EXPLORATION OF FAVOURABLE TRAITS FOR NITROGEN USE EFFICIENCY IN ANCIENT WHEAT SPECIES

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Abstract

Nitrogen (N) fertiliser is one of the main inputs of intensive wheat cropping systems in the UK. The average application of N fertiliser in the UK for winter wheat was 220 kg N ha^{-1} in 2011, but typically only 50% of this applied N is taken up by the crop. Breeding crops for high N use efficiency (NUE; grain dry matter yield per unit availability of N from soil and/or fertiliser) while maintaining acceptable yield, is widely accepted as one of the approaches to maximise farmers' revenue and minimise pollution risk by reducing N fertiliser application. NUE is divided into two components; N uptake efficiency (NUpE; crop N uptake per unit availability of N from soil and/or fertiliser) and N utilisation efficiency (NUtE; grain dry matter yield per crop N uptake). NUE can be improved by improving NUpE and /or NUtE. Targeted integration of favourable traits into breeding programmes needs adequate diversity of the existing genotypes. However, genetic diversity of modern bread wheat is narrowed due to chance hybridisation events during wheat evolution and selective breeding for high yield. Hence, it has become necessary to search for novel sources of genetic variation for NUE. One of the sources could be the ancient wheat species, which provide novel resources of genes to improve NUE of modern bread wheat.

The overall objective of the current project is to explore favourable physiological traits for NUE in ancient wheat species. Three field experiments, four glasshouse experiments and three growth room experiments were conducted at Sutton Bonington Campus, University of Nottingham. The current work can be divided into four main components; (i) investigation of general plant growth and development, (ii) quantification of NUE and its components, (iii) exploration of root architectural traits of seedlings and mature plants, and (iv) identification of seedling root QTLs related to NUpE in a wheat x spelt population.

Significant variation for plant establishment and development was identified among wheat species where bread wheat had high plant establishment and faster plant development compared to ancient wheat species. Above-ground biomass production of bread wheat, spelt and emmer was either similar or bread wheat was slightly higher at maturity under high N levels (HN). However, high above-ground biomass

production at zero N (no N fertiliser; NN) was observed in spelt genotypes. High grain yield of bread wheat is due to improved harvest index when compared to tall ancient wheat species. Delayed onset of flag leaf senescence, slow senescence rate and prolonged leaf greenness were observed in some spelt genotypes, especially spelt cv. Oberkulmer and were positively associated with biomass production. Flag leaf length, width, green area, specific leaf area and SPAD values were significantly different between genotypes used in the study. The greatest green area, SPAD value and maximum width were recorded in bread wheat.

NUE was higher in bread wheat followed by spelt, emmer and einkorn. Emmer 2 had higher NUpE than spelt, bread wheat and einkorn genotypes. Delayed senescence hence extended green area duration and deep and vigorous root system might have influenced NUpE of spelt genotypes. High fertiliser N recovery rate of emmer 2 may be associated with well distributed, shallow, horizontally grown root system, especially at early plant growth. NUtE of bread wheat is higher than ancient wheat species and closely related to harvest index and reduced plant height. NUtE is more controlled by genetic factors than NUpE. N supply has a negative relationship with NUpE and its components. Relationships between NUE and its components were not consistent between experiments in the study. However, NUpE and NUtE explained more of the variation in NUE under HN than NN.

Root system architecture varied significantly between wheat species used in the study. Seminal root characters of the seedling such as tip angle, number of seminal roots, seminal root length and total root length are closely related to mature root systems. Spelt had long seminal roots with narrow tip angles and hence develops deep root system while having a well-developed superficial root system due to nodal roots. Therefore, both top soil scavenging and deep soil foraging occurs efficiently. A large and deep root system contributes significantly to greater above-ground biomass and green area production of spelt. A greater number of seminal roots with wide tip angles help emmer to develop a root system architecture well adapted to take up fertiliser N from the top soil layers, especially in early stage of the plant growth. There is a possibility of using seedling root traits such as total root length and average length of the seminal root to predict NUpE at maturity. Therefore, these traits might be used as selection criteria for crop breeding for efficient N uptake. Emmer had favourable

seedling root traits related to NUpE while spelt showed more favourable root traits at for N uptake at maturity than modern bread wheat and einkorn. Root N uptake efficiency and specific absorption rate of N were higher in emmer than spelt, bread wheat and einkorn.

Phenotypic evaluation and genetic analysis of recombinant inbred lines (RILs) produced from the cross between Swiss winter wheat variety Forno (*Triticum aestivum*) and Swiss winter spelt cv. Oberkulmer (*T. spelta*) was carried out. Two parents of the population were significantly different for a number of traits, including: number of seminal roots, average length of seminal roots, total root length, maximum width, width to depth ratio, tip angle and emergence angle of seminal root. All measured root traits of seedlings of the RILs varied significantly. A total of 26 significant QTLs were identified for seedling root traits. These QTLs were located on ten different chromosomes; 1BS, 2A, 2D, 3A, 3B, 4A, 5A, 5B, 7AL and 7D. QTL coincidence was found for total root length either with number of seminal roots or seminal root length of the seedlings which showed strong phenotypic relationship. RILs F_5-10, F_5-36, F_5-134, F_5-146, F_5-230 and F_5-234 could be efficient genotypes for N uptake at maturity. The contribution of the *A* genome to the phenotypic variation observed within this cross for the development of the seedling root system is more important than the *B* and *D* genomes, as assessed by QTL analysis.

Further studies are needed to identify QTLs associated with NUpE and root architectural traits of emmer and spelt. Favourable root traits in emmer and spelt related to NUpE could be introduced to bread wheat through direct crossing or creating synthetic wheat. The introgression of spelt into modern bread wheat will be useful for molecular studies to develop marker assisted breeding for high NUpE.

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List of Abbreviations

AFNR	Apparent fertiliser N recovery rate
AGB	Above ground biomass production
ANOVA	Analysis of Variance
DAS	Days after sowing
DAT	Days after transplanting
DEFRA	Department of Environment, Food and Rural Affairs
DF	Degrees of freedom
DH	Double haploid lines
DM	Dry matter
FAO	Food and Agriculture Organisation of the United Nations
GAD	Green area duration
GAI	Green area index
GS	Growth stage
GT	Genotype
HGCA	Home Grown Cereals Authority
HI	Harvest index
IFA	International Fertiliser Industry Association
LTM	Long term mean
LOD	Logarithm of Odds
QTLs	Quantitative trait loci
r	Correlation coefficient
R^2	Coefficient of determination
Rht	Semi-dwarfing genes
RILs	Recombinant inbred lines
RLD	Root length density
RSA	Root system architecture
SD	Standard deviation
SE	Standard error of means
SED	Standard error of difference of means
SLA	Specific leaf area
SLN	Specific leaf Nitrogen
SMN	Soil mineral N

SR	Solar radiation
SRL	Specific root length
$S_{AB}R_N$	Specific absorption rate of nitrogen
SPAD	Soil Plant Analysis Development meter
SS	Sub-sample
TGW	Thousand grain weight
N	Nitrogen
N_{ava}	Crop N availability
N_{off}	Total above-ground plant N uptake
NUE	N use efficiency
NUpE	N uptake efficiency
NUtE	N utilisation efficiency
$NUpE_R$	N uptake efficiency of roots
NDVI	Normalised difference vegetation index
NIR	Near-infrared
VIS	Visible red

Chapter 1

Introduction and literature review

1.1 World demand for food

Global population is projected to increase up to 9.6 billion by 2050 and further increase to 10.9 billion by 2100 (UN, 2014). Demand for food is expected to be doubled by 2030 due to increase in per capita consumption from 12 Teracalories day^{-1} at present to 25 Teracalories day^{-1} in 2030. Estimated rate of crop yield to fulfil the demand of growing population in 2050 is 2.4% per year (Ray *et al*., 2013). However the rate of yield improvement for the major four crops; maize, rice, wheat and soybean, which provide more than 60% of the calories globally, is only 1.6%, 1.0%, 0.9%, and 1.3% per year, respectively. Several studies have shown that increased productivity is the viable solution to increase crop yield rather than expanding cultivated lands (Ray *et al*., 2013). Therefore, in order to produce food for the growing population, productivity of the land needs to be improved by approximately 50% by 2050 (Murchie *et al*., 2009). However, increasing population leads to use lands for non-agricultural purposes hence food production may be significantly affected. World food prices increased massively from 2000 to 2008 and then began to decline from 2008. Multiple causes may be responsible for the unprecedented rise of global food prices such as changes in land-use pattern, economy and climate (Murchie *et al*., 2009). Rapid growth in demand for biofuel (Hubert *et al*., 2010) and growing concern of biodiversity conservation have had a significant effect on the changes of land-use pattern (Campbell *et al*., 2008; Gahukar *et al*., 2009). Urbanisation and rapid economic growth, mainly in Asia, has changed food consumption patterns of the people where demand for meat increased significantly hence the increased use of maize and soybean as livestock feed. Further, demand of wheat and rice has increased as a staple food in Africa in relation to improved economy.

Changing climate will have a significant impact on future food production. For example, regions near the equator, where many developing countries are located, will suffer from severe drought, while countries in high latitudes may benefit from increasing temperature (Easterling *et al*., 2007). Water scarcity has become problematic in agriculture and reduced food production therefore leads to increased food prices. Unfortunately, the majority of people hit by rising food prices are poor and food-insecure (Hubert *et al*., 2010). Estimated yield loss per year is about 10% due to crops grown in water limited conditions experiencing drought stress. Further,

30% of wheat cultivated areas in the UK are affected by at least 10 days of drought (Foulkes *et al*., 2002, 2007). Changing intensity and the seasonality of the rainfall in the UK is associated with climate change. More frequent winter flooding is expected in south and south-west regions while having drought and water stress in summer (Jenkins *et al*., 2008). Based on the climate change models, it was predicted that severe soil moisture deficits due to reduction of summer rainfall in many cropping regions may occur (Dodd *et al*., 2011).

1.2 Global wheat production

Wheat was the first cereal known to be domesticated ten thousand years ago. For the past eight thousand years, wheat has being a staple food for people living in Europe, West Asia and North Africa (Curtis, 2002). About 90% of cereal demand all over the world is supplied by wheat, rice and maize. Wheat is one of the major cereals providing 21% of food calories and 20% of the dietary protein for more than 3.5 billion people in the developing world (Braun *et al*., 2010). Demand for wheat is expected to increase 60% by 2050 while climate change is likely to depress yield (Easterling *et al*., 2007; Hubert *et al*., 2010). Wheat provides 35 to 60% of the daily calories for people in North Africa, Central and West Asia.

Total global wheat production in 2013 was 716 million tonne (Figure 1.1). Average world wheat yield of 2.5 t ha^{-1} must be increased up to 3.5 t ha^{-1} by 2020 to supply food for the growing population (Rosengrant *et al*., 1995). It is predicted that, in order to fulfil the demand, annual global wheat production should be increased from 716 million tonnes to 840 million tonnes by 2050 of which more than 70% will be consumed by people who live in developing countries. However, at present, the estimated rate of yield increment of wheat is 0.9% per year (Ray *et al*., 2013).

Production of wheat increased 3.6% per year from 1966 to 1979, after the Green Revolution, providing food for millions of people around the world. Introduction of high yielding semi-dwarf varieties, together with improved irrigation and crop management practices, significantly increased wheat production both in the developing and developed world. However, productivity rate has slowed steadily in wheat during 1984 to 1994 by 2.8% and further during 1995 to 2005 by 1.1% (CIMMYT, 2011). In some countries, wheat yield seems to be reaching a plateau, possibly due to reduced application of N fertiliser due to high price, environmental

concerns and/or existing varieties having reached their maximum theoretical yield (Calderini and Slafer, 1998).

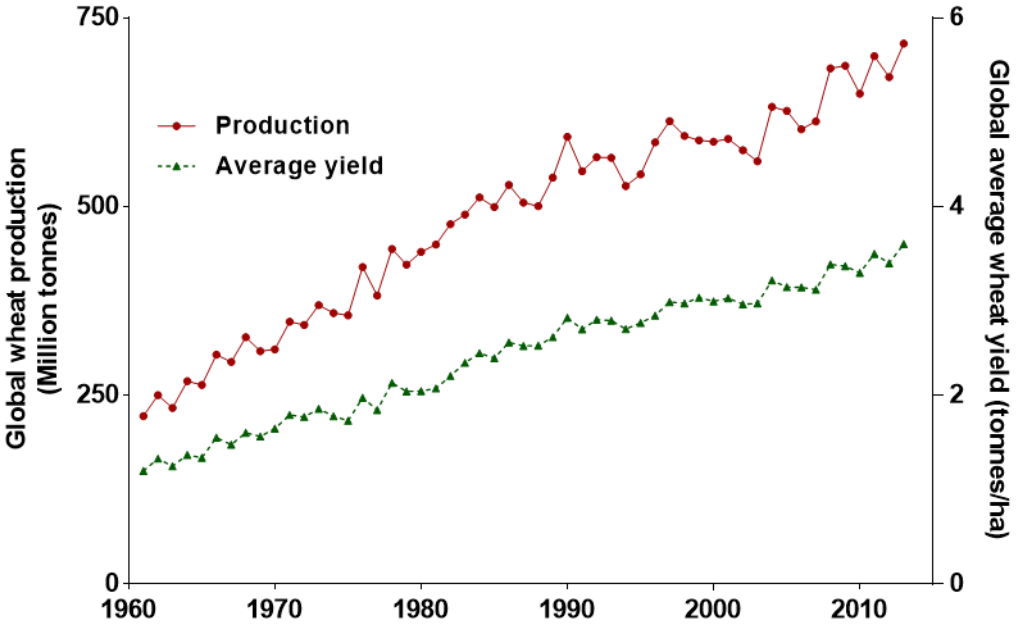


Figure 1.1 Trends in global wheat production and average grain yield (1961-2013) (Source: FAOSTAT 2015)

In 2013, contribution from Asia for global wheat production was 44.7% followed by 31.5% from Europe. America, Africa and Oceania contributed 16.6, 4 and 3.3% of world wheat production, respectively. Percentage of annual growth rate of the area harvested, production and yield of wheat is shown in Figure 1.2 (FAOSTAT 2015).

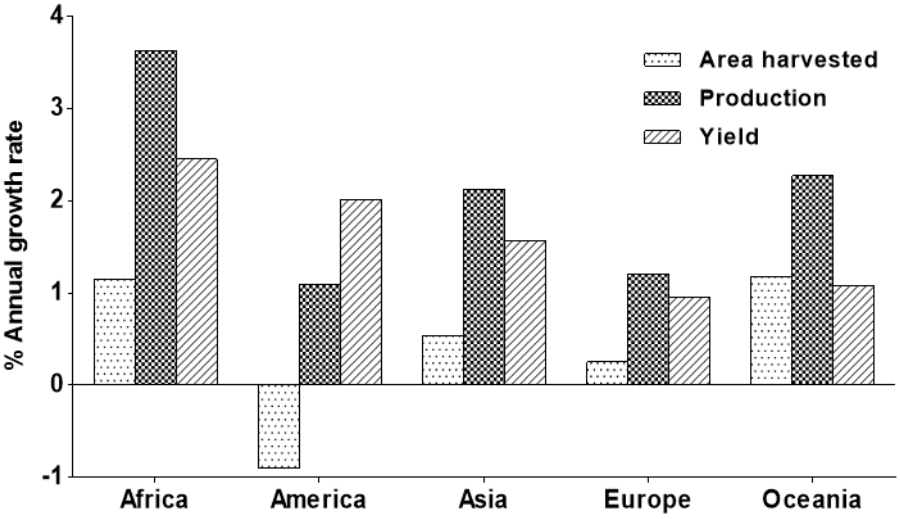


Figure 1.2 Annual growth rate (%) of the area harvested, production and yield of wheat (2000-2013) (growth rate calculated based on the least-square method (Source: FAOSTAT 2015)

Wheat production in the UK

Total wheat production in the UK for the year 2013 was 11.9 million tonnes; 1.7% of the global wheat production. Average yield in the UK for last five years was 7.8 t ha^{-1} (Figure 1.3) (FAOSTAT 2015) and total harvested area of wheat was 1.97 million ha. On farm wheat yield has been static from 1996 despite the yield increment of 0.5 t ha^{-1} per year due to potential of new varieties (Clarke *et al*., 2012). This static yield may be due combination effects of changing climate, soil condition deterioration, lack of good quality seeds and new policies (Sylvester-Bradley 2010).

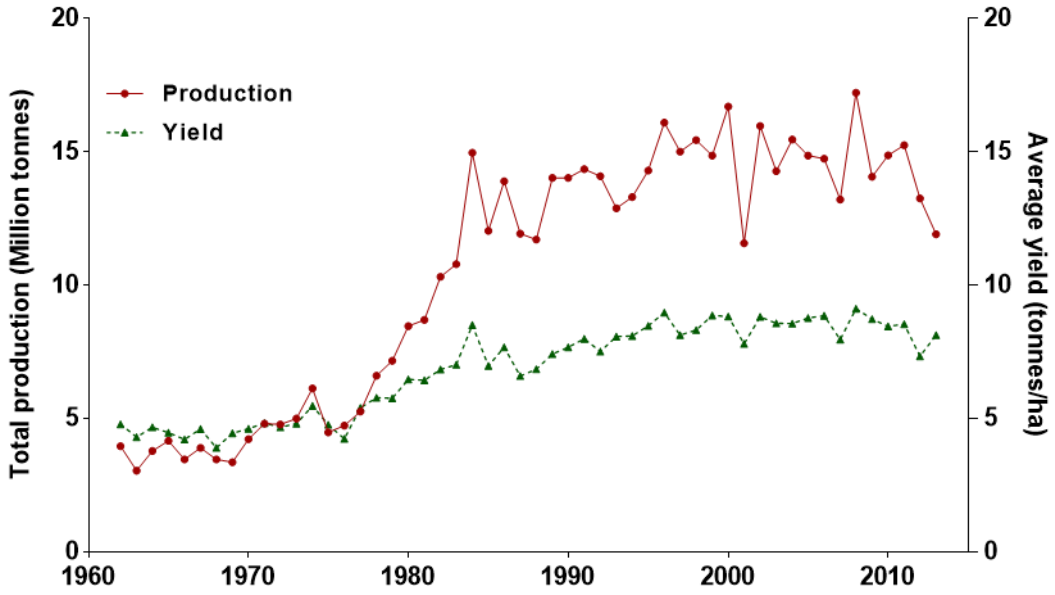


Figure 1.3 Total production and average yield of wheat in the UK (1961-2013) (Source: FAOSTAT 2015)

1.3 Green Revolution and food production

1.3.1 Benefits of Green Revolution

Introduction of high yielding new varieties of wheat and rice to the farmers in Latin America and Asia occurred in late-1950s to mid-1960s. Also, over the following years the release of modern varieties by breeders had a significant impact on farmers' economies. The success stories of the performance of newly released varieties were termed the "Green Revolution" (Evenson and Gollin, 2003).

Breeders incorporated semi-dwarfing genes resulting in shorter plants which allowed more biomass allocation towards grain production hence high harvest index. Plants

use relatively less energy to produce straw and leaf materials. These varieties responded better to fertiliser than old varieties. Under irrigation or reliable rainfall, together with good husbandry, they produced substantial grain yield (Evans, 1998). Increased grain production reduced food price globally and average calorie intake has risen significantly due to low price of food (Evenson and Gollin, 2003). However, sub-Saharan Africa did not receive notable benefits from the early Green Revolution (1960-1980) due to introduction of unsuitable varieties which were unable to produce sustainable yield under local conditions (Evenson and Gollin, 2003).

1.3.2 Green Revolution and N fertiliser application

1.3.2.1 Role of N in plant growth

Nitrogen (N) is an essential element for crop growth and development (Kraiser *et al*., 2011) and the availability of N determines the rate of plant growth and development massively in natural ecosystems (Marschner, 1995; Epstein and Bloom, 2005) and in agricultural cropping systems (Galloway and Cowling, 2002). Under N limited conditions, plants reduce the rate of leaf production and the final leaf number (Sagan *et al*., 1993), number of nodes, and the number of tillers (Tràpani and Hall 1996), depending on the species.

1.3.2.2 Demand for fertiliser N in agriculture

Globally, the application of N fertiliser in agriculture has increased significantly during the last five decades (Hirel *et al*., 2007), with the influence of the Green Revolution. Currently, half of global food production depends on synthetic N fertiliser. Accelerated population growth during past 150 years shows a close relationship with the production of industrial scale N fertiliser (Dawson and Hilton, 2011). It is estimated that two-thirds of global N fertiliser will be used by developing countries by 2025 (Galloway *et al*, 1995) where the main objective is subsistence rather than environmental concern (Campbell *et al*., 1995). It is estimated that 57.5 million tonnes of N were applied to cereals in 2010-2011, representing 55.2% of world fertilizer N consumption. Wheat was the main crop receiving N fertilizers, with 18.1% of global use, followed by maize with 16.8% and rice with 15.4%. Other cereals accounted for 4.8% of the world total (Heffer, 2013). In 2001-2011, China consumed more than 31% of N fertiliser followed

by India which was about 16%. Contribution of global N fertiliser consumption from USA and EU was 11% and 10%, respectively and rest was from all other countries.

Every year, 100 million tonnes of ammonia, a main ingredient of N fertiliser, are produced (Palme *et al*., 2014). Fossil fuel consumption for producing ammonia by pressurising air using the "Haber-Bosch" process is very high. Since 1962 annual global N fertiliser consumption in agriculture has increased from 11.8 million tonnes to 111 million tonnes in 2013 (Figure 1.4) (IFA Database, 2015) and is expected to increase up to about 120 million tonnes in 2018/2019 (Heffer and Prud'homme, 2014).

In 1951, average application of N fertiliser to UK wheat was only 28 kg N ha^{-1}. Amount of N application increased up to 73 kg N ha^{-1} by 1975. It further increased up to 185 kg N ha^{-1} during 1975 and 1985 (Austin, 1999). However, since 1985, relatively a stable amount of N fertiliser was applied to wheat at about 190 kg N ha^{-1} until 2005 (IFA database, 2015). Consumption of N fertiliser in the UK decreased from 2005 possibly due to farmers' concern on the significant price rise of N fertilisers compared to the price of the wheat grain (Figure 1.4). As most plants only take up *c.* 50% of the N fertiliser received, the rest may be lost to the environment or immobilised by microbial population creating problems. On average, in the UK, nitrogen uptake efficiency (NUpE; total plant N uptake (N_{off}) per unit availability of N from the soil/fertiliser) of winter wheat is 60% (Sylvester-Bradley and Kindred *et al*., 2009) while overall nitrogen use efficiency (NUE; grain dry matter yield per unit of N availability) of cereals globally is approximately 33% (Raun and Johnson 1999). Enormous quantities leach into ground water table or lost to surface water bodies resulting in eutrophication. Creation of "dead zones" in oceans, with death and decomposition of massive algae blooms, is one of the examples of harmful impact of excess N fertiliser application in agriculture. Microbial denitrification may have significant contribution in producing greenhouse gasses making agriculture the second largest industrial contributor of greenhouse gas emission (Palme *et al*., 2014).

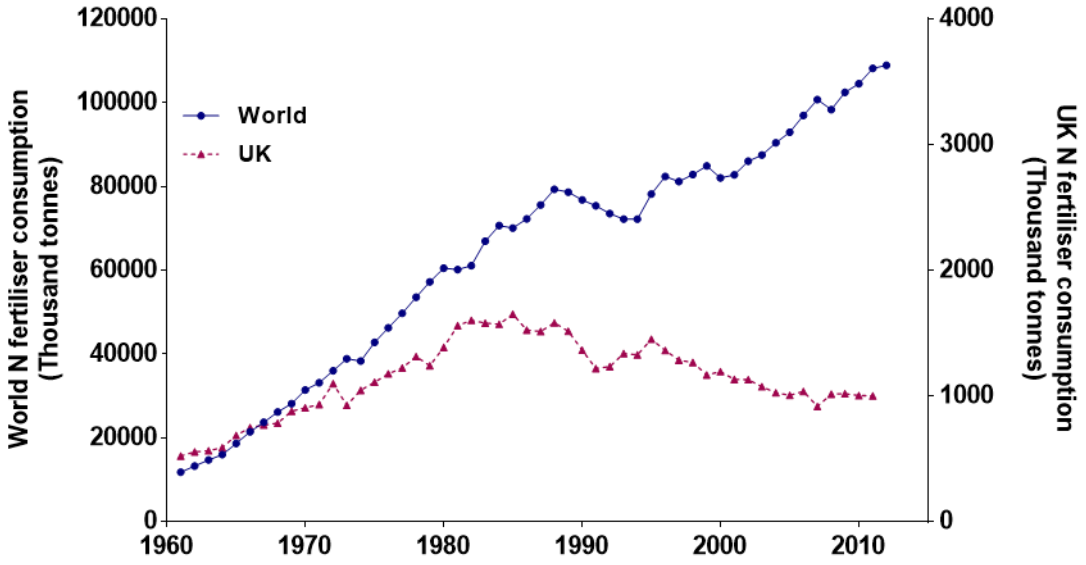


Figure 1.4 N fertiliser consumption in agriculture globally and in the UK (1961-2013) (Source: IFA database, 2015)

1.4 Efficient use of N fertiliser

Dependence of food production on N fertiliser increases significantly with the influence of global population growth. It is estimated that, without N fertiliser in agriculture, it is not possible to fulfil the current demand of food energy and protein (Dawson and Hilton 2011). At present, one of the many challenges faced by plant breeders is to release cultivars that minimize pollution risk while maintaining farmers' revenue by producing acceptable yield. Minimising pollution can be achieved either by applying low N fertiliser or improving plant uptake of N. Improving yield and quality per unit N fertiliser applied would help to maximise farmers' revenue (Le Gouis *et al*., 2000). The negative impact on environment, health and economy due to application of N fertiliser highlights the necessity of having efficient genotypes for N fertiliser use. High NUE can be achieved by improving management strategies and/or breeding advancement.

Effective crop management strategies can be used to minimise fertiliser N losses and increase NUE hence reduce environmental pollution. Crop N demand and supply can be synchronised (Shanahan *et al*., 2008) by applying N fertiliser as two or three splits (Addiscott *et al*., 1991) at major growth stages. Previous findings suggested that, N fertiliser application at booting stage increased harvest index (HI; the proportion of

grain in above-ground dry matter at harvest) but low N harvest index (NHI; the proportion of grain N in total above-ground plant N uptake) in wheat. However, in contrast, improved HI and NHI were observed in wheat by applying N fertiliser at booting stage (Spiertz and Ellen, 1978). Whereas early supply of N fertiliser increased grain yield while constant or slightly increased HI was observed (Cassman *et al*., 1992). N fertiliser rate can be calculated considering growing environment such as soil type, average rainfall, previous crop, and residual N availability of the soil (Raun and Johnson 1999). In the last 30 years, NUE at farm level has improved in the UK due to synchronisation of N demand of crop and fertiliser N supply rather than the genetic effects of the varieties (Sylvester- Bradley and Kindred, 2009).

Development of new varieties/cultivars with high NUE through traditional plant breeding and modern biotechnology is an alternative approach to improve NUE. Desirable traits could be identified using existing varieties/ ancient wheat germplasm. For example, ancient wheat species of einkorn, emmer, and spelt could be used to strengthen the variation of traits related to NUE in wheat breeding. Appropriate crosses between selected varieties or species may be implemented to introduce new traits to a segregation population. Further, QTL studies can be performed to identify useful lines having high NUE within the segregation population (Hirel *et al*., 2007).

1.5 Nitrogen use efficiency and components

1.5.1 Definition of nitrogen use efficiency

NUE has been defined in different ways in the literature (Barraclough *et al*., 2010). Researchers used different methods to calculate NUE by changing the way to calculate availability of N from the soil. N availability for the crop comes from a combination of N fertiliser and soil N mineralisation. Estimation of mineral N availability in the soil is complex and standard methods have not yet been established. In some studies, soil mineral N (SMN) was measured at sowing or before N fertiliser application for 90 cm of soil depth (Muurinen *et al*., 2006), although within season N supply by mineralisation of organic matter was not considered. Sylvester-Bradley and Kindred (2009) suggested that N supply by residual N pool plus N supply by mineralisation of organic matter is equivalent to total above-ground plant N uptake (N_{off}) at harvest at zero N fertiliser supplied plots. Whereas, N_{off} plus residual ammonium and nitrate at

harvest at zero N fertiliser applied plots were used as total soil N supply to the crop (Paponov *et al*., 1996). Bingham *et al*. (2012) calculated total N supply to spring barley as a sum of soil mineral N shortly after sowing/before crop emergence, N_{off} and residual soil mineral N at zero fertilised plots at harvest, and amount of N fertiliser applied.

Most studies have adopted the definition of Moll *et al*. (1982), in which NUE can be defined as grain dry matter yield per unit of N availability (N_{ava}) or supply (Equation 1.1). N supply for the crop is from fertiliser and/or soil N mineralisation.

$$\text{NUE} = \frac{\text{Grain yield (kg ha}^{-1})}{\text{N availability (kg N ha}^{-1})} \qquad \textbf{Equation 1.1}$$

NUE includes the above-ground plant N_{off} and the utilisation of N to produce grain. Several physiological processes associated with the absorption, translocation, assimilation, and redistribution of N are responsible for the utilisation of N within the plant (Moll *et al*., 1982). However, N contained in root tissues has been excluded from the calculation.

NUE can be divided into two components; (i) N-uptake efficiency (NUpE; N_{off} per unit availability of N from the soil/fertiliser) (Equation 1.2), and (ii) N-utilisation efficiency (NUtE; grain yield per unit plant N_{off}) (Equation 1.3) (Le Gouis *et al.*, 2000). N_{off} does not include root N in this calculation.

$$\text{NUpE} = \frac{N_{\ off}\text{(kg N ha}^{-1})}{N_{\ ava}\ \text{(kg N ha}^{-1})} \qquad \textbf{Equation 1.2}$$

$$\text{NUtE} = \frac{\text{Grain yield (kg ha}^{-1})}{N_{\ off}\ \text{(kg N ha}^{-1})} \qquad \textbf{Equation 1.3}$$

Therefore;

$$\text{NUE} = \frac{N_{\,off}\ (\text{kg N ha}^{-1})}{N_{\,ava}(\text{kg N ha}^{-1})}\ \text{x}\ \frac{\text{Grain yield (kg ha}^{-1})}{N_{\,off}\ (\text{kg N ha}^{-1})}$$

Or

$$\text{NUE} = \text{NUpE x NUtE} \qquad \textbf{Equation 1. 4}$$

These definitions of NUE and its two components are used hereafter in this thesis. According to Equation 1.4, NUE could be increased by improving either NUpE or NUtE.

1.5.2 Available genetic variation in NUE

Responses of NUE to different levels of N supply are well documented. Additional supply of N fertiliser always decreases NUE (Chamorro *et al*., 2002; Gaju *et al*, 2011). The level of N in the soil plays an important role in the expression of NUpE and NUtE (Ortiz-Monasterio *et al*., 1997). Low NUE is associated with high N loss and cost of production. Genetic variation in NUE and the interaction between varieties and N regimes were reported in major cereal crops like wheat (Ortiz-Monasterio *et al*., 1997), maize (Moll *et al*., 1982) and rice (Borrell *et al*., 1998). However, genotype and/or environment contribute significantly to the expression of either NUpE or NUtE under different soil N regimes (Coque and Galliais, 2006). Gaju *et al*. (2011) reported significant genetic variation for NUE, NUpE and NUtE in bread wheat.

Dhugga and Waines (1989) found that the NUpE to be the dominant component of NUE at low and high N levels for field grown spring bread wheat and durum wheat (Ehdaie *et al*., 2001). NUpE showed a strong relationship with NUE under low N while NUtE was more important under high N for 10 wheat varieties grown in Mexico (Ortiz-Monasterio *et al*., 1997). Gaju *et al*. (2011) found a stronger relationship between genetic variation of NUE in winter wheat with NUtE under high N and low N conditions than NUpE. The lack of relationship between NUpE and NUtE in barley

suggests that improvement of each component can be done independently (Bingham *et al*., 2012). Further, NUtE has a strong relationship with HI while increased NUpE of some barley varieties may be associated with a large grain sink. Contribution of components of NUE may be different though similar levels of NUE are found between varieties suggesting that potential traits related to each component could be used to improve NUE in wheat. Fischer (1981), more than 30 years ago in his review of water and N use efficiency, concluded that there is a possibility to improve NUpE in wheat though NUtE automatically increased with improved yield.

1.5.3 Nitrogen uptake efficiency

The ability of the plant to remove N from the soil as nitrate and ammonium ions is defined as NUpE. Inorganic N in the soil is from natural mineralisation of organic matter and N fertiliser. It is well known that the significant genetic variability exists among rice (Borrell *et al*., 1998) and wheat (Halloran and Lee, 1979; Ortiz-Monasterio *et al*., 1997; Le Gouis *et al*., 2000) varieties for NUpE. Genotypes, which have the ability to uptake more N when N supply is abundant, and store in vegetative plant parts, can be used to reduce N losses into the soil. The physiological and genetic basis of such variability in NUpE is not yet fully investigated although root morphology and stay-green properties are thought to be important (Christopher *et al*., 2008).

Application of N fertiliser in large quantities and inappropriate timing may lead to reduced NUpE hence reduced NUE. Water availability and soil texture may also have significant influence on NUpE. Less availability of N for plants may occur due to drought while N leaching and deep drainage take place with high rainfall. Cereal crops are genetically less efficient for NUE hence full recovery of N fertiliser in cereals is never achieved (Vaidyanathan, 1984). Previous studies have found that average fertiliser N recovery efficiency of cereals was about 50% (Ladha *et al*., 2005).

1.5.4 Nitrogen utilisation efficiency

The ability of plants to use the N taken up to produce grains is defined as NUtE. NUtE may be related to harvest index hence genetically controlled, therefore more related to yield determining process. Genotypes with same NUpE or even low NUpE but high NUtE produce more grain yield. Several studies have reported genetic variation in NUtE in wheat (Dhugga and Waines, 1989; May *et al*., 1991, Gaju *et al*., 2011). NUtE

increased from 34 at HN to 52 kg DM kg^{-1} N at LN for 16 bread making and feed wheat varieties in the UK and France (Gaju *et al*., 2011). NUtE among 39 elite UK winter wheat cultivars ranged from 27 to 77 kg DM kg^{-1} N depending on the amount of N fertiliser applied (Barrclough *et al*., 2010). Further, grain yield was positively correlated NUtE under each N regime (Barrclough *et al*., 2010). Pask *et al*. (2012) reported 33 to 51 kg DM kg^{-1} N of NUtE for Istabraq, a high yielding UK winter wheat cultivar, when averaged across site and seasons under optimum and zero N fertiliser supply, respectively.

