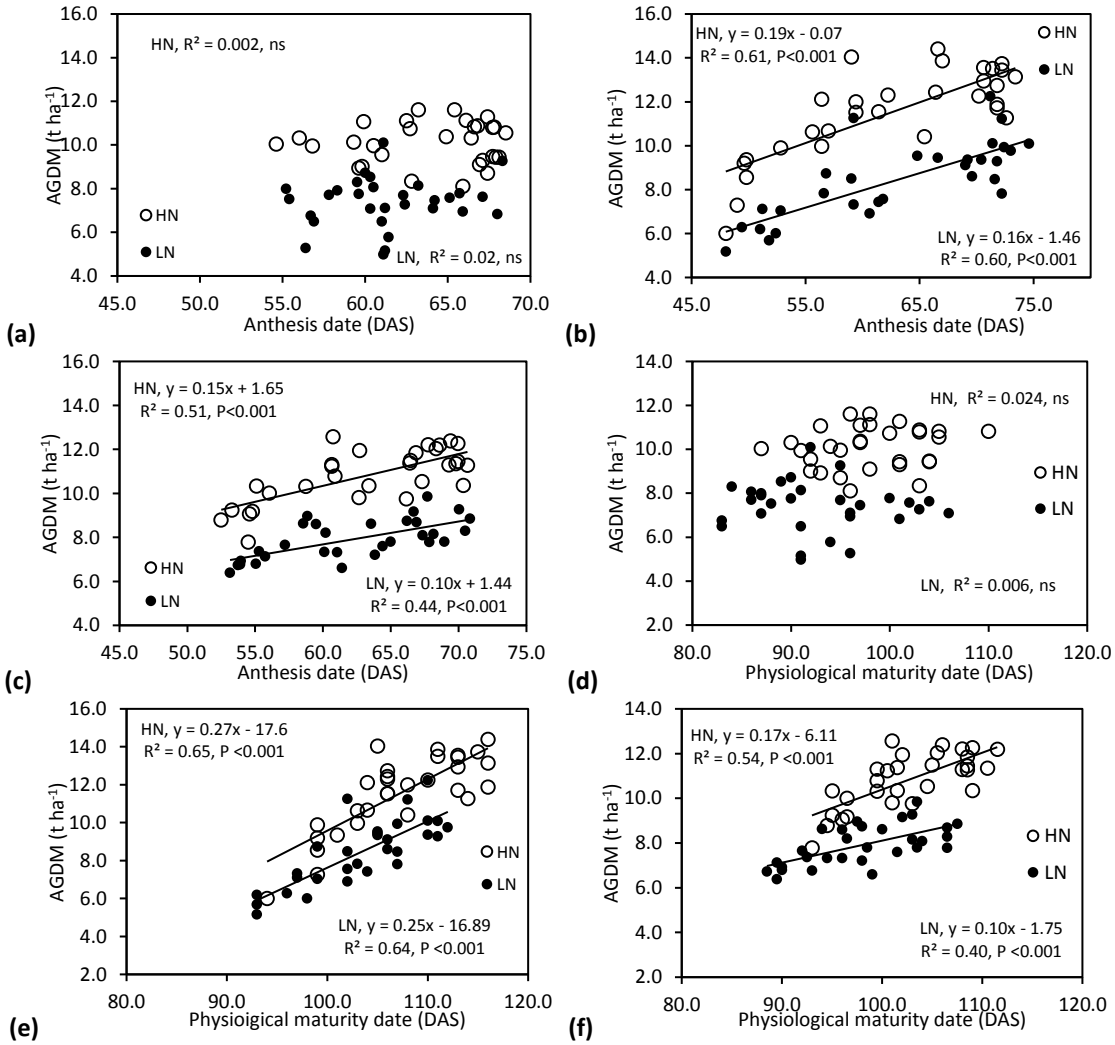


Figure 4. 4 Linear regression of anthesis date on above-ground dry matter (100% DM) in (a) 2014 (b) 2016 (c) across the year; and physiological maturity date on above-ground dry matter (100% DM) in (d) 2014 (e) 2016 (f) across the year for 30 wheat cultivars.

Across years, AD and PMD were also positively associated with AGDM under HN (R^2= 0.51, P<0.001 and R^2=54, P<0.001, respectively) and under LN (R^2= 0.44, P<0.001; R^2= 40, P<0.001, respectively) conditions (Fig. 4.4. c and f).

In 2014, harvest index (HI) did not differ between the HN (0.50) and LN (0.48) treatments (Table 4.5). The cultivars ranged from 0.43 to 0.55 under HN and 0.41 to 0.54 under LN conditions (P<0.001). There was an N x G interaction (P=0.01), with decreases under LN ranging from -10.4 (KHARCHIA- 65) to 19.6% (WH- 1021). There was a positive linear relationship between GY and HI under HN (R^2=50, P<0.001) and LN (R^2=0.17, P=0.026) conditions (Fig. 4.5. a). In 2016, there was again no significant difference in HI between the two N treatments. The cultivars ranged from 0.37 (KHARCHIA- 65) to 0.55 (KRL-1-4) under HN and 0.41 (KHARCHIA- 65) to 0.54 (DBW- 51) under LN conditions (P<0.001). There was a N x G interaction (P= 0.008), with responses to N limitation ranging from -13.3% (MACS-2496) to 14.8% (RAJ- 4238). There was no correlation between GY and HI under HN whereas under LN there was a trend for a negative linear relationship (R^2=0.10; P=0.09) (Fig. 4.5. b).

Averaging across years, HI did not differ under HN (0.48) and LN (0.47) conditions. The cultivars ranged from 0.40 to 0.53 under HN conditions and 0.43 to 0.55 under LN conditions (P<0.001). There was an N x G interaction with response to LN range from -10.1 (KHARCHIA-65) to 12.8% (WH- 1021) reduction but there was no Y x N x G interaction. There was a positive trend between HI and GY under HN (R^2=0.10, P=0.096).

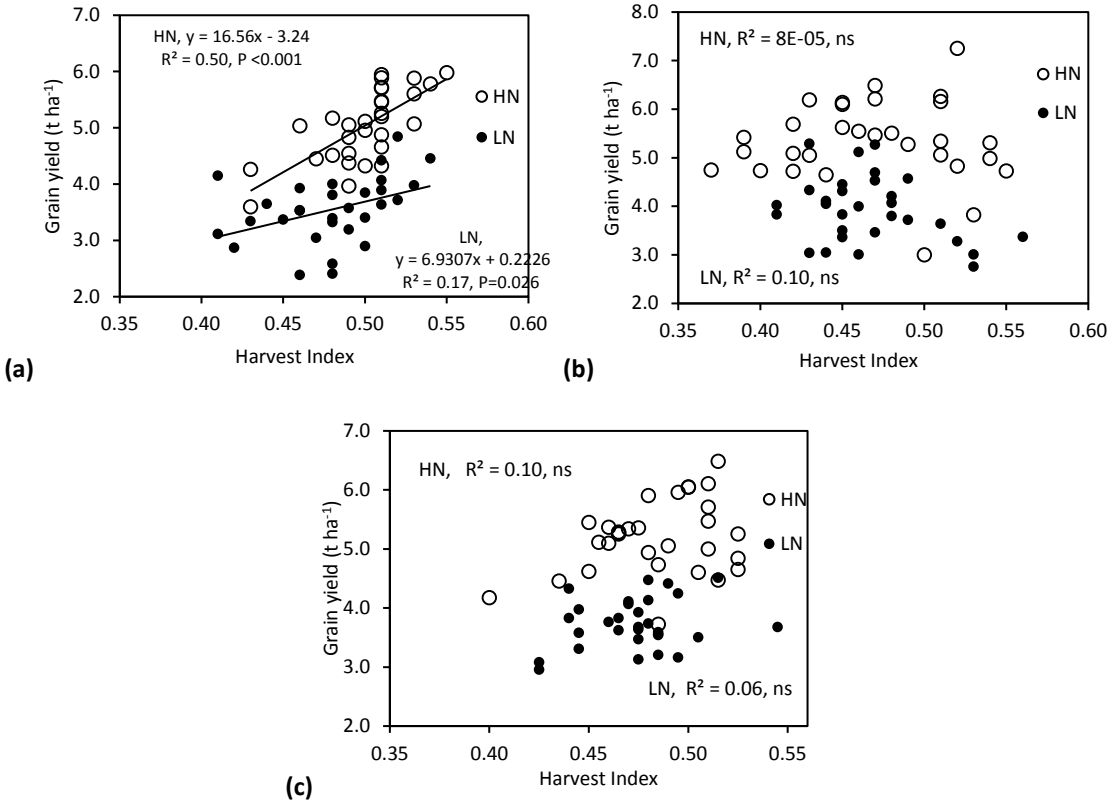


Figure 4. 5 Linear regression of grain yield (100% DM) on harvest index (HI) in (a) 2014 (b) 2016, and (c) mean across the years in 30 cultivars.

Table 4. 2 Plant height (PH), anthesis date (AD) and physiological maturity date (PMD) of 30 cultivars in the year 2014, 2016 and mean of two years.

	Plant Height (cm)						AD (DAS)						PMD (DAS)					
	2014		2016		Mean		2014		2016		Mean		2014		2016		Mean	
Cultivars	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**
BH- 1146	108.6	105.8	59.9	60.3	84.2	83.0	54.6	56.7	55.6	51.2	55.1	54.0	87.0	83.0	103.0	97.0	95.0	90.0
CBW- 38	88.8	81.9	65.5	64.0	77.2	73.0	66.4	62.3	66.4	64.8	66.4	63.6	97.0	95.0	106.0	105.0	101.5	100.0
DBW- 14	79.3	71.6	47.1	48.7	63.2	60.1	61.0	59.5	48.0	48.0	54.5	53.8	92.0	84.0	94.00	93.0	93.0	88.5
DBW- 16	81.8	75.0	64.9	62.3	73.4	68.6	68.1	68.0	72.6	73.0	70.4	70.5	104.0	101.0	114.0	112.0	109.0	106.5
DBW- 17	75.5	68.2	56.8	55.0	66.2	61.6	66.6	65.1	70.6	69.6	68.6	67.4	110.0	102.0	113.0	106.0	111.5	104.0
DBW- 39	83.7	77.0	70.5	65.4	77.1	71.2	67.4	61.2	71.4	72.2	69.4	66.7	101.0	96.0	111.0	108.0	106.0	102.0
DBW- 46	92.3	89.9	76.5	73.1	84.4	81.5	66.8	64.2	71.8	71.2	69.3	67.7	103.0	97.0	113.0	110.0	108.0	103.5
DBW- 51	88.0	80.7	69.5	66.8	78.8	73.8	67.1	65.9	66.6	70.4	66.9	68.2	101.0	96.0	116.0	110.0	108.5	103.0
DBW- 71	78.0	75.3	54.0	50.6	66.0	63.0	60.5	55.2	57.0	59.2	58.8	57.2	95.0	87.0	104.0	97.0	99.5	92.0
DPW- 621-50	85.1	76.7	67.5	63.7	76.3	70.2	62.7	61.0	70.2	69.0	66.5	65.0	100.0	91.0	110.0	106.0	105.0	98.5
GW- 322	82.8	77.4	67.5	66.8	75.2	72.1	66.1	63.2	70.6	69.2	68.4	66.2	98.0	91.0	113.0	105.0	105.5	98.0
HD- 2733	83.7	73.7	66.1	59.9	74.9	66.8	67.7	67.1	72.2	74.6	70.0	70.9	103.0	104.0	115.0	111.0	109.0	107.5
HD- 2967	83.4	80.5	65.0	63.6	74.2	72.1	67.7	65.7	72.2	72.2	70.0	70.0	104.0	100.0	113.0	107.0	108.5	103.5
HD-2932	85.0	82.7	52.2	48.5	68.6	65.6	65.4	60.3	56.4	56.8	60.9	58.6	96.0	89.0	103.0	99.0	99.5	94.0
HI- 8498	80.7	73.7	59.9	62.9	70.3	68.3	66.9	61.1	65.4	66.6	66.2	63.9	98.0	91.0	108.0	105.0	103.0	98.0
HW- 2044	81.6	76.6	54.1	46.9	67.9	61.8	56.0	56.9	49.0	49.4	52.5	53.2	90.0	83.0	99.00	96.0	94.5	89.5
K- 0307	82.2	81.8	55.4	49.3	68.8	65.6	65.9	61.2	59.4	59.2	62.7	60.2	96.0	91.0	106.0	102.0	101.0	96.5
KHARCHIA- 65	116.8	99.4	101.7	97.5	109.2	98.5	62.8	56.4	71.8	72.4	67.3	64.4	103.0	96.0	106.0	107.0	104.5	101.5
KRL- 19	74.8	71.5	41.5	45.9	58.2	58.7	59.8	57.8	49.8	52.8	54.8	55.3	92.0	86.0	101.0	99.0	96.5	92.5
KRL- 210	82.6	75.4	58.9	53.7	70.7	64.6	59.9	59.6	61.4	60.6	60.7	60.1	93.0	90.0	106.0	102.0	99.5	96.0
KRL-1-4	84.0	78.1	48.6	49.5	66.3	63.8	56.8	55.4	49.8	52.4	53.3	53.9	91.0	88.0	99.0	98.0	95.0	93.0
KRL-213	78.1	74.5	65.5	60.1	71.8	67.3	68.5	62.4	67.0	71.4	67.8	66.9	105.0	103.0	111.0	110.0	108.0	106.5
MACS- 6222	85.0	79.6	59.7	51.3	72.4	65.5	63.2	60.3	62.2	61.8	62.7	61.1	98.0	87.0	106.0	102.0	102.0	94.5
MACS-2496	79.1	80.0	65.0	62.2	72.1	71.1	67.9	64.1	73.4	71.6	70.7	67.9	101.0	106.00	116.0	107.0	108.5	106.5
MACS-6478	78.2	74.3	54.5	53.8	66.3	64.1	62.5	60.0	59.0	59.0	60.8	59.5	97.00	90.0	105.0	102.0	101.0	96.0
NW-1067	79.3	71.3	48.6	50.3	64.0	60.8	67.4	61.4	59.4	61.4	63.4	61.4	95.0	94.0	108.0	104.0	101.5	99.0
PDW- 314	79.6	78.2	62.1	60.9	70.9	69.6	67.8	68.3	71.8	71.8	69.8	70.1	105.0	95.0	116.0	111.0	110.5	103.0
RAJ- 4229	78.6	76.6	44.5	42.7	61.5	59.7	59.6	58.3	49.6	51.8	54.6	55.1	93.0	87.0	99.0	93.0	96.0	90.0
RAJ- 4238	77.1	77.6	47.5	45.8	62.3	61.7	59.3	60.5	52.8	51.0	56.1	55.8	94.0	86.0	99.0	93.0	96.5	89.5
WH- 1021	89.8	84.2	53.1	52.8	71.5	68.5	64.9	61.1	56.4	56.6	60.7	58.9	97.0	92.0	104.0	103.0	100.5	97.5
Mean	84.1	79.0	60.1	57.8	72.1	68.4	63.9	61.3	62.7	63.0	63.3	62.2	98.0	92.7	107.2	103.3	102.6	98.0
Min	74.8	68.2	41.5	42.7	58.2	58.7	54.6	55.2	48.0	48.0	52.5	53.2	87.0	83.0	94.0	93.0	93.0	88.5
Max	116.8	105.8	101.7	97.5	109.2	98.5	68.5	68.3	73.4	74.6	70.7	70.9	110.0	106.0	116.0	112.0	111.5	107.5
	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df
N	0.18 *	1	0.79 ns	2	0.5 **	3	0.05 *	1	0.79 ns	2	0.5 ns	3	6.4 ns	1	1.61 ns	2	0.9 *	3
G	1.88 ***	58	2.65 ***	116	1.8 ***	174	1.1 ***	58	1.19 ***	116	0.9 ***	174	20.53 ns	58	1.51 ***	116	1.2 ***	162
N*G	2.61 ns	58.5	3.77 ns	113.1	2.6 ns	172.3	1.54 **	58.1	1.83 ns	42.8	1.3 ns	84.3	29.25 ns	55.8	2.65 ns	14	1.9 ns	34.1
Y					0.7 ***	3					0.9 ns	3					0.7 ***	3
Y*N					0.9 ns	6					1 ns	4.7					1.3 ns	5.4
Y*G					2.7 ***	150.1					1.5 ***	21.5					1.8 ***	78.1
Y*N*G					3.7 ns	175.7					2 *	53					2.7 *	76.3
H^2					0.78	0.67					0.73	0.69					0.90	0.95

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level.

Table 4. 3 Grain yield (GY, 100% DM), above-ground dry matter (AGDM, 100%DM) and harvest Index (HI) of 30 cultivars in the year 2014, 2016 and mean of two years.

	GY (t ha^{-1})						AGDM (t ha^{-1})						HI					
	2014		2016		Mean		2014		2016		Mean		2014		2016		Mean	
Cultivars	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**
BH- 1146	4.26	2.87	4.65	3.04	4.46	2.95	10.0	6.8	10.6	7.1	10.3	6.9	0.43	0.42	0.44	0.43	0.44	0.43
CBW- 38	5.05	3.34	5.63	4.31	5.34	3.83	10.3	7.7	12.4	9.5	11.4	8.6	0.49	0.43	0.45	0.45	0.47	0.44
DBW- 14	4.45	3.65	3.00	2.76	3.72	3.20	9.6	8.3	6.0	5.2	7.8	6.7	0.47	0.44	0.50	0.53	0.49	0.49
DBW- 16	4.51	3.40	4.72	4.45	4.62	3.93	9.4	6.8	11.3	9.8	10.4	8.3	0.48	0.50	0.42	0.45	0.45	0.48
DBW- 17	5.78	3.53	6.14	4.00	5.96	3.76	10.8	7.6	13.6	8.6	12.2	8.1	0.54	0.46	0.45	0.46	0.50	0.46
DBW- 39	5.71	3.71	6.10	5.12	5.90	4.42	11.3	7.1	13.5	11.2	12.4	9.2	0.51	0.52	0.45	0.46	0.48	0.49
DBW- 46	5.17	3.37	5.05	5.29	5.11	4.33	10.9	7.5	11.7	12.2	11.3	9.8	0.48	0.45	0.43	0.43	0.46	0.44
DBW- 51	4.55	3.32	6.19	3.83	5.37	3.58	9.3	6.9	14.4	9.4	11.8	8.2	0.49	0.48	0.43	0.41	0.46	0.45
DBW- 71	4.83	3.81	5.28	3.47	5.05	3.64	10.0	8.0	10.7	7.3	10.3	7.7	0.49	0.48	0.49	0.47	0.49	0.48
DPW- 621-50	5.46	3.19	5.09	4.05	5.27	3.62	10.7	6.5	12.3	9.1	11.5	7.8	0.51	0.49	0.42	0.44	0.47	0.47
GW- 322	5.88	4.46	6.21	4.57	6.05	4.51	11.1	8.1	12.9	9.4	12.0	8.7	0.53	0.54	0.47	0.49	0.50	0.52
HD- 2733	5.47	3.89	5.42	4.33	5.45	4.11	10.8	7.6	13.7	10.1	12.3	8.9	0.51	0.51	0.39	0.43	0.45	0.47
HD- 2967	4.88	3.85	5.69	3.50	5.28	3.67	9.5	7.8	13.4	7.8	11.5	7.8	0.51	0.50	0.42	0.45	0.47	0.48
HD-2932	5.89	3.93	5.05	4.21	5.47	4.07	11.6	8.5	10.0	8.7	10.8	8.6	0.51	0.46	0.51	0.48	0.51	0.47
HI- 8498 ł	4.66	2.41	5.34	4.53	5.00	3.47	9.1	5.0	10.4	9.4	9.8	7.2	0.51	0.48	0.51	0.47	0.51	0.48
HW- 2044	5.12	3.05	3.83	3.28	4.47	3.16	10.3	6.5	7.3	6.3	8.8	6.4	0.50	0.47	0.53	0.52	0.52	0.50
K- 0307	3.97	2.38	5.51	5.27	4.74	3.83	8.1	5.2	11.5	11.2	9.8	8.2	0.49	0.46	0.48	0.47	0.49	0.47
KHARCHIA- 65	3.60	2.59	4.75	4.03	4.17	3.31	8.3	5.3	12.7	9.9	10.5	7.6	0.43	0.48	0.37	0.41	0.40	0.45
KRL- 19	4.38	3.53	4.83	3.64	4.60	3.59	9.0	7.7	9.4	7.0	9.2	7.4	0.49	0.46	0.52	0.51	0.51	0.49
KRL- 210	5.03	3.11	5.47	3.05	5.25	3.08	11.1	7.8	11.5	6.9	11.3	7.3	0.46	0.41	0.47	0.44	0.47	0.43
KRL-1-4	4.95	3.98	4.73	3.37	4.84	3.67	9.9	7.5	8.5	6.0	9.2	6.8	0.50	0.53	0.55	0.56	0.53	0.55
KRL-213	5.61	3.57	6.49	4.69	6.05	4.13	10.6	7.3	13.9	10.1	12.2	8.7	0.53	0.49	0.47	0.47	0.50	0.48
MACS- 6222	5.95	3.39	6.27	3.72	6.11	3.55	11.6	7.1	12.3	7.6	11.9	7.3	0.51	0.48	0.51	0.49	0.51	0.49
MACS-2496	5.07	3.64	5.12	3.83	5.10	3.74	9.4	7.1	13.1	8.5	11.3	7.8	0.53	0.51	0.39	0.45	0.46	0.48
MACS-6478	5.72	4.42	7.25	4.07	6.48	4.24	11.1	8.7	14.0	8.5	12.6	8.6	0.51	0.51	0.52	0.48	0.52	0.50
NW-1067	4.33	2.90	5.55	3.36	4.94	3.13	8.7	5.8	12.0	7.4	10.3	6.6	0.50	0.50	0.46	0.45	0.48	0.48
PDW- 314 ł	5.98	4.84	4.74	4.11	5.36	4.47	10.8	9.3	11.9	9.3	11.3	9.3	0.55	0.52	0.40	0.44	0.48	0.48
RAJ- 4229	4.32	4.00	4.99	3.01	4.65	3.50	8.9	7.9	9.2	5.7	9.1	6.8	0.51	0.48	0.54	0.53	0.53	0.51
RAJ- 4238	5.20	4.07	5.31	3.01	5.25	3.54	10.1	8.1	9.9	6.2	10.0	7.1	0.51	0.51	0.54	0.46	0.53	0.49
WH- 1021	5.26	4.15	6.16	3.80	5.71	3.97	10.4	10.1	12.1	7.8	11.2	9.0	0.51	0.41	0.51	0.48	0.51	0.45
Mean	5.03	3.54	5.35	3.92	5.19	3.73	10.1	7.4	11.5	8.4	10.8	7.9	0.5	0.5	0.5	0.5	0.5	0.5
Min	3.60	2.38	3.00	2.76	3.72	2.95	8.1	5.0	6.0	5.2	7.8	6.4	0.4	0.4	0.4	0.4	0.4	0.4
Max	5.98	4.84	7.25	5.29	6.48	4.51	11.6	10.1	14.4	12.2	12.6	9.8	0.6	0.5	0.6	0.6	0.5	0.5
	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df
N	0.25 ns	1	0.21 *	2	0.16 **	3	0.46 ns	1	0.39 **	2	0.3 **	3	0.005 ns	1	0.006 ns	2	0.004 ns	3
G	0.35 ***	58	0.44 ***	116	0.31 ***	174	0.72 ***	58	0.86 ***	116	0.6 ***	174	0.013 ***	58	0.014 ***	116	0.01 ***	174
N*G	0.55 ns	18.05	0.64 *	80.4	0.45 *	103.2	1.1 ns	23.47	1.25 *	84.6	0.89 ns	113.1	0.019 **	53.9	0.021 **	90.06	0.015 ***	141.2
Y					0.25 ns	3					0.37 *	3					0.01 ns	3
Y*N					0.3 ns	5.2					0.48 ns	5.8					0.011 ns	4.1
Y*G					0.5 ***	41.1					0.93 ***	83					0.018 ***	26.4
Y*N*G					0.68 ns	90.9					1.3 ns	134.9					0.024 ns	64.2
H^2					0.65	0.09					0..30	NA					0.31	0.17

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level. (ł) durum wheat cultivars.

4.3.4 Nitrogen-use efficiency

Overall available soil N values were 196.9 kg ha^{-1} in 2013-4 and 141 kg ha^{-1} in 2015-6. These values seemed higher and may have been overestimated by laboratories. Because of this NUpE values were in several cases higher under HN than LN which was unexpected. Also, there were uncertainties about N leached from the soil associated with the flood irrigation. Therefore, N uptake at anthesis and at harvest are given more importance than NUpE and results are focused on N uptake as it was more reliable estimate of N uptake capacity.

4.3.4.1 NUE and N uptake 2014

In 2014, NUE was increased in the LN (17.8 g GDM g^{-1} N) compared with the HN treatment (15.76 g GDM g^{-1} N) (Table 4.7) and cultivars ranged from 11.3 to 18.8 g GDM g^{-1} N under HN and 11.7 to 24.3 g GDM g^{-1} N under LN conditions (P=0.001). The interaction of N x G was not significant.

Above-ground N-uptake at anthesis (AGN_A) was reduced from 144.7 kg N ha^{-1} under HN to 78.4 kg N ha^{-1} under LN conditions but this reduction was not significant (P=0.15; Table 4.8). Cultivars ranged from 100.9 (HI- 8498) to 205.5 (MACS- 6222) kg N ha^{-1} under HN and 48.8 (KHARCHIA- 65) to 117.6 (RAJ- 4229) kg N ha^{-1} under LN conditions (P=0.10). There was no significant N x G interaction.

Above-ground N uptake at harvest (AGN_H) was reduced from 148.9 kg N ha^{-1} under HN to 83.0 kg N ha^{-1} under LN conditions (P= 0.09; Table 4.8). Cultivars ranged from 114.9 kg N ha^{-1} (KHARCHIA- 65) to 187.1 kg N ha^{-1} (MACS- 6222) under HN and 52.8 kg N ha^{-1} (KHARCHIA-65) to 114.2 kg N ha^{-1} (RAJ- 4229) under LN conditions (P<0.001). There was no significant N x G interaction.

Post-anthesis N-uptake (PANU) was overall 0.57 kg N ha^{-1} in the HN and 0.99 kg N ha^{-1} in the LN treatment (Table. 4.8). Cultivars ranged from -44.1 (KHARCHIA- 65) to 35.4 (KRL-19) kg N ha^{-1} under HN conditions and from -20.7 kg N ha^{-1} (RAJ- 4229) to 27.3 kg N ha^{-1} (HD-2932) under LN conditions (P=0.33). There was no interaction for N x G.

Overall, GY showed stronger association with AGN_A and AGN_H under LN conditions than HN and there was no association with PANU under both N conditions (Fig. 4.6).

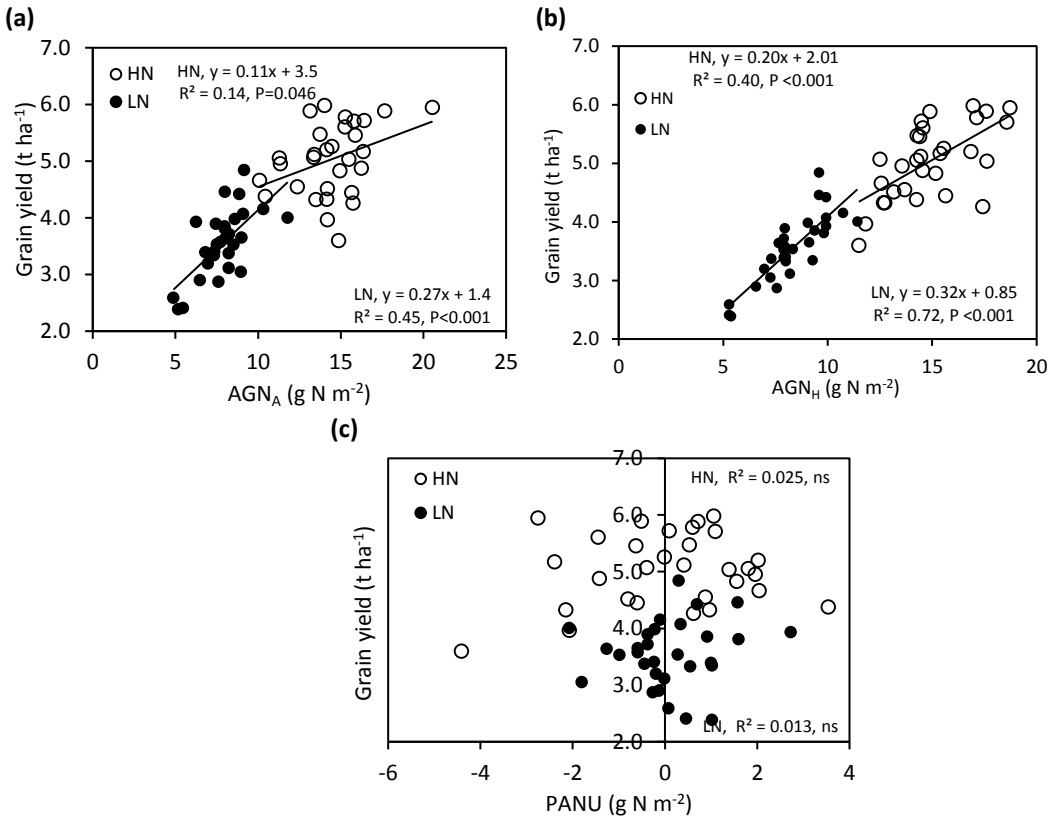


Figure 4. 6 Linear regression (a) above-ground N at anthesis (AGN_A) vs. grain yield (100% DM), (b) above-ground N at harvest (AGN_H) vs. grain yield (100% DM), (c) post-anthesis N uptake vs. grain yield (100% DM) for 30 wheat cultivars in 2014.

4.3.4.2 NUE and N uptake 2016

In 2016, NUE was increased in the LN (21.7 g DM g^{-1} N) compared with the HN treatment (20.5 g DM g^{-1} N) (Table 4.7) and cultivars ranged from 11.5 to 27.8 g DM g^{-1} N under HN and 15.2 to 29.2 g DM g^{-1} N under LN condition (P<0.001). The interaction of N treatment and genotype was not significant.

AGN_A was reduced from 154.6 kg N ha^{-1} under HN to 85.3 kg N ha^{-1} under LN conditions (P=0.011; Table. 4.8). Cultivars ranged from 76.6 (DBW- 14) to 242.4 (DBW- 51) kg N ha^{-1} under HN and 49.7 (KRL- 210) to 130.7 (DBW- 46) kg N ha^{-1} under LN conditions (P<0.001). There was an N treatment x genotype interaction (P=0.001), with cultivar reduction under N limitation ranging from 8.9 to 68%.

AGN_H was reduced from 174.9 kg N ha^{-1} under HN to 86.7 kg N ha^{-1} under LN conditions (P=0.004; Table 4.8). Cultivars ranged from 86.9 (DBW- 14) to 232.5 (MACS-6478) kg N ha^{-1} under HN and 65.0 (KRL- 210) to 117.3 (DBW- 46) kg N ha^{-1} under LN conditions (P=0.004). There was an N treatment x genotype interaction (P=0.029), with cultivar reduction under N limitation ranging from 18.2 to 65.1%.

PANU was overall 21.9 kg N ha^{-1} under HN and 3.1 kg N ha^{-1} under LN treatment (P=0.02; Table. 4.8). Cultivars ranged from -24.3 (HD- 2967) to 63.2 (HI- 8498) kg N ha^{-1} under HN conditions to -28.9 (KHARCHIA- 65) to 23.4 (RAJ- 4229) kg N ha^{-1} under LN conditions (P<0.001). The cultivar increases under N limitation ranged from -78.5 to 47.8%.

In 2016 also GY showed stronger association with AGN_A and AGN_H under LN conditions than HN. There was a negative association between GY and PANU under LN conditions and no association under HN (Fig. 4.7).

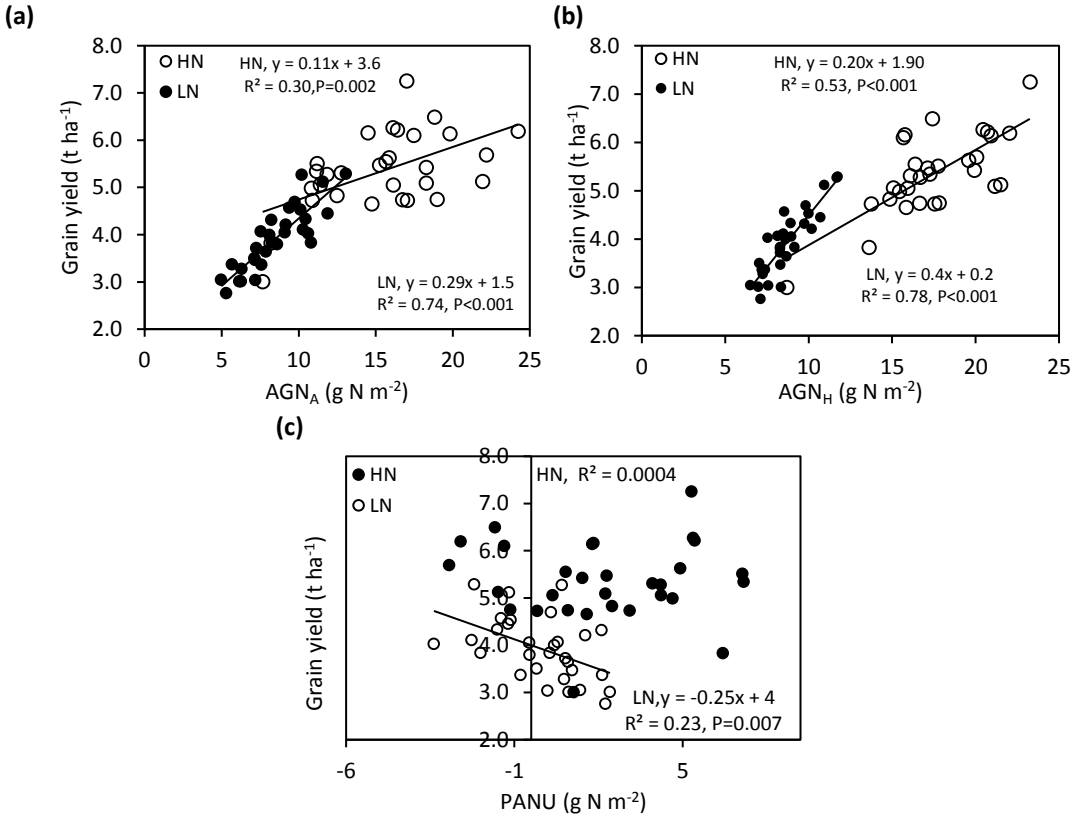


Figure 4. 7 Linear regression (a) above-ground N at anthesis (AGN_A) vs. grain yield (100% DM), (b) above-ground N at harvest (AGN_H) vs. grain yield (100% DM), (c) post-anthesis N uptake vs. grain yield (100% DM) for 30 wheat cultivars in 2016.

4.3.4.3 NUE and N uptake 2014-16

Averaging across years, NUE was increased in the LN (19.7 g DM g^{-1} N) compared with the HN treatment (18.1 g DM g^{-1} N) (Table. 4.7) and cultivars ranged from 12.7 to 22.7 g DM g^{-1} N under HN and 15.6 to 23.8 g DM g N^{-1} under LN conditions (P<0.001). There was no interaction of N treatment x genotype or year x N treatment x genotype.

AGN_A was reduced from 150.6 kg N ha^{-1} under HN to 82.5 kg N ha^{-1} under LN conditions (P=0.003; Table 4.8). Cultivars ranged from 102.8 (HW- 2044) to 198.1 (HD- 2967) kg N ha^{-1} under HN and from 62.8 (KRL- 210) to 111.3 (DBW- 46) kg N ha^{-1} under LN conditions (P=0.001). Cultivar decrease under LN ranging from 22.9 to 62.3% (P=0.001), whereas the year x N treatment x genotype interaction was not significant.

AGN_H was reduced from 161.9 kg N ha^{-1} under HN to 84.9 kg N ha^{-1} under LN conditions (P<0.001; Table 4.8). Cultivars ranged from 121.6 (DBW- 14) to 195.8 (MACS- 6222) kg N ha^{-1} under HN and 64.1 (KHARCHIA- 65) to 100.6 (HD-2932) kg N ha^{-1} under LN conditions (P=0.006). There was an N treatment x genotype interaction with cultivar responses to LN range from 29.7 to 58.7% reductions. There was no year x N treatment x genotype interaction.

PANU decreased from HN (13.3 kg N ha^{-1}) to LN (2.3 kg N ha^{-1}) conditions (P=0.039; Table 4.8). Cultivars ranged from -21.4 (KHARCHIA- 65) to 46.1 (HI- 8498) kg N ha^{-1} under HN conditions and from -17.1 (KHARCHIA- 65) to 20.5 (HD-2932) kg N ha^{-1} under LN conditions (P<0.001). There was no significant N treatment x genotype or year x N treatment x genotype interaction.

Across years, GY showed stronger association with AGN_A and AGN_H under LN conditions than HN and there was no association with PANU under both N conditions (Fig. 4.8).

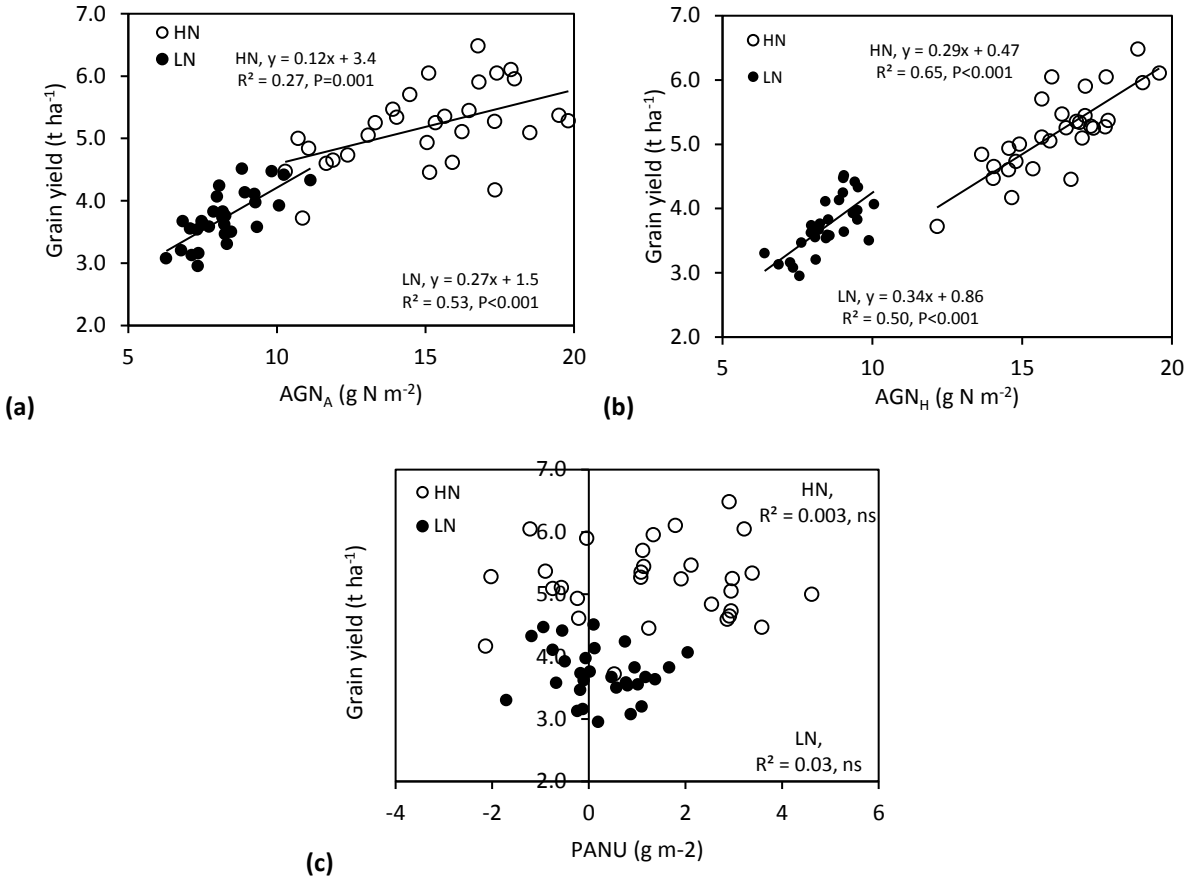


Figure 4. 8 Linear regression (a) above-ground N at anthesis (AGN_A) vs. grain yield (100% DM), (b) above-ground N at harvest (AGN_H) vs. grain yield (100% DM), (c) post-anthesis N uptake vs. grain yield (100% DM) for 30 wheat cultivars. Values are mean of 2014 and 2016.

4.3.5 Nitrogen-Utilization efficiency:

In 2014, N-utilization efficiency (NUtE) increased from 34.0 g DM g^{-1} N under HN to 43.3 g DM g^{-1} N under LN conditions (P=0.09; Table 4.7). The cultivars ranged from 24.4 (BH-1146) to 39.6 (GW- 322) g DM g^{-1} N under HN and 35.71 (CBW- 38) to 50.59 (PDW- 314, durum wheat) g DM g^{-1} N under LN conditions (P<0.001). Cultivar responses to HN ranged from -2.0 to 35.6% reduction (P= 0.01). Overall under HN, there was a less strong positive association amongst cultivars between NUtE and GY (R^2=0.18, P=0.019; Fig. 4.12. b) than between N-uptake at harvest and GY (R^2=0.40; P<0.001; Fig. 4.7. b); whereas under LN, the association between NUtE and GY was not significant and that of N-uptake at harvest and GY was significant (R^2=0.72; P<0.001; Fig. 4.6. b).

In 2016, NUtE increased from 31.4 under HN to 45.9 g DM g^{-1} N under LN conditions (P=0.009, Table 4.7). Cultivars ranged from 23.9 (MACS-2496) to 40.3 (DBW- 39) under HN

and 35.7 (RAJ- 4229) to 54.1 (KHARCHIA- 65) g DM g^{-1} N under LN conditions (P=0.03). There was an N treatment x genotype interaction (P<0.001; Table 4.5). Overall a smaller proportion of the genetic variation in GY was accounted for by NUtE under both HN (R^2=0.02) and LN (R^2=0.16, P=0.03) conditions than by N-uptake at harvest (R^2=0.53 and 0.78, respectively) (Fig. 4.7. b).

Averaging over years, NUtE increased from 32.7 under HN to 44.6 g GDM g^{-1} N under LN conditions (P=0.001, Table 4.7). Cultivars ranged from 27.2 (BH- 1146) to 38.3 (KRL-213) under HN and 37.3 (RAJ- 4229) to 51.2 (KHARCHIA- 65) g GDM g^{-1} N under LN conditions (P<0.001). There was an N treatment x genotype (P<0.001) interaction with increase under N limitation ranging from 13.9 to 41.4%. There was no year x N treatment x genotype interaction. Overall a smaller proportion of the genetic variation in GY was accounted for by NUtE under both HN (R^2=0.26, P=0.004) and LN (R^2=0.22, P=0.009) conditions than by N-uptake at harvest (R^2=0.65 and 0.50, respectively) (Fig. 4.8. b).

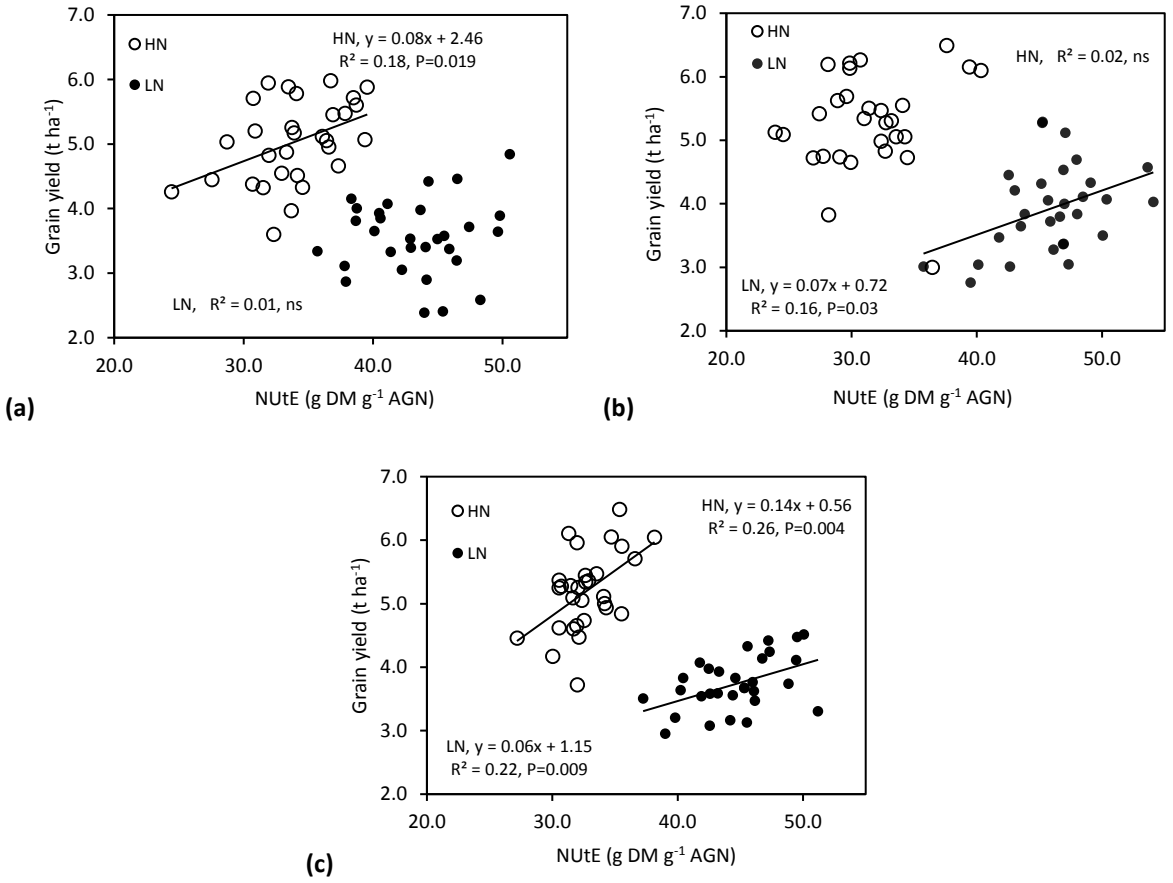


Figure 4. 9 Linear regression of N-utilization efficiency on grain yield (100% DM) (a) in 2014 (b) in 2016 and (c) mean across years 2014-16 for 30 wheat cultivars.

Table 4. 4 N-use efficiency (NUE), N-uptake efficiency (NUpE) and N-utilization efficiency (NUtE) in 30 cultivars in year 2014, 2016 and mean of two years.

	NUE (g DM g^{-1} av. N)						NUtE (g DM g^{-1} AGN)					
	2014		2016		Mean		2014		2016		Mean	
	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN
BH- 1146	13.4	14.4	17.8	16.8	15.6	15.6	24.4	37.9	29.9	40.1	27.2	39.0
CBW- 38	15.9	16.8	21.6	23.8	18.7	20.3	36.4	35.7	28.9	45.2	32.6	40.4
DBW- 14	14.0	18.3	11.5	15.2	12.7	16.8	27.6	40.1	36.5	39.5	32.0	39.8
DBW- 16	14.1	17.1	18.1	24.6	16.1	20.8	34.2	44.1	26.9	42.5	30.5	43.3
DBW- 17	18.3	17.6	23.5	22.1	20.9	19.9	34.1	45.0	29.9	47.0	32.0	46.0
DBW- 39	17.8	18.5	23.4	28.3	20.6	23.4	30.8	47.4	40.3	47.1	35.5	47.2
DBW- 46	16.3	16.8	19.4	29.2	17.8	23.0	33.9	45.9	34.3	45.3	34.1	45.6
DBW- 51	14.4	16.7	23.7	21.2	19.0	18.9	33.0	41.4	28.1	43.8	30.5	42.6
DBW- 71	14.9	19.0	20.2	19.1	17.6	19.1	32.0	38.7	32.7	41.8	32.4	40.2
DPW- 621-50	17.0	16.0	19.5	22.4	18.3	19.2	36.9	46.5	24.5	45.7	30.7	46.1
GW- 322	18.7	22.3	23.8	25.2	21.3	23.7	39.6	46.5	29.8	53.6	34.7	50.1
HD- 2733	17.3	19.6	20.8	23.9	19.0	21.8	37.9	49.8	27.4	49.1	32.6	49.5
HD- 2967	15.2	19.3	21.8	19.3	18.5	19.3	33.3	40.6	29.6	50.1	31.4	45.3
HD-2932	18.5	19.7	19.4	23.3	18.9	21.5	33.5	40.5	33.6	43.0	33.5	41.8
HI- 8498 ł	14.7	11.9	20.5	25.0	17.6	18.5	37.3	45.4	31.0	46.9	34.2	46.2
HW- 2044	16.2	15.2	14.7	18.1	15.4	16.7	36.1	42.3	28.2	46.1	32.1	44.2
K- 0307	12.3	11.7	21.1	29.1	16.7	20.4	33.7	44.0	31.4	45.2	32.5	44.6
KHARCHIA- 65	11.3	13.0	18.2	22.2	14.7	17.6	32.3	48.3	27.7	54.1	30.0	51.2
KRL- 19	13.7	17.8	18.5	20.1	16.1	18.9	30.7	42.9	32.7	43.5	31.7	43.2
KRL- 210	15.7	15.5	21.0	16.8	18.3	16.2	28.7	37.8	32.3	47.3	30.5	42.6
KRL-1-4	15.5	20.0	18.1	18.6	16.8	19.3	36.6	43.7	34.5	47.0	35.5	45.3
KRL-213	17.6	18.0	24.9	25.9	21.3	22.0	38.7	45.5	37.6	48.0	38.2	46.7
MACS- 6222	18.8	16.9	24.0	20.5	21.4	18.7	31.9	42.9	30.7	45.9	31.3	44.4
MACS-2496	15.9	18.2	19.6	21.2	17.8	19.7	39.4	49.7	23.9	48.0	31.6	48.8
MACS-6478	17.6	22.1	27.8	22.5	22.7	22.3	38.5	44.3	32.2	50.4	35.4	47.3
NW-1067	13.6	14.5	21.3	18.6	17.4	16.5	34.6	44.2	34.1	46.9	34.3	45.5
PDW- 314 ł	18.8	24.3	18.2	22.7	18.5	23.5	36.7	50.6	29.0	48.5	32.9	49.5
RAJ- 4229	12.5	21.8	19.1	16.6	15.8	19.2	31.5	38.8	32.4	35.7	31.9	37.2
RAJ- 4238	16.4	20.4	20.3	16.6	18.4	18.5	30.9	41.1	33.2	42.7	32.0	41.9
WH- 1021	16.6	20.8	23.6	21.0	20.1	20.9	33.7	38.3	39.4	46.6	36.6	42.5
Mean	15.8	17.8	20.5	21.7	18.1	19.7	34.0	43.3	31.4	45.9	32.7	44.6
Min	11.3	11.7	11.5	15.2	12.7	15.6	24.4	35.7	23.9	35.7	27.2	37.2
Max	18.8	24.3	27.8	29.2	22.7	23.7	39.6	50.6	40.3	54.1	38.2	51.2
	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df
N	1.04 ns	1	1.22 ns	2	0.86 ns	3	1.22 ns	1	1.37 **	2	0.97 ***	3
G	1.5 ***	58	2.11 ***	116	1.45 ***	174	1.57 ***	58	2.61 *	116	1.75 ***	174
N*G	2.33 ns	19.7	3.18 ns	58.3	2.19 ns	82.3	2.5 **	15	3.88 ***	69.9	2.62 ***	94.2
Y					1.21 *	3					1.21 ns	3
Y*N					1.54 ns	5.4					3.85 ns	5.8
Y*G					2.41 **	35.7					5.51 *	62.7
Y*N*G					3.28 ns	80.4					7.6 ns	114.5
H^2					0.62	0.09					NA	0.75

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level. (ł) durum wheat cultivars

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Table 4. 5 Above-ground N at anthesis (AGN_A), above-ground N at harvest (AGN_H) and post-anthesis N-uptake (PANU) of 30 cultivars in 2014, 2016 and two years mean.

	AGN_A (g N m^{-2})						AGN_H (g N m^{-2})						PANU (g N m^{-2})					
	2014		2016		Mean		2014		2016		Mean		2014		2016		Mean	
Cultivars	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**
BH- 1146	15.7	7.6	14.8	7.2	15.1	7.3	17.4	7.6	15.8	7.6	16.6	7.6	0.62	-0.26	1.66	0.49	1.24	0.19
CBW- 38	11.3	7.3	15.9	8.2	14.0	7.9	14.3	9.3	19.6	9.7	16.9	9.5	1.81	1.02	4.43	2.09	3.38	1.66
DBW- 14	15.7	9.0	7.7	5.3	10.9	6.8	15.6	9.1	8.7	7.1	12.2	8.1	-0.60	-0.59	1.27	2.21	0.52	1.09
DBW- 16	14.2	7.4	17.1	11.9	15.9	10.1	13.2	8.0	17.6	10.7	15.4	9.4	-0.80	-0.23	0.18	-0.68	-0.21	-0.50
DBW- 17	15.3	8.5	19.8	8.1	18.0	8.3	17.1	7.9	20.9	8.6	19.0	8.3	0.60	-0.98	1.82	0.69	1.33	0.02
DBW- 39	15.8	8.3	17.5	11.6	16.8	10.2	18.6	7.9	15.7	10.9	17.1	9.4	1.09	-0.37	-0.80	-0.66	-0.05	-0.55
DBW- 46	16.4	8.2	16.2	13.1	16.2	11.1	15.4	7.3	15.9	11.7	15.7	9.5	-2.39	-0.44	0.64	-1.69	-0.57	-1.19
DBW- 51	12.4	7.2	24.2	10.8	19.5	9.3	13.7	8.0	22.0	9.2	17.9	8.6	0.88	0.55	-2.09	-1.50	-0.90	-0.68
DBW- 71	14.9	8.0	11.8	7.2	13.1	7.5	15.2	9.8	16.7	8.3	15.9	9.1	1.56	1.60	3.85	1.22	2.94	1.37
DPW- 621-50	15.9	7.0	18.3	9.1	17.3	8.2	14.4	7.0	21.2	9.0	17.8	8.0	-0.63	-0.19	2.21	-0.06	1.07	-0.11
GW- 322	13.2	8.0	16.4	9.4	15.1	8.8	14.9	9.6	20.7	8.5	17.8	9.1	0.72	1.58	4.86	-0.89	3.21	0.10
HD- 2733	13.8	7.5	18.3	10.4	16.5	9.3	14.3	8.0	19.9	8.9	17.1	8.4	0.53	-0.37	1.52	-1.01	1.13	-0.75
HD- 2967	16.3	8.0	22.2	7.1	19.8	7.5	14.5	9.4	20.1	7.0	17.3	8.2	-1.42	0.92	-2.43	0.16	-2.03	0.47
HD-2932	17.7	6.3	11.4	9.2	13.9	8.0	17.6	9.9	15.1	10.2	16.3	10.1	-0.51	2.73	3.86	1.61	2.11	2.05
HI- 8498 ł	10.1	5.5	11.2	10.1	10.7	8.3	12.6	5.3	17.3	10.0	14.9	7.6	2.04	0.46	6.32	-0.61	4.61	-0.18
HW- 2044	13.4	9.0	8.2	6.3	10.3	7.4	14.4	7.3	13.6	7.3	14.0	7.3	0.41	-1.80	5.70	0.98	3.58	-0.13
K- 0307	14.2	5.2	11.2	10.2	12.4	8.2	11.8	5.4	17.8	11.7	14.8	8.5	-2.07	1.02	6.28	0.91	2.94	0.95
KHARCHIA- 65	14.9	4.9	19.0	10.6	17.3	8.3	11.5	5.3	17.8	7.5	14.7	6.4	-4.41	0.08	-0.62	-2.89	-2.14	-1.71
KRL- 19	10.4	7.5	12.5	7.9	11.7	7.7	14.2	8.3	14.9	8.7	14.5	8.5	3.54	0.28	2.41	1.09	2.86	0.77
KRL- 210	15.5	8.2	15.3	5.0	15.3	6.3	17.6	8.2	17.1	6.5	17.4	7.4	1.39	-0.01	2.25	1.46	1.91	0.87
KRL-1-4	11.4	8.6	10.9	5.7	11.1	6.8	13.6	9.1	13.7	7.4	13.7	8.2	1.96	-0.22	2.93	2.11	2.54	1.17
KRL-213	15.3	7.7	18.8	9.7	17.4	8.9	14.6	8.0	17.4	9.8	16.0	8.9	-1.45	-0.59	-1.07	0.59	-1.22	0.12
MACS- 6222	20.6	6.8	16.1	7.3	17.9	7.1	18.7	7.9	20.4	8.3	19.6	8.1	-2.75	1.00	4.81	1.02	1.79	1.01
MACS-2496	13.4	8.0	21.9	8.3	18.5	8.2	12.5	7.7	21.5	8.3	17.0	8.0	-0.39	-1.26	-0.98	0.55	-0.75	-0.17
MACS-6478	16.4	8.9	17.0	7.5	16.8	8.1	14.5	9.9	23.3	8.1	18.9	9.0	0.09	0.70	4.77	0.79	2.90	0.75
NW-1067	14.2	6.5	15.7	7.6	15.1	7.1	12.7	6.6	16.4	7.2	14.6	6.9	-2.15	-0.13	1.03	-0.31	-0.24	-0.24
PDW- 314 ł	14.0	9.2	16.7	10.3	15.7	9.8	17.0	9.6	16.7	8.5	16.8	9.0	1.06	0.30	1.09	-1.77	1.08	-0.94
RAJ- 4229	13.5	11.8	10.8	6.2	11.9	8.5	12.7	11.4	15.4	8.3	14.1	9.9	0.97	-2.07	4.20	2.34	2.91	0.57
RAJ- 4238	14.2	9.1	12.8	6.1	13.3	7.3	16.8	9.9	16.1	7.0	16.5	8.5	2.02	0.34	3.61	1.11	2.97	0.80
WH- 1021	14.5	10.3	14.5	8.6	14.5	9.3	15.6	10.7	15.8	8.3	15.7	9.5	-0.01	-0.10	1.86	-0.05	1.11	-0.07
Mean	14.5	7.8	15.5	8.5	15.1	8.3	14.9	8.3	17.5	8.7	16.2	8.5	0.06	0.10	2.19	0.31	1.33	0.22
Min	10.1	4.9	7.7	5.0	10.3	6.3	11.5	5.3	8.7	6.5	12.2	6.4	-4.41	-2.07	-2.43	-2.89	-2.14	-1.71
Max	20.6	11.8	24.2	13.1	19.8	11.1	18.7	11.4	23.3	11.7	19.6	10.1	3.54	2.73	6.32	2.34	4.61	2.05
	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df
N	1.6 ns	1	0.7 *	2	0.8 **	3	0.95 ns	1	0.58 **	2	0.51 ***	3	0.7 ns	1	0.3 *	2	0.3 *	3
G	1.5 ns	58	1.7 ***	116	1.2 ***	174	1.03 ***	58	1.67 **	116	1.12 **	174	1.3 ns	58	1.3 ***	116	1.0 ***	174
N*G	2.6 ns	6.58	2.5 ***	90.69	1.9 ***	75.48	1.72 ns	9.98	2.39 *	106.6	0.86 *	129.7	2.0 ns	34.54	1.9 ns	117.5	1.4 ns	164.8
Y					0.8 ns	3					1.64 ns	3					0.5 ns	3
Y*N					1.1 ns	5.99					1 ns	4.9					0.6 ns	5.4
Y*G					1.9 ***	69.65					1.81 **	48.8					1.4 ns	126
Y*N*G					2.7 ns	112.67					2.46 ns	104.2					2.0 ns	167.3

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level. (ł) durum wheat cultivars.

4.3.6 Nitrogen Nutrition Index

In 2014, NNI was higher under HN (1.00) than under LN (0.62), although the N treatment effect was not significant (P=0.13). The cultivars ranged from 0.84 (CBW- 38) to 1.27 (MACS- 6222) under HN and under LN from 0.48 (CBW- 38) to 0.77 (RAJ- 4229). There was no significant interaction for N treatment x cultivar. There was no relationship between GY and NNI under HN or LN conditions. In 2016, NNI was higher under HN (1.19) than under LN conditions (0.77) (P=0.016). The cultivars ranged from 0.98 (HI- 8498, durum wheat) to 1.49 (BH- 1146) under HN and from 0.60 (HD- 2967) to 0.98 (K- 0307) under LN conditions (P=0.12, ns). There was no significant interaction for N treatment x genotype. There was no relationship between GY and NNI under either HN or LN conditions. Across years, NNI was higher under HN (1.09) than under LN conditions (0.70) (P=0.003). Cultivars ranged from 0.92 (CBW- 38) to 1.33 (BH- 1146) under HN and 0.58 (CBW- 38) to 0.81 (RAJ- 4229) under LN conditions (P=0.016). There was no significant N treatment x genotype or N treatment x genotype x year interaction. There was no relationship between GY and NNI under either HN or LN conditions.

4.3.7 Biomass production efficiency

In 2014, overall BPE increased from 68.1 to 90.4 kg DM kg N^{-1} under HN compared to LN conditions (P=0.09). Cultivars ranged from 57.4 to 75.1 under HN and from 79.4 to 102.2 kg DM kg N^{-1} under LN conditions (P=0.001). There was no significant N treatment x genotype interaction. In 2016, overall BPE similarly increased from 67.4 to 99.2 kg DM kg N^{-1} under HN compared to LN conditions (P=0.021). Cultivars ranged from 53.6 to 89.2 kg DM kg N^{-1} under HN and from 67.55 to 132.45 kg DM kg N^{-1} under LN conditions (P<0.001). The increase for cultivars under N limitation ranged from 0.75 to 44.5%. Averaging across years, BPE increased from 67.7 under HN to 94.8 kg DM kg N^{-1} under LN conditions (P=0.003). Cultivars ranged from 60.9 to 76.6 under HN and from 74.3 to 115.6 kg DM kg N^{-1} under LN conditions (P<0.001). There was no N treatment x genotype interaction or year x N treatment x genotypes interaction.

4.3.8 Nitrogen remobilization efficiency

In 2014, there was a slightly increase in post-anthesis N remobilization efficiency (NRE) from 0.82 under HN to 0.84 under LN conditions (P=0.004). Cultivars ranged from 0.73 to 0.88 under HN and 0.79 to 0.89 under LN conditions (P<0.001). The N treatment x genotype interaction was not significant. In 2016, post-anthesis NRE increased from 0.76 under HN to 0.83 under LN conditions (P=0.008). Cultivars ranged from 0.67 to 0.85 under HN and 0.77 to 0.87 under LN conditions (P=0.06). The N treatment x genotype interaction was not significant. Averaging across years, post-anthesis NRE increased from 0.79 under HN to 0.84 under LN conditions (P=0.001). Cultivars ranged from 0.74 to 0.84 under HN and 0.78 to 0.87 under LN conditions (P=0.006). There was no N treatment x genotype and a year x N treatment x genotype interaction. Overall, NRE showed positive association with GY under HN in 2014, under LN in 2016 and under both N conditions across year (Fig. 4.10).

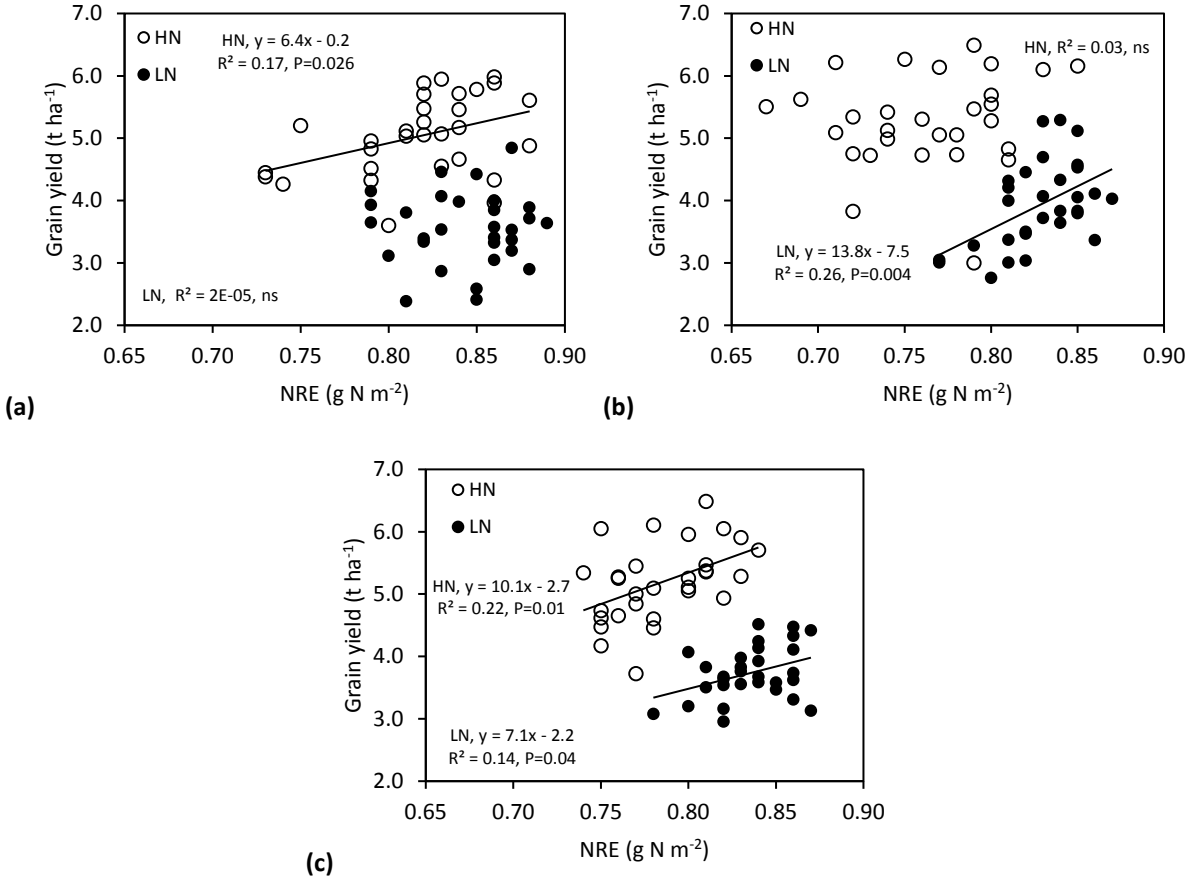


Figure 4. 10 Linear regression of N remobilization efficiency on grain yield (100% DM) for 30 cultivars in (a) 2014 (b) 2016 and (c) mean 2014 and 2016.

4.3.9 Nitrogen harvest index

In 2014, N harvest index (NHI) increased slightly from 0.85 under HN to 0.87 under LN conditions (P=0.11). Cultivars ranged from 0.78 (KHARCHIA- 65 and DBW- 14) to 0.89 (HI-8498, KRL-213 and PDW- 314) under HN and 0.82 (DBW- 14) to 90 (NW-1067) under LN conditions (P<0.001, Table 8.9). The N treatment x genotype interaction was not significant. In 2016, NHI overall increased from 0.80 under HN to 0.83 under LN conditions (P=0.018). Cultivars differed in the range of 0.71 (KHARCHIA- 65) to 0.86 (WH- 1021) under HN treatment and 0.79 (RAJ- 4238) to 85 (DBW- 71, DPW- 621-50, HI- 8498, K- 0307, KRL- 19 and WH- 1021) under LN conditions (P<0.001). There was a N treatment x genotype interaction (P=0.006). Averaging across 2014 and 2016, NHI increased from 0.82 under HN to 0.85 under LN conditions (P=0.003). Cultivars differed in the range 0.75 (KHARCHIA- 65) to 0.86 (WH- 1021) under HN and 0.83 (KRL- 210) to 0.87 (DBW- 39 and NW-1067) under LN conditions (P<0.001). Cultivar responses to N limitation ranged from -1.8 to 12.4% increase (P<0.001) but there was no significant year x N treatment x genotype interaction.

Table 4. 6 Grain N% and N Harvest Index (NHI) in 30 cultivars under HN and LN conditions in year 2014, 2016 and mean of two years.

	Grain N%						NHI					
	2014		2016		Mean		2014		2016		Mean	
	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN
BH- 1146	2.99	2.16	2.89	2.06	2.93	2.10	0.77	0.83	0.84	0.83	0.81	0.83
CBW- 38	2.27	2.27	2.71	1.92	2.53	2.06	0.84	0.84	0.77	0.84	0.80	0.84
DBW- 14	2.49	1.85	2.42	2.22	2.45	2.07	0.72	0.77	0.82	0.86	0.78	0.82
DBW- 16	2.28	1.85	2.69	1.97	2.52	1.93	0.78	0.85	0.73	0.81	0.75	0.83
DBW- 17	2.47	1.84	2.69	1.80	2.60	1.82	0.85	0.86	0.80	0.83	0.82	0.84
DBW- 39	2.46	1.82	2.22	1.82	2.32	1.82	0.84	0.88	0.82	0.85	0.83	0.86
DBW- 46	2.29	1.90	2.46	1.80	2.39	1.84	0.82	0.87	0.78	0.82	0.79	0.84
DBW- 51	2.62	2.05	2.77	1.95	2.71	1.99	0.85	0.87	0.79	0.83	0.81	0.85
DBW- 71	2.65	2.15	2.54	2.05	2.59	2.09	0.82	0.85	0.85	0.85	0.83	0.85
DPW- 621-50	2.31	1.83	2.99	1.90	2.72	1.87	0.83	0.87	0.74	0.85	0.78	0.86
GW- 322	2.16	1.80	2.55	1.58	2.40	1.67	0.84	0.85	0.78	0.84	0.80	0.84
HD- 2733	2.26	1.72	2.70	1.72	2.52	1.72	0.83	0.88	0.76	0.83	0.79	0.85
HD- 2967	2.57	2.06	2.76	1.71	2.68	1.85	0.87	0.87	0.78	0.84	0.82	0.85
HD-2932	2.54	1.99	2.51	2.08	2.52	2.04	0.85	0.86	0.84	0.84	0.84	0.84
HI- 8498 ł	2.34	1.89	2.63	1.84	2.51	1.86	0.87	0.87	0.82	0.85	0.84	0.86
HW- 2044	2.29	1.91	2.93	1.81	2.67	1.85	0.81	0.83	0.83	0.82	0.82	0.82
K- 0307	2.45	1.94	2.60	1.88	2.54	1.90	0.84	0.85	0.79	0.86	0.81	0.85
KHARCHIA- 65	2.16	1.77	2.74	1.52	2.51	1.62	0.73	0.85	0.71	0.83	0.72	0.83
KRL- 19	2.57	1.92	2.60	2.01	2.59	1.97	0.81	0.84	0.85	0.86	0.83	0.85
KRL- 210	2.71	2.08	2.63	1.76	2.66	1.89	0.82	0.80	0.82	0.83	0.82	0.82
KRL-1-4	2.22	1.87	2.37	1.86	2.31	1.87	0.82	0.84	0.82	0.86	0.82	0.85
KRL-213	2.20	1.83	2.07	1.77	2.12	1.79	0.86	0.85	0.77	0.84	0.81	0.84
MACS- 6222	2.54	1.92	2.62	1.89	2.59	1.90	0.80	0.85	0.81	0.85	0.81	0.85
MACS-2496	2.16	1.63	2.96	1.93	2.64	1.81	0.82	0.87	0.73	0.85	0.77	0.85
MACS-6478	2.30	1.89	2.55	1.71	2.45	1.78	0.84	0.86	0.84	0.84	0.84	0.85
NW-1067	2.36	1.97	2.42	1.82	2.40	1.88	0.84	0.88	0.81	0.85	0.82	0.86
PDW- 314 ł	2.27	1.77	2.91	1.71	2.65	1.73	0.87	0.87	0.80	0.84	0.82	0.85
RAJ- 4229	2.61	2.23	2.49	2.35	2.54	2.30	0.81	0.83	0.82	0.84	0.82	0.84
RAJ- 4238	2.51	2.01	2.45	1.91	2.48	1.95	0.78	0.84	0.82	0.82	0.80	0.82
WH- 1021	2.34	2.01	2.26	1.86	2.29	1.92	0.82	0.80	0.87	0.85	0.85	0.83
Mean	2.41	1.93	2.60	1.87	2.53	1.90	0.82	0.85	0.80	0.84	0.81	0.84
Min	2.16	1.63	2.07	1.52	2.12	1.62	0.72	0.77	0.71	0.81	0.72	0.82
Max	2.99	2.27	2.99	2.35	2.93	2.30	0.87	0.88	0.87	0.86	0.85	0.86
	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df
N	0.05 ns	1	0.06 **	2	0.04 ***	3	0.01 ns	1	0.01 *	2	0.004 **	3
G	0.10 ***	58	0.15 *	116	0.10 ***	174	0.02 ***	58	0.02 ***	116	0.01 ***	174
N*G	0.15 ns	42	0.22 *	95.84	0.15 *	141.5	0.02 ns	55.5	0.03 **	113.9	0.02 ***	169.9
Y					0.07 ns	3					0.01 ns	3
Y*N					0.09 ns	4.9					0.01 ns	4.4
Y*G					0.16 ns	59.8					0.02 ***	85.9
Y*N*G					0.23 ns	118.9					0.03 ns	145
H^2					0.15	0.68					0.21	NA

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level. (ł) durum wheat cultivars.

4.3.10 Trait associations

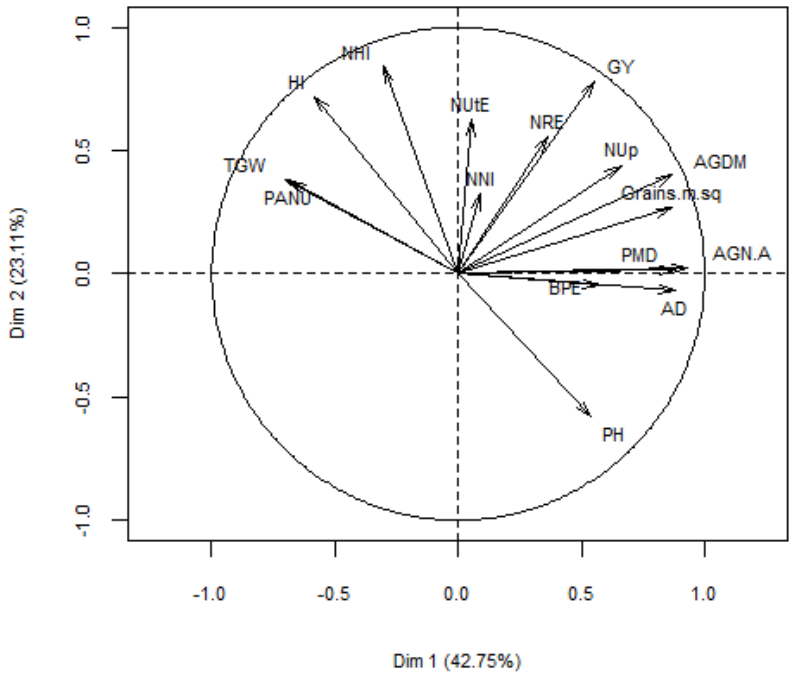
The association between grain yield and NUE and their components under the HN and LN condition is shown in the biplots in Fig. 4.14.

Under HN, PCA showed that the first two principal components (PCs) explained 65.9% of total variation. The first principal component (PC1) explained 42.75% variation and the

group of traits explaining this variation were AGN_A, PMD, AD, BPE, grains m^{-2}, and N-uptake at maturity with positive effects whereas PANU and TGW had negative effects. The second principal component (PC2) explained 23.1% of the variation and the group of traits explaining this variation included NUtE, NHI, GY, HI and NRE. Under LN, the first two PCs explained 61.5% of total variation. PC1 explained 42.2% variation and traits associated with positive effects were AD, PMD, NRE, NUtE, AGN_A, grains m^{-2}, BPE, PH and GY and PANU was associated with a negative effect. PC2 explained 19.3% of variation and traits showing association with positive effects were NUp_H, NNI, HI and TGW. There was no trait showing any negative effect.

Overall under HN, GY showed a positive association with NRE, NUp_H, and NUtE. AGDM showed a positive association with NUp_H, grains m^{-2}, AGN_A, PMD, and AD. PH showed a negative association with PANU, TGW, NHI and HI. Under LN, GY showed a positive association with AGDM, grains m^{-2}, AGN_A and NUp_H. NUtE showed a positive association with AD, PMD, NRE, NUtE, BPE, and PH and negative association with PANU and NNI. Overall, the traits that showed strongest association with GY were NRE under HN, and AGN_A and AGDM under LN conditions.

a) High N



b) Low N

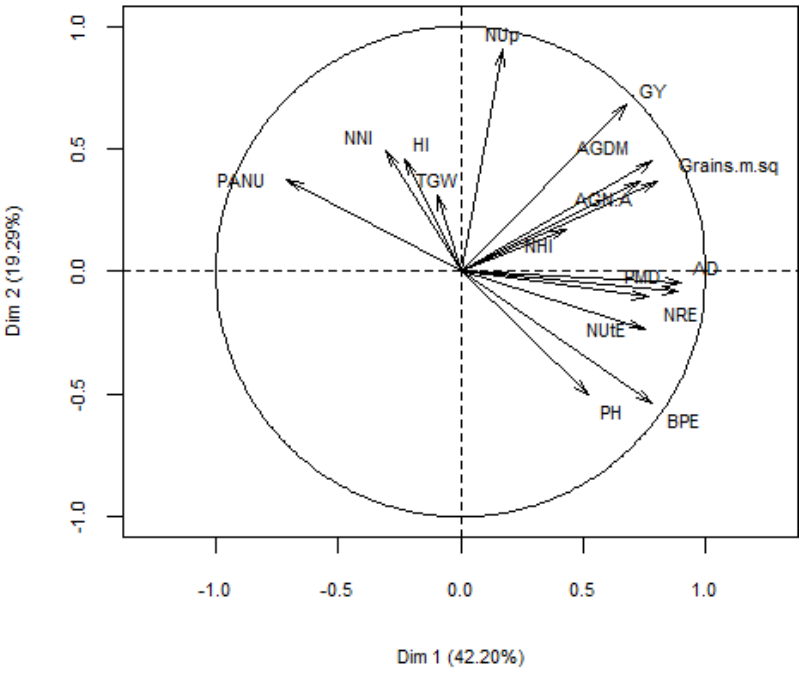


Figure 4. 11 Biplot for grain yield (GY), above-ground dry matter (AGDM) at harvest, grains m^{-2} (grains.m.sq), thousand grain weight (TGW), N-utilization efficiency (NUtE), N-uptake at harvest (NUp), N remobilization efficiency (NRE), above-ground N at anthesis (AGN.A), harvest index (HI), N nutrition index (NNI), N harvest index (NHI), biomass production efficiency (BPE), post-anthesis N-uptake (PANU), plant height (PH) and days to anthesis (AD) for 30 cv. (a) HN and (b) LN (values based on mean of year 2014 and 2016).

4.3.11 Breeding zone effect

In this experiment, the 30 cultivars were selected from six breeding zones in India. This experiment was conducted at Hol farm near Pune which is in the Peninsular Zone (PZ) of India. In India, wheat growing area is divided in to six climatic zones (Fig 1.3) and breeding programs in each zone are dedicated to breed wheat cultivars suitable for that zone. Therefore, an analysis was carried out to test for the effect of the breeding zone within the overall genotype effect. The main effect of the breeding zone was significant for all the traits except NRE, NHI, and NNI (Table 4.10). Also, there was a significant interaction for N x Breeding zone for GY, AGDM, NUpE, NUtE, NNI, HI, TGW, NHI, AGN_A and AGN_H.

The main effects of the breeding zone were that plant height was relatively higher in AZ cultivars than cultivars from other zones. Cultivars from PZ and NWPZ were late flowering under both N conditions than those from other zones. GY and AGDM was highest for the cultivars from PZ and NWPZ and lowest for cultivars from the All Zone group. Also, PZ cultivars showed better NUpE and NUtE compared to other zones. Amongst breeding zones GY was positively associated with AGN_A, AGN_H and PANU under both N conditions.

The GY interaction of N x breeding zone was mainly associated with yield potential as higher yielding zones lost relatively more yield under LN conditions; the interaction for GY was associated with AGN_H effects rather than NUtE effects. Also, cultivars from higher yielding zones overall showed lower NNI indicating higher N deficiency and indicating a requirement for higher N for better performance.

4.3.12 Broad-sense heritability

Broad-sense heritability (H^2) for important yield traits was calculated for 30 cultivars under HN and LN conditions and for most of the traits it was higher under HN condition than LN conditions (Table 4.1, 4.2 and 4.3). GY, HI, TGW, PH, AD and NUE showed H^2 higher under HN (0.65, 0.31, 0.65, 0.78, 0.73, and 0.62 respectively) than LN (0.09, 0.17, 0.46, 0.67, 0.69, 0.09 respectively).

Table 4. 7 Mean of breeding zone for plant height, anthesis date (AD), physiological maturity date (PMD), grain yield (GY), above-ground dry matter (AGDM), N-use efficiency (NUE), N-uptake efficiency (NUpE), N-utilization efficiency (NUtE), N nutrition index (NNI), harvest index (HI), thousand grain weight (TGW), biomass production efficiency (BMPE), N remobilization efficiency (NRE), N harvest index (NHI), above-ground N at anthesis (AGN_A), above-ground N at harvest (AGN_H) and post-anthesis N uptake (PANU).

	BZ	No of cultivars	Plant Height (cm)		AD (DAS)		PMD (DAS)		GY (t ha^{-1})		AGDM (t ha^{-1})		NUE (g DM g^{-1} N)		NUpE (g AGN g^{-1} N)		NUtE (g DM g^{-1} AGN)		NNI	
			HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN
1	AZ	3	77.9	73.66	58.47	57.87	98.67	95.67	4.54	3.52	9.65	7.24	15.88	18.63	0.51	0.41	32.42	46.57	1.09	0.71
2	CZ	3	67.1	65.19	61.03	59.38	99.67	93.83	5.24	3.69	10.18	7.66	18.29	19.48	0.56	0.46	33.24	43.27	1.04	0.69
3	NEPZ	11	73.4	69.46	63.86	62.89	102.59	98.77	4.94	3.71	10.62	8.02	17.23	19.70	0.55	0.46	32.17	43.16	1.08	0.71
4	NWPZ	8	70.9	67.08	65.33	64.30	105.38	100.13	5.49	3.79	11.44	8.20	19.17	20.00	0.60	0.45	33.08	44.87	1.11	0.68
5	PZ	4	71.5	68.19	65.61	63.65	104.25	98.75	5.93	4.01	11.96	8.11	20.79	21.12	0.65	0.45	33.25	47.66	1.18	0.68
6	SHZ	1	67.9	61.78	52.50	53.15	94.50	89.50	4.47	3.16	8.79	6.38	15.41	16.66	0.49	0.38	32.12	44.19	0.98	0.76
			SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df
	N		0.5 **	3	0.5 ns	3	1 *	3	0.16 **	3	0.3 **	3	0.86 ns	3	0.025 *	3	0.97 ***	3	0.045 **	3
	Y		0.71 ***	3	0.9 ns	3	0.7 ***	3	0.25 ns	3	0.37 *	3	1.26 *	3	0.042 *	3	1.21 ns	3	0.055 *	3
	BZ		1.34 ***	174	0.6 ***	174	0.9 ***	162	0.23 ***	174	0.45 ***	174	1.07 ***	174	0.036 ***	174	1.29 **	174	0.049 ns	174
	N*BZ		1.9 ns	151.38	1 ns	40.7	1.6 ns	16.2	0.35 **	53.1	0.68 *	60.78	1.7 ns	39.5	0.055 *	53.3	2.02 *	46.9	0.081 *	29.5
	N*G*BZ		2.57 ns	172.25	1.3 ns	84.3	1.9 ns	34.1	0.45 ns	103.2	0.89 ns	113.06	2.19 ns	82.3	0.072 ns	103.4	2.62 ***	94.2	0.103 ns	63.7
	N*G*Y*BZ		3.74 ns	175.73	2 *	53	2.7 *	76.3	0.68 ns	90.9	1.3 ns	134.85	3.28 ns	80.4	0.109 ns	83.1	3.84 ns	114.5	0.15 ns	83.7

			HI		TGW g		BPE		NRE		NHI		AGN_A (g N m^{-2})		AGN_H (g N m^{-2})		PANU (g N m^{-2})	
			HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN
1	AZ	3	0.48	0.49	36.56	38.68	68.44	96.49	0.77	0.84	0.81	0.85	13.36	7.63	14.28	7.70	1.09	0.08
2	CZ	3	0.52	0.48	44.10	45.21	64.64	91.08	0.78	0.82	0.84	0.85	12.65	7.86	15.90	8.72	3.23	0.89
3	NEPZ	11	0.47	0.47	37.06	41.74	68.56	93.30	0.78	0.84	0.82	0.85	14.94	8.71	15.65	8.71	0.92	0.05
4	NWPZ	8	0.48	0.46	36.58	40.16	68.56	97.24	0.81	0.83	0.83	0.85	16.38	8.23	16.98	8.53	0.77	0.22
5	PZ	4	0.50	0.49	37.27	42.28	66.71	96.81	0.78	0.84	0.82	0.86	17.07	8.03	18.31	8.53	1.79	0.42
6	SHZ	1	0.52	0.50	42.28	41.15	63.36	90.06	0.75	0.82	0.835	0.830	10.28	7.36	14.03	7.26	3.58	-0.13
			SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df
	N		0.004 ns	3	0.16 ***	3	3.12 **	3	0.004 ***	3	0.045 **	3	0.8 **	3	0.51 ***	3	0.31 *	3
	Y		0.01 ns	3	1.1 **	3	4.43 ns	3	0.009 *	3	0.055 *	3	0.8 ns	3	0.86 ns	3	0.45 ns	3
	BZ		0.008 ***	174	1.03 ***	174	2.93 *	174	0.02 ns	174	0.049 ns	174	0.9 ***	174	0.83 ***	174	0.71 **	174
	N*BZ		0.011 ***	89.8	1.42 ***	176.8	5.08 ns	20.2	0.02 ns	173	0.081 **	29.5	1.5 ***	35.6	1.24 **	76.3	1.0 ns	130.5
	N*G*BZ		0.015 **	141.2	1.94 *	175.9	6.33 *	43.7	0.03 ns	176.9	0.103 **	63.7	1.9 *	75.5	1.64 ns	129.7	1.38 ns	164.8
	N*G*Y*BZ		0.024 ns	64.2	3.01 ns	97.5	9.64 *	46.7	0.04 ns	168.9	0.15 ns	83.7	2.7 ns	112.7	2.46 ns	104.2	2.0 ns	167.3

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level.

4.4 DISCUSSION

Although in the results section, results of each year are presented separately, the discussion will be mainly based on mean results across the two years. If there is a large genotype interaction in the two years, then each case will be discussed separately.

4.4.1 Effect of plant height, anthesis and physiological maturity date on grain yield and NUE

Overall in 2016 mean plant height was 24 cm shorter under HN and 21 cm shorter under LN than in 2014. In 2016, plant height may have been affected due to soil depth and/or short periods of water stress at around stem elongation (GS32) or during the overall growing season. Experiments were conducted in the different field in the two years. In 2014 soil depth was about 90 to 100 cm and in 2016 about 60 to 90 cm. In 2016, because of shallower soil depth roots may not have been able to penetrate below 90 cm affecting water uptake. Also, in 2016, the total amount of irrigated water was less despite the total frequency of irrigation was more (seven in 2016 vs. four in 2014) due to longer growing season. Also, because of overall higher temperature during the crop growing season, may affects development during stem extension in 2016 affecting plant height (Foulkes *et al.*, 2007).

On average in both years AD was similar (64 and 61 DAS in 2014 and 63 and 63 DAS in 2016 under HN and LN respectively) whereas there was approximately a 7d difference in PM. In 2016, PM was later (107 and 103 DAS under HN and LN respectively) than in 2014 (98 and 93 DAS respectively). A longer time from AD to PMD in 2016 was mainly associated with an early sowing date in 2016. Generally, spring wheat in the PZ is sown from early November to mid-December. If sown early crop can profit from early or mid-cold weather and escape from heat stress at the end of season. Also, in 2016, in the initial two months there was higher temperature compared to 2014 followed by a decrease in temperature which favored the wheat crop in this region constituting to longer grain feeling period (Table 3.1). On average crop duration from sowing to PMD was 94 and 105 DAS in 2014 and 2016, respectively. This could also be the reason of slightly higher yield in 2016.

Cultivars also responded differently to N treatment (P=0.046) under HN and LN conditions. Also, cultivars from different breeding zones responded differently to N treatment (P=0.01). There was a significant positive association between each of GY and AGDM with AD and PMD under both N conditions which showed that later developing cultivars were able to produce higher AGDM. The association between biomass and flowering under LN could be explained by later flowering cultivars developing deeper roots and extracting more N from the soil. Alternatively, as longer duration for AD and PMD may have favored the accumulation of higher N-uptake per-anthesis and post-anthesis respectively and grain number per m^2 increasing post-anthesis demand for N, and may not be associated with root traits. A similar association between anthesis and GY explained by pre-anthesis N uptake was also reported by Gaju *et al.* (2014) in UK winter wheat cultivars.

There was a stronger positive association of AD with NRE and NUtE under LN than HN indicating that under N limiting conditions NRE and NUtE could be improved through effects of the stay-green trait. Generally, NUtE increases as the NHI increase and as N remobilized from different plant part to grain more efficiently. NUtE is a yield determining process which is affected by canopy formation and survival, construction and maintenance of photosynthetic processes, grain filling through carbohydrate and N remobilization and grain storage capacity. Crops with higher NUtE produce higher yield from the same amount of N uptake or lower N uptake under N limiting conditions. Also, there is the possibility that higher NRE was linked to higher N-uptake at anthesis under LN as there was a positive association with NRE and N-uptake at anthesis under LN than under HN. Higher H-uptake at anthesis was also associated with higher green m^{-2} and GY. If later flowering cultivars have higher N-uptake at anthesis, they may allocate relatively more above-ground N as 'reserve' N rather than 'photosynthetic' N or 'structural' N, with 'reserve' N being remobilized post-anthesis with a higher efficiency than the 'structural' or 'photosynthetic N' pools. Pask *et al.* (2012) reported similar association under HN for winter wheat. Overall under both N condition, NUtE showed a positive association with BPE (data not shown) which shows that improvement in BPE could provide potential physiological avenues to increase HI above the theoretical maximum of about 0.62 for winter wheat (Austin, 1980) and therefore increase in NUtE and grain yield under N limiting conditions. NUtE also have showed positive association

with NHI and negative association with grain N% and these associations were stronger under LN conditions indicating lower grain N content could increase grain yield under N limiting conditions.

Most of the AD effect amongst the 30 cultivars, however, was associated with the breeding zone effect. Cultivars selected for this experiment are elite cultivars from different breeding zones and have different physiology. As the experiment was conducted in the PZ, check cultivars from this zone seemed to perform better as expected. These cultivars may have adaptation to local climatic conditions due to presence or absence of genes like photoperiod-insensitive (*Ppd*) or earliness per se (eps) genes which affect flowering date (Kumar *et al.*, 2012). As monsoon rain, availability of irrigation and terminal drought are the major factors that affect grain yield in Indian wheat, modern wheat varieties are selected to be adaptable to varying climatic conditions. Kumar *et al.* (2012) showed that most of the Indian wheat cultivars have one or more of *Ppd*, *eps* or vernalization (*Vrn*) genes and depending on the frequency of these genes, these cultivars showed variation in flowering date. This may be associated with extended photosynthesis resulting in higher grain yield (Gaju *et al.*, 2016). The incorporation of Ppd-D1a allele in the post-green revolution Indian cultivars is related to the use of germplasm gained from CIMMYT (International Maize and Wheat Improvement Centre) (Borlaug, 1983).

4.4.2 Genetic variation in NUE in relation to NUpE and NUtE components.

There was genetic variation in NUE in both N treatments. The genetic variation in NUE and grain yield was more strongly associated with NUpE than NUtE under both N conditions. Furthermore, the NUE and NUpE association was stronger under LN than under HN conditions in individual years. The stronger association between NUE and NUpE under LN than HN conditions were reported for wheat by Le Gouis *et al.* (2000) in France and by Barraclough *et al.* (2010) and Gaju *et al.* (2011) in the UK. Present results showed some consistency with previous investigations in that the association between NUpE and NUE tended to increase as the degree of N limitation increased. Overall, NUtE also showed association amongst cultivars with GY and NUE under both HN and LN conditions showing NUtE is an important trait under both conditions.

Association of GY and NUE with NUtE was also reported by Barraclough *et al.* (2010) and Gaju *et al.* (2011) in UK winter wheat cultivars.

Cultivars rankings changed in the HN and LN treatment with GY reduction under N limitation ranging amongst cultivars from MACS- 6222 (42%) to DBW- 14 (14%) indicating N x G interaction. The apparent ability of some of the genotypes to maintain GY better under LN conditions was associated with a significant N x cultivar interaction for N uptake. It can be speculated that better maintenance of N uptake was associated with a more effective rooting system, but rooting traits were not measured in the present field experiments. Deeper rooting might be favored by later AD (Foulkes *et al.*, 2004) and/or a relatively deeper distribution of roots or a lower specific root mass (King *et al.*, 2003). There is also possibility of early seedling root vigor associated with N uptake and to test this a 2D seedling root phenotyping experiment was conducted on a subset of the 30 cultivars of which results are presented in chapter 7.

The apparent different responses of the cultivars to N limitation may have been partly influenced by their wheat breeding center in India. Results showed that genetic variation in AGN was mainly due to breeding zone, although there was also a trend for an effect of cultivars within a zone. There was no interaction for N x genotype (within the zone) for GY, AGDM and AGN_H, therefore the N x cultivar interaction for GY appeared to be mainly explained by the different responses of the cultivars according to breeding zone. However, there was an N x G x Breeding zone interaction for NUtE. The effect of the breeding zone on NUtE could be mainly explained by the variation in anthesis date. Overall the two durum wheat cultivars (HI-8498 and PDW-314) in this experiment showed similar performance as that of the bread wheat cultivars.

NUtE showed a positive correlation with NUE and GY under both N conditions. If NHI (grain N / AGN_H) remains unchanged, then increasing NUtE will result in decreased grain N% (Foulkes *et al.*, 2009). MACS-6478 and MACS-6222 were high yielding cultivars in this experiment and both come from PZ showing adaptation to this climatic zone. High yield of these cultivars was associated with overall better performance for important traits like AGDM and N-uptake at anthesis. The two highest yielding cultivars under LN (PDW-314, GW-322) also maintained GY better than other genotypes under LN associated with increasing NUtE and relatively greater

decreases in grain N% under LN conditions. Therefore, it may be inferred that the ability of a genotype to produce a viable grain with a minimal grain N% may be related to increased grain yield under LN conditions. Van Keulen (1977) reported that the minimum level of protein dilution in small grain cereals was 9.2% and in the present experiment the minimum value observed was 9%. Although selection for low grain N% may not be possible where high grain N% is required for the end-use market, e.g. for bread-making cultivars; this strategy may be possible for wheat that is used for animal feed, distillation or biofuel market. The Indian cultivars studied in this experiment were bread making cultivars and high protein content is an important trait for Indian cultivars as 90% wheat in India use for *chapatti* making where high protein content (above 9%) increases nutritional value (Bedekar, 2001). In this experiment, average protein content was 12.6% and 10.3% under HN and LN conditions respectively assuming conversion factor of 5 and 5.4 which was estimated respectively based on first-year protein estimation using NMR. These values are in the range of hard wheat (>12%), medium hard (>10%) and soft wheat (<9.5%) used for chapatti making (Nagarajan, 2006).

Overall AGN_A and AGN_H was lower under LN conditions as N supply was limiting. However, for some of the cultivars (around 10%) AGN_A values were higher than AGN_H and therefore PANU was negative for these cultivars. This could be due to an underestimation of AGN_H for these cultivars during analysis of crop components. N estimation at anthesis was done for all plant components (flag-leaf, stem with leaf lamina and ear) whereas at harvest N estimation was not done for chaff and assumed to be same as that of stem and leaf lamina N. In such case, if some of the cultivars have less N at harvest in stem and leaves than in chaff, this may have under estimated the AGN_H.

There was a positive association between GY and AGN_A and AGN_H under both the N levels but the association was stronger between GY and AGN_H. The stronger association between GY and AGN_H shows that N accumulation at the end of growth cycle as expected was more responsible for yield than at anthesis. There was no association of crop NRE with grain m^{-2} (an indicator of sink-strength) under HN but there was under LN ($P=0.03$); and crop NRE showed stronger positive association with NUp_A under LN ($P<0.001$) then HN ($P=0.02$) which indicates

that under N limiting conditions NRE may be partly driven by sink (grain m^{-2}) as well as source strength (NUp_A).

Crop NRE showed a negative association with PANU under HN and LN and this could be explained by the fact that N accumulation in non-grain crop components may have occurred during grain filling. Also, there is possibility of feedback mechanism involved between PANU and NRE where if PANU is higher, then, there is less demand from grain for N to be remobilized from vegetative tissues.

4.5 CONCLUSION

Overall high yielding cultivars were mainly associated with later flowering, and these cultivars lost more yield under LN conditions which also showed the yield response was related to the yield potential of the individual cultivars. Mostly genetic variation in yield appeared to be due to N uptake. A similar result was also reported by Gaju *et al.* (2011) in UK and French winter wheat cultivars. Also, grain yield was strongly related to N uptake at maturity which was related to the maturity date. Effect of the zone on the difference on HI showed that NUtE was positively associated with HI. Also, results showed that there was a consistent effect of the breeding zone in the different years. Generally, amongst the cultivars there was no association found between NUpE and NUtE.

Overall, in this chapter, genetic variation in GY seems to be due to variation in N uptake at harvest under both N conditions and NUpE was playing more important role in determining GY than NUtE efficiency. Also, higher N-uptake both pre-anthesis and post-anthesis seems related to AD and PMD and these results implied a role of stay-green traits determining yield. Moreover, greater N-uptake could be associated with root architectural traits as deeper or denser root system could extract more N from soil under N limited conditions (Foulkes *et al.*, 2009). Therefore, in next two chapters, above-ground senescence related (chapter 5) and below ground root system architecture related (chapter 6) traits that could be explaining genetic variation in N-uptake will be discussed in detail.

Summary

In summary, the findings in relation to the hypotheses tested in this chapter were:

"Under LN conditions NUpE plays a more important role in determining genetic variation in NUE than NUtE".

This hypothesis was partially supported as results showed that genetic variation in N uptake was more strongly correlated with NUE than NUtE under both N conditions. However, the strength of the correlation between N uptake and NUE was similar under LN and HN conditions.

"There was genetic variation for N-use efficiency and its components traits that are related to NUpE and NUtE in the panel of 30 elite Indian spring wheat cultivars."

As discussed there was genetic variation for N-use efficiency and its component traits that was significantly related to N uptake and NUtE in the panel of 30 elite Indian spring wheat cultivars. The strongest correlation with NUE was for N uptake at anthesis under both N conditions.

"There is a N x genotype interaction for grain yield in Indian cultivars which can be explained by physiological traits."

Significant N x G interaction was observed for GY, HI, NUtE, AGN_A, AGN_H, grain N% and NHI. The main trait explaining the interaction for GY was NUpE at anthesis and at harvest.

CHAPTER 5

Field Experiments: Effects during pre- and post-anthesis growth

5.1 INTRODUCTION

This chapter presents the results for the physiological determinants of crop growth in the period from emergence to physiological maturity and investigates the sequence of yield forming processes pre- and post-anthesis in relation to N treatments and cultivars and their interaction.

Wheat crops during growth accumulate N in plant components and remobilize N to grain during grain filling. Therefore, accumulation and remobilization of N are important processes affecting final grain yield and quality of grain (Barraclough *et al.*, 2010; Gaju *et al.*, 2011). N accumulation before anthesis provides the major source of grain N in wheat and it has been reported that approximately 50 to 95% of the grain N at harvest comes from the remobilization of N that is stored in the shoots and roots before anthesis (Kichey *et al.*, 2007). The important source of such stored N to grain N mainly comes from leaves and stems (Critchley, 2001) and the roots and chaff contribute about 10 and 15%, respectively (Dalling, 1985).

N-remobilization efficiency (NRE; the proportion of N in the crop or crop component at anthesis which is not present in the crop or crop component at harvest) depends on the amount of N remobilized to the grain in the post-anthesis period and on the amount of N stored in vegetative parts at anthesis. Previous studies on several different crops found that genetic control of N remobilization is linked to the regulation of leaf senescence (Masclaux *et al.*, 2001; Uauy *et al.*, 2006). In such cases, the stay-green trait, that is, the ability of the plant to retain green canopy during grain filling, may be associated with N remobilization (Gaju *et al.*, 2014). In sorghum, the stay-green trait has been related to the N supply-demand balance during grain filling (Van Oosterom *et al.*, 2010). Martre *et al.* (2003) suggested that it's not the grain N demand that is responsible for driving N from vegetative tissues to grain but N relocation is driven by the

N source. Developing cultivars that stay-green and photosynthesize for a longer time by modifying NRE could be advantageous to produce a higher yield. However, longer photosynthesis for same amount N available could eventually reduce the grain N concentration affecting grain N quality. In that case, such a strategy could be used for feed wheat cultivars rather than bread-making wheat where grain N is an important trait. Gaju *et al.* (2011) reported that the onset of post-anthesis senescence amongst 16 wheat cultivars grown in the field in France and the UK was negatively correlated with the crop NRE under low N supply, whereas under high N supply there was no correlation. This result suggested that under N-limiting conditions grain growth of wheat crops may be mainly source-limited; under non-N-limiting, optimal conditions, grain growth is reported to mainly sink-limited (Fischer, 2008). Therefore, under N limiting conditions stay-green traits may be responsible for higher yield. Also, post-anthesis, a deep root system can potentially increase the stay-green effect by taking up more N after flowering and from soil at depth (Foulkes *et al.*, 2009).

The wheat growing season in the Peninsular zone of India, is one of the shortest wheat growing seasons in the world and terminal heat stress is one of the common and major abiotic cause of wheat yield reduction. Under heat stress, the photosynthetic process is affected especially during grain filling stage when demand for assimilates is the greatest and grain filling period become shorter with higher temperature. Hence, the stay-green trait, which is believed to affect radiation-use efficiency, could be important under heat stress. Kumari *et al.* (2007) have reported a significant association between heat tolerance and stay-green in 936 Indian and CIMMYT spring wheat lines. Kumar *et al.* (2010) suggested that delay in leaf senescence near physiological maturity stage may promote higher translocation and grain filling rates with direct effect on yield components. Kumar *et al.* (2016) reported an association between QTLs of a stay-green trait with heat tolerance trait in Indian wheat lines. Genetic variation for functional stay-green (delayed senescence associated with extended photosynthesis) has reported in bread wheat (Silva *et al.*, 2001; Verma *et al.*, 2004; Foulkes *et al.*, 2007; Gaju *et al.*, 2011). Although, there are some recent experiments conducted on Indian wheat cultivars where traits related to NUE were studied (Kaur *et al.*, 2016; Sathisha and Desai, 2016) and these experiments did not include detailed physiological analysis and NRE and stay-green traits were not measured.

Stay-green traits can be assessed in the field using different techniques. Visual stay-green scoring can be rapid and doesn't required any specific equipment other than diagnostic chart. The negative side of this technique is that scoring is subjective and could vary between observers. Visual assessments have been performed for the number of green leaves per culm (Haussmann *et al.*, 1999), the greenness of the flag leaf of all fertile shoots (Foulkes *et al.*, 2007) and greenness of just the flag leaf and peduncle (Joshi *et al.*, 2007a). More objective methods have also been applied, with measurements of individual leaves using instruments such as the Minolta SPAD meter (Christopher *et al.*, 2008). However, such methods can be time-consuming and impractical for measurements of large numbers of genotypes, as they require repeated measurements of multiple leaves to provide reliable information for a single experimental plot. In such cases, the normalized difference vegetative index (NDVI) measured using a spectroradiometer such as the hand-held Greenseeker (NTech Industries, Ukiah, CA, USA) (Kipp *et al.*, 2014) could be a better option. In this experiment, visual senescence scoring, and SPAD were used for flag-leaf senescence whereas NDVI was used to measure the canopy senescence kinetics.

The main hypotheses examined were that:

(i) N accumulation at anthesis in the flag-leaf, stem and remaining leaves and ear is a determinant of genetic variation in post-anthesis N remobilization from these respective plant components.

(ii) Leaf-lamina N remobilization is a determinant of genetic variation in flag-leaf senescence.

(iii) Different techniques of leaf and canopy senescence measurement give similar results for relative genotype performance and responses to N availability.

(iV) Stay-green traits show a stronger association with genetic variation in yield under N limitation compared to optimal N conditions.

The specific objectives were to:

(i) investigate the physiological basis of genetic variation in N accumulation in plant components (flag-leaf lamina, stem-and-remaining-leaves and ear) at anthesis and N remobilization and responses to N availability and

(ii) examine associations between N accumulation and N partitioning in plant components (flag-leaf lamina, stem-and-remaining-leaves and ear) at anthesis and N remobilization and flag-leaf senescence kinetics and grain yield under HN and LN conditions. The hypotheses were tested in two field experiments examining 30 Indian cultivars grown under high and low N conditions at ARI, Pune India.

5.2 MATERIAL AND METHODS

This section describes the methodology used in physiological measurements in the two field experiments conducted in 2014 and in 2016 at ARI, Pune, India. The experimental design and field plot management practices were described in the previous chapter.

5.2.1 N nutrition index and N partitioning index

N nutrition index (NNI) was estimated according to the ratio of the actual above-ground crop N% at anthesis (GS61) and the critical N% at anthesis estimated according to the 'critical dilution curve' described for wheat by (Justes *et al.*, 1994). The N partitioning index (NPI) is the proportion of above-ground N in the crop component.

5.2.2 Flag-leaf senescence parameters

Senescence kinetics were assessed visually every 7 days in each sub-plot by recording the percentage of the flag-leaf green area senesced after anthesis (GS61) for main shoot on five tagged plants in 2013-4 or for the whole flag-leaf layer in 2015-6 using a standard diagnostic key based on a scale of 0 to 10 (0 to 100% senesced, Fig. 5.1). For each sup-plot, the regression analysis was done by fitting senescence score against thermal time (base temperature of 0°C) by using a modified version of an equation with five parameters consisting of a monomolecular and a logistic function (Eq.3.7) (Génard *et al.*, 1999), as described by Gaju *et al.* (2011) and as described in Chapter 3 (section 3.1.2.11).

5.2.3 Flag-leaf chlorophyll content

The relative chlorophyll content of the flag-leaf was measured from anthesis (GS61) to flag-leaf 100% senescenced using a hand-held meter (model SPAD 502, Minolta, Japan) as an indicator of the relative chlorophyll content (Miller *et al.*, 1999; Lopez-Bellido *et al.*, 2004). In the year 2014 five fertile shoots were tagged whereas in 2016 three fertile shoots in each sub-plot

were randomly selected. The measurements were made every 7 days for each flag-leaf at three different positions (basal, middle and apical). Modified Gompertz curves (Eq 5.2) were fitted to the flag-leaf SPAD values against thermal time (t) (base temp. 0^{o}C after anthesis, GS61) for each replication under each sup-plot treatment as described in Chapter 3 (section 3.1.2.12). Values of thermal time when SPAD values were 90% and 10% were taken as the Onset (SS.Onset) and end (SS.End) of flag-leaf senescence.