In spring barley, NUtE expressed on a grain basis ($NUtE_g$) was more associated with HI than NUtE expressed on biomass basis. Further, percentage contributed by variety on total variance for NUE and $NUtE_g$ was larger than year x site interaction (Bingham *et al*., 2012).

1.6 Photosynthesis and N use efficiency

Green area of the canopy and leaf area index can be used to determine crop N status. Application of N fertiliser increases leaf area index and specific leaf N (SLN; amount of N per unit leaf area), then biomass production of the plant. Therefore strong relationships can be found between N supply, leaf N content, leaf N distribution and photosynthesis activities. Further, N content of the leaf determines radiation use efficiency (RUE) of the plant (Gastal and Lemaire, 2002). Therefore chlorophyll content, photosynthetic activities and spectral reflectance serve as major indices for N content of the leaf.

Above-ground biomass and leaf area index are increased with high N fertilizer in spring barley and wheat varieties at anthesis resulting in high grain yield. Ahamad *et al*. (1999) found that chlorophyll meter/SPAD-502 coupled with global positioning systems can be used to identify N stress levels for maize. According to Gholizadeh (2009), SPAD readings are closely related to leaf N content of the rice plants. Therefore, SPAD meter can be used to evaluate N status of the crop.

1.7 Stay-green phenotyping

A genotype with the ability to retain leaf green area for longer than an ordinary genotype is called a "stay-green" phenotype (Thomas and Smart, 1993). They are a potential source of assimilating more carban (C) during the post-anthesis grain filling period. There are two types of "stay-green" properties seen among cereal crops called "functional stay-green" and "non-functional stay-green". In functional stay-green, plants produce more C assimilates through active photosynthesis during post anthesis grain filling while in non-functional stay-green, leaves remain in green due to lesions of chlorophyll catabolism, which lack photosynthetic capacity (Jiang, *et al*., 2004).

Thomas and Howarth (2000) described five types of stay-green phenotypes among plants; (i) plants with late initiation of senescence with normal rate, (ii) plants with normal initiation of senescence but slow rate, (iii) plants having lesions of chlorophyll degradation, leaving rest of the leaf to continue normal senescence (iv) rapid senescence or death (v) enhanced greenness with normal initiation and rate of senescence. However, only type one, two and perhaps type five were recognised as functional stay-green phenotypes.

1.7.1 Functional stay-green

Functional stay-green phenotypes have been identified within many crop species. Stay-green mutants of durum wheat having higher NUE than wild types were identified under glasshouse conditions (Spano *et al*., 2003) while rice mutants with functional stay-green properties had a positive relationship with grain yield, especially due to improved seed setting rate (Fu and Lee, 2008; Yoo *et al*., 2007). Borrell and Hammer (2000) found that the stay-green phenotypes were directly associated with grain yield in sorghum. In maize, stay-green varieties had prolonged active photosynthesis under both high and low N conditions; hence dry matter accumulation and grain yield were higher than ordinary varieties (Echarte *et al*., 2008). Also, functional stay-green properties were associated with active photosynthesis during post-anthesis grain filling in spelt (Parmar, 2014). However, functional stay-green properties are not always associated with improved grain yield. In rice, it was found that stay-green varieties under irrigation do not produce high yield (Jiang *et al*., 2004). Previous studies have identified wheat lines with functional stay-green although

physiological processes underlying the results were not extensively studied (Foulkes, *et al*., 2007). However, Christopher *et al*. (2008), working in Australia, found that stay-green property of spring wheat SeriM82 was due to extraction of water from deep layers of the soil during post-anthesis grain filling period.

1.7.2 Canopy senescence and N remobilisation

Flag leaf senescence is a complex phenomenon controlled by genetics and the environment surrounded by the crop (Christopher *et al*., 2008). In general, during senescence, nutrients such as N available in the vegetative parts of the plant are recycled to develop grains (Lim *et al*., 2007; Gregersen *et al*., 2008; Guiboileau *et al*., 2010). In wheat, leaf senescence has a strong relationship with remobilisation of N from leaf to grain resulting in 40-90% of grain N from leaf (Van Sanford and Mackown, 1986; Kichey *et al*., 2007). Further, it is estimated that over 70% of the remobilised N was stored during pre-anthesis in wheat (Kichey *et al*., 2007). Canadian red spring wheat contributed about 18% of flag leaf N for grain development (Wang *et al*., 2008) while winter wheat contributed about 75% of N remobilisation from leaf N (Pask *et al*., 2012).

N supply and demand controls post-anthesis flag leaf senescence (Van Oosterom *et al*., 2010). Accelerated or delayed senescence may be a result of imbalance of N supply and demand (Rajcan and Tollenaar, 1999). Increased temperature induces the onset of senescence (Spiertz, 1977) while water and nutrient deficiencies, especially N, may have a negative effect on flag leaf green area duration (Gregory, *et al*., 1981). NUpE is significantly influenced by water availability post-anthesis. Limited water supply may disturb the balance of N supply and demand; hence accelerated leaf senescence can be seen (Gan and Amasino, 1977). The date of onset and the rate of senescence determine C assimilation during post-anthesis grain filling (Thomas and Howarth, 2000). Hirel *et al*. (2007) suggested that C is as important as N since plant needs energy and favourable structure (shoot and root) for N uptake post-anthesis (Dreccer, 2005). Hence C and N metabolism is equally important for the senescence process (Spano *et al*., 2003). Delaying senescence provides an opportunity to produce more C assimilates by prolonging photosynthesis. However, delayed senescence is not always beneficial, in terms of yield, if straw has more unused carbohydrate when the season terminates (Yang, *et al*., 1997). Therefore, remobilisation of C and N is crucial in the senescence

process. Some studies suggested that storage capacity of N in the canopy controlled N remobilisation during grain filling (Martre *et al*., 2003) rather than demand for N from the grains. Further, N remobilisation was halted by limited capacity of sink organs (Hirel *et al*., 2007). If post-anthesis N remobilisation is a sink-driven process, delayed senescence and post-anthesis N uptake would have to contribute less N for grain filling while if it is a source-driven process grain N concentration should be high. Under field conditions, at low N, winter wheat showed positive correlation with onset of senescence and NUtE suggesting that N remobilisation is a source-driven process under N limited conditions (Gaju *et al*., 2011). The relationships between leaf senescence, N remobilisation, grain yield and grain N content are very complicated and not easy to manipulate (Derke, 2013).

A simulation model proposed by Martre *et al*. (2007) observes negative relationship between grain yield and grain N concentration. This is a well-recognised phenomenon known as the dilution effect. This can be partially explained by delayed leaf senescence due to slow remobilisation efficiency of N hence improved C assimilation during grain filling and grain yield. Delayed senescence is expected to be related to post-anthesis root N uptake (Bogard *et al*., 2010; Mi *et al*., 2000). However, there was no direct effect of improved efficiency of post-anthesis N uptake on N remobilisation and grain filling duration or grain yield but post-anthesis N uptake rate encouraged grain protein content hence grain N concentration in model simulations (Martre *et al*., 2007). Spano *et al*. (2003) based on experimental results concluded that stay green mutants of durum wheat had a higher NUE than wild types but grain N content was not different. Therefore, grain protein concentration of mutant was lower than the wild type. There is a possibility to break the negative relationship between grain yield and grain protein concentration by improving storage capacity of non- protein N in leaves and stem. Indirectly, this helps to reduce detrimental effects of excess N fertiliser on environment by reducing leaching, denitrification and volatilisation (Martre *et al*., 2007). Grain yield was positively correlated with stem N concentration in UK winter wheat (Shearman *et al*., 2005).

1.8 Necessity of Second Green Revolution

Most of the wheat varieties released since the Green Revolution are highly sensitive to N fertiliser availability (Earl and Ausubel, 1983). It is hard to achieve theoretical optimum yield under N limited cropping systems such as cultivated regions in sub-Saharan Africa. Only farmers who have access to high inputs, especially N fertiliser, have been benefited from the Green Revolution. Since most of the cultivars of wheat and rice have responded under optimum growing conditions. Similarly, most of the wheat varieties in the UK, as a result of variety selection under optimum conditions (Foulkes *et al*., 1998; Sylvester-Bradley and Kindred, 2009), could not produce sustainable yield under resource limited cropping systems. We now need wheat varieties that have the ability to produce reasonable yield in marginal conditions and are also able to uptake N fertiliser efficiently under optimum availability.

The necessity of a Second Green Revolution was highlighted by several researchers during the past few years. Wollenweber *et al*. (2005) emphasised that, due to the declining rate of yield improvement in major food crops, a Second Green Revolution is needed to boost crop production. Lynch (2007) suggested that development of new crop varieties which can withstand infertile soil conditions and provide food for poor farmers, who have limited or no access to fertiliser N, would be the basis of a Second Green Revolution. This can be achieved by improving root system architecture hence efficient below-ground resource capture. Additionally, this helps to protect the environment by avoiding over application of N fertiliser to intensive cropping systems (Foulkes *et al*., 2009). Most of the candidate physiological traits of above ground plant, which can be used to breed new varieties for low N soil, have been identified for major cereal crops such as wheat (Le Gouis *et al*., 2000; Gaju *et al*., 2011). Further, the need for crops that require little applied N fertiliser for optimum yield output was suggested as the Second Green Revolution by Palme *et al*. (2014).

1.9 Root system architecture

Understanding of the behaviour and the function of root system architecture (RSA; the spatial configuration of a root system) is critically important to improve water and nutrient uptake of the plant. The root system plays a major role in determining soil exploration and therefore nutrient acquisition including N and provides anchorage to

the plant while maintaining plant-soil microbe interactions (Hirel *et al*., 2007). Since plants are sessile organisms, plants must adjust their morphology, physiology, growth and development with the influence of the adverse environmental conditions such as N depletion in the soil. Modification of RSA is one of the adaptations to survive under resource limited conditions. Many plant species modify RSA enabling them to uptake nutrients by proliferating lateral roots hence increase foraging capacity within nutrient rich soil layers (Walch-Liu *et al*., 2006). Consideration of plant productivity and RSA under different environmental conditions is therefore important (Wu *et al*., 2005). Recently, there has been a growing interest in exploration of root architectural traits and breeding crops for selected root traits (Price *et al*., 2002) relevant to uptake of water, nitrogen and phosphorus. One of the major concerns of present wheat breeding programmes is to develop genotypes with efficient root systems to uptake more water and nutrients under resource limited conditions. Several studies pointed out the importance of studying quantitative and qualitative associations between root traits under N limited soil (Kamara *et al*., 2003; Coque and Gallais, 2006).

1.9.1 Approaches to study root system architecture

Studying the RSA in terms of size and volume is difficult under field conditions since extraction of roots from the soil is extremely laborious and time consuming (Kondo *et al*., 2003; Garnett *et al*., 2009). Therefore, RSA has not directly been considered in wheat breeding programmes (Waines and Ehdaie 2007). Soil cores, soil columns ((Hund *et al*., 2008; Carvalho, 2009), gel chambers (Bengough *et al*., 2006), soil filled chambers (Manschadi *et al*., 2006, Manschadi *et al*., 2008), glass-walled growth boxes (Liao *et al*., 2006) and rhizotrons (Devienne-Barret *et al*., 2006; Laperche *et al*., 2007) are some of the most popular methods to study root systems and provide considerably high level of information. However, those methods are unsuitable for studying root system architecture for a large number of replicates or mapping populations with a large number of genotypes. Therefore, high-throughput phenotyping techniques have been used to study root system architecture under controlled environmental conditions for mapping populations (Hund *et al*., 2009, Atkinson *et al*., 2015). Bonser *et al*. (1996) developed root phenotyping systems based on germination paper pouches to study basal root angle in common beans (*Phaseolus vulgaris*). This method was successfully adapted for root phenotyping in maize seedlings (Hund *et al*., 2009).

Further, Atkinson *et al*. (2015) used growth pouches to grow seedlings of doubled haploid progeny derived from a cross between the winter wheat cultivars Rialto and Savannah followed by digital imaging. Also, root QTLs (Atkinson *et al*., 2015) were identified based on the results of image analysis using *RootNav* software (Pound *et al*., 2013).

1.9.2 Role of root system architecture in improving NUE

The response of RSA to N supply by fertiliser was reported in previous studies (Kondo *et al*., 2003; Wang *et al*., 2004) although root system investigation in relation to biomass production and grain yield are very limited. However, the direct impacts of RSA in grain yield have already been discussed (Lynch, 2007; Smith and Smet, 2012). Root traits such as length, number and branching pattern may be affected by the type of soil N (Maizlich *et al*., 1980). N uptake of the soil may be varied in the field condition due to uneven distribution of roots and NO_3^- concentration (Wiesler and Horst, 1994). Soil physical conditions have to be considered when studying root system architecture in relation to N availability (Bengough *et al*., 2006). There was a significant, but weak, relationship found between root traits related to size and the shape with biomass production under sub-optimal N supply for a maize RIL population (Guingo *et al*., 1998). Furthermore, the correlation between root number and grain yield was negative at low N supply (Gallais and Coque, 2005), possibly due to competition between two sinks, roots and shoots, for assimilated resources when N is limited (Oaks, 1992).

1.9.3 Root traits and N uptake efficiency

1.9.3.1 Rooting depth

Root architectural traits which encourage top soil foraging are useful for acquisition of phosphorus in infertile soils and N for high input cropping systems. NO_3^- is a highly mobile nutrient and important to uptake before being transported to deeper layers of the soil horizon. Deep and narrow root systems provide opportunities to uptake more N from deeper layers of the soil and useful in infertile soil (Garnett *et al*., 2009). However, deep rooting comes at a carbon cost to the plant and is highly dependent on the soil type. Bowman *et al*. (1998), experimenting on creeping bent grass (*Agrostis palustris*), concluded that plants with large and deep root systems reduce NO_3^-

leaching compared to those with small and shallow root systems. Rooting depth of UK- field grown winter wheat varies from 140 to 200 cm under optimum growing conditions and is highest at anthesis (Gregory *et al*., 1978; Barraclough, 1989, Lucas *et al*., 2000) while rooting depth has a positive correlation with water extraction. Whereas, Australian spring wheat recorded root depth 80 to 120 cm under field condition (Siddique *et al*., 1990). Rooting depth of wheat and barley, like other crops, depends on soil texture, structure, nutrition and depth of the ground water table though as a general rule deeper root systems may be related to long growing period (Barraclough *et al*., 1991). Furthermore, plants with higher root surface area due to lateral branches and root hairs have the ability to take up more N than plants with sparse root systems (Liao *et al*., 2004; Gahoonia *et al*., 2007; Smith and Smet, 2012). Barraclough *et al*. (2010) mentioned that deep rooting enables plants to uptake residual N from the subsoil which was leached during previous cropping cycles.

Nakomota and Oyangi (1994) who, based on the variation of the angular spread of seminal roots of Japanese wheat germplasm, found that genotypes with narrower angles of seminal roots ended up with deep root systems while shallow root systems had much wider angle and horizontally grown seminal roots. Similarly, Manschadi *et al*. (2008) based on the results of 27 Australian and three CIMMYT wheat genotypes, suggested that seedling seminal root angles is a major influence on mature root architecture. Genotypes with narrow seminal root angles, at seedling stage developed deep and compact root systems at maturity when compared to shallow seminal root angled seedlings. In common beans, root systems are shallow when basal root angle is wide, which facilitates top soil foraging hence greater phosphorous acquisition (Lynch and van Beem 1993). Growth angle of nodal roots in rice was associated with vertical distribution and depth of the root system which was described as a useful trait under drought for upland rice (Kato *et al*., 2006).

1.9.3.2 Root length and root length density

Extraction of all roots in a field experiment is extremely difficult for all crops. However, alternative techniques have provided better opportunity to calculate total root length of a given plant. The roots are extracted commonly using soil cores followed by root washing then scanned using *WinRHIZO* scanner. Total root length can be calculated using *WinRHIZO* software. Then extrapolated estimates of root

length (β) for 1m depth is done. Gale and Grigal's (1987) model is widely used to determine β as follows (Equation 1.5).

$$\beta = (1 - Y)^{1/d} \qquad \textbf{Equation 1.5}$$

Where *Y* is the cumulative proportion of total roots from the shallowest level sampled to the reported level and *d* is depth (cm).

Hoad *et al*. (2004) experimenting on wheat in the field reported 7- 28 km m^{-2} total root length, while Barraclough *et al*. (1989) reported about 22-35 km m^{-2}. Total root length varied from 2.9-18.2 km m^{-2} (average 9.8 km m^{-2}) in 17 winter wheat experiments carried out during 2007 to 2013 in the UK (White *et al*., 2015). Wheat breeding in the UK has reduced the ability of the root system to uptake N, derived from mineralisation, at the bottom layers of the soil (Foulkes *et al*., 1998; Sylvester-Bradley *et al*., 2001).

Root length density (RLD) is defined as root length per unit of soil volume and used extensively to describe soil exploration ability of the plant (Kramer and Boyer, 1995; Atkinson 2000; White *et al*., 2015) (Equation 1.6).

$$\text{RLD (cm cm}^{-3}) = \frac{\text{Total root length (cm)}}{\text{Root volume (cm}^{-3})} \qquad \textbf{Equation 1.6}$$

RLD decreases with rooting depth and the highest value is observed at anthesis. Critical RLD to absorb all available NO_3^- from the soil is 0.1 to 1 cm cm^{-3} (van Noordwijk, 1983). At top 20 cm of the soil horizon, RLD of field grown wheat varies between 5.6 to 10 cm cm^{-3} while in the 80 to 100 cm layer it was 0.4 to 1.5 cm cm^{-3} at or after anthesis (Gregory *et al*., 1978; Barraclough 1989 & 1991). Further, Pillinger *et al*. (2005) reported 7 cm cm^{-3} of RLD in the topsoil while 0.2 cm cm^{-3} in the 80-100 cm soil layer for winter wheat. Also, RLD in the plough layer in the field ranged from 3 to 6 cm cm^{-3} though it was less than 1 cm cm^{-3} below 40 cm (Hoad *et al*., 2004). Atta *et al*. (2013) found RLD ranging from 0.6-15 cm cm^{-3} in the top 15 cm of the soil for 15 wheat genotypes grown in Australia. Old wheat varieties reported greater RLD and root biomass in Western Australia in sand over clay soil when compared with modern

varieties in the top 40 cm of soil (Siddique *et al*., 1990). Elsewhere, reduction of subsoil NO_3^- was closely related RLD in maize (Wiesler and Horst, 1994) due to high N uptake. Main driving forces for high N uptake of a breeding line, Vigour 18, were associated with vigorous early root growth (Liao *et al*. 2004). As a result of that high RLD, root biomass and root surface area was reported.

Winter wheat genotypes increased RLD at anthesis under both irrigation and drought with additional supply of N fertiliser (Barraclough, 1989). As a compound effect, water uptake also increased with high N supply due to increased RLD and green canopy development. Gregory and Brown (1989) observed a relationship between additional N supply and root growth during grain filling period under well watered conditions. In order to acquire sufficient N, plants attempt to increase RLD to explore more soil volume hence increase N uptake (Liu *et al*., 2009).

1.9.3.3 Specific root length

The ratio between root length and root biomass is called specific root length (SRL) (Equation 1.7). In other words, SRL is economy of root length production due to biomass allocation. Root length production is a trade-off between photosynthetic assimilate allocation to shoots and roots.

$$\text{Specific root length (SRL; cm g}^{-1}) = \frac{\text{Total root length (cm)}}{\text{Root biomass (g)}} \qquad \textbf{Equation 1.7}$$

N fertiliser increased total root length without increasing root biomass hence high SRL (Barraclough, 1989). According to previous studies, efficiency of SRL under resource limited conditions showed mixed responses (Ryser, 1998). Relatively high SRL can be observed among young barley and wheat plants which then decrease as plant grow due to thickening of the roots in the field (Wilhelm *et al*., 1982). SRL depends on root fineness (root length per unit root volume) and tissue density (root biomass per unit root volume) (Ryser and Lambers, 1995). Genotypes with high growth rate generally have high SRL due to rapid production of root length hence efficient resource capture (Eissenstat, 1992).

1.9.3.4 Root biomass and N uptake efficiency of root

The effect of genotypic variation of root biomass on nutrient and water uptake together with leaching potential of bread wheat is not well documented (Ehdaie *et al*., 2010). Mian *et al*. (1993) reported significant variation between 40 bread wheat genotypes grown in hydroponic culture for fresh root weight. When grown in pots, with and without irrigation, spring bread wheat genotypes showed genetic variation for root biomass (Ehdaie *et al*., 1991). There was a strong correlation between N_{off} and root biomass for bread wheat 'Pavon 76' and its three near-isogenic translocation lines: Pavon 1RS.1AL, Pavon 1RS.1BL and Pavon 1RS.1DL (Ehdaie *et al*., 2010) where grain yield also had a positive correlation with root biomass. Root biomass is considered as a favourable root trait under drought in wheat (Bangal *et al*., 1988). Root length and root biomass of wheat have been shown to correlate strongly with N uptake suggesting the contribution of those two traits towards the efficiency of N uptake (Brady *et al*., 1993; Bowman *et al*., 2002). Root biomass is considered as a favourable root trait under drought in wheat (Bangal *et al*., 1988). As a result of more investment of biomass in roots, greater N uptake can be observed in some wheat genotypes (Liao *et al*., 2004).

N uptake efficiency of roots ($NUpE_R$) is defined as N_{off} per unit root biomass (Equation 1.8)

$$\mathrm{NU_PE_R} = \frac{N_{off}\ (\mathrm{kg\ N\ ha^{-1}})}{\text{Root biomass}\ (\mathrm{kg\ ha^{-1}})} \qquad \textbf{Equation 1. 8}$$

1.9.4 Reduced height gene (*Rht*) alleles in modern bread wheat

The reduced height gene alleles (*Rht-B1b* and *Rht-D1b*; previously termed as *Rht1* and *Rht2*), semi-dwarfing alleles from Japanese wheat 'Norin 10', have reduced sensitivity to gibberellic acid which induce stem elongation of plants (Murase *et al*., 2008). Severe dwarfism (> 30% of height reduction) could be achieved by introducing *Rht-B1c* (former *Rht3*) which was found in 'Tom Thumb' (Börner *et al*., 1997). Therefore, *Rht* genes, together with many other minor genes control plant height (Ahmed *et al.*, 2000) while increased grain yield and HI are also associated with introduction of *Rht* alleles

(Chapman *et al*., 2007). Lodging risk was significantly reduced by reduction of plant height in wheat hence N fertiliser application was greatly encouraged. Since 1970, *Rht-D1b* has been widely used in the UK wheat breeding programmes. Green Revolution wheat varieties have non-optimal root systems as Waines and Ehdaie (2007) reported that root biomass of semi-dwarf varieties is less than two- thirds of some landraces. Further, they have reduced root proliferation (Bai *et al*., 2013). However, reduced above-ground biomass production but increased root length and root biomass per area was recorded as a result of *Rht* genes in spring wheat grown the field (Miralles *et al*., 1997). McCaig and Morgan (1993) reported that above-ground biomass production was increased with plant height though there was no relationship between plant height and root biomass production. *Rht* near-isogenic lines (NILs) containing *Rht-B1c*, *Rht-D1c*, *Rht-8c*, and *Rht-12*, significantly reduce total root length and other root traits when compared with the control of Mercia (*rht*) (Bai *et al*., 2013). Further, some NILs reported reduced root biomass responding to *Rht* but root: shoot ratio was not different from the control. Figure 1.5 shows the tall wheat variety of Mercia as a control and NILs carrying *Rht1*, *Rht2*, *Rht3* and *Rht12*. The association was found between dwarfing and NUpE (Austin *et al*., 1977). Severe dwarfing reduced rooting of the crop under field conditions (Wojciechowske *et al*., 2009). However, *Rht*-B1b and *Rht*-D1b had no effect on crop N yield.

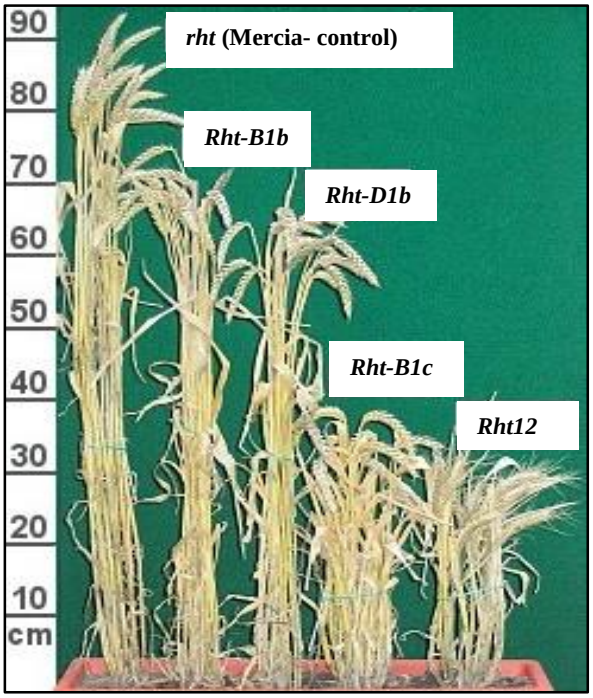


Figure 1.5 Tall variety of Mercia (*rht*) as a control together with semi-dwarf and dwarf varieties of wheat carrying *Rht-B1b*, *Rht-D1b*, *Rht-B1c* and *Rht12* (http://www.newhallmill.org.uk)

1.9.5 QTLs for root traits

Areas of the chromosome which control complex traits by contributing many genes with a small genetic effect are called quantitative trait loci (QTL) (Sharma *et al*., 2011). Identification of QTLs based on high-density genetic maps has strengthened the understanding of the genetic basis of root traits in cereals. Recently, the root QTLs were detected which were co-located with QTLs responsible for nutrient uptake of wheat (An *et al*., 2006), maize (Tuberosa *et al*., 2002) and rice (Steele *et al*., 2013). In Eastern India, as a result of marker-assisted backcrossing, four QTLs for root traits have been introgressed into an upland rice cultivar which has increased root length during tillering and grain yield by 1 t ha^{-1} (Steele *et al*., 2013). Several root studies have shown a correlation between deep rooting and NUpE (Bowman *et al*., 1998). Grain yield of rice can be increased up to 10% by introducing a deep rooting QTLs (*DRO1*) into shallow rooted cultivars (Uga, 2013; Arai-Sanoh *et al*., 2014). A study on maize recombinant inbred lines showed QTL coincidences between N uptake and root architectural traits highlighting the possibility to breed genotypes for a root system efficient for N uptake (Coque *et al*., 2008). Similarly, Atkinson *et al*. (2015) detected

some QTLs for seedling root traits co-located with N uptake and grain yield of field grown plants of Savannah x Rialto wheat doubled haploid (DH) mapping population. Bai *et al*. (2013) identified root QTLs co-located with thousand grain weight while some QTLs related to seminal root based traits overlapped with QTLs for plant height in winter wheat (Avalon; short) x spring wheat (Cadenza; tall) DH population.

1.10 Wheat domestication in ancient agriculture

The discovery of agriculture, about 12,000 years ago, in the Near East, changed human culture by shifting from hunter-gatherer to cultivating plants for food (Lev-Yadun *et al*., 2000; Salamini *et al*., 2002). The Fertile Crescent, along the Tigris and Euphrates rivers, at present Israel, Jordan, Lebanon, western Syria, southwest Turkey, Iraq and western flanks of Iran, is the place where the first pioneer farmers lived (Moore *et al*., 2000; Gopher *et al*., 2002) (Figure 1.6). Contribution of the discovery of agriculture on the sedentary lifestyle of early farmers was enormous. According to the archaeological evidence, they were fed on naturally grown wild cereal species, before cereals were cultivated and domesticated deliberately (Moore *et al*., 2000; Gopher *et al*., 2002; Zohary and Hopf, 2000). Domestication of cereals was the first experiment of early farmers.

Wild wheats (*Triticum urartu, T. boeoticum and T. dicoccoides*), wild barley (*Hordeum spotaneum*) and wild rye (*Secale vavilovii*) were three ancestor cereals which were among the founder crops during the Neolithic Revolution, cultivated forms of all, still can be seen in agriculture around the world (Lev-Yadun *et al*., 2000; Salamini *et al*., 2002). Domestication is generally known as a process of genetic improvement by selecting key harvesting traits which have been altered from wild forms of crops. For example in cereals, seed size, stiffness of ear rachis and glume tenacity were used as selection criteria (Salamini *et al*., 2002).

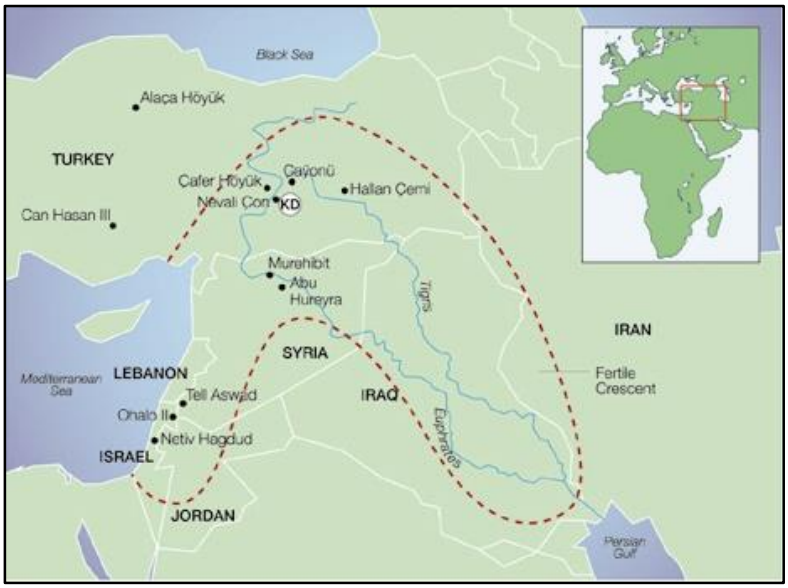


Figure 1.6 Fertile Crescent: a birth place of present agriculture about 12, 000 years ago (adapted from Salamini *et al*., 2002)

1.10.1 Evolution of modern bread wheat

Triticum aestivum (modern bread wheat) is a result of a number of random hybridisation events between progenitors of different ploidy levels. One of the most important steps in the evolution of modern wheat was domestication of emmer (*T. dicoccum*), a tetraploid wheat derived from its wild progenitor of *T. dicoccoides* (2n = 4x = 28, genome A^uA^uBB). *A* genome for wild emmer was from *T.urartu*, a diploid wild progenitor (Dvorak *et al*., 1993) and *B* genome was from the closest ancestor of goat grass (*Aegilops speltoides*) (2n = 2x = 14, genome SS) (Peng *et al*., 2011). This hybridisation occurred 300,000-500,000 years ago (Huang *et al*., 2002; Dvoral and Akhunov 2005). Around 9,000 years ago, cultivated emmer hybridised with another goat grass *Ae. tauschii* (2n = 2x = 14, genome *D*) and gave rise to a hexaploid spelt wheat (*T. spelta*, A^uA^uBBDD) (Dvorak *et al*., 1998; Matsuoka and Nasuda 2004; Peng *et al*., 2002). However, archaeological evidence suggested that the geographical distribution of emmer and goat grass (*Ae. tauschii*) is not common. Therefore, it is likely that few accessions of both species were involved in formation of hexaploid wheat, resulting in relatively narrow genetic variation among hexaploid wheat (Feldman 2001). Natural mutation changed the hard ears to more easily threshed types for both cultivated emmer and spelt forming durum wheat (*T. durum*) and bread wheat

(*T. aestivum*), respectively about 8,500 BP and was the final step of *Triticum* domestication (Figure 1.7). However, some researchers suggested, based on the results of recent experiments, that spelt (*T. spelta*) is not the ancestor of modern bread wheat (Dvorak *et al*., 2006). It seems that the genetic background of ancestry of modern wheat is complicated due to several factors such as gene flow from wild cereals (Dvorak *et al*., 2011).

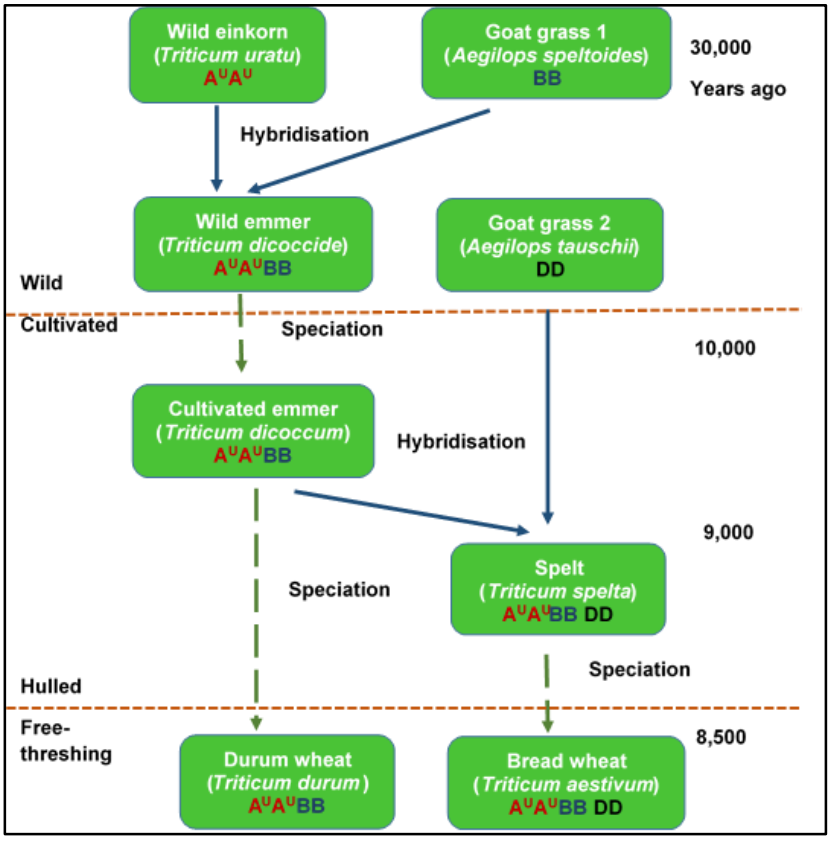


Figure 1.7 Evolution of wheat from 30,000 years ago until to modern hexaploid wheat present today (Peng *et al*., 2011, reproduced from http://www.newhallmill.org.uk/wht-evol.htm)

1.10.2 Ancient wheat species

1.10.3 Einkorn

Triticum boeoticum (wild einkorn, $2n = 2x = 14$, genome A^mA^m) was the first wheat cultivated in the Fertile Crescent 12,000 years ago. The first domesticated diploid is *T. monococcum*, a cultivated form of einkorn from its wild progenitor of *T. boeoticum,* present today (Harlan *et al*., 1966); Salamini *et al*., 2002; Kilian *et al*., 2007). During domestication brittle rachis of *T. boeoticum* has transformed to tough, which make it

easy to harvest. As a result of interbreeding between wild einkorn and domesticated einkorn, fertile progenies were produced. Einkorn started to be abandoned in the Bronze Age. It is a relic crop in present agriculture and hard to find production figures for recent years. However, it is still produced on a small scale in Mediterranean countries (Nesbitt and Samuel 1996; Perrino *et al*., 1996). Gluten in einkorn is not suitable for making breads but other products like biscuits, pasta and un-yeasty bread (Moudry *et al*., 2011). There is no contribution from first domesticated and cultivated diploid wheat of *T. monococcum* (cultivated einkorn) or its progenitor of *T. boeoticum* (wild einkorn) for wheat evolution. *T. monococcum* (or its wild progenitor) is not the donor of *A* genome of modern hexaploid wheat.

1.10.3.1 Emmer

Wild emmer (*T. dicoccoides*), a progenitor of cultivated emmer (*T. dicoccum*) did participate in the evolution of hexaploid wheat (Dvorak *et al*., 2011). Wild emmer grows all over the Fertile Crescent naturally. Wild emmer has a brittle rachis which allows seed shatter at maturity and large seeds when compared to cultivated emmer. However, all tetraploid wheat, unlike its progenitor, has a non-brittle rachis which increases harvestable yield (Salamini *et al*., 2002; Gill *et al*., 2007; Tzarfati *et al*., 2013). Although, durum wheat (*T. durum*) is derived from the same progenitor, it has free threshing naked seeds which mainly produce flour for pasta production. Cultivated emmer was the main crop of the Fertile Crescent until the early Bronze Age (Bar-Yosef 1998). In ancient Egypt, domesticated emmer is considered as only one source of flour having bread making quality (Samuel 1994). Emmer cultivation is not common in the world at present and only 10% of the total wheat area is covered by emmer in 2013. It is cultivated mainly in Ethiopia, Iran, Turkey, Italy, Spain and India (Stallknecht *et al*., 1996). Grains of emmer are used to prepare traditional food in Ethiopia, India and Yemen hence must still be considered as an important crop (Damania 1998). However, due to its nutritional value and distinctive taste, there is a growing interest of cultivating emmer in some European countries (Zaharieva *et al*., 2010).

1.10.3.2 Spelt

Spelt (*Triticum spelta*) is hexaploid hulled wheat with AABBDD genome. In Roman times and the Middle Age, spelt was an important cereal crop in Europe (Dvorak *et al*., 2006). Today, spelt is grown in a limited area of the world and on a small scale. However, spelt is still a major cereal in isolated regions throughout Middle Europe such as Germany, the Czech Republic, Austria, Hungary and Switzerland (Troccoli and Codianni 2005). Spelt, together with emmer, is in low input cropping systems in Europe due to its nutritional values when compared to bread wheat and their high resistance to the unfavourable environmental factors like low fertilisation (Suchowilska *et al*., 2009). Grain yield of spelt is lower than bread wheat but superior than einkorn and emmer (Troccoli and Codianni 2005). However, above-ground biomass production of spelt and bread wheat are either similar or bread wheat is higher under favourable conditions (Ruegger and Winzeler 1993). Moudry *et al*. (2011) found that flour of spelt has the same qualities as that of bread wheat and therefore able to develop products which are similar to bread wheat. Figure 1.8 shows ears, spikelets and seeds of different wheat species.



Figure 1.8 Appearances of ears, spikelets and seeds of several wheat species from left to right: cultivated einkorn (*Triticum monococcum*), cultivated emmer (*T. dicoccum*), spelt (*T. spelta*) and bread wheat (*T. aestivum*)

1.10.4 Ancient wheat: as a genetic resource for wheat improvement

Novel genetic diversity has had an enormous impact on wheat breeding and wheat cultivation since the inception of the Green Revolution in the 1960s. Most of this impact has been realized through improved disease resistance, particularly for the rusts, derived from variability both within and external to the primary wheat gene pool.

The genetic variation of tetraploid wheat can also be enhanced by crossing both cultivated and wild tetraploid forms with *T. urartu*, the *A* genome donor (Brandolini *et al*., 2008), or with *T. monococcum* to produce tetraploids with new A-genome variation, suitable for introgression into hexaploid wheat. Emmer is already in the breeding programmes for its favourable traits for drought tolerance (Damania *et al*., 1992), pest (Liu *et al*., 2005) and disease resistance (Oliver *et al*., 2008). Although, emmer is a self-pollinated (Golenberg 1987) tetraploid wheat (Zohary and Hopf 2000), it is fully compatible to be crossed successfully with durum wheat (Yanchenko 1985) and bread wheat. Emmer has accumulated high genetic diversity for drought stress adaptations during the long evolution history under Mediterranean conditions (Peleg *et al*., 2005). *T. dicoccum*, *T. polonicum*, and *T. carthlicum* have also been used to improve the drought tolerance of durum wheat (Al-Hakimi 1998). Primary synthetic wheat produced by crossing *T. dicoccum* and *Ae. tauschii* provides plant breeders with new genetic diversity for drought/stress tolerance (Mujeeb-Kazi *et al*., 2000).

Wheat x spelt crosses are mainly made for lodging resistance and disease resistance (Schmid and Winzeler 1990; Schmid *et al*., 1994). A higher level of genetic diversity is expected to be generated by crossing spelt and bread wheat than within spelt or bread wheat only crossess (Liu *et al*., 1990, Siedler *et al*., 1994). Ancient wheat species have been used as genetic materials to improve modern bread wheat for disease resistance, drought tolerance and lodging resistance but haven't yet been used to improve NUE and its components.

1.11 Rationale

Wheat is one of the globally important cereals due to its nutritional value and economical value which contribute significantly to world food trade. However, as in other cereals NUE of wheat is significantly low when compared to other food crops. In globally, only 33% of applied fertiliser N being recovered in the grains of cereals. Under farming practices, apparent fertiliser N recovery rate of root crops such as cassava, sweet potato, potato and sugar beet were 10, 25, 40 and 50%, respectively (Neeteson, 1989; Hartemink *et al*., 2000), while it was about 30% for sugar cane (Basanta *et al*., 2003). In the UK, N fertiliser recovery rate of winter wheat is about 50%. Therefore application of N fertiliser in intensive farming systems contributes significantly to environmental pollution and farmers' revenue. Therefore, this is

critical time to develop new varieties with high NUE while maintaining sustainable yield to fulfil food demand in the future. Targeted integration of favourable traits into breeding programmes needs adequate diversity of the existing genotypes. However, genetic diversity of modern bread wheat is narrowed due to chance hybridisation events and selective breeding for high yield. Hence, it has become necessary to search for novel sources of genetic variation for NUE. One of the sources could be the ancient wheat genotypes, which provide novel resources of genes/candidate traits (Trethowan and Mujeeb-Kazi, 2008; Sparkes, 2010) to improve NUE of modern bread wheat.

1.12 Aims of the thesis

The overall aim of the PhD is to investigate favourable physiological traits of ancient wheat species related to NUE which could be used to improve modern bread wheat.

1.13 Objectives and hypotheses

1.13.1 Objectives

Four main objectives of the PhD are,

1. Identify physiological variation such as crop establishment, plant morphology, biomass production and grain yield between ancient wheat species of einkorn, emmer, spelt with modern bread wheat.
2. Quantify NUpE, NUtE and NUE of ancient wheat species when compared to modern bread wheat and to identify physiological traits of above-ground plant which are associated with NUE and its components under field and controlled environmental conditions.
3. Identify and quantify the variation of root architectural traits of ancient wheat species and explain relationships between seedling root traits and NUpE at maturity.
4. Explore the phenotypic variation present in a wheat x spelt cross and to determine the genetic locations of genes controlling seedling root architectural traits in this population.

1.13.2 Hypothesis

The main hypothesis tested in this thesis is; 'ancient wheat species can be used as novel resources of genes to improve NUE of modern bread wheat'.

Specific hypotheses

1. Plant growth and development of ancient wheat species are significantly different from modern bread wheat.
2. Above-ground biomass production is higher in spelt but grain yield is higher in bread wheat due to improved harvest index.
3. Stay-green traits can be identified among ancient wheat species.
4. Flag leaf properties are significantly different between genotypes.
5. NUE and its components are different between genotypes used in the study and decrease with N supply.
6. NUpE of ancient wheat species are higher than bread wheat and associated with plant height and root system architecture.
7. NUtE of bread wheat is higher than ancient wheat species and closely related to HI.
8. NUpE is closely related to NUE at low N supply while NUtE is closely related to NUE at high N supply.
9. Root system architecture of ancient wheat species is significantly different from modern bread wheat.
10. Early seedling root architecture has a significant influence on mature root systems.
11. A large root biomass would enhance N uptake, which contributes to the greater green area and above-ground biomass production.
12. Seedling root traits could be used as selection criteria for NUpE at maturity.
13. Two parents of wheat x spelt population are significantly different for seedling root traits.
14. Seedling root system architecture varies significantly between RILs of this population and significant segregation of root traits among RILs is observed.
15. QTLs can be identified for seedling root architectural traits.
16. The introgression of spelt into modern bread wheat could provide novel genetic lines with improved traits for modern bread wheat for high NUpE.

1.14 Structure of the thesis

This thesis is based on the title of exploration of favourable traits for nitrogen use efficiency in ancient wheat species and comprises seven main chapters followed by references and appendices. There are four results chapters and each chapter concludes with a discussion relating to the specific hypotheses tested and the results obtained with previous findings in the literature. General discussion and conclusions focused on authors' overview and potential use of the results in breeding programmes. Limitations of the study together with future research possibilities are also discussed. Figure 1.9 shows schematic diagram of the structure of the thesis.

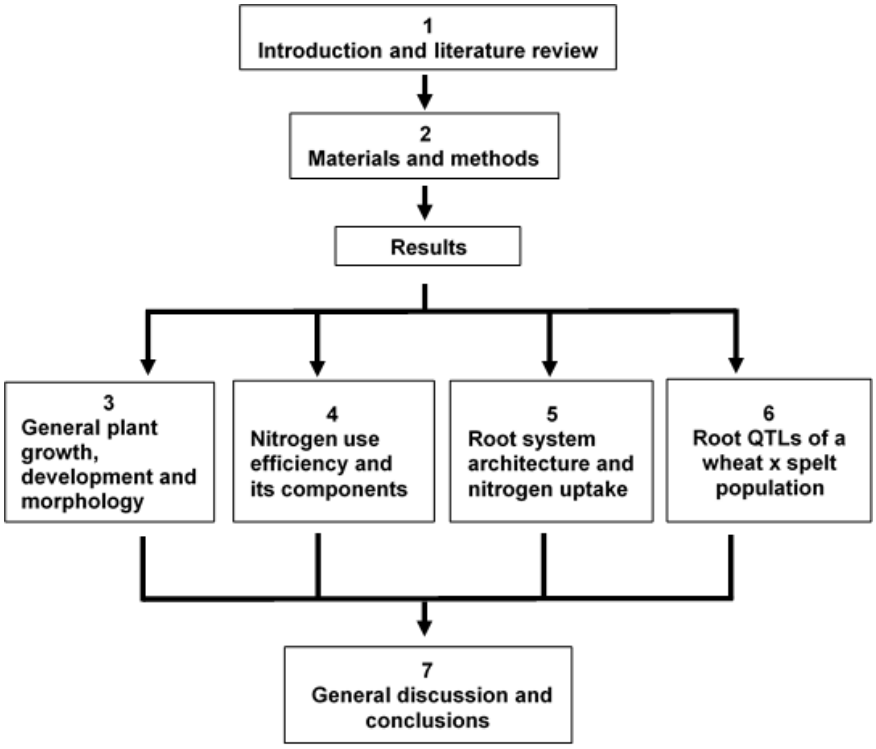


Figure 1.9 Schematic diagram of the structure of the thesis

Chapter 2

Materials and Methods

2.1 Introduction

In this chapter the materials and methods used in the field experiments, glasshouse experiments and growth room experiments are described. Three field experiments, four glasshouse experiments and three growth room experiments were conducted during three years of the study from October 2011 to October 2014.

2.2 Genetic materials

Three hulled wheat species with different ploidy levels were used as genetic materials for the experiments. They included cultivated einkorn (*Triticum monococcum* L., a diploid with AA genome), cultivated emmer (*T. dicoccum*, a tetraploid with AABB genome) and spelt (*T. spelta* L., a hexaploid with AABBDD genome), together with modern bread wheat (*T. aestivum*, a hexaploid with AABBDD genome). Ten genotypes were used, three einkorn (1, 2 and 3), two emmer (1 and 2), three cultivars of spelt (SB, Oberkulmer and Tauro) and two cultivars of modern bread wheat (Xi 19 and JB Diego). Seeds were obtained from a previous field experiment at Sutton Bonington. Originally, seed for einkorn, emmer and spelt genotypes were collected from the archaeological department at the University of Nottingham in 2007. Modern bread wheat cultivars were chosen from the Home Grown Cereals Authority (HGCA) recommended list released in 2008/2009.

2.3 Field experiments

All field experiments were established at Sutton Bonington Campus, School of Biosciences, University of Nottingham, UK (52^0 50' N, 1^0 15' W). Experiments were conducted under rain-fed conditions where, annual average rainfall and temperature are 606 mm and 13.5^0C, respectively. Thousand grain weight (TGW) of all genotypes together with seed rate applicable for three field experiments are shown in Table 2.1. Plant growth regulators were applied in all three experiments to minimise lodging and all other crop management followed best agricultural practices for UK wheat production.

Table 2.1 Thousand grain weight (TGW) and total seed rate of three field experiments established in 2011, 2012 and 2013

GT	TGW (g)			Seed rate (kg ha^{-1})		
	2011	2012	2013	2011	2012	2013
JB Diego	54.0	35.5	45.0	135.0	75.0	135.0
Xi 19	37.0	31.5	41.0	93.0	66.0	123.0
Spelt Tauro	58.9	57.0	53.0	152.0	118.0	159.0
Spelt SB	59.6	52.5	48.0	149.0	110.0	144.0
Spelt Oberkulmer	58.9	57.0	54.0	147.0	120.0	162.0
Emmer 1	53.4	37.5	43.7	134.0	79.0	132.0
Emmer 2	39.8	34.0	34.9	100.0	71.0	105.0
Einkorn 1	34.5	44.0	40.0	86.0	92.0	120.0
Einkorn 2	44.2	43.5	45.0	110.0	91.0	135.0
Einkorn 3	43.5	37.0	40.0	109.0	78.0	120.0

2.3.1 2011/2012 field experiment

Field site was planted with winter oats in the previous cropping season. The sowing density used in the experiment was 250 seeds m^{-2} and sowing date was 18 October 2011. The soil was a sandy loam with pH 7.6 containing 67.8 mg l^{-1} of P, 510, mg l^{-1} of K and 241 mg l^{-1} of Mg in the top 30 cm and 78.2 kg N ha^{-1} at 90 cm in February 2012. A randomised complete block design was used in the experiment with four replicates. Thus, the experiment was divided in to 40 plots (Appendix 1A). The length and width of the each plot were 24 m and 1.625 m, respectively while row width was 0.125 m. Each plot was divided into two halves and first half was used to collect samples throughout the season while second half was combined at maturity. Fertiliser N, a total of 140 kg N ha^{-1}, was applied as ammonium nitrate (NH_4NO_3, 34.5 N %) in three splits at early tillering, stem elongation and flag leaf emergence at 40, 40 and 60 kg N ha^{-1} , respectively (Appendix 1B). All plots received plant growth regulators (PGR) at 0.2 l ha^{-1} at early tillering and stem elongation.

2.3.2 2012/2013 field experiment

The sowing density of the experiment was reduced to 210 seeds m^{-2} due to limitation of the seeds and sown on 17 October 2012. The previous crop was winter oats. The

soil type of the experimental site was similar to the previous year, where pH was 7.4. However, nutrient availability of the soil was different. On average P, K and Mg availability of top 30 cm soil layer was 67.5 mg l^{-1}, 414 mg l^{-1} and 213.7 mg l^{-1}, respectively. N availability of the soil in top 90 cm was 45.2 kg N ha^{-1}, 63.2 kg N ha^{-1} and 58.9 kg N ha^{-1} in block one, two and three correspondingly in February 2013. Experiment was set up according to split-plot design where, main plot factor was N level and sub-plot factor was genotype (Appendix 2A). Treatment combinations were replicated three times. Three N levels of 0 kg N ha^{-1} (zero N; NN), 100 kg N ha^{-1} (low N; LN) and 200 kg N ha^{-1} (high N; HN) were used. NH_4NO_3 was applied as N fertiliser at 40 and 60 kg N ha^{-1} at early tillering and stem elongation for LN treatment while 40, 80 and 80 kg N ha^{-1} was applied at early tillering, stem elongation and flag leaf emergence for HN treatment. PGR was applied three times from stem elongation to flag leaf emergence at 1.4, 0.2 and 0.5 l ha^{-1} (Appendix 2B). There were 90 plots in total which were 12 m long and 1.625 m wide. The spacing between two rows was 0.125 m. Three quadrat (1.2 m x 0.6 m) samples were collected throughout the season and rest of the plot was combined at maturity. Due to poor establishment, emmer 1 and einkorn 3 were removed from the experiment.

2.3.3 2013/2014 field experiment

Spring oil seed rape was cultivated in the field in the previous season. The sowing density of the experiment was 300 seeds m^{-2} and sowing date was 19 November 2013. Soil type of the experiment site was sandy loamy and pH was 6.8. The experimental design was a split-plot in which N treatment was randomised on main plot, genotypes were randomised on the sub-plot and each treatment was replicated three times (Appendix 3A). Sub-plot size was 12 m x 1.625 m with an inter-row spacing of 0.125 m. On average P, K and Mg availability of the soil in top 30 cm of the depth was 72 mg l^{-1}, 216.7 mg l^{-1} and 221 mg l^{-1}, respectively. Soil mineral N availability in top 90 cm was 73.9, 74.9 and 65.8 kg N ha^{-1} in first, second and third block, respectively in February. Three N levels of 0 kg N ha^{-1} (NN), 100 kg N ha^{-1} (LN) and 150 kg N ha^{-1} (HN) were used based on soil mineral N analysis in February. LN plots received 40 and 60 kg N ha^{-1} at early tillering and stem elongation while 40, 80 and 30 kg N ha^{-1} for HN plots at early tillering, stem elongation and flag leaf emergence. All plots were treated twice with PGR at 1 l ha^{-1} during stem elongation to flag leaf emergence

(Appendix 3B). Three einkorn genotypes were excluded from the field experiment due to their poor establishment and growth performances throughout the experiment. Figure 2.1 shows (A) einkorn 1 and (B) einkorn 2 in June 2014.

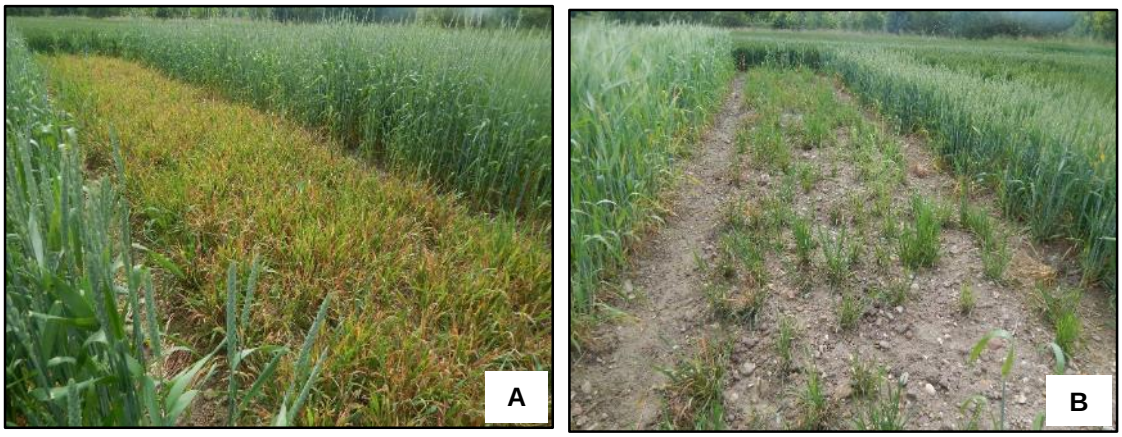


Figure 2.1 (A) Einkorn 1 and (B) einkorn 2 in June 2014

2.3.4 Population count

Plant population was calculated on 9 December 2012, 15 January 2013 and 8 February 2014 for first, second and third field experiments, respectively. A quadrat (0.5 m x 0.5 m) was used to count plant population in five different places within the plot and an average value was calculated. Percentage establishment was calculated from these values. Same method was applied in 2011/2012 and 2013/2014 field experiments but in 2012/2013 plant establishment was very poor for some genotypes hence plant number was counted in three rows of 1 m of the plot and then expressed as a percentage.

2.3.5 Developmental stages

Zadoks' scale was used to assess the crop growth based on the development of the main stem (Zadoks *et al*., 1974). The date when each genotype reached stem elongation (growth stage (GS); GS31), flag leaf emergence (GS39), ear emergence (GS59) and at anthesis (GS61), was recorded, when 50% of the plants within the genotype reached that particular growth stage. First internode of the true stem detectable and not less than 2 cm was identified as GS31. The point when flag leaf all visible was identified as GS39 and emergence of ear was GS59 while onset of flowering/ anthesis was GS61. Physiological maturity was recorded when the entire

leaf lamina senesced. Close observation is needed to identify growth stages accurately hence, plants were observed carefully at 1-2 day intervals. Duration from sowing up to GS61 was considered as pre-anthesis growth phase and the period after GS61 to physiological maturity was identified as the post-anthesis growth phase.

2.3.6 Growth analysis

2.3.6.1 Early growth

Early growth of the genotypes was evaluated only in the 2011/2012 field experiment. Samples were collected four months after sowing to determine N content of the plants in parallel to the soil mineral N analysis. A quadrat (0.5m x 0.5 m) was used to collect samples from the field. The outer rows of the plot were not sampled, and a buffer of at least 50 cm was left between successive sample areas and at the plot ends. Plants were kept in a plastic bag immediately to avoid drying. Then plants were taken into the laboratory and temporarily stored in a cold room (4^oC) maximum for four days to avoid plant tissue deterioration. Plants were washed thoroughly to remove all soil particles, and then dried on paper towel in a cool environment. Later, plants were weighed as whole sample and roots were separated using a sharp blade. After that, the above ground material was divided in to two subsamples (subsample 1; SS_1 and subsample 2; SS_2) assuring equal weight of two subsamples. SS_1 was used to calculate above-ground biomass production while all plants in SS_2 were separated into leaf lamina and leaf sheath in order to measure total green area. A leaf area meter (LI-3100, LI-COR inc, Lincoln, Nebraska, USA) was used to measure green area of the SS_2 in cm^2. Finally, SS1, green leaves and stems, together with root sample, were oven dried at 80^oC for 48 hours.

2.3.6.2 GS31, 39 and 61

When 50% of the genotypes and 50% of the plants within the genotype reached the particular developmental stage, samples were collected. A quadrat of 0.25 m^2 (0.5 m x 0.5 m) was used in 2011/2012 and 2013/2014 field experiments while 0.72 m^2 (1.2 m x 0.6 m) used in 2012/2013 field experiment due to uneven establishment of the genotypes. Plants were uprooted in all sampling points and placed directly into plastic bags before being transferred to the cold room. Samples were collected at GS31, GS39 and GS59 in 2011/2012 field experiment and GS39 and GS61 in 2012/2013 field

experiment while only at GS61 in 2013/2014 field experiment. Total fresh weight of the quadrat sample was measured without roots. Then 50% of the sample was used to calculate above-ground biomass production (SS_1) and 25% measured as SS_2. SS_2 was partitioned into green lamina, green stem and sheath and dead leaves in GS31, while flag leaf area and dry weight of non-green were recorded at GS39. Green area of flag leaf, 2nd leaf, 3rd leaf, remaining leaves and ears were recorded when partitioning SS_2 at GS59 and GS61. SS1 and all segregated parts of the SS_2 were oven dried at 80°C for 48 hours. Figure 2.2 shows (A) bread wheat and (B) emmer at GS31 in April 2014.

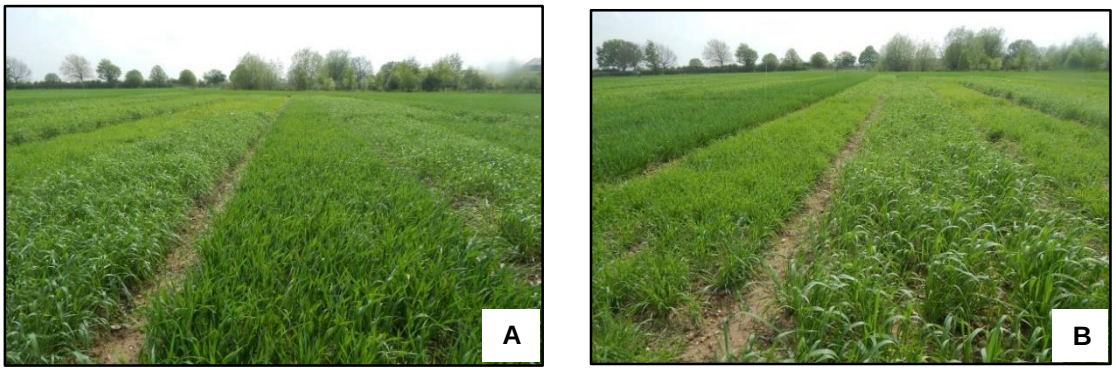


Figure 2.2 GS 31 (A) bread wheat cv. Xi 19 and (B) emmer 2 in April 2014

2.3.6.3 Pre-harvest sampling

The pre-harvest sampling was done at maturity when all plants were fully senesced (Figure 2.3). Quadrat samples were taken from non-lodged areas of the plot for lodging-prone genotypes in all experiments. The quadrat (as in 2.3.6.2) sample was carefully placed in pre-labelled paper sacks in the field to avoid grain losses during transport. Ears of all uprooted plants were put inside the bag. In the laboratory, all roots were separated and discarded. Then, fresh weight of the quadrat sample was recorded before being divided into two subsamples (as in 2.3.6.2). SS_1 was used to calculate biomass production while SS_2 was partitioned into ears, leaves and stem plus sheath and oven dried to obtain dry weight (as in 2.3.6.2). The dried ears were hand threshed carefully and separated grains from the chaff. The grains were re-dried to achieve dry weight of the grain sample. The difference between dry weight of the ears and grains was taken as chaff dry weight. Harvest index (HI) was calculated based on the result of pre-harvest sample by using the Equation 2.1.

$$\mathrm{HI} = \frac{\text{Grain yield (kg ha}^{-1})}{\text{Total above ground biomass (kg ha}^{-1})}$$ **Equation 2. 1**



Figure 2.3 Matured plants of (A) spelt cv. SB (B) emmer 1 in August 2013

2.3.7 Chlorophyll content

2.3.7.1 Chlorophyll concentration index

A Soil Plant Analysis Development meter (SPAD-502, Minolta, Osaka, Japan) was used to measure chlorophyll concentration index of the green leaves avoiding leaf mid rib due to thickness and paleness which could affect readings. The fully developed, upper most leaf was used to take SPAD measurements and average across the leaf was recorded. Ten leaves were used to measure SPAD per plot and the average value was used as a mean value per genotype. After anthesis, SPAD values were taken from flag leaf until maturity. The principle of the SPAD meter is transmittance based on 650 nm (green) and 940 nm (infrared) wavelengths (Minolta, 1989).

2.3.7.2 Chlorophyll extraction

The fully developed flag leaf was used to take samples for chlorophyll extraction and at the same time the SPAD value was recorded. The leaf samples were taken using cork borer, 1.4 cm in diameter. Collected samples were temporarily stored in liquid N before being stored in -80^{o}C until extraction. Two leaf discs were ground using mortar and pestle with 50 ml of 80% acetone. The ground leaf sample was centrifuged for 5 minutes at 3000 rpm in order to remove debris. A Spectrophotometer (Cary 50) was

used to analyse chlorophyll content of the sample. Chlorophyll (a + b) is calculated as in Porra *et al*., (1989) in 80% acetone; (µg/ml) (Equation 2.2).

$$\text{Chl a} = 12.25 \text{ x } (A663.6) - 2.55 \text{ x } (A646.6)$$

$$\text{Chl a} = 20.31 \text{ x } (A646.6) - 4.91 \text{ x } (A663.6)$$

$$\text{Chl a + b} = 17.768 \text{ x } (A646.6) + 7.34 \text{ x } (A663.6) \qquad \textbf{Equation 2. 2}$$

2.3.8 NDVI measurement

The normalised difference vegetation index (NDVI) was measured in the field experiment in 2013/2014 using GreenSeeker Handheld Crop Sensor (Model HCS-100) (Figure 2.4). It was continued from the beginning of the stem elongation until flag leaf senescence at weekly intervals. Average NDVI along the sub-plot was taken at above the canopy. The principle of the NDVI is based on spectral reflectance of red and near-infrared wavelength as shown in Equation 2.3.



Figure 2.4 GreenSeeker Handheld Crop Sensor (Model HCS-100)

$$\text{NDVI} = (\text{NIR} - \text{VIS}) / (\text{NIR} + \text{VIS}) \qquad \textbf{Equation 2. 3}$$

Where, VIS and NIR stand for the spectral reflectance measurements acquired in visible (red) and near-infrared regions, respectively.

2.3.9 Flag leaf characteristics

2.3.9.1 Length and width

Ten fully developed flag leaves at GS61 (GS59 in 2011/2012 field experiment) were taken, randomly, from the main shoot. The leaf length was measured from the base of the leaf to the tip of the leaf. Leaf width was taken by averaging three measurements along the length of the leaf at 30%, 50% and 70% of the length from the base.

2.3.9.2 Flag leaf greenness

Flag leaf greenness was recorded starting from anthesis. Two approaches were used to assess canopy senescence by evaluating flag leaf; visual scoring and SPAD values. A scale ranging from 10 (fully green) to 0 (fully senesced) was used to estimate declining of greenness based on the whole plot (Figure 2.5). Also, SPAD values were recorded on 10 flag leaves, which were clean and disease free. This was continued at 4 day intervals until flag leaf fully senesced.



Figure 2.5 Different stages of flag leaf greenness (10 = fully green) to (0 = fully senesced) (Modified image based on Gaju *et al,* 2011)

Visual score of flag leaf greenness was plotted against thermal time (base temperature at anthesis 0^{o}C) using a four parameter logistic model (Equation 2.4). GenStat 15th edition were used to estimate the four parameters.

$$Y = A + \frac{C}{1 + e^{-B(t-M)}}$$ **Equation 2. 4**

Where, *Y* is greenness score, *A* is lower asymptote in the unit of Y axis, *C* is the difference between upper and lower asymptote in the unit of *Y* axis, *B* is doubled relative senescence rate at the time *M*, *M* is the time when the absolute senescence rate is at maximum and *t*; accumulated thermal time after anthesis in degree days (^{o}Cd) (Karadavut *et al*., 2008).

Green area duration was estimated by calculating the trapezoidal area between assessment dates (area under the curve).

2.3.9.3 Specific leaf area (SLA)

Ten fully developed flag leaves were taken from the main shoot at GS61, except in 2011/2012 field experiment, when taken at GS59. Green area of ten leaves was measured using leaf area meter and dry weight was recorded after oven drying them at 80^{o}C for two days. SLA was calculated using Equation 2.5.

$$\text{SLA } (\text{cm}^{-2}\ \text{g}^{-1}) = \frac{\text{Leaf area } (\text{cm}^2)}{\text{leaf dry weight } (\text{g})}$$ **Equation 2. 5**

2.3.10 Plant height

Plant height was measured at maturity in the laboratory. Ten plants were randomly selected from pre-harvest sample to measure plant height. Main shoots were measured from the base to the tip of the spike, excluding awns.

2.3.11 Lodging assessment

Lodging was observed in the field just after anthesis in all field experiments. Lodging was defined as the leaning of plants from the vertical position (between 45^{o} to 90^{o}). The percentage of the plant in the plot that was lodged was recorded just after anthesis and two times afterwards. Both stem lodging and root lodging were observed in the

field depending on the genotypes (Figure 2.6A and B), but the total of both types of lodging was recorded. However, lodged areas of the plot were not used for quadrat sampling, measurements of SPAD, visual senescence score and pre-harvest sampling in all years.



Figure 2.6 (A) Stem lodging of emmer and (B) root lodging of einkorn in July 2012 during grain filling

2.3.12 Disease assessment

During the latter part of the growing season in 2011/2012 field experiment, fusarium head blight (*Fusarium* spp.) and powdery mildew (*Blumeria graminis* f.sp. *tritici*) were observed in the field and therefore, disease severity was assessed (Figure 2.7A, B and C). Ten ears were selected randomly and observed carefully for brown lesions

spread on the ear. When brown lesions were found all over the ear the disease severity was 100%.



Figure 2.7 Symptoms of fusarium head blight on (A) einkorn 1 (B) emmer 2 and (C) bread wheat cv. Xi 19

2.3.13 Threshability

Threshability is defined as a proportion of grain in the combine sample on weight basis. Twenty five grams of combine sample was used to calculate the threshability of the genotype. Both threshed and un-threshed grains were separated manually and counted carefully. Threshability was calculated using Equation 2.6.

$$\text{Threshability (\%)} = \frac{\text{Grain weight (g)}}{\text{combined sample (g)}} \text{ x } 100 \qquad \textbf{Equation 2. 6}$$

2.3.14 Combine yield and harvest index

Combine grain yield was measured in all three field experiments. Only the second half of the plot was combined in the 2011/2012 field experiment while all the sub-plots were combined in 2012/2013 and 2013/2014 field experiments. Empty area of the plot due to collection of quadrat samples during growth season accounted for 14% and 3% of the total plot area in 2013 and 2014 experiments, respectively. Therefore, combine yield was adjusted based on the area of the quadrat. Length and width of the plot except

tramline were measured just before harvesting. Moisture content of the grain and grain yield were automatically recorded in the combine harvester. Combine yield was corrected using threshability % of the ancient wheat genotypes. Combine grain yield was expressed as tonnes per hectare (t ha^{-1} at 100% DM).

2.3.15 Plant N content

Plant materials were ground to achieve a particle size of < 200 µm. Then, 45 to 50 mg of samples were weighed and encapsulated in tin capsules. The encapsulated samples were then analysed for N% according to the Dumas method using a Fisons NA-2000 elemental analyser (Fisons, Ipswich, UK) calibrated against Methyl-N standard (N content = 9.28%). N content in straw, grain and chaff was analysed at maturity and presented as a percentage. Plant N uptake, nitrogen use efficiency (NUE), nitrogen uptake efficiency (NUpE), nitrogen utilisation efficiency (NUtE) and nitrogen harvest index (NHI) were calculated using Equations 2.7 to 2.11. N in roots was not considered when calculating total plant N uptake.