5.2.4 Normalised difference vegetation index (NDVI)

The NDVI was measured in each experiment in each sub-plot approximately every 7 days from booting (GS41) until late grain-filling using a FieldScout CM 1000 NDVI Chlorophyll Meter (Spectrum Technologies, USA). The measurements were taken between 10.00 and 15.00 on clear sunny days. The NDVI chlorophyll meter sensor was held 50 cm above the crop. NDVI was calculated from measurements of light reflectance in the red and near infrared (NIR) regions of the spectrum as:

$$NDVI = (R_{900} - R_{680})/(R_{900} + R_{680}) \qquad \text{Equation 5.1}$$

where R_{680} and R_{900} are the reflectances at 680 and 900 nm, respectively. Similarly, to SPAD analysis, modified Gompertz curves (Eq 5.1) were fitted to NDVI values over thermal time (t) post-anthesis (GS61) for each replication in each treatment combination. NDVI values before thermal time 400 °Cd (NDVI pre-tt400) and after thermal time 400 °Cd (NDVI post-tt400) post-anthesis (GS61) were averaged and used as indicators of stay-green.

5.2.5 Canopy temperature

Canopy temperature was recorded in each experiment on a sub-plot basis using a hand-held infrared thermometer (sixth Sense LT300 Infrared Thermometer) on bright sunny days between 11.00 and 14.00 hours, at seven days intervals, starting from anthesis (GS61) until the late milk stage (GS79).

5.2.6 Leaf photosynthesis (A_{max}), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and maximum efficiency of PSII (Fv'/Fm') in HN treatment

Leaf photosynthesis measurements were taken only in 2015-6 under HN conditions. Light-saturated photosynthetic rate (A_{max}), stomatal conductance (g_s), intercellular carbon dioxide concentration (C_i) and maximum efficiency of PSII (Fv'/Fm') of the flag leaf were measured using a Li-Cor LI-6400XT Portable Photosynthesis System (Lincoln, NE, USA) (Fig. 3.1 e). In each sub-plot, a single reading on a single flag-leaf of two randomly selected fertile primary shoots were taken. The instrument was calibrated for humidity at 50–60% relative humidity and the settings for the A_{max} readings were: photosynthetically active radiation (PAR) 1500 µ mol $m^{-2}s^{-1}$, sample chamber CO_2 concentration 360 µl l^{-1} and flow rate 400 µ mol s^{-1}. The measurements were taken between 10.00 and 15.00 around 1-2 weeks after anthesis. Chlorophyll fluorescence of the flag leaf was also measured at the same time of A_{max} measurements.

5.3 RESULTS

In this section results of physiological traits studied in 30 cultivars from the field experiments in 2014 and in 2016 are presented. In this chapter results on N-uptake at anthesis and at harvest in crop components, N partitioning at anthesis, N remobilization efficiency and flag-leaf and canopy senescence related traits will be presented.

5.3.1 N accumulation in crop components at anthesis

Overall, above-ground N at anthesis (AGN_A) date for individual years was presented in chapter 4. Here only across years data for AGN_A is again presented. Across years, above-ground N at anthesis (AGN_A) was reduced from 150.6 kg N ha^{-1} under HN to 82.5 kg N ha^{-1} under LN conditions (P=0.003; Table 4.6). Cultivars ranged from 102.8 (HW- 2044) to 198.1 (HD- 2967) kg N ha^{-1} under HN and from 62.8 (KRL- 210) to 111.3 (DBW- 46) kg N ha^{-1} under LN conditions (P=0.001). Cultivar decrease under LN ranged from 22.9 to 62.3% (P=0.001), whereas the year x N treatment x genotype interaction was not significant.

5.3.1.1 Flag-leaf N uptake at anthesis

In 2014, as N supply increased flag-leaf N uptake (N_A) increased from 0.52 (LN) to 1.35 (HN) g m^{-2} but this increase was not significant (Table 5.1, Fig. 5.4 a and b). Under HN, flag-leaf N_A ranged amongst the 30 cultivars from 0.7 to 2.0 g m^{-2} and under LN conditions from 0.30 to 0.88 g m^{-2} (P<0.001). There was no interaction for N treatment x genotype. In 2016, as N supply increased flag-leaf N_A increased from 0.92 (LN) to 1.87 (HN) g m^{-2} (P=0.009; Table 5.1, Fig. 5.4 c and d). Under HN, flag-leaf N_A ranged from 1.1 to 2.8 g m^{-2} and under LN conditions from 0.6 to 1.4 g m^{-2} (P<0.001). There was no interaction between N treatment and genotype. Across years, as N supply increased flag-leaf N_A increased from 0.8 (LN) to 1.7 (HN) g m^{-2} (P<0.001; Table 5.1, Fig. 5.4 e and f). Under HN, flag-leaf N_A ranged from 1.1 to 2.2 g m^{-2} and under LN conditions from 0.5 to 1.0 g m^{-2} (P<0.001). There was no N treatment x genotype or year x N treatment x genotype interaction.

5.3.1.2 Stem and remaining leaf N uptake at anthesis

In 2014, as N supply increased, the uptake of N at anthesis in the stem and remaining leaves (i.e. excluding flag-leaf) (StL N_A) increased from 4.7 (LN) to 9.3 (HN) g m^{-2} (Table 5.1, Fig. 5.4 a and b). Under HN, StL N_A ranged amongst the 30 cultivars from 5.9 to 13.4 g m^{-2} and under LN conditions from 2.9 to 7.0 g m^{-2} (P=0.15). There was no significant interaction between N treatment and genotype. In 2016, as N supply increased StL N_A increased from 4.6 (LN) to 8.9 (HN) g m^{-2} (P=0.018; Table 5.1, Fig. 5.4 c and d). Under HN, StL N_A ranged from 4.4 to 13.5 g m^{-2} and under LN conditions from 2.5 to 7.3 g m^{-2} (P<0.001). There was an interaction between N treatment and genotype with responses to N limitation ranging from 3.2 to 73.5% reduction (P=0.019). Across years, as N supply increased StL N_A increased from 4.6 (LN) to 9.1 (HN) g m^{-2} (P=0.004; Table 5.1, Fig. 5.4 e and f). Under HN, StL N_A ranged from 5.8 to 12.0 g m^{-2} and under LN conditions from 3.6 to 6.3 g m^{-2}. There was an N treatment x genotype (P=0.007) but no year x N treatment x genotype interaction.

5.3.1.3 Ear N uptake at anthesis

In 2014, as N supply increased ear N uptake at anthesis (ear N_A) increased from 2.7 (LN) to 3.8 (HN) g m^{-2} (Table 5.1, Fig. 5.4 a and b). Under HN, ear N_A ranged from 2.4 to 5.5 g m^{-2} and under LN conditions from 1.6 to 4.0 g m^{-2} (P=0.001). There was no significant interaction between N treatment and genotype. In 2016, as N supply increases ear N_A increased from 3.0 (LN) to 4.7 (HN) g m^{-2} (P=0.004; Table 5.1, Fig. 5.4 c and d). Under HN, ear N_A ranged from 2.1 to 8.0 g m^{-2} and under LN conditions from 1.7 to 5.1 g m^{-2} (P<0.001). There was an interaction between N treatment and genotype (P<0.001) with reduction under N limitation ranging from -8.6 to 60.2%. Across years, as N supply increased ear N_A increased from 2.9 (LN) to 4.3 (HN) g m^{-2} (P=0.002; Table 5.1, Fig. 5.4 e and f). Under HN, ear N_A ranged amongst cultivars from 2.9 to 6.1 g m^{-2} and under LN conditions from 2.1 to 4.2 g m^{-2} (P<0.001). There was an N treatment x genotype (P<0.001) and year x N treatment x genotype (P=0.006) interaction.

Table 5. 1 Flag-leaf N (FL N_A), Stem and leaves N (StL N_A) and Ear N (Ear N_A) at anthesis (GS61) in 30 cultivars under HN and LN conditions in 2014, 2016 and mean across years.

	FL N_A (g m^{-2})						StL N_A (g m^{-2})						Ear N_A (g m^{-2})					
	2014		2016		Mean		2014		2016		Mean		2014		2016		Mean	
	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN
Mean	1.35	0.52	1.87	0.92	1.66	0.76	9.33	4.66	8.94	4.63	9.10	4.64	3.80	2.66	4.65	2.97	4.31	2.85
Min	0.74	0.30	1.14	0.57	1.10	0.50	5.92	2.86	4.43	2.52	5.78	3.61	2.43	1.55	2.09	1.66	2.91	2.06
Max	1.99	0.88	2.81	1.37	2.23	1.04	13.40	6.96	13.48	7.27	11.97	6.33	5.50	3.96	7.95	5.13	6.13	4.23
	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df
N	0.11 ns	1.0	0.09 **	2.0	0.07 ***	3.0	1.14 ns	1	0.59 *	2	0.56 **	3	0.36 ns	1	0.12 **	2	0.15 **	3
G	0.17 ***	58.0	0.23 ***	116.0	0.16 ***	174.0	1.06 ns	58	1.17 ***	116	0.84 ***	174	0.45 **	58	0.49 ***	116	0.35 ***	174
N*G	0.26 ns	22.0	0.33 ns	99.5	0.23 ns	132.5	1.87 ns	6.82	1.73 *	74.47	1.29 **	64.2	0.72 ns	14.29	0.69 ***	117.57	0.51 ***	139.8
Y					0.05 **	3.0					0.54 ns	3					0.24 ns	3
Y*N					0.09 ns	5.2					0.79 ns	6					0.28 ns	5.07
Y*G					0.23 **	170.0					1.3 ***	71.5					0.55 ***	65.4
Y*N*G					0.33 ns	170.2					1.85 ns	107.2					0.76 **	124.9

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level

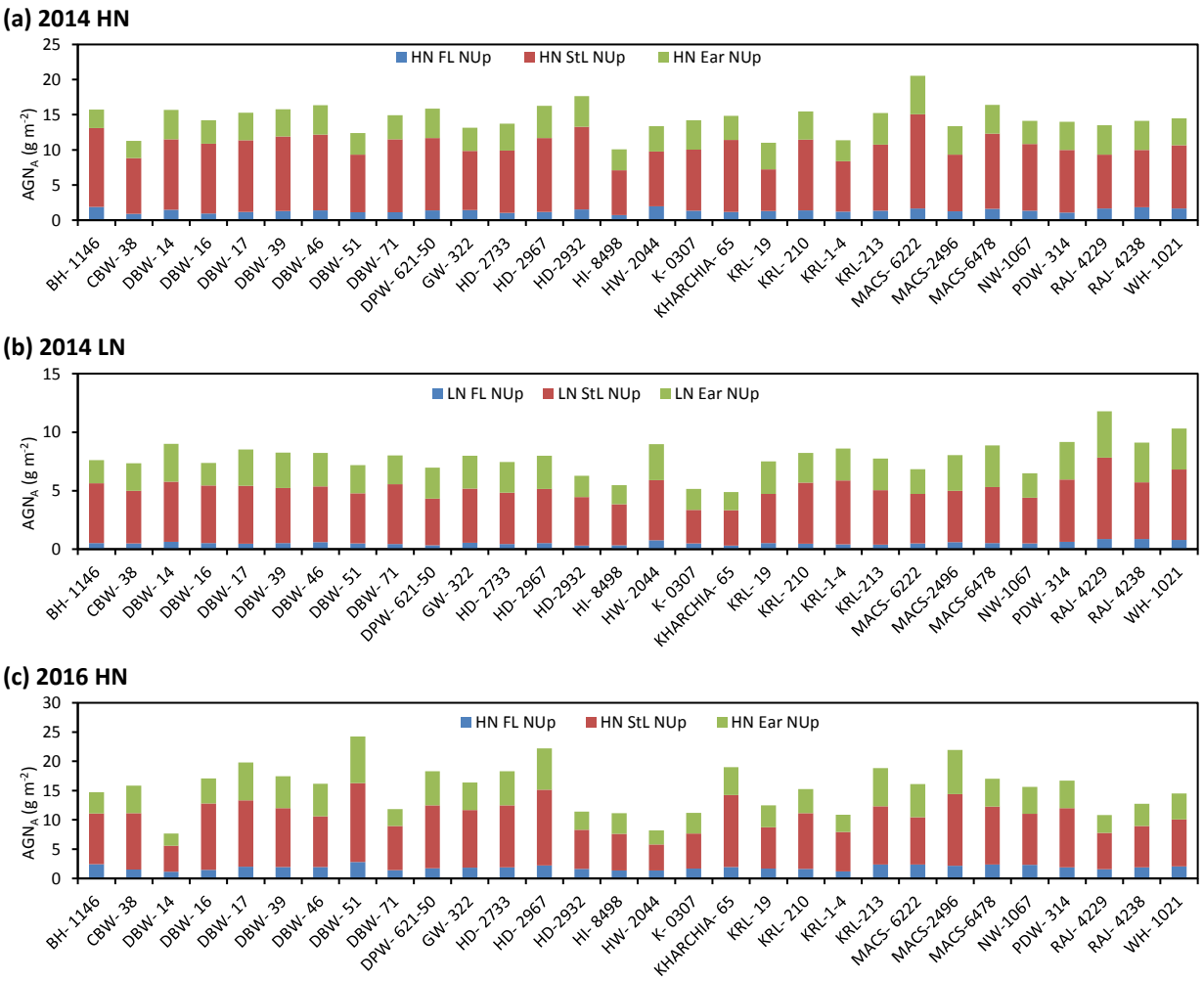
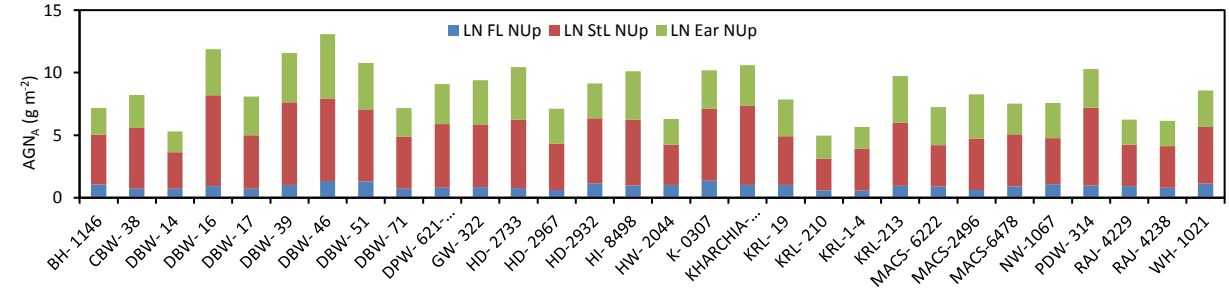
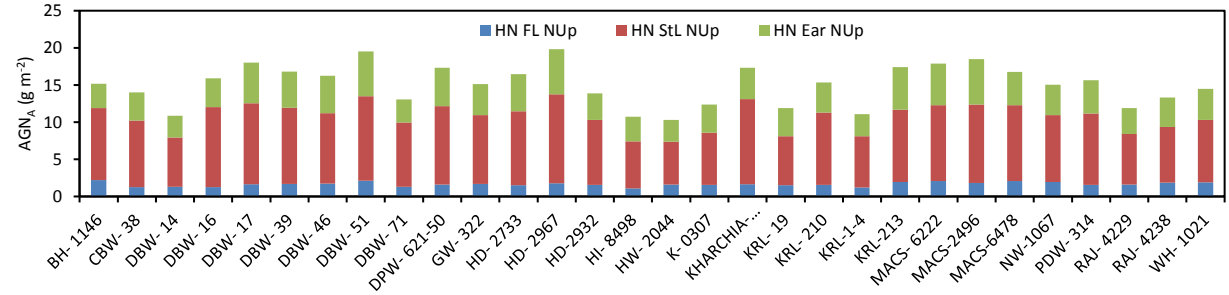


Figure 5. 1 Above-ground N uptake at anthesis under high N (HN) and low N (LN) in flag-leaf (FL), stem and remaining leaf (StL) and Ear at anthesis in 30 cultivars in (a,b) 2014, (c,d) 2016 and (e,f) across years. (Stat in Table 5.1)

(d) 2016 LN



(e) Across years HN



(f) Across years LN

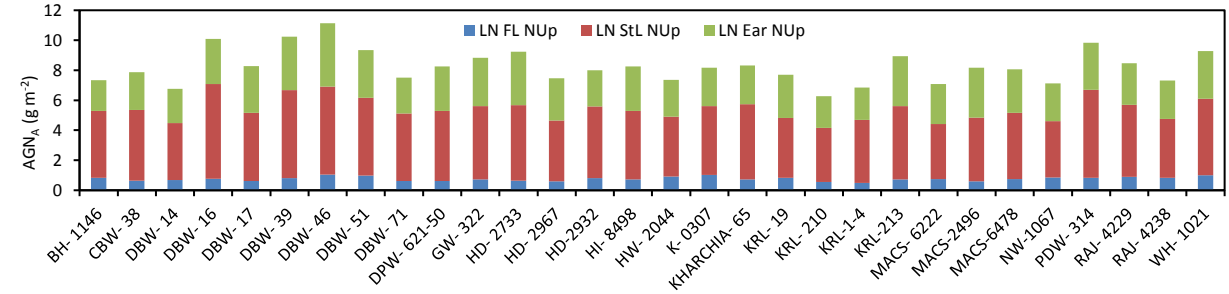


Figure 5. 1 Contd. Above-ground N uptake at anthesis under high N (HN) and low N (LN) in flag-leaf (FL), stem and remaining leaf (StL) and Ear at anthesis in 30 cultivars in (a,b) 2014, (c,d) 2016 and (e,f) across years. (Stat in Table 5.1)

5.3.2 N partitioning Index at anthesis

Overall, amongst 30 cultivars, the proportion of N in the flag-leaf and stem and leaves increased with N supply while the proportion of N in the ear decreased (Fig. 5.5). Overall, the amount of above-ground N in different crop components was reduced with N limitation in the 30 cultivars. Distribution of N in crop components was 11%, 60%, 29% under HN conditions and under LN conditions it was 10%, 56%, 34% for FL, StL and Ear, respectively. Overall, under LN conditions partitioning of N to the ear was relatively higher (34%) than under HN conditions (29%).

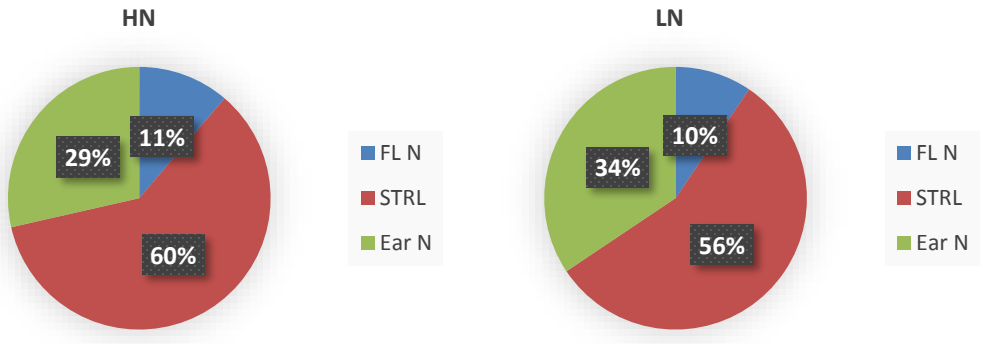


Figure 5. 2 Percentage (%) of total N uptake at anthesis in flag-leaf, stem + leaf, and ear (mean of 2013-4 and 2015-6) among 30 Indian cultivars under high N (HN) and low N (LN) conditions.

5.3.2.1 Flag-leaf NPI

In 2014, flag-leaf N partitioning index at anthesis (FL NPI_A) decreased overall with N deficiency from 0.10 (HN) to 0.07 (LN) (P=0.008; Table 5.2, Fig. 5.6 a and b). Under HN, FL NPI_A ranged from 0.07 to 0.15 and under LN conditions from 0.05 to 0.10 (P<0.001). There was no significant interaction between N treatment and genotype. In 2016, FL NPI_A did not decrease overall significantly with N deficiency: 0.12 (HN) vs 0.11 (LN) (Table 5.2, Fig. 5.6 c and d). Under HN, FL NPI_A ranged from 0.09 to 0.17 and under LN conditions from 0.07 to 0.16 (P<0.001). There was no significant interaction between N treatment and genotype. Across years, FL NPI_A decreased overall with N deficiency from 0.11 (HN) to 0.09 (LN) (P=0.012; Table 5.2, Fig. 5.6 e and f). Under HN, FL NPI_A ranged from 0.08 to 0.16 and under LN conditions from 0.07 to 0.13 (P<0.001). There was no N treatment x genotype or year x N treatment x genotype interaction.

5.3.2.2 Stem and remaining leaf NPI

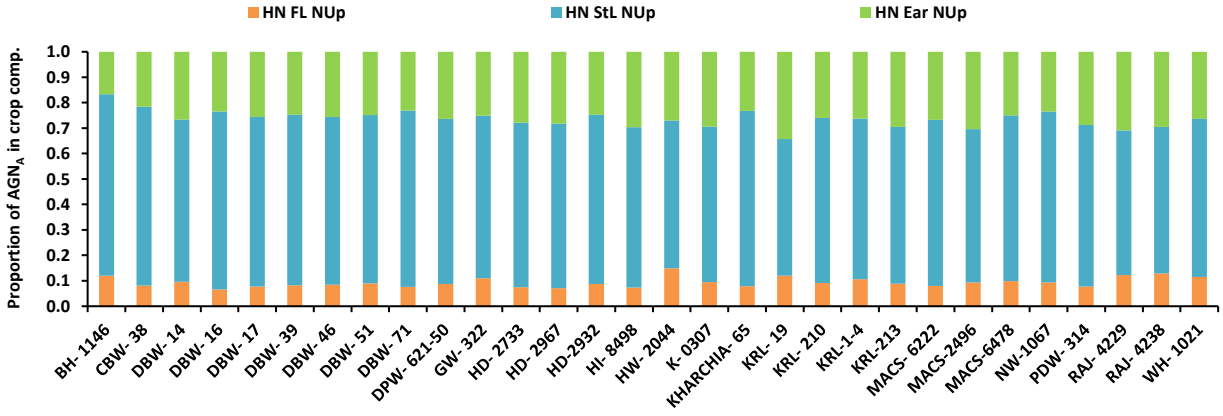
In 2014, stem and remaining leaf N partitioning index at anthesis (StL NPI_A) decreased overall with N deficiency from 0.64 (HN) to 0.59 (LN) (Table 5.2, Fig. 5.6 a and b). Under HN, StL NPI_A ranged from 0.50 to 0.72 and under LN conditions from 0.53 to 0.67 (P=0.003). There was no interaction between N treatment and genotype. In 2016, StL NPI_A decreased overall with N deficiency from 0.58 (HN) to 0.54 (LN) but this decrease was not significant (Table 5.2, Fig. 5.6 c

and d). Under HN, StL NPI_A ranged from 0.50 to 0.66 and under LN conditions from 0.45 to 0.62 ($P<0.001$). There was no interaction between N treatment and genotype. Across years, StL NPI_A decreased overall with N deficiency from 0.61 (HN) to 0.56 (LN) ($P=0.037$; Table 5.2, Fig. 5.6 e and f). Under HN, StL NPI_A ranged from 0.54 to 0.68 and under LN conditions from 0.52 to 0.65 ($P<0.001$). There was no N treatment x genotype or year x N treatment x genotype interaction.

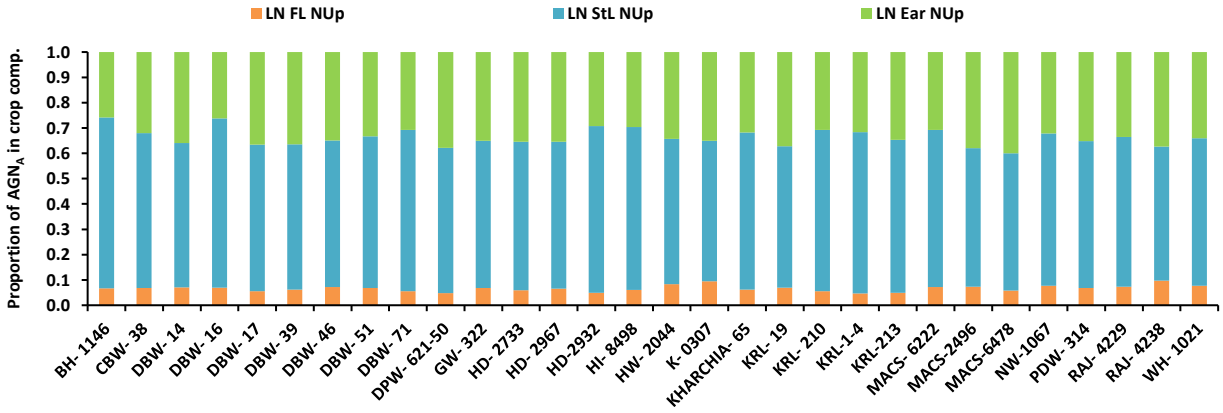
5.3.2.3 Ear NPI

In 2014, ear NPI_A overall increased with N deficiency from 0.26 under HN to 0.34 under LN conditions but this increase was not significant (Table 5.2, Fig. 5.6 a and b). Under HN, ear NPI_A ranged from 0.16 to 0.37 and under LN conditions from 0.26 to 0.40 ($P=0.019$). There was no interaction between N treatment and genotype. In 2016, ear NPI_A overall again increased with N deficiency from 0.30 under HN to 0.35 under LN conditions but also not significantly (Table 5.2, Fig. 5.6 c and d). Under HN, ear NPI_A ranged from 0.24 to 0.35 and under LN conditions from 0.29 to 0.44 ($P<0.001$). There was no interaction between N treatment and genotype. Averaging across years, ear NPI_A increased with N deficiency from 0.28 under HN to 0.35 under LN conditions ($P=0.01$; Table 5.2, Fig. 5.6 e and f). Under HN, ear NPI_A ranged from 0.21 to 0.35 and under LN conditions from 0.28 to 0.41 ($P<0.001$). There was no N treatment x genotype or year x N treatment x genotype interaction.

(a) 2014 HN



(b) 2014 LN



(c) 2016 HN

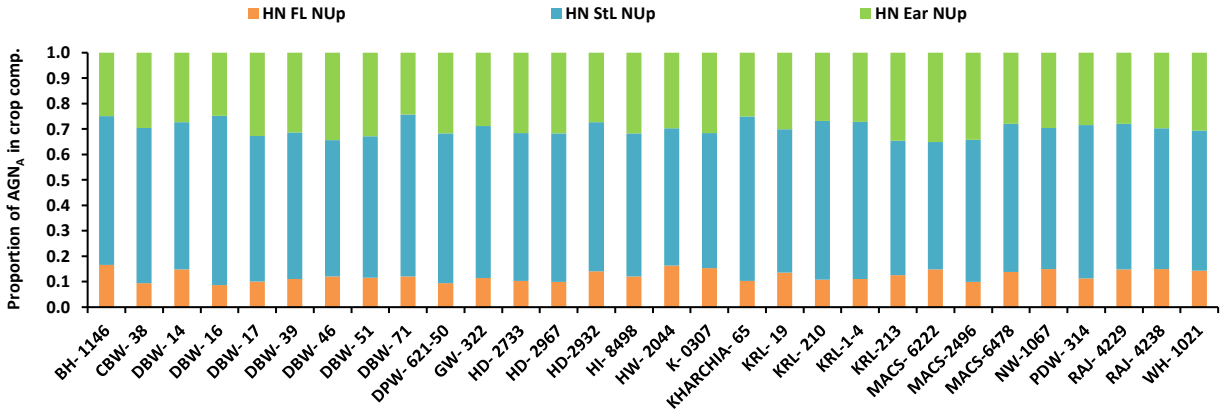
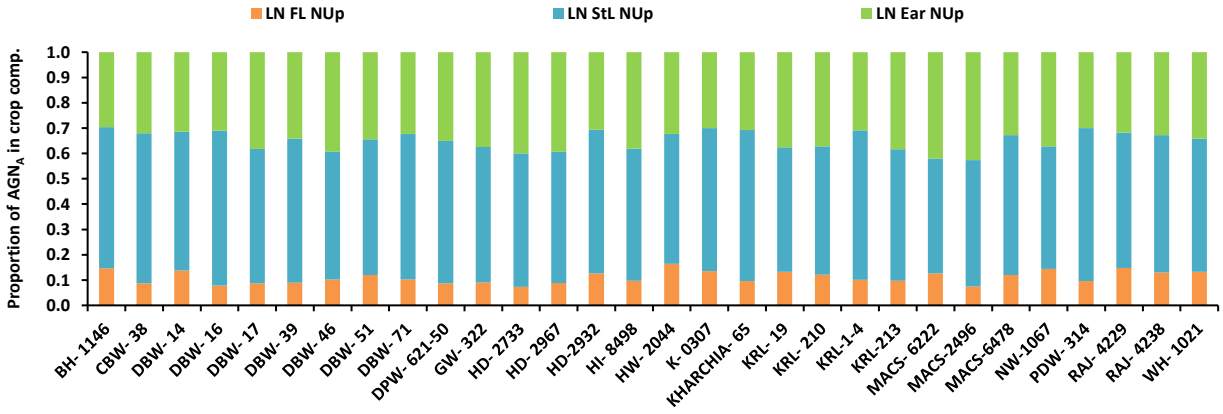
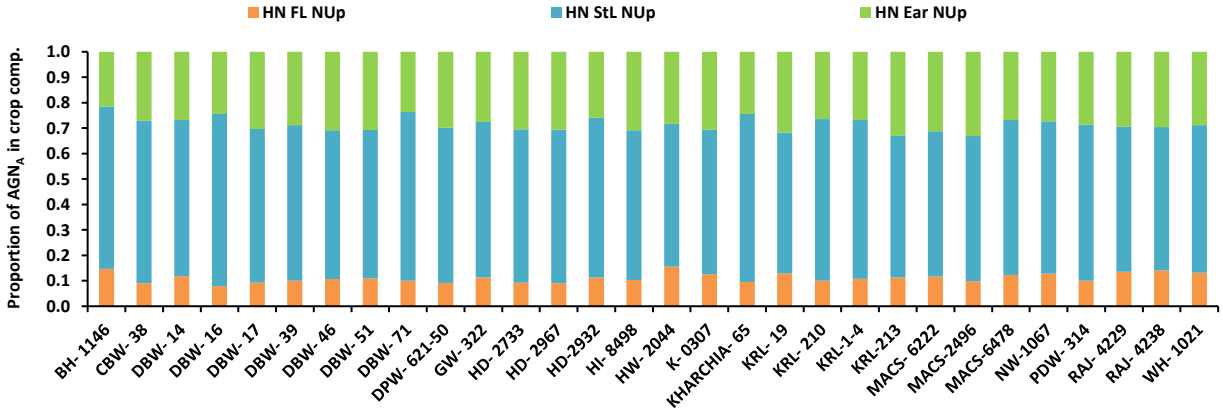


Figure 5. 3 Proportion of N uptake under high N (HN) and low N (LN) conditions in flag-leaf (FL), stem and remaining leaf (StL) and Ear at anthesis in 30 cultivars in (a,b) 2014, (c,d) 2016 and (e,f) across years. (Stat in Table 5.2).

(d) 2016 LN



(e) Across years HN



(f) Across years LN

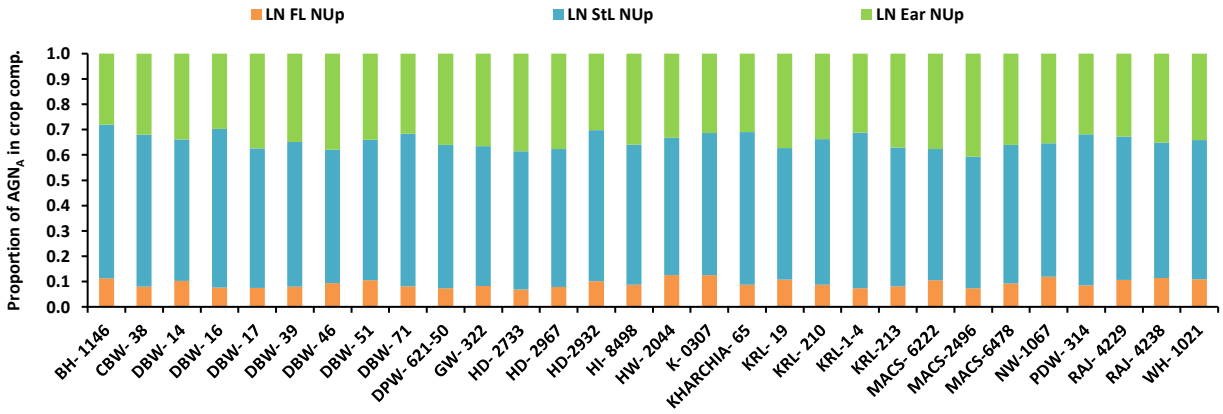


Figure 5. 3 Contd. Proportion of N uptake under high N (HN) and low N (LN) conditions in flag-leaf (FL), stem and remaining leaf (StL) and Ear at anthesis in 30 cultivars in (a,b) 2014, (c,d) 2016 and (e,f) mean across years. (Stat in Table 5.2).

Table 5. 2 Flag-leaf N partitioning index (FL NPI_A), stem and remaining leaf N partitioning index (StL NPI_A) and Ear N partitioning index (Ear NPI_A) at anthesis in 30 Indian cultivars under HN and LN conditions in 2014, 2016 and mean of 2014 and 2016.

	FL NPI_A						StL NPI_A						Ear NPI_A					
	2014		2016		Mean		2014		2016		Mean		2014		2016		Mean	
	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN
Mean	0.10	0.07	0.12	0.11	0.11	0.09	0.64	0.59	0.58	0.54	0.61	0.56	0.26	0.34	0.30	0.35	0.28	0.35
Min	0.07	0.05	0.09	0.07	0.08	0.07	0.50	0.53	0.50	0.45	0.54	0.52	0.16	0.26	0.24	0.29	0.21	0.28
Max	0.15	0.10	0.17	0.16	0.16	0.13	0.72	0.67	0.66	0.62	0.68	0.65	0.37	0.40	0.35	0.44	0.34	0.41
	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df
N	0 **	1	0.01 ns	2	0.004 **	3	0.01 ns	1	0.02 ns	2	0.01 *	3	0.01 ns	1	0.02 ns	2	0.01 **	3
G	0.01***	58	0.01***	116	0.01***	174	0.03 **	58	0.02 ***	116	0.02 ***	174	0.03 *	58	0.02 ***	116	0.02 ***	174
N*G	0.02 ns	58.1	0.02 ns	60.9	0.01 ns	120	0.05 ns	51.8	0.04 ns	33.01	0.03 ns	75.6	0.05 ns	50.7	0.04 ns	42.8	0.03 ns	88.7
Y					0.01 **	3					0.01 **	3					0.01 *	3
N*Y					0.01 ns	4.8					0.02 ns	6					0.01 ns	4.4
G*Y					0.01 ***	37					0.03 **	90.6					0.03 ns	171.6
N*G*Y					0.016 ns	85.3					0.042 ns	123					0.04 ns	147.1

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level.

5.3.3 N uptake at harvest

5.3.3.1 Flag leaf N uptake at harvest

In 2014, as N supply increased flag-leaf N uptake at harvest (FL N_H) increased from 0.08 (LN) to 0.24 (HN) g m^{-2} (Table 5.3, Fig. 5.4 a and b). Under HN, FL N_H ranged amongst the 30 cultivars from 0.14 to 0.44 g m^{-2} and under LN conditions from 0.05 to 0.14 g m^{-2} ($P<0.001$). The reduction under N limitation ranging from 42.9 to 82.1% ($P=0.006$). In 2016, as N supply increased FL N_H uptake increased from 0.13 (LN) to 0.34 (HN) g m^{-2} ($P=0.004$; Table 5.3, Fig. 5.7 c and d). Under HN, FL N_H ranged from 0.20 to 0.57 g m^{-2} and under LN conditions from 0.07 to 0.22 g m^{-2} ($P<0.001$). Reduction under N limitation ranged from 14.3 to 86.0% ($P<0.001$). Across years, as N supply increased FL N_H uptake increased from 0.11 (LN) to 0.29 (HN) g m^{-2} ($P<0.001$; Table 5.3, Fig. 5.7 e and f). Under HN, FL N_H ranged from 0.20 to 0.43 g m^{-2} and under LN conditions from 0.07 to 0.17 g m^{-2} ($P<0.001$). There was an N treatment x genotype ($P<0.001$) and year x N treatment x genotype interaction ($P<0.001$).

5.3.3.2 Stem and remaining leaf N uptake at harvest

In 2014, stem and remaining leaf N uptake at harvest (StL N_H) increased from 0.8 (LN) to 1.6 (HN) g m^{-2} (Table 5.3, Fig. 5.4 a and b). Under HN, StL N_H ranged from 1.0 to 2.5 g m^{-2} and under LN conditions from 0.5 to 1.2 g m^{-2} ($P<0.001$). Response of genotype to N limitation ranged from 22.1 to 73.4% ($P=0.013$). In 2016, StL N_H uptake increased from 0.8 under LN to 2.2 g m^{-2} under HN conditions ($P=0.001$; Table 5.3, Fig. 5.7 c and d). Under HN, StL N_H ranged from 0.9 to 3.7 g m^{-2} and under LN conditions from 0.5 to 1.3 g m^{-2} ($P<0.001$). Decrease for genotypes under N limitation ranged from 27.2 to 76.1% ($P=0.004$). Averaging across years, StL N_H increased from 0.8 under LN to 1.9 g m^{-2} under HN conditions ($P<0.001$; Table 5.3, Fig. 5.7 e and f). Under HN, StL N_H ranged from 1.3 to 2.8 g m^{-2} and under LN conditions from 0.5 to 1.0 g m^{-2} ($P<0.001$). Decreases under low N ranged from 29.7% to 75.1% ($P=0.004$) but there was no year x N treatment x genotype interaction.

5.3.3.3 Grain N at harvest

In 2014, grain N_H increased from 6.7 under LN to 11.9 g m^{-2} under HN conditions but this increase was not significant (Table 5.3, Fig. 5.4 a and b). Under HN, grain N_H ranged from 7.7 to 14.6 g m^{-2} and under LN conditions from 4.2 to 8.3 g m^{-2} ($P<0.001$). There was no interaction between N treatment and genotype. In 2016, grain N_H increased from 7.4 under LN to 14.0 g m^{-2} under HN conditions ($P=0.01$; Table 5.3, Fig. 5.7 c and d). Under HN, grain N_H ranged from 7.4 to 18.1 g m^{-2} and under LN and from 5.3 to 9.5 g m^{-2} ($P=0.01$). Decrease under N limitation ranged amongst genotype from 11.8 to 62.7% ($P=0.03$). Across the years, as N supply increased grain N_H increased from 7.1 (LN) to 13.0 (HN) g m^{-2} ($P<0.001$; Table 5.3, Fig. 5.7 e and f). Under HN, grain N_H ranged from 9.1 to 16.1 g m^{-2} and under LN conditions from 5.3 to 8.4 g m^{-2} ($P<0.001$). There was an N treatment x genotype ($P=0.023$) but no year x N treatment x genotype x interaction.

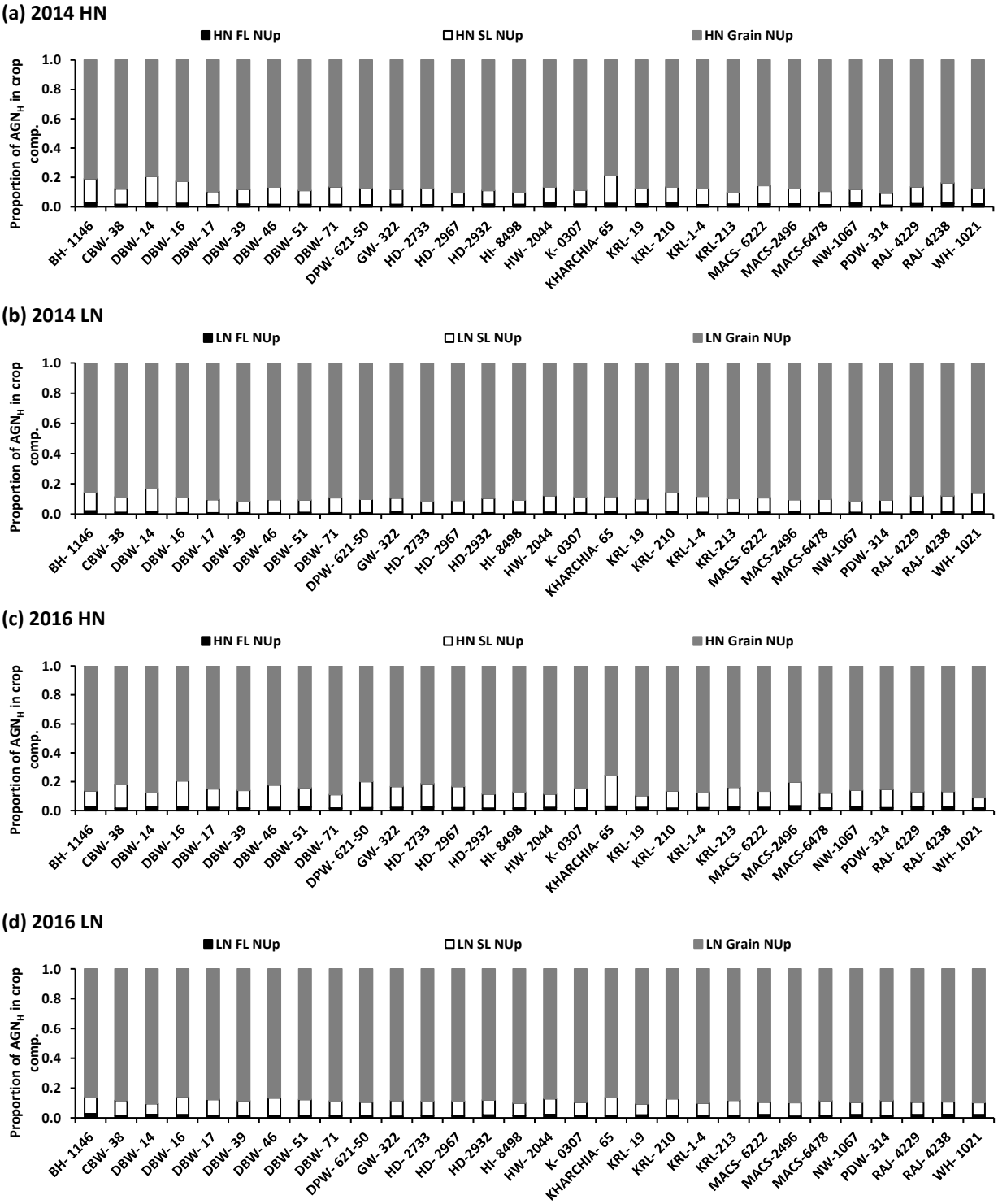


Figure 5. 4 Proportion of N accumulation in flag-leaf (FL), stem and leaves (SL) and Grain at harvest in 30 cultivars in a) 2014 b) 2016 and c) mean across years. (Stat in Table 5.3).

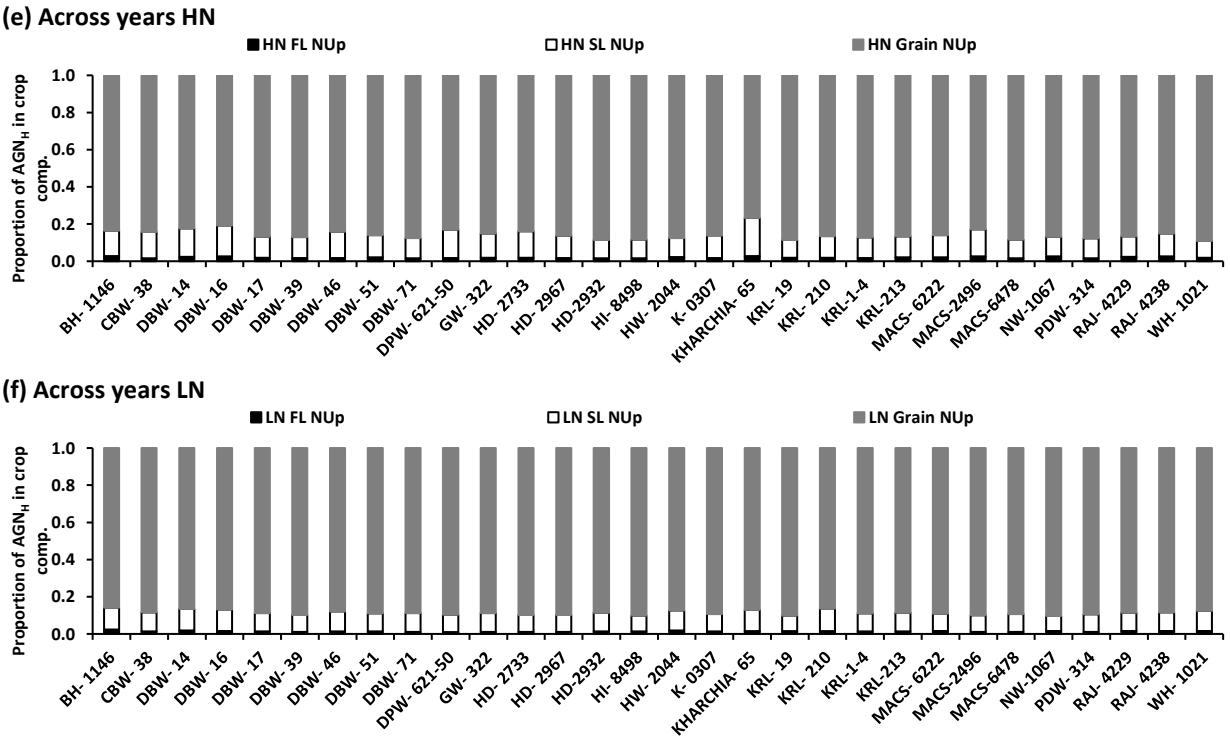


Figure 5. 4 Contd. Proportion of N accumulation in flag-leaf (FL), stem and leaves (SL) and Grain at harvest in 30 cultivars in a) 2014 b) 2016 and c) mean across years. (Stat in Table 5.3).

Table 5. 3 Flag-leaf N (FL N_H), Stem and remaining leaf N (StL N_H) and Grain N (Grain N_H) at harvest in 30 cultivars under HN and LN conditions in 2014, 2016 and across years.

	FL N_H (g m^{-2})						StL N_H (g m^{-2})						Grain N_H (g m^{-2})					
	2014		2016		Mean		2014		2016		Mean		2014		2016		Mean	
	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN
Mean	0.24	0.08	0.34	0.13	0.29	0.11	1.57	0.75	2.19	0.84	1.88	0.80	11.9	6.7	14.0	7.4	13.0	7.1
Min	0.14	0.05	0.20	0.07	0.20	0.07	0.97	0.47	0.85	0.54	1.30	0.53	7.7	4.2	7.4	5.3	9.1	5.3
Max	0.44	0.14	0.57	0.22	0.43	0.17	2.53	1.20	3.70	1.31	2.79	1.00	14.6	8.3	18.2	9.5	16.1	8.3
	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df
N	0.03 ns	1	0.01 **	2	0.01 ***	3	0.13 ns	1	0.05 ***	2	0.06 ***	3	0.7 ns	1	0.50 **	2	0.41 ***	3
G	0.03 ***	58	0.04 ***	116	0.03 ***	174	0.17 ***	58	0.34 ***	116	0.22 ***	174	0.89 ***	58	1.27 **	116	0.87 ***	174
N*G	0.05 ns	9.9	0.06 ***	106.4	0.04 ***	123.6	0.28 *	15.1	0.48 **	117.8	0.32 **	173.9	1.42 ns	14.7	1.84 *	98.7	1.3 *	123.7
Y					0.01 ***	3					0.05 **	3					0.8 ns	3
N*Y					0.01 ns	3.8					0.08 *	5.7					0.9 ns	4.5
G*Y					0.04 ***	177					0.32 ***	176.8					1.5 ns	31.4
N*G*Y					0.06 ***	165.8					0.46 ns	179.6					1.96 ns	74.8

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level.

5.3.4 Post-anthesis N Remobilization efficiency

5.3.4.1 Flag-leaf N remobilization efficiency

In 2014, flag-leaf N remobilization efficiency (FL NRE) increased overall with N deficiency from 0.82 (HN) to 0.85 (LN) but it was not significant (P=0.11, Table 5.4). Under HN, FL NRE ranged from 0.70 to 0.87 and under LN conditions from 0.72 to 0.90 (P=0.035). There was no significant interaction between N treatment and genotype. In 2016, FL NRE increased overall with N deficiency from 0.81 (HN) to 0.85 (LN) (P=0.027, Table 5.4). Under HN, FL NRE ranged from 0.72 to 0.87 and under LN conditions from 0.75 to 0.91 (P<0.001). Increase of genotype under N limitation ranged from -3.6 to 17.2% (P=0.027). Across years, FL NRE increased overall with N deficiency from 0.82 (HN) to 0.85 (LN) (P=0.005; Table 5.4). Under HN, FL NRE ranged from 0.71 to 0.87 and under LN conditions from 0.78 to 0.89 (P<0.001). There was a no N treatment x genotype and year x N treatment x genotype interaction.

5.3.4.2 Stem and remaining leaf NRE

In 2014, stem and remaining leaf N remobilization efficiency (StL NRE) did not change with N deficiency and was 0.83 under both the conditions (Table 5.4). Under HN, StL NRE ranged from 0.74 to 0.89 and under LN conditions from 0.76 to 0.88 (P=0.014). There was no interaction between N treatment and genotype. In 2016, StL NRE showed a trend to increase with N deficiency from 0.75 (HN) to 0.81 (LN) (P=0.056, Table 5.4). Under HN, StL NRE ranged from 0.62 to 0.85 and under LN conditions from 0.71 to 0.86 (P=0.029). Increases of genotypes under N limitation ranged from -10.1 to 24.4% (P=0.035). Across years, StL NRE increased overall with N deficiency from 0.78 (HN) to 0.82 (LN) (P=0.027; Table 5.4). Under HN, StL NRE ranged from 0.78 to 0.88 and under LN conditions from 0.83 to 0.90 (P=0.046). There was no interaction for N treatment x genotype or year x N treatment x genotype.

Table 5. 4 Flag-leaf N remobilization efficiency and stem and remaining leaf N remobilization efficiency for 30 wheat cultivars in 2014 and 2016 and mean across years.

	FL NRE						StL NRE					
	2014		2016		Mean		2014		2016		Mean	
Cultivars	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN
BH- 1146	0.75	0.72	0.83	0.81	0.80	0.78	0.77	0.83	0.79	0.79	0.78	0.80
CBW- 38	0.80	0.85	0.80	0.82	0.80	0.83	0.83	0.80	0.64	0.78	0.72	0.79
DBW- 14	0.78	0.81	0.83	0.80	0.81	0.80	0.75	0.76	0.81	0.81	0.78	0.79
DBW- 16	0.70	0.90	0.72	0.75	0.71	0.81	0.81	0.86	0.75	0.82	0.77	0.84
DBW- 17	0.83	0.86	0.78	0.82	0.80	0.84	0.86	0.88	0.75	0.77	0.80	0.81
DBW- 39	0.78	0.88	0.87	0.88	0.83	0.88	0.84	0.88	0.81	0.83	0.83	0.85
DBW- 46	0.86	0.90	0.85	0.89	0.86	0.89	0.85	0.86	0.73	0.80	0.78	0.83
DBW- 51	0.83	0.85	0.81	0.89	0.82	0.88	0.84	0.85	0.77	0.83	0.80	0.84
DBW- 71	0.81	0.83	0.82	0.84	0.82	0.84	0.81	0.81	0.81	0.80	0.81	0.81
DPW- 621-50	0.86	0.85	0.80	0.87	0.83	0.86	0.84	0.85	0.69	0.84	0.75	0.84
GW- 322	0.86	0.80	0.78	0.91	0.81	0.87	0.82	0.81	0.70	0.82	0.75	0.82
HD- 2733	0.84	0.89	0.77	0.83	0.80	0.86	0.83	0.88	0.72	0.81	0.76	0.84
HD- 2967	0.84	0.86	0.85	0.86	0.85	0.86	0.89	0.85	0.77	0.79	0.81	0.81
HD-2932	0.81	0.82	0.86	0.84	0.84	0.83	0.87	0.78	0.78	0.79	0.81	0.79
HI- 8498	0.80	0.84	0.78	0.86	0.79	0.85	0.85	0.85	0.70	0.82	0.76	0.83
HW- 2044	0.84	0.89	0.79	0.86	0.81	0.87	0.80	0.85	0.72	0.76	0.75	0.80
K- 0307	0.86	0.90	0.85	0.88	0.85	0.89	0.86	0.79	0.62	0.82	0.71	0.81
KHARCHIA- 65	0.82	0.79	0.75	0.88	0.77	0.84	0.81	0.84	0.71	0.86	0.75	0.85
KRL- 19	0.82	0.86	0.83	0.85	0.82	0.85	0.75	0.84	0.83	0.83	0.80	0.83
KRL- 210	0.73	0.72	0.85	0.86	0.80	0.81	0.82	0.81	0.79	0.71	0.80	0.75
KRL-1-4	0.86	0.80	0.80	0.78	0.83	0.79	0.80	0.84	0.78	0.80	0.79	0.81
KRL-213	0.84	0.88	0.84	0.82	0.84	0.85	0.88	0.85	0.77	0.80	0.81	0.82
MACS- 6222	0.82	0.83	0.83	0.82	0.83	0.82	0.84	0.80	0.72	0.79	0.77	0.79
MACS-2496	0.82	0.87	0.72	0.87	0.76	0.87	0.83	0.87	0.74	0.79	0.78	0.82
MACS-6478	0.87	0.87	0.86	0.87	0.87	0.87	0.85	0.82	0.78	0.80	0.81	0.81
NW-1067	0.81	0.89	0.82	0.87	0.82	0.88	0.88	0.88	0.79	0.84	0.83	0.86
PDW- 314	0.86	0.86	0.84	0.89	0.85	0.88	0.86	0.85	0.78	0.85	0.81	0.85
RAJ- 4229	0.84	0.86	0.75	0.82	0.79	0.84	0.80	0.86	0.76	0.77	0.77	0.80
RAJ- 4238	0.80	0.87	0.79	0.82	0.80	0.84	0.74	0.80	0.77	0.81	0.76	0.81
WH- 1021	0.86	0.83	0.86	0.86	0.86	0.85	0.83	0.78	0.85	0.84	0.84	0.82
Mean	0.82	0.85	0.81	0.85	0.82	0.85	0.83	0.83	0.75	0.81	0.78	0.82
Min	0.70	0.72	0.72	0.75	0.71	0.78	0.74	0.76	0.62	0.71	0.71	0.75
Max	0.87	0.90	0.87	0.91	0.87	0.89	0.89	0.88	0.85	0.86	0.84	0.86
	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df
N	0.01 ns	1	0.01 *	2	0.004 **	3	0.01 ns	1	0.013 ns	2	0.01 *	3
G	0.04 **	58	0.03 ***	116	0.02 ***	174	0.03 *	58	0.03 *	116	0.02 *	174
N*G	0.05 ns	58.8	0.04 *	117	0.03 ns	176.8	0.04 ns	58.6	0.05 *	101	0.03 ns	158
Y	0.75	0.72	0.83	0.81	0.01 ns	3	0.77	0.83	0.79	0.79	0.01 ns	3
Y*N					0.01 ns	4.7	0.83	0.80	0.64	0.78	0.02 ns	5.4
Y*G					0.03 **	155.7	0.75	0.76	0.81	0.81	0.04 ns	111.2
Y*N*G					0.04 ns	176.7					0.05 ns	159.9

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level.