Crop N_{off} in February (Feb), parallel to soil mineral N (SMN) analysis, was carried out only in 2011/2012 field experiment. Plant growth and development in 2012/2013 and 2013/2014 experiments were very poor in Feb, therefore N_{off} was not calculated. However in the 2011/2012 experiment, NUpE at maturity was calculated using two methods varying the way calculate N availability for the crop. SMN in Feb and fertliser N supply were used as components of N supply in method 1, while crop N_{off} and SMN in Feb together with fertiliser N supply in method 2. SMN has been estimated as available N up to 90 cm depth of the soil. N released from organic matter during the growth season is not considered when calculating N_{ava} in the field experiments. For glasshouse experiments, only one source of N was fertiliser N.

$$\text{N uptake (g N)} = \text{Plant DW (g) x N content of the plant (\%)} \qquad \textbf{Equation 2.7}$$

$$\text{NUE} = \frac{\text{Grain yield (kg ha}^{-1})}{N_{ava}\ (\text{kg N ha}^{-1})} \qquad \textbf{Equation 2.8}$$

Method 1

$$\text{NUpE} = \frac{N_{off}\ (\text{kg N ha}^{-1})}{N_{ava}1\ (\text{kg N ha}^{-1})} \qquad \textbf{Equation 2. 9a}$$

N_{ava} = SMN in Feb + fertiliser N

Method 2

$$\text{NUpE} = \frac{N_{off}\ (\text{kg N ha}^{-1})}{N_{ava}\ 2(\text{kg N ha}^{-1})} \qquad \textbf{Equation 2. 9b}$$

N_{ava} = SMN in Feb + crop N_{off} in Feb + fertiliser N

$$\text{NUtE} = \frac{\text{Grain yield (kg ha}^{-1})}{N_{off}\ (\text{kg N ha}^{-1})} \qquad \textbf{Equation 2. 10}$$

$$\text{NHI} = \frac{\text{Grain N (kg N ha}^{-1})}{N_{off}(\text{kg N ha}^{-1})} \qquad \textbf{Equation 2. 11}$$

2.3.16 Apparent fertiliser N recovery rate

The proportion of the applied fertiliser N which is recovered by the crop at harvest in comparison with the N zero is defined as apparent fertiliser N recovery rate (AFNR) (Bloom *et al*., 1988). AFNR was calculated only in second field experiment where one treatment was 0 kg N ha^{-1} of applied N fertiliser. The only source of N for those plots was from soil mineral N. Fertiliser N recovery rate was calculated using Equation 2.12.

$$\text{AFNR (\%)} = \frac{(\text{AG N}_{\text{fr}} - \text{AG N}_0)}{\text{N}_{\text{fr}}} \quad \text{x } 100 \qquad \textbf{Equation 2. 12}$$

Where AG N_{fr}; total plant N_{off} at fertiliser N applied plots (eg: 200 kg N ha^{-1}), AG N_0; total plant N_{off} at zero N fertiliser applied plot, N_{fr}; amount of N fertiliser applied (eg: 200 kg N ha^{-1}).

2.4 Controlled environment experiments

2.4.1 Pot experiment

2.4.1.1 Experimental design and layout

The experiment was arranged according to a split-plot design with N levels as the main factor and genotypes as the sub-plot factor. Ten genotypes were used in this experiment (section 2.2). All treatment combinations were replicated three times (Appendix 4). Two N levels were used equivalent to 50 and 200 kg N ha^{-1} calculated based on pot surface area. The experiment was conducted from February to August 2013 under glasshouse conditions.

2.4.1.2 Materials and growth conditions

Seeds were sown in modular trays, one per hole, filled with sand compost medium (Levington F2 +S) on 27 February 2013. Trays were kept at 6^{o}C in a growth room for 8 weeks to fulfil the vernalisation requirement. Eight weeks after germination, selected healthy plants were transplanted into 2 litre black plastic pots, one per pot, filled with low N peat compost (P 125 g m^{-3} and K 300 g m^{-3}). Altogether, 240 pots with 10 different genotypes, hence 24 plants per genotype, were kept in a glasshouse under natural daylight and maximum temperature of 30^{o}C. Temperature of the glasshouse was adjusted by opening roof vents regularly. Twenty pots were allocated for 1 m^2 of the floor. All plants received 0.73 g N $plant^{-1}$ (equivalent to 50 kg N ha^{-1}) as liquid form of ammonium nitrate (NH_4NO_3) two days after transplanting while in addition to that plants treated equivalent to 200 kg N ha^{-1} received 1.1 g N $plant^{-1}$ each at stem elongation and flag leaf emergence. N content in low N peat compost did not consider when calculate N availability for plant. Leachate of the pot did not analyse for NO_3^- or other elements.

2.4.1.3 Sample collection

Destructive sampling was done at GS31, GS39, GS61 and at maturity. Samples were collected when five out of ten genotypes reached a particular growth stage. SPAD values were taken from the upper most leaf at GS31 and GS39 but flag leaf at GS61, before plants removed from the pot. Three plants per genotype (each from a block) for

high N (HN) and low N (LN) were taken. Plants were partitioned into ears, leaves and stems based on their growth stage. Green area of the leaves, flag leaf, ear and stem with sheath were measured. Number of tillers and plant height were recorded at maturity. Also, grains were separated from the ears by hand threshing them carefully. All samples were oven dried at 80ºC for two days to establish dry weight. Figure 2.8 shows the plants at (A) GS31 (B) GS39 and (C) GS61 in the glasshouse.

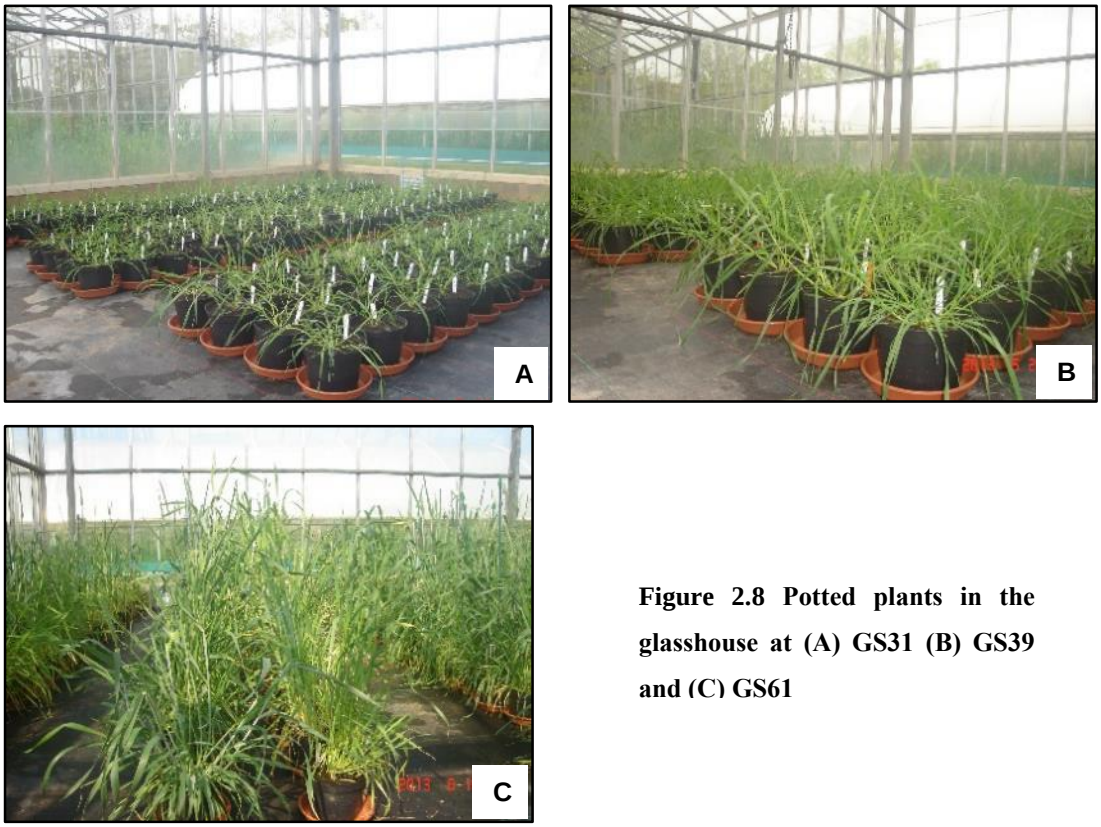


Figure 2.8 Potted plants in the glasshouse at (A) GS31 (B) GS39 and (C) GS61

2.4.1.4 N analysis

N was analysed at maturity for straw, grain and chaff using the Dumas method. NUpE, NUtE, NUE and NHI were calculated as in the section of 2.3.15.

2.4.2 Root experiments

2.4.2.1 2D root phenotyping experiment – I- Ancient wheat

2.4.2.1.1 Experimental design and layout

The experiment was set up as a completely randomised design with 27 replicates (Appendix 5). Ten genotypes were used in the experiment (section 2.2). Uniform seeds

were obtained using a set of sieves. The seed size of two bread wheat cvs, three spelt cvs, two emmer genotypes and einkorn 2 were 2.8-3.35 mm while einkorn 1 and einkorn 3 were 2-2.8 mm in diameter. The experiment was conducted in March 2013.

2.4.2.1.2 Materials and growth conditions

There were three tanks available for the experiment with nine plastic containers in each tank (Figure 2.9A). Ninety plastic holders were placed in each tank and each holder was uniquely numbered. Seeds were surface sterilised by dipping them in 70% (v/v) ethanol for 60 seconds followed by submerging in 5% (v/v) sodium hypochlorite solution for 10 minutes. Seeds were then washed thoroughly with sterilised deionised water and placed in the plastic tray covered by wet blotting paper (Figure 2.9B). They were kept in a dark room at 4^{o}C for two days to synchronise germination. Based on the length of radicle (> 5 mm), selected germinated seeds were placed, one seed per pouch, on to the growth pouches (Figure 2.9C). Each growth pouch consisted of strong black polythene sheet (240 mm x 280 mm), blue germination paper (240 mm x 300 mm), plastic rod and two 19 mm foldback clips (Figure 2.9D). The black polythene sheet and germination paper were attached to the rod with two foldback clips. Growth pouches, with seeds placed with the embryo facing the bottom of the paper, hung into plastic containers with lower 2 cm dipped in nutrient solution (Figure 2.9E) (1/4 strength of Hoagland's No. 2, Basal Salt Mixture). Each container was filled with 3 l of nutrient solution at the beginning of the experiment. The composition of the basal salt mixture was KNO_3 (606.6 mg l^{-1}), Ca $(NO_3)_2$ (656.4 mg l^{-1}), $MgSO_4$ (240.76 mg l^{-1}), $NH_4H_2PO_4$ (115.03 mg l^{-1}), $MnCl_2.4H_2O$ (1.81mg l^{-1}), H_3BO_3 (2.86 mg l^{-1}), MoO_3 (0.016 mg l^{-1}), $ZnSO_4.7H_2O$ (0.22 mg l^{-1}), $CuSO_4.5H_2O$ (0.08 mg l^{-1}) and $Fe_2(C4H4O_6)_3$ (5 mg l^{-1}). The water level of the plastic containers was topped up by using deionised water at two days intervals.

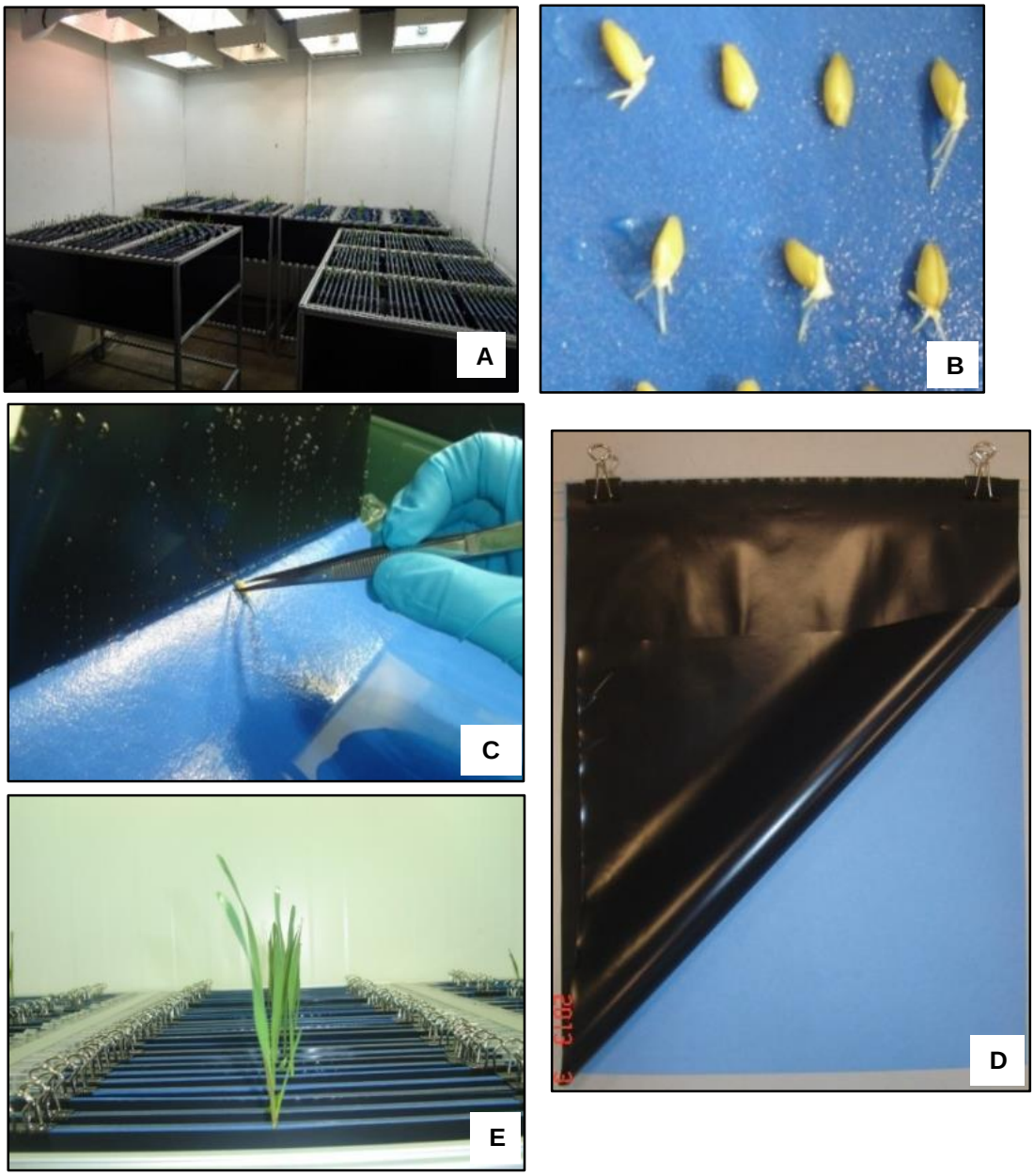


Figure 2.9 (A) Growth room with specially designed tanks (B) two days old germinated seeds (C) growth pouch consisted of blue germination paper, black polythene sheet, plastic rod and fold back clips (D) germinated seeds placed on the growth pouch and (E) seedlings at 14 days after transfer to the germination paper

The PAR of the growth room was 400 µmol m^{-2} s^{-1}. The photoperiod of the growth room was 12/12 and 18ºC/13ºC day and night temperature. Contaminated seeds within the growth pouches were discarded immediately after being identified.

2.4.2.1.3 Image analysis

Two weeks after transferral to the germination paper, vertically grown root systems were imaged using a Nikon D5100 DSLR camera and modified copy stand. Contaminated seedlings were discarded without taking images. The images were analysed using root phenotyping software *RootNav* (Pound *et al*., 2013). The *RootNav* software was chosen due to its quality and quantity of obtainable measurements. The software produced readings of number of seminal and lateral roots, average length seminal and lateral roots, total root length, tip angles and emergence angles of seminal root, maximum width, depth, width to depth ratio and convex hull (Figure 2.10). Data were stored in a database until analysis. True images (Figure 2.11A, B), probability images (Figure 2.11 C, D) and thumbnail images (Figure 2.11 E, F) of bread wheat cv. Xi 19 and emmer 2 produced by *RootNav* software at 14 day after transfer (DAT) to the germination paper are shown in Figure 2.11.

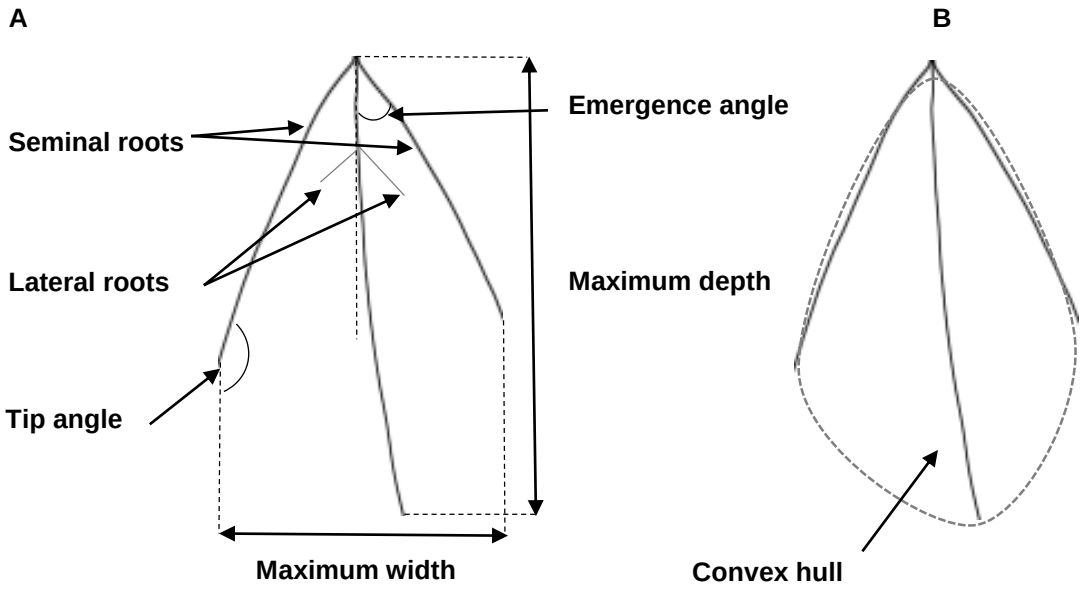


Figure 2.10 Schematic diagrams of seedling root traits measured at 14 day after transfer (DAT) to the germination paper (A) number of seminal roots and lateral roots, maximum width, maximum depth, tip angle and emergence angle of seminal root (B) convex hull (the area covered by all root materials)

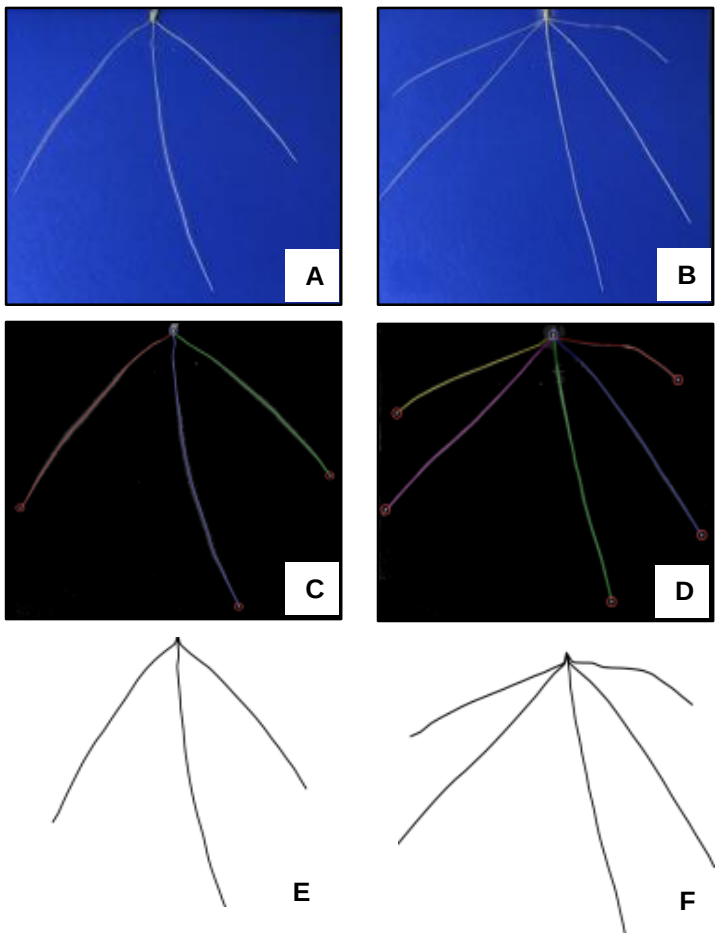


Figure 2.11 (A) and (B); true images of bread wheat cv. Xi 19 and emmer 2, (C) and (D); root system extraction and quantification by *RootNav* software of bread wheat cv. Xi 19 and emmer 2, (E) and (F); reconstruction of root image of bread wheat cv. Xi 19 and emmer 2 produced by *RootNav* at 14 DAT

2.4.2.2 Modular tray experiment

2.4.2.2.1 Experimental design and layout

Fifteen genotypes, including ten genotypes used in previous experiments, and five bread wheat cvs. (Hobbit, Glasgow, Gallant, Forno, and Beaver) were used in this experiment. The experiment was set up according to a completely randomised design with 20 replicates and conducted under glasshouse conditions (Appendix 6) under natural day light.The experiment was conducted in June 2014.

2.4.2.2.2 Materials and growth conditions

The seed size of seven bread wheat cvs, three spelt cvs, two emmer cvs and einkorn 2 were 2.8-3.35 mm while einkorn 1 and einkorn 3 were 2-2.8 mm. The seeds were

soaked in deionised water for 24 hours and kept at 13ºC under dark conditions to synchronise germination. Germinated seeds were placed in to modular trays filled with sharp washed sand and compost (John Inns No 2) using a pair of forceps. The volume of a single hole in the tray was 15 cm^3. Seedlings were watered every other day and no artificial fertiliser was applied throughout the experiment. The experiment was conducted under natural day light and maximum temperature of 30ºC. Temperature of the glasshouse was maintained by opening vents automatically during the day. The plants were kept in the glasshouse under 16 /8 hour light/dark cycle.

2.4.2.2.3 Sample collection and root screening

Two week old seedlings were carefully removed from the tray and washed thoroughly with tap water. Number of seminal roots was counted manually before the root system was scanned. Root samples were spread in an acrylic box (A4 size) (Figure 2.12A) with tap water to minimize the overlaps. Root samples were digitised at 400 dpi resolution and 256 grey contrasts (Tiff format) with a scanner (Figure 2.12B) with a transparency adapter (*WinRHIZO* STD 1600+, Regent Instruments Inc., Quebec; Canada). The scanned images (Figure 2.12C) of the root systems were analysed using *WinRHIZO* regular V.2002c software (*WinRHIZO* STD 1600 +, Regent Instruments Inc., Quebec; Canada). Following scanning, roots together with shoots were oven-dried at 80ºC for 48 hours before determining their dry weight.

Total root length, root volume, average root diameter and root length in different diameter classes (less than 0. 5 m, 0.5 to 1 mm, 1 to 1.5 mm, 1.5 to 2 mm, 2 to 2.5 mm, 2.5 to 3mm, 3 to 3.5 mm, 3.5 to 4 mm, 4 to 4.5 mm and more than 4.5 mm) were recorded. However, due to complexity, only root length at less than 0.5 mm diameter (very fine roots), 0.5 to 1 mm (fine roots) and sum of the values of all other classes as more than 1 mm (thick roots) were considered. Specific root length (SRL) was calculated based on original data of the root scanning results (Equation 2.13).

$$\text{Specific root length (SRL; cm g}^{-1}) = \frac{\text{Total root length (cm)}}{\text{Root biomass (g)}} \qquad \textbf{Equation 2.13}$$

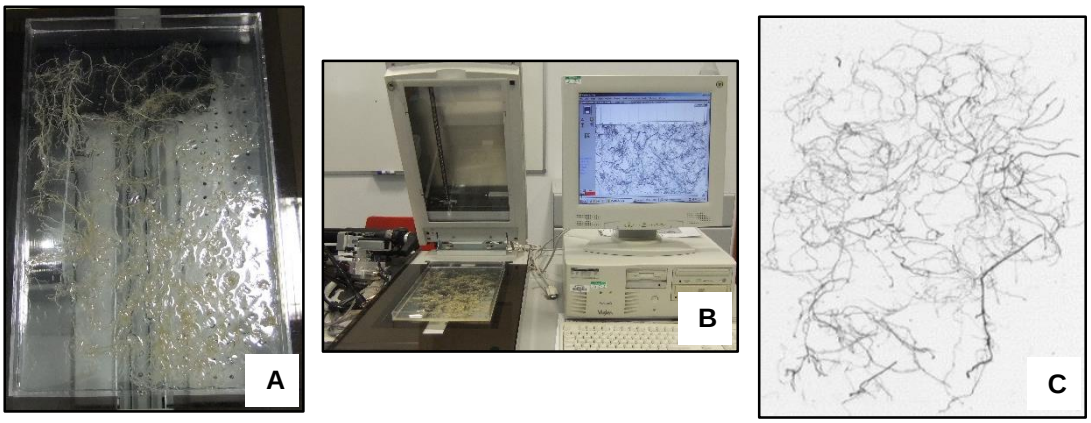


Figure 2.12 (A) Submerged root sample in acrylic box and (B) *WinRHIZO* root scanner (C) scanned image

2.4.2.3 Root phenotyping pot experiment

Ten genotypes were used in this experiment (section 2.2). A ceptometer was employed to determine photosynthetic photon flux density (PPFD) in the glasshouse to quantify photosynthetically active radiation (PAR; µmol m^{-2} s^{-1}) in different sections of the glasshouse (Table 2.2). Clear differences were observed in the various sections at morning, noon and evening. The experiment was set up according to a randomised complete block design with three replicates, with the blocks oriented to account for the gradient in PAR (Appendix 7). The experiment was conducted from May to July 2013.

Table 2.2 PAR values depicting light gradient in the glasshouse

Time	Section 1 (µmol m^{-2} s^{-1})	Section 2 (µmol m^{-2} s^{-1})	Section 3 (µmol m^{-2} s^{-1})
9:00am	936	864	635
1:00pm	530	616	557
5:00pm	149	200	186

2.4.2.3.1 Materials and growth conditions

After being kept with some moisture in petri dishes for two days to enhance synchronization of germination, the seeds were sown, a single seed per cell, in sand compost medium (Levington F2+S) having N 150 g m^{-3}, P 200 g m^{-3}, K 350 g m^{-3} and pH 5.3-5.7. Seed trays were kept in a glasshouse at 20^{o}C and watered every day (Figure 2.13A). Ten day old healthy seedlings were transplanted into 3 litre pots with 4

drainage holes, filled with hydroleca clay pellets (Figure 2.13B, C and D). Hydroleca clay pellets are produced by expanding clay granules at over 1200^{o}C. All plants were treated with a complete nutrient solution (Standard HortiMix 1.0 g l^{-1}) twice daily for the first two weeks and once daily for the remaining period of the experiment. The composition of the HortiMix was NO_3^- (850 mg l^{-1}), NH_4^- (140 mg l^{-1}), $(NH_2)_2CO$ (510 mg l^{-1}), P_2O_5 (700 mg l^{-1}), K_2O (300 mg l^{-1}), MgO(160 mg l^{-1}), Fe EDTA (15.3 mg l^{-1}), Mn (10 mg l^{-1}), B (3 mg l^{-1}), Zn (2.7 mg l^{-1}), Cu (2 mg l^{-1}) and Mo (1.2 mg l^{-1}). Adequate water was supplied throughout the experiment. The experiment was conducted under natural sunlight (16/8 hour light/dark cycle) and maximum temperature of the glasshouse was 30^{o}C.

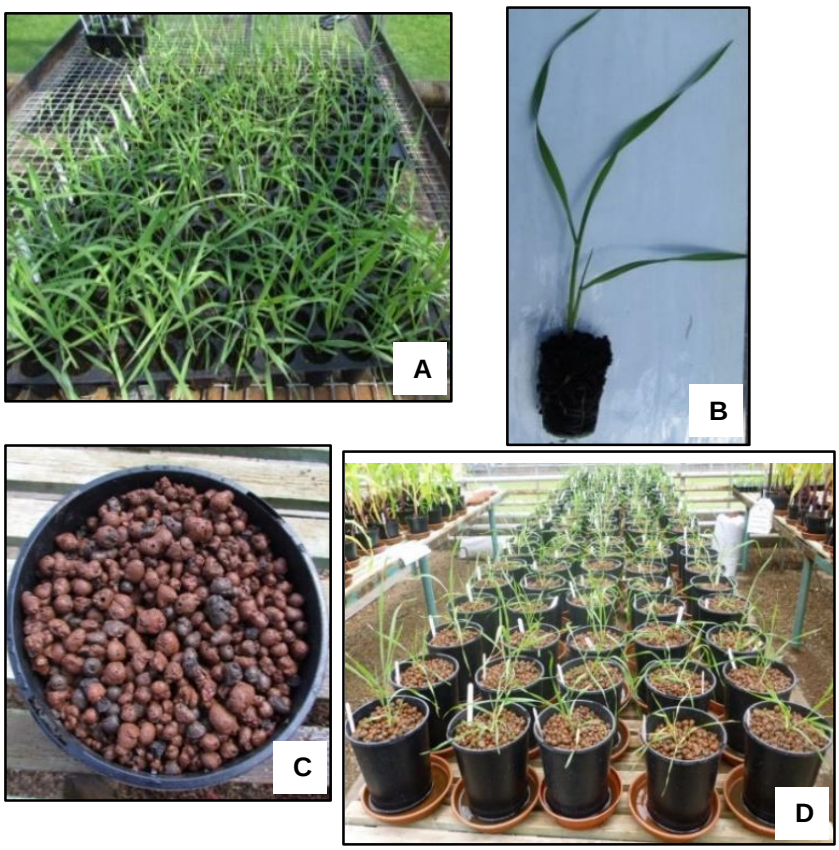


Figure 2.13 (A) 10 day old seedlings in the germination tray filled with sand compost medium (B) single seedling at transplanting (C) Hydroleca clay pellets filled in 3 litre plot (D) transplanted seedlings

2.4.2.3.2 Sample collection

Four destructive samples, a single plant per genotype, were taken at nine day intervals to screen root systems and above-ground biomass together with green area production at 14, 23, 32 and 41 days after transplanting (DAT). SPAD values of the genotypes were recorded before plants were removed from the pot (section 2.3.7.1). Above-ground plant materials were oven dried at 80^{o}C for 48 hours.

2.4.2.3.3 Root screening

At each sampling, the roots were gently extracted from the pots and the hydroleca removed carefully (Figure 2.14A). Roots were extracted by washing them thoroughly with tap water to remove soil particles and debris of the medium (Figure 2.14B). Roots were kept in water until scanning to avoid drying the root systems which would alter the root diameter. Cleaned root systems were divided into 2-3 segments depending on their size, before being scanned to ensure all roots were captured (section 2.4.2.2.3).

Scanned images were analysed to obtain root parameters (section 2.4.2.2.3). In addition, root elongation rate (RER) and root N uptake efficiency ($NUpE_R$) were calculated (Equation 2.14 and 2.15).

$$\text{RER (cm day}^{-1}) = \frac{\text{TRL at 41 DAT} - \text{TRL at 14 DAT}}{(41 - 14)\ \text{days}} \qquad \textbf{Equation 2.14}$$

$$\text{NU}_\text{P}\text{E}_\text{R} = \frac{N_{off}\ (\text{g N plant}^{-1})}{\text{Root biomass (g plant}^{-1})} \qquad \textbf{Equation 2.15}$$

Where; TRL; total root length, DAT; days after transplanting, N_{off}; total plant N uptake

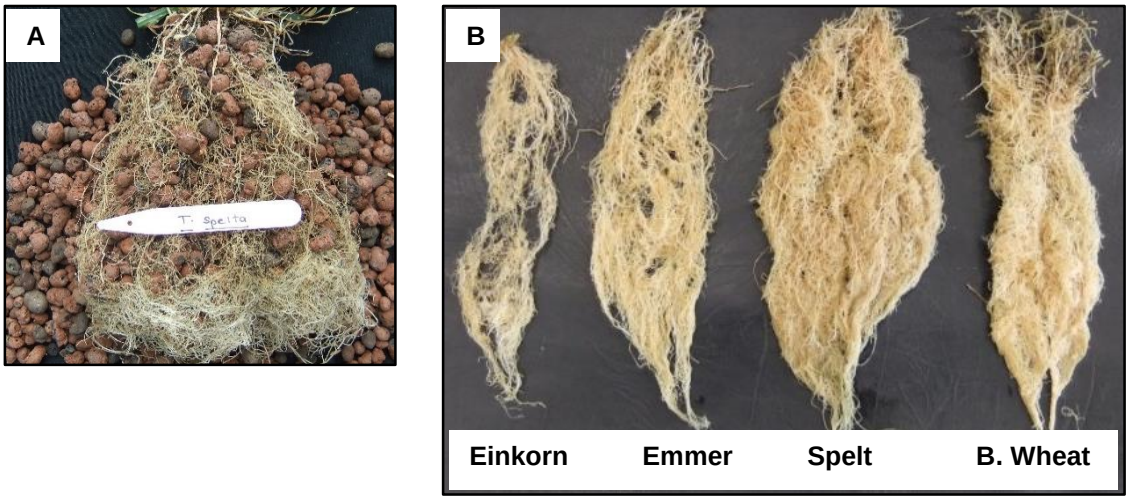


Figure 2.14 (A) Uprooted spelt plant at 41 DAT (B) cleaned entire root systems of einkorn, emmer, spelt and bread wheat at 41 DAT

2.4.2.3.4 N uptake

Above-ground shoots were analysed for N% (see section 2.3.15). Based on N% of the plant materials, N_{off} of the plant was calculated using Equation 2.8.

The specific absorption rate of N ($S_{AB}R_N$) was calculated based on the relationship described by Hackett *et al*. (1969) and Liao *et al*. (2004) for the period between 1st sampling at 14 DAT and last sampling at 41 DAT (Equation 2.16).

$$S_{AB}R_N = \frac{PN_2 - PN_1}{T_2 - T_1} \text{ x } \frac{\ln R_2 - \ln R_1}{R_2 - R_1} \qquad \textbf{Equation 2.16}$$

Where, PN; total plant N (mg), (T_2-T_1); time between 1st and last sampling, R; root biomass (mg) or total root length (m).

2.4.2.4 Column experiment

2.4.2.4.1 Experimental design and layout

Seven genotypes, except einkorn genotypes and JB Diego from previous experiments while including Forno; a bread wheat cultivar, were used in this experiment. A split plot design was used in the experiment where main plot factor was N levels and sub-plot factor was genotypes. Three levels of N equivalent to 50, 100 and 200 kg N ha^{-1}

were applied. N availability for plants at maturity was calculated as a sum of N supplied manually through NH_4NO_3 plus N supplied through compound fertiliser by fertigation system (Table 2.3). All plants received 0.05 g N plant^{-1} equivalent to 50 kg N ha^{-1} two days after transplanting. In addition to that, plants treated equivalent to 100 and 200 kg N ha^{-1} received 0.05 and 0.16 g N plant^{-1}. Fertiliser were applied at stem elongation and flag leaf emergence. Three blocks were arranged to accommodate for variation in PAR along the glasshouse. The experiment was conducted under natural day light from January 2014 to August 2014. All plants were labelled by giving them a unique number (Appendix 8).

Table 2.3 N availability for the plants in 2014 glasshouse column experiment. Fertiliser N was applied manually as NH_4NO_3 for N50, N100 and N200 treatments at 0.054, 0.11 and 0.22 g plant^{-1} for all genotypes while additional N was supplied through fertigation system which varied depending on anthesis date of the genotype

Genotype	Fertigation system			Total N_{ava} per plant		
	No of days	Solution (mL)	Only N (g)	N50	N100	N200
Emmer 2 Xi 19	34	3120	0.37	0.43	0.49	0.60
Emmer 1 Forno	40	3960	0.48	0.53	0.59	0.70
Spelt Tauro	41	4080	0.49	0.54	0.60	0.71
Spelt Oberkulmer	48	4920	0.59	0.64	0.70	0.81
Spelt SB	53	5520	0.66	0.72	0.77	0.89

2.4.2.4.2 Materials and growth conditions

One seed, per hole was sown in modular trays on 28 January 2014 filled with sand compost medium (Levington F2 + S). Seedlings were subjected to a vernalisation period of 10 weeks in glasshouse at 4^{o}C and under natural daylight. After vernalisation (31 March), the seedlings were transplanted into the columns, one plant per column, 10 cm in diameter and 100 cm in height. All columns were split into two halves longitudinally (Figure 2.15A), which were then taped together with brown parcel tape (Figure 2.15B) and the bottom was closed by using piece of black shading net gaged 2 (20 cm x 20 cm) to allow drainage (Figure 2.15C). All the columns were filled with

hydroleca and washed thoroughly to remove soil and broken parts as much as possible. Plants were provided with plant supports from GS 39 to avoid lodging. The experiment was conducted under natural daylight with maximum day and night temperatures of 18 and 13ºC for first two weeks and then increased up to 25 to 18ºC. Maximum temperature of the glasshouse was regulated by opening roof vents automatically.

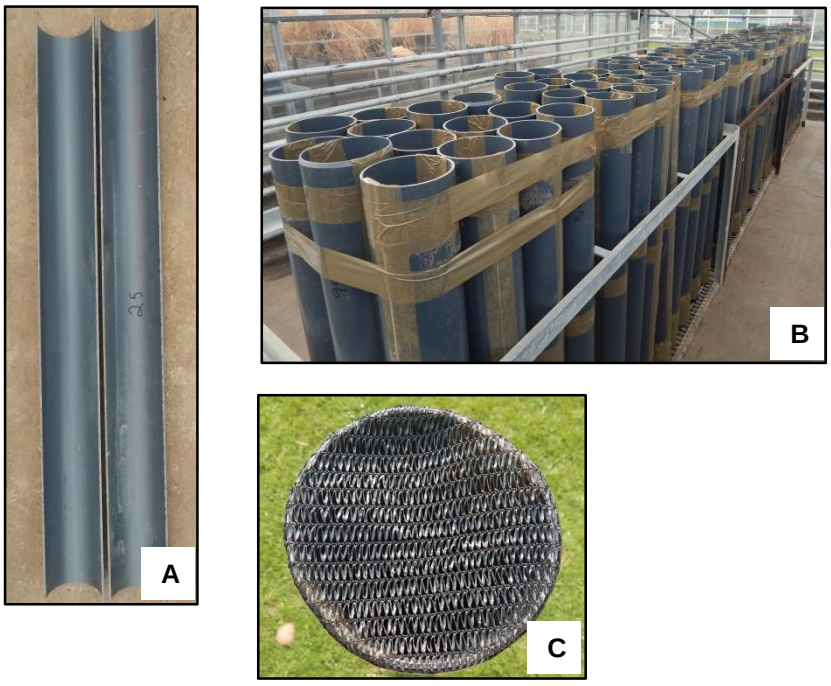


Figure 2.15 (A) Split column of 1m (B) two halves of the column tapped together and (C) bottom of the column covered by a piece of shading net

Drip irrigation was installed two weeks after transplanting with the flow rate of 120 ml day^{-1} of standard compound fertiliser mixture, Kristalon Red (12:12:36 + micro; at 1 mg l^{-1}). The fertiliser mixture consisted of 12% of N, 12% of P_2O_5, 36% K_2O, 2.5% of water soluble sulphur trioxide, 1 % of water soluble sulphur, water soluble B (0.025%), Cu (0.01%), Fe (0.07%), Mn (0.04%), Zn (0.025%) and Mo (0.004%) and all micro nutrients were chelated by EDTA, except Mo. Calcium (Ca) is not in this mixture. Fertigation system had been shut down for 10 days to reduce N sypply and was replaced with water. Leachate of the column did not analyse for NO_3^- or other elements. Figure 2.16A and B show plants at anthesis having drip irrigation system and plant supports.

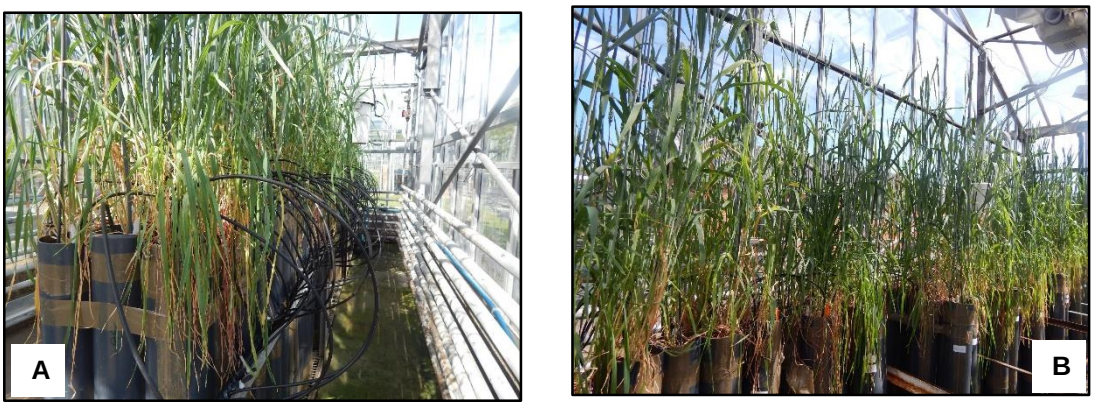


Figure 2.16 (A) Drip irrigation (B) plants at anthesis

2.4.2.4.3 Data collection

Number of tillers, number of leaves per main shoot and SPAD values were recorded (section 2.3.7.1) at two weeks intervals from two weeks after transplanting until flag leaf senescence. Plant height was measured at GS31, GS39, at anthesis and at maturity.

2.4.2.4.4 Sample collection

Two destructive samplings were done at anthesis and at maturity. At anthesis, green area and biomass production were calculated based on individual plants. Green area of the flag leaf, 2nd leaf, 3rd leaf and remaining leaves together with ear, stem and sheath were recorded. Above-ground biomass was calculated based on the dry weight of different parts of the plant. At maturity, ear, leaf and stem dry weight was established by oven drying them at 80^{o}C for 48 hours. Ears were hand threshed and separated carefully from the chaff. Weight of re-dried grain was subtracted from ear weight to obtain chaff weight of the particular genotype.

2.4.2.4.5 Root screening

Columns were split into two halves (Figure 2.17) using a sharp blade along the length through taped area to collect root samples. The root system was divided into five segments from 0-100 in 20 cm parts such as 0- 20 cm, 20- 40 cm, 40- 60 cm, 60- 80 cm and 80- 100 cm and separated from hydroleca and washed using tap water. Root samples were then brought to the laboratory in water to avoid dehydration. For statistical analysis, a scoring number was given for each layer from score 1 (reaching

20 cm depth) until score 5 (reaching 100 cm depth) thus deeper root was identified by higher score.

The procedure used in section 2.4.2.2.3 was applied to scan root samples and to calculate root parameters. Root elongation rate, root N uptake efficiency and specific absorption rate of N were calculated. In addition, RLD was calculated for different root depths at anthesis and at maturity (Equation 2.17).

$$\text{RLD (cm cm}^{-3}) = \frac{\text{Total root length (cm)}}{\text{Root volume (cm}^3)} \qquad \textbf{Equation 2. 17}$$

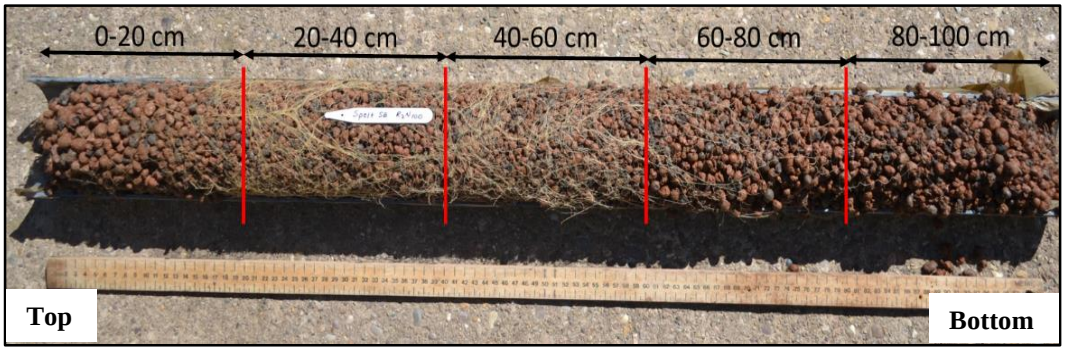


Figure 2.17 Root distribution of 1m long column split into 2 halves and divided further to five sections

2.4.2.4.6 N analysis

N was analysed at maturity for straw, grains and chaff using the Dumas method. NUpE, NUtE, NUE and NHI were calculated as in the section of 2.3.15.

2.4.2.5 2D root phenotyping experiment- II- wheat x spelt population

2.4.2.5.1 Genetic materials and source

A total of 226 F_5 recombinant inbred lines (RILs) were produced from the cross between Swiss winter wheat variety Forno (*T. aestivum*) and Swiss winter spelt variety Oberkulmer (*T. spelta*) (Messmer *et al*., 1999). Forno is a modern bread wheat variety released in 1986 and Oberkulmer is an old Swiss land variety which was released in 1948. Wheat and spelt are both hexaploid species with three genomes (AABBDD) and seven homologous chromosome groups. Spelt is commonly called hexaploid hulled wheat. When compared to bread wheat, spelt is a taller plant having long lax ears,

brittle rachis and tight glumes. Also, thousand grain weight and above-ground biomass of spelt is higher than bread wheat (Winzeler *et al*., 1994).

2.4.2.5.2 Experimental design and layout

Seeds were obtained by hand threshing ears collected from field experiment conducted in 2013 at Sutton Bonington Campus. The RILs (202 lines) of a wheat x spelt population, together with parents, were arranged in a completely randomised design with 10 replicates. However, due to complexity of the experiment, it was conducted as four runs accommodating three replicates in each line on run one, two and three, and one in run four (204 x 10 = 2040). Four tanks with 360 place holders were available for the experiment. In total there were 720 test positions available for a single run due to modified version of growth pouch (section 2.4.2.1.2). It contained two black polythene sheets, two blue germination papers, a plastic rod and two 19 mm foldblack clips. This allowed 45 unused test positions per run which were used to retest any seed which failed to germinate or were contaminated by fungi/ bacteria. Runs were considered as blocks when analysing phenotypic data. This experiment was repeated while maintaining same growth room conditions and number of replicates.

2.4.2.5.3 Materials and growth room conditions

Seeds were not sterilised due to a large number of lines used in the experiment. Seeds were placed on both sides of the growth pouch, having saturated germination papers, hung into plastic containers filled with 3 l of deionised water. Water level of the containers was topped up at two days intervals to ensure germination paper remained saturated throughout the experiment. The PAR of the growth room was 240 µmol m^{-2} s^{-1}. The photoperiod of the growth room was 12/12 and 17ºC/13ºC day and night temperature.

2.4.2.5.4 Image analysis

Images were taken 13 days after seeding using Nikon D500 with copy stand. Images were analysed using *RootNav* V1.7.5 software. Root traits measured in the experiment were similar to section 2.4.2.1.3.

2.4.2.5.5 QTL analysis

The genetic map of Forno x Oberkulmer was based on 182 RFLP (restricted fragment length polymorphism and simple sequence repeats), and 230 segregating loci (Messmer *et al*., 1999). Due to the high degree of heterozygous bands, 24 RILs were excluded from the population. Hence, JoinMap v4 (Van Ooijen, 2006) was used to carry out linkage map analysis with 202 RILs and 23 linkage groups were obtained, covering 2469 cM with an average marker density of 13.6 cM. General interval mapping was performed by using MapQTL 6.0 software (Van Ooijen, 2009). Based on that, the locations of the significant QTLs, LOD (logarithm of odds) score, additive effects and percentage of phenotypic variance explained by individual QTLs (R^2) was identified. The charts of linkage groups and locations of significant QTLs were drawn using MapChart software (Voorrips, 2002).

The threshold for significant QTL detection was determined with 1000 permutations at $\alpha = 0.05$ (Churchil and Doerge, 1994). The significant QTLs were declared when the LOD score was at or above the significant threshold value for a given root trait. Also, QTLs with LOD value less than the threshold but higher than or equal 2, was identified as indicative QTLs. QTL symbol was designed according to the standard nomenclature of the catalogue of gene symbols of wheat, proposed by McIntosh *et al*. (2008) (http://wheat.pw.usda.gov/GG2/Triticum/wgc/2008/).

Phenotypic data of traits for quantitative trait loci (QTL) analysis were the pooled mean values of two experiments hence total number of replicates were twenty.

2.5 Meteorological data/microclimatic data

Data on maximum and minimum air temperature, rainfall and solar radiation were downloaded from the meteorological station data base from October 2011 to August 2014. Long term mean (LTM) was calculated based on the data from 1959 to 2011. Mean air temperature, monthly average rainfall and daily solar radiation are presented with field experiment results.

Microclimatic conditions such as maximum and minimum temperature and relative humidity were recorded in the glasshouse using TinyTag data logger (Tinytag Ultra 2 TGU4500, Meaco, UK) throughout the experiment.

2.6 Statistical analysis

Data was analysed using GenStat statistical package 15th edition. Analysis of variance (ANOVA) appropriate to the design of each experiment was performed. Data were tested for nomal distribution before apply parametric statistic analysis. Mean differences were tested using LSD and Duncan's multiple range test 5% probability level depending on the nature of the experiment. Based on the results of ANOVA, effect of genotype, N level, GT x N and error on total sums of squares was calculated for NUE and its components. Pearson correlation analysis, simple linear regression analysis and comparisons of regression were used to examine the relationships between continuous variables. Comparison of regressions/ parallel regression analysis (linear regression with groups) was used to compare the difference between treatments in parameters estimated by linear regression. The most parsimonious line fit for each regression, either; (i) a common line, (ii) separate lines for each group using a common intercept, or (iii) separate lines allowing both slope coefficients and intercepts were estimated.

Chapter 3

General plant growth, development and morphology

Chapter 3

3.1 Introduction

This chapter describes above-ground biomass production, green area index, flag leaf properties, stay-green traits, and grain yield of three ancient hulled wheat species of einkorn (*Triticum monococcum* L., a diploid with AA genome), cultivated emmer (*T. dicoccum*, a tetraploid with AABB genome) and spelt (*T. spelta* L., a hexaploid with AABBDD genome), together with modern bread wheat (*T. aestivum*, a hexaploid with AABBDD genome) under field conditions. Meteorological data for the three seasons are presented for the most important variables affecting crop growth such as air temperature, rainfall and solar radiation. A field experiment in 2011/12 compared ancient wheat species with modern bread wheat in terms of crop growth, grain yield and nitrogen use efficiency (NUE). Two experiments in 2012/13 and 2013/14 compared plant growth, yield and NUE under three different N regimes. Results are presented and discussed, where possible, side by side in order to compare seasons, allowing some indication as to whether the characteristics are consistent across growing seasons.

Objective

The aim of the current chapter is to identify and quantify physiological traits of ancient wheat species with special reference to plant development, growth, structure, stay-green properties and grain yield under different N levels when compared to modern bread wheat.

Specific hypotheses

1. Plant growth and development of ancient wheat species are significantly different from modern bread wheat.
2. Above-ground biomass production is higher in spelt but grain yield is higher in bread wheat due to improved harvest index.
3. Stay-green properties can be identified among ancient wheat species.
4. Flag leaf properties are significantly different between genotypes used in the study.

3.2 Results

The results of the field experiments carried out during the years 2011/12, 2012/13 and 2013/14 are presented in this chapter. All experiments were conducted at University of Nottingham Farm, Sutton Bonington, UK (52^{o} 50' N, 1^{o} 15' W). The experimental years are referred to hereafter by their harvest year (eg: 2011/12 is referred as 2012).

3.2.1 Meteorological data of the experimental site

The meteorological data for Sutton Bonington, UK are presented as temperature, rainfall and solar radiation for 3 seasons, together with the long-term means (LTM).

3.2.1.1 Temperature

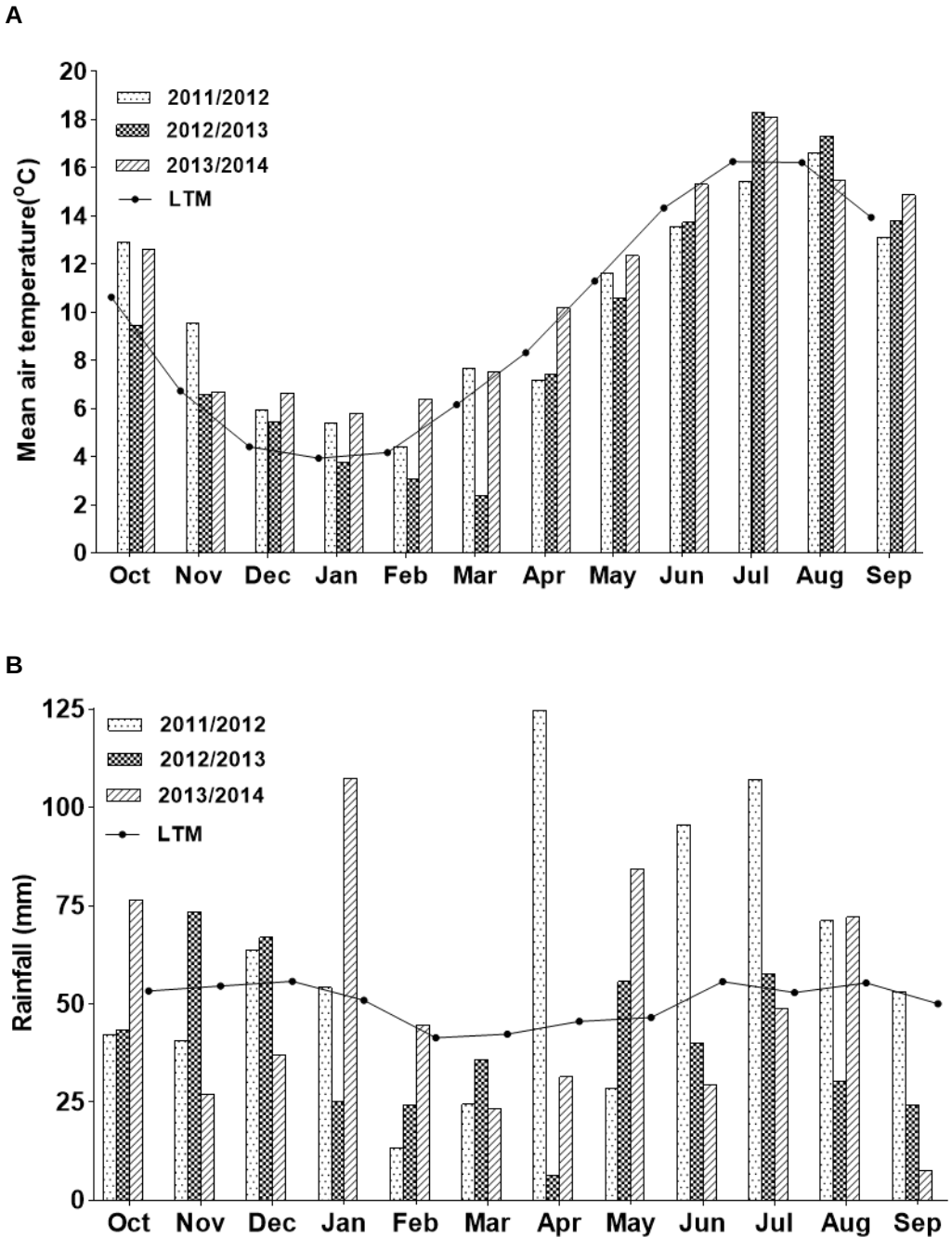
In 2012, the average annual temperature of the site from sowing (October 2011) to harvest (August 2012) was 10.3^{o}C which was 5.6% higher than the LTM for annual temperature of 9.7^{o}C. The warmer than average annual temperature was associated with high temperatures from sowing to stem elongation in March. However, in 2013, annual average temperature was 0.38^{o}C less than the LTM. In 2014, monthly temperature from sowing (November 2013) to harvesting (August 2014) was higher than LTM leading to an annual temperature 11.65% higher than the LTM (Figure 3.1A).

3.2.1.2 Rainfall

LTM for annual rainfall in Sutton Bonington is 604 mm. In 2012, annual rainfall at the experimental site was 718 mm, which was 114 mm more than the LTM. This wet weather was due to the rainfall between stem elongation (April 2012) to harvest (August 2012) which was 59% of the annual rainfall. Annual rainfall in 2013 growth season was 17% less than the LTM. Therefore, overall, this season was dry for rain-fed crops. In 2014, January rainfall was more than double the LTM while in May it was 81% greater than the LTM. However, total seasonal rainfall of 2014 was less than the long term total (Figure 3.1B).

3.2.1.3 Solar radiation

The total incident solar radiation from sowing to harvest for 2012, 2013 and 2014 was 2863, 3050 and 2836 MJ m^{-2}, respectively. However, solar radiation from anthesis to the end of grain filling (June to August) was higher in 2014 followed by 2013 and 2012. It was 10% less than the LTM in 2012 while 9 and 14% higher than the LTM in 2013 and 2014, respectively (Figure 3.1C). When compared to the 2012 season 2013 and 2014 seasons had more solar radiation and brighter conditions from anthesis to the end of grain filling.



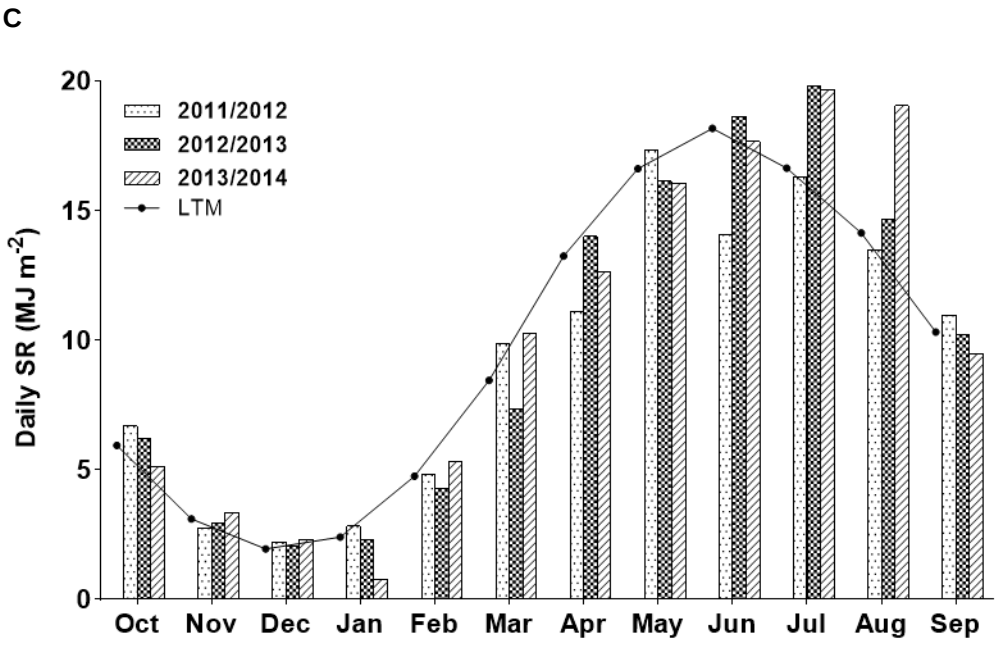


Figure 3.1 (A) Monthly air temperature (ºC) (B) monthly rainfall (mm) (C) daily solar radiation (MJ m^{-2}) in 2011/ 2012, 2012/2013 and 2013/2014 growth seasons at Sutton Bonington. LTM of mean temperature (1959-2011), rainfall (1961-2011) and Daily SR (2000-2011) are shown as a line graph (Source: www.metoffice.gov.uk)

3.2.2 Field establishment

Plant establishment of the genotypes varied significantly across growing seasons ($P < 0.001$). Establishment of bread wheat cv. JB Diego and cv. Xi 19 was significantly higher than all other genotypes for all seasons. In 2012, emmer 2 showed poorer plant establishment than others. Plant establishment in the 2013 experiment was significantly lower than 2012 and 2014. This may be due to the quality of the seed which were collected from previous field experiment where fusarium head blight (*Fusarium* spp.) was reported. Emmer 1 and einkorn 3 had very poor plant establishment in 2013. Therefore, these two genotypes were excluded from further analysis.

In 2014, all genotypes had high plant establishment with bread wheat again showing better performance than others. However, plant establishment in 2014 was poorer than 2012. Nevertheless, plant establishment of einkorn 3 was 20% less than that of cv. Xi 19 (Table 3.1). There was no effect of N treatment or genotype x N interaction (GT x N) on plant establishment either in the 2013 or 2014 season.

Table 3.1 Plant establishment (%) and plant density (plants m^{-2}) of ten genotypes in 2012, 2013 and 2014 field experiments

GT	2012		2013		2014	
	Plant establishment (%)	Plant density (Plants m^{-2})	Plant establishment (%)	Plant density (Plants m^{-2})	Plant establishment (%)	Plant density (Plants m^{-2})
JB Diego	83.52	208.81	40.47	106.44	59.52	178.56
Xi 19	91.68	229.28	39.33	103.47	62.26	186.78
Spelt Tauro	70.80	177.02	23.49	61.78	55.81	167.44
Spelt SB	79.84	199.64	24.00	63.11	55.44	166.33
Spelt Oberkulmer	77.76	194.42	24.55	64.56	57.33	172.02
Emmer 1	79.60	199.05	11.45	30.11	53.15	159.44
Emmer 2	64.24	160.62	23.07	60.67	53.59	160.78
Einkorn 1	77.84	194.65	21.80	57.33	50.26	150.78
Einkorn 2	81.52	203.88	17.74	46.67	54.30	162.89
Einkorn 3	87.04	217.62	12.93	34.05	49.74	149.22
SED; GT (*df*)	3.524(27)***	8.813(27)***	1.691(54)***	4.448(54)***	1.477(54)***	4.430(54)***

3.2.3 Plant development

The date, when each genotype reached stem elongation (GS31), flag leaf emergence (GS39), ear emergence (GS59) and anthesis (GS61) is shown in Table 3.2 for 2012, 2013 and 2014 field experiments. Bread wheat reached a particular growth stage more rapidly than the ancient wheat species. Einkorn showed slow growth rate throughout the growing season. The gap between bread wheat and einkorn at GS31 was nearly a month in 2012. However, the gap narrowed gradually with plant development.

In 2013, at the beginning, all genotypes spent more time to reach to their key stages when compared to 2012. This may be due to wet weather and prolonged winter in early 2013 hence low temperature. Average temperature from January to April in 2013 was 33% less than in 2012. Therefore, bread wheat took almost 20, 11, 4 and 3 more days to reach to GS31, GS39, GS59 and GS61, respectively, than in 2012.

Similar to previous years, throughout the season bread wheat reached each particular growth stage earlier than all other genotypes in 2014. In general, plant development rate of four species could be ranked as bread wheat > emmer > spelt > einkorn. There was no effect of N and GT x N interaction on the dates of GS31, GS39, GS 59 and GS61 in either experiment. Abnormal growth and development was observed for all einkorn genotypes in 2014. They took much more time to reach their key stages when compared to previous seasons. Plants of einkorn genotypes showed stunted growth with red leaves together with dead tillers. Most of the symptoms are similar to barley yellow dwarf virus infection (BASF, 2004) but this was not confirmed. Due to poor growth performances, three einkorn genotypes were excluded from further analysis.

Matured plants were sampled on 22 August for both 2012 and 2013 experiments and on 11 August in 2014. Therefore, the duration from anthesis to maturity of the genotypes were considerably shorter in 2014 season. On the other hand, sowing date of 2014 experiment was 5 weeks later than 2012 and 2013 experiments. However, above average temperature and below average rainfall in June and July in 2014 may have had a significant influence on early crop maturity.

3.2.4 Plant growth

3.2.4.1 Above-ground biomass production

Year 2012

In 2012, at GS26, the highest above-ground biomass production (AGB) was recorded in emmer 2 (66 g m^{-2}) despite the fact that it recorded the lowest establishment rate. This may be due to less competition among seedlings for available resources. Einkorn 3 had lowest AGB of 26.8 g m^{-2} ($P < 0.001$).

As the season progressed, the highest AGB was recorded in bread wheat cv. JB Diego and the lowest value was recorded in einkorn 3 ($P < 0.001$). All genotypes were sampled when 50% of the genotypes reached GS31. Since all einkorn genotypes and some of the spelt and emmer genotypes had slow growth rate at the beginning of the season, they may not have reached GS31 when sampling was done (Figure 3.2).

At GS39, bread wheat cv. JB Diego had the highest AGB of 1152 g m^{-2} while einkorn 1 had 285 g m^{-2} ($P < 0.001$). AGB of spelt and emmer was between bread wheat and einkorn (Figure 3.2). Bread wheat accumulated the greatest amount of AGB (1492 g m^{-2}) compared to all other species at GS59 where emmer, spelt and einkorn species accumulated an average of 1267 g m^{-2}, 1251 g m^{-2} and 577 g m^{-2}, respectively ($P < 0.001$) (Figure 3.2).

Averaged across the species, AGB was highest in spelt followed by emmer, bread wheat and einkorn at maturity ($P < 0.001$) (Figure 3.2). It is noteworthy that spelt and emmer had increased AGB accumulation late in the growing season when compared to the other two species. Figure 3.3 shows AGB accumulation of ten genotypes throughout growth season in 2011/2012 against thermal time.