5.3.5 Genetic variation in flag-leaf visual senescence score related parameters

In 2014 and 2016, for flag-leaf visual senescence score, onset of senescence of rapid phase (VS.OnsetRP), senescence duration of rapid phase (VS.DurRP) and end of rapid phase (VS.EndRP) in the post-anthesis period were estimated.

5.3.5.1 VS.OnsetRP

In 2014, VS.OnsetRP was earlier under LN (651.3 °Cd) than under HN (713.8 °Cd) (Table 5.1). Under HN, VS.OnsetRP ranged from 575.0 (DBW- 14) to 1055.0 (PDW- 314) °Cd and under LN conditions from 394.5 (BH- 1146) to 896.5 (HW- 2044) °Cd (P<0.001). There was no significant interaction between N treatment and genotype (Fig. 5.4 a). In 2016, VS.OnsetRP was earlier under LN (789.6 °Cd) than under HN (984.5 °Cd) (P=0.015, Table 5.1). Under HN, VS.OnsetRP ranged from 510.0 (DBW- 14) to 1501.0 (HD-2932) °Cd and under LN conditions from 407.0 (RAJ-4229) to 1061.0 (PDW- 314) °Cd (P<0.001). There was no significant interaction between N treatment and genotype (Fig. 5.4 b). Across years, VS.OnsetRP was earlier under LN (720.4) than HN (849.1) (P=0.003, Table 5.1). Under HN, VS.OnsetRP ranged from 542.5 (DBW- 14) to 1135.8 (HD-2932) °Cd post anthesis and under LN conditions from 480.9 (BH- 1146) to 940.0 (PDW- 314) °Cd post anthesis (P<0.001). There was no significant N treatment x genotype or N treatment x genotype x year interaction (Fig. 5.4 c). Overall VS.OnsetRP showed a positive association with GY under HN in 2014, under both N conditions in 2016 and across years (Fig. 5.8 a, b and c)

5.3.5.2 End of rapid phase of senescence

In 2014, VS.EndRP was earlier under LN (814.7 °Cd) than under HN (927.9 °Cd) conditions (P=0.014; Table 5.1). Under HN, VS.EndRP ranged from 743.0 (BH- 1146) to 1136.5 (DBW- 16) °Cd and under LN conditions from 621.0 (HW- 2044) to 1016.0 (DBW- 16) °Cd (P<0.001). There was an interaction between N treatment and genotype (P<0.001) (Fig. 5.4 g). In 2016, VS.EndRP was earlier under LN (1079.2) than under HN (1187.7 °Cd) (Table 5.1). Under HN, VS.EndRP ranged from 937.3 (DBW- 14) to 1408.7 (KRL- 210) °Cd and under LN conditions from 738.0 (RAJ- 4229) to 1363.3 (DBW- 16) °Cd (P<0.001). The decrease under LN ranged from -15.0% to 34.2% (P<0.001) (Fig. 5.4 h). Averaging across years, VS.EndRP was earlier under LN (946.9) than under HN (1057.8) conditions (P=0.027, Table 5.1). Under HN, VS.EndRP ranged from 874.2 (DBW- 14) to 1186.8 (DBW- 17) °Cd and under LN conditions from 700.4 (DBW- 14) to 1189.7 (DBW- 16) °Cd (P<0.001). Decreases under LN ranged from -2.5% to 26.0% (P<0.001). There was also an N treatment x genotype x year interaction (P<0.001) (Fig. 5.4 i). Overall VS.EndRP showed no

association with GY under both N conditions in 2014 and showed a positive association with both conditions in 2016 and across years (Fig. 5.8 g, h and i).

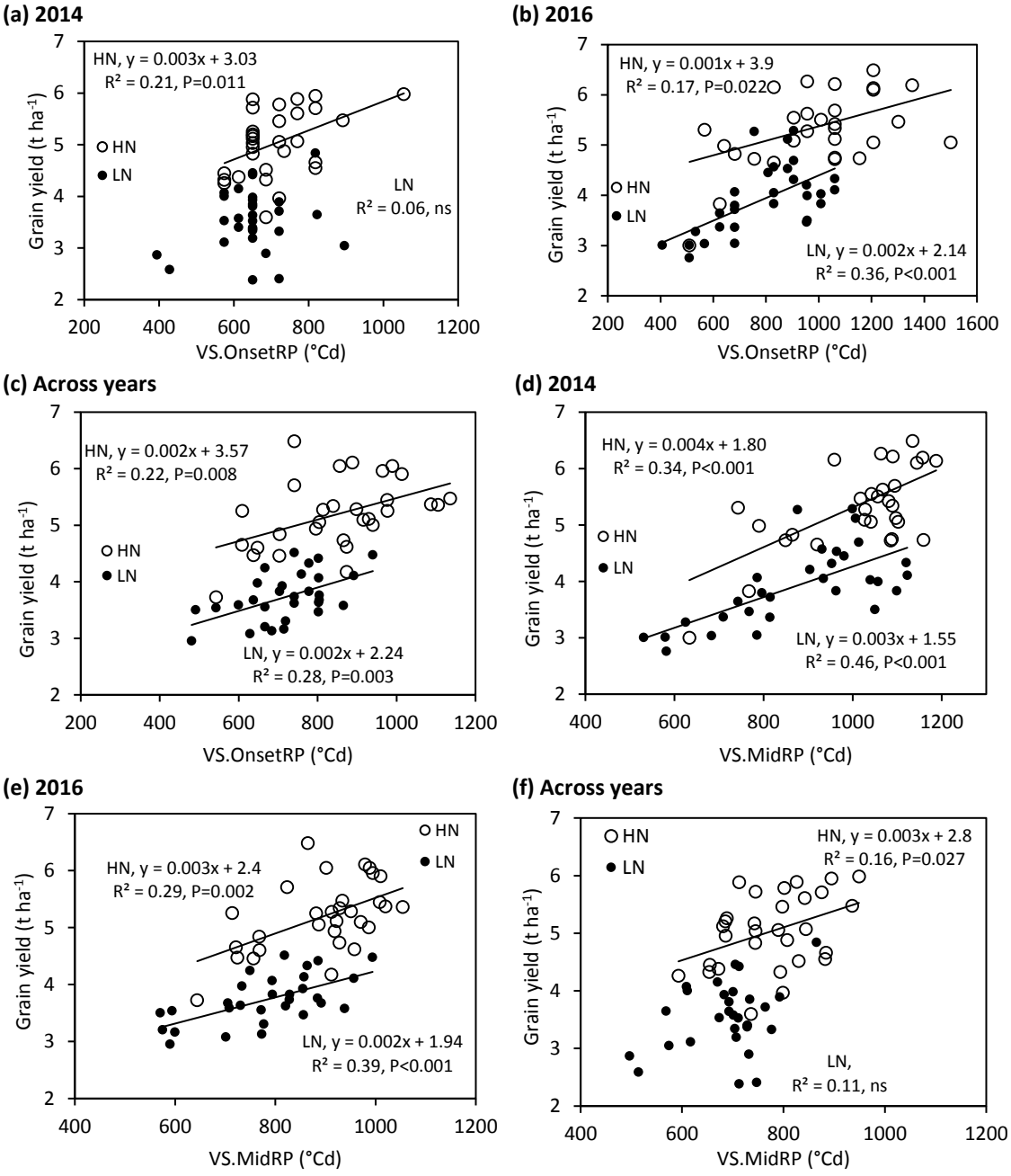


Figure 5. 5 Linear regression amongst 30 cultivars under HN and LN treatment of grain yield (100% DM, GY) on onset of senescence of rapid phase (VS.OnsetRP) in a) 2014 b) 2016 and c) average across 2014-16; and GY on midpoint of rapid phase of senescence (VS.MidRP) in d) 2014 e) 2016 and f) average across 2014-16; and GY on end of rapid phase of senescence (VS.EndRP) in g) 2014 h) 2016 and i) average across 2014-16.

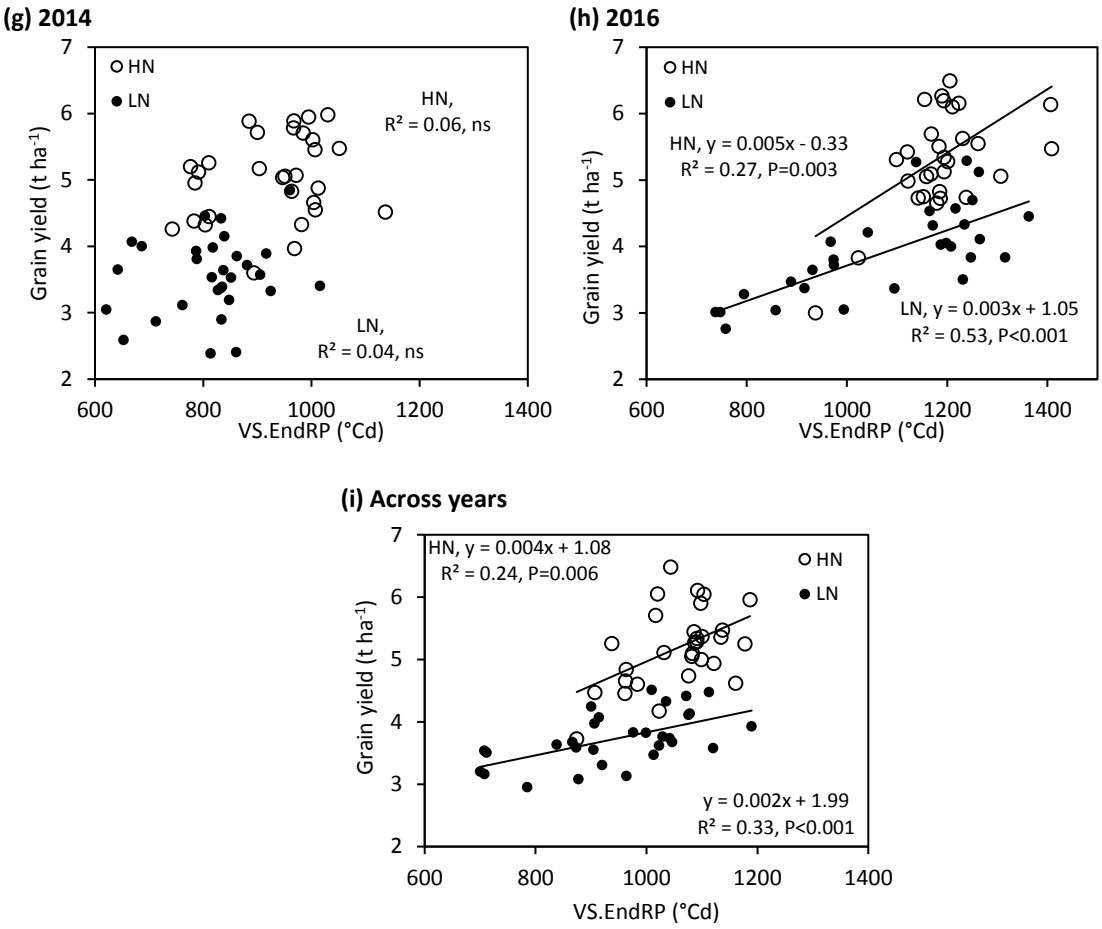


Figure 5. 5 Contd. Linear regression amongst 30 cultivars under HN and LN treatment of grain yield (100% DM, GY) on onset of senescence of rapid phase (VS.OnsetRP) in a) 2014 b) 2016 and c) average across 2014-16; and GY on midpoint of rapid phase of senescence (VS.MidRP) in d) 2014 e) 2016 and f) average across 2014-16; and GY on end of rapid phase of senescence (VS.EndRP) in g) 2014 h) 2016 and i) average across 2014-16.

Table 5. 5 Thermal time (base temp. 0 °C) post-anthesis (GS61) duration of senescence of slow phase (VS.OnsetRP), flag-leaf visual senescence mid-point (VS.MidRP), and duration of end of senescence of rapid phase (VS.EndRP) in °C for 30 Indian cultivars under HN and LN conditions in 2014, 2016 and mean of 2014 and 2016.

	VS.OnsetRP (°Cd)						VS.MidRP (°Cd)						VS.EndRP (°Cd)					
	2014.0		2016.0		Mean		2014.0		2016.0		Mean		2014.0		2016.0		Mean	
Cultivars	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN
BH- 1146	575.0	394.5	830.7	567.3	702.8	480.9	593.0	496.5	920.0	683.3	756.5	589.9	743.0	712.5	1179.3	857.7	961.2	785.1
CBW- 38	722.0	651.0	957.0	905.0	839.5	778.0	790.0	704.0	1067.3	953.0	928.7	828.5	951.0	827.5	1231.0	1171.7	1091.0	999.6
DBW- 14	575.0	823.5	510.0	510.0	542.5	666.8	655.0	568.5	633.3	581.7	644.2	575.1	811.0	642.5	937.3	758.3	874.2	700.4
DBW- 16	686.5	613.0	1061.0	808.3	873.8	710.7	830.0	729.0	1085.7	980.7	957.8	854.8	1136.5	1016.0	1186.0	1363.3	1161.3	1189.7
DBW- 17	722.0	651.0	1207.7	957.0	964.8	804.0	801.5	711.0	1187.7	1057.3	994.6	884.2	967.0	851.5	1406.7	1207.7	1186.8	1029.6
DBW- 39	819.0	722.0	1207.7	882.7	1013.3	802.3	875.5	764.0	1144.0	1007.0	1009.8	885.5	985.0	881.0	1210.7	1263.3	1097.8	1072.2
DBW- 46	651.0	651.0	1207.7	905.0	929.3	778.0	742.5	728.5	1102.3	999.3	922.4	863.9	904.0	832.5	1159.0	1239.0	1031.5	1035.8
DBW- 51	819.0	722.0	1354.3	1009.0	1086.7	865.5	882.5	777.0	1157.0	1099.3	1019.8	938.2	1007.5	925.0	1193.3	1315.7	1100.4	1120.3
DBW- 71	651.0	651.0	957.0	955.0	804.0	803.0	744.5	693.0	1028.3	767.7	886.4	730.3	963.5	788.5	1200.7	888.7	1082.1	838.6
DPW- 621-50	722.0	651.0	905.0	830.7	813.5	740.8	798.0	707.0	1026.7	934.3	912.3	820.7	1006.5	848.0	1168.0	1198.7	1087.3	1023.3
GW- 322	651.0	651.0	1061.0	830.7	856.0	740.8	712.5	705.5	1090.0	931.3	901.3	818.4	885.0	803.0	1155.0	1216.7	1020.0	1009.8
HD- 2733	892.0	722.0	1061.0	1061.0	976.5	891.5	935.5	792.5	1080.7	1120.3	1008.1	956.4	1051.5	917.0	1120.7	1235.0	1086.1	1076.0
HD- 2967	735.0	651.0	1061.0	957.0	898.0	804.0	807.5	733.5	1094.7	1050.0	951.1	891.8	1012.5	862.5	1168.7	1231.3	1090.6	1046.9
HD-2932	770.5	651.0	1501.0	955.0	1135.8	803.0	826.0	683.0	1041.0	904.3	933.5	793.7	967.5	787.5	1306.7	1041.7	1137.1	914.6
HI- 8498	819.0	722.0	1061.0	882.7	940.0	802.3	884.0	747.5	1089.3	964.3	986.7	855.9	1004.5	861.0	1193.7	1165.0	1099.1	1013.0
HW- 2044	651.0	896.5	624.7	533.0	637.8	714.8	681.0	574.0	767.3	625.3	724.2	599.7	791.5	621.0	1023.0	794.7	907.3	707.8
K- 0307	722.0	651.0	1009.0	756.3	865.5	703.7	799.0	712.5	1056.3	876.0	927.7	794.3	969.0	814.0	1183.7	1138.3	1076.3	976.2
KHARCHIA- 65	686.5	429.0	1061.0	1009.0	873.8	719.0	736.0	514.0	1088.0	1039.7	912.0	776.8	893.5	653.0	1152.3	1187.7	1022.9	920.3
KRL- 19	613.0	575.0	682.0	624.7	647.5	599.8	672.0	673.5	864.7	742.7	768.3	708.1	783.0	816.0	1185.0	931.7	984.0	873.8
KRL- 210	651.0	575.0	1302.3	682.0	976.7	628.5	745.0	617.0	1018.0	785.3	881.5	701.2	947.0	761.5	1408.7	994.0	1177.8	877.8
KRL-1-4	651.0	651.0	756.3	624.7	703.7	637.8	686.0	701.0	849.3	710.0	767.7	705.5	785.0	818.0	1142.0	915.7	963.5	866.8
KRL-213	770.5	613.0	1207.7	905.0	989.1	759.0	842.0	701.5	1134.7	1013.7	988.3	857.6	1002.5	905.5	1205.7	1251.0	1104.1	1078.3
MACS- 6222	819.0	651.0	957.0	682.0	888.0	666.5	895.0	728.5	1063.3	814.7	979.2	771.6	995.0	835.0	1189.3	974.3	1092.2	904.7
MACS-2496	770.5	651.0	1061.0	830.7	915.8	740.8	844.0	693.0	1097.0	963.0	970.5	828.0	972.0	837.5	1193.7	1247.3	1082.8	1042.4
MACS-6478	651.0	651.0	830.7	682.0	740.8	666.5	745.0	712.5	985.0	786.0	865.0	749.3	900.0	833.5	1187.3	968.0	1043.7	900.8
NW-1067	686.5	686.5	905.0	682.0	795.8	684.3	794.0	732.0	1042.0	814.3	918.0	773.2	982.0	834.0	1261.7	1094.7	1121.8	964.3
PDW- 314	1055.0	819.0	1155.7	1061.0	1105.3	940.0	949.0	865.5	1159.3	1122.7	1054.2	994.1	1030.5	960.5	1238.0	1265.7	1134.3	1113.1
RAJ- 4229	575.0	575.0	641.7	407.0	608.3	491.0	653.5	610.5	789.7	530.7	721.6	570.6	803.5	686.5	1122.0	738.0	962.8	712.3
RAJ- 4238	651.0	575.0	567.3	510.0	609.2	542.5	685.5	609.0	742.3	579.3	713.9	594.2	776.5	668.5	1099.0	747.7	937.8	708.1
WH- 1021	651.0	613.0	830.7	682.0	740.8	647.5	688.0	670.0	959.0	796.3	823.5	733.2	810.0	839.0	1223.0	973.7	1016.5	906.3
Mean	713.8	651.3	984.5	789.6	849.2	720.4	776.4	688.5	1012.1	874.5	894.3	781.5	927.9	814.7	1187.7	1079.2	1057.8	946.9
Min	575.0	394.5	510.0	407.0	542.5	480.9	593.0	496.5	633.3	530.7	644.2	570.6	743.0	621.0	937.3	738.0	874.2	700.4
Max	1055.0	896.5	1501.0	1061.0	1135.8	940.0	949.0	865.5	1187.7	1122.7	1054.2	994.1	1136.5	1016.0	1408.7	1363.3	1186.8	1189.7
	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df
N	11.3 ns	1.0	23.8 *	2.0	15.6 **	3.0	7.3 ns	1.0	11.2 **	2.0	7.6 ***	3.0	2.4 *	1.0	43 ns	2.0	27.2 *	3.0
G	73 ***	58.0	88.7 ***	116.0	62.1 ***	174.0	14.4 ***	58.0	31.8 ***	116.0	20.8 ***	174.0	19.9 ***	58.0	47.7 ***	116.0	31 ***	174.0
N*G	102.1 ns	58.9	125.6 ns	115.5	87.7 ns	174.7	21.4 ***	36.9	45.6 ***	105.9	29.9 ***	155.9	27.8 ***	58.7	79.1 ***	20.8	51 ***	33.5
Y					16.8 ***	3.0					14.3 ***	3.0					26.9 **	3.0
N*Y					23.2 **	6.0					16.2 *	4.6					38.7 ns	6.0
G*Y					89.7 ***	173.6					32.7 ***	63.8					51.6 ***	36.1
N*G*Y					126.7 ns	180.0					44.7 ***	124.2					73.3 ***	62.8

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level.

5.3.6 Genetic variation in flag-leaf SPAD senescence-related parameters

5.3.6.1 Flag-leaf SPAD at anthesis ($SPAD_A$)

In 2014, $SPAD_A$ was lower under LN (44.2) than under HN (48.3) (P=0.079; Table 5.6) and ranged from 44.5 to 51.7 and from 36.1 to 50.5 under LN and HN conditions, respectively (P<0.001). There was no significant interaction between N treatment and genotype. In 2016, $SPAD_A$ was lower under LN (44.5) than under HN (50.5) (P=0.03; Table 5.6) and ranged from 45.6 to 55.1 and from 32.1 to 55.4 (P<0.001) under HN and LN conditions, respectively. There was an interaction between N treatment and genotype (P<0.001). Averaging across years, $SPAD_A$ was lower under LN (44.4) than under HN (49.4) (P=0.005, Table 5.6) and ranged amongst cultivars from 45.4 to 52.8 and from 34.1 to 50.1, respectively (P<0.001). Decrease under N limitation ranged from 3.0 to 26.3% (P<0.001) and there was a year x N treatment x genotype interaction (P=0.008).

5.3.6.2 Onset of SPAD-Senescence (SS.Onset)

In 2014, SS.Onset was earlier under LN (337.0 °Cd) than under HN (451.6 °Cd). Under HN, SS.Onset ranged amongst cultivars from 327.3 to 533.6 °Cd and under LN conditions from 215.7 to 484.5 °Cd (P=0.036). There was no significant interaction between N treatment and genotype. In 2016, SS.Onset was earlier under LN (261.1 °Cd) than under HN (460.8 °Cd) (P=0.016) and ranged amongst cultivars from 219.5 to 681.7 °Cd and from 85.6 to 494.9 °Cd respectively (P<0.001). There was a significant interaction between N treatment and genotype (P=0.021). Across years, SS.Onset was earlier under LN (299.0 °Cd) than under HN (456.2 °Cd) conditions (P=0.003). Under HN, SS.Onset ranged amongst genotype from 312.6 to 591.6 °Cd and under LN conditions from 190.8 to 444.0 °Cd (P<0.001). There was a significant interaction between N treatment and genotype (P=0.005) but not N treatment x genotype x year interaction.

5.3.6.3 SPAD-senescence duration (SS.Dur)

In 2014, SS.Dur was shorter under HN (146.3 °Cd) than under LN (168.3 °Cd) (Table 5.6). Under HN, SS.Dur ranged from 68.83 to 264.8 °Cd and under LN conditions from 95.9 to 278.1 °Cd. There was no significant interaction between N treatment and genotype. In 2016, SS.Dur was shorter under HN (228.2 °Cd) than under LN (345.8 °Cd) (P=0.024; Table 5.6). Under HN, SS.Dur ranged from 91.8 to 514.4 °Cd and under LN conditions from 163.1 to 685.0 °Cd (P<0.001). There was no significant interaction between N treatment and genotype. Across years, SS.Dur was shorter under HN (193.4) than under LN (259.7 °Cd) (P=0.025, Table 5.6). Under HN, SS.Dur ranged from 88.6 to 514.4 °Cd and under LN conditions from 150.1 to 429.7 °Cd (P<0.001). Decrease under N limitation ranged amongst cultivars from -36.0 to 59.7% (P<0.001) but there was no year x N treatment x genotype interaction.

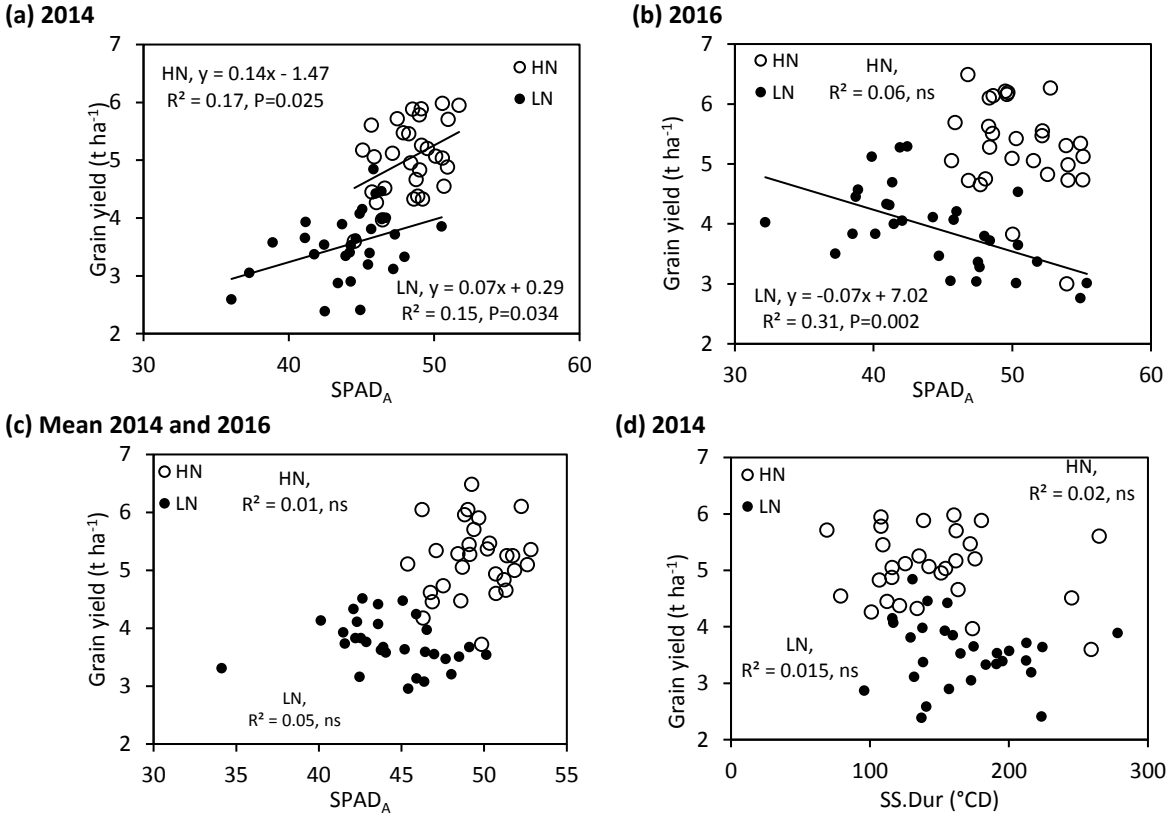


Figure 5. 6 Linear regression amongst 30 cultivars under high N (HN) and low N (LN) conditions of grain yield (100% DM) on flag-leaf SPAD at anthesis in (a) 2014 (b) 2016 and (c) mean 2014 and 2016; and grain yield (100% DM) on SPAD senescence duration in thermal time (base temp. 0°C) (SS.Dur) in (d) 2014 (e) 2016 and (f) mean 2014 and 2016.

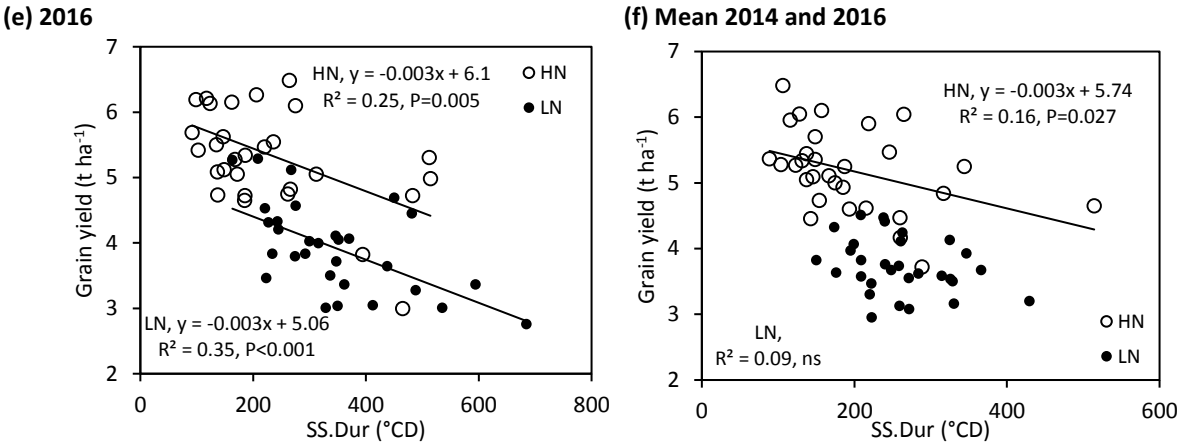


Figure 5. 6 Contd. Linear regression amongst 30 cultivars under high N (HN) and low N (LN) conditions of grain yield (100% DM) on flag-leaf SPAD at anthesis in (a) 2014 (b) 2016 and (c) mean 2014 and 2016; and grain yield (100% DM) on SPAD senescence duration in thermal time (base temp. 0°C) (SS.Dur) in (d) 2014 (e) 2016 and (f) mean 2014 and 2016.

5.3.7 Genetic variation in NDVI-senescence-related parameters

5.3.7.1 NDVI pre-tt400

In 2014, NDVI (averaged over measurements from 0 to 400 °Cd post-anthesis, NDVI pre-tt400) was slightly higher under HN (83.7) than under LN (82.7) but this difference was not significant (Table 5.6) and ranged amongst cultivars from 73.2 to 88.8 and from 78.3 to 86.5, respectively (P<0.001). There was no significant interaction between N treatment and genotype. In 2016, NDVI pre-tt400 was higher under HN (88.9) than under LN (81.3) (P=0.01; Table 5.6) and ranged amongst cultivars from 83.0 to 93.0 and from 69.2 to 89.2, respectively (P<0.001). There was an interaction between N treatment and genotype (P=0.10). Averaged across years, NDVI pre-tt400 was higher under HN (86.3) than under LN conditions (82.0) (P=0.014, Table 5.6). Under HN, NDVI pre-tt400 ranged from 80.3 to 89.1 and under LN conditions from 74.6 to 85.8 (P<0.001). Decreases under LN ranged amongst cultivars from -0.3% to 11.7% (P=0.017) but there was no year x N treatment x genotype interaction.

5.3.7.2 NDVI post-tt400

In 2014, NDVI (averaged over measurements from 400 to 800 °Cd post-anthesis; NDVI post-tt400) was slightly higher under HN (77.8) than under LN but it was also not significant (74.8) (Table 5.1) and ranged amongst cultivars from 61.5 to 89.2 and from 71.5 to 79.2, respectively. There was no significant interaction between N treatment and genotype. In 2016, NDVI post-tt400 was again higher under HN (67.4) than under LN (54.5) (P=0.022; Table 5.1) and ranged amongst cultivars from 54.3 to 79.2 and from 43.4 to 73.1 (P<0.001), respectively. There was no significant interaction between N treatment and genotype. Averaged across years, NDVI post-tt400 was higher under HN (72.6) than under LN (65.0) (P=0.014, Table 5.1) and ranged amongst cultivars from 62.8 to 84.2 and from 58.1 to 83.5 (P<0.001), respectively. Decreases under LN ranged amongst cultivars from -6.4% to 22.1% (P=0.029), but there was no year x N treatment x genotype interaction.

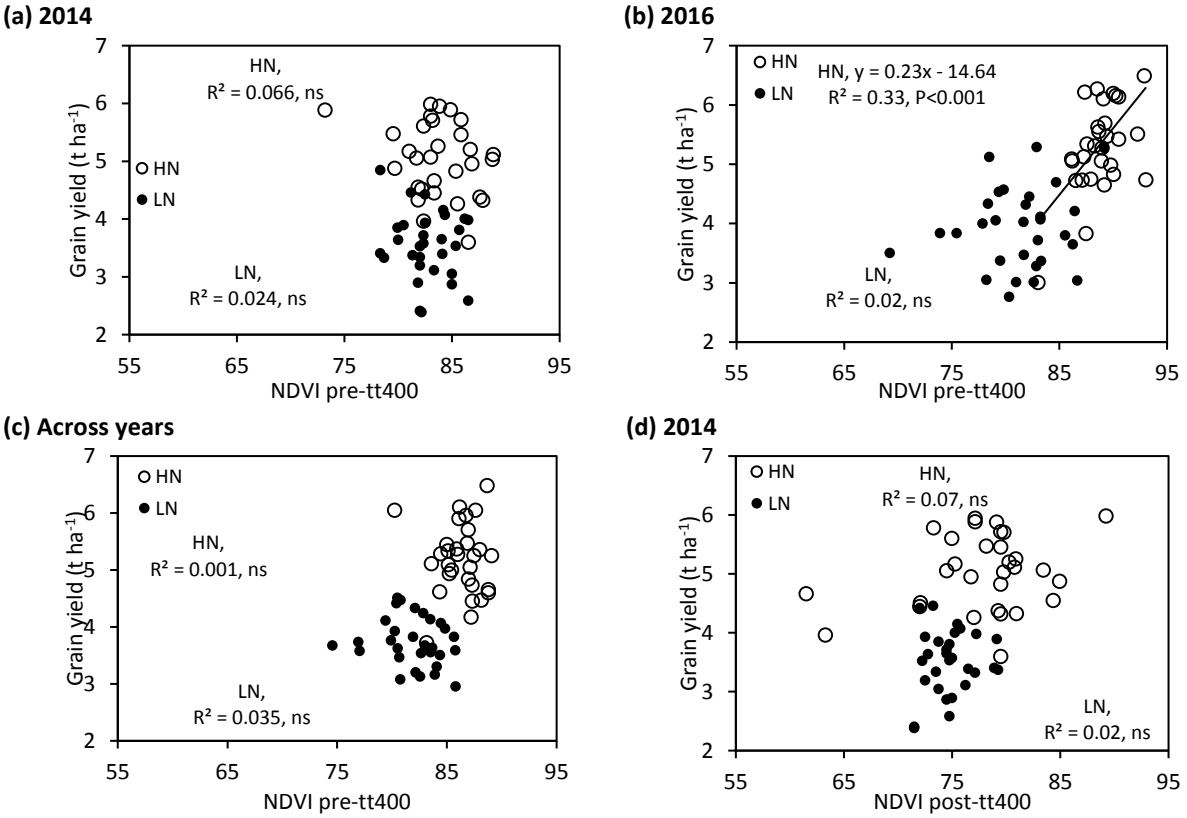


Figure 5. 7 Linear regression amongst 30 cultivars under high N (HN) and low N (LN) conditions of grain yield (100% DM) on mean NDVI for 0 to 400°Cd (base temp. 0°C) post-anthesis (GS61, NDVI pre-tt400) in (a) 2014 (b) 2016 and (c) mean 2014 and 2016; and grain yield (100% DM) on mean NDVI from 400 to 800 °Cd (NDVI post-tt400) in (d) 2014 (e) 2016 and (f) mean 2014 and 2016.

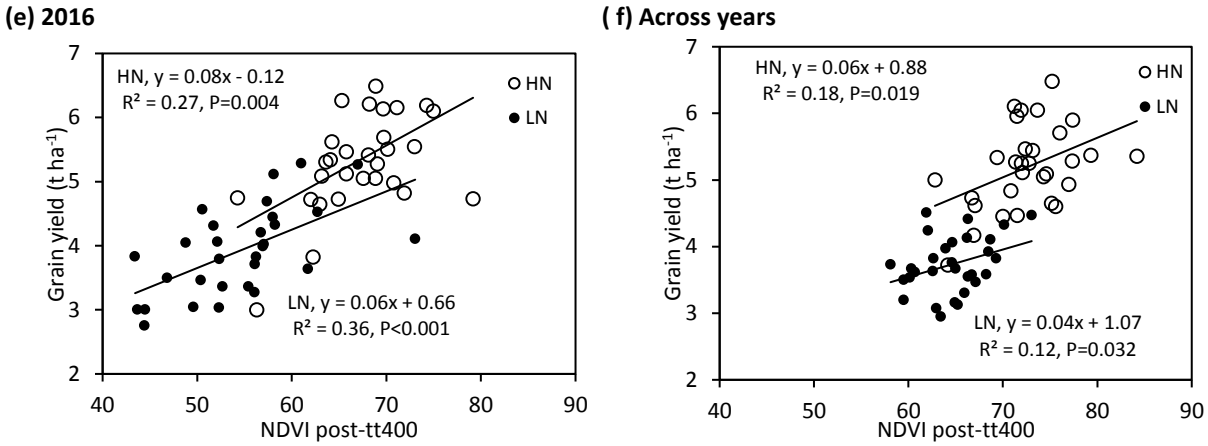


Figure 5. 7 Contd. Linear regression amongst 30 cultivars under high N (HN) and low N (LN) conditions of grain yield (100% DM) on mean NDVI for 0 to 400°Cd (base temp. 0°C) post-anthesis (GS61, NDVI pre-tt400) in (a) 2014 (b) 2016 and (c) mean 2014 and 2016; and grain yield (100% DM) on mean NDVI from 400 to 800 °Cd (NDVI post-tt400) in (d) 2014 (e) 2016 and (f) mean 2014 and 2016.

Table 5. 6 Duration (thermal time, base temp. 0 °C) of flag-leaf SPAD-senescence (SS.Dur), SPAD at anthesis ($SPAD_A$), average NDVI from 0 to 400 °Cd post-anthesis (GS61, NDVI pre-tt400) and from 400 to 800 °Cd (NDVI post-tt400) in 30 Indian cultivars under high N (HN) and low N (LN) conditions in 2014, 2016 and mean of 2014 and 2016.

	SS.Dur (°Cd)						$SPAD_A$						NDVI pre-tt400						NDVI post-tt400					
	2014		2016		Mean		2014		2016		Mean		2014		2016		Mean		2014		2016		Mean	
Genotype	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN
BH- 1146	100.8	95.9	184.5	349.9	142.7	222.9	46.0	43.4	47.7	47.4	46.9	45.4	85.5	85.0	89.2	86.6	87.3	85.8	77.0	74.5	63.0	52.3	70.0	63.4
CBW- 38	115.9	190.8	146.8	227.1	131.4	209.0	45.9	43.9	48.3	41.1	47.1	42.5	81.7	82.0	88.6	81.9	85.1	81.9	74.5	73.5	64.3	51.7	69.4	62.6
DBW- 14	112.2	174.5	464.9	685.0	288.6	429.7	45.7	41.1	53.9	54.9	49.8	48.0	83.3	84.0	83.0	80.3	83.2	82.2	72.0	74.5	56.3	44.4	64.2	59.5
DBW- 16	244.9	212.4	185.3	481.7	215.1	347.0	46.6	44.2	46.9	38.7	46.7	41.5	82.2	78.3	86.5	82.2	84.3	80.3	72.1	78.9	62.0	58.0	67.0	68.4
DBW- 17	107.8	165.1	123.7	316.1	115.7	240.6	49.0	44.3	48.6	41.5	48.8	42.9	83.0	82.0	90.5	77.8	86.7	79.9	73.3	72.2	69.7	57.0	71.5	64.6
DBW- 39	162.0	212.6	275.1	267.8	218.6	240.2	51.0	47.3	48.4	39.9	49.7	43.6	83.2	82.3	89.1	78.5	86.1	80.4	79.8	74.5	75.0	58.1	77.4	66.3
DBW- 46	161.7	138.3	171.5	208.8	166.6	173.5	45.1	41.8	45.6	42.4	45.4	42.1	81.0	81.3	86.2	82.9	83.6	82.1	75.3	79.2	68.9	61.0	72.1	70.1
DBW- 51	78.7	183.2	98.5	234.4	88.6	208.8	50.7	48.0	49.7	40.1	50.2	44.0	81.8	78.7	90.0	75.4	85.9	77.1	84.3	77.1	74.3	56.2	79.3	66.7
DBW- 71	106.7	129.2	167.7	223.5	137.2	176.4	49.0	45.7	48.4	44.7	48.7	45.2	85.3	85.7	88.9	81.7	87.1	83.7	79.5	74.8	69.1	50.4	74.3	62.6
DPW- 621-50	109.1	216.1	136.5	351.6	122.8	283.9	48.3	45.5	50.0	42.1	49.1	43.8	85.8	82.0	86.1	79.1	86.0	80.5	79.5	72.5	63.2	48.8	71.3	60.7
GW- 322	138.5	141.5	117.5	275.6	128.0	208.6	48.5	46.4	49.5	38.9	49.0	42.6	73.2	81.2	87.3	79.8	80.3	80.5	79.1	73.2	68.2	50.6	73.7	61.9
HD- 2733	172.0	278.1	102.7	243.4	137.3	260.8	47.9	43.7	50.3	40.9	49.1	42.3	79.5	80.5	90.5	78.4	85.0	79.4	78.2	79.1	68.1	58.2	73.1	68.7
HD- 2967	115.5	159.6	91.8	337.1	103.7	248.3	50.9	50.5	45.9	37.3	48.4	43.9	79.7	79.9	89.2	69.2	84.4	74.6	84.9	73.8	69.7	46.8	77.3	60.3
HD-2932	180.1	153.8	311.8	244.9	246.0	199.3	49.1	41.2	51.5	46.0	50.3	43.6	84.8	82.5	88.9	86.4	86.9	84.5	77.1	72.5	67.6	56.8	72.3	64.6
HI- 8498	163.2	223.4	186.2	221.3	174.7	222.4	48.8	44.9	54.9	50.4	51.8	47.7	83.3	82.0	87.6	79.4	85.4	80.7	61.5	71.5	64.1	62.7	62.8	67.1
HW- 2044	125.2	172.8	394.0	488.3	259.6	330.5	47.1	37.3	50.0	47.6	48.6	42.5	88.8	85.0	87.4	82.8	88.1	83.9	80.8	73.8	62.3	56.1	71.5	64.9
K- 0307	173.6	137.2	134.8	163.1	154.2	150.1	46.4	42.5	48.6	41.9	47.5	42.2	82.3	82.2	92.2	89.2	87.3	85.7	63.3	71.5	70.1	67.0	66.7	69.3
KHARCHIA- 65	259.1	140.6	261.3	300.1	260.2	220.4	44.5	36.1	48.1	32.2	46.3	34.1	86.5	86.5	87.9	81.7	87.2	84.1	79.5	74.8	54.3	57.1	66.9	65.9
KRL- 19	121.2	191.5	265.7	437.7	193.4	314.6	48.9	42.4	52.6	50.4	50.7	46.4	87.6	85.3	90.0	86.2	88.8	85.8	79.3	74.8	71.9	61.7	75.6	68.2
KRL- 210	154.4	131.8	220.2	412.3	187.3	272.0	50.6	47.2	52.2	45.6	51.4	46.4	88.8	83.3	89.4	78.2	89.1	80.8	79.8	76.3	65.8	49.6	72.8	62.9
KRL-1-4	151.1	137.8	482.6	594.5	316.8	366.2	48.4	46.4	54.0	51.8	51.2	49.1	86.8	86.5	87.1	79.5	87.0	83.0	76.8	77.3	64.9	52.7	70.8	65.0
KRL-213	264.8	200.1	264.4	450.3	264.6	325.2	45.7	38.9	46.8	41.4	46.2	40.1	82.3	82.3	92.9	84.7	87.6	83.5	75.0	75.0	68.9	57.4	71.9	66.2
MACS- 6222	107.9	195.2	206.0	347.8	156.9	271.5	51.7	45.6	52.8	48.4	52.2	47.0	83.8	84.1	88.5	83.0	86.2	83.5	77.1	76.5	65.3	56.1	71.2	66.3
MACS-2496	142.3	224.1	148.8	292.8	145.5	258.5	50.1	44.6	55.1	38.5	52.6	41.6	83.0	80.0	87.3	73.9	85.1	76.9	83.4	72.8	65.8	43.4	74.6	58.1
MACS-6478	68.8	155.8	143.4	370.5	106.1	263.1	47.5	46.0	51.0	45.8	49.2	45.9	85.8	82.5	91.5	83.2	88.7	82.9	79.5	72.0	70.9	52.1	75.2	62.1
NW-1067	133.9	156.7	235.9	361.9	184.9	259.3	49.2	44.3	52.2	47.5	50.7	45.9	81.8	81.8	88.6	83.3	85.2	82.6	81.0	75.0	73.0	55.4	77.0	65.2
PDW- 314	160.3	130.5	137.1	346.3	148.7	238.4	50.6	45.8	55.1	44.3	52.8	45.1	83.0	78.3	93.0	83.2	88.0	80.8	89.2		79.2	73.1	84.2	83.5
RAJ- 4229			514.4	329.1	514.4	329.1	48.6	46.7	54.0	50.3	51.3	48.5	87.8	86.2	89.7	82.6	88.8	84.4	79.5	75.3	70.8	43.7	75.1	59.5
RAJ- 4238	175.5	116.8	512.2	535.3	343.8	326.0	49.5	44.9	53.9	55.4	51.7	50.1	86.7	84.3	88.3	81.0	87.5	82.6	80.3	75.8	63.6	44.5	71.9	60.1
WH- 1021	135.2	116.1	162.3	274.4	148.7	195.2	49.1	45.1	49.6	48.0	49.4	46.5	83.7	84.2	90.2	85.5	86.9	84.8	80.9	75.5	71.1	52.3	76.0	63.9
Mean	146.3	168.3	228.2	345.8	193.4	259.7	48.3	44.2	50.5	44.5	49.4	44.3	83.7	82.7	88.8	81.3	86.3	82.0	77.8	74.8	67.4	54.5	72.6	64.9
Min	68.8	95.9	91.8	163.1	88.6	150.1	44.5	36.1	45.6	32.2	45.4	34.1	73.2	78.3	83.0	69.2	80.3	74.6	61.5	71.5	54.3	43.4	62.8	58.1
Max	264.8	278.1	514.4	685.0	514.4	429.7	51.7	50.5	55.1	55.4	52.8	50.1	88.8	86.5	93.0	89.2	89.1	85.8	89.2	79.2	79.2	73.1	84.2	83.5
	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df
N	30.5 ns	1	20.4 *	2	17 *	3	0.5 ns	1	1.1 *	2	0.7 **	3	2.3 ns	1	0.8 **	2	1 *	3	3.2 ns	1	2 *	2	1.7 *	3
G	40 ns	51	56.7 ***	84	38 ***	135	1.2 ***	51	1.8 ***	84	1.2 ***	135	1.7 ***	58	2.1 ***	116	1.5 ***	174	1.9 ns	31	4.5 ***	116	3.2 ***	147
N*G	63.4 ns	15.4	81.4 ns	80.5	55.4 ***	110.8	1.8 ns	40.5	2.7 ***	50.2	1.8 ***	78.3	3.3 ns	4.3	3 **	103.2	2.3 *	67.4	4.1 ns	2.8	6.6 ns	90.4	4.7 *	93.2
Y					18.5 **	3					0.7 ns	3					0.5 *	3					0.6 ns	3
N*Y					25.3 ns	6					1 ns	6					1.1 *	4.4					1.8 ***	3.8
G*Y					56.9 ***	104.1					1.9 ***	81.3					2.1 ***	165.9					4.5 *	150
N*G*Y					80.3 ns	130.5					2.6 **	111.5					3.1 ns	128.3					6.6 ns	135.51

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level.

5.3.8 Flag-leaf A_{max}, g_s, C_i and Fv'/Fm' in HN treatment in 2016

Under HN conditions in 2016, 7 days and 15 days (07 Jan and 23 Jan) post-anthesis two assessment dates, flag-leaf photosynthesis (A_{max}), stomatal conductance (g_s), intrasellar CO_2 concentration (C_i) and maximum efficiency of PSII (Fv'/Fm') were measured (Table 5.7). Averaging over assessment dates, flag-leaf photosynthesis rate ranged amongst cultivars from 13.2 to 23.4 µmol m^{-2} s^{-1} (P<0.001) (Table 5.7) and stomatal conductance from 0.15 to 0.42 mol m^{-2} s^{-1} (P<0.001) (Table 5.7). Maximum efficiency of PSII ranged amongst cultivars from 0.55 to 0.64 (P=0.047) (Table 5.7).

Flag-leaf A_{max} showed a positive linear relationship amongst genotypes with AGDM (R^2=0.19, P=0.02; Fig. 5.11 a) and non-significant a positive association with GY (P=0.14, Fig. 5.11 a). There was a positive linear association of each of GY and AGDM with g_s (R^2=0.22, P=0.01 and R^2=0.19, P=0.02 respectively; Fig. 5.7 b) and with Fv'/Fm' (R^2=0.21, P=0.01 and R^2=0.21, P=0.01 respectively; Fig. 5.7 d).

Overall there was no association between A_{max} or C_i or g_s or Fv'/Fm' with flag-leaf specific leaf N (SLN_A) at anthesis (Data not shown). There was a positive linear association between A_{max} and conductance (R^2= 0.52, P<0.001) (Data not shown). There was no association between A_{max} and flag-leaf area.

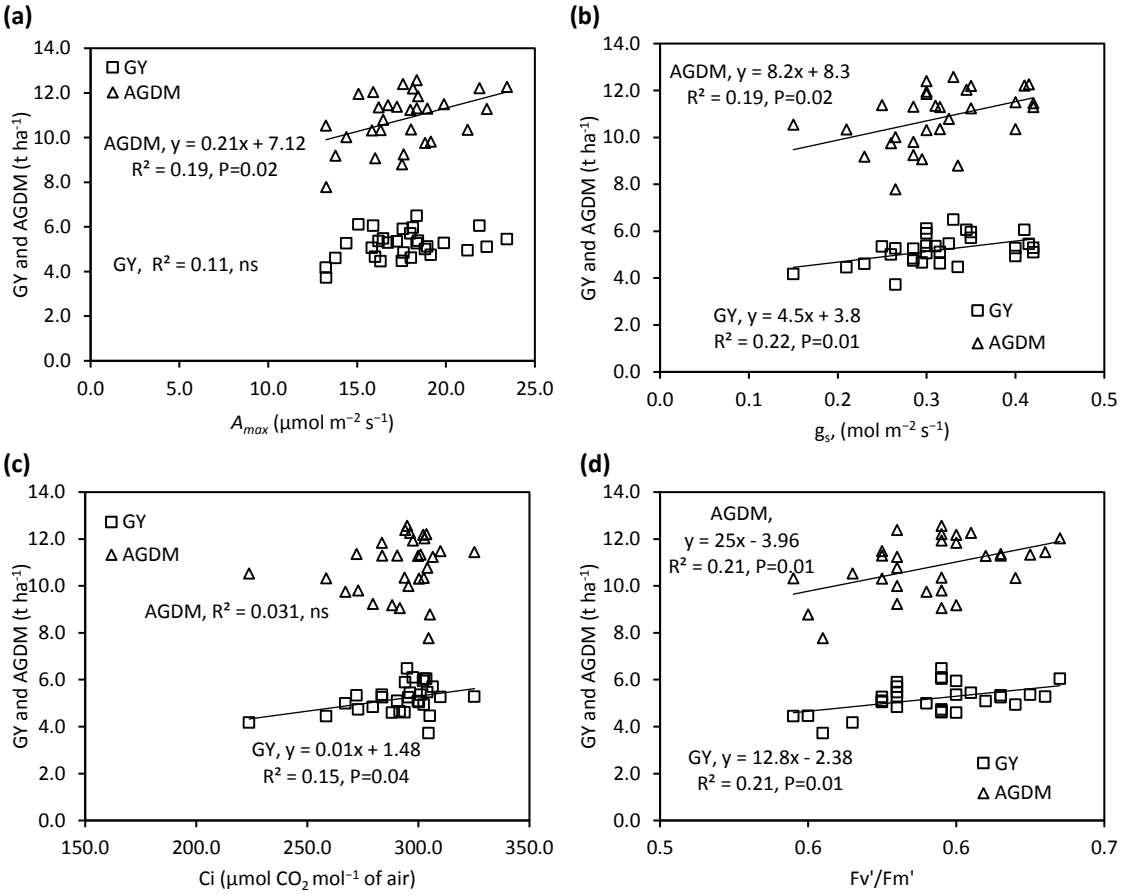


Figure 5. 8 Simple linear regression of grain yield (100% DM) and above-ground dry matter on light-saturated maximum photosynthesis (A_{max}), stomatal conductance (g_s), internal CO_2 concentration (C_i), and efficiency of PSII (Fv'/Fm') under high N (HN) conditions in 2016 for 30 wheat cultivars.

Table 5. 7 Mean of two post-anthesis assessment date of Flag-leaf photosynthesis (A_{max}), stomatal conductance (g_s), intracellular CO_2 concentration (C_i) and PSII efficiency (Fv'/Fm') for 30 wheat cultivars.