Table 3.2 The date genotype reached stem elongation (GS31), flag leaf emergence (GS39), ear emergence (GS59) and anthesis (GS61) in 2012, 2013 and 2014. (Note: The date which genotype reached GS59 was not recorded in 2014)

GT	GS31			GS39			GS59			GS61		
	2012	2013	2014	2012	2013	2014	2012	2013	2014	2012	2013	2014
JB Diego	19-Apr-12	08-May-13	24-Apr-14	20-May-12	31-May-13	24-May-14	10-Jun-12	14-Jun-13	-	15-Jun-12	18-Jun-13	15-Jun-14
Xi 19	17-Apr-12	07-May-13	26-Apr-14	24-May-12	30-May-13	26-May-14	09-Jun-12	12-Jun-13	-	14-Jun-12	17-Jun-13	14-Jun-14
Spelt Tauro	22-Apr-12	14-May-13	29-Apr-14	20-May-12	30-May-13	26-May-14	11-Jun-12	16-Jun-13	-	15-Jun-12	18-Jun-13	17-Jun-14
Spelt SB	22-Apr-12	14-May-13	30-Apr-14	28-May-12	29-May-13	28-May-14	14-Jun-12	16-Jun-13	-	17-Jun-12	20-Jun-13	17-Jun-14
Spelt Ober	20-Apr-12	12-May-13	28-Apr-14	30-May-12	01-Jun-13	26-May-14	10-Jun-12	17-Jun-13	-	14-Jun-12	18-Jun-13	17-Jun-14
Emmer 1	27-Apr-12	10-May-13	29-Apr-14	30-May-12	07-Jun-13	30-May-14	16-Jun-12	20-Jun-13	-	27-Jun-12	20-Jun-13	20-Jun-14
Emmer 2	30-Apr-12	12-May-13	26-Apr-14	28-May-12	06-Jun-13	29-May-14	18-Jun-12	18-Jun-13	-	24-Jun-12	21-Jun-13	21-Jun-14
Einkorn 1	19-May-12	31-May-13	03-Jun-14	18-Jun-12	17-Jun-13	30-Jun-14	01-Jul-12	30-Jun-13	-	07-Jul-12	03-Jul-13	29-Jul-14
Einkorn 2	15-May-12	28-May-13	07-Jun-14	18-Jun-12	14-Jun-13	27-Jun-14	01-Jul-12	04-Jul-13	-	07-Jul-12	08-Jul-13	21-Jul-14
Einkorn 3	17-May-12	28-May-13	08-Jun-14	07-Jun-12	14-Jun-13	25-Jun-14	18-Jun-12	21-Jun-13	-	25-Jun-12	25-Jun-13	27-Jul-14

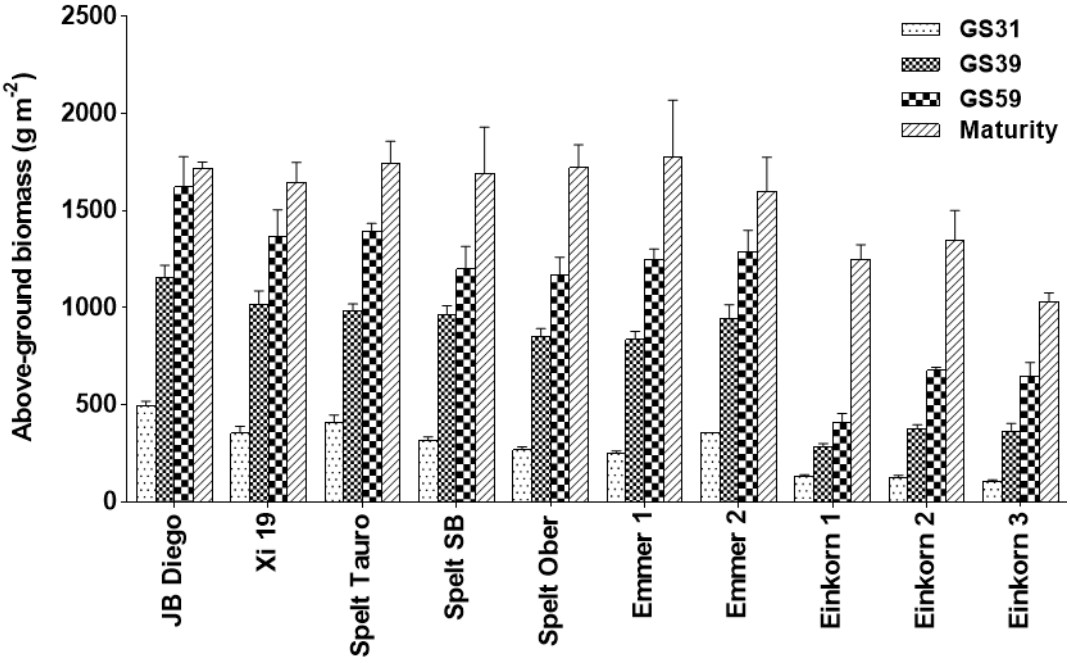


Figure 3.2 Above-ground biomass production at GS31, GS39, GS59 and at maturity in 2012. Error bars represent SE of the genotype (*df* = 27)

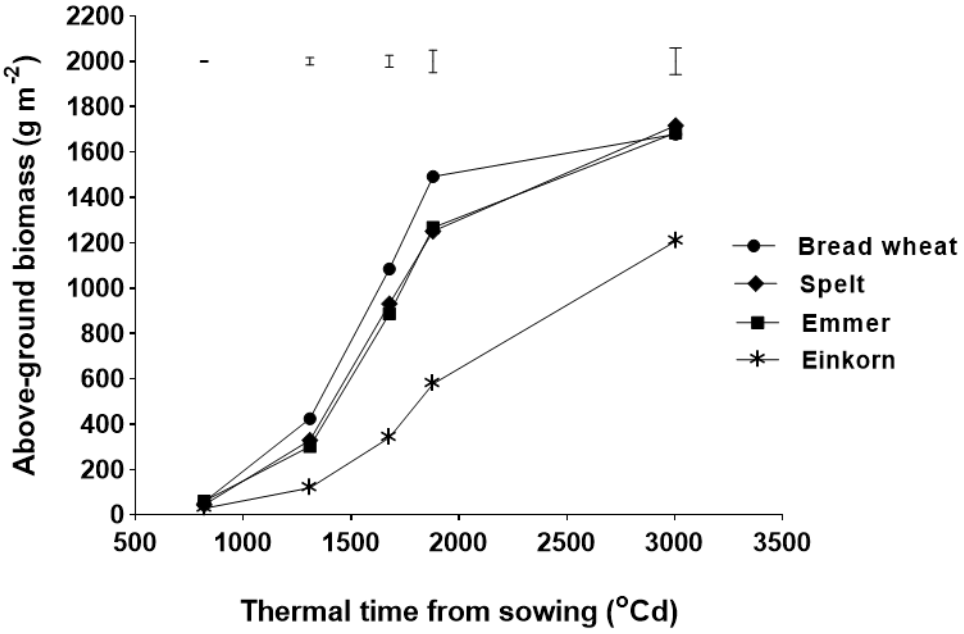


Figure 3.3 Above-ground biomass accumulation of bread wheat, spelt, emmer and einkorn species against thermal time from sowing at GS31, GS39, GS59 and pre-harvest in 2012. Error bars represent SED of the genotype (*df* = 27)

Year 2013 and 2014

Three N regimes equal to zero N (no fertiliser N applied; NN), 100 kg N ha^{-1} (Low N; LN) and 200 kg N ha^{-1} (High N; HN) were used in 2013 while 100 kg N ha^{-1} and 150 kg N ha^{-1} was applied to LN and HN plots, respectively in 2014. These rates were calculated based on the results of soil mineral N analysis in February of the respective year. Genotypes were sampled at GS39, GS61 and at maturity for 2013 and only GS61 and at maturity for 2014 for AGB calculation.

In 2013, at GS39, the highest AGB was recorded in bread wheat cv. JB Diego for HN treated plants but spelt Tauro had highest AGB under NN condition. Spelt SB had highest AGB production under LN than NN and HN (Figure 3.4). At GS61, wheat cv. Xi 19 and emmer 2 had the highest AGB in 2013 and 2014, respectively (Table 3.3).

At maturity in 2013, effect of genotype and N level was significant on AGB ($P < 0.001$, $P < 0.01$, respectively). When averaged across the species, bread wheat had the highest AGB followed by emmer, spelt and einkorn in NN while spelt had more AGB than emmer and einkorn in LN and HN. In 2014, genotypic effect on AGB was not significant while N level was significant ($P < 0.001$) (Table 3.4).

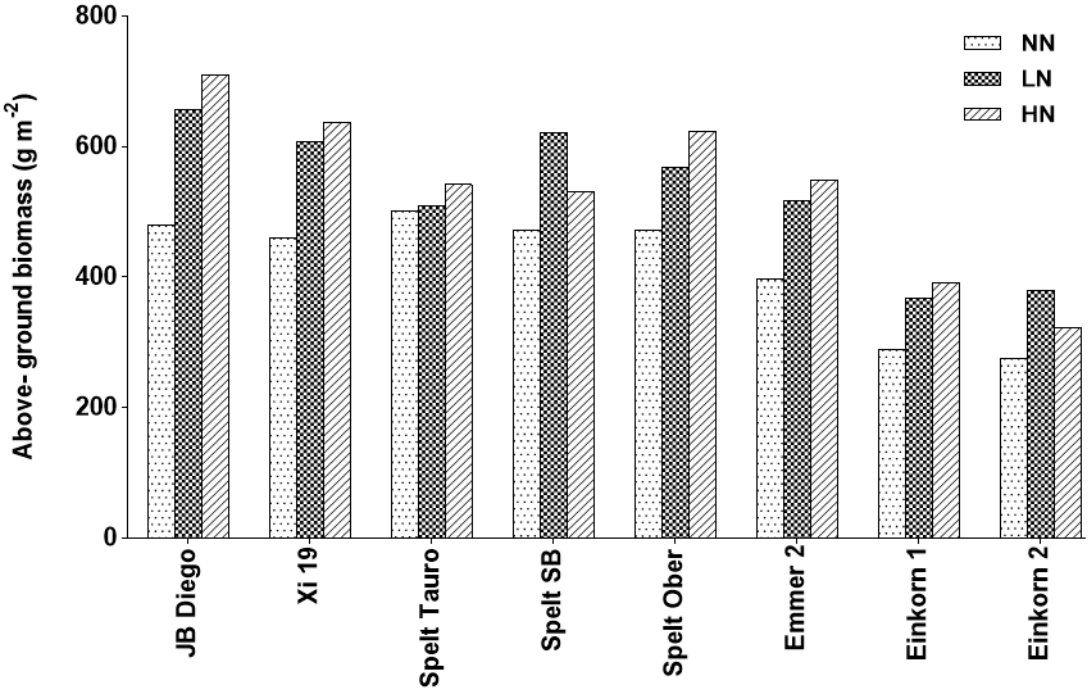


Figure 3.4 Above- ground biomass production at GS39 under NN, LN and HN in 2013. SED for GT x N was 59.84 (*df* = 42)

Table 3.3 Above-ground biomass production (g m^{-2}) in 2013 and 2014 field experiments under zero N (NN), low N (LN) and HN (high N) at GS61

GT	GS61 (AGB g m^{-2})					
	2013			2014		
	NN	LN	HN	NN	LN	HN
JB Diego	715.59	1016.17	1105.66	1115.12	1294.05	1491.76
Xi 19	758.57	1080.38	1127.06	861.43	1176.17	1202.43
Spelt Tauro	776.15	886.72	962.07	1084.54	1243.11	1513.51
Spelt SB	673.77	956.91	1078.01	1246.32	1302.17	1405.87
Spelt Oberkulmer	667.51	948.34	916.09	856.05	1245.93	1045.44
Emmer 1	-	-	-	1069.31	1277.04	1124.51
Emmer 2	744.68	943.09	894.05	1136.43	1359.88	1540.45
Einkorn 1	361.04	517.67	474.75	-	-	-
Einkorn 2	309.51	373.22	376.70	-	-	-
SED; GT (*df*)	53.1 (42)***			28.4 (36)***		
N (*df*)	47.2 (4)*			63.2 (4)**		
GT x N (*df*)	98.1 (36.45)^{NS}			105.3 (32.39)^{NS}		

Table 3.4 Above- ground biomass production (g m^{-2}) in 2013 and 2014 field experiments under zero N (NN), low N (LN) and HN (high N) at maturity

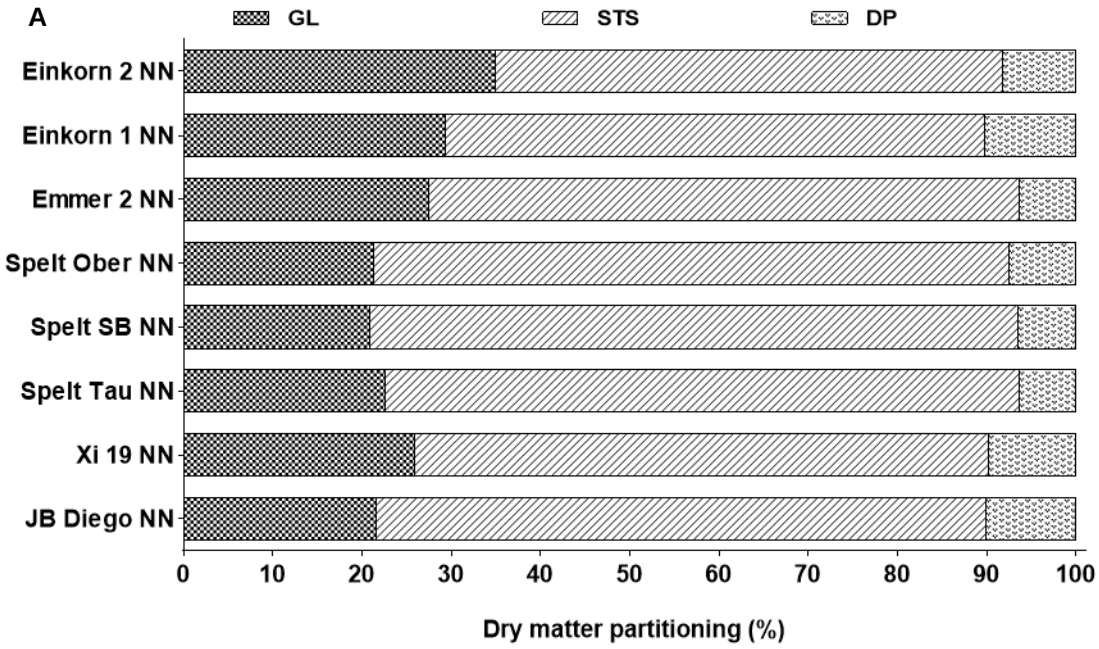
GT	At maturity (AGB g m^{-2})					
	2013			2014		
	NN	LN	HN	NN	LN	HN
JB Diego	1127.34	1610.54	1651.55	1623.32	2335.44	2477.85
Xi 19	1139.65	1569.32	1599.04	1552.17	1933.05	1872.54
Spelt Tauro	980.21	1384.87	1487.43	1669.04	1851.53	2302.22
Spelt SB	988.59	1453.58	1461.59	1866.33	2073.56	2058.32
Spelt Oberkulmer	1013.14	1479.03	1557.32	1616.21	1904.72	2103.67
Emmer 1	-	-	-	1667.05	2056.66	1783.87
Emmer 2	1126.88	1386.32	1500.05	1880.54	1951.09	1979.92
Einkorn 1	764.12	901.33	949.91	-	-	-
Einkorn 2	817.17	1072.71	1228.00	-	-	-
SED; GT (*df*)	80.254 (42)***			127.644(36)^{NS}		
N (*df*)	77.114 (4)*			50.211 (4)**		
GT x N (*df*)	151.142 (33.39)^{NS}			210.835 (32.54)^{NS}		

3.2.4.2 Above-ground biomass partitioning

AGB was partitioned into three crop components; (i) green lamina, (ii) true stem plus leaf sheath and (iii) dead parts at GS31 and GS39. At GS59/GS61 green ears were also separated. At maturity, true stem plus leaf sheath, leaf lamina and ear was considered.

In the 2012 experiment, the genotypic effect of dry matter partitioning into different components at any growth stage was highly significant ($P < 0.001$) (Table 3.5). At GS31, most of the genotypes partitioned more AGB towards green lamina production while at GS39 more towards true stem plus leaf sheath production. At maturity, bread wheat cvs. allocated more AGB to develop ears while ancient tall genotypes (spelt, emmer and einkorn) for true stem plus leaf sheath.

Genotype ($P < 0.001$), N treatment ($P < 0.05$) and GT x N ($P < 0.05$) interaction was significant for crop component partitioning at GS39 in 2013 experiment (Figure 3.5). All species partitioned a high proportion of AGB toward green lamina production at the higher N levels than low N. It was observed that einkorn genotypes allocated more AGB for green lamina production than other genotypes despite the N application. Similarly, spelt genotypes used more AGB for stem plus leaf sheath growth. The dead proportion of AGB was higher under NN for all genotypes. This may be due to high rate of nutrient remobilisation from older leaves to newly developed leaves under N limited condition.



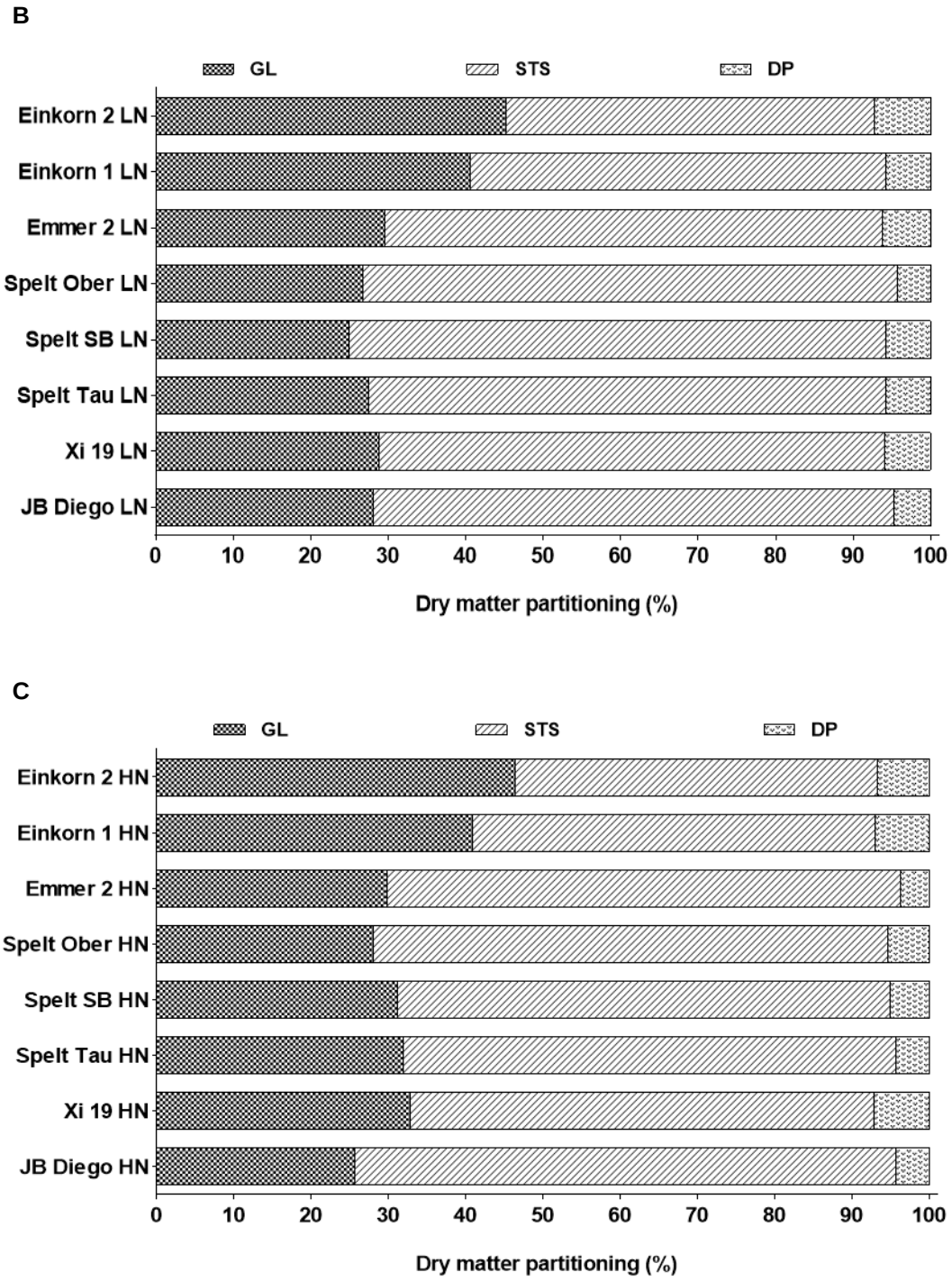


Figure 3.5 Proportion of AGB partitioning into three crop components (GL; green lamina, STS; true stems plus leaf sheath, DP; dead parts) at GS39 under three N treatments (A) NN (B) LN and (C) HN in 2013 field experiment (SED of GT x N for GL, STS and DP were 1.166, 1.319 and 0.682, respectively at *df* = 14)

Table 3.5 Proportion of AGB partitioned into diffrent plant components (Stem plus leaf sheath, green leaf lamina, dead parts and ear) at GS31, GS39, GS59 and pre-harvest in 2012 experiment

GT	AGB partitioning (%)												
	GS31			GS39			GS59				Maturity		
	STS	GL	DP	STS	GL	DP	STS	GL	EAR	DP	STS	LVS	EAR
JB Diego	45.73	41.88	12.38	59.82	28.20	11.98	57.16	19.34	8.87	14.63	40.60	8.53	50.87
Xi 19	37.39	42.37	20.24	60.51	27.41	12.08	54.96	16.88	10.92	17.24	40.00	7.76	52.24
Spelt Tauro	43.67	44.67	11.66	60.61	28.32	11.07	58.36	20.43	7.28	13.93	51.56	11.05	37.38
Spelt SB	38.66	43.14	18.20	58.58	26.15	15.28	57.93	17.59	10.06	14.42	47.54	9.63	42.83
Spelt Oberkulmer	39.40	46.88	13.72	58.09	28.02	13.89	60.44	20.95	8.21	10.40	48.50	9.44	42.06
Emmer 1	37.49	46.28	16.23	56.60	30.51	12.89	58.35	20.73	10.46	10.45	48.96	9.08	41.96
Emmer 2	43.34	38.74	17.91	58.61	27.28	14.11	61.63	18.16	8.38	11.83	57.58	7.61	34.81
Einkorn 1	21.25	53.92	24.84	37.05	40.76	22.19	57.74	33.93	8.33	0.00	56.15	6.92	36.93
Einkorn 2	15.51	53.59	30.90	35.40	41.41	23.19	58.83	30.04	11.13	0.00	62.85	7.40	29.75
Einkorn 3	17.34	39.53	43.12	41.77	36.68	21.55	65.76	22.44	8.51	3.29	67.40	6.04	26.56
SED (*df* = 27)	2.001***	2.864***	3.171***	1.908***	1.912***	2.621***	2.277***	1.975***	2.246^{NS}	1.619***	3.166*	1.178***	3.576*

STS; stem plus sheath, GL; green lamina, DP; dead parts, LVS; dried leaves and EAR; dried ear

***** Significant at *P* < 0.001, **significant at *P* < 0.01, *significant at *P* < 0.05, *NS* - Not significant**

At GS61, AGB partitioned more to true stem plus leaf sheath followed by ear, green lamina and dead parts in both experiments (Table 3.6 and 3.7). Remarkably, it is observed that, plants allocate more assimilates to produce green leaf lamina at HN while true stem plus sheath in NN. Proportion of AGB for dead parts was higher under NN than other N levels for most of the genotypes.

In 2013, at maturity, only genotype ($P < 0.001$) had significant effect on the proportion of AGB for leaf lamina development at maturity and there were no effect of N and/or GT x N. However in 2014, GT x N ($P < 0.01$) was significant. Allocation of assimilates for true stem plus leaf sheath and ear was significantly different between genotypes ($P < 0.001$), N ($P < 0.05$) and GT x N ($P < 0.01$) in 2013 while it was only significant for genotypes ($P < 0.001$) in 2014 (Data not shown). In general, all genotypes allocated more assimilates toward ear followed by true stem plus sheath and then leaf lamina regardless of N level in both years.

Table 3.6 Proportion of AGB partitioned into four crop components (green lamina plus sheath, true stem, ear and dead leaves) at GS61 under three N treatments in 2013 experiment

GT	AGB partitioning (%) – 2013 (GS61)											
	NN	LN	HN	NN	LN	HN	NN	LN	HN	NN	LN	HN
	GL			STS			EAR			DP		
JB Diego	13.03	14.31	15.24	64.06	65.17	63.52	19.29	18.06	19.56	3.62	2.46	1.68
Xi 19	13.18	15.88	17.36	60.03	61.41	59.20	22.01	20.14	20.65	4.79	2.57	2.79
Spelt Tauro	13.16	16.23	17.19	63.53	62.72	61.22	18.77	19.39	19.36	4.55	1.66	2.22
Spelt SB	12.29	14.85	15.85	64.08	63.70	61.74	19.43	18.57	19.17	4.19	2.88	3.24
Spelt Oberkulmer	12.35	14.28	15.73	64.74	63.70	62.18	19.17	18.76	19.30	3.75	3.26	2.79
Emmer 2	15.59	16.60	17.99	68.99	67.05	65.87	12.37	12.08	12.23	3.04	4.27	3.91
Einkorn 1	18.43	20.60	27.43	64.07	65.06	61.90	9.36	10.10	6.58	8.13	4.24	4.10
Einkorn 2	22.98	27.36	28.73	67.56	55.23	66.49	3.05	0.00	0.52	6.41	17.40	4.27
SED; GT (*df*)	0.776 (42)***			0.821 (42)^{NS}			0.941 (42)***			0.993(42)***		
N (*df*)	0.995 (4)*			0.998 (4)***			0.592 (4)^{NS}			0.532 (4)*		
GT x N (*df*)	1.603 (21.67)^{NS}			1.181 (39.18)***			1.634 (44.90)^{NS}			1.694 (45.92)***		

NN; zero N fertiliser, LN; low N fertiliser, HN; high N fertiliser, GL; green lamina, STS; true stem plus sheath, DP; dead parts

***** Significant at *P* < 0.001, **significant at *P* < 0.01, *significant at *P* < 0.05, *NS* - Not significant**

Table 3.7 Proportion of AGB partitioned into four crop components (green lamina plus sheath, true stem, ear and dead leaves) at GS61 under three N treatments in 2014 experiment

GT	AGB partitioning (%) – 2014 (GS61)											
	NN	LN	HN	NN	LN	HN	NN	LN	HN	NN	LN	HN
	GL			STS			EAR			DP		
JB Diego	10.30	14.66	18.89	60.25	60.12	56.93	21.33	19.83	19.42	8.12	5.40	4.77
Xi 19	9.53	15.18	17.27	58.70	56.84	55.22	18.79	18.56	20.74	12.98	9.42	6.77
Spelt Tauro	8.29	14.38	16.79	64.10	61.67	59.60	19.31	19.93	18.25	8.30	4.02	5.37
Spelt SB	7.44	13.74	12.78	65.36	62.34	62.84	17.97	17.72	18.67	9.23	6.20	5.70
Spelt Oberkulmer	10.93	15.96	16.23	66.53	62.03	64.26	17.26	16.80	15.30	5.28	5.21	4.21
Emmer 1	12.05	12.18	14.35	65.10	61.64	60.21	16.80	18.50	17.65	6.05	7.68	7.78
Emmer 2	7.33	10.06	15.13	68.04	66.52	63.90	12.87	15.53	15.68	11.76	7.89	5.28
SED; GT (*df*)	0.819 (36)***			0.768 (36)***			0.754 (36)***			0.621 (36)***		
N (*df*)	1.582 (4)*			0.667 (4)*			0.923 (4)^{NS}			0.645 (4)*		
GT x N (*df*)	2.056 (8.17)*			1.401 (26.99)^{NS}			1.521 (17.09)^{NS}			1.187 (21.89)***		

NN; zero N fertiliser, LN; low N fertiliser, HN; high N fertiliser, GL; green lamina, STS; true stem plus sheath, DP; dead parts

***** Significant at P < 0.001, **significant at P < 0.01, *significant at P < 0.05, *NS* - Not significant**

3.2.4.2.1 Ear partitioning

Ear dry matter allocation into grain and chaff was significantly different between genotypes and species in 2012 ($P < 0.001$) at maturity (Figure 3.6). In 2013 and 2014, genotype ($P < 0.001$), species ($P < 0.001$), GT x N ($P < 0.05$) and species x N ($P < 0.01$) interaction was significant for the proportion of accumulated assimilates in the ear partitioned into grain and chaff (Figure 3.7). In general, bread wheat partitioned more ear dry matter towards grain production than spelt, emmer and einkorn.

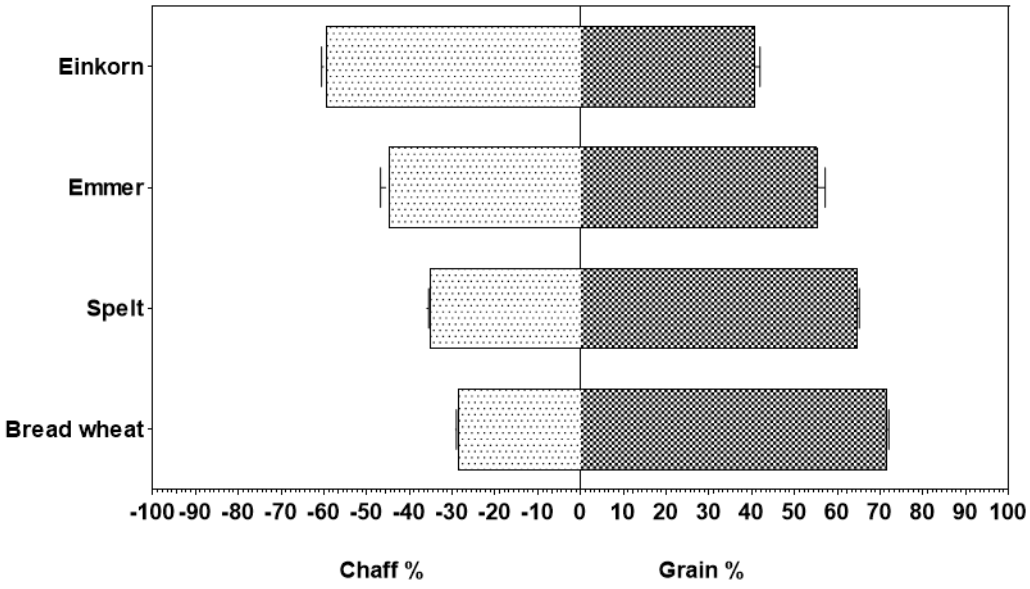


Figure 3.6 Proportion of ear biomass partitioning into grain and chaff for bread wheat, spelt, emmer and einkorn in 2012 experiment. Error bars represent SE (*df* = 33)

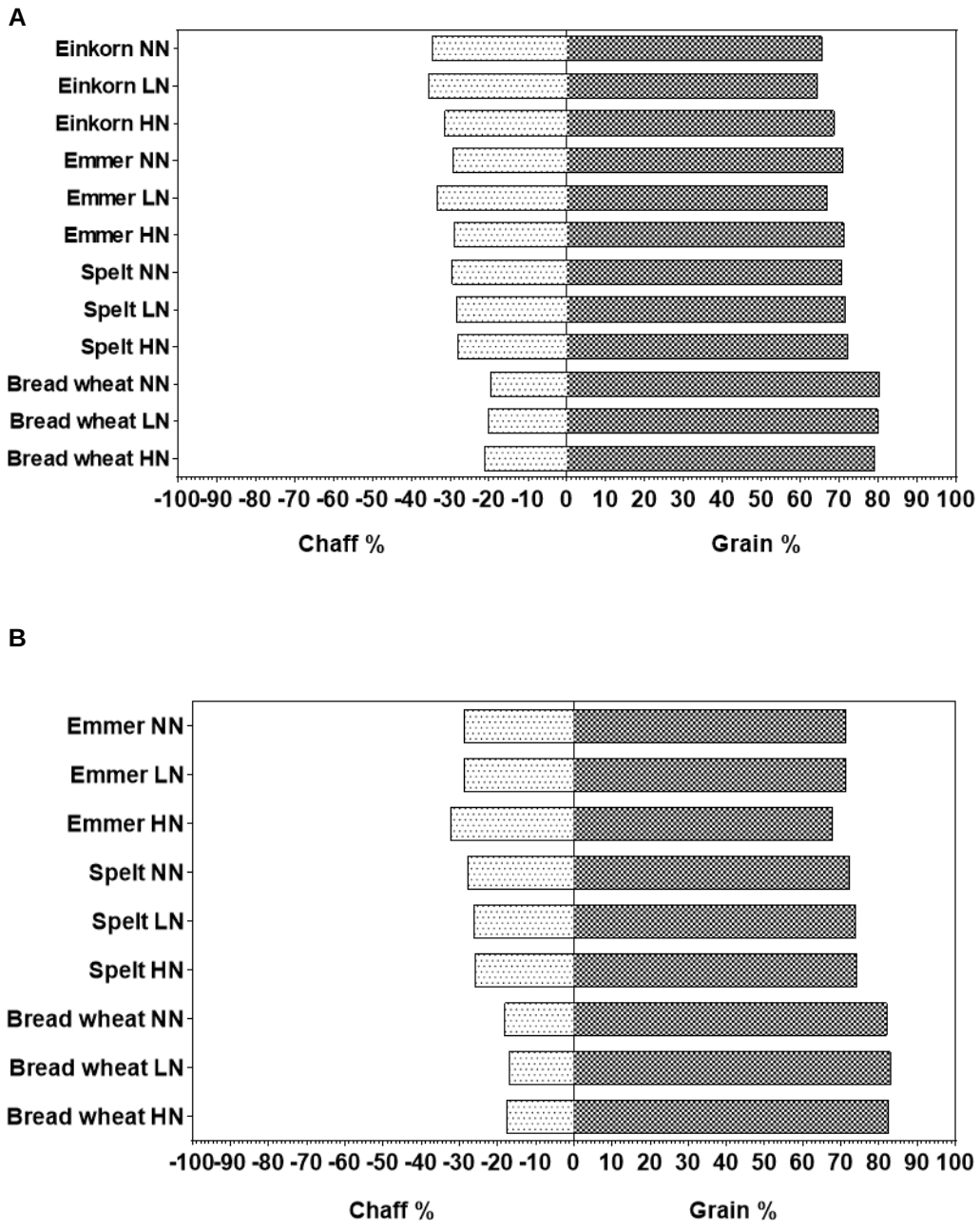


Figure 3.7 Proportion of ear biomass partitioning into grain and chaff for bread wheat, spelt, emmer and einkorn in (A) 2013 and (B) 2014 experiments. SED for Species x N in 2013 = 2.091 (*df* = 19.83), 2014 = 0.917 (*df* = 11.65)

3.2.4.2.2 Grain yield

Grain yield significantly differed between genotype ($P < 0.001$) for all experiments. Yields are expressed on a 100% dry matter basis. On average, bread wheat cv. JB Diego produced the highest yield while einkorn species had less grain yield. N application ($P < 0.05$) significantly increased grain yield in 2013 and 2014 (Table 3.8)

although yield difference between LN and NN was greater than HN and LN. Grain yield was not always highest at HN.

Table 3.8 Grain yield in 2012, 2013 and 2014 experiments (Grains were obtained by hand threshing ears of quadrat samples) (NN; zero N fertiliser, LN; low N fertiliser, HN; high N fertiliser, DM; 100% dry matter basis)

GT	Grain yield (t ha^{-1} at 100% DM)						
	2012	2013			2014		
		NN	LN	HN	NN	LN	HN
JB Diego	6.53	5.62	7.82	7.46	6.11	8.84	9.52
Xi 19	5.83	5.50	6.93	7.75	5.21	7.15	7.07
Spelt Tauro	4.02	4.22	5.11	6.18	4.83	5.85	6.67
Spelt SB	4.89	3.86	6.02	6.31	5.52	6.88	6.46
Spelt Oberkulmer	4.71	3.51	6.34	5.72	4.51	5.92	6.39
Emmer 1	4.22	-	-	-	4.72	5.65	4.91
Emmer 2	3.46	4.55	5.45	6.42	4.93	5.71	5.24
Einkorn 1	2.61	2.68	3.42	3.87	-	-	-
Einkorn 2	1.45	3.00	3.68	4.89	-	-	-
Einkorn 3	0.92	-	-	-	-	-	-
SED; GT (*df*)	0.882 (27)***	0.445 (42)***			0.445 (36)***		
N (*df*)	-	0.413(4)*			0.228 (4)*		
GT x N (*df*)	-	0.824 (34.36)^{NS}			0.75 (33.1)^{NS}		

***** Significant at *P* < 0.001, **significant at *P* < 0.01, *significant at *P* < 0.05, *NS*- Not significant**

3.2.4.3 Green area index (GAI)

Year 2012

GAI of the genotypes at early plant growth (GS26) was significantly different between genotypes ($P < 0.001$). Bread wheat cv. JB Diego and emmer 2 had the highest GAI of 0.7 and einkorn 3 had 0.2. When averaged across the species, GAI of bread wheat and emmer was not significantly different and spelt and einkorn was less than bread wheat and emmer. After that, the highest GAI was recorded in bread wheat cv. JB Diego at all times while the lowest values were recorded in einkorn 1. GAI for modern bread wheat cv. JB Diego was 5.04 at GS31, 10.5 at GS39 and 10.27 at GS59. The

high GAI of bread wheat may have a relationship with high seed rate and greater plant establishment.

However, as a species, spelt was not significantly different from bread wheat for GAI at GS31 while emmer and einkorn were significantly lower. Also, bread wheat, spelt and emmer had high GAI which were not significantly different at GS39 and 59. Figure 3.8 shows GAI of ten genotypes throughout growth season in 2012 against thermal time.

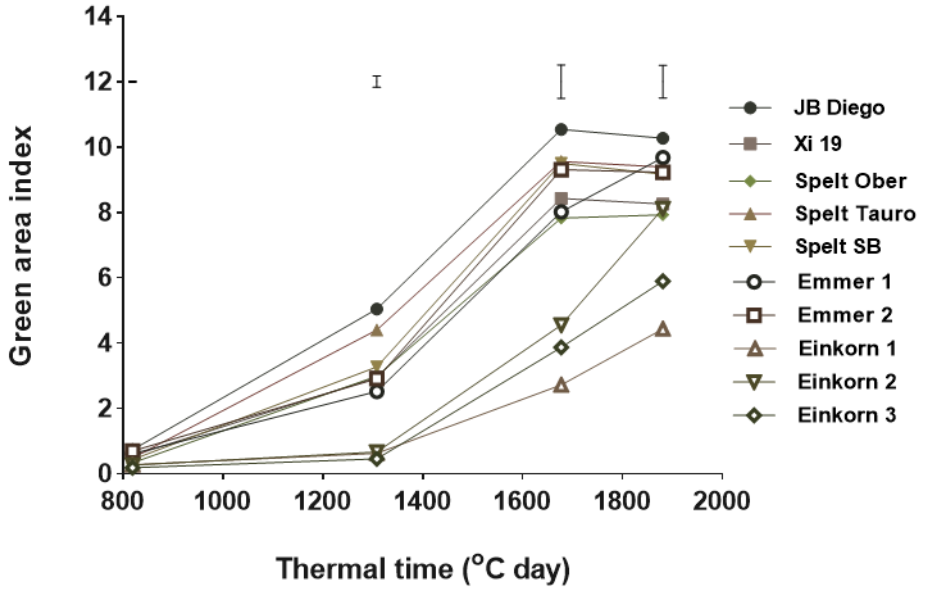


Figure 3.8 GAI at GS26, GS31, GS39 and GS59 in 2012 experiment. Error bars represent SED of the genotype

In 2013, GAI was significantly affected by genotype ($P < 0.001$) and N ($P < 0.001$) but not GT x N at GS39. At NN and HN, spelt Tauro and emmer 2 had the highest GAI, respectively. At LN, GAI was highest in cv. JB Diego and spelt SB (Figure 3.9).

Generally speaking, GAI increased significantly with N application ($P < 0.05$) and differed between genotypes ($P < 0.001$) in both experiments in 2013 and 2014 at GS61. In 2013, emmer 2 recorded the highest GAI of 3.7 and 5.9 under NN and LN condition while emmer 1 had GAI of 4.7 and 6.1 in 2014. Nevertheless, under HN bread wheat cv. JB Diego recorded the highest GAI though it was not significantly different from emmer 1 and 2. GAI of einkorn was significantly less than all other genotypes (Table 3.9).

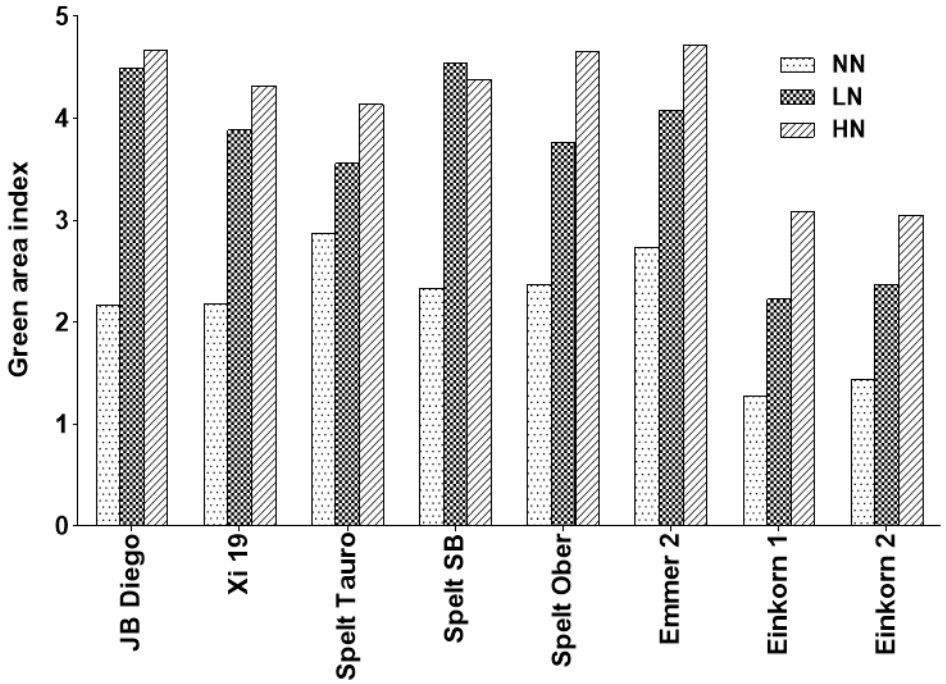


Figure 3.9 GAI at GS39 in 2013 experiment (SED for GT x N = 0.428 at *df* = 45.86)

Table 3.9 GAI at GS61 in 2013 and 2014 experiments under NN; zero N fertiliser, LN; low N fertiliser, HN; high N fertiliser conditions

GT	GS61					
	2013			2014		
	NN	LN	HN	NN	LN	HN
JB Diego	2.63	5.03	6.41	3.25	5.80	7.66
Xi 19	2.68	4.91	5.69	2.82	4.67	5.77
Spelt Tauro	3.16	4.56	5.29	4.23	5.81	6.93
Spelt SB	2.81	4.79	4.93	4.27	5.67	6.21
Spelt Oberkulmer	2.77	4.66	4.80	3.85	5.81	6.21
Emmer 1	-	-	-	4.71	6.09	7.19
Emmer 2	3.70	5.91	5.83	4.46	5.89	7.54
Einkorn 1	1.77	3.10	3.18	-	-	-
Einkorn 2	1.95	2.83	3.10	-	-	-
SED; GT (*df*)	0.320 (42)***			0.301 (36)***		
N (*df*)	0.370 (4)**			0.682 (4)*		
GT x N (*df*)	0.639 (25.41)^{NS}			0.836 (8.79)^{NS}		

***** Significant at *P* < 0.001, **significant at *P* < 0.01, *significant at *P* < 0.05, *NS*- Not significant**

3.2.4.4 Green area partitioning

It was observed that green area partitioned into green lamina and true stem plus leaf sheath was significantly affected by genotype ($P < 0.001$) at GS39 in 2012. On average, leaf lamina contributed more than 80% of the GAI. In 2013, contribution of green area from leaf lamina was significantly affected by genotype ($P < 0.001$), N level and GT x N interaction ($P < 0.05$). Contribution of leaf lamina for GAI was greatest in bread wheat followed by spelt, emmer and einkorn at GS39.

In 2012, all genotypes, at GS59, had more than 70% of their green area in green lamina while 20% was from true stem plus leaf sheath. Contribution from green ears for total GAI was less than 10%. The similar trend was observed in 2013 and 2014 at GS61. There was no effect of N or a GT x N interaction on green area partitioning. Only genotype ($P < 0.001$) was significantly different for the proportion of green area from different plant components.

3.2.5 Disease assessment

During the latter part of the growing season in 2012, fusarium head blight (*Fusarium spp*. and *Microdochium nivale*) and powdery mildew (*Blumeria gtaminis f.sp. tritici*) were observed in the field. Fusarium head blight significantly affected yield of susceptible genotypes. Bread wheat cv. Xi 19 recorded the highest disease infection rate of 52% while spelt Tauro recorded 5% ($P < 0.001$) (Figure 3.10). In 2013 and 2014, fusarium head blight was not observed at the experimental site.

3.2.6 Lodging assessment

Lodging was observed in the field just after anthesis in all seasons but was especially severe in 2012. At the end of the 2012 season, emmer and einkorn genotypes showed highest susceptibility for lodging recording 90% of lodging in einkorn 2 and 89% for emmer 2 (Figure 3.11). Bread wheat cv. Xi 19 did not lodge while in cv. JB Diego only 6% lodged. Lodging score was recorded in spelt genotypes of between 7 to 21%. In 2013 and 2014, N application ($P < 0.001$) increased lodging which differed between genotypes ($P < 0.001$). Emmer 2, einkorn 1, einkorn 2 and spelt Tauro recorded 90, 70, 75 and 20% of lodging at anthesis, respectively in 2013 at HN while only emmer

1 and emmer 2 genotypes lodged in LN (48%) and HN (77%) treated plots in 2014 at anthesis. Genotypes did not lodge at NN in both seasons.

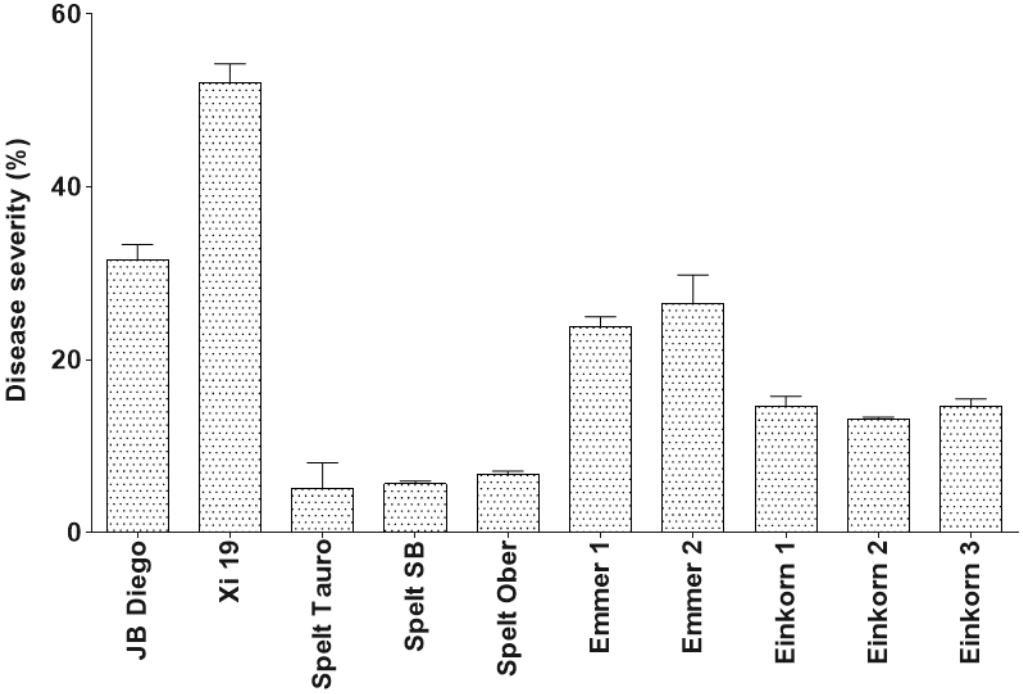


Figure 3.10 Fusarium head blight severity (%) in 2012 field experiment. Error bars represents SE of the genotype (*df* = 27)

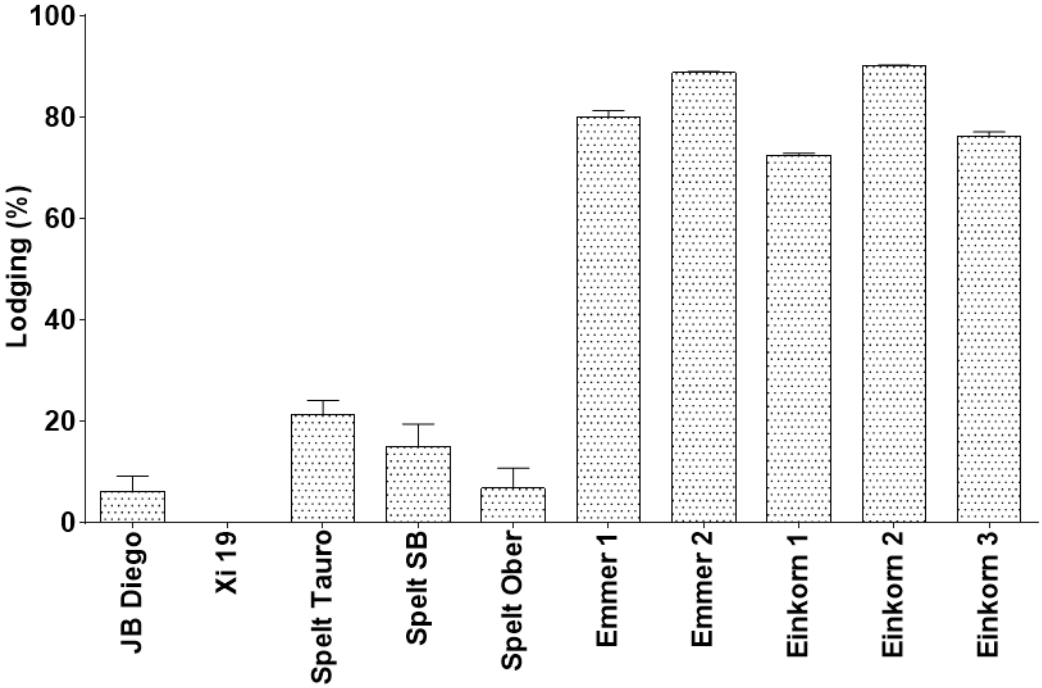
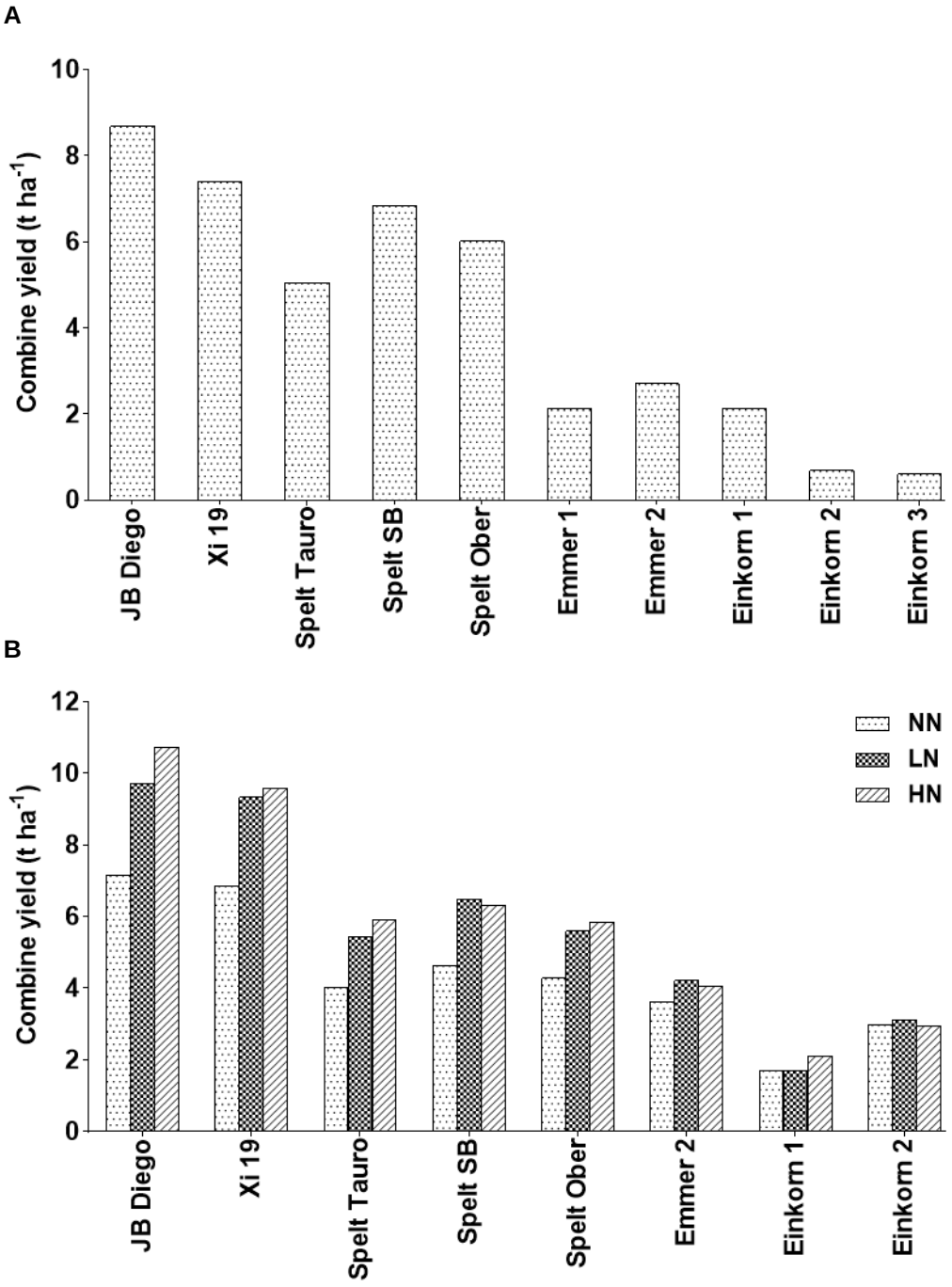


Figure 3.11 Lodging (%) in 2012. Error bars represent SE of the genotype (*df* = 27)

3.2.7 Combine yield

Bread wheat cv. JB Diego had the highest combine yield in 2012 of 8.6 t ha^{-1} at 100% DM while einkorn 3 recorded the lowest value of 0.6 t ha^{-1} ($P < 0.001$) (Figure 3.12 A). Combine yields were adjusted for threshability for all experiments. Averaged across the species, bread wheat produced higher yield followed by spelt, emmer and einkorn. In 2013 and 2014, genotype ($P < 0.001$), N ($P < 0.05$) and GT x N ($P < 0.05$) was significant for combine yield (Figure 3.12 B & C).



C

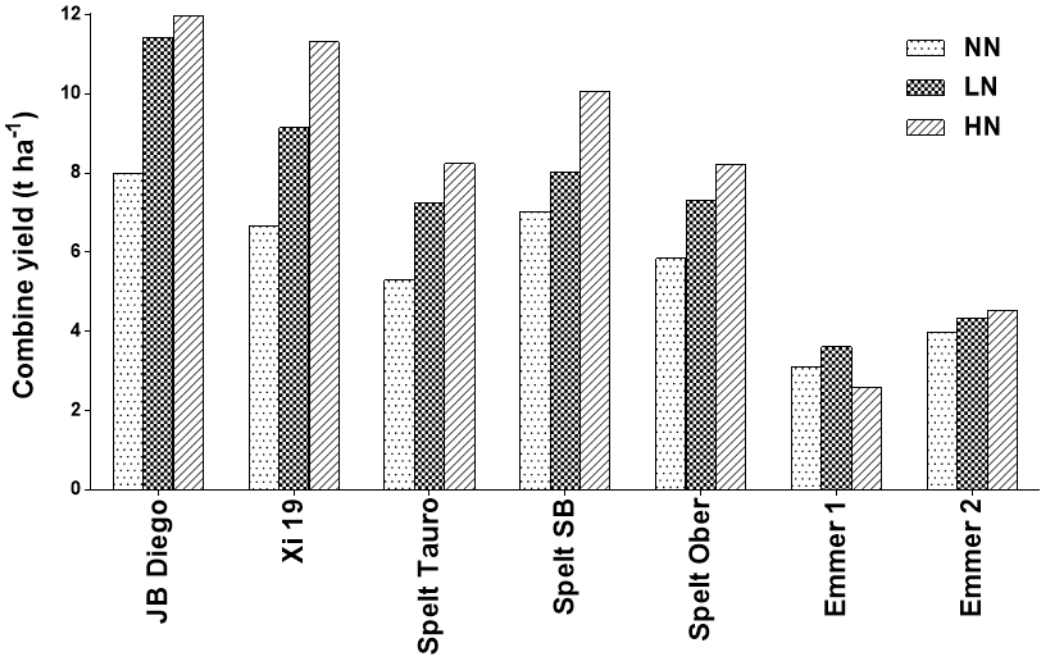


Figure 3.12 Combine yield at 100% DM in (A) 2012 (B) 2013 and (C) 2014 field experiments. (SED of GT in 2012 = 0.391 (*df* = 27), SED of GT x N in 2013 and 2014 was 0.433 (*df* = 38.06) and 0.893 (*df* = 19.93), respectively

In 2012, a strong correlation was found between sample yield and combine yield (r = 0.89) (Figure 3.13A). Bread wheat and spelt genotypes had higher combine yield than sample yield while emmer and einkorn genotypes recorded higher sample yield than combine yield. Correlation analysis revealed that the relationship between combine yield and sample yield was highly significant for all N treatments in 2013 and 2014 (r = 0.9 & r = 0.8, respectively) (Figure 3.13 B & C).

In 2012, a strong negative correlation was found between combine yield and lodging % (r = 0.91, $P < 0.001$). A similar relationship was found between combine yield and lodging % in 2013 (r = 0.79, $P < 0.001$) and 2014 (r = 0.83, $P < 0.001$) field experiments under HN.

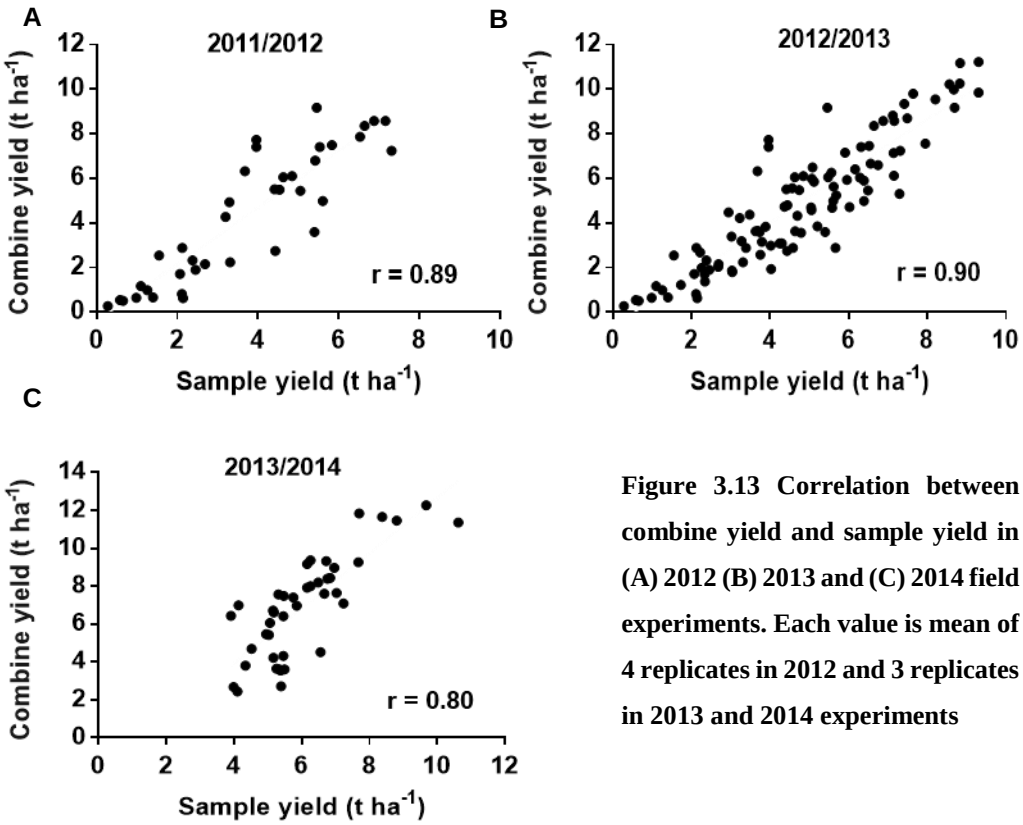


Figure 3.13 Correlation between combine yield and sample yield in (A) 2012 (B) 2013 and (C) 2014 field experiments. Each value is mean of 4 replicates in 2012 and 3 replicates in 2013 and 2014 experiments

3.2.8 Threshability

Threshability of the genotype ($P < 0.001$) varied from 2 to 100% in 2012. Two bread wheat cvs. had 100% threshability while all emmer and einkorn genotypes had less than 3% of threshability while threshability of spelt genotypes was between 42 to 62%. Threshability was significantly affected by genotype ($P < 0.001$) and N ($P < 0.001$) in 2013 experiment while GT x N ($P < 0.05$) also significant in 2014. In general, application of N significantly reduced threshability of all ancient wheat genotypes.

3.2.9 Harvest index (HI)

In 2012, effect of genotype on HI was significant ($P < 0.001$) (Table 3.10). When averaged across species, bread wheat recorded the highest HI (0.37) followed by spelt (0.26), emmer (0.21) and einkorn (0.13). In 2013, HI was higher under NN than LN and HN for most of the genotypes. The effect of main factors (GT; $P < 0.001$ and N; $P < 0.01$) and the interaction was significant in 2013. A similar trend was reported in the 2014 experiment though N level ($P = 0.057$) and GT x N was not significant in that year (Table 3.10).

Table 3.10 Harvest index of genotypes in 2012, 2013 and 2014 field experiments under NN; zero N fertiliser, LN; low N fertiliser, HN; high N fertiliser conditions

GT	Harvest Index						
	2012	2013			2014		
		NN	LN	HN	NN	LN	HN
JB Diego	0.38	0.57	0.53	0.50	0.38	0.38	0.38
Xi 19	0.36	0.55	0.52	0.52	0.34	0.37	0.38
Spelt Tauro	0.23	0.43	0.39	0.41	0.29	0.32	0.29
Spelt SB	0.29	0.42	0.41	0.43	0.29	0.33	0.31
Spelt Oberkulmer	0.27	0.38	0.40	0.41	0.28	0.31	0.30
Emmer 1	0.21	-	-	-	0.28	0.27	0.27
Emmer 2	0.21	0.36	0.34	0.34	0.26	0.29	0.27
Einkorn 1	0.21	0.32	0.26	0.38	-	-	-
Einkorn 2	0.10	0.36	0.35	0.39	-	-	-
Einkorn 3	0.09	-	-	-	-	-	-
SED; GT (*df*)	0.037 (27)***	0.012 (42)***			0.007 (36)***		
N (*df*)	-	0.004 (4)**			0.005 (4)^{NS}		
GT x N (*df*)	-	0.021 (49.49)**			0.013(31.02)^{NS}		

***** Significant at *P* < 0.001, **significant at *P* < 0.01, *significant at *P* < 0.05, *NS* – Not significant**

3.2.10 Thousand grain weight (TGW)

Thousand grain weight (TGW) of the genotypes were significantly different in 2012 and 2013 experiments ($P < 0.001$). There was no effect of N or interaction of GT x N for TGW in 2013. TGW was higher in spelt than other species in both experiments where it was 40, 35 and 20% higher than bread wheat, emmer and einkorn, respectively in 2012 while 17, 24 and 27% in 2013.

3.2.11 Plant morphology and structure

3.2.11.1 Plant height

Plant height at maturity was significantly different between genotypes ($P < 0.001$) in 2012. Average height of bread wheat was 86 cm while spelt, emmer and einkorn were 129, 124 and 123 cm, respectively. Therefore bread wheat was 30 to 33% shorter than ancient wheat species (Figure 3.14). In 2013 and 2014, plant height of most of the

genotypes, regardless of N level, was shorter than plants in the 2012 experiment. Plant height was significantly affected by genotype ($P < 0.001$) and N ($P < 0.05$) (Table 3.11).

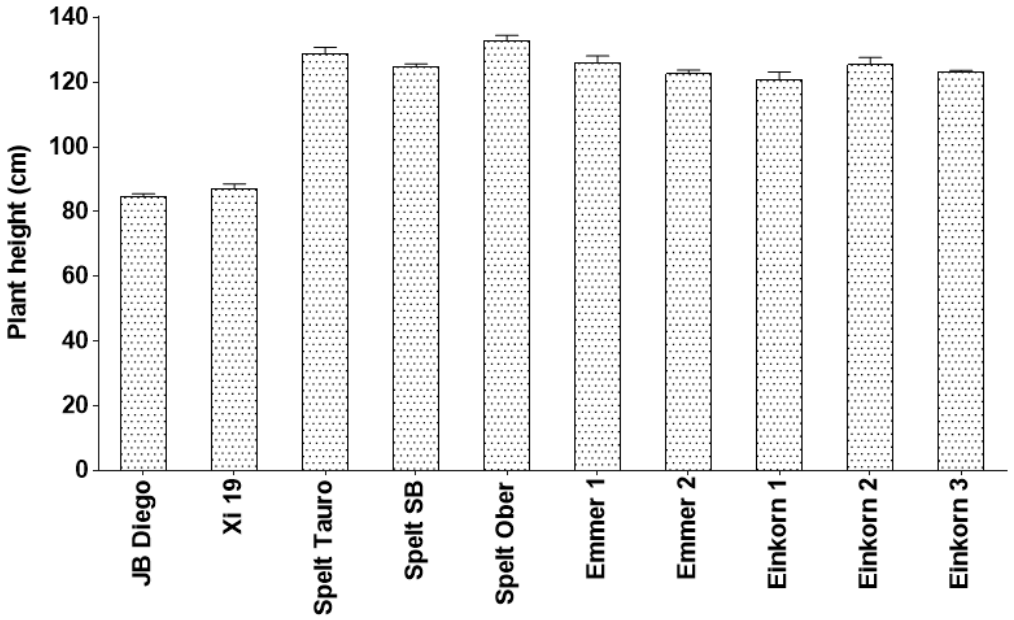


Figure 3.14 Plant height at maturity in 2012 field experiment (SED = 2.26, *df* = 27)

Table 3.11 Plant height at maturity in 2013 and 2014 experiments under NN; zero N fertiliser, LN; low N fertiliser, HN; high N fertiliser conditions

GT	Plant height (cm)					
	2013			2014		
	NN	LN	HN	NN	LN	HN
JB Diego	63.97	78.60	77.52	62.33	75.52	76.73
Xi 19	59.97	79.59	76.77	65.17	75.67	76.27
Spelt Tauro	96.07	113.02	111.87	105.17	116.27	116.17
Spelt SB	101.11	107.07	115.56	113.17	122.83	132.67
Spelt Oberkulmer	91.37	110.07	120.44	101.00	115.17	126.17
Emmer 1	-	-	-	121.51	127.55	132.52
Emmer 2	108.13	125.71	120.44	105.67	128.52	129.54
Einkorn 1	95.59	112.19	103.37	-	-	-
Einkorn 2	109.31	116.88	118.86	-	-	-
SED; GT (*df*)	2.902 (42)***			2.514 (36)***		
N (*df*)	2.702 (4)**			3.645 (4)*		
GT x N (*df*)	5.422 (34.64)^{NS}			5.435 (16.94)^{NS}		

***** Significant at *P* < 0.001, **significant at *P* < 0.01, *significant at *P* < 0.05, *NS* - Not significant**

3.2.11.2 Flag leaf properties

Flag leaf green area index (FLGAI), flag leaf SPAD (FLSPAD), length (FLL) width (FLW) and specific flag leaf area (SFLA) were significantly different between genotypes ($P < 0.001$) in all experiments while N had a significant influence on all of the parameters in 2013. However, neither N nor the GT x N interaction was significant for flag leaf properties in 2014. Table 3.12 and 3.13 show flag leaf traits in 2012 and 2013 field experiments.

Table 3.12 Flag leaf properties of bread wheat, spelt, emmer and einkorn genotypes at GS59 in 2012 experiment. [FLGAI; flag leaf green area index, FLSPAD; SPAD of flag leaf, FLL; flag leaf length (cm), FLW; flag leaf width (cm), SFLA; specific flag leaf area ($cm^2\ g^{-1}$)]

GT	FGAI	FLSPAD	FLL (cm)	FLW (cm)	SFLA ($cm^2\ g^{-1}$)
JB Diego	2.21	51.86	19.98	2.02	214.01
Xi 19	1.66	53.91	18.89	1.79	189.84
Spelt Tauro	1.70	52.20	21.44	1.87	215.81
Spelt SB	1.84	49.24	24.49	1.62	230.74
Spelt Oberkulmer	1.61	51.99	19.87	1.78	222.59
Emmer 1	2.12	43.68	22.23	1.84	241.69
Emmer 2	1.88	49.35	19.15	1.61	261.08
Einkorn 1	0.50	40.14	11.24	0.83	271.43
Einkorn 2	0.84	35.37	14.48	0.85	334.57
Einkorn 3	0.87	42.78	13.23	0.89	313.43
SED (*df* = 27)	0.256***	1.190***	1.314***	0.075***	26.735***

***** Significant at $P < 0.001$**

Table 3.13 Flag leaf properties of bread wheat, spelt, emmer and einkorn genotypes at GS61 in 2013 experiment. [FLGAI; flag leaf green area index, FLSPAD; SPAD of flag leaf, FLL; flag leaf length (cm), FLW; flag leaf width (cm), SFLA; specific flag leaf area (cm^2 g^{-1})]

GT	FLGAI			FLSPAD			FLL (cm)			FLW (cm)			SFLA (cm g^{-1})		
	NN	LN	HN	NN	LN	HN	NN	LN	HN	NN	LN	HN	NN	LN	HN
JB Diego	0.42	0.93	1.33	41.52	52.30	53.12	14.09	18.56	18.86	1.59	1.76	1.86	180.80	192.80	201.30
Xi 19	0.51	0.98	1.20	48.62	56.33	57.15	14.97	18.26	19.95	1.78	1.80	1.99	162.90	183.80	184.10
Spelt Tauro	0.66	1.03	1.19	48.29	51.06	53.67	19.21	23.56	23.96	1.73	1.88	1.90	201.20	209.50	210.40
Spelt SB	0.45	1.09	0.70	42.89	49.81	50.37	18.38	22.46	23.57	1.47	1.56	1.66	184.10	185.60	194.90
Spelt Oberkulmer	0.45	1.02	0.93	42.58	50.61	51.41	16.99	20.33	23.25	1.55	1.74	1.78	189.30	199.50	211.70
Emmer 2	0.70	1.35	1.29	39.98	49.02	53.28	20.72	24.62	25.73	1.68	1.84	1.92	205.40	221.70	249.10
Einkorn 1	0.16	0.34	0.31	42.77	47.96	48.34	13.21	17.02	18.33	0.93	1.05	1.09	191.70	194.60	198.60
Einkorn 2	0.12	0.21	0.23	32.61	33.71	38.20	14.65	17.38	17.82	0.88	0.94	1.02	211.50	211.60	234.20
SED; GT (*df*)	0.105(42)***			0.900(42)***			0.765(42)***			0.037(42)***			7.180(42)***		
N (*df*)	0.080(4)**			0.532(4)**			0.910(4)*			0.039(4)*			4.980(4)*		
GT x N (*df*)	0.188(41.26)^{NS}			1.553(45.47)*			1.538(24.57)^{NS}			0.071(29.06)^{NS}			12.660(43.48)^{NS}		

NN; zero N fertiliser, LN; low N fertiliser, HN; high N fertiliser

***** Significant at *P* < 0.001, **significant at *P* < 0.01, *significant at *P* < 0.05, *NS* - Not significant**

3.2.12 Canopy persistence and stay-green

3.2.12.1 SPAD

SPAD value can be used as an indicator of the greenness of the leaf lamina, which is related to the N status of the plant and chlorophyll concentration [chl] of the leaf (Montemurro *et al*., 2006). However, the relationship between [chl] of the leaf lamina and SPAD varied significantly with plant species (Uddling *et al*., 2007). Therefore, a calibration of SPAD based on actual chlorophyll content of the species of bread wheat, spelt, emmer and einkorn was done in 2014. Figure 3.15 shows the results of a comparison of regressions between SPAD and [chl] (a + b) of flag leaf of bread wheat, spelt, emmer and einkorn ($R^2 = 0.82$, $P < 0.001$). When SPAD value increased by one total [chl] increased by 1.35, 1.84, 0.51 and 2.42 µg cm^2 for bread wheat, spelt, emmer and einkorn, respectively.