Cultivars	A_{max} (µmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	C_i (µmol CO_2 mol^{-1} of air)	Fv'/Fm'
BH- 1146	16.33	0.21	258.5	0.55
CBW- 38	17.24	0.25	272.2	0.62
DBW- 14	13.26	0.27	304.6	0.56
DBW- 16	18.03	0.32	293.9	0.60
DBW- 17	18.13	0.35	302.2	0.60
DBW- 39	17.57	0.30	294.1	0.58
DBW- 46	18.94	0.32	290.4	0.58
DBW- 51	18.43	0.30	283.5	0.60
DBW- 71	15.84	0.30	300.2	0.58
DPW- 621-50	19.89	0.40	309.9	0.58
GW- 322	15.90	0.35	302.8	0.64
HD- 2733	23.44	0.42	296.2	0.61
HD- 2967	16.74	0.42	325.2	0.63
HD-2932	16.47	0.33	304.0	0.58
HI- 8498	18.82	0.26	267.1	0.59
HW- 2044	17.53	0.34	305.1	0.55
K- 0307	19.15	0.29	272.9	0.60
KHARCHIA- 65	13.24	0.15	223.8	0.57
KRL- 19	13.78	0.23	288.2	0.60
KRL- 210	18.36	0.29	283.7	0.62
KRL-1-4	17.61	0.29	279.6	0.58
KRL-213	21.89	0.41	303.6	0.60
MACS- 6222	15.08	0.30	297.5	0.60
MACS-2496	22.30	0.42	299.9	0.61
MACS-6478	18.35	0.33	295.0	0.60
NW-1067	21.22	0.40	302.4	0.62
PDW- 314	16.21	0.31	301.0	0.63
RAJ- 4229	16.02	0.30	291.6	0.60
RAJ- 4238	14.39	0.27	295.5	0.58
WH- 1021	17.99	0.35	306.5	0.58
Mean	17.60	0.31	291.7	0.59
Min	13.24	0.15	223.8	0.55
Max	23.44	0.42	325.2	0.64
	SED, Pro (df)	SED, Pro (df)	SED, Pro (df)	SED, Pro (df)
Ass	0.41 *** (40)	0.013 ** (41)	3.89 ** (41)	0.006 * (40)
Genotypes	1.59 *** (40)	0.05 *** (41)	15.06 *** (41)	0.025 * (40)
Ass*Genotypes	2.25 *** (40)	0.07 *** (41)	21.29 * (41)	0.035 ns (40)

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level.

5.3.10 Associations between GY, N-uptake, NUtE and leaf and canopy senescence-related parameters

To investigate trait associations, principal component analysis was conducted for 21 traits related to yield, N uptake and senescence for the 30 cultivars in each year and for the mean of the two years under HN and under LN conditions.

5.3.10.1 PCA of traits in 2014

In 2014 under HN, PCA showed that the first two principal components (PCs) explained 56.4% of total variation (PC1, 32.5% and PC2, 23.9%) (Fig. 5.12 a) and under LN, first two PCs explained 56.8% of total variation (PC1, 34.3% and PC2, 22.5%) (Fig. 5.12 b).

Under HN: GY showed a positive association with AGDM ($P<0.001$), grain N ($P<0.001$), and N uptake components at anthesis like NUp An, StL N An and Ear N An. NUtE showed a positive association with AD, senescence traits (VS.OnsetRP and VS.EndRP) and N remobilization components (FL NRE, StL NREand Crop NRE) and showed negative association with FL NPI An and FL N An.

Under LN: GY showed a positive association with NUpAn ($P<0.001$) along with above-ground N components (Ear N An, FL N An, StL N An), grain N ($P<0.001$), AGDM ($P<0.001$) and NUpH. NUtE showed a positive association with N remobilization components (FL NRE, StL NRE and crop NRE), AD, senescence traits (VS.OnsetRP and VS.EndRP) and HI. PANU showed negative association with FL NRE.

5.3.10.2 PCA of traits in 2016

In 2016 under HN, PCA showed that the first two PCs explained 60.8% of total variation (PC1, 42.5% and PC2, 18.3%) (Fig. 5.12 c) and under LN, first two PCs explained 69.7% of total variation (PC1, 54.4% and PC2, 15.3%) (Fig. 5.12 d). In summary

Under HN: GY showed a positive association with FL N An, AGDM, NUp An and overall above-ground N components (Ear N An, Grain N An, StL N An) and senescence traits (VS.OnsetRP and

VS.EndRP). NUtE showed a positive association with N remobilization components like FL NRE (P<0.001), StL NRE (P=0.02) and Crop NRE (P<0.001), SLN, and photosynthesis. PANU showed negative association with above-ground N components (FL N An, StL N An and Ear N An), AD, senescence traits (VS.OnsetRP and VS.EndRP) and showed positive association with HI.

Under LN: GY showed a positive association with NUp An along with above-ground N components (FL N An, StL N An and Ear N An and grain N), AGDM, NRE traits (FL NRE, StL NRE and crop NRE) and senescence traits (VS.OnsetRP and VS.EndRP) and AD. NUtE showed a positive association with AD, senescence traits (VS.OnsetRP and VS.EndRP) and negative association with FL NPI An and PANU. PANU showed negative association with above-ground N components (FL N An, StL N An and Ear N An) AD, and senescence traits (VS.OnsetRP and VS.EndRP) and positive association with HI.

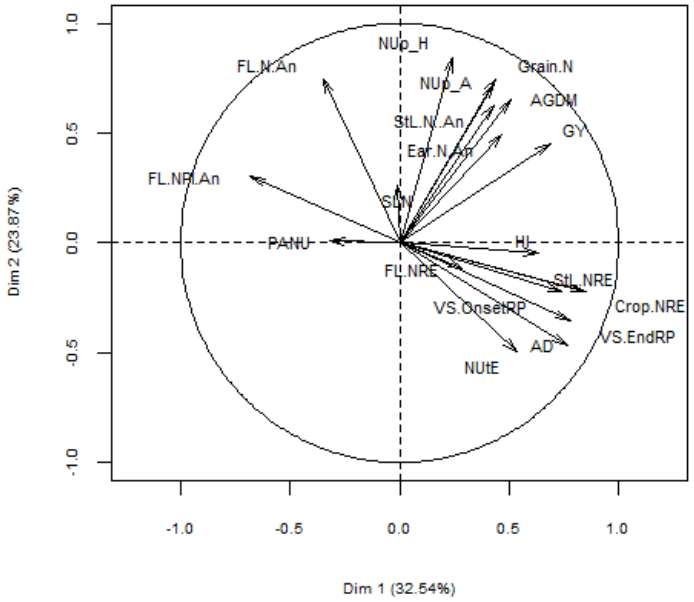
5.3.10.3 PCA analysis of traits across years

Across years, under HN, PCA showed that the first two PCs explained 62.9% of total variation (PC1, 43.4% and PC2, 19.5%) (Fig. 5.12 e) and under LN, first two PCs explained 62.7% of total variation (PC1, 45.1% and PC2, 17.6%) (Fig. 5.12 f). In summary

Under HN: GY showed a positive association with NRE traits (FL, StL and Crop NRE) and above-ground N at anthesis traits (FL N, Ear N, SLN and Grain N), NUp H and AGDM. NUp An showed a positive association with AD, StL N An and senescence traits (VS.OnsetRP and VS.EndRP). NUtE showed a positive association with FL NRE and StL NRE and HI. PANU showed negative association with AD, senescence traits (VS.OnsetRP and VS.EndRP) and NUp An.

Under LN: GY showed a positive association with NUp An and its components (StL N and Ear N and grain N), AGDM and NUpH. NUtE was positively associated with NRE traits (FL, StL and crop NRE) and senescence traits (VS.OnsetRP and VS.EndRP) along with AD and negatively with PANU. GY showed stronger association with NUpH than NUtE as compared to HN conditions.

a) 2014 HN



(b) 2014 LN

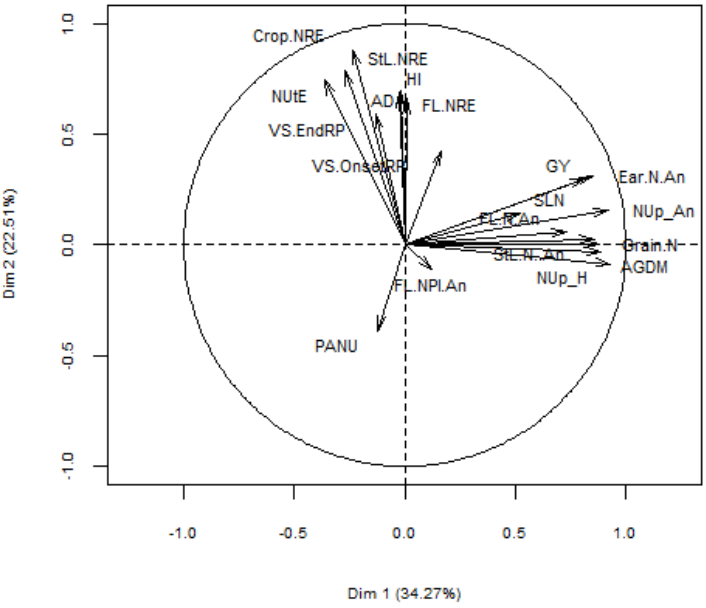
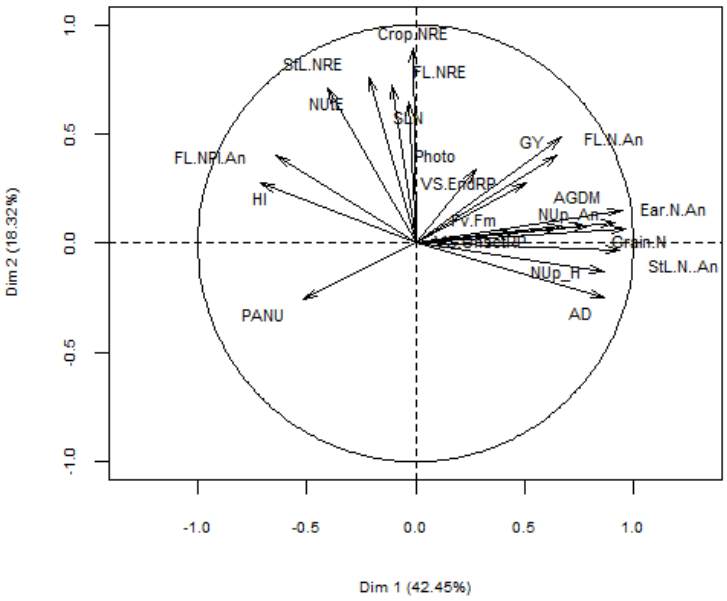


Figure 5.9 Principal component analysis for grain yield (GY), harvest index (HI), anthesis date (AD), flag-leaf visual senescence onset of rapid phase (VS.OnsetRP) and end of rapid phase (VS.EndRP), N remobilization efficiency of flag-leaf (FL NRE), stem and remaining leaf (StL NRE) and crop (Crop NRE), flag-leaf N partitioning index at anthesis (FL NPI AN), Fla-leaf N at anthesis (FL N An), stem and remaining leaf N at anthesis (StLN AN) and ear N at anthesis (Ear N An), grain N (Grain N), post-anthesis N uptake (PANU), N-uptake efficiency at anthesis (NUp An) and at harvest (NUp H), N-utilization efficiency (NUtE) and specific leaf N (SLN) at anthesis.

(c) 2016 HN



(d) 2016 LN

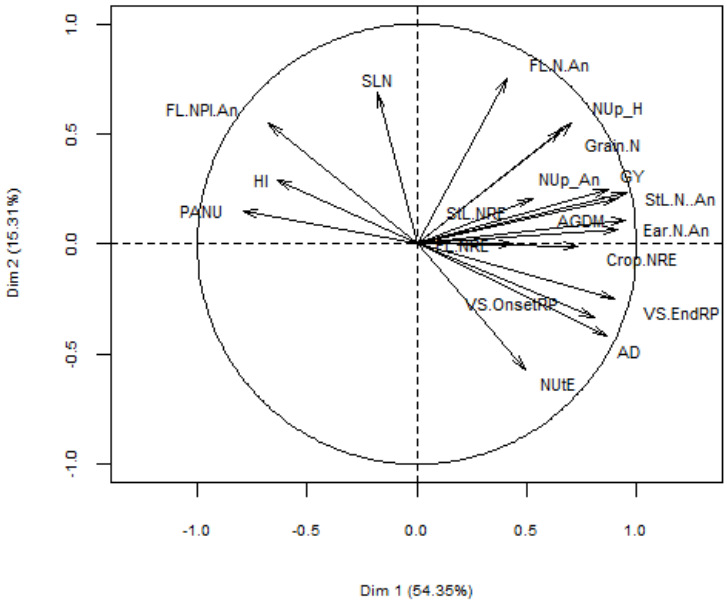
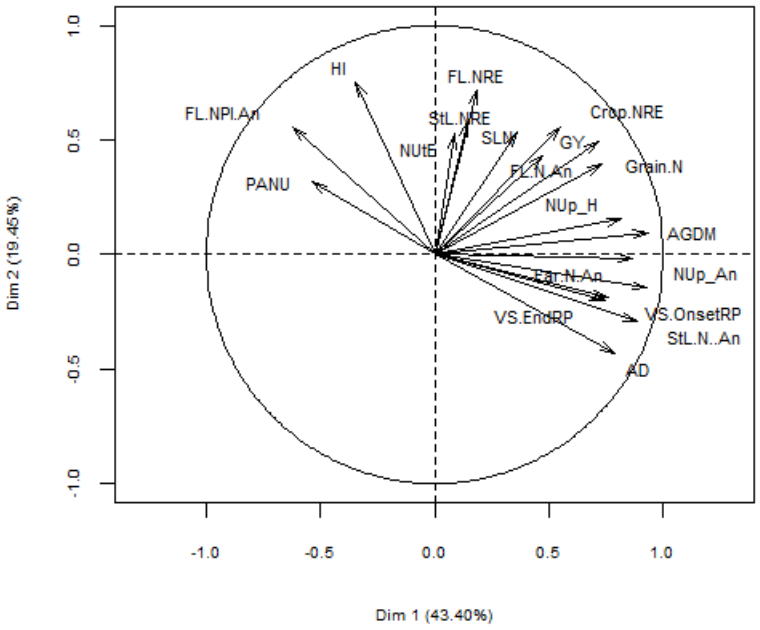


Figure 5.9 Contd. Principal component analysis for grain yield (GY), harvest index (HI), anthesis date (AD), flag-leaf visual senescence onset of rapid phase (VS.OnsetRP) and end of rapid phase (VS.EndRP), N remobilization efficiency of flag-leaf (FL NRE), stem and remaining leaf (StL NRE) and crop (Crop NRE), flag-leaf N partitioning index at anthesis (FL NPI AN), Fla-leaf N at anthesis (FL N An), stem and remaining leaf N at anthesis (StLN AN) and ear N at anthesis (Ear N An), grain N (Grain N), post-anthesis N uptake (PANU), N-uptake efficiency at anthesis (NUp An) and at harvest (NUp H), N-utilization efficiency (NUtE) and specific leaf N (SLN) at anthesis.

(e) Cross years HN



(f) Cross years LN

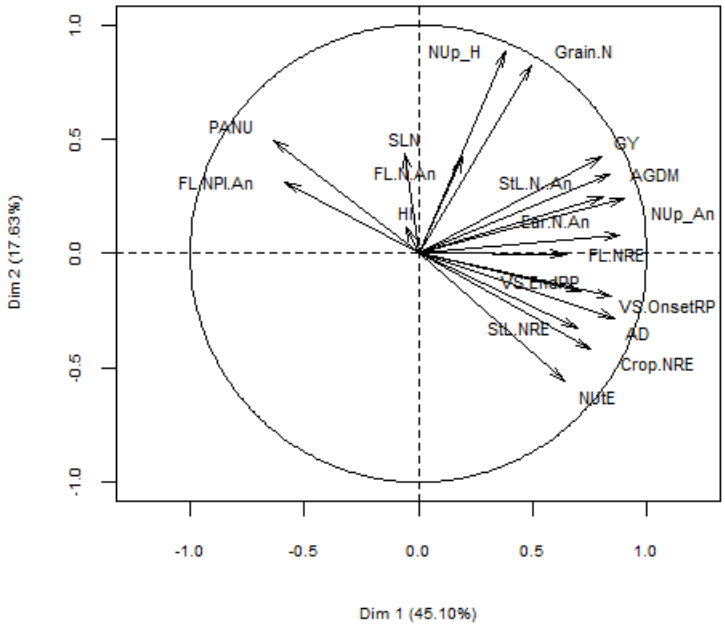


Figure 5. 9 Contd. Principal component analysis for grain yield (GY), harvest index (HI), anthesis date (AD), flag-leaf visual senescence onset of rapid phase (VS.OnsetRP) and end of rapid phase (VS.EndRP), N remobilization efficiency of flag-leaf (FL NRE), stem and remaining leaf (StL NRE) and crop (Crop NRE), flag-leaf N partitioning index at anthesis (FL NPI AN), Fla-leaf N at anthesis (FL N An), stem and remaining leaf N at anthesis (StLN AN) and ear N at anthesis (Ear N An), grain N (Grain N), post-anthesis N uptake (PANU), N-uptake efficiency at anthesis (NUp An) and at harvest (NUp H), N-utilization efficiency (NUtE) and specific leaf N (SLN) at anthesis.

5.3.11 Effects of breeding zone

Effects of the breeding zone on yield and harvest traits were explained in chapter 4. In this chapter traits related to N uptake, plant component N remobilization and senescence are explained. The effect of the breeding zone was significant for FL N_A, StL N_A, Ear N_A, VS.OnsetRP, VS.EndRP, and grains m^{-2} (Table 5.7). There was N x breeding zone interaction for FL N_A, StL N_A, Ear N_A, VS.OnsetRP, VS.EndRP and grains m^{-2}. AD was positively associated amongst breeding zone with NUp_A and VS.OnsetRP and VS.EndRP under HN and LN conditions but there was no strong association with NRE or SLN. Concerning the N*G*BZ interaction (i.e. interaction of N and genotype within a breeding zone), FL NRE and Crop NRE only showed interaction. There was Y*N*G*BZ interaction for FL NRE, StL NRE, Crop NRE and VS.EndRP.

Overall NWPZ and PZ cultivars were late flowering and maturing and showed stay-green traits with a higher yield than other zones. The main effect of the breeding zone was that PZ cultivars showed relatively higher N-uptake (FL N_A, StL N_A, Ear N_A), SLN and grains m^{-2} under HN and higher Ear N_A and grains m^{-2} under LN conditions than cultivars from other zones. There was a positive association amongst breeding zones between GY and STRL NRE, Crop NRE, FL N_A, STRL N_A, Ear N_A, VS.OnsetRP, VS.EndRP, Grains m^{-2} and SLN_A under HN and Ear N_A, VS.OnsetRP, VS.EndRP, Grains m^{-2}, SLN_A under LN conditions. GY showed a negative association with FL NRE, StL NRE Crop NRE, FL N_A, and StL N_A under LN conditions and FL NRE under HN conditions.

Results also showed an effect of the breeding zone on photosynthesis traits and their associated with other traits. Amongst breeding zones, it was found that SLN (g N m^{-2}) under HN showed a positive association with g_s (R^2=0.84, P=0.01) and with C_i (R^2=0.93, P=0.002) and under LN showed a positive association with A_{max} (R^2=0.85, P=0.01) and g_s (R^2=0.75, P=0.03) (data not shown).

5.3.12 Performance of individual cultivars

Overall, MACS-6478 was the highest yielding cultivar under HN conditions and GW-322 was highest yielding cultivar under LN condition. MACS-6478 also showed highest ranking under

HN conditions for AGDM, NUE, AGN_A, NRE, StL N_A (15.3 g m^{-2}) and grain N_H (16.1 g m^{-2}). GW-322 showed highest NUE under LN. HD-2932 showed highest NUpE (0.53), grain N (8.4 g m^{-2}) and AGN_H (10.1 G M^{-2}) under LN conditions and highest VS.OnsetRP (1135.8 CD) under HN conditions. Kharchia-65 was the tallest cultivar under both N treatments. It also showed highest NUtE and BMPE under LN and StL N_H (2.8 g m^{-2}) under HN. However, it showed the lowest performance for NUpE, AGN_H (g m^{-2}), grain N_H (5.3 g m^{-2}) and $SPAD_A$ (34.1) under LN conditions and NRE and NHI under HN conditions. KRL-19 was the shortest cultivar under both conditions.

HW-2044 had earliest AD under both conditions. It also showed lowest AGN_A (14.72 g m^{-2}) under HN and highest FL NPI_A under both HN and LN conditions (0.16 and 0.13). K-0307 under LN conditions showed the highest FL N_A (1.7 g m^{-2}), StL N_A (7.4 g m^{-2}) and AGN_A and lowest SS.Dur (150.1 °CD). Under HN it showed highest grain N_H (9.5 g m^{-2}). BH-1146 was lowest yielding cultivar under LN and had the lowest HI, NUE, VS.OnsetRP (480.9 CD) under LN conditions and the lowest NUtE under HN. It also showed lowest Ear NPI_A under both N treatments (0.21 and 0.28). DBW-14 under HN conditions showed lowest GY, AGDM, NUE, NUpE, AGN_H, grain N (9.12 g m^{-2}), VS.MidRP (644.2 CD) and VS.OnsetRP (542.5 CD). Under LN condition it had lowest AGN_A and StL N_A. It also had the earliest PMD and lowest for Ear N_A under both HN and LN conditions (4.07 and 2.85 g m^{-2}, respectively).

Table 5. 8 Mean of breeding zones for grain yield (GY), above-ground dry matter (AGDM), anthesis date (AD), N-utilization efficiency (NUtE), flag-leaf N remobilization efficiency (FL NRE), stem and remaining leaf N remobilization efficiency (StL NRE), crop N remobilization efficiency (Crop NRE), flag-leaf N at anthesis (FL N_A), stem and remaining leaf N at anthesis (StL N_A), ear N at anthesis (Ear N_A), visual senescence onset of rapid phase (VS.OnsetRP), visual senescence end of rapid phase (VS.EndRP), grains m^{-2} and specific leaf N at anthesis (SLN_A) under HN and LN conditions. Values represent the mean of 2014 and 2016.

	No of	GY (t ha^{-1})		AGDM (t ha^{-1})		AD (DAS)		NUtE (g GDM g AGN^{-1})		FL NRE		StL NRE		Crop NRE	
	cultivars	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN
AZ	3	4.58	3.55	9.77	7.33	58	58	32.26	46.9	0.86	0.87	0.82	0.87	0.83	0.89
CZ	3	5.24	3.74	10.16	7.75	60	59	33.1	43.46	0.86	0.89	0.84	0.85	0.85	0.88
NEPZ	11	4.99	3.78	10.79	8.22	64	63	32.1	43.26	0.85	0.88	0.83	0.87	0.84	0.88
NWPZ	8	5.52	3.8	11.64	8.23	65	65	32.84	45.27	0.86	0.87	0.85	0.86	0.85	0.88
PZ	4	5.99	4.02	12.19	8.18	66	64	32.43	48.02	0.86	0.89	0.83	0.85	0.84	0.89
SHZ	1	4.34	3.18	8.49	6.36	52	52	31.33	44.58	0.87	0.91	0.84	0.87	0.85	0.89
		SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df
	N	0.16 *	3	0.30 **	3	0.50 ns	3	0.97 ***	3	0.004 **	3	0.01 *	3	0.01 *	3
	Y	0.25 ns	3	0.37 *	3	0.90 ns	3	1.21 ns	3	0.005 **	3	0.01 *	3	0.006 *	3
	BZ	0.23 ***	174	0.45 ***	174	0.60 ***	174	1.29 **	174	0.02 ns	174	0.01 ns	174	0.011 ns	174
	N*BZ	0.35 **	53.1	0.68 *	60.8	1.00	40.7	2.02	46.9	0.02 ns	175.4	0.02 ns	88.5	0.016 ns	133.4
	N*G*BZ	0.45 ns	103.2	0.89	113.1	1.30 ns	84.3	2.62	94.2	0.03 *	177	0.03 ns	140.2	0.021 **	166
	N*G*Y*BZ	0.68 ns	90.9	1.30	134.9	2.0 *	53	3.84	114.5	0.04 *	179	0.04 *	163.2	0.031 *	173.6

	No of	FL N_A (g m^{-2})		StL N_A (g m^{-2})		Ear N_A (g m^{-2})		VS.OnsetRP		VS.EndRP		Grains m^{-2}		SLN_A (g N m^{-2})	
	cultivars	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN
AZ	3	2.23	1.06	12.13	6.32	5.46	3.7	759.9	672.3	1024.1	911.9	12989.6	9438.7	2.22	1.71
CZ	3	2.33	1.2	11.06	6.5	5.47	3.83	924.6	729.3	1086.3	899.8	12126.6	8538.3	2.34	1.78
NEPZ	11	2.39	1.23	12.18	6.76	5.76	4.01	866.9	725.5	1073.5	994.6	14200.4	9547.9	2.33	1.81
NWPZ	8	2.28	0.99	13.01	6.16	6.35	3.9	944.9	788.4	1138.4	1016.7	16200.1	9888.5	2.39	1.83
PZ	4	2.81	1.02	13.98	5.85	7.19	4.18	875.6	714.2	1084	991.9	17128.5	9908.6	2.44	1.79
SHZ	1	2.42	1.52	8.44	5.81	4.43	3.58	635.2	678.4	930.4	725.2	10374.6	7999.7	2.38	1.82
		SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df
	N	0.12 **	3	0.73 **	3	0.19 **	3	15.6 **	3	27.2 *	3	383.5 ***	3	0.103 *	3
	Y	0.084 ***	3	0.89 *	3	0.39 **	3	16.7 ***	3	26.9 **	3	444.7 **	3	0.07 ns	3
	BZ	0.172 *	174	0.84 *	174	0.36 ***	174	45.8 ***	174	22.9 ***	174	649.4 ***	174	0.108 ns	173
	N*BZ	0.265 *	56.5	1.36 *	32.6	0.52 **	98.4	64.7 ns	160.5	41.5 ***	15.9	969.1 ***	81.5	0.18 ns	26.1
	N*G*BZ	0.346 ns	107.7	1.74 ns	69.7	0.70 ns	147.4	87.7 ns	174.7	51.0 ***	33.5	1281.51 *	134.4	0.227 ns	56.6
	N*G*Y*BZ	0.491 ns	158.2	2.56 ns	91.9	1.06 ns	97.4	126.7 ns	180	73.3 ***	62.8	1861.7 ns	156.8	0.319 ns	113.1

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level. (AZ=All Zones, CZ=Central Zone, NEPZ=North Eastern Plain Zone, NWPZ=North Western Plain Zone, PZ=Peninsular Zone, SHZ=Southern Hills Zone.)

5.4 DISCUSSION

The present results allow, firstly, discussion of the physiological basis of genetic variation in N accumulation, N partitioning and N remobilization and their responses to N supply. Secondly, the associations of senescence parameters with N accumulation, N partitioning, NRE and grain yield and the implications of the present results for breeding more N-efficient crops will be discussed. Emphasize is given to traits explaining or related to N uptake as there was stronger linear association with GY for N uptake than for NUtE in Chapter 4 (Fig. 4.8 and 4.9).

5.4.1 Genetic variation in N accumulation and N partitioning and response to N availability.

Overall, above-ground N at anthesis in the FL and StL was reduced under LN conditions but the N partitioning to the ear was relatively higher (34%) under LN conditions than under HN conditions (29%) suggesting that under N limitation the plant prioritizes allocation of N to the ear to produce viable grains and use limited N efficiently. Similar results were also reported by Pask *et al.* (2012) and Gaju *et al.* (2014) in winter wheat France and the UK. Above-ground N accumulation in crop components at anthesis (FL N_A, StL N_A and Ear N_A) under both N conditions showed a positive association with GY and AGDM, except FL N_A under LN conditions, indicating the importance of N accumulation at anthesis for higher yield at maturity. Under both N conditions later anthesis favored higher StL N_A and Ear N_A which was associated with higher GY and AGDM. It is possible that under LN, later anthesis was indirectly responsible for higher NUtE by creating ear sink strength (grains m^{-2}) which may have driven more ear N uptake at anthesis and in turn upregulating post-anthesis photosynthesis (Reynolds *et al.*, 2000). Effects of sink strength of upregulating photosynthesis are reported in sugarcane (McCormick *et al.*, 2006) and in wheat (Reynolds *et al.*, 2005).

FL N partitioning at anthesis showed a negative association with AD under both HN and LN conditions and with GY under LN conditions indicating earlier cultivars allocate more N to flag-leaf. Higher FL N at anthesis could be explained by the fact second dose of N was applied 25 days and 20 days after sowing in 2014 and 2016 respectively, which may have come close to flowering time of early cultivars given more 'luxury N' allocated to FL. This could also be seen through flag-leaf SPAD at anthesis which showed a positive association in 2014

but a negative association in 2016 with GY. This association also may have been influenced by the fact that, in 2014, the growing season was shorter (94 DAS) so the second dose of N fertilizer applied after 25 days of anthesis may have come relatively closer to flowering time of early cultivars.

Proportionally more N in the ear and less in the leaf lamina and stem at anthesis under N limitation indicates the ear to be a priority sink for N as N supply became limiting. The genetic range in ear NPI in the present study was slightly higher than that reported by Gaju *et al.* (2014) of 0.18-0.22 under HN and 0.22-0.30 under LN or by Pask *et al.* (2012) 0.18-0.22 under HN and 0.23-0.28 under LN. The greater range may be due to greater genetic diversity amongst thirty cultivars and a greater range in anthesis data relative to the total growth period of the plant. Under LN conditions, cultivars with later anthesis date tended to partition more N to the ear. This may have been because under LN later anthesis date favored deeper rooting and an enhanced N uptake capacity during ear growth from booting to anthesis or alternatively there was a longer time for soil N mineralization with later anthesis enhanced N supply from booting to anthesis. Similar results were reported by Gaju *et al.* (2014) in UK winter wheat cultivars. Under LN conditions, the increased ear N partitioning could also be associated with an increase in ear N concentration rather than an increase in ear dry mass partitioning. The prioritization of the ear for N partitioning under LN raises the question of whether N concentration in the ear at anthesis is important for floral development (Sinclair and Jamieson, 2006). Present results, however, indicated no association between N concentration in the ear at anthesis (ear N%) and grains per gram of ear dry mass at anthesis (fruiting efficiency) amongst cultivars (data not shown) suggesting that assimilate supply to the ear rather than N concentration in the ear was the critical determinant of floret survival prior to anthesis in these experiments. Fischer (2008) concluded that there was no evidence for effects of N on grain number apart from those operating via dry matter accumulation; and González *et al.* (2011) demonstrated in wheat that onset of floret death was associated with the beginning of spike growth at the maximum rate consistent with assimilate availability regulating grain number formation.

5.4.2 N accumulation, N partitioning and N remobilization and senescence duration amongst cultivars.

In this study, stay-green variables were estimated allowing comparison of adaptation of different genotypes to the specific climate of the ARI field site in the peninsular zone of India.

In general, in wheat crops senescence occurs earlier if there is limited N reserve and in this experiment all the senescence parameters showed relatively reduced senescence duration and/or earlier onset of senescence under LN compared to HN. Similar results were also reported by Pask (2009) and Gaju *et al.* (2014). Results showed that StL N_A and Ear N_A were positively associated with onset (VS.OnsetRP) and end of flag-leaf senescence (VS.EndRP) under both N conditions indicating more N accumulation in these plant components at anthesis may be responsible for buffering N remobilization hence a longer senescence indicating source control on senescence. Ear NPI_A showed a negative association with SPAD senescence traits (SS.Onset and SS.End) under LN indicating greater N allocated to ears at anthesis may affect faster chlorophyll depletion in the flag-leaf resulting in faster senescence. There was no association of SPAD at anthesis with Ear NPI_A under both N conditions. However, flag-leaf SPAD at anthesis showed a positive association with FL NPI_A under LN conditions only.

Overall, visual senescence scoring parameters (VS.OnsetRP and VS.EndRP) showed a positive association with crop NRE under HN conditions and FL NRE, StL NRE and crop NRE under LN conditions. Generally, senescence traits show negative association with NRE but in this experiment this association was positive (Gaju *et al.*, 2016). These results are in agreement with the 'self-destruction' hypothesis of canopy senescence as N is extracted from the vegetative tissues post-anthesis and remobilized to the grain which is reported it in wheat (Sinclair and DeWit, 1975) and in sorghum (Van Oosterom *et al.*, 2010). The experiment of cloning of the NAM-B1 gene from durum wheat also showed the genetic variation in the pattern of canopy senescence linked to canopy N remobilization (Uauy *et al.*, 2006). These present results are contrary to the results in the winter wheat experiments of Gaju *et al.* (2011) and Pask *et al.* (2012) which found a negative association between NR traits and senescence traits under HN; whereas under LN Gaju *et al.* (2014) found no association

between NRE and senescence traits. A positive association between flag-leaf senescence and NRE may be associated with timing of NR in that later N remobilization may have been associated with greater N accumulated at anthesis also simultaneously associated with greater NRE and later senescence. Therefore, there may have been an autocorrelation between greater NRE and later senescence.

Overall PCA trait analysis showed that under HN, NUp_A, senescence traits and GY were major components of PC1 and PC2. Most of the associations showed by PCA analysis were also apparent through correlation analysis. Primarily PCA showed that under HN, GY and crop NRE were positively associated with each other and with flag-leaf senescence traits. This could be interpreted as higher N uptake at anthesis could increase NRE per se and GY through the stay-green trait. A similar association of GY, NUp_A and senescence traits was also found under LN conditions. It is possible that increased expression of rooting traits may have contributed to the stay-green trait under low N; and therefore, rooting traits were screened in a subset of the 6 cultivars and results are presented Chapter 7. Another strategy to increase N uptake at anthesis could be an increase duration of from GS31 to G61 which may optimize crown root production and N accumulation.

Currently, in Indian wheat breeding programs cultivars are selected mostly under HN levels and it would not be cost-effective to select NUE traits under both LN and HN conditions at multi-location trials, although it has been shown that direct selection at LN is more efficient (Brancourt-Hulmel *et al.*, 2005). In the present study, in the LN environments overall N uptake at anthesis and at harvest was associated with phenotypic variation amongst cultivars in NUE and GY. In order to improve NUp_A, optimizing the rooting system will be an important consideration; a root architectural model indicated that the deeper the relative distribution of the roots the greater the N capture as a proportion of that available to the plant (King *et al.*, 2003). Positive associations between increased root density at depth and N-uptake in wheat have also been demonstrated in field investigations, where variation in rooting traits was due to either different sowing dates and soil types (Barraclough, 1986) or genotypes e.g. (Ford *et al.*, 2006) and present findings indicated it may be important to consider rooting traits in breeding programs for NUE. Although root traits were not measured in the field experiment in present study, cultivars selected for their best and worst performance under LN conditions were studied in a 2D seedling root system architecture and results from this experiment are

presented in chapter 7 as mentioned above. It can be speculated that cultivars with the higher yield under LN conditions may have had greater root mass and/or relatively deeper root systems favoring N uptake deeper in the soil profile. It is possible that the delayed canopy senescence (as indicated by flag-leaf visual and SPAD senescence traits) allowed the photosynthetic activity to be prolonged enhancing the post-anthesis N uptake, as reported for stay-green genotypes in sorghum (Borrell *et al.*, 2001a). Alternatively, increased N uptake for these cultivars under LN conditions may have led to delayed senescence particularly if this was associated with greater post-anthesis N uptake (Bogard *et al.*, 2011). In present investigation, higher amount of N uptake at anthesis showed a positive association with senescence traits under both N conditions indicating higher N in flag-leaf takes longer time to remobilize. Whilst grain growth of modern wheat cultivars is considered to be mostly sink-limited under optimal conditions (Borrás *et al.*, 2004), there is some evidence that the extent of sink limitation has decreased with breeding in recent years and the grain growth of recent cultivars may be co-limited by source and sink (Acreche and Slafer, 2009). In the present study, grain growth of the elite Indian wheat cultivars seems have been co-limited even under HN conditions according to the association between senescence traits and GY.

5.4.3 Effect of photosynthesis on grain yield.

Under HN conditions amongst 30 cultivars, results show that there was a positive effect of higher photosynthesis on AGDM and a weaker association with GY. The association of GY with post-anthesis A_{max} was expected and may have reflected upregulation in relation to grain numbers per unit area or extended photosynthesis due to stay-green effect. The genetic variation in pre-anthesis A_{max} on GY needs to be tested in future experiments. Gaju *et al.* (2016) reported an association between GY and A_{max} on 15 genotypes which included landraces, synthetic-derived lines and UK modern cultivars in field experiment in over two years.

5.4.4 Effects of cultivar adaptation to breeding zone on grain yield.

With regards to breeding zone, results showed that individual performance of cultivars from the Peninsular Zone (PZ) was generally better than other zones which was expected. Cultivars from North Western Plain Zone (NWPZ) and PZ were later flowering and maturing than other cultivars. The possible role of *Vrn*, *Ppd* or earliness per se was discussed in chapter 4. Generally, in North India the wheat growing season is longer than in southern India. Also, the climate during wheat growing season in north India is colder than central or south India. So, the wheat yield is higher than in central or south India. In the PZ where the experiment was conducted, the wheat growing season is shorter and cultivars from northern India are not able to perform to their potential even though they were late maturing. Overall the cultivars performing relatively better than other cultivars were MACS-6478 under HN and GW-322 under LN and both these cultivars were recommended and suitable for cultivars in PZ. GW-322 is adopted to the central zone and comes from breeding center, State Agriculture University of Junagar, Gujrat and has the salt tolerance, drought tolerance with increased water use-efficiency (IIWBR, Karnal, ISBN: 0972-6098). Traditionally cultivars from these breeding zones were bred for drought tolerance due to scarcity of water in this part of India. These cultivars, therefore, may have a deeper root system which may help to uptake more N from deep soil. This cultivar showed the best performance for NUE under LN conditions which may be because of a deep root system and able to take more N from the soil.

Overall two different techniques of measurement of flag-leaf senescence were used in this experiment, that is, visual scoring and SPAD chlorophyll measurements and visual senescence scoring seemed the more reliable as it showed stronger associations with yield traits. Also, this method has advantages over SPAD senescence as it took less time. For example for visual senescence scoring of 100 plot take approximately half hour and SPAD measurements (3 readings per flag-leaf on three shoots per plot) took approximately 3 hours.

Summary

In summary, the findings in relation to the hypotheses tested in this chapter were:

"N accumulation at anthesis in the flag-leaf, stem and remaining leaves and ear is a determinant of genetic variation in post-anthesis N remobilization from these respective plant components".

The hypothesis was partially supported. Averaging across years, N accumulation at anthesis in i) flag-leaf, ii) stem and remaining leaves and iii) the whole crop was positively associated with whole crop N remobilization under HN (for the whole crop, r^2=0.41) and with under LN from flag-leaf, stem and remaining leaves and the whole crop N remobilization (r^2= 0.45, r^2= 0.55, r^2=0.68, respectively).

"Leaf-lamina N remobilization is a determinant of genetic variation in flag-leaf senescence".

Leaf-lamina N remobilization efficiency showed positive association with onset of flag-leaf senescence only under LN conditions therefore this hypothesis was supported under LN conditions but not under HN conditions.

"Different techniques of leaf and canopy senescence measurement give similar results for relative genotype performance and responses to N availability".

Overall this hypothesis was not supported as flag-leaf senescence score timing showed a stronger association with GY than NDVI post-anthesis and flag-leaf SPAD senescence timing which was not associated with GY under LN conditions.

"Stay-green traits show a stronger association with genetic variation in yield under N limitation compared to optimal N conditions".

This hypothesis was supported as there was slightly stronger association of GY with VS.OnsetRP and VS.EndRP under LN (r^2=0.53, r^2=0.58) than under HN (r^2=0.47, r^2=0.49) conditions.

CHAPTER 6

N-Use Efficiency And Associated Traits For Amphidiploid Lines And Bread Wheat Parents In Glasshouse Experiments

6.1 INTRODUCTION

Bread wheat originated around 10,000 BC and since then, during domestication, wheat has been selected by humans for improved grain yield, and adapted to diverse environmental conditions and agriculture practices. This process has led to the loss of genetic diversity (Dubcovsky and Dvorak, 2007) and modification in traits like vernalization requirement, photoperiod requirement, and adaptation to abiotic stresses including water-limiting conditions, low temperature, salt and soil toxicity tolerance. Development of semi-dwarf cultivars worldwide and photoperiod-insensitive wheat cultivars in many regions that responded to higher N fertilizer doses in the 1960s and 1970s has narrowed the genetic base affecting the selection procedure for new wheat cultivars for N-use efficiency. Moreover, wheat is a naturally a self-pollinated crop with less heterozygosity and heterosis than outcrossing crops (Longin *et al.*, 2012). Therefore, the option of hybrid seed production to enhance diversity and break the yield plateau seems difficult.

However, genetic diversity can be created by incorporating genes into cultivated wheat from wild wheat relatives (Hawkesford, 2014; Nemeth *et al.*, 2015). Genetic diversity can be created by introgression including production of interspecific hybrids by crossing ancestral or wild relatives of bread wheat to bread wheat cultivars and then repeated back crossing to one of the parents (Tiwari *et al.*, 2014) or by producing amphidiploids by crossing wheat as the female parent with a range of related species to produce F1 interspecific hybrids and subsequent chromosome doubling to complete the diploid chromosome set from each parent. Normally, recombination in wheat is restricted to identical homologous chromosomes from the same genome due to the presence of the *Ph1* locus located on the long arm of chromosome 5B (Al-Kaff *et al.*, 2007). Thus, the *Ph1* locus normally has to be removed before

homoeologous recombination between the chromosomes of a wild relative and wheat can occur (with the exception of crosses with *Aegilops speltoides* and *Aegilops mutica*; both of these wild relatives carry genes which themselves induces gene transfer). The F1 hybrids can then be backcrossed to wheat to produce advanced backcross populations (Reynolds *et al.*, 2012; Jiang *et al.*, 2014; Nemeth *et al.*, 2015; King *et al.*, 2017).

Wheat wild relatives and cultivated grass species could provide genetic diversity in breeding programs to improve specific traits which are not present in cultivated wheat. For example, *Triticum urartu* (the wheat A genome donor) is implicated in photosynthetic capacity and disease resistance (Austin *et al.*, 1982; Sybesma, 2013), *Thinopyrum bessarabicum* is a highly salt tolerant species (King *et al.*, 1997), and a potential donor of genes for heat tolerance, drought and disease resistance, rye (*Secale cereale*) has resistance to rust, acid soils, heat stress, drought stress (Roux *et al.*, 2004), *Aegilops speltoides* (the putative wheat B-genome donor) is a potential donor of disease resistance and insect resistance (Elek *et al.*, 2014), *Ae. umbellulata* has leaf rust resistance (Sears, 1956), and *Ambylopyrum muticum* (=*Ae. mutica*) has powdery mildew resistance (Eser, 1998).

The use of ancestral-derived germplasm, introgressing genes from the ancestral wheat species, and landraces has been suggested as a source of variation for biomass and NUE in wheat breeding programs (Moore, 2015; Gaju *et al.*, 2016). Wild relatives of bread wheat have been reported to have higher leaf photosynthetic rates than modern cultivars (Evans and Dunstone, 1970; Johnson *et al.*, 1987; Carver *et al.*, 1989) suggesting that wheat breeding may have resulted in lower photosynthetic rate. Austin *et al.* (1982) reported that the rate of net photosynthesis was in general highest in the diploid wheat species, intermediate for the tetraploid species and lowest for hexaploid *T. aestivum* L. Synthetic lines (*Triticum durum* × *Triticum tauschi*) and synthetic derivatives developed by CIMMYT have been associated with higher leaf photosynthetic rate (Del Blanco *et al.*, 2000) and grain yield (Ogbonnaya *et al.*, 2007) than recurrent parents under optimal conditions. In addition, primary synthetic spring wheats have been shown to have greater root biomass compared to recurrent parents in Australia (Dreccer *et al.*, 2004) and CIMMYT synthetic-derived wheat lines expressed increased partitioning of root mass to deeper soil profiles and an ability to extract moisture from those depths and higher grain yield under drought compared with the recurrent parents in NW Mexico (Reynolds *et al.*, 2006; Lopes and Reynolds, 2011). There is also evidence that

root system of landraces is larger compared with that of modern cultivars (Ehdaie and Waines, 1997; Waines and Ehdaie, 2007).

In the present study, the amphidiploids studied were produced by crossing wild relatives of wheat (*Ae. mutica, Ae. speltoides, Ae. umbellalata, Ae. comosa, Th. turcicum. Th. bessarbicum*) with bread wheat carrying the *ph1* mutation (Chinese Spring mutants P208/535) and non-mutant wheat cultivars (Chinese Spring euploid 94, Paragon, Pavon 76, Highbury) and for first time these amphidiploids are characterized for NUE and associated traits.

Therefore, the objectives of this experiment were to

(i) Identify novel wheat lines (amphidiploids) expressing higher biomass and N-uptake than the recurrent bread wheat parents under high and low N conditions

(ii) Understand the physiological mechanisms underlying the improved biomass and N-uptake in the novel ancestral amphidiploid lines

The hypotheses tested were:

1. There is genetic variation for above-ground dry matter and N-use efficiency and it's components traits that are related to NUpE and NUtE in the panel of 18 amphidiploid lines of winter wheat.

2. There is novel genetic variation for increased leaf photosynthesis traits (rate and duration) in amphidiploid lines under low N conditions compared to the bread wheat parental lines.

6.2 MATERIALS AND METHODS

This section explains the methodology used including the physiological measurements in two glasshouse experiments conducted in 2014 and 2015 at Sutton Bonington, University of Nottingham, UK campus.

6.2.1 Experimental design

For the two glasshouse experiments, the sowing dates were 23 June 2014 and 6 July 2015 (Fig. 6.2). The experimental procedures and measurements were the same in both experiments unless stated otherwise (Fig. 3.4 a). Seeds were sown in plastic modules filled with F2 + S compost (Levington Advance Seed & Modular F2S). After seed germination (~6 days after sowing) seedlings of 2 to 4 cm length were transferred to a cold room for vernalization for 6 weeks at 6 °C with a 12 h photoperiod and then transplanted into 2 L pots in the glasshouse. Twenty amphidiploid lines along with five bread wheat parental cultivars were grown in the experiment (Table 6.1). Among bread wheat parent cultivars, Paragon (PAR) and Highbury (HB) were UK spring cultivars whereas Pavon was a Mexican a CIMMYT cultivar. Chinese Spring Euploid was the Chinese spring wheat cultivar and Chinese Spring mutant was the Chinese Spring wheat cultivar having the mutant allele at the *ph1* locus which facilitates recombination and gene transfer between the homeologous chromosomes of wheat and its wild relatives. The experimental design used was a split-plot where two levels of N treatment (high N and low N) were randomized on the main-treatment and 25 genotypes were randomized on the sub-plot treatment. A single seedling of each line was transplanted into each pot and represented one replicate. For each treatment combination, there were four replicates. Pot size was 2 L with 16.8 cm diameter and a F block low N peat-compost (Klasmann Medium Peat 818) was used as the soil medium and supplemented with nutrients as described in Table 6.1.

Table 6. 1 Composition of low N peat-compost and nutrient solution used in glasshouse experiments in 2014 and 2015.

Peat-composts F blocks low N (winter)	Amount	Units
Peat: Sand	100:0	% volume
Single super phosphate	1.5	Kg m^{-3}
Potassium sulphate	0.75	Kg m^{-3}
Ground lime chalk	2.25	Kg m^{-3}
Ground magnesian limestone	2.25	Kg m^{-3}
Fritted trace elements WN225	0.4	Kg m^{-3}
P_2O_5 and K_2O	125 and 300	g m^{-3} = mg $litre^{-1}$
Nutrient solution NPK:0,36,36	100	g per 2L diluted for a stock solution 1 FEED/100 water (1 g/L)

6.2.2 Nitrogen treatment

Nitrogen was applied manually by dissolving ammonium nitrate (NH_4NO_3) granules (34% N) in water. Two levels of N were applied, low N 60 kg ha^{-1} equivalent and high N 200 kg ha^{-1} equivalent (0.25 and 1.27 g NH_4NO_3 pot^{-1} under LN and HN, respectively). For low N, N application was split into two doses each of 30 kg N ha^{-1} equivalent and for high N into three doses of 50 kg N ha^{-1}, 50 kg ha^{-1} and 100 kg ha^{-1} equivalents. The first application was applied immediately after transplanting of seedlings in pots and the second at GS31 for both treatments. The last application for the high N treatment was at flag-leaf emergence (GS39).

6.2.3 Glasshouse environmental conditions

Plants were grown in a glasshouse with a light cycle of 16 h at 20 °C and dark cycle of 8 h at 15 °C. Plants was irrigated with a complete nutrient solution (minus N) regularly with an automatic irrigation system to maintain plants free from water stress. The composition of the complete nutrient solution is described in Table 6.1. Daily minimum and the maximum air temperature was measured using a tiny tag temperature data logger (Gemini data loggers) (Fig. 6.1). On average, during the wheat growing season in the glasshouse from June to December, PAR was 194 µ mol $m^{-2}s^{-1}$ between 10.00 to 13.00 and 153 µ mol m^{-2} s^{-1} between 13.00 to 16.00. Overall ambient solar radiation during the experiments was 11501 KJ m^{-2} between June to December in 2014 and 11743 KJ m^{-2} between July to December 2015, respectively.

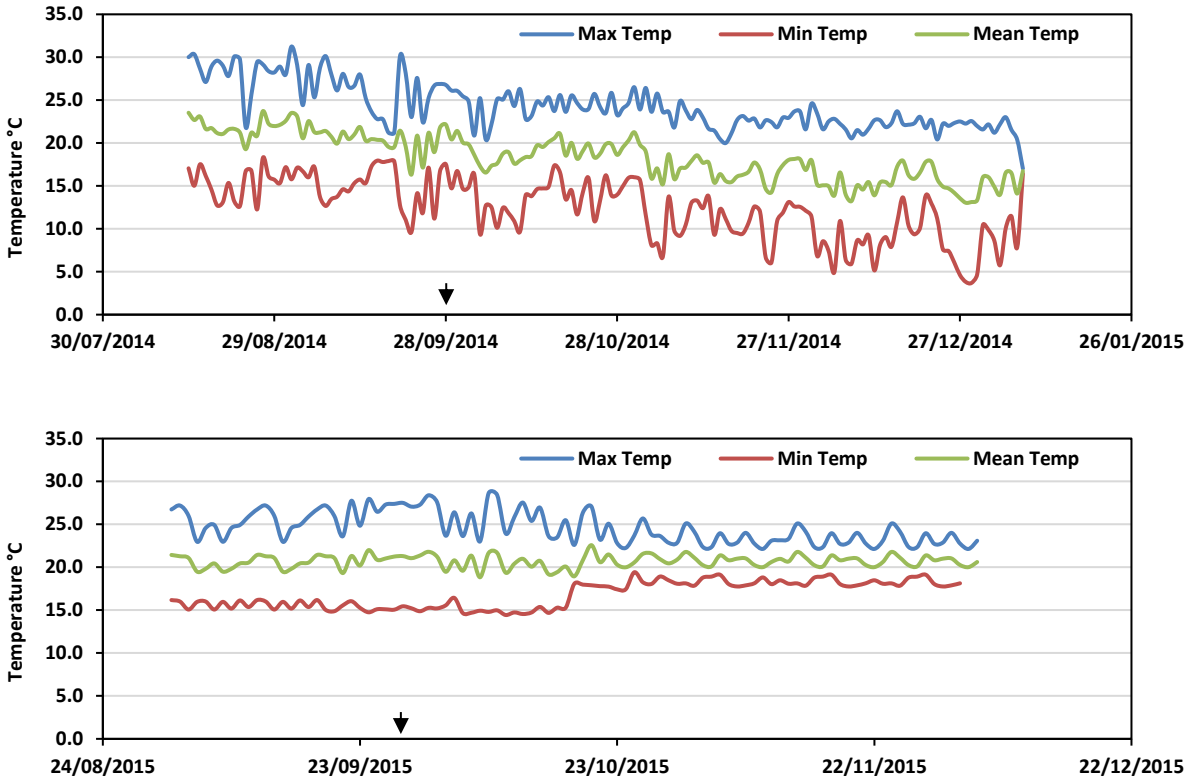


Figure 6. 1 Minimum, maximum and mean ambient temperature in glasshouse experiment in 2015 and 2016 after seedling transplanting. (arrow indicating anthesis date).

6.2.4 Genotype treatment

Twenty amphidiploid lines (Table 6.2) derived from crosses between ancestral grass accessions of wheat wild relatives (Fig. 3.4 b, c, d) and bread wheat cultivars along with five bread wheat parents were planted.

Table 6. 2 Amphidiploid lines along with bread wheat parents used to produce amphidiploid lines.

Bread wheat parent 2n = 6x =42 (AABBDD)	Wild grass parent	Grass species genome	Abbreviations
CS Eup 94	Aegilops mutica 2130004	2n = 2x = 14 (TT)	Ae. mut4 x CSE
CS Eup 94	Aegilops mutica 2130008	2n = 2x = 14 (TT)	Ae. mut8 x CSE
Paragon	Aegilops mutica 2130012	2n = 2x = 14 (TT)	Ae. mut12 x Par
Highbury	Aegilops mutica 2130012	2n = 2x = 14 (TT)	Ae. mut12 x HB
CS Eup 94	Aegilops mutica 2130012	2n = 2x = 14 (TT)	Ae. mut12 x CSE
CS Eup 94	Aegilops speltoides 2140008	2n = 2x = 14 (SS)	Ae. spe8 x CSE
Pavon 76	Aegilops speltoides 2140008	2n = 2x = 14 (SS)	Ae. spe8 x Pav
Pavon 76	Aegilops speltoides 449340	2n = 2x = 14 (SS)	Ae. spelt40 x Pav
Pavon 76	Aegilops umbellulata 542377	2n = 2x = 14 (UU)	Ae. umb77 x Pav
CS Eup 94	Aegilops umbellulata 554410	2n = 2x = 14 (UU)	*Ae. umb*10/0 x CSE
CS Eup 94	Aegilops umbellulata 554410	2n = 2x = 14 (UU)	*Ae. umb*10/3 x CSE
Paragon	Aegilops comosa var. comosa 276970	2n = 2x = 14 (MM)	Ae. com70 x Par
CS Eup 94	Thynopyrum turcicum P208/201	2n=10x=70 (EE)	*Th. tur*201 x CSE
Highbury	Secale anatolicum P208/142	2n = 2x = 14 (RR)	Se. ana142 x HB
CS Eup 94	Secale anatolicum P208/142	2n = 2x = 14 (RR)	Se. ana142 x CSE
CS Eup 94	Secale anatolicum P208/141	2n = 2x = 14 (RR)	Se. ana141 x CSE
CS Eup 94	Secale anatolicum P208/142	2n = 2x = 14 (RR)	Se. ana142/ x CSE
CS Eup 94	Secale anatolicum P208/142	2n = 2x = 14 (RR)	NA
CS Mutant P208/535	Thinopyrum bessarabicum 531712	2n = 2x = 14 (JJ)	NA
Chinese Spring	Thinopyrum bessarabicum Abers.	2n = 2x = 14 (JJ)	20 Th. bes x CS
CS Euploid 94		2n = 6x =42 (AABBDD)	CSE
Paragon		2n = 6x =42 (AABBDD)	PAR
Highbury		2n = 6x =42 (AABBDD)	HB
Pavon 76		2n = 6x =42 (AABBDD)	PAV
CS Mutant P208/535		2n = 6x =42 (AABBDD)	CSM

6.2.5 Plant development

Regular monitoring of plant growth stages was done following the decimal code of Zadoks growth stages (Zadoks *et al.*, 1974). The growth stages were assigned when the main shoots were at the specific growth stage. In both years, anthesis date (AD) was recorded when the ears on the main shoot carried visible stamens (GS61). Date of heading (GS55) was taken when half of ear was visible on the main shoot and physiological maturity (GS89) when the peduncle of the main shoot was 100% senesced. Depending on growth stage, data were recorded once or twice a week. Plant height to the tip of the ear was measured on the main shoot at harvest.

6.2.6 Leaf photosynthesis, stomatal conductance, intercellular CO_2 concentration and maximum efficiency of PSII

Gas-exchange photosynthesis measurements were taken on the flag-leaf of each plant on the main shoot using a Li-Cor 6400 XT Portable Photosynthesis System with chlorophyll fluorescence attachment (Li-Cor Biosciences, NE, USA) under HN and LN conditions. Light-saturated photosynthetic rate (A_{max}), stomatal conductance (g_s), intercellular carbon dioxide concentration (C_i) and maximum efficiency of PSII (Fv'/Fm') of the flag leaf were measured. Measurements were taken on the flag leaf twice a week in between 10.00 and 15.00 h from flag-leaf emergence (GS37) to mid-grain filling (GS85). Li-cor settings were: flow rate 400 µmol s^{-1}, block temperature 20°C with ambient relative humidity. The sample (cuvette) CO_2 concentration was set to 400 µmol mol^{-1} and PAR set to 1500 µmol m^{-2} s^{-1} (10% blue). All parameters were analyzed by taking average values per plant during each of the pre-anthesis and post-anthesis periods.

6.2.7 Flag-leaf senescence measurements

Senescence kinetics were assessed visually every 7 days in each plant by recording the percentage of green area senesced of the flag-leaf after anthesis (GS61) of main shoots in both years using a standard diagnostic key based on a scale of 0 to 10 (0 to 100% senesced). Flag-leaf SPAD senescence parameters for SS.Onset, SS.Dur and SS.End were assessed as described in Chapter 3 (section 3.1.2.12). All other methodology used and the visual senescence parameter calculations were as described in section 3.1.2.11 to measured VS.OnsetRP, VS.Dur, VS.MidRP, and VS.EndRP.

6.2.8 Harvest and growth analysis

Harvesting was done at physiological maturity by cutting the whole plant at ground level from each pot. The fresh weight of the plant was recorded and the plant divided into the main shoot, remaining fertile shoots (those with ear) and infertile shoots (those without an ear), for growth analysis. Plant components from each fertile shoot category were divided into: i) ear, ii) leaf lamina and iii) stem and leaf sheath and each component weighed after

oven drying at 80°C for 48 h. The ears were threshed. After drying at 80°C for 48 h the grain was weighed and the grains counted. Plant N% of: i) grain ii) leaf lamina and iii) stem and leaf sheath of main shoot and the rest of the shoots was determined using the Dumas method as described for the field experiments. The weight of the infertile shoots was recorded after oven drying at 80°C for 48 h.

GY, NUE and their components were calculated as described in Chapter 3 (section 3.2). Data of GY, AGDM and N-uptake was also calculated per shoot basis. Anthesis date, physiological maturity date, and plant height were measured on the main shoot of each plant.

6.2.9 Statistical analysis

ANOVA for a split-plot experimental design and correlation, regression and PCA analysis of data was carried out as described in section 3.2.3 using Gestat 18th Edition. Results are presented for individual years if there was a significant effect for year otherwise mean values across years from the cross-year ANOVA are presented. Analysis of variance (ANOVA) procedures for a split-plot design was used to analyze N treatments and cultivar effects and test their interaction in individual years assuming replicates were random effects and genotypes were fixed effects. A cross-year ANOVA was applied to analyze N treatments and genotypes effects across years and the interaction with year, assuming N treatments and genotypes were fixed effects and replicates and year were random effects. ANOVA was used to test the significance of differences between years (Y), N treatments (N), genotypes (G) and their interactions and treatment combination difference were tested using lest significant difference of the means. A probability value of less than or equal to 0.05 was considered as significant, although values between 0.05 and 0.10 may be included in comments. Correlation and regression analysis using the mean values across replicated was carried out using GenStat 18th. Biplots were created using R programs (https://www.r-project.org/). Skewed data was transformed and probability values were used from transformed data for significance.

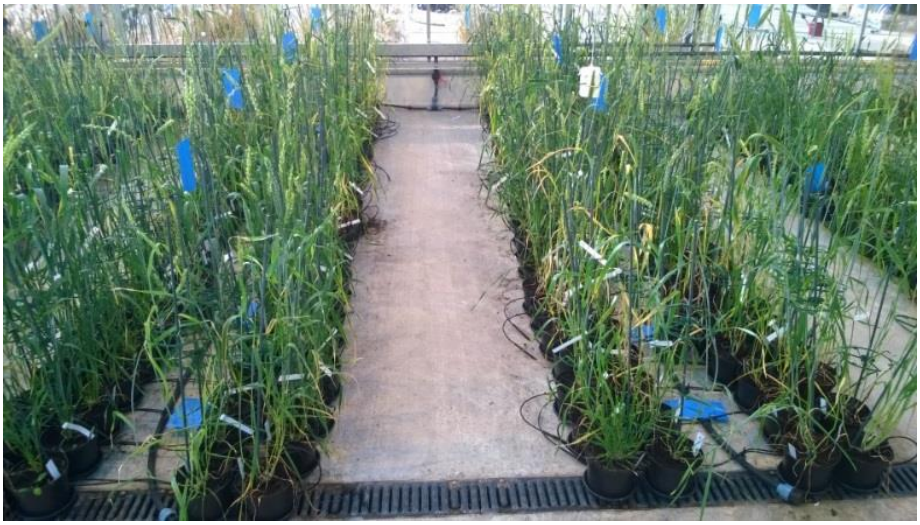


Figure 6. 2 Glasshouse experiment in 2016

6.3 RESULTS

Overall there was no significant interactions for year x N treatment x genotype for the majority of the traits including GY, AGDM and N-uptake per plant. Therefore, most of the results presented are based on the mean of two years' data. If there is a Y x N x G interaction then results of each year are presented separately. Out of 25 genotypes two of the amphidiploids *Secale anatolicum* P208/142 and *Thinopyrum bessarabicum* 531712 did not grow properly and therefore data for these amphidiploids were omitted from the analysis.

6.3.1 Plant height and anthesis and physiological maturity date

Overall plant height (PH) increased from 71.0 cm under HN to 74.0 cm under LN conditions (P = 0.033; Table 6.3). Plant height ranged amongst cultivars from 42.3 (*Ae. spelt*40 x Pav) to 105.8 cm (*Se. ana*141 x CSE) under HN and 46.8 (*Ae. spelt*40 x Pav) to 108.7 cm (*Se. ana*141 x CSE) under LN conditions (P<0.001). There was no N x G interaction but there was Y x N x G interaction (P=0.001). Anthesis date (AD), was slightly earlier under LN (94 DAS) than under HN conditions (96 DAS) (P=0.02; Table 6.3). AD ranged from 84 to 114 DAS under HN and 82 to 106 DAS under LN conditions (P<0.001). There was no N x G interaction or Y x N x G interaction. Overall there was no difference for PH between the amphidiploids and their respective parents.

Averaging cross years, under LN plants reached physiological maturity 10 days earlier (131 DAS) than under HN (141 DAS) conditions (Table 6.3). Physiological maturity date (PMD) ranged from 128 to 163 DAS under HN and 119 to 146 DAS under LN conditions (P<0.001). There was an N x G interaction (P=0.004) with advancement in PM in response to N limitation ranging from 0 – 22 days and there was a Y x N x G interaction (P<0.001).

6.3.2 Grain Yield

In 2015, grain yield (GY) reduced by 25% under LN (2.6 g plant^{-1}) compared to HN (3.4 g plant^{-1}) conditions (P=0.045; Table 6.4). Genotypes ranged from 0.3 (*Ae. spe*40 x Pav) to 9.3 (*Th. tur*201 x CSE) g plant^{-1} under HN and 0.4 (*Ae. spe*40 x Pav) to 6.5 (CSE) g plant^{-1} under LN conditions (P<0.001). The N x G interaction was significant (P<0.001) with reduction under LN

ranging from -47% (*Ae. mut*12 x HB) to 61.4% (*Ae. mut*4 x CSE). In 2016, GY reduced by 51% under LN (1.8 g plant^{-1}) compared to HN (3.7 g plant^{-1}) conditions (P=0.002; Table 6.4). Genotypes ranged from 1.0 (*Ae. spe*8 x Pav) to 8.7 (*Th. tur*201 x CSE) g plant^{-1} under HN and 0.5 (*Se. ana*142 x CSE) to 3.7 (PAR) g plant^{-1} under LN conditions (P<0.001). The N x G interaction was significant (P<0.001) with reduction under LN ranging from -32% (*Se. ana*142/ x CSE) to 81.9% (*Se. ana*142 x CSE). Averaged across years, GY reduced by 38% under LN (2.2 g plant^{-1}) compared to HN (3.6 g plant^{-1}) conditions (P=0.004; Table 6.4; Fig 6.3 a). Genotypes ranged from 0.8 (*Ae. spe*40 x Pav) to 9.0 g plant^{-1} (*Th. tur*201 x CSE) under HN and 0.6 (*Th. tur*201 x CSE) to 4.7 g p^{-1} (*Ae. spe*40 x Pav) under LN conditions (P<0.001). The N x G interaction was significant (P<0.001) (Table 6.1), with reduction under LN ranging from -35% (*Ae. mut*12 x HB) to 62% (*Se. ana*142 x CSE) and there was a Y x N x G interaction (P=0.002). Overall there was a positive linear relationship among the cultivars under HN and LN conditions (R^2= 0.83; P<0.001; Fig. 6.1). Only the line *Th. tur*201 x CSE showed significant transgressive segregation (TS) above its bread wheat parent under HN and there was no TS under LN conditions.

6.3.3 Above-ground dry matter and harvest Index

In 2015, AGDM reduced by 29.1% under LN (10.2 g plant^{-1}) compared to HN (14.3 g plant^{-1}) conditions (P=0.003; Table 6.4). Genotypes ranged from 3.8 (*Ae. spe*40 x Pav) to 21.5 (*Th. tur*201 x CSE) g plant^{-1} under HN and 3.8 (*Ae. spe*40 x Pav) to 15.4 (PAR) g plant^{-1} under LN conditions (P<0.001). The N x G interaction was significant (P<0.006) with reduction under LN ranging from -6.7% (*Ae. mut*12 x Par) to 51.5% (*Ae. spe*8 x Pav). In 2016, above-ground dry matter (AGDM) reduced by 49.2% under LN (7.3 g plant^{-1}) compared to HN (14.5 g plant^{-1}) conditions (P<0.001; Table 6.4). Genotypes ranged from 2.9 (*Ae. spe*40 x Pav) to 23.7 (*Th. tur*201 x CSE) g plant^{-1} under HN and 2.9 ([10] *Ae. umb*10/0 x CSE) to 10.8 (*Th. tur*201 x CSE) g plant^{-1} under LN conditions (P<0.001). The N x G interaction was significant (P<0.001) with reduction under LN ranging from -10% (*Ae. spe*40 x Pav) to 69.6% (*Ae. umb*10/0 x CSE). Across years, AGDM was reduced by 39% under N limitation (P<0.001; Table 6.4). Genotypes ranged from 3.1 (*Ae. spe*40 x Pav) to 22.5 (*Se. ana*141 x CSE) g plant^{-1} under HN and from 3.5 (*Ae. spe*40 x Pav) to 12.7 (Paragon) g plant^{-1} under LN conditions (P<0.001). There was N x G interaction (P<0.001) but no Y x N x G interaction. Genotype responses to N limitation ranged from -5.9 (*Ae. spe*40 x Pav) to 51.3% (*Se. ana*141 x CSE). Overall there was a positive linear

relationship among the genotypes under HN and LN conditions (R^2=0.78; P<0.001; Fig. 6.3 b). Three lines (*Th. tur*201 x CSE, *Se. ana*142 x HB and *Se. ana*141 x CSE) showed significant TS above their wheat parent under HN. There was no TS under LN conditions (Fig. 6.4). Overall there was no association between AGDM and AD or PMD under both HN and LN conditions.

Averaging across years, HI did not differ between the HN (0.25) and LN (0.25) treatments and was generally much lower as expected in amphidiploid lines in the range 0.11 to 0.41 under HN and 0.10 to 0.38 under LN conditions, than the breads wheat parents (range 0.25 to 0.43 under HN and 0.29 to 0.38 under LN conditions). The genotypes overall ranged from 0.11 (*Ae. mut*12 x HB) to 0.43 (CSM and PAV) under HN and 0.10 (*Se. ana*142 x CSE) to 0.38 (*Ae. umb*10/0 x CSE and CSE) under LN conditions (P<0.001). There was no N x G and Y x N x G interaction.

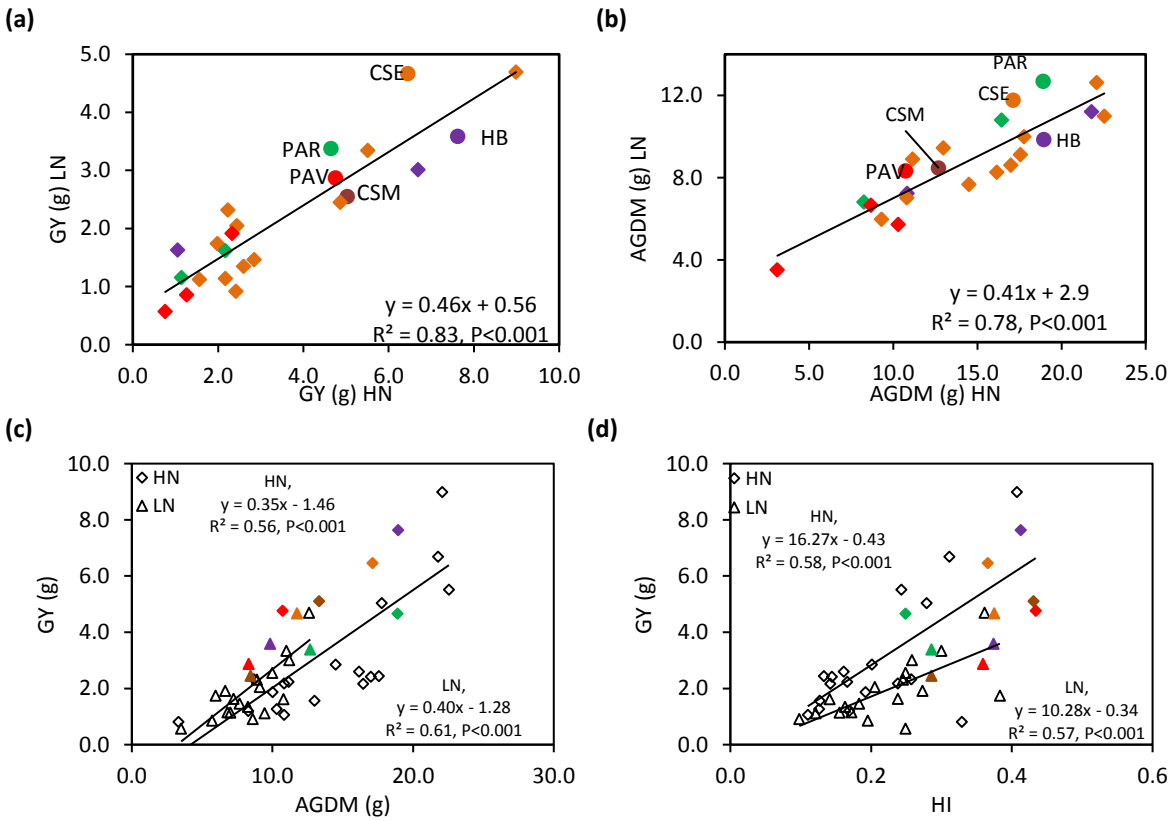


Figure 6. 3 Linear regression of (a) grain yield (GY, 100% DM) and (b) above-ground dry matter (AGDM, 100%) under high N (HN) vs. low N (LN), (c) grain yield vs. above-ground dry matter and (d) grain yield vs. harvest index (HI) under high N (HN) and low N (LN) in 23 genotypes (18 amphidiploids and 5 parents) (mean of 2015 and 2016).

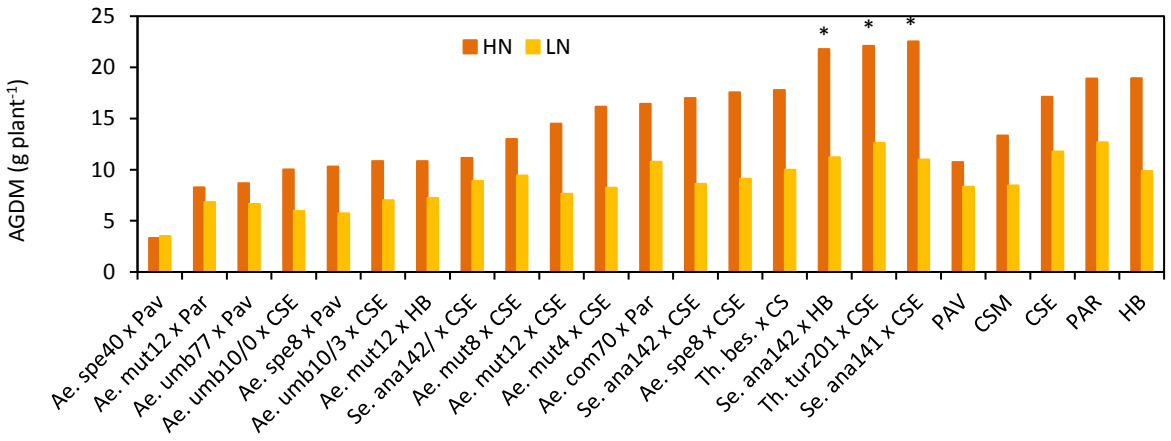


Figure 6. 4 Above-ground dry mater (AGDM g plant^{-1}) for 23 genotypes (18 amphidiploids and 5 parents) under HN and LN condition (mean of 2015 and 2016) (* TS with 5% level of significance).

Table 6. 3 Plant height, anthesis date (AD), and physiological maturity date (PMD) in 23 genotypes (18 amphidiploids and 5 parents) under high (HN) and low N (LN) condition (mean of 2015 and 2016).

	Plant height (cm)		AD (DAS)		PMD (DAS)	
Genotypes	HN	LN	HN	LN	HN	LN
*Ae. mut*4 x CSE	80.4	84.2	94	95	140	134
*Ae. mut*8 x CSE	73.1	83.6	90	88	139	126
*Ae. mut*12 x Par	57.0	51.6	106	103	141	139
*Ae. mut*12 x HB	59.9	60.8	102	104	163	141
*Ae. mut*12 x CSE	79.7	83.5	89	88	141	131
*Ae. spe*8 x CSE	80.9	83.5	92	90	140	127
*Ae. spe*8 x Pav	44.1	52.5	94	96	149	142
*Ae. spe*40 x Pav	42.3	46.8	95	91	137	135
*Ae. umb*77 x Pav	59.1	61.9	84	82	129	123
*Ae. umb*10/0 x CSE	63.8	55.3	110	106	150	135
*Ae. umb*10/3 x CSE	67.7	72.8	97	96	140	130
*Ae. com*70 x Par	58.4	60.4	114	104	162	146
*Th. tur*201 x CSE	84.2	88.0	86	84	128	119
*Se. ana*142 x HB	85.2	83.9	99	99	146	133
*Se. ana*142 x CSE	85.0	77.2	99	101	139	139
*Se. ana*141 x CSE	105.8	108.6	89	88	136	122
*Se. ana*142/ x CSE	74.9	88.3	93	91	149	134
Th. bes x CS	93.3	89.5	91	91	150	139
CSE	82.2	86.8	88	85	132	119
CSM	72.7	82.1	93	92	134	127
HB	60.9	62.0	87	86	131	129
PAR	67.0	74.3	89	88	134	128
PAV	57.5	66.4	86	86	133	124
Mean	71.08	74.08	94	93	141	131
	SED	df	SED	df	SED	Df
N	0.80 *	3.0	0.31 *	3.0	0.81 ***	3.0
G	3.85 ***	132.0	1.90 ***	131.0	2.59 ***	128.0
N*G	5.39	135.0	2.64	133.6	3.67 **	127.3
N*G*Y	7.55 ***	272.8	3.64	246.4	5.10 ***	234.8

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level.

Table 6. 4 Grain yield (GY) per plant, above-ground dry matter (AGDM) per plant, and N-utilization efficiency (NUtE) in 23 genotypes (18 amphidiploids and 5 parents) under HN and LN condition in 2014 and 2015 and mean of 2014-15.

	GY (g plant^{-1})						AGDM (g plant^{-1})						NUtE (g DM g^{-1} AGN)					
	2014		2015		Mean		2014		2015		Mean		2014		2015		Mean	
Genotypes	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**
Ae. mut4 x CSE	2.36	0.91	2.84	1.78	**2.60**	**1.35**	15.3	7.9	17.0	8.6	**16.2**	**8.3**	8.06	12.28	11.27	34.00	**9.66**	**23.14**
Ae. mut8 x CSE	1.85	1.39	1.27	0.85	**1.56**	**1.12**	15.7	11.1	10.3	7.8	**13.0**	**9.4**	5.30	8.67	9.83	13.69	**7.56**	**11.18**
Ae. mut12 x Par	0.98	1.24	1.41	1.07	**1.20**	**1.16**	7.1	7.6	9.4	6.0	**8.3**	**6.8**	8.54	13.16	8.23	19.15	**8.38**	**16.16**
Ae. mut12 x HB	0.61	1.16	1.50	2.10	**1.06**	**1.63**	14.5	8.4	7.2	6.0	**10.8**	**7.2**	2.82	6.99	8.19	36.63	**5.50**	**21.81**
Ae. mut12 x CSE	3.68	2.08	2.03	0.85	**2.85**	**1.46**	13.5	8.2	15.5	7.2	**14.5**	**7.7**	12.40	16.84	7.81	14.65	**10.11**	**15.75**
Ae. spe8 x CSE	3.58	3.16	1.30	0.93	**2.44**	**2.05**	19.2	10.8	16.0	7.5	**17.6**	**9.1**	9.69	18.53	7.80	9.29	**8.75**	**13.91**
Ae. spe8 x Pav	1.53	0.78	1.01	0.94	**1.27**	**0.86**	11.1	5.4	9.5	6.1	**10.3**	**5.7**	7.71	8.32	6.36	15.16	**7.03**	**11.74**
Ae. spe40 x Pav	0.32	0.35	1.30	0.79	**0.81**	**0.57**	3.8	3.8	2.9	3.2	**3.3**	**3.5**	5.24	5.53	15.45	16.93	**10.35**	**11.23**
Ae. umb77 x Pav	1.64	2.18	3.01	1.64	**2.33**	**1.91**	7.4	7.4	9.9	6.0	**8.7**	**6.7**	9.25	17.05	12.96	19.99	**11.11**	**18.52**
*Ae. umb*10/0 x CSE	1.58	2.47	2.17	1.01	**1.87**	**1.74**	10.6	9.1	9.4	2.9	**10.0**	**6.0**	8.01	19.01	29.18	12.00	**18.59**	**15.51**
*Ae. umb*10/3 x CSE	1.95	1.38	2.40	0.89	**2.17**	**1.14**	11.9	8.4	9.8	5.7	**10.8**	**7.0**	7.06	10.97	14.66	14.05	**10.86**	**12.51**
Ae. com70 x Par	1.71	2.29	2.63	0.94	**2.17**	**1.61**	16.7	13.0	16.2	8.6	**16.4**	**10.8**	6.11	16.20	10.50	13.86	**8.30**	**15.03**
*Th. tur*201 x CSE	9.31	6.01	8.66	3.38	**8.99**	**4.69**	21.5	14.4	22.7	10.8	**22.1**	**12.6**	17.31	33.16	18.50	35.07	**17.91**	**34.12**
Se. ana142 x HB	5.63	3.85	7.75	2.17	**6.69**	**3.01**	20.2	13.2	23.4	9.2	**21.8**	**11.2**	11.24	22.40	19.49	26.59	**15.36**	**24.50**
*Se. ana*142 x CSE	2.30	1.38	2.54	0.46	**2.42**	**0.92**	16.8	10.6	17.2	6.7	**17.0**	**8.6**	7.74	12.66	10.37	8.75	**9.05**	**10.71**
*Se. ana*141 x CSE	6.00	4.07	5.03	2.62	**5.52**	**3.34**	21.4	12.1	23.7	9.9	**22.5**	**11.0**	13.64	24.87	13.27	28.08	**13.46**	**26.47**
*Se. ana*142/ x CSE	3.29	2.92	1.17	1.72	**2.23**	**2.32**	12.9	10.6	9.4	7.2	**11.2**	**8.9**	11.32	19.31	4.54	23.52	**7.93**	**21.42**
Th. bes. x CS	5.26	3.08	4.81	2.01	**5.03**	**2.55**	17.1	11.8	18.5	8.2	**17.8**	**10.0**	15.79	18.83	14.29	21.63	**15.04**	**20.23**
CS Euploid 94	5.81	6.49	7.10	2.84	**6.45**	**4.67**	16.8	14.5	17.5	9.0	**17.1**	**11.8**	14.16	33.04	16.69	30.67	**15.42**	**31.86**
CS Mutant P208/535	4.03	2.61	6.16	2.28	**5.10**	**2.45**	10.2	9.1	16.5	7.8	**13.3**	**8.5**	13.75	13.21	16.95	24.04	**15.35**	**18.63**
Highbury	8.90	3.78	6.35	3.39	**7.63**	**3.59**	18.9	11.1	19.0	8.6	**18.9**	**9.9**	20.11	26.04	16.44	37.87	**18.28**	**31.95**
Paragon	1.91	3.01	7.40	3.74	**4.65**	**3.38**	16.5	15.4	21.3	10.0	**18.9**	**12.7**	8.07	14.96	18.45	40.63	**13.26**	**27.80**
Pavon 76	4.82	2.91	4.69	2.82	**4.76**	**2.87**	10.9	10.2	10.6	6.5	**10.7**	**8.3**	17.50	17.69	18.23	31.79	**17.87**	**24.74**
Mean	**3.44**	**2.59**	**3.68**	**1.79**	**3.56**	**2.19**	**14.3**	**10.2**	**14.5**	**7.3**	**14.4**	**8.8**	**10.47**	**16.94**	**13.45**	**22.96**	**11.96**	**19.95**
	SED, Pro (df)		SED, Pro (df)		SED, Pro (df)		SED, Pro (df)		SED, Pro (df)		SED, Pro (df)		SED, Pro (df)		SED, Pro (df)		SED, Pro (df)	
N	0.260 * (3)		0.17 ** (3)		0.153 *** (6)		0.45 ** (3)		0.28 *** (3)		0.265 *** (6)		1.69 * (3)		0.84 *** (3)		0.941 *** (6)	
G	0.650 *** (130)		0.57 *** (130)		0.435 *** (260)		1.48 *** (131)		1.38 *** (130)		1.011 *** (261)		2.46 *** (130)		3.71 *** (127)		2.222 ***(257)	
N*G	0.940 *** (118.6)		0.81 *** (130.3)		0.621 * (249.1)		2.1 ** (130.3)		1.9 *** (132.97)		1.424 *** (265.2)		3.8 ** (55.96)		5.2 *** (129.96)		3.214 **(223.4)	
Y					0.252 ns (6)						0.572 ns (6)						1.105 * (6)	
Y*N					0.295 * (9.93)						0.630 *** (8.47)						1.451 ns (11.7)	
Y*G					0.652 *** (154.2)						1.511 * (160.7)						3.265 *** (190.9)	
Y*N*G					0.900 (236.7)						2.076 ns (240.4)						4.582 * (249.4)	

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level.

6.3.4 Above-ground N-uptake at harvest

Plant above-ground N-uptake at harvest was reduced from 0.28 g N under HN to 0.11 g N under LN conditions (P<0.001; Table 6.5). Genotypes ranged from 0.08 (*Ae. spe*40 x Pav) to 0.50 (*Th. tur*201 x CSE) g N plant^{-1} under HN and from 0.04 (*Ae. spe*40 x Pav) to 0.16 (PAR) g N plant^{-1} under LN conditions (P<0.001). There was an N x G interaction (P<0.001) with genotype response to LN ranging from 38.7 to 73.7% and a Y x N x G interaction (P=0.006). There was a positive association between PL AGN_H and AGDM in the HN (R^2=0.82, P<0.001) and LN (R^2=0.63, P<0.001) treatment. Two lines *Th. tur*201 x CSE and *Se. ana*142 x HB showed TS under HN conditions.

The number of fertile shoots per plant reduced from 8.6 plant^{-1} under HN to 4.3 plant^{-1} under LN (P=0.002). Genotypes ranged from 4.3 (*Ae. spe*40 x Pav) to 14.4 (*Ae. spe*8 x CSE) plant^{-1} under HN and from 2.6 (*Ae. mut*12 x HB) to 6.4 (*Ae. umb*10/3 x CSE) plant^{-1} under LN conditions (P<0.001). There was an N x G interaction (P<0.001) with reduction in tiller number per plant under LN ranging from 17.7 to 64.8% but not a Y x N x G interaction. Five lines under HN and four lines under LN conditions showed TS.

Main-shoot above-ground N-uptake at harvest was reduced from 0.042 g N under HN to 0.028 g N under LN conditions (P<0.001; Table 6.5). Genotypes ranged from 0.015 (*Ae. spe*8 x Pav) to 0.086 (*Se. ana*142 x HB) g N under HN and from 0.012 (*Ae. spe*40 x Pav) to 0.043 (PAR) g N under LN conditions (P<0.001). There was an N x G interaction (P=0.015) with genotype response to LN ranging from -16.7 to 53.5% but not a Y x N x G interaction. There was a positive association between MS AGN_H and AGDM in the HN (R^2=0.58, P<0.001) and LN (R^2=0.43, P<0.001) treatment. Three lines under HN conditions showed TS over parents.

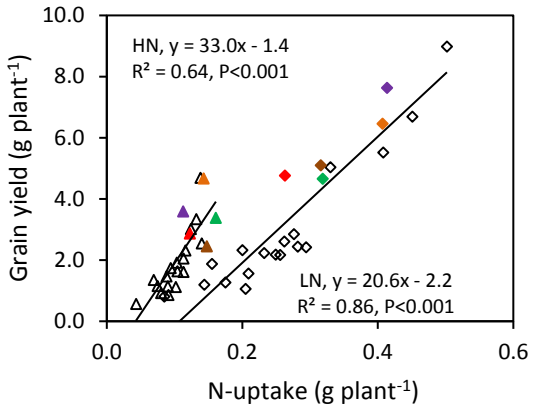


Figure 6. 5 Linear regression of grain yield (100% DM) on N-uptake at harvest under high N (◊) and low N (△) condition in 23 genotypes (18 amphidiploids and 5 parents in color symbols) (mean data of 2015 and 2016) .

6.3.5 N-utilization efficiency

In 2015, $NUtE_{plant}$ increased from 10.5 g DM g^{-1} N under HN to 16.9 g DM g^{-1} N under LN conditions (P=0.03; Table 6.4). The genotypes ranged from 2.8 (*Ae. mut*12 x HB) to 20.1 (HB) g DM g^{-1} N under HN and 5.5 (*Ae. spe*40 x Pav) to 33.2 (*Th. tur*201 x CSE) g DM g^{-1} N under LN conditions (P<0.001). There was an N x G interaction (P=0.008) with reduction under HN ranging from -3.9 (CSM) to 62.3% (*Ae. com*70 x Par). In 2016, $NUtE_{plant}$ increased from 13.5 g DM g^{-1} N under HN to 23.0 g DM g^{-1} N under LN conditions (P=0.001; Table 6.4). The genotypes ranged from 4.5 (*Se. ana*142/ x CSE) to 29.2 (*Ae. umb*10/0 x CSE) g DM g^{-1} N under HN and 8.8 (*Se. ana*142 x CSE) to 40.6 (PAR) g DM g^{-1} N under LN conditions (P<0.001). There was a N x G interaction (P< 0.001) with reduction under HN ranging from –58.9 (*Ae. umb*10/0 x CSE) to 80.7% (*Se. ana*142/ x CSE). Across years, $NUtE_{plant}$ increased from 12.0 under HN to 20.0 g DM g^{-1} N under LN conditions (P<0.001; Table 6.4). The genotypes ranged from 5.5 (*Ae. mut*12 x HB) to 19.5 (*Ae. umb*10/0 x CSE) g GDM g^{-1} N under HN and 10.7 (*Se. ana*142 x CSE) to 34.1 (*Th. tur*201 x CSE) g DM g^{-1} N under LN conditions (P<0.001). There was an N x G interaction (P<0.001) with increase under LN ranging from -23.0 to 74.8% and a Y x N x G interaction (P=0.015). There was no significant TS over the recurrent parents under both the N conditions.

$NUtE_{MS}$ increased from 11.7 g DM g^{-1} N under HN to 18.3 g DM g^{-1} N under LN conditions (P=0.006; Table 6.5). The genotypes ranged from 4.3 (*Ae. mut*12 x HB) to 21.1 (PAV) g GDM g^{-1} N under HN and 6.8 (*Ae. spe*40 x Pav) to 33.0 (HB) g GDM g^{-1} N under LN conditions (P<0.001). There was an N x G interaction (P<0.001) with increase under LN ranging from -

25.0 to 59.8%. The linear relation between NUtE and NUE was significant under both HN (R^2=0.58; P=0.007) and LN (R^2=0.86; P<0.001) conditions (Fig. 6.3).

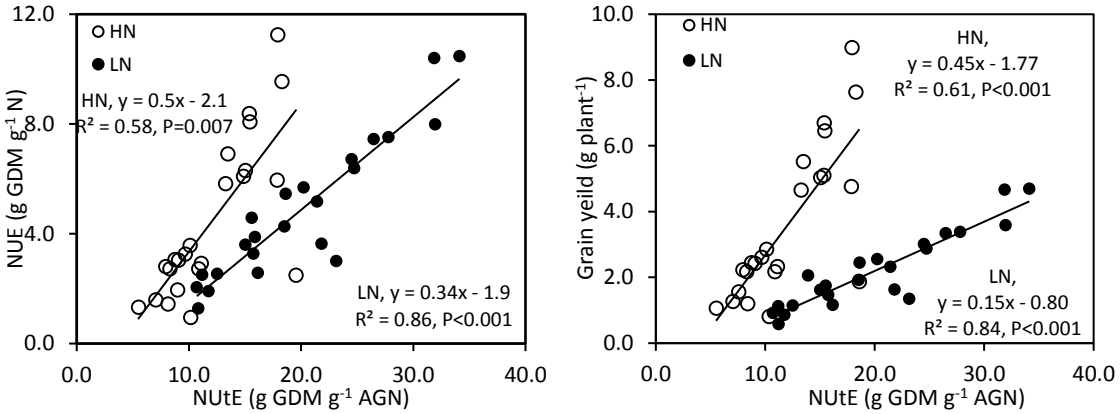


Figure 6. 6 Linear regression of N-use efficiency and grain yield (100% DM) on N-utilization efficiency under HN and LN condition in 23 genotypes (18 amphidiploids and 5 parents) (mean data of 2015 and 2016).

Averaging across years, NHI increased from 0.41 under HN to 0.55 under LN conditions (P<0.001, Table 6.5). Genotypes differed in the range 0.17 (*Ae. mut*12 x HB) to 0.64 (CSE) under HN and 0.26 ([8] *Ae. spe*40 x Pav) to 0.73 (CSE) under LN conditions (P<0.001). Percent reduction under HN ranged from -19.9 to 59.8% but there was no N x G or Y x N x G interaction. There was no significant TS over the recurrent parents under either N treatments. There was no association between NHI and AGDM under HN but there was a positive association under LN conditions (R^2=0.31, P=0.006).

6.3.6 Main-shoot N partitioning indices (NPIs) at harvest

Averaging across years and genotypes, the proportion of AGN at harvest in the leaf and stem decreased with decreasing N supply while it increased in the grain (Fig. 6.7). Averaging across years, MS leaf NPI_H decreased with N deficiency from 0.18 (HN) to 0.14 (LN) (P=0.034; Fig. 6.5). Under HN, MS leaf NPI_H ranged from 0.07 (PAV) to 0.26 (*Ae. mut*12 x HB) and under LN conditions from 0.08 (CSE) to 0.27 (*Ae. mut*12 x HB) (P<0.001). There was N x G interaction with response to N limitation ranged from -24.7 to 51.0% reduction and there was no Y x N x G interaction. There were 10 lines under HN and 7 lines under LN showing TS.

Averaging across years, MS stem NPI_H decreased with N deficiency from 0.21 (HN) to 0.17 (LN) (P=0.003; Fig. 6.7). Under HN, MS stem NPI_H ranged from 0.12 (*Th. tur*201 x CSE) to 0.33 (*Ae. mut*12 x HB) and under LN conditions from 0.09 (HB) to 0.26 (*Se. ana*142 x CSE) (P<0.001). There was no significant N x G but there was a Y x N x G interaction (P=0.02). There were 14 lines under HN and 9 lines under LN showing TS. In contrast to MS leaf and MS stem NPI_H, MS grain NPI_H increased with N deficiency from 0.46 (HN) to 0.57 (LN) (Fig. 6.7). Under HN, MS grain NPI_H ranged from 0.18 to 0.70 and under LN conditions from 0.33 to 0.76 (P<0.001). There was no significant N x G or Y x N x G interaction. There were no lines showing TS under either N treatment.

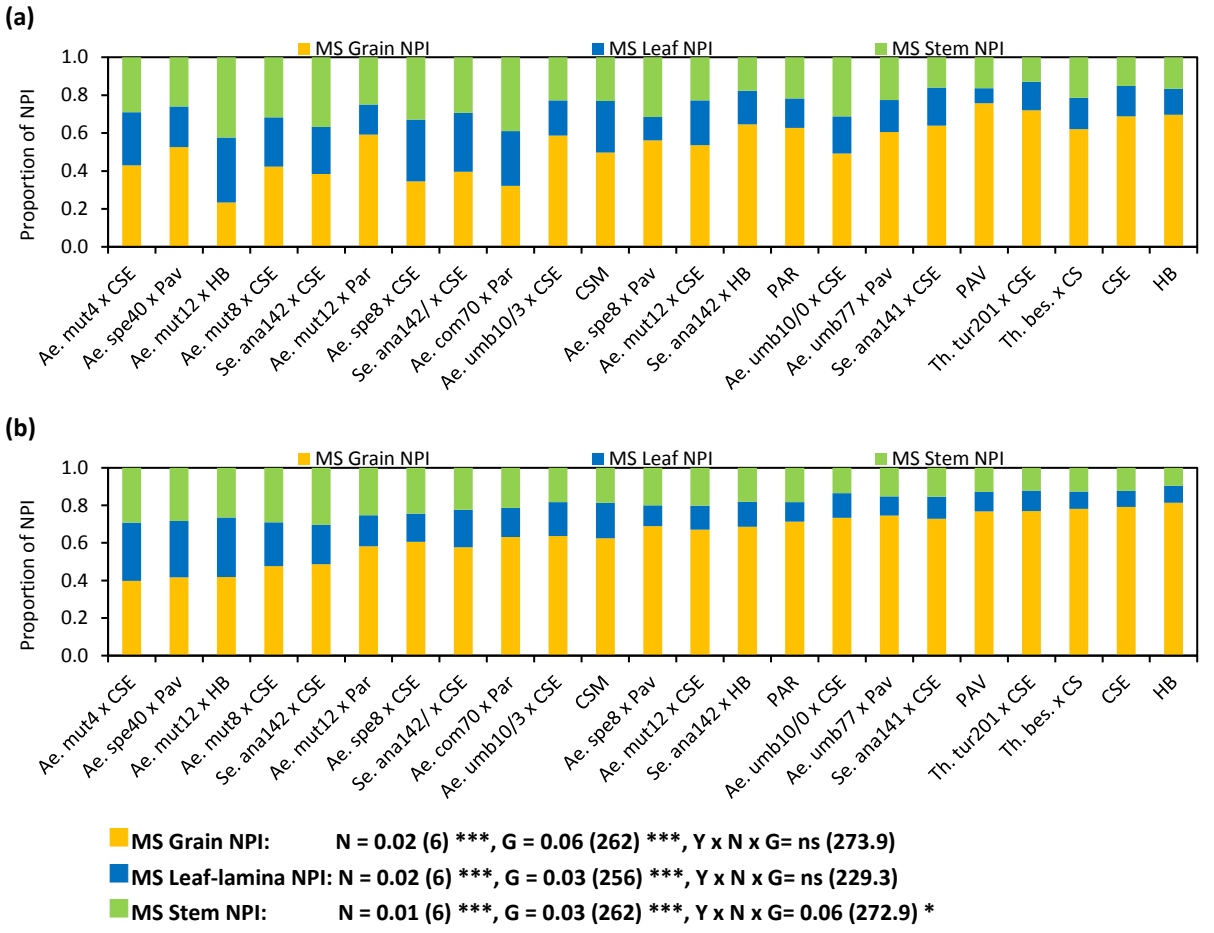


Figure 6. 7 Proportion of above-ground N in main-shoot at harvest in grain (Grain NPI), leaf-lamina (Leaf-lamina NPI) and stem with leaf sheath (Stem NPI) under (a) HN and (b) LN conditions in 23 genotypes (18 amphidiploids and 5 parents) (mean data of 2014 and 2015). SED (df) for N, G and Y x N x G interaction. *Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level.

6.3.7 Flag-leaf A_{max}, g_s and Fv'/Fm'

Overall there was no significant effect of N for pre-anthesis flag-leaf photosynthesis rate (Pre-An A_{max}): HN (22.8 µmol m^{-2} s^{-1}) versus LN (22.6 µmol m^{-2} s^{-1}) conditions (Table 6.5, Fig. 6.8 a). Pre-An A_{max} ranged from 18.9 (CSM) to 26.6 (*Ae. mut*12 x HB) µmol m^{-2} s^{-1} under HN and 19.0 (*Ae. spe*8 x CSE) to 26.1 (*Ae. umb*77 x Pav) µmol m^{-2} s^{-1} under LN conditions (P<0.001). There was no N x G interaction but there was a Y x N x G interaction (P=0.002). Amongst amphidiploids, two (*Ae. mut4* x CSE and *Ae. mut*12 x CSE) genotypes under HN and three (*Ae. umb*10/3 x CSE, *Se. ana*142 x CSE and *Th. bes* x CS) under LN showed transgressive segregation for A_{max} (Fig. 6.8 a). There was no association between pre-An A_{max} and flag-leaf area under both N conditions.

Overall there was no significant effect of N for post-anthesis flag-leaf A_{max}: HN (16.5) versus LN (16.2) µmol m^{-2} s^{-1} (Table 6.5, Fig. 6.8 b). Results showed that post-An A_{max} ranged from 12.0 (CSE) to 20.2 (*Ae. mut*4 x CSE) µmol m^{-2} s^{-1} under HN and 12.3 (CSM) to 23.2 (*Ae. umb*10/3 x CSE) µmol m^{-2} s^{-1} under LN conditions (P<0.001). There was no N x G or Y x N x G interaction. Amongst amphidiploids, 12 lines under HN and 7 lines under LN showed significant transgressive segregation above their recurrent parents (Fig. 6.8 b). There was a positive association of post-An A_{max} with a flag-leaf area under HN (R^2=0.42, P=0.046).

Three amphidiploid lines (*Th. tur*201 x CSE, *Se. ana*142 x HB and *Se. ana*141 x CSE) which showed the highest grain yield amongst amphidiploids and also showed significant TS over their bread wheat parents for AGDM under HN (Fig. 6.4). Three lines also maintain photosynthesis better than parents during late grain filling perhaps associated with stay-green effect (Fig. 6.9).

Overall there was no significant effect of N for pre-anthesis flag-leaf stomatal conductance (pre-AN g_s): HN (0.37 mol m^{-2} s^{-1}) and LN (0.41 mol m^{-2} s^{-1}) conditions (Table 6.4). Pre-AN g_s ranged from 0.24 to 0.53 mol m^{-2} s^{-1} under HN and 0.27 to 0.61 mol m^{-2} s^{-1} under LN conditions (P<0.001). There was an N x G (P=0.017) and a Y x N x G interaction (P=0.007).

The pre-anthesis maximum efficiency of PSII (Pre-AN Fv'/Fm') also overall did not differ significantly under HN (0.57) and LN (0.55) conditions (Table 6.4). Pre-AN Fv'/Fm'

ranged from 0.53 to 0.60 under HN and 0.48 to 0.63 under LN conditions (P<0.001). There was an N x G interaction (P=0.006) with reduction under N limitation ranging from -4.26 (*Th. tur*201 x CSE) to 17.2% (*Ae. mut*4 x CSE) and also a Y x N x G interaction (P=0.002).

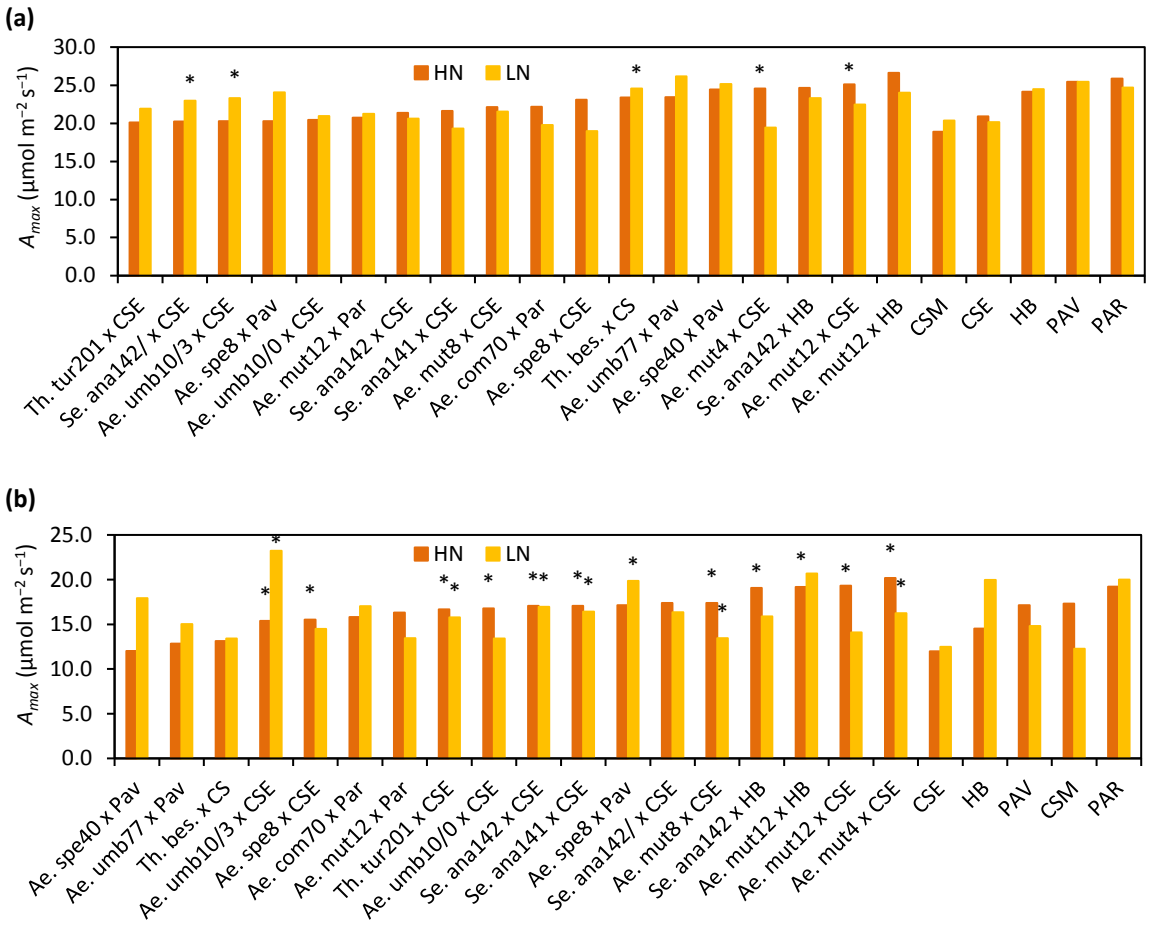
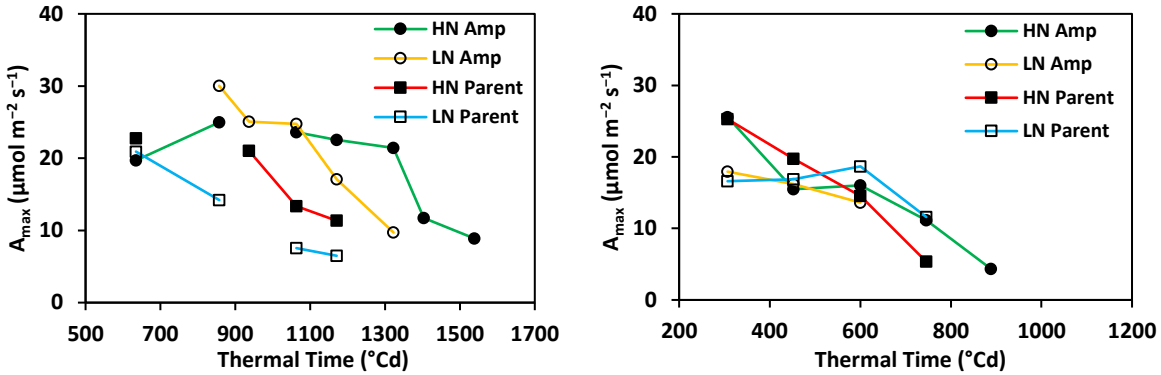
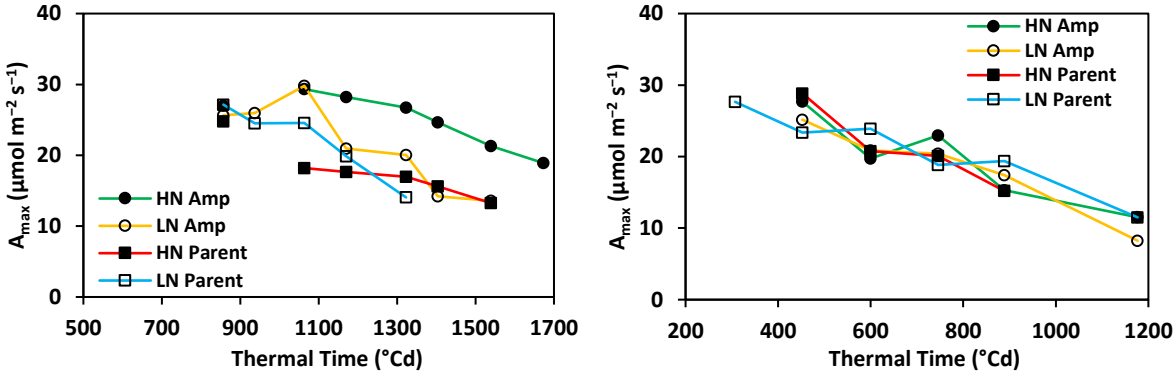


Figure 6. 8 (a) Pre-anthesis flag-leaf photosynthesis rate (Pre-AN A_{max}) and (b) Post-anthesis flag-leaf photosynthesis rate (Post-AN A_{max}) for 23 genotypes (18 amphidiploids and 5 bread wheat parents) under high N (HN) and low N (LN) conditions (mean of 2014 and 2015) (* indicates variation above respective recurrent bread wheat parent at 5% level of significance).

(a) *Th. tur*201 x Chines Spring Euploid in 2015 and 2016



(b) *Se. ana*142 x Highbury in 2015 and 2016



(c) *Se. ana*141 x Chines Spring Euploid in 2015 and 2016

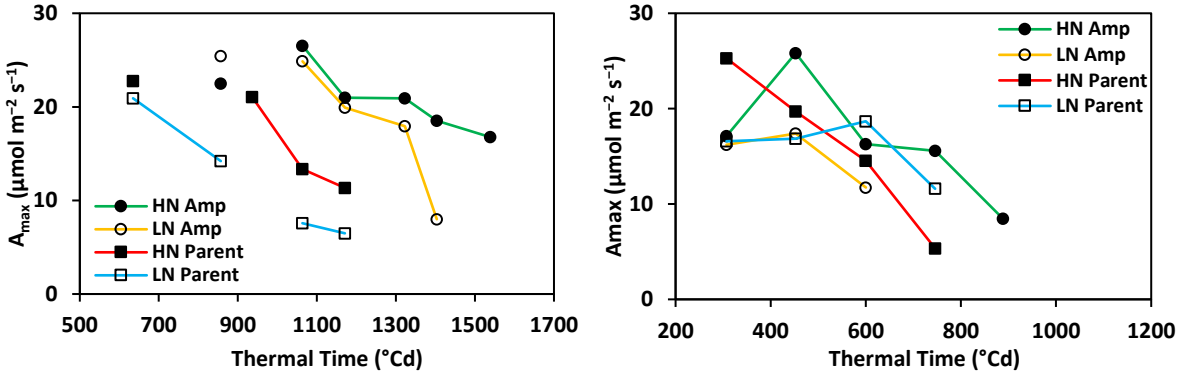


Figure 6. 9 Flag-leaf A_{max} against thermal time since GS41 for top 3 high yielding amphidiploids lines (a) *Th. tur*201 x CSE (b) *Se. ana*142 x HB and (c) *Se. ana*141 x CSE and their bread wheat parents in 2015 and 2016.

Table 6. 5 Main-shoot N-utilization efficiency (MS NUtE), N harvest index (NHI), above-ground N per main-shoot (MS AGN), above-ground N per plant (PL AGN), pre- and post- anthesis flag-leaf photosynthesis (pre-An A_{max} and post-An A_{max}), stomatal conductance (pre-An cond. and post-An cond.) and maximum efficiency of PSII (pre-An Fv'/Fm' and post-An Fv'/Fm') in 23 genotypes (18 amphidiploids and 5 bread wheat parents) under high N (HN) and low N (LN) conditions (mean of 2015 and 2016).

	MS NUtE (g DM g^{-1} AGN)		NHI		MS AGN (g $shoot^{-1}$)		PL AGN (g $plant^{-1}$)		Pre-An Amax (µmol m^{-2} s^{-1})		Pre-An Cond. (mol m^{-2} s^{-1})		Pre-An Fv'/Fm'		Post-An Amax (µmol m^{-2} s^{-1})		Post-An Cond. (mol m^{-2} s^{-1})		Post-An Fv'/Fm'	
Genotypes	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**
Ae. mut4 x CSE	7.40	12.40	0.27	0.30	0.032	0.019	0.26	0.07	24.6	19.8	0.39	0.29	0.58	0.49	20.2	16.2	0.32	0.29	0.56	0.44
Ae. mut8 x CSE	6.80	8.50	0.23	0.45	0.030	0.022	0.21	0.10	22.1	22.7	0.35	0.42	0.54	0.54	17.4	13.4	0.28	0.22	0.53	0.50
Ae. mut12 x Par	11.50	12.90	0.30	0.47	0.028	0.019	0.14	0.08	21.2	21.4	0.35	0.31	0.54	0.54	16.3	13.4	0.31	0.21	0.58	0.50
Ae. mut12 x HB	4.00	8.70	0.17	0.37	0.044	0.034	0.21	0.10	27.2	23.2	0.57	0.49	0.58	0.54	19.2	20.7	0.46	0.48	0.60	0.56
Ae. mut12 x CSE	9.80	13.30	0.45	0.64	0.038	0.023	0.28	0.09	24.8	22.3	0.45	0.37	0.53	0.55	19.3	14.1	0.28	0.22	0.55	0.47
Ae. spe8 x CSE	5.80	13.50	0.27	0.48	0.029	0.021	0.28	0.11	23.6	19.1	0.36	0.27	0.59	0.54	15.5	14.5	0.23	0.30	0.53	0.56
Ae. spe8 x Pav	9.90	13.80	0.29	0.48	0.015	0.018	0.18	0.09	20.4	25.1	0.46	0.53	0.59	0.56	17.1	19.9	0.31	0.42	0.61	0.62
Ae. spe40 x Pav	9.30	6.80	0.35	0.26	0.022	0.012	0.08	0.04	24.4	25.6	0.37	0.45	0.58	0.57	12.0	17.9	0.17	0.37	0.57	0.59
Ae. umb77 x Pav	12.20	18.60	0.47	0.65	0.028	0.022	0.20	0.10	24.2	25.8	0.53	0.60	0.61	0.53	12.8	15.0	0.18	0.31	0.54	0.55
*Ae. umb*10/0 x CSE	9.10	15.00	0.48	0.63	0.024	0.027	0.16	0.10	22.0	21.9	0.30	0.44	0.55	0.55	16.8	13.4	0.22	0.17	0.57	0.50
*Ae. umb*10/3 x CSE	10.10	13.80	0.39	0.47	0.031	0.020	0.25	0.09	20.2	23.1	0.37	0.50	0.61	0.56	15.4	23.2	0.23	0.49	0.51	0.54
Ae. com70 x Par	5.30	13.30	0.20	0.47	0.031	0.020	0.26	0.11	22.3	19.8	0.41	0.38	0.60	0.57	15.8	17.0	0.27	0.41	0.58	0.55
*Th. tur*201 x CSE	19.30	31.60	0.62	0.73	0.059	0.029	0.50	0.14	20.1	22.7	0.24	0.35	0.54	0.57	16.7	15.8	0.24	0.24	0.55	0.56
Se. ana142 x HB	16.90	22.80	0.54	0.65	0.086	0.040	0.45	0.12	24.7	22.3	0.37	0.42	0.55	0.55	19.1	15.9	0.29	0.32	0.55	0.54
*Se. ana*142 x CSE	7.20	10.10	0.24	0.33	0.039	0.021	0.29	0.08	21.4	20.0	0.37	0.37	0.57	0.57	17.0	16.9	0.29	0.28	0.53	0.61
*Se. ana*141 x CSE	16.10	25.90	0.50	0.68	0.066	0.038	0.41	0.13	19.9	21.1	0.29	0.36	0.59	0.54	17.1	16.4	0.25	0.34	0.56	0.50
*Se. ana*142/ x CSE	8.10	18.60	0.25	0.48	0.038	0.032	0.23	0.12	20.4	23.5	0.27	0.40	0.57	0.55	17.4	16.3	0.29	0.29	0.58	0.52
Th. bes Abers. x CS	15.30	24.90	0.53	0.69	0.061	0.037	0.33	0.14	23.4	24.9	0.38	0.34	0.51	0.52	13.1	13.4	0.20	0.24	0.54	0.56
Chinese Spring E	17.50	31.30	0.64	0.73	0.053	0.035	0.41	0.14	20.9	20.8	0.30	0.36	0.55	0.52	12.0	12.5	0.16	0.17	0.50	0.52
Chinese Spring M	13.10	17.90	0.50	0.56	0.038	0.033	0.32	0.15	19.6	19.9	0.29	0.33	0.56	0.57	17.3	12.3	0.24	0.24	0.56	0.40
Highbury	17.40	33.00	0.63	0.73	0.077	0.042	0.41	0.11	26.4	25.2	0.44	0.55	0.56	0.58	14.5	20.0	0.20	0.51	0.57	0.58
Paragon	15.20	27.80	0.43	0.64	0.059	0.043	0.32	0.16	25.3	24.8	0.44	0.39	0.60	0.59	19.2	20.0	0.29	0.40	0.59	0.57
Pavon	21.10	25.90	0.61	0.67	0.046	0.035	0.26	0.12	25.4	25.4	0.49	0.51	0.59	0.64	17.1	14.8	0.28	0.26	0.58	0.54
Mean	**11.70**	**18.30**	**0.41**	**0.55**	**0.042**	**0.028**	**0.28**	**0.11**	**22.8**	**22.6**	**0.38**	**0.41**	**0.57**	**0.55**	**16.5**	**16.2**	**0.26**	**0.31**	**0.56**	**0.53**
	SED	df	SED	Df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df
N	0.96 **	3	0.007 ***	3	0.002 **	3	0.01 ***	3	0.50 ns	3	0.021 ns	3	0.006 ns	3	0.86 ns	5	0.02 *	5	0.01 *	5
G	1.92 ***	132	0.06 ***	132	0.004 ***	132	0.021 ***	132	1.59 ***	121	0.05 ***	121	0.019 ***	115	1.63 ***	118	0.03 ***	118	0.02 ***	119
N*G	2.82 ***	97.1	0.1	133.9	0.006 ***	93.4	0.03 ***	95.8	2.26 ns	121.1	0.08 ns	101	0.03 *	114.4	2.41 **	103.3	0.05 ***	104.3	0.03 ***	113.9
Y*N*G	4.08 **	227.8	0.1	269.7	0.008 ns	208.3	0.04 **	222.4	2.94 *	204.1	0.09 ns	184	0.04 **	173.2	4.23 ns	29.8	0.08 *	51.7	0.05 ns	48.2

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level.

6.3.8 Genetic variation in flag-leaf senescence parameters

As in the field experiments (Chapter 5), flag-leaf visual senescence score data were fitted against thermal time to estimate senescence parameters in the post-anthesis phase: onset of senescence (VS.OnsetRP), senescence duration (VS.DurRP) and end of senescence (VS.EndRP). In summary, GY showed a negative association with VS.OnsetRP and VS.EndRP under HN and LN conditions, respectively (Table 6.6). VS.Mid showed a negative association with GY under both N conditions (data not shown).

VS.OnsetRP was earlier under LN (730.4 °Cd) than HN (826.6 °Cd) (P=0.016, Table 6.6). Under HN, VS.OnsetRP ranged from 505.0 (CSM) to 1308.7 (*Ae. mut*12 x Par) °Cd and under LN conditions from 378.8 (CSM) to 1061.5 (*Ae. mut*12 x Par) °Cd (P<0.001). There was no N x G interaction but there was a Y x N x G interaction (P=0.006). Out of 18 amphidiploid lines, most of the amphidiploids showed TS (14 under HN and 12 under LN) under both N conditions.

VS.EndRP was earlier under LN (1070.3 °CD) than under HN (1265.7 °CD) (P<0.001, Table 6.6). Under HN, VS.EndRP ranged from 867.4 (CSM) to 1594.2 (*Ae. mut*12 x Par) °Cd and under LN conditions from 717.6 (*Th. tur*201 x CSE) to 1481.7 (*Ae. mut*12 x Par) °Cd (P<0.001). There was N x G (P=0.008) but no Y x N x G interaction. Similarly to VS.OnsetRP, most the lines showed TS (16 under HN and 14 under LN).

6.3.9 Genetic variation in flag-leaf SPAD senescence-related parameters

SS.Onset was earlier under LN (294.5 °Cd) than under HN conditions (381.0 °Cd) (P=0.013, Table 6.6). Under HN, SS.Onset ranged from 231.5 to 566.8 °Cd and under LN conditions from 152.9 to 426.5 °Cd (P<0.001). There was an N x G (P=0.013) interaction with advance under LN ranging from -15.5 (PAV) to 55.56 (*Ae. mut*4 x CSE) ^{o}Cd and there was a Y x N x G interaction (P<0.001).

SS.Mid was earlier under LN (525.2 °Cd) than HN conditions (686.7°Cd) (P<0.001, Table 6.6). Under HN, SS.Mid ranged from 425.1 to 932.8 °Cd and under LN conditions from 354.9 to 715.5 °Cd (P<0.001). There was an N x G (P<0.001) interaction with advance under LN ranging from -1.4 (PAV) to 47.6 ^{o}Cd (*Ae. mut*4 x CSE) but no Y x N x G interaction.

Table 6. 6 Flag-leaf visual senescence parameters: onset of rapid phase (VS.OnsetRP), end of rapid phase (VS.EndRP), and SPAD senescence parameters: mid-point (SS.Mid) and onset (SS.Onset) in 23 genotypes (18 amphidiploids and 5 parents) under high N (HN) and low N (LN) conditions (mean of 2014 and 2015).

	VS.OnsetRP °Cd		VS.EndRP °Cd		SS.Onset °Cd		SS.Mid °Cd	
Genotypes	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**
*Ae. mut*4 x CSE	868.9	596.3	1205.3	880.6	344.0	152.9	677.7	354.9
*Ae. mut*8 x CSE	768.4	578.3	1419.1	1164.0	356.4	204.3	710.0	449.1
*Ae. mut*12 x Par	1308.7	1061.5	1594.1	1481.7	455.0	326.4	742.2	620.9
*Ae. mut*12 x HB	1091.7	1034.3	1498.5	1354.8	558.0	302.2	797.5	582.7
*Ae. mut*12 x CSE	821.8	642.5	1284.7	946.4	295.3	293.9	651.0	501.7
*Ae. spe*8 x CSE	638.2	652.9	1176.5	1003.1	248.4	248.6	598.9	426.6
*Ae. spe*8 x Pav	1043.4	902.0	1405.3	1262.5	566.8	346.4	878.3	658.0
*Ae. spe*40 x Pav	827.6	887.6	1237.5	1131.5	394.7	377.2	758.1	619.0
*Ae. umb*77 x Pav	583.3	595.8	884.5	818.8	342.9	324.8	584.7	479.3
*Ae. umb*10/0 x CSE	941.4	817.6	1324.6	1079.1	397.2	276.9	743.3	505.3
*Ae. umb*10/3 x CSE	713.8	723.3	1121.6	1024.1	373.5	229.5	549.6	448.8
*Ae. com*70 x Par	951.3	754.3	1552.8	1252.5	231.5	200.8	445.1	379.4
*Th. tur*201 x CSE	658.0	581.3	1017.9	717.6	363.2	260.2	631.6	465.9
*Se. ana*142 x HB	996.8	955.1	1518.6	1283.3	402.9	426.5	760.4	623.3
*Se. ana*142 x CSE	907.4	835.0	1339.9	1386.7	407.0	288.4	637.5	557.1
*Se. ana*141 x CSE	849.9	628.8	1325.4	848.9	418.3	290.3	757.2	475.7
*Se. ana*142 x CSE	960.1	793.4	1368.0	1054.0	464.4	334.6	908.3	505.3
Th. bes x CS	828.4	692.0	1561.3	1256.6	417.7	363.0	932.8	715.5
CSE	555.3	519.8	919.0	727.0	517.4	348.7	703.7	592.6
CSM	505.0	378.8	867.4	727.0	267.1	159.6	528.6	384.6
HB	709.0	739.4	1198.1	976.8	342.9	364.9	572.4	510.2
PAR	780.3	837.8	1344.7	1314.4	318.4	320.7	604.1	612.3
PAV	703.1	590.7	945.9	925.2	280.5	331.8	620.5	611.8
Mean	**826.6**	**730.4**	**1265.7**	**1070.3**	**381.0**	**294.5**	**686.7**	**525.2**
Corr. with GY plant^{-1}	-0.47 *	-0.38 ns	-0.35 ns	-0.51 *	-0.16 ns	0.23 ns	-0.16 ns	0.06 ns
Corr. with AGDM plant^{-1}	-0.22 ns	-0.31 ns	0.05 ns	-0.18 ns	-0.20 ns	-0.04 ns	-0.15 ns	-0.10 ns
	SED	df	SED	SED	Df	Df	SED	df
N	17.9 *	3.0	16.3 ***	10.5 ***	3.0	3.0	14.8 **	3.0
G	65.9 ***	132.0	69.0 ***	60.9 ***	116.0	132.0	62.1 ***	116.0
N*G	92.8 ns	133.7	96.8 ns	84.9 *	118.5	134.8	87.2 ns	118.9
N*G*Y	130.0 *	241.1	134.0 ns	104.9 **	166.7	242.8	113.7 ***	158.7

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level.

6.3.10 Trait associations for yield, N-uptake and flag-leaf senescence

To investigate the trait associations, principal component analysis was conducted for 16 traits related to yield, and N-uptake plant^{-1} and flag-leaf senescence for the 18 amphidiploids and 5 bread wheat parents for mean data over the two years under HN and LN conditions (Fig. 6.10 a and b).

Under HN, the first two PCs explained 71.4% of the variation. PC1 explained 54.2% of the variation and the group of traits associated were GY, grains plant^{-1}, grain N plant^{-1}, NUp H

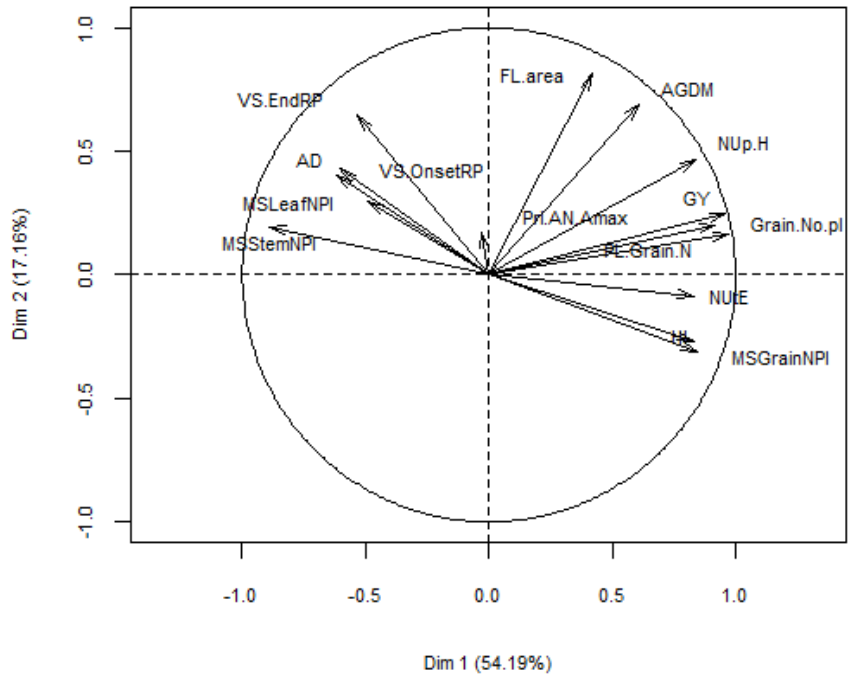
plant^{-1}, NUtE, HI, MS Grain NPI showing a positive effect, whereas MS stem NPI, MS leaf NPI, AD, VS.EndRP and VS.OnsetRP showed a negative effect. PC2 explained 17.2% of variation and traits associated with it were AGDM and FL area. Under HN, GY plant^{-1} was positively associated with grains plant^{-1} ($P<0.001$), grain N plant^{-1} ($P<0.001$) and NUpE ($P<0.001$) and negatively associated with MS stem NPI ($P<0.001$). NUtE showed a positive association with MS Grain NPI and HI and negative association with MS stem NPI, MS leaf NPI, AD, PMD. AGDM plant^{-1} showed a positive association with FL area and NUpE.

Under LN, the first two PCs explained 69.6% of phenotypic variation. PC1 explained 55.6% of the variation and the group of associated traits were GY, grain N plant^{-1}, grains plant^{-1}, NUtE, NUp H, HI and MS grain NPI with positive effect, whereas MS stem NPI, MS leaf NPI, and AD showed a negative effect. PC2 explained 13.9% of variation and the main trait associated with it was Pre-anthesis A_{max} with a negative effect. GY plant^{-1} showed a positive association with grain N plant^{-1}, grains plant^{-1}, NUtE, NUp H, HI and MS Grain NPI. HI and MS grain NPI showed a negative association with AD, MS stem NPI and MS leaf NPI and senescence traits (VS.OnsetRP and VS.EndRP).

6.3.11 Performance of individual amphidiploids

Among the amphidiploids, *Th. tur*201 x CSE showed the best performance and transgressive segregation (TS) for GY and AGDM plant^{-1} under both HN and LN conditions. This amphidiploid under both N conditions also showed TS for NUtE and MS stem NPI and under HN conditions showed highest AGN plant^{-1} and TS AGN plant^{-1} for MS Grain NPI, MS Leaf NPI. Under LN conditions, this line also showed highest NUtE and TS for NUtE and PL Grain NPI. Two other lines, *Se. ana*142 x HB and *Se. ana*141 x CSE, also showed significant TS above their bread wheat parent cultivar under HN for AGDM. Amphidiploid Ae. umb77 x Pav showed earliest AD, the lowest number of spikelets per ear and highest TGW under both N treatments. Among the parents, Highbury, had the highest GY and AGDM, spike no./ear, PL AGN, NUE, NUpE, pre-AN Amax under HN conditions and the highest NUtE under both N conditions. Under LN, Highbury had the latest PM date and the highest TGW, MS Grain NPI, MS NUtE and pre-AN stomatal conductance.

(a)



(b)

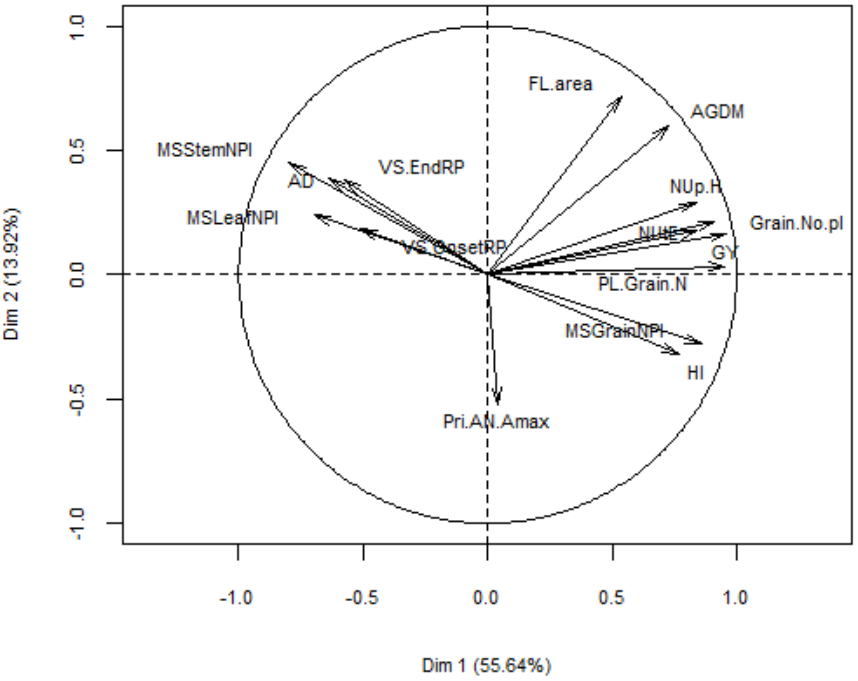


Figure 6. 10 Biplot for 23 genotypes (18 amphidiploids and 5 parents) under (a) high N (HN) and (b) low N (LN) condition (mean data of 2015 and 2016) for grain yield (GY), above-ground dry matter (AGDM), harvest index (HI), grain No. per plant (Grain No.pl), plant grain N (PL.Grain.N), N-utilization efficiency (NUtE), N-uptake at harvest (NUp H), days to anthesis (AD), flag-leaf area (FL area), visual senescence onset of rapid phase (VS.OnsetRP) and end of rapid phase (VS.EndRP), main-shoot N portioning index of stem (MSStemNPI), leaf (MSLeafNPI) and grain (MSGrainNPI) and Pre-anthesis Amax (Pre-AN Amax).

6.4 DISCUSSION

In many of the amphidiploid lines harvest index was lower than in the elite bread wheat parents as expected so that grain yield plant^{-1} was usually significantly lower in amphidiploid lines than the bread wheat parental cultivars. Therefore, in relation to the potential of the amphidiploid lines for use in NUE breeding this discussion focuses mainly on genetic variation in AGDM and N uptake plant^{-1} and associated traits, assuming that HI can be subsequently increased by modern breeding in this material with higher biomass and N uptake.

Overall there was no significant interaction of year x N treatment x genotype for the majority of traits including grain yield, above-ground dry matter (AGDM) and N uptake per plant. This was probably due to the fact that the experimental conditions of glasshouse were similar in both years.

6.4.1 Effect of plant height and development and AGDM

Interestingly plant height under LN (74.1 cm) was slightly higher than HN (71.1) conditions. Under LN, overall there was almost a 50% reduction in a number of fertile shoots plant^{-1} which may have resulted more N available per main shoot, or that main shoots were less shaded and received more incident light per shoot, than under HN conditions. Hendriks *et al.* (2016) reported greater root to shoot ratio in a de-tillering experiment showing reduced tiller number increased root length and root biomass per shoot. As plants were grown in 2 L pots, roots were not able to grow deeper but may have grown longer in length under LN. Overall, GY and AGDM increased with increasing PH indicating that increasing PH by breeding could potentially increase GY. Overall, amphidiploids *Se. ana*142 x HB, and *Se. ana*141 x CSE showed TS over bread wheat parent for PH under both N conditions and *Th. bes* x CS showed TS over bread wheat parent only under HN conditions showing heterosis which could exploit to increase grain yield. Moreover, there was no association between plant height and HI. There are similarly recent trends in CIMMYT spring wheat cultivars from 1966 to 2009 with genetic gain in plant height and biomass and a positive linear association between AGDM and plant height (R^2=0.43, P<0.05). There is also some evidence showing seedling root traits are associated with PH. Seedling root traits based on paper-based germination screens were

shown to be positively linked to mature plant traits such as plant height and yield in recent studies on a Savannah x Rialto DH winter wheat population (Atkinson *et al.*, 2015). Also, seedling root traits (total root length; seminal laterals length; seminal axes length; seminal laterals surface area; seminal laterals volume; root dry weight; shoot dry weight) were associated with plant height in a winter wheat Avalon x Cadenza DH population (Bai *et al.*, 2013). Overall amphidiploids may also have a deeper root than the parent, but in this glasshouse experiment root traits were not measured. However, four amphidiploids which showed contrasting performance under LN along with two bread wheat parents were studied for root seedling architectural traits in hydroponic conditions and results are presented in chapter 7.

During the Green Revolution, wheat breeders introduced reduced height (*Rht*) semi-dwarf genes which increased HI and decreased susceptibility to lodging, especially under high N inputs (Borojevic and Borojevic, 2005; Foulkes *et al.*, 2009). Taller plants are susceptible to lodging and reduced height was a key target for wheat breeding. However, the relationship between PH and root architecture traits is not clear (Foulkes *et al.*, 2009). Some studies showed that reduced height has a negative effect on root length and depth (Siddique *et al.*, 1990; Haberle *et al.*, 1995), so increased PH could potentially increase root length favoring increased grain yield under LN conditions. In this study three amphidiploids (*Th. tur*201 x CSE, *Se. ana*142 x HB and *Se. ana*141 x CSE) showed TS for PH along with AGDM under both N conditions (except for *Se. ana*141 x CSE under LN for AGDM) and could have potential to increase PH, root length, AGDM and GY under N limitation. However, this association is less interesting if PH is not in the range of ca. 70 – 90 cm as this is likely the optimum range for PH in winter wheat (Flintham *et al.*, 1997). Overall across years, there was no N x G interaction for either PH or AD in this study.

On average, anthesis date was one day earlier under LN than HN conditions. In terms of PMD, under LN on average it was 7 DAS earlier than HN conditions (P=0.001). Earliness under stress condition could be related to the plant's ability to adapt to environmental conditions. In N limited conditions, plants tend to finish their life cycle earlier and use the limited resources for the production of seeds for next generation. Overall N stress has effect on plant development but a larger effect on senescence. There was a strong association between AD and senescence paraments (VS.OnsetRP and VS.EndRP) under both N conditions

with later anthesis date associated with delayed senescence, extended photosynthesis and higher grain yield. Gaju *et al.* (2016) also reported earlier AD under LN but treatment effect was not significant.

6.4.2 Genetic variation on leaf photosynthesis

Overall pre-anthesis photosynthesis (A_{max}), stomatal conductance (g_s) and efficiency of PSII (Fv'/Fm') were not affected by N treatment. This could be partly explained by the fact that there was only a small extent of N stress before anthesis. Post-An A_{max} was also not affected by N treatment but there was a significant effect on stomatal-conductance and efficiency of PSII. However, genetic variation in A_{max} did not show any association with GY or AGDM. Overall, biomass per plant was strongly linked to tillers per plant and there was a significant reduction in tiller number per plant under LN (~50% under LN conditions) reducing total photosynthetic green canopy.

However, several amphidiploids showed higher photosynthesis than their bread wheat parents. The three highest AGDM producing amphidiploids were able to maintain better post-anthesis photosynthesis for a longer time than their parent cultivars as compared to other amphidiploid lines under both N treatments. Overall there was more TS for post-An A_{max} than pre-An A_{max}. Present results therefore showed specific amphidiploids had potential to increase leaf photosynthesis rate. Encouragingly, there was no negative association between pre-An or post-An A_{max}, stomatal conductance and PSII efficiency with a flag-leaf area under both N conditions (except for post-AN Amax under HN conditions), which showed that higher photosynthesis, was independent of flag-leaf area. The lack of a GY association with A_{max} could be explained by the fact that there may be sink limitation of grain growth and even with higher leaf photosynthesis potential plants were not able to produce higher GY. Higher leaf photosynthesis rate was reported in synthetic-derived hexaploid lines compared to the Paragon UK spring wheat parental cultivar (Gaju *et al.*, 2016). Greater photosynthetic capability than bread wheat cultivars and synthetic hexaploid wheat lines was reported in hexaploid triticale, octoploid triticale, and Chinese Spring-rye disomic addition lines with rye chromosomes associated with a Rubisco large subunit gene (at heading and grain-filling stages) and a Rubisco small subunit gene (at grain-filling stage) (Song *et al.*, 2017).

6.4.3 Genetic variation in N uptake and NUtE

Overall N uptake plant^{-1} at maturity showed a strong association with GY (HN, R^2=0.93, P<0.001; LN, R^2=0.91, P<0.001) and AGDM (HN, R^2=0.76, P<0.001; LN, R^2=0.75, P<0.001) under both N treatment showing the important role of N accumulation in maintaining greener canopy for higher photosynthesis and GY and AGDM production. Overall NUtE was higher under LN showing under N limitation with plants use available N more efficiently to produce maximum dry matter. Similar results were also reported by Gaju *et al.* (2016). Also, as expected there was a negative relation between grain N % and NUtE (data not shown).

Numerous previous studies of cultivars and segregating populations have shown inverse relationships between GY and grain N% (Kibite and Evans, 1984; Fowler, 2003; Triboi *et al.*, 2006). The N × G interaction for NUtE was statistically significant with genotypes which increased NUtE relatively more under low N, e.g., *Th. tur*201 x CSE, maintaining NUE and GY relatively better under LN. Therefore, an enhanced ability to produce viable grains at a low grain N% may be a trait associated with high GY under LN conditions. In other words, the minimum grain N% in the biomass or grain at harvest will largely determine how much biomass or grain can be produced per unit of absorbed N. Van Keulen (1977) concluded that the minimum level to which grain N% can be diluted in small grains is approximately 9.2/5.7%. Raising NUtE associated with lower grain N% is feasible in end-use markets for which a high grain starch to protein ratio is desirable, e.g. the feed, distilling or biofuel markets. NUtE in the present study was correlated with grain N% (data not shown), so under moderate to low N conditions a possible constraint on raising GY may be the minimum grain N% for a viable grain. A lower grain N% could imply more photosynthesis per unit of absorbed N which may, in turn, be linked to a reduced efficiency of post-anthesis N remobilization to the grain. N remobilization was not measured in the glasshouse experiment but correlations between senescence-related traits and NUtE and GY are discussed further below.

GY showed a slightly stronger positive association with N-uptake than NUtE under both HN and LN conditions. Furthermore, these association between GY and N uptake was stronger under LN conditions than under HN conditions. There are previous studies which also showed similar results where NUpE accounted a greater proportion of genetic variation in NUE under LN than under HN conditions, (Ortiz-Monasterio *et al.*, 1997; Le Gouis *et al.*,

2000; Muurinen *et al.*, 2006; Gaju *et al.*, 2016). This also indicates that, if NUpE is important in NUE, then root architecture may be playing a key role in NUE by extracting N from deep soil. This hypothesis will be examined discussed in the next chapter. Overall, there was an expectation of higher N uptake in amphidiploids as compared to their parents under LN condition but this was not generally the case. This perhaps could be partly explained by the lower HI in the amphidiploids.

Comparing NUE in parents and amphidiploid lines, parents had 52 and 55% higher average values over amphidiploids under HN and LN conditions, respectively, but there was a small difference for AGDM (parents were only 11 and 18% higher for AGDM plant^{-1} under HN and LN, respectively). It was hypothesized that the relative reduction in AGDM under LN conditions may be less in amphidiploids (40.4%) than bread wheat parent (35.4%) but this was not the case. The is small difference in AGDM may have been partly due to the fact that in this experiment, root depth traits of the amphidiploids were not fully expressed.

Amongst 23 genotypes, Paragon showed highest AGDM plant^{-1} under LN as well as highest N-uptake plant^{-1} under LN. Also, this cultivar was able to produce highest AGN plant^{-1} under LN conditions amongst 23 genotypes. *Th. tur*201 x CSE which showed TS over its recurrent parent (CSE) for GY and AGDM along with highest GY and second highest AGDM under HN conditions also showed TS and higher NUE and NUpE under HN conditions suggesting that this amphidiploid has potential to exploit in breeding cultivars suitable for optimum N condition and higher yield. Previously, there is no any published evidence of *Thinopyrum turcicum* showing any abiotic or abiotic stress tolerance. However, in this experiment this amphidiploid produce by crossing *Thinopyrum turcicum* with Chinese spring euploid showed promising performance for yield traits and may have potential for future breeding for yield improvement.

The amphidiploid (*Th. tur*201 x CSE) also showed highest GY and second highest AGDM under LN conditions along with highest NUtE. Higher NUtE may link to senescence rate as this line had a short senescence duration (136.4 °CD). Considering that there was an N x G interaction for AGDM plant^{-1}, this amphidiploid also showed potential for use in breeding for N limited conditions. Furthermore, this amphidiploid also showed highest grain NPI under LN which could further explain its high NUtE.

6.4.4 Relationship between senescence traits with other yield traits

In terms of visual senescence score, under HN conditions NUE and NUtE showed a negative association with VS.Onset.RP. Under LN conditions NUE and NUtE also showed a negative association with VS.EndRP and GY showed a negative association with VS.Mid. These results showing negative associations were unexpected as NUE and NUtE should increase with extended photosynthesis and stay-green traits. This negative association possibly suggested that the amphidiploids may lack functional stay-green traits. Alternativly, there may be sink-limitation of grain growth in amphidiploids and this may have limited the GY or AGDM even though there was potentially extended photosynthesis in some of the amphidiploids. This was also suggested by the association of GY and AGDM with senescence traits, which only positive within the five parent cultivars with higher HI.

Summary

In summary, the findings in relation to the hypotheses tested in this chapter were:

"There is genetic variation for grain yield and above ground dry matter and N-use efficiency and it's components traits that are related to NUpE and NUtE in the panel of 18 amphidiploid lines of winter wheat".

This hypothesis was supported as there was significant genetic variation for GY, AGDM and NUE and its component traits related to NUpE and NUtE in the amphidiploid lines and GY showed association with NUpE and NUtE under HN (R^2= 0.64, 0.61) and LN (R^2= 0.86, 0.84) conditions amongst the lines.

"There is novel genetic variation for increased leaf photosynthesis traits (rate and duration) in amphidiploid lines under low N conditions compared to the bread wheat parental lines".

This hypothesis was supported as amphidiploid lines were identified which showed significantly higher and more prolonged photosynthesis than their recurrent parents under LN conditions.

CHAPTER 7

2D Seedling Root Phenotyping Of Cultivars And Amphidiploids

7.1 INTRODUCTION

Since 1970, agriculture food production has been doubled to fulfill the demand of increasing population which was also associated with a 7-fold increase in fertilizer inputs. Out of total fertilizer applied in the field, worldwide only 33% of it is take up by the plant and the rest of its loss in soil (Raun and Johnson, 1999) causing soil and water pollutions or emitted as NO_3 or nitrous oxides (Foulkes *et al.*, 2009; Yao *et al.*, 2017). Root system architecture (RSA, the spatial configuration of a root system in soil) is critical to N uptake from the soil to increase NUE (Atkinson *et al.*, 2015). Thus RSA is directly responsible for higher GY under water and/or nutrient limitation (Smith and Smet, 2012). However, to date there are few breeding programs that include root system architectural traits (RSAT) as selection criteria and one of the reasons is the difficulty in phenotyping RSAT in field conditions (Waines and Ehdaie, 2007). This limitation together with selection under high fertilizer N inputs in breeding programs may have led to selection of cultivars which are not optimized for N uptake from soil. Especially after the 'Green Revolution' there is evidence that root biomass of elite cultivars is less than two-thirds the mean of some wheat landraces (Waines and Ehdaie, 2007). Also, most modern wheat cultivars were produced by introducing reduced height (*Rht*) genes which may have reduced root proliferation (Bai *et al.*, 2013). Therefore, it may be possible to increase grain yield by optimizing the root system architectural traits (RSAT) which could lead to a second 'Green Revolution' with improved efficiency of below-ground resource capture (Lynch, 2007) and help to reduce leaching of N fertilizer to ground water and its adverse environmental and economic effects (Foulkes *et al.*, 2009). Wild wheat could provide genetic diversity for RSAT responsible for higher N uptake as they reported to have drought tolerance because of their deeper root system. Wild wheat relative, *Agropyron elongatum*, was reported to be a promising donor for enhanced root architecture and water stress adaptation (Placido *et al.*, 2013). Introgression of 7AS QTL from wild emmer wheat in to bread wheat induced a deeper

root system under progressive water stress, which may enhance abiotic stress resistance and productivity of domesticated wheat (Merchuk-Ovnat *et al.*, 2017).

Wheat has two main root systems, namely the seminal (embryonal) roots and the nodal (crown or adventitious) roots (Chochois *et al.*, 2015). Seminal roots in cultivated wheat include one primary root, two pairs of symmetric roots, and, at times, a sixth central root. Nodal roots usually become visible when the fourth leaf emerges at the tillering stage. Seminal roots penetrate the soil earlier and more deeply than nodal roots and remain functional for the entire plant cycle, hence contributing to moisture extraction from deeper soil layers (Christopher *et al.*, 2013).

To date, root system analysis was primarily conducted using techniques such a root extraction/imaging of roots from soil cores or soil columns or rhizotrons which give high levels of detailed information about the root system but these techniques are labor intensive and time-consuming. To overcome these limitations, researchers have developed various artificial media systems to study roots such as hydroponic culture (Kabir *et al.*, 2015), agarose gel chambers (Manschadi *et al.*, 2008), and systems using germination paper (Bai *et al.*, 2013). Recently, systems based on germination paper pouches were successfully used to study seedling root systems to detect QTL for RSAT which co-located for grain yield and N-uptake under N limitation (Atkinson *et al.*, 2015) and grain yield (Xie *et al.*, 2017) in wheat.

Even though there are obvious limitations to study rooting traits at the seedling stage, the early vigor of root growth has been shown to play an important role in N-uptake (Liao *et al.*, 2004b). Also, other traits related to the early stage of seedling development like the angle of seminal root emergence have been linked to root distribution with depth at later growth stages and hence drought tolerance (Manschadi *et al.*, 2008).

In this study, a high-throughput seedling phenotyping platform was used to quantify wheat seedling RSA traits in 12 genotypes selected on their performance of grain yield and N-uptake under N-limiting conditions with the aim to quantify genetic variation and an association of seedling root traits with N-uptake and yield under N limitation in the field and glasshouse experiments. In field and glasshouse experiments (Chapter 4, 5 and 6), it was shown that NUpE is playing a more important role determining genetic variation in GY than

NUtE under N limitation which indicates that root architecture may be playing an important role in determining the genetic variation in NUE in the experiment.

Therefore, the objectives were to:

i) Quantify genetic variation in RSA traits in six Indian, two European spring bread wheat cultivars, and four amphidiploid lines under high N and low N and responses of RSA to N availability.

ii) Determine correlations between genetic variation RSA traits and genetic variation in grain yield, biomass, and N-uptake and associated above-ground physiological traits under high N and low N conditions and the N x G interactions in field/glasshouse experiments.

The specific hypotheses of this experiment were:

1. Genetic variation in early seedling root vigor is associated with RSAT of the mature root system uptake of N from soil and increases yield under N limiting conditions.

2. Genetic variation in 2D seedling screen traits is related to N uptake and NUE in the field under N limiting conditions.

7.2 MATERIALS AND METHODS

7.2.1 Plant material

The genotypes used in this experiment were selected based on their contrasting performance for GY and NUpE in the two field experiments (2013 and 2015) and two glasshouse experiments (2014 and 2015) under LN conditions (Table 7.1). For comparison of field and glasshouse traits, yield traits of the amphidiploids were calculated on the basis of pot area or per shoot basis in both the experiment. Out of 12 genotypes, four were amphidiploids and eight were bread wheat cultivars. One of the bread wheat cultivars (Chinese Spring Euploid) used in this experiment was the parent of four of the selected amphidiploid lines for this 2D seedling RSA study (Table 3.6) whereas the second was a check UK spring wheat cultivar (Paragon).

Table 7. 1 List of genotypes studied for 2D seedling RSA under high N (HN) and low N (LN) conditions. (HY=high yielding, LY=low yielding under low N treatment in field or in glasshouse experiment).

No.	Genotypes	Rationale for selection (HY/LY = high/low yield)	Seed size (mm)
1	GW- 322	HY, Field expt.	3.35
2	HD-2932	HY, Field expt.	2.8
3	DBW- 39	HY, Field expt.	3.35
4	DBW- 14	LY, Field expt.	3.35
5	KHARCHIA- 65	LY, Field expt.	2.8
6	HW- 2044	LY, Field expt.	2.8
7	*Th. turcicum* P208/201 x CS Eup 94	HY, Glasshouse expt.	2.8
8	*Se. anatolicum* P208/141 x CS Eup 94	HY, Glasshouse expt.	2.8
9	*Ae. umbellulata* 554410 x CS Eup 94	LY, Glasshouse expt.	2.0
10	*Se. anatolicum* P208/142 x CS Eup 94	LY, Glasshouse expt.	2.0
11	CS Euploid 94	Parent, Glasshouse expt.	2.8
12	Paragon	Parent, Glasshouse expt.	2.8

7.2.2 Root system architecture phenotyping

Seeds were sieved through a set of calibrated graduated sieves (Scientific Laboratory Supplies Ltd, Hessle, UK). All seeds used in the experiment were taken from the fraction collected between 2 mm and 3.35 mm mesh sizes (Table 7.1). This size was selected as it represented the average seed size of all the genotypes. Seeds were surface sterilized by rinsing in 70% (v/v) ethanol for 30 s, followed by transfer to 5% (v/v) sodium hypochlorite

solution for 10 min, and finally washed three times with sterile water. Sterilized seeds were placed onto moistened germination paper crease-side down and incubated at 4°C for 5 days to synchronize germination on 22 March 2017. Following cold treatment, seeds were transferred to a light-impermeable box for 48 h to complete germination. The box was placed inside the controlled environment room where phenotyping was conducted (12 h photoperiod: 20°C day, 15°C night, with a light intensity of 400 µmol m^{-2} s^{-1} PAR). Uniformly germinated seeds with roots ~5 mm in length were transferred to individual growth pouches on 27 March 2017. The growth pouch system was based on the design developed for maize (Hund *et al.*, 2009). Each pouch consisted of a sheet of germination paper (24 × 30 cm; Anchor Paper Company, St Paul, MN, USA) covered with a black polyethene film of equal area (75 µm thick; Cransford Polythene Ltd, Suffolk, UK) (Fig. 7.1). The germination paper and film were fixed to an acrylic rod (316 × 15 × 5 mm; Acrylic Online, Hull, UK) using two 18 mm fold back clips. A matrix barcode label affixed to the rod allowed identification of each seedling. A single seedling was placed in each pouch centered 2 cm from the top edge and held in place by the adhesion of the polyethene sheet to the wet germination paper. Growth pouches were fitted into four aluminum and polypropylene frame assemblies (Fig. 7.1 A) in the controlled environment chamber. Each assembly consisted of an aluminum profile frame (104 × 62 × 102 cm; KJN Ltd, Leicester, UK) supporting toothed acrylic holders to suspend each pouch in a set position. Black polypropylene side panels (101 × 31 × 0.3 cm and 63 × 31 × 0.3 cm; Cut Plastic Sheeting, Devon, UK) maintained the pouches in darkness.

The base of each frame held a black polypropylene tray (99 × 61 × 10 cm; Stansa Plastic Fabrication Ltd, Suffolk, UK) containing 18 L modified one-quarter Hoagland's solution (Hoagland and Arnon, 1950) with HEDTA as the iron chelator (Piñeros *et al.*, 2005). The composition (mg l^{-1}) of the nutrient solution for HN and LN was as follows:

High N: $(NH_4)_3PO_4$, 29 mg; $Ca(NO_3)_2$, 165 mg; $MgSO_4$, 251.87 mg; KNO_3, 151.99 mg; H_3BO_3, 28.44 mg; Cu_2SO_4, 75 mg; $MnCl_2(H_2O)_4$, 10.1 mg; MoO_3, 0.2 mg; $ZnSO_4$, 2.29 mg; FeHEDTA, 25.49 mg.

Low N: $(NH_4)_3PO_4$, 29 mg; $CaSO_4 \cdot ½H_2O$, 101.15 mg; KCL, 112.09 mg); H_3BO_3, 28.44 mg; Cu_2SO_4, 75 mg; $MnCl_2(H_2O)_4$, 10.1 mg; MoO_3, 0.2 mg; $ZnSO_4$, 2.29 mg; FeHEDTA, 25.49 mg.

The solutions were adjusted to pH 6 using KOH. The volume of nutrient solution in each tray was maintained automatically via a float valve system and header tank containing deionized water. Each frame assembly consisted of three rows of 30 pouches allowing 90 plants per frame. Pouches were suspended so that the bottom 3 cm of the pouch was submerged in the nutrient solution.

A split-plot design was used with two levels of N (HN and LN) as the main treatment level and 12 genotypes randomized as the split plot treatment level. There were 15 replicates. Phenotyping of all genotypes was done in one run of the experiment. Nine days after emergence (leaf 2 stage), individual pouches were transferred to a copy stand (model number SGCS-920; Speed Graphic, Hampshire, UK) for imaging using a Nikon D600 DSLR camera (Fig. 7.1 B) controlled using NKRemote software (Breeze Systems Ltd, Camberley, UK). The copy stand was modified with two draw slides (RS UK, Northants, UK), a Nylatron block (600 × 260 × 22 mm), and white acrylic sheets (330 × 290 × 9 mm and 290 × 290 × 9 mm; Cut Plastic Sheeting, Devon, UK) to form a template to ensure consistent placement of each pouch. The polythene film covering each pouch was carefully peeled back leaving the roots fixed to the blotting paper for imaging. The draw slides then enabled the template block to be repositioned allowing shoots to be imaged without moving the pouch. Images were taken of all 15 replicate seedlings of the 12 genotypes in two N treatments, resulting in 360 RSA images.

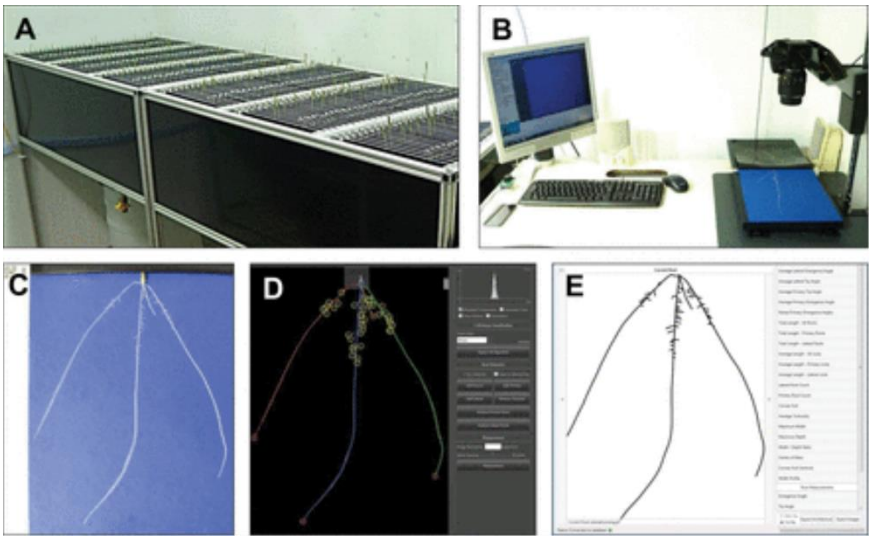


Figure 7. 1 Phenotyping pipeline. (A) Growth assembly. (B) Image acquisition. (C) Example root image. (D) Root system extraction and quantification using RootNav software. (E) Reconstruction of root system in silico and trait measurement (Atkinson *et al.*, 2015).

7.2.3 Image analysis

Root system images were processed using RootNav software (Pound *et al.*, 2013). Traits were quantified from extracted RSA using RootNav's standard functions (Table 3.8) as detailed by Pound *et al.* (2013). For this study, RootNav functionality was extended to measure additional traits describing the distribution of the root system (the co-ordinates of the center of mass of the root system and the coordinates of the centroid of the convex hull), determined as follows.

Centroid of convex hull: The convex hull of the root system is calculated using the standard definition, that of the smallest convex set of X points containing the entire root system. This results in a set of points $(x_1, y_1), \ldots, (x_n, y_n)$, the centroid (geometric centre) of which is calculated as:

$$C_x = \frac{1}{6A}\sum_{i=0}^{n}(x_i + x_{i+1})(x_i y_{i+1} - x_{i+1} y_i) \qquad \text{Equation 7.1}$$

$$C_y = \frac{1}{6A}\sum_{i=0}^{n}(y_i + y_{i+1})(x_i y_{i+1} - x_{i+1} y_i) \qquad \text{Equation 7.2}$$

where A is the signed area of the convex hull,

$$A = \frac{1}{2}\sum_{i=0}^{n}(x_i y_{i+1} - x_{i+1} y_i) \qquad \text{Equation 7.3}$$

It should be noted that the point (x_{n+1}, y_{n+1}) is equal to (x_1, y_1) in the above formulae.

As root angle varies with position along the root and the angle between pairs of seminal roots has been shown to be significant (Christopher *et al.*, 2013), additional angle measurements were calculated at a fixed point 15 mm along each root, and at 50, 75, and 100% of root length (Fig. 7.1 C). The RSA traits quantified by the RootNav functions are listed in Table 7.2.

Table 7. 2 Root system architecture traits measured in 2D seedling platform.

Acronym	Description (units)
TotLegAllRo	Total length of all roots (mm)
TotLegSemRo	Total length of seminal roots (mm)
TotLegLatRo	Total length of lateral roots (mm)
	Number of seminal roots
	Number of lateral roots
	Maximum width of the root system (mm)
	Maximum depth of the root system (mm)
W/D ratio	Width-depth ratio (RMW/RMD)
	Angle of emergence between the outermost seminal roots measured at the first quartile of the total length (degrees)
	Angle of emergence between the second pair of seminal roots measured at the first quartile of the total length (degrees)
	Angle of emergence between the outermost seminal roots measured at the second quartile of the total length (degrees)
	Angle of emergence between the second pair of seminal roots measured at the first quartile of the total length (degrees)
	Angle of emergence between the outermost seminal roots measured at the third quartile of the total length (degrees)
	Angle of emergence between the second pair of seminal roots measured at the third quartile of the total length (degrees)
	Angle of emergence between the outermost seminal roots measured at the root tip (degrees)
	Angle of emergence between the second pair of seminal roots measured at root tip (degrees)

7.2.4 Statistical analysis

ANOVA for a split-plot experimental design, correlation, and regression analysis of data was carried out using Genstat version 17 as described in section 3.1.4. PCA analysis was carried out using R program as described in section 3.1.4. ANOVA was carried out for a split-plot design, where two N treatments were the main treatment level and genotypes were the split-plot treatment level. In addition, an unbalanced ANOVA to test amphidiploid (AMP) and cultivars (CV) group effect was carried out using GenStat version 17.

For regression analysis grain yield per unit area for the 4 amphidiploids and Pargon and Chines Spring Euploid was estimated on an per unit area equivalent calculated based on the pot area.

7.3 RESULTS

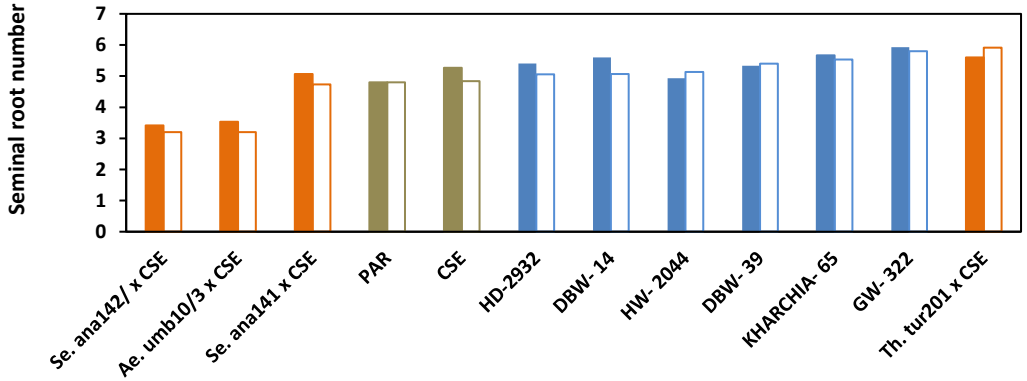
7.3.1 Phenotypic variation in seedling RSA traits between genotypes

Overall seminal root number per plant decreased slightly from HN (5.0) to LN (4.9) conditions (P=0.027, Table 7.3, Fig. 7.2.a). Seminal root number ranged amongst genotypes from 3.4 to 5.9 under HN and 3.2 to 5.9 under LN conditions (P<0.001). There was no N treatment x genotype interaction. However, in contrast to seminal root number, the total length of seminal roots increased with N deficiency by 11.3% (HN 5117.7 and LN 5769.8 mm) (P<0.001, Table 7.3, Fig. 7.2.b). The total length of all seminal roots per plant ranged from 3035 to 7170 mm under HN and 3075 to 7704 mm under LN conditions. There was an N treatment x genotype interaction (P=0.002).

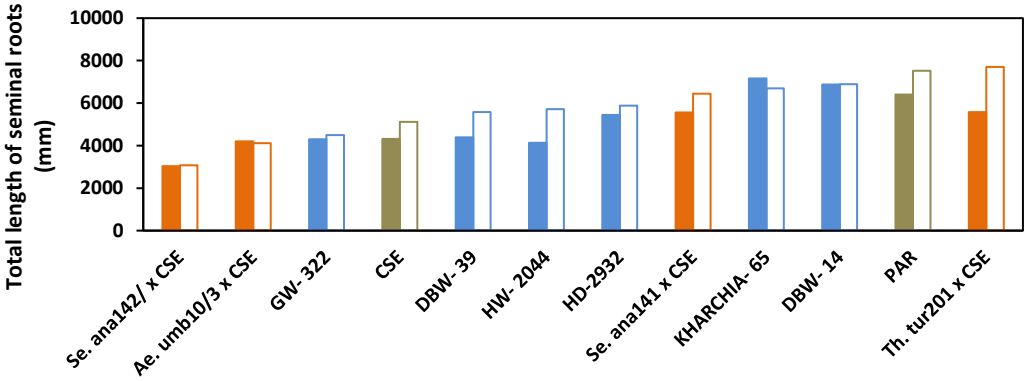
Lateral root number increased by 56% under LN conditions compared to HN conditions (P<0.001, Table 7.3). The total length of lateral roots ranged from 4.2 to 266.7 mm under HN and 3.6 to 734.1 mm under LN conditions, and there was an N treatment x genotype interaction (P<0.001). Moreover, the total length of lateral roots also increased by 62% under LN conditions compared to HN conditions (P<0.001, Table 7.3). The total length of lateral roots ranged from 4.2 to 266.7 mm under HN and 3.6 to 734.1 mm under LN conditions, and there was an N treatment x genotype interaction (P<0.001).

The total length of all roots also showed an increase of 13.3% under LN compared to HN conditions (HN 5206 and LN 6002 mm) (P<0.001, Table 7.3, Fig 7.2.c). The total length of all roots ranged from 3039 to 7436 mm under HN and 3095 to 8258 mm under LN conditions, and there was an N treatment x genotype interaction (P=0.002).

(a)



(b)



(c)

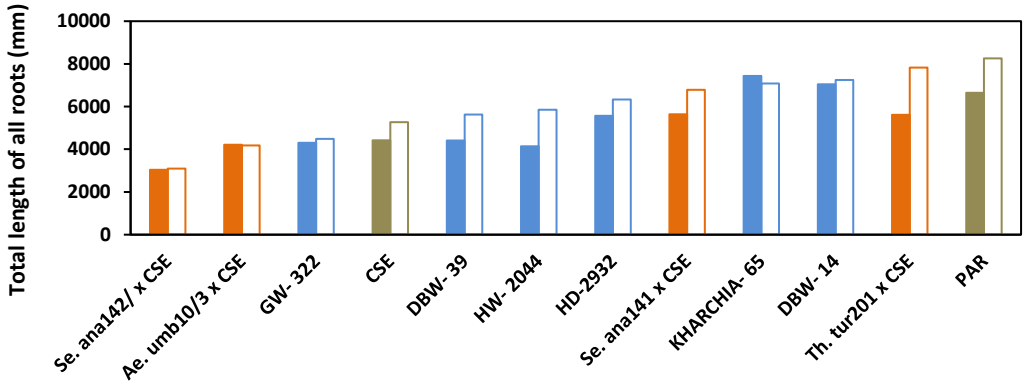


Figure 7. 2 Phenotypic variation in 12 genotypes (8 bread wheat cultivars and 4 amphidiploids) under high N (HN) and low N (LN) (a) seminal root number per seedling (b) total length of seminal roots per seedling (c) total length of all roots per seedling. (HN, solid columns and LN empty columns) (SED, Pro. and d.f. for N, G and N x G are shown in Table 7.3).

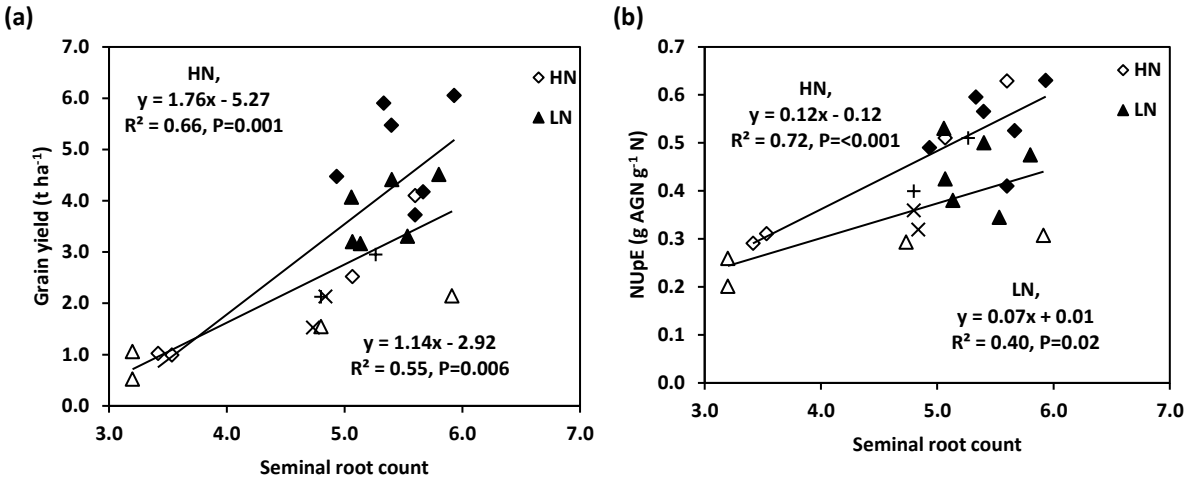


Figure 7. 3 Linear regression of (a) grain yield (t ha^{-1}, 100% DW) for genotypes in the field experiments (mean 2014 and 2016) and in glasshouse experiments (mean 2015 and 2016) and (b) NUpE for genotypes in the field experiments (per unit area equivalent) (mean 2014 and 2016) and in glasshouse experiments (mean 2015 and 2016) on seminal root number in 12 genotypes (4 amphidiploids and 8 bread wheat). (♦ ▲ Indian cultivars; ◊ △ Amphidiploids, and + x UK cultivars under HN and LN respectively).

Table 7. 3 Seminal root number, total length of seminal roots, lateral root number, total length of lateral roots and, total length of all roots per plant, center of mass X, maximum width of root system, maximum depth of root system for 12 genotypes (8 bread wheat cultivars and 4 amphidiploids) for groups (mean of 15 replications).

	Seminal root number		Tot Leg seminal roots (mm)		Lateral root number		Tot Leg Lateral roots (mm)		Tot Leg all roots		Centre Of mass X		Max width (mm)		Max depth (mm)	
Bread wheat cultivars	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**	**HN**	**LN**
GW- 322	5.93	5.80	4297	4489	0.33	0.13	6.50	3.60	4304	4492	0.00	20.40	526	532	1125	1223
HD-2932	5.40	5.06	5445	5875	5.87	17.23	122.90	451.30	5568	6326	14.00	47.10	1193	1280	1748	1836
DBW- 39	5.33	5.40	4387	5575	0.93	2.27	24.50	48.20	4411	5623	10.40	43.20	483	862	1247	1514
Chinese Spring Euploid	5.27	4.84	4317	5119	3.93	6.25	102.50	151.00	4420	5270	40.00	40.40	738	1047	1205	1412
Paragon	4.80	4.80	6405	7524	10.60	22.87	240.90	734.10	6646	8258	38.10	73.40	985	1123	2138	2340
DBW- 14	5.60	5.07	6879	6894	6.33	14.70	166.10	351.30	7045	7246	18.10	43.10	891	901	1834	1814
KHARCHIA- 65	5.67	5.53	7170	6700	10.73	14.00	266.70	382.40	7436	7083	41.30	56.70	1419	1210	2027	1871
HW- 2044	4.93	5.13	4135	5720	0.13	5.10	5.70	125.90	4140	5846	29.00	-1.10	586	997	1369	1681
Cultivars group mean	**5.37**	**5.20**	**5379**	**5987**	**4.86**	**10.32**	**116.98**	**280.98**	**5496**	**6268**	**23.86**	**40.40**	**852.63**	**994**	**1586**	**1711**
Amphidiploids																
Th. tur201 x CSE	5.60	5.91	5578	7704	1.47	4.16	36.70	122.70	5615	7827	34.10	91.90	1011	1290	1530	2295
Se. ana141 x CSE	5.07	4.73	5562	6446	3.00	11.73	76.80	340.00	5639	6786	-4.40	2.30	937	940	1776	2122
Ae. umb10/3 x CSE	3.53	3.20	4202	4116	0.47	2.00	10.20	64.20	4213	4180	55.50	114.30	916	753	1701	1698
Se. ana142/ x CSE	3.42	3.20	3035	3075	0.15	0.66	4.20	20.60	3039	3095	50.20	82.40	578	556	1387	1490
Amphidiploid group Mean	**4.41**	**4.26**	**4594**	**5335**	**1.27**	**4.64**	**31.98**	**136.88**	**4626**	**5472**	**33.85**	**72.73**	**860**	**884**	**1598**	**1901**
Overall Mean	**5.00**	**4.90**	**5118**	**5770**	**3.70**	**8.40**	**88.60**	**232.90**	**5206**	**6003**	**27.20**	**51.20**	**855**	**958**	**1591**	**1775**
	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df	SED	df
N	0.06 *	14	89.9 ***	14	0.7 ***	14	21.5 ***	14	102.5 ***	14	5.29 ***	14	44.1 *	14	43.4 ***	14
G	0.2 ***	302	324.6 ***	302	1.4 ***	302	46.0 ***	302	339.8 ***	302	23.61 **	302	97.5 ***	302	88.6 ***	302
N*G	0.28 ns	315.6	448.6 **	315.9	2.03 ***	293.05	65.8 ***	289.77	471.4 **	315.93	32.4 ns	313.69	139.2 **	294.12	127.5 ***	281.97
N (Groupe)	0.107 ns		184.9***		0.809***		24.91 ns		197.7***				48.1*		49.81***	
Groupe	0.114 ***		196.5***		0.860***		26.48 ns		210.1***				51.1 ns		52.93 x	
Treat x Groupe	0.186 ns		321 ns		1.41 ns		43.32 ns		343.7 ns				83.6 ns		86.6 x	

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level and x 10% level (P=0.10).

7.3.2 Phenotypic correlation between seedling RSA traits

Significant correlations between root traits were found. Total root length of all roots (seminal and crown) was positively correlated with both the number (HN, r=0.54, P=0.07 and LN r=0.60, P=0.04) and length of seminal roots (r=0.99, P<0.001 and LN, r=0.99, P<0.001) under both N conditions. Root system shape represented by the ratio of width to depth was closely associated with the maximum width (HN, r= 0.80, P=0.002, and LN, r= 0.67, P=0.017) rather than depth under both N conditions. Under both N conditions average primary emerging angle was associated with the Width/Depth ratio (HN, r= 0.68, P=0.016 and LN, r= 0.77, P=0.004).

7.3.3 PCA showing association between yield traits in field experiment and seedling RSAT

In PCA analysis a total of 19 traits which include traits related to yield, N-uptake and RSAT from the field, glasshouse and 2D seedling root phenotyping hydroponic experiment was studied.

PCA shows that first two PCs explain 70.3% of variation under HN and 63.5% of variation under LN. Under both the N conditions, majority of yield traits were associated with PC1 and having a major contribution to overall variation. As the association between yield, biomass and yield component traits were discussed in chapter 4 and 5, in this section results of the association between yield and biomass and RSA traits are only discussed. Overall correlation analysis showed that yield traits per shoot were more strongly associated with the seedling RSA traits than yield per unit area, therefore, traits per shoot only were considered for PCA. Also, yield per shoot can be calculated directly in both field and glasshouse experiments.

Under HN conditions (Fig. 7.4.a), seminal root count (SemRoCount) showed a positive association with NUp Sh^{-1} (r=0.74, P=0.006) and AGDM Sh^{-1} (r=0.67, P=0.018) and Grain No. Sh^{-1} (r=0.84, P<0.001). All these traits grouped under PC1 with positive effects. On the other hand, AD and PMD associated with AvgPriEmAng (Average primary emerging angle) and center of MassX (Center of mass horizontal co-ordinates) and grouped under PC1 with

negative effects. In terms of PC2 he traits with positive effects were PH, width/depth ratio and TotLegLatRo (total length of lateral roots) showing positive associations with each other.

Under LN conditions (Fig. 7.4.b), seminal root count was more strongly associated with GY Sh^{-1} (r=0.74, P=0.006), Grain No. Sh^{-1} (r=0.81, P=0.001) and Grain NUp Sh^{-1} (r=0.61, P=0.035) and these traits grouped under PC1 with positive effects. Furthermore, seminal root count was negatively associated with center of mass horizontal co-ordinates (CentorofMassX) (P=ns). In contrast to HN conditions, Width/Depth ratio was negatively associated with PH. Furthermore, NUp Sh^{-1} (NUp.Sh), AGDM Sh^{-1} (AGDM.Sh) and the total length of all roots (TotLegAllRo) were negatively associated with average length of all roots (AvgLegAllRo) and average primary emerging angles (AvgPriEmAng). Overall, NUp Sh^{-1} along with AGDM Sh^{-1} were the traits most strongly associated with total length of all roots (TotlegAllRo), lateral root count (LatRoCount) and the total length of lateral roots (TotLegLatRo).

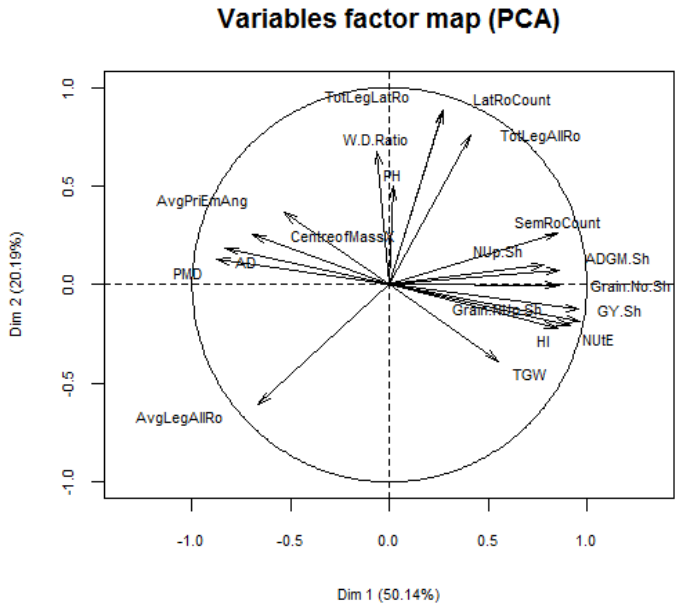
7.3.4 Association of seminal root number with other traits

Seminal root number was the single most promising trait as it shows association with GY, GY/Sh, AGDM, AGDM/Sh (not under LN), HI, AD, PMD, NUpE, NUtE, NUp/Sh (not under LN), Grain NUp/Sh, Grain No./Sh under both N treatment. Seminal root length and the total length of all roots did not show any association with other traits (Table 7.4 a). If compared association only between 6 Indian cultivars, seminal root number showed positive association with AD and NUtE and primary emerging angle, width and depth of root system. Under LN conditions primarily emerging angle, maximum depth and width and total length of lateral roots showed negative association with HI (Table 7.4 b).

7.3.5 RSAT comparison amongst amphidiploid and cultivar group

Comparing trait variation between amphidiploids and cultivars group, amphidiploid showed relatively higher maximum depth of root system than cultivars (P=0.06) and also showed relative higher increase in root depth under N limiting conditions (P=0.11, Table 7.3).

(a) HN



(b) LN

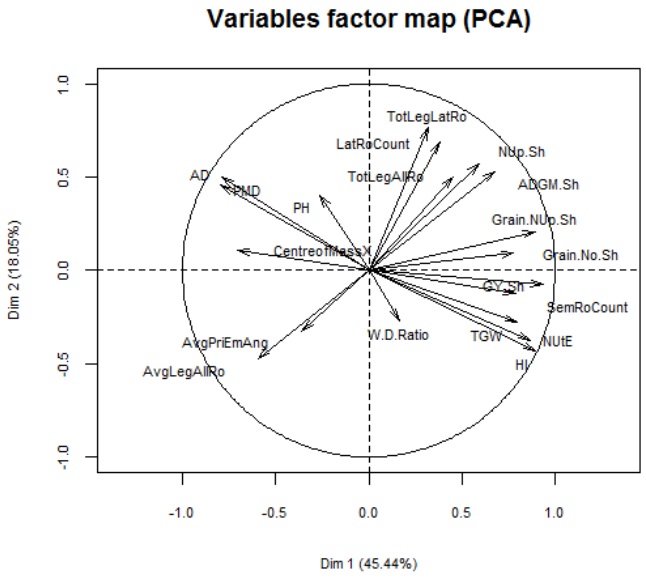


Figure 7. 4 Principal component analysis of grain yield per shoot (GY Sh^{-1}), above-ground dry matter per shoot (AGDM Sh^{-1}), thousand grain weight (TGW), harvest index (HI), anthesis date (AD), physiological maturity date (PMD), plant height (PH), N-uptake per shoot (NUp Sh^{-1}), grain N-uptake per shoot (Grain NUp Sh^{-1}), grain number per shoot (Grain No. Sh^{-1}), N-utilization efficiency (NUtE), average primary emerging angles (AvgPriEmAng), average length of all roots (AvgLegAllRo), total length of all roots (TotlegAllRo), seminal root count (SemRoCount), lateral root count (LatRoCount), total length of lateral toots (TotLegLatRo), center of mass horizontal co-ordinates (CentorofMassX) and With/Depth ratio under (a) HN and (b) LN in 12 genotypes (4 amphidiploids and 8 bread wheat).

Table 7. 4 Pearson's Correlation coefficient

(a) between seminal root count, seminal root length and total length of all roots under HN and LN and grain yield (GY t ha^{-1}), grain yield per shoot (GY/Sh), above-ground dry matter (AGDM, t ha^{-1}), above-ground dry matter per shoot (AGDM/Sh), harvest index (HI), thousand grain weight (TGW), plant height (PH), anthesis date (AD), physiological maturity date (PMD), N-uptake Efficiency (NUpE), N-utilization efficiency (NUtE), N-uptake per shoot (NUp/Sh), grain N-uptake per shoot (Grain NUp/Sh), grain number per shoot (Grain No./Sh) among 12 genotypes. (Mean of 15 replications).

	Seminal root number		Seminal root length		Total length of all root	
	HN	LN	HN	LN	HN	LN
GY	0.81 **	0.74 **	0.14 ns	0.15 ns	0.14 ns	0.13 ns
GY/Sh	0.76 **	0.74 **	0.20 ns	0.23 ns	0.20 ns	0.22 ns
AGDM	0.83 ***	0.76 **	0.31 ns	0.25 ns	0.30 ns	0.24 ns
AGDM/Sh	0.67 *	0.45 ns	0.37 ns	0.30 ns	0.37 ns	0.32 ns
HI	0.76 **	0.79 **	0.19 ns	0.26 ns	0.18 ns	0.23 ns
TGW	0.23 ns	0.42 ns	0.13 ns	0.12 ns	0.12 ns	0.12 ns
PH	0.26 ns	-0.06 ns	0.30 ns	0.07 ns	0.30 ns	0.07 ns
AD	-0.59 *	-0.58 *	-0.26 ns	-0.24 ns	-0.29 ns	-0.22 ns
PMD	-0.68 *	-0.63 *	-0.36 ns	-0.25 ns	-0.35 ns	-0.22 ns
NUpE	0.85 ***	0.63 *	0.18 ns	0.20 ns	0.16 ns	0.21 ns
NUtE	0.71 *	0.83 ***	0.24 ns	0.29 ns	0.23 ns	0.27 ns
NUp/Sh	0.74 **	0.24 ns	0.27 ns	0.23 ns	0.27 ns	0.25 ns
Grain NUp/Sh	0.84 ***	0.61 *	0.20 ns	0.34 ns	0.19 ns	0.35 ns
Grain No/Sh	0.84 ***	0.81 **	0.20 ns	0.34 ns	0.20 ns	0.33 ns

(b) between primary emerging angle, total length of all roots, seminal root count, maximum width and depth of root system, total length of lateral roots and grain yield (GY), above-ground dry matter, harvest index, plant height, anthesis date, N-utilization efficiency, N harvest index, N-uptake at anthesis and at harvest amongst 6 Indian cultivars (Mean of 15 replication).

	Primary emerging angle		Total length of all roots		Seminal root number		Maximum width		Maximum depth		Total length of lateral roots	
	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN
GY	-0.37 ns	-0.45 ns	-0.72 ns	-0.75 ns	0.17 ns	0.52 ns	-0.47 ns	-0.46 ns	-0.73 ns	-0.72 ns	-0.64 ns	-0.49 ns
AGDM	-0.1 ns	-0.18 ns	-0.43 ns	-0.52 ns	0.33 ns	0.48 ns	-0.21 ns	-0.2 ns	-0.49 ns	-0.49 ns	-0.31 ns	-0.26 ns
HI	-0.61 ns	-0.92**	-0.7 ns	-0.79 ns	-0.37 ns	0.24 ns	-0.62 ns	-0.87*	-0.6 ns	-0.83*	-0.78 ns	-0.80*
TGW	-0.14 ns	-0.5 ns	-0.17 ns	0.11 ns	-0.74 ns	-0.64 ns	-0.16 ns	0.05 ns	0.04 ns	0.12 ns	-0.25 ns	0.04 ns
PH	0.54 ns	0.72 ns	0.45 ns	0.19 ns	0.32 ns	0.58 ns	0.55 ns	0.26 ns	0.41 ns	0.14 ns	0.6 ns	0.18 ns
AD	0.09 ns	0.01 ns	-0.08 ns	-0.46 ns	0.57 ns	0.83*	0 ns	-0.31 ns	-0.23 ns	-0.53 ns	0.03 ns	-0.39 ns
NUtE	-0.6 ns	0.24 ns	-0.74 ns	-0.41 ns	0.05 ns	0.91*	-0.72 ns	-0.26 ns	-0.81 ns	-0.46 ns	-0.77 ns	-0.43 ns
NHI	-0.48 ns	-0.03 ns	-0.75 ns	-0.36 ns	-0.49 ns	0.47 ns	-0.56 ns	-0.07 ns	-0.59 ns	-0.35 ns	-0.76 ns	-0.28 ns
NUp An	0.32 ns	-0.2 ns	0.11 ns	-0.54 ns	0.47 ns	0.58 ns	0.23 ns	-0.28 ns	0 ns	-0.54 ns	0.24 ns	-0.54 ns
NUp H	-0.21 ns	-0.51 ns	-0.59 ns	-0.46 ns	0.28 ns	-0.08 ns	-0.31 ns	-0.2 ns	-0.62 ns	-0.37 ns	-0.47 ns	-0.14 ns

*Significance at the 5% (P = 0.05) level. **1% (P = 0.01) level. ***0.1% (P = 0.001) level.

7.4 DISCUSSION

Significant links of the seedling RSAT with field and glasshouse yield and yield components

In the previous chapters, results in the field and in glasshouse experiments showed N-uptake was playing a more important role in determining genetic variation in grain yield than NUtE which suggested that root traits play an important role in determining genetic variation in NUE through N-uptake. Therefore, to test this hypothesis genotypes contrasting for GY and N-uptake under LN conditions from both the field and glasshouse experiments were screened in the 2D seedling RSA pipeline to see if these traits correlate with field or glasshouse data.

In the 2D seeding root phenotyping experiment germination paper based 'pouch and wick' phenotyping pipeline was successfully used as a high-throughput, cost-effective way to characterize the 12 genotypes which were selected based on their performance under LN conditions for grain yield and NUpE. Although this 'pouch and wick' platform could not provide a 3D growing environment similar to the field experiment root traits, it could be applied to compare the different genotypes under similar experimental conditions and associate these root traits with actual field data. Comparison and association between indoor seedling RSA traits with yield traits measured under field conditions was done in many experiments and crops, for example, in bread wheat (Liu *et al.*, 2013; Atkinson *et al.*, 2015; Xie *et al.*, 2017) durum wheat (*Triticum durum*)(Cane *et al.*, 2014), maize (*Zea mays*) (Li *et al.*, 2015) and oilseed rape (*Brassica napus*) (Thomas *et al.*, 2016).

In this experiment amongst 12 genotypes, association was found between seminal root number per seedling and most of the yield traits per shoot e.g. GY, ADGM, and N uptake under HN and LN conditions. These results show that under both HN and LN conditions the total number of roots per plant was the single most important trait responsible for the better performance of these traits. As initial root vigor is important for inter-plant competition and nutrient uptake it could explain these associations in that initial root number could enhance N-uptake and result in higher GY and AGDM. There is evidence that vigorous seedling roots are beneficial to take up more soil resources (e.g. N and P) for early plant growth (Zhu *et al.*, 2005; An *et al.*, 2006; Cao *et al.*, 2014) leading to a release of the inter-plant competition. The resultant availability of more resources for the development of above-ground biomass would increase grain yield. However, seminal root number was negatively associated with AD and

PMD. This association could be partly explained by the fact that early genotypes may develop seminal roots relatively quicker roots.

Comparing amphidiploids with bread wheat, it has found that, amphidiploid showed relative higher increase in maximum depth of root system under N limiting conditions than bread wheat. Previous, higher root mass in landraces than modern bread wheat cultivars is reported by Waines and Ehdaie (2007). Recently, a near-isogenic line, introgressed with a QTL on chromosome 7AS from wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) into the background of bread wheat cv. BarNir, showed a deeper root system under progressive water stress, which may enhance abiotic stress resistance and productivity of domesticated wheat (Merchuk-Ovnat *et al.*, 2017).

Overall, amongst 12 genotypes studied there was no association found for seedling root angle or root length with GY traits under both N conditions (data not shown). This could be explained by the fact that at seedling stage these traits could not express at their full potential and at maturity these traits may change. A limitation with seedling screens is that the results may not correlate to mature plant measurements taken in the field. A variety of factors may be responsible, such as the lack of adventitious roots at seedling growth stages, that account for a significant proportion of the final root system and are critical for nutrient and water uptake (Boatwright and Ferguson, 1967). Other factors such as seed size and endosperm composition are known to influence seedling growth (Maydup *et al.*, 2013), although in this study seeds were sieved to remove any size effect. However, there are some study's where seedling RSAT showed association with field traits. Liu *et al.* (2013) reported positive association between root system size of seminal roots with grain yield. Bai *et al.* (2013) and Atkinson *et al.* (2015) showed association between QTLs identified for seedling RSAT and field traits likes yield and N-uptake. Xie *et al.* (2017) found association of field traits like grains m^{-2}, grains per spike, spikes m^{-2}, above-ground biomass m^{-2} and grain yield with seedling RSAT like seminal root number and total root length and maximum width of the root system.

Amongst 6 Indian cultivars, there was a negative association between HI and maximum depth and width of root system, primary emerging angle, and total length of lateral roots. Seminal root number showed positive association with AD and NUtE. Overall these

associations were encouraging and association with important traits like GY and N uptake may need screening of all Indian cultivars and amphidiploid lines.

Overall in this experiment, amongst 12 genotypes and amongst 6 Indian genotypes we have reported a significant association between seedling RSAT and field yield, N-uptake and NUtE related traits (Table 7.4 a and B) showing that the RSAT measured after 9 days of seedling growth on pouches could be useful to predict or use as selection criteria for breeding yield or N uptake related traits in field breeding program.

Summary

In summary, the findings in relation to the hypotheses tested in this chapter were:

"Genetic variation in early seedling root vigour is associated with RSAT of the mature root system and N uptake which increases yield under N limiting conditions".

This hypothesis was supported as there was a positive association between seedling seminal root number in the controlled environmental experiment and yield per shoot in glasshouse and field experiments for 12 genotypes (8 wheat cultivars and 4 amphidiploid lines).

"Genetic variation in 2D seedling screen traits is related to N uptake and NUE in the field under N limiting conditions".

This hypothesis was partially sported as there was association between seminal root number and NUpE and NUtE for 12 genotypes (8 wheat cultivars and 4 amphidiploid lines) under HN (R^2=0.85, 0.63) and LN (R^2=0.71, 0.83) conditions respectively.

CHAPTER 8

General Discussion

8.1 Field, glasshouse and 2D seedling root phenotyping experiment

The Indian wheat grain yield revolution in the 1970s involved modification in harvest index due to the introduction of the Norin dwarfing genes which reduced plant height and changed source-sink balance, thereby promoting greater partitioning of photoassimilates to grain and reducing lodging under favorable conditions of fertilized and irrigated fields. This brought an era of intensive agriculture where N fertilizer was the most essential and central element of this revolution. However, increased N application also increases risk of foliar diseases and lodging and is indirectly responsible for pollution and increasing production cost. Therefore, there is economic and environmental pressure on plant breeders to develop new wheat cultivars which need less N input but at the same time maintain high grain yield and quality. However, to develop new N efficient wheat varieties requires knowledge of traits determining NUE and genetic diversity. Therefore, in this study two different genetic materials, 30 Indian elite spring wheat cultivars and 18 amphidiploid lines where studied.

8.1.1 Yield responses to N

Positive yield responses to N supply were observed associated with effects on both numerical (e.g. grains m^{-2}) and physiological (e.g. above-ground biomass) yield components. Under N limiting conditions in both field and glasshouse experiments the grain yield reduction was ca. 30%. Post Green Revolution varieties have typically been selected under non-limiting fertilizer inputs to breed high N responsive cultivars (Foulkes *et al.*, 1998a; Hawkesford, 2017). This selection procedure tends to select genotypes that are efficient in N uptake when it is abundant, but has provided little or no selection pressure to improve either N-utilization or N-uptake efficiency when N availability is limited (Cormier *et al.*, 2016). Moreover, traits that are responsive to added N may have been selected leading to cultivars able to exploit fertilizer N efficiently. The analysis of a panel of UK winter wheat varieties indicated that among modern varieties there was a clear correlation of performance irrespective of N input

(Barraclough *et al.*, 2010), and a broader analysis of 195 varieties in a multisite trial came to a similar conclusion (Cormier *et al.*, 2013). A comparison of two tall and eight semi-dwarf CIMMYT bread wheat varieties released between 1950 and 1985, and grown at multiple N rates, indicated gains in grain yield N responses in the more modern varieties, in which were associated with increased N-uptake and NUtE (Ortiz-Monasterio *et al.*, 1997). However, selection under high N supply may mask efficiency differences among genotypes in ability to recover and utilize soil N to produce grain and the highest ranking genotypes selected under high N may not perform relatively as well under limited N supply (Moll *et al.*, 1982). On the other hand, in some studies in maize, it has been found that the genotypes selected under low N fertilization input could not perform better under N-rich soil conditions (Muruli and Paulsen, 1981). Therefore, Gallais and Coque (2005) suggested that when the plant material performs relatively well under low N input, it should be selected under N deficiency conditions for which yield reduction does not exceed 35–40%. In the present investigation, in both field and glasshouse experiment the ca. 30% reduction under LN conditions represents ideal conditions to study the traits related to NUE and its components.

8.1.2 Genetic diversity and association with NUE and NUE components

In the beginning of this study one of the hypothesis was that "There is genetic variation for N-use efficiency and its components traits that are related to NUpE and NUtE in the panel of 30 elite Indian spring wheat cultivars and the panel of 18 amphidiploid lines of winter wheat". In both the field and glasshouse experiment genetic diversity was identified which could be exploited to develop new N efficient cultivars. In the field, for the 30 Indian elite cultivars although there was a positive linear association between GY under HN and LN conditions; some cultivars performed relatively better under LN conditions (eg. GW-332 and PDW-314) than other cultivars and could potentially be exploited for NUE breeding. Relatively better performance for GW-332 and PDW-314 than other cultivars seemed to be mainly associated with higher N uptake under LN conditions and greater reduction of N% in grain. Similarly, in the glasshouse experiment amphidiploid lines were identified which performed relatively better under LN conditions (eg. *Th. tur*201 x CSE and *Se. ana*141 x CSE) than their bread wheat parent.

The next hypothesis was "Under LN conditions NUpE plays a more major role in determining NUE than NUtE". Breeding efforts worldwide have shown that genetic variation in NUE has been positively correlated with both N-uptake and N-utilization efficiency (Hirel *et al.*, 2007). High N-uptake efficiency is a desirable trait particularly in reducing N leaching losses and acquiring N from deep soil. Present results showed that overall N-uptake was more strongly associated with NUE than NUtE under both HN and LN conditions in 30 elite Indian cultivars. Positive associations between NUE and NUpE under low N conditions in wheat were reported in Mexico (Ortiz-Monasterio *et al.*, 1997), in France (Le Gouis *et al.*, 2000), in Finland (Muurinen *et al.*, 2006) and in Australia (Sadras and Lawson, 2013) and under both HN and LN in the UK (Gaju *et al.*, 2016), but NUE was most strongly associated with NUtE in France (Brancourt-Hulmel *et al.*, 2003) and in the UK (Foulkes *et al.*, 1998a; Barraclough *et al.*, 2010; Gaju *et al.*, 2011). It is possible that root system architecture traits like early seedling root vigor, seminal root number, root length and root angle play an important role in determining the genetic variation in N uptake. Similar results to the 30 elite India wheat cultivars were also reported in the present glasshouse experiments on the amphidiploids. For the amphidiploids, the relative importance of NUpE in determining NUE could be explained by the fact that wild wheat species may have a more extensive and deeper root system. However, amphidiploids were grown in 2 L pots and expression of rooting traits may have been reduced under these conditions.

In soil, nitrate is one of the readily available nutrients and it can be easily leached down in the soil profile so root depth is an important attribute for soil N acquisition. Palta and Watt (2009) demonstrated in a ^{15}N labelling experiment on wheat that more vigorous root systems capture ca. 42-60% more N in top 0.2 m of soil profile than non-vigorous root systems. Moreover, greater root length density at depth could increase water uptake and N uptake from deep soil, so breeding for water uptake can also increase N-uptake in wheat (Cormier *et al.*, 2016). Narayanan *et al.* (2014) found a significant diversity in rooting depth in the Cultivated Wheat Collection (CWC) of spring wheat germplasm and reported that genotypes from Australia, Mediterranean, and west Asia regions possessed greater rooting depth compared with those from south Asia, Latin America, Mexico, and Canada. In wheat, there is large genetic variation for RSAT between domesticated and wild forms, and within each of the diploid, tetraploids and hexaploid wheat species (Manschadi *et al.*, 2008; Ayalew

et al., 2015; Bektas *et al.*, 2016). Genetic studies show that root traits are complex and controlled by many genes, each with small genetic effect. In bread wheat, quantitative traits loci (QTL) for different root traits were detected on the short arm of rye chromosome 1 (1RS) (Sharma *et al.*, 2011). QTLs for seedling root traits in winter wheat in a doubled-haploid population were detected by Bai *et al.* (2013). Twenty nine QTLs for seedling root traits were reported in a study on a doubled-haploid population derived from a cross between winter wheat cultivars Savanath x Rialto by Atkinson *et al.* (2015).

Considering these previous studies, one of the hypotheses in this study was that "Genetic variation in GY and N-uptake is linked to RSAT, post-anthesis N-uptake, source-sink strength, and stay-green traits under LN conditions". In the present study, twelve genotypes were selected for root seedling phenotyping from the field and glasshouse experiments based on contrasting performance under LN conditions. The seedling root phenotyping experiment also tested the hypothesis that "Early seedling root vigor is responsible for uptake of N from soil and increases yield under N limiting conditions" and "Genetic variation in 2D seedling screen traits is related to N-uptake in field and glasshouse experiments". In this study, the amphidiploids produced from crossing hexaploid wheat with wild wheat relatives demonstrated genetic variation for RSAT. Amphidiploids overall showed a relatively higher increase in seminal and lateral root length under N limiting conditions than the parental bread wheat cultivars and could therefore have potential to exploit for NUE breeding. Results also showed that, seminal root number was the trait which showed strongest association with field and glasshouse grain yield and associated traits (GY, AGDM, HI, AD, PMD, *NUpE*, NUtE, grain N uptake and grains m^{-2}) amongst the 12 genotypes under both N treatments. Just considering the 6 Indian cultivars in the field experiment a positive association between seminal root number and NUtE was encouraging and indicates that testing associations between seedling root traits and field NUE traits in future experiments seems justified. The amphidiploid (*Th. tur*201 x CSE) showing best performance in the glasshouse experiments also showed the highest number of seminal roots per plant under LN conditions in the 2D seedling root phenotyping experiment. The root system of wheat is complex and a series of quantitative traits were used to describe the root spatial configuration in the soil. In this experiment when considering above-ground traits per shoot for the 12 genotypes in the root phenotyping experiment only seminal root number showed correlation with N uptake.

However, in previous studies more root seedling traits were reported to show correlation with N uptake, e.g. root number, length, root angle, rooting width and depth (Pound *et al.*, 2013; Atkinson *et al.*, 2015). In wheat, root traits are particularly important for post-anthesis N uptake as extended grain filling duration associated with the stay-green effect will increase grain yield under N limiting conditions.

In relation to N-utilization efficiency, one of the hypotheses in this study was that "Genetic variation in NUtE is linked to source-sink strength, stay-green traits, and N remobilization efficiency under LN conditions". The present field experiments results showed genetic variation in NUtE was associated with NRE under both N treatments and with flag-leaf senescence traits only under LN conditions. Association of NUtE and flag-leaf senescence traits under LN conditions could be explained by the fact that the grain growth was source limited and the stay-green effect therefore contributed to increased grain dry matter yield.

Also, NUtE showed a strong negative association with grain N% under both N treatments. If NHI (grain N / AGN_H) remains unchanged, then increasing NUtE will result in decreased grain N% (Foulkes *et al.*, 2009). The two highest yielding cultivars under LN (PDW-314, GW-322) also maintained GY better than other genotypes under LN associated with relatively greater increases in NUtE and decreases in grain N% under LN conditions. Therefore, it may be concluded that the ability of a genotype to produce a viable grain with a minimal grain N% may be related to increased grain yield under LN conditions. Van Keulen (1977) reported that the minimum level of protein dilution in small grain cereals was 9.2% and in the present experiment the minimum value observed was 9% (assuming a conversion factor of 5.0 and 5.4 for grain N% under HN and LN treatment, respectively). Although selection for low grain N% may not be possible where high grain N% is required for the end-use market, e.g. for bread or *chapatti* making cultivars; this strategy may be possible for wheat that used for animal feed, distillation or biofuel market. Indian cultivars studied in this experiment were bread making cultivars and high protein content is an important trait for Indian cultivars as 90% wheat in India is used for *chapatti* making where high protein content increases nutritional value (Bedekar, 2001). In the present experiment, average protein content was 12.6% and 10.3% under HN and LN conditions, respectively. These values are in the range of hard wheat (>12%), medium hard (>10%) and soft wheat (<9.5%) used for *chapatti* making (Nagarajan, 2006).

There are many studies demonstrating N fertilizer application timing or number of split applications changes the bread-making quality of wheat and also affects the gliadin to glutenin ratio and high molecular weight glutenin subunits (HMW-GS) to low molecular weight glutenin subunits (LMW-GS) ratio (Fuertes-Mendizábal *et al.*, 2010; Zörb *et al.*, 2010; Xue *et al.*, 2016; García-Molina and Barro, 2017). Delayed N application mostly increases protein over starch in the grain and prolongs the duration of grain-filling and also increasing grain yield by reducing grain protein (Sowers *et al.*, 1994). So, it may be possible that identifying genetic variation in the gliadin to glutenin ratio and selecting for a lower ratio could allow grain quality to be maintained with lower grain protein content.

8.1.3 Genetic diversity in flag-leaf photosynthesis traits to improve NUE

In this study light-saturated maximum photosynthesis rate (A_{max}) of the flag-leaf was measured in the field experiments for one year (2016) under HN conditions and in glasshouse experiments for two years (2015 and 2016) under both HN and LN conditions. In field experiments, results showed that under HN conditions genetic variation in post-anthesis A_{max} was positively associated with AGDM and showed a trend for a positive association with GY. These results demonstrated that improvement in leaf photosynthesis rate may be an important trait to enhance biomass. Previously, the importance of improvement in leaf photosynthesis for wheat for biomass and yield enhancement was reported by Long *et al.* (2006), Parry *et al.* (2010) and Carmo-Silva *et al.* (2017). Parry *et al.* (2010) reported in a review of the literature that previous genetic increases in wheat yield potential were primarily associated with increases in harvest index rather than increase biomass. In many wheat breeding programmes, it seems that HI has been optimized through breeding efforts and is now approaching the theoretical maximum of ca. 0.64 for wheat, although still there is an opportunity for small increases. Also, a trade-off has been reported between increases in productive biomass and HI in some wheat breeding programmes so efforts are needed to maintain HI in high biomass backgrounds (Foulkes *et al.*, 2007; Foulkes *et al.*, 2011; Aisawi *et al.*, 2015).

Photosynthetic capacity and efficiency are bottlenecks to raising productivity and there is strong evidence that increasing photosynthesis will increase crop yields provided that

other constraints do not become limiting (Gaju *et al.*, 2016; Carmo-Silva *et al.*, 2017). Even small increases in the rate of net photosynthesis can translate into large increases in biomass and hence yield, since carbon assimilation is integrated over the entire growing season and crop canopy. Leaf photosynthetic rate, however, was historically poorly correlated with grain yield when different genotypes of a crop species are compared (Parry *et al.*, 2010). This led to the viewpoint that the scope for improvement of leaf photosynthesis to improve yield potential may be limited. By contrast, more recent experiments comparing the growth of a genotype in current and future projected elevated [CO_2] environments showed that increased in leaf photosynthesis is closely associated with similar increases in yield of major crops including wheat (Parry *et al.*, 2010; Simkin *et al.*, 2015). In C3 plants theoretical photosynthetic energy conversion efficiency is about 4.6% (Zhu *et al.*, 2008), while under field conditions, recorded energy conversion efficiency is less than one-third of this value. This suggests that there is scope for to increase energy conversion efficiency in photosynthesis processes (Reynolds *et al.*, 2000; Zhu *et al.*, 2008). Association between grain yield and photosynthesis was reported by Gaju *et al.* (2016) in UK synthetic lines, land races and modern wheat cultivars. These more recent-associations between A_{max} and AGDM are encouraging and could potentially be exploited for future breeding to increase yield.

In the present glasshouse experiments, there was no association amongst genotypes between flag-leaf A_{max} and AGDM or GY plant^{-1}; this could possibly be explained by the fact that amphidiploids had lower HI possibly associated with sink limitation despite having variation in A_{max} and transgressive segregation for this trait. Thus, amphidiploid lines (12 lines under HN and 7 lines under LN) showed higher post-anthesis flag-leaf A_{max} than their bread wheat parents. The three amphidiploid lines (*Th. tur*201 x CSE, *Se. ana*142 x HB and *Se. ana*141 x CSE) that showed the highest grain yield also showed significant TS over their bread wheat parents for AGDM plant^{-1} under HN. Previously, wild relatives of wheat were reported to have higher photosynthesis rate than modern wheat cultivars (Evans and Dunstone, 1970; Johnson et al., 1987; Carver et al., 1989) suggesting that wheat breeding may have resulted in lower photosynthetic rate. Austin et al. (1982) reported that rate of net photosynthesis was highest in the diploid wheat species, intermediate for the tetraploid species and lowest for hexaploid *T. aestivum* L. They also found there was overall a positive association with smaller leaf size in these species. Synthetic lines (*Triticum durum* × *Triticum tauschi*) and synthetic

derivatives developed by CIMMYT have also been associated with higher leaf photosynthetic rate (Del Blanco et al., 2000) and grain yield (Ogbonnaya et al., 2007) than recurrent parents under optimal conditions. Overall, in the present field and glasshouse experiments encouragingly there was no association between flag-leaf area and A_{max}.

8.1.4 Genetic diversity in flowering date in NUE breeding

In the field experiments, the cultivars were control cultivars for each of five wheat breeding zones and were adapted to the local climate of the respective breeding zones. Two of the original hypotheses regarding effects of AD related to the cultivar's origin of breeding zone: "Anthesis and physiological maturity date show G*E interaction affecting grain yield under LN conditions" and "Grain yield in Indian cultivars depends on cultivars adaptation to climatic zone according to the interaction of G x breeding zone". Results showed that association between traits across breeding zones was strongly correlated with effects of anthesis date. Previously, studies on Indian wheat cultivars showed that most Indian wheat cultivars have genes, e.g. vernalization (*Vrn* genes) or photoperiod-insensitive (*Ppd* genes) or earliness per se (*eps* genes), affecting flowering date (Kumar *et al.*, 2012). This variation in anthesis date was seen in the experiments as well as associations between AD and grain yield. In India, wheat is grown in the *rabi* (winter) season from around November to March, but the exact sowing dates and growing seasons vary from north to south. Generally, in the north the winter season is longer than in central or southern India giving more time for wheat growth resulting in higher yield. Today most rainfed wheat in India is in the Central and Peninsular regions, and accounts for ca. 30% of India's total production with little access to irrigation (Wasson *et al.*, 2012). Availability of irrigation is one of the important factors that plays a role in decision making for selecting cultivars as terminal drought is a common problem. This experiment was conducted in the Peninsular Zone and in this zone the wheat growing period is one of shortest in the world (around 100 days). In these conditions, an earlier flowering whilst maintaining physiological maturity date, to extend grain filling duration, could be one strategy for increasing yield. Results indicate that cultivars from PZ were able to perform relatively better because of early flowering combined with a later physiological maturity. However, early root vigor will be necessary in early flowering cultivars to capture maximum N pre-anthesis. The genetic diversity reported in this experiment in the amphidiploids

(Chapter 7) could contribute to such improvement. Alternatively, introducing traits like a deeper root system from later maturing winter wheat cultivars could be useful strategy. In the past there are some examples where some traits from winter wheat have been introduced in spring wheat. For example, in CIMMYT Mexico, using Russian winter wheat variety *Kavkaz*, a very high yielding disease resistant variety *Veery* was developed through the transfer of the short arm of chromosome 1B of wheat with 1R of rye with IB of wheat and further used in breeding program to produce varieties like *Attila, Kauz, Pastor, Baviacora* which now commonly occur in the pedigree of CIMMYT releases (Mishra *et al.*, 2013). So, transferring genes from the amphidiploids with predominantly winter backgrounds into spring wheat programs seems feasible.

8.2 Application of physiological traits in breeding for increase N-uptake and N-utilization efficiency

NUE, N-uptake, NUtE are complex traits and their physiology and genetic regulation is not well understood. There are no previous detailed investigations on NUE and its components traits in Indian wheat cultivars. In the present study, under both HN and LN conditions genetic variation in NUE and GY was found to be more strongly associated with variation in N uptake than NUtE. In order to improve N-uptake, optimizing the rooting system will be an important target trait for NUE breeding. Root modeling experiments have shown that the deeper the root system, the greater is the N capture in the soil profile as a proportion of that available to the plant (King *et al.*, 2003). Moreover, the 2D seedling root phenotyping experiment showed a positive association between seminal root number at seedling stage and GY in field and glasshouse conditions under both N conditions suggesting that number of seminal root at early stage could be a possible selection criteria for increasing NUpE.

In the present field experiments under LN conditions variation in NUtE and GY amongst genotypes was associated with flag-leaf senescence traits (VS.OnsetRP and VS.EndRP) and genetic variation in senescence traits appeared to be associated with crop NRE. However, the physiological reasons for the positive relation between NRE and stay-green traits is unclear and needs further investigation. These results suggest that identifying the genetic and physiological basis of NRE will be important in future strategies to increase

photosynthetic capacity during grain filling and NUtE in wheat breeding programs under low to medium N level. Measuring stay-green traits in field conditions is laborious and time consuming. The present study showed that visual flag-leaf senescence scoring was less time consuming and more reliable than SPAD senescence measurements. Stronger GY correlations with visual senescence score than SPAD parameters may be because the measurement was based on a greater number of shoots. To improve spectral reflectance NDVI measurement at the canopy level one strategy may the use of Unmanned Aerial Vehicle (UAV) to reduce the diurnal variation over the time course of the measurements (Araus and Cairns, 2014; Kyratzis *et al.*, 2017).

There were some associations with senescence traits that were only apparent under low N indicating an association with N treatment x genotype interaction (e.g. between senescence and NUtE). Thus, to be representative of low N performance, phenotypic assessment of this trait must be carried out at low N supply. Further studies are required combining genetics and physiology to identify the location of QTLs for senescence-related traits linked to NUtE and QTL interactions with N availability.

In this study, positive association has been reported between NUtE and NRE and N-uptake at anthesis. This suggests that cultivars that have higher N uptake at anthesis, they may allocate relatively more above-ground N as 'reserve' N which is remobilized post-anthesis with a higher efficiency than the 'structural' or 'photosynthetic N' pools (Pask *et al.*, 2012). Therefore, to breed higher NUtE cultivars another physiological avenue may be to select genotypes which have higher N uptake at anthesis and a capacity to accumulate 'reserve' N when N is available. Overall under both N treatments, NUtE showed a positive association with BPE which indicates scope to improve NUtE and grain yield through BPE under N limiting conditions. Traits indicated by the present results in the field, glasshouse, and controlled environment experiments for increasing NUE are summarized in Table 8.1.

Table 8. 1 Optimized ideotype for NUE under LN conditions

Trait	Mode of impact	Importance	Effects relevant for N-use efficiency
Early anthesis and late maturity	UpE, NUtE	High	Extend grain filling, increase TGW
Root length density at depth	NUpE	High	Increased root length density at depth leads to improve below-ground resource capture under N stress
Leaf photosynthetic rate at anthesis per unit N	NUtE	Medium	Enhanced photosynthetic rate in the post-anthesis period, TGW
N remobilization efficiency post-anthesis	NUtE	Medium	Higher N remobilization efficiency associated with stay-green phenotype
Post-anthesis N uptake	NUpE	Low	Fleaf-leaf stay green; PANU correlated negatively with N remobilization efficiency
Stay-green	NUtE	High	Delayed onset/rate canopy senescence associated with increased leaf photosynthesis and/or yield hence increased NUE
Lower grain N%	NUtE	High	Increase NUtR and grain yield for feed wheat
Gliadin to glutenin ratio	NUtE	Medium	Increase yield with minimum grain N%
Low flag leaf SLN	NUpE, NUtE	Low	Increase photosynthesis per unit N; increase flag-leaf green area per unit N
Lower FL NPI	NUpE, NUtE	Low	Increase stay-green effect

8.3 Future work

In the present investigation, genetic diversity was identified in Indian wheat cultivars for NUE and its components (N uptake and NUtE) traits. Genotypes selected for study in these experiments were elite cultivars and amphidiploids; a wider range of genotypes should be screened in future work to identify further for diversity for NUE traits, e.g. primary synthetic wheats and synthetic derivatives

In this experiment, the N uptake was more important than NUtE in explaining genetic variation in grain yield in both field and glasshouse experiments. N uptake appeared to be related to RSAT in this experiment. Results were encouraging and in future there is a need to screen the material for the RSATs in field conditions to obtain more reliable information. In this future work shovelomics techniques for measuring crown root traits (crown root angle, crown root length and seminal root length) could be deployed (Trachsel *et al.*, 2013).

In the field experiment flag-leaf senescence was assessed using visual senescence scoring key and the SPAD chlorophyll meter and the canopy senescence was assessed using NDVI. Visual senescence scoring was found to be effective and less time and labor consuming than the other two techniques. To screen a large number of genotypes using present techniques with ground-based spectral reflectance radiometers would have several limitations e.g. it is time consuming and the environment changes during the day which may affect the results. In the future with technological advancements in high-throughput phenotyping platforms (HTPPs), rapid screening on a large scale may be developed with the use of UAVs (Kyratzis *et al.*, 2017) or Phenomobiles (Deery *et al.*, 2014; Fernandez *et al.*, 2017) carrying spectroradiometers (Araus and Cairns, 2014). Also, different types of imaging systems for remote sensing of plots like hyperspectral radiometers or cameras could be used to develop new signatures for biomass, nutrient status, pigment degradation, photosynthetic efficiency and water content (Araus and Cairns, 2014). Use of UAVs could also be combined with thermography for rapid canopy temperature measurement under N stress conditions (Deery *et al.*, 2016). In this experiment, the LI-COR IRGA system was used for photosynthesis measurement which has limitations for screening large numbers of genotypes especially in high temperature area.

In present investigation in both field and glasshouse experiments identified genotypes were identified which showed contrasting performance for NUE and related traits with similar anthesis date and plant height under low N conditions which could be used as parents in future crosses to produce segregating populations for QTL detection and marker development for marker–assisted selection (Table 3.6). In future with availability of rapid screening HTPPs, it will also be possible to develop and phenotype large genetically diverse populations from different wheat growing zones of India in association mapping or genome-wide association study. Further identification and transfer of interesting genes to develop new cultivars could utilize new chip-based marker platforms for ultra-high-throughput genotyping. Field phenotyping of NUE traits is laborious and costly therefore use of linked molecular markers could assist in breeding. Biparental recombinant inbred and doubled haploid populations have previously has been used to detect QTLs regions associated with NUE traits (Cormier *et al.*, 2016; Guttieri *et al.*, 2017). Association mapping (Cormier *et al.*, 2014) approaches also have been applied to NUE traits. Similar studies to date have not been

widely reported in Indian spring wheat cultivars, although research is ongoing under the BBSRC Newton Virtual Joint Centres with India for Agricultural Nitrogen. Therefore, in future there is a need to conduct genome-wide association analysis with high-density SNP markers to identify genomic regions that may be associated with NUE traits in Indian spring wheat cultivars.

In the present investigation amphidiploids which have introgressed segments from wild wheat relatives showed some evidence for improved flag-leaf photosynthesis rate and RSAT. Future work to identify the interesting introgressions and associated genes could utilize the recently developed high-density SNP genotyping array (35 K Breeders array) for hexaploid wheat and its secondary and tertiary gene pool which has been recently developed (Winfield *et al.*, 2016). This enables the rapid and cost-effective characterization of introgressions. In the glasshouse experiment three amphidiploid lines showed higher AGDM than parent breed wheat cultivars and these were planted in field conditions for seed multiplication and in future they could be use in pre-breeding programmes as source of parental genotypes for photosynthesis traits. At ARI Pune, India, best performing amphidiploid lines under N limiting conditions are already been used to cross with Indian local cultivars and in future there will be a need to evaluate them on genetic studies for improvement in physiological traits under N stress conditions.

In the present investigation amphidiploids from *T. urartu* as a parent were not available. *T. urartu* is reported to be have higher photosynthesis (Sybesma, 2013). So, in future there is a need to use in *T. urartu* for amphidiploid production and evaluation for photosynthesis traits.

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