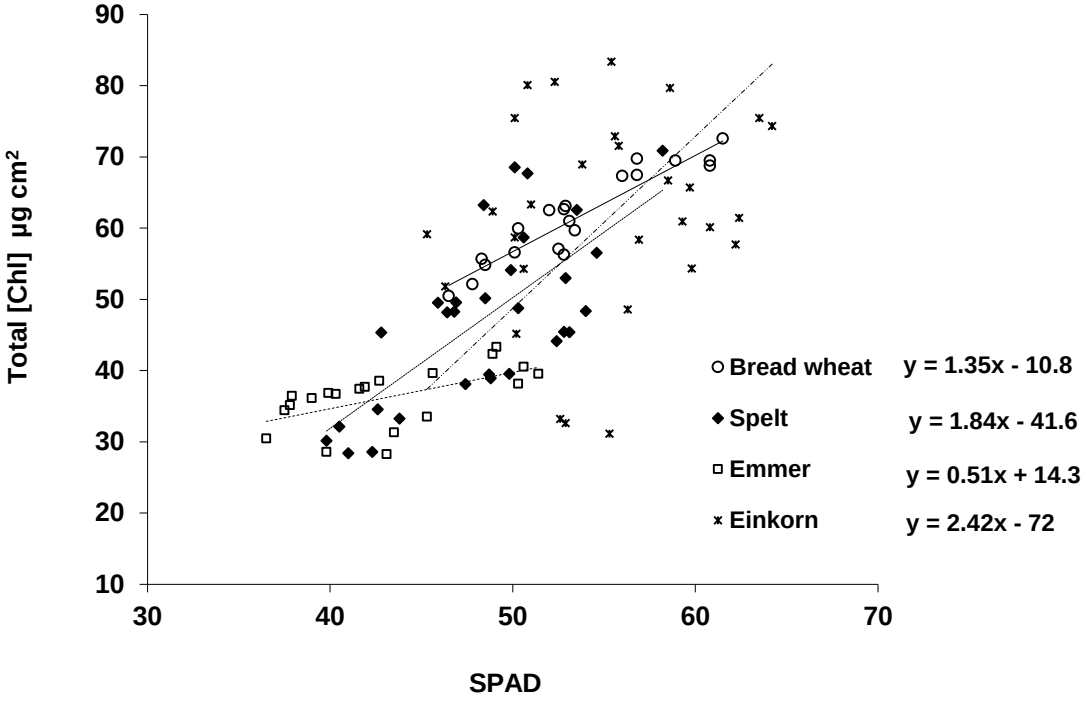


Figure 3.15 Comparison of regressions between chlorophyll concentration [chl] and SPAD values of four species; bread wheat, spelt, emmer and einkorn ($R^2 = 0.82$, $P < 0.001$)

In 2012, at GS31, 39, 59 and 61, bread wheat cv. Xi 19 had the highest SPAD value while einkorn 2 and 3 had the lowest values ($P < 0.001$). When averaged across species, bread wheat recorded the highest SPAD value followed by spelt, emmer and einkorn throughout the growth pre-anthesis ($P < 0.001$) (Table 3.14).

During post-anthesis, bread wheat cv. Xi 19 declined SPAD rapidly when compared to ancient wheat species. However, this might be influenced by fusarium and powdery mildew diseases in 2012 though, bread wheat cv. JB Diego also senesced rapidly hence declined its SPAD values. SPAD values of spelt genotyeps at late-grain filling period were higher than all other genotypes (Table 3.15) ($P < 0.001$) due to the lowest rate of SPAD decline. Figure 3.16 shows SPAD distribution throughout the growth period of bread wheat, spelt, emmer and einkorn against thermal time in 2012 field experiment.

Table 3.14 SPAD values and chlorophyll concentrations at GS31 (16- Apr- 12), GS39 (25-May-12), GS56 (13-Jun-12) and GS61 (20-Jun-12) for ten genotypes in 2012 experiment. Regression equations were used to calculate respective chlorophyll concentrations [chl] µg cm^2 for four species. (Bread wheat [chl] = 1.35 x SPAD – 10.8; spelt [chl] = 1.84 x SPAD – 41.6; emmer [chl] = 0.51 x SPAD + 14.4; einkorn [chl] = 2.42 x SPAD – 72)

GT	16-Apr-12		25-May-12		13-Jun-12		20-Jun-12	
	SPAD	[Chl] µg cm^2	SPAD	[Chl] µg cm^2	SPAD	[Chl] µg cm^2	SPAD	[Chl] µg cm^2
JB Diego	51. 62	58.88	54.41	62.65	51.86	59.21	51.29	58.44
Xi 19	52.49	60.06	56.80	65.87	53.96	62.04	55.38	63.96
Spelt Tauro	48.66	47.93	54.25	58.22	52.20	54.44	52.48	54.97
Spelt SB	49.16	48.85	50.64	51.58	49.25	49.01	48.98	48.51
Spelt Oberkulmer	49.04	48.63	53.02	55.96	51.99	54.06	51.12	52.46
Emmer 1	44.02	36.75	46.56	38.05	43.68	36.58	40.87	35.14
Emmer 2	48.98	39.28	50.57	40.09	49.36	39.47	46.97	38.25
Einkorn 1	43.64	33.61	48.99	46.55	40.15	25.16	48.31	44.91
Einkorn 2	40.78	26.68	42.72	31.39	35.38	13.61	40.23	25.34
Einkorn 3	38.55	21.30	43.87	34.15	42.79	31.54	47.87	43.84
SED (*df* = 27)	1.829***	3.984***	0.986***	1.893***	1.189***	2.356***	1.124***	1.984***

***** Significant at *P* < 0.001**

Table 3.15 SPAD values and chlorophyll concentrations during post-anthesis flag leaf fully senescence for ten genotypes in 2012 experiment. Regression equations were used to calculate respective chlorophyll concentrations [chl] µg cm^2 for four species. (Bread wheat [chl] = 1.35 x SPAD – 10.8; spelt [chl] = 1.84 x SPAD – 41.6; emmer [chl] = 0.51 x SPAD + 14.4; einkorn [chl] = 2.42 x SPAD – 72)

GT	11-Jul-12		17-Jul-12		22-Jul-12		26-Jul-12	
	SPAD	[Chl] µg cm^2	SPAD	[Chl] µg cm^2	SPAD	[Chl] µg cm^2	SPAD	[Chl] µg cm^2
JB Diego	47.93	53.91	42.76	46.92	29.47	28.99	20.22	16.50
Xi 19	40.40	43.74	32.87	33.57	16.24	11.12	9.22	1.65
Spelt Tauro	49.35	49.20	44.86	40.93	38.74	29.69	32.96	19.05
Spelt SB	44.52	40.32	42.59	36.76	40.24	32.45	33.75	20.50
Spelt Oberkulmer	42.83	37.19	42.33	36.28	38.43	29.11	33.44	19.93
Emmer 1	41.17	35.29	36.23	32.77	32.32	30.78	26.69	27.91
Emmer 2	44.10	36.79	43.53	36.50	30.84	30.03	26.20	27.66
Einkorn 1	45.21	37.41	42.36	30.49	40.68	26.44	38.20	20.46
Einkorn 2	38.80	21.91	35.45	13.79	32.70	7.15	30.52	1.87
Einkorn 3	38.74	21.76	36.85	17.16	34.26	10.90	30.14	2.64
SED (*df* = 27)	2.134***	4.124***	1.811***	3.351***	2.432***	3.426***	2.176***	3.617***

***** Significant at *P* < 0.001**

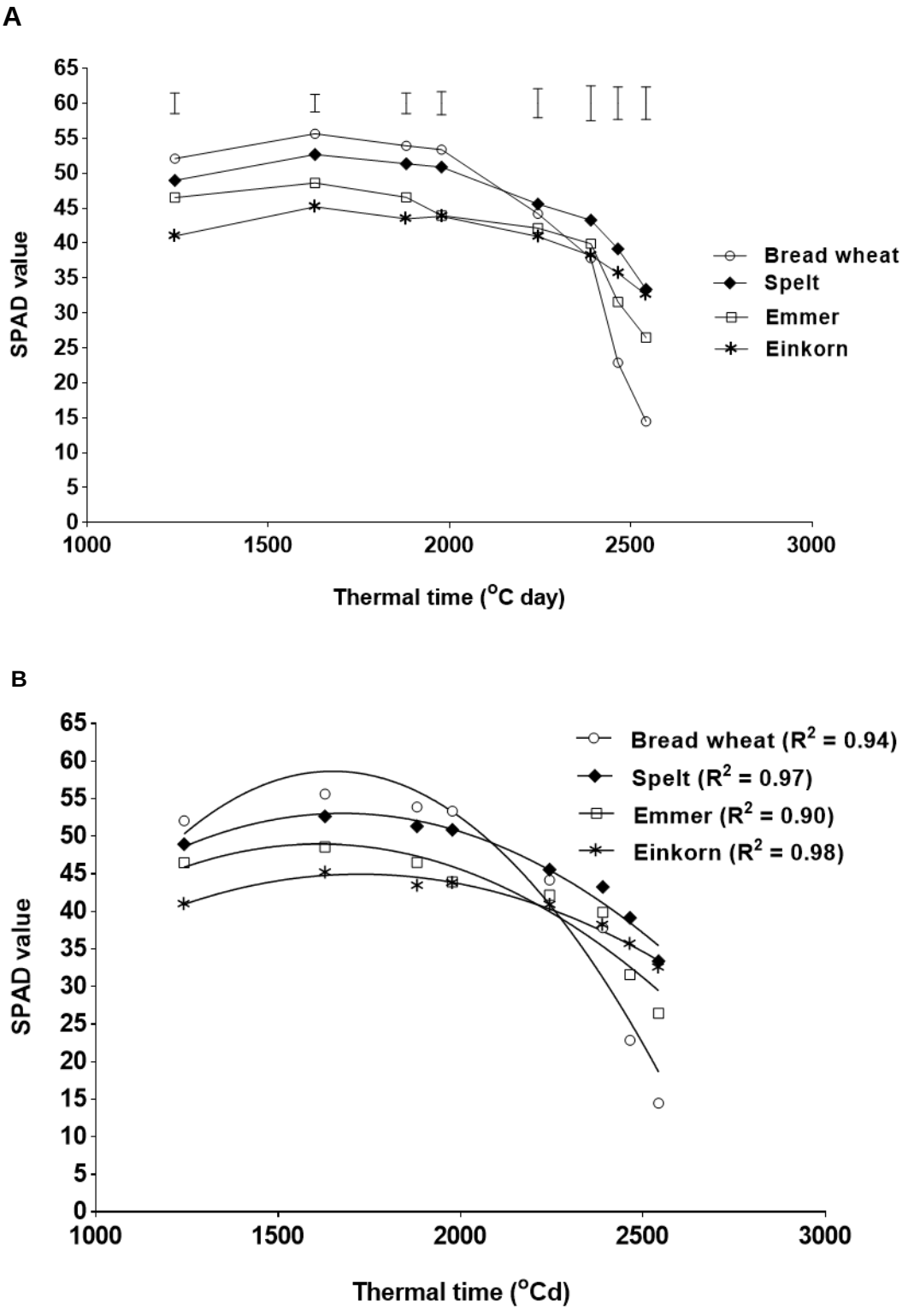


Figure 3.16 (A) Observed values of SPAD throughout the growth period of bread wheat, spelt, emmer and einkorn against thermal time in 2012 experiment (error bars represent SED for species) and (B) fitted curves to second order polynomial quadratic relationship ($Y = B_0 + B_1*X + B_2*X*X$) between SPAD and thermal time (B_0, B_1 and B_2 are coefficients)

SPAD values were measured on 14-May, 05-Jun, 24-Jun, 11-Jul, 21-Jul, 26-Jul, 01-Aug and 07-Aug in 2013. Effect of genotype and N level on SPAD values was significant ($P < 0.001$) throughout the experiment while the interaction between the two main factors was also significant on most of the assessment days ($P < 0.001$). Bread wheat genotypes had the highest SPAD during plant growth for all N levels.

SPAD depletion rate was higher in NN plots for all genotypes. Figure 3.17 illustrates SPAD distribution throughout the growing season against thermal time for NN (A), LN (B) and HN (C) for species. In 2014, SPAD values were measured at GS31, GS39, GS61 and four times after anthesis. Except at GS31, SPAD values were significantly affected by N while GT was significant for all measurements. Three out of seven occasions interaction of GT x N was highly significant for SPAD. Bread wheat had highest SPAD followed by spelt and emmer for all N levels (Figure 3.18).

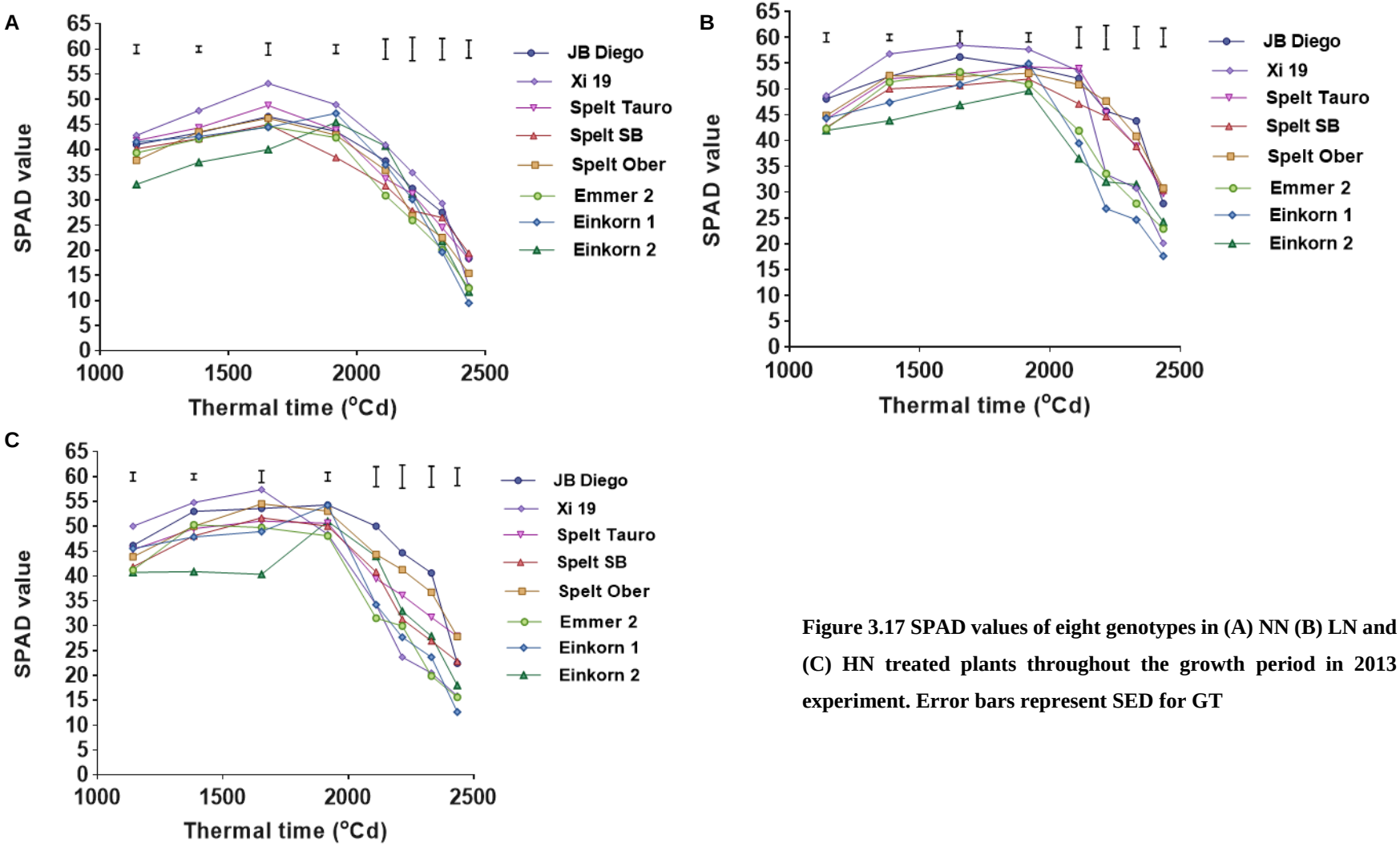


Figure 3.17 SPAD values of eight genotypes in (A) NN (B) LN and (C) HN treated plants throughout the growth period in 2013 experiment. Error bars represent SED for GT

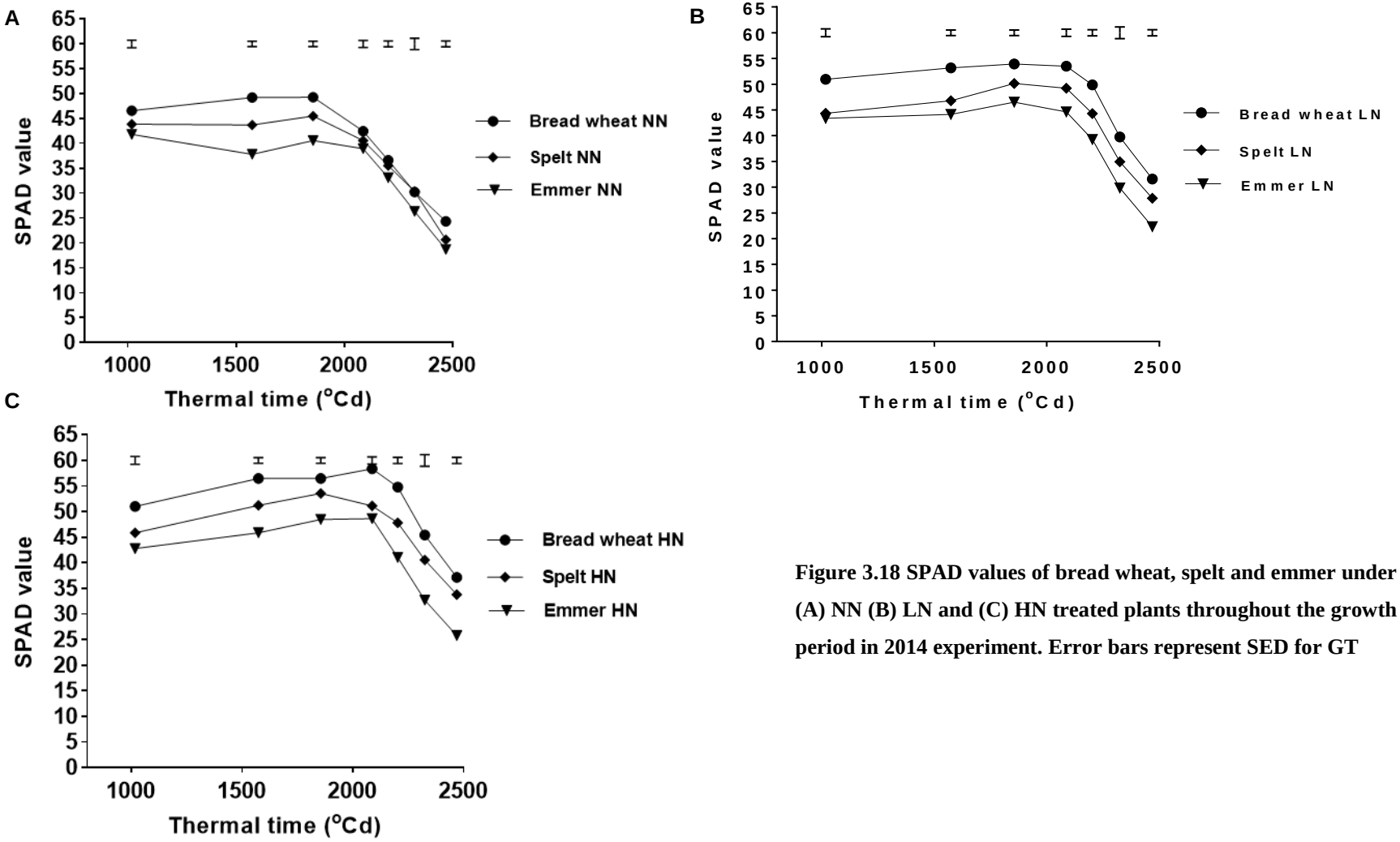


Figure 3.18 SPAD values of bread wheat, spelt and emmer under (A) NN (B) LN and (C) HN treated plants throughout the growth period in 2014 experiment. Error bars represent SED for GT

3.2.12.2 Visual greenness score

At anthesis, none of the genotypes had started to senesce hence the greenness score was 10 for all genotypes, but over time, all genotypes showed a decline of flag leaf 'greenness'. Visual greenness score was significantly different between genotypes at all sampling points in the 2012 season ($P < 0.001$). Spelt genotypes performed particularly well, showing the slowest decline in leaf greenness throughout the assessment period. Visual score of flag leaf greenness plotted against thermal time (base temperature at anthesis 0^{o}C) using four parameter logistic model (standard *S*-shaped curve) (Figure 3.19). GenStat 15th edition was used to estimate four parameters of values of lower asymptote, the difference between upper and lower asymptote, doubled relative senescence rate and the time when the absolute senescence rate is at maximum. Greenness score at anthesis was considered as ten (10 = fully green). Table 3.16 shows estimated parameters of logistic model for flag leaf greenness of ten genotypes in 2012.

Table 3.16 Estimated parameters for logistic model for flag leaf greenness of ten genotypes in 2012 experiment. Y; greenness score, A; lower asymptote in the unit of Y axis, C; the difference between upper and lower asymptote in the unit of Y axis, B; doubled relative senescence rate at the time M, M; the time when the absolute senescence rate is at maximum, t; accumulated thermal time after anthesis in degree days (^{o}Cd)

GT	Y=A+ C/(1+e^(-B(t-M)))				R^2 (%)	Green area duration
	A	C	B	M		
JB Diego	-0.56	10.68	-0.0115	539.80	99.0	5166
Xi 19	-0.18	10.00	-0.0155	534.90	99.6	5091
Spelt Tauro	0.15	9.92	-0.0127	526.00	99.5	5240
Spelt SB	0.19	9.92	-0.0138	496.50	99.1	5048
Spelt Ober	-0.24	10.12	-0.0146	606.40	99.2	5886
Emmer 1	-0.47	11.06	-0.0103	364.20	98.3	3690
Emmer 2	-1.21	11.65	-0.0087	440.20	99.2	4163
Einkorn 1	-6.21	16.51	-0.0083	399.10	97.9	2762
Einkorn 2	-3.54	13.54	-0.0106	363.20	98.5	2825
Einkorn 3	-0.28	10.42	-0.0122	392.20	99.6	3860
SED (*df* = 27)	0.906***	1.086***	0.001252***	15.341***	*NA*	62.9***

*****Significant at *P* < 0.001, *NA*; not applicable**

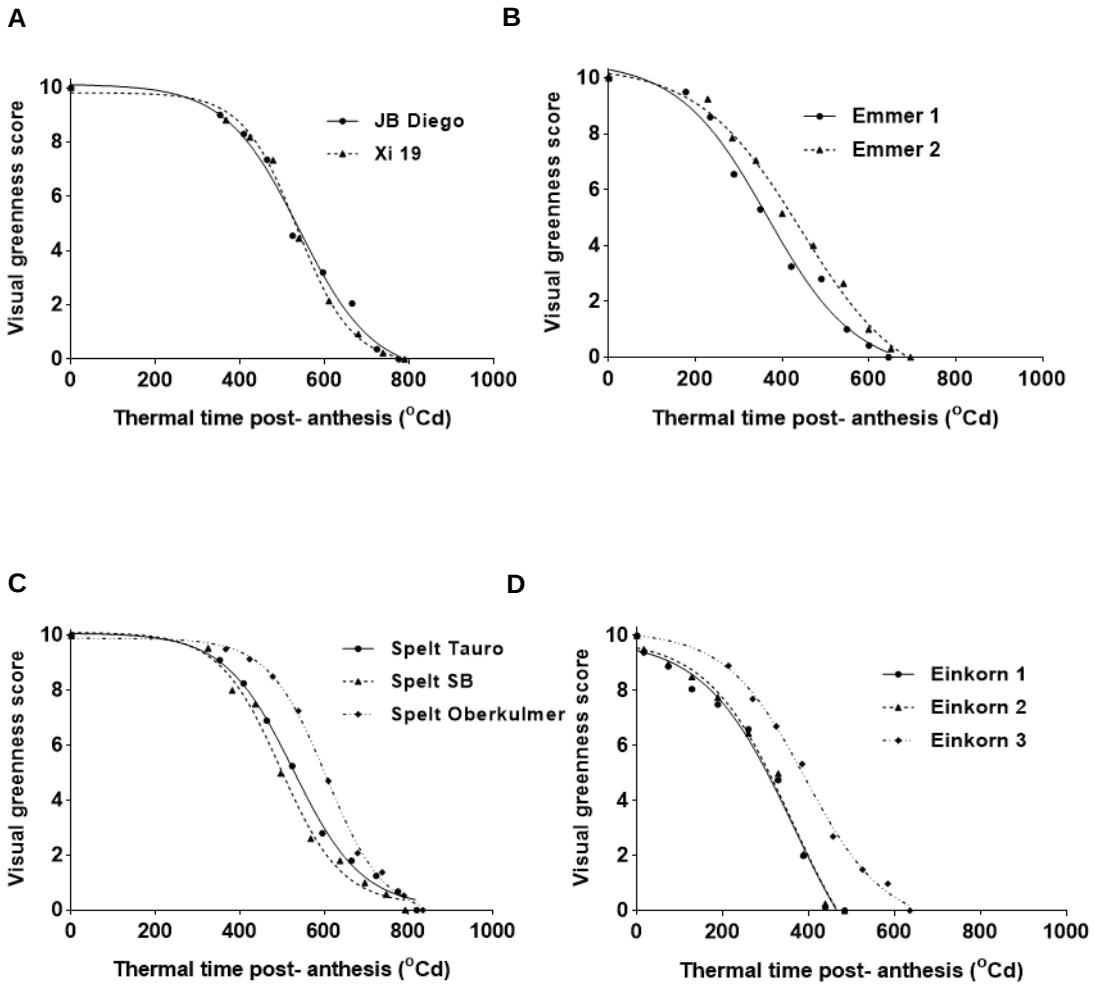


Figure 3.19 Observed values and fitted curves (estimated parameters are presented in Table 3.16) for post-anthesis flag leaf greenness in the mean of visual score for (A) Bread wheat cv. JB Diego and cv. Xi 19 (B) Emmer 1 and 2 (C) Spelt cv. Tauro, cv. SB and Oberkulmer (D) Einkorn 1, 2 and 3 in 2012 experiment

According to Table 3.16, timing of rapid flag leaf senescence (M) which describes the time (°Cd) when absolute rate of flag leaf senescence is maximum, was higher in spelt cv. Oberkulmer followed by bread wheat cv. JB Diego. Statistical differences between spelt cv. Oberkulmer and bread wheat cv. JB Diego was significant though spelt cv. SB and cv. Tauro was not different from either bread wheat cv. JB Diego or Xi 19 in 2012 field experiment.

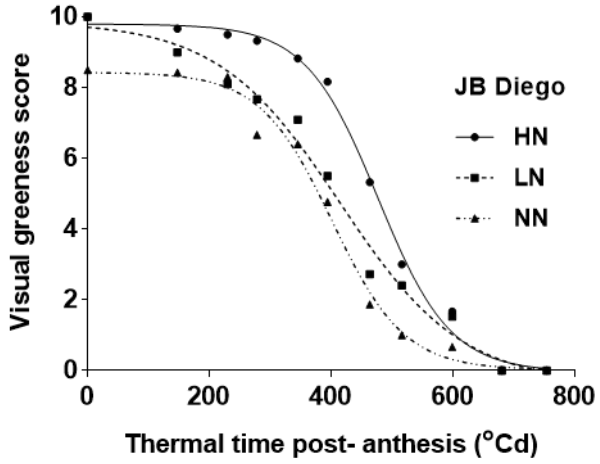
Figure 3.20 shows fitted curves for seven genotypes in 2014 under three N regimes while Table 3.17 for detail parameters of fitted model. Emmer genotypes together with bread wheat cv. JB Diego at NN started to senesce before anthesis while greenness score of all other genotypes was 10 at LN and HN. According to table 3.17, timing of rapid flag leaf senescence (M) was significantly high in spelt cv. Oberkulmer at any level of N though other spelt genotypes had relatively low value for M. Timing of rapid flag leaf senescence of spelt cv. Tauro was not statistically different from either bread wheat cv. Xi 19 at HN while bread wheat cv. JB Diego at NN in 2014 field experiment.

Table 3.17 Estimated parameters for logistic model for flag leaf greenness of seven genotypes in 2014 experiment under three N regimes. Y; greenness score, A; lower asymptote in the unit of Y axis, C; the difference between upper and lower asymptote in the unit of Y axis, B; doubled relative senescence rate at the time M, M; the time when the absolute senescence rate is at maximum (or time at ½ C), t; accumulated thermal time after anthesis in degree days (ºCd)

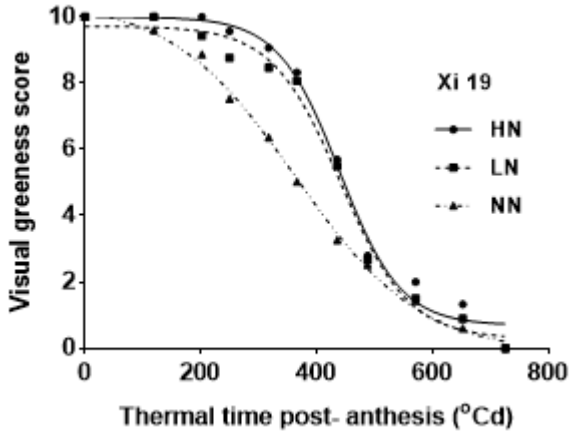
GT	Y=A + C/(1+e^(-B(t-M)))											
	A			B			C			M		
	HN	LN	NN	HN	LN	NN	HN	LN	NN	HN	LN	NN
JB Diego	-0.051	-0.377	-0.026	-0.01689	-0.00901	-0.00833	8.41	10.25	10.96	401.50	414.40	381.80
Xi 19	0.693	0.290	-0.147	-0.01899	-0.01818	-0.00929	9.31	9.44	10.66	439.00	440.40	363.00
Spelt Tauro	0.443	-0.109	0.069	-0.01665	-0.01382	-0.01424	9.64	10.04	9.95	437.30	444.80	386.90
Spelt SB	0.321	-0.037	0.006	-0.01436	-0.01337	-0.01359	9.88	10.13	10.03	426.90	429.60	377.50
Spelt Oberkulmer	0.015	-0.078	-0.138	-0.02010	-0.02006	-0.01488	9.86	9.71	10.07	490.60	481.90	425.10
Emmer 1	-0.093	-0.096	-0.087	-0.02004	-0.01738	-0.01607	8.55	9.59	9.50	324.57	330.10	312.10
Emmer 2	-0.014	-0.118	-0.083	-0.01999	-0.01314	-0.01518	8.65	10.00	9.79	322.80	301.00	255.80
SED; GT (*df* = *36)*		0.098***			0.00112***			0.155*			3.87***	
N (*df* = 4)		0.059**			0.00072*			0.077*			2.38***	
GT x N (*df* = 39.7)		0.167*			0.00193***			0.261***			6.66***	

***** Significant at *P* < 0.001, **significant at *P* < 0.01, *Significant at *P* < 0.05**

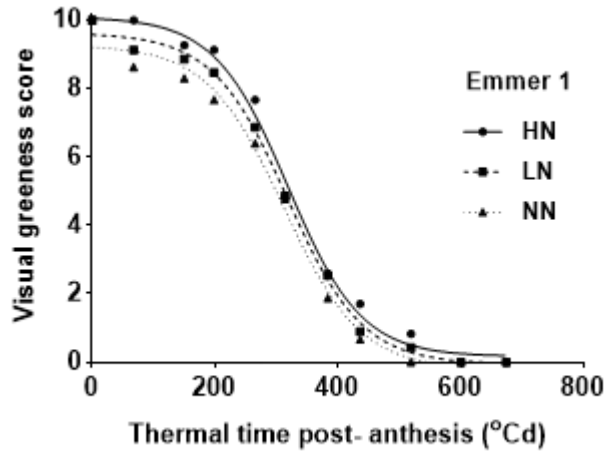
A


JB Diego
HN
LN
NN
Visual greeness score
Thermal time post- anthesis (°Cd)
0
2
4
6
8
10
0
200
400
600
800

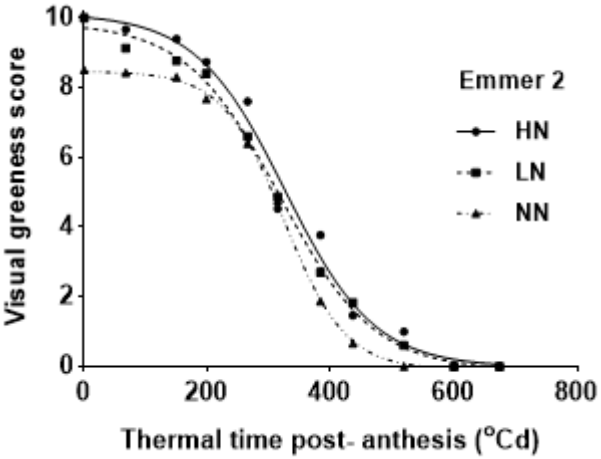
B


Xi 19
HN
LN
NN
Visual greeness score
Thermal time post- anthesis (°Cd)
0
2
4
6
8
10
0
200
400
600
800

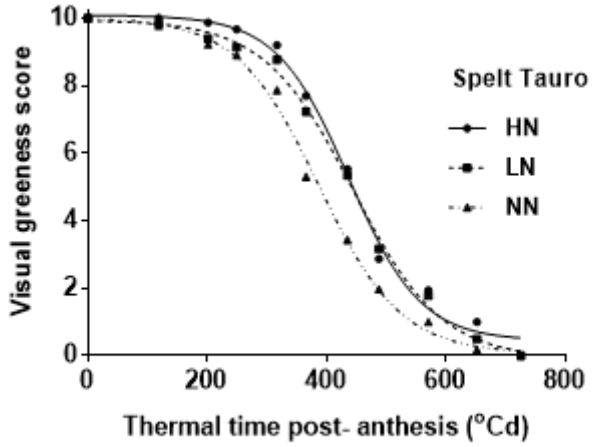
C


Emmer 1
HN
LN
NN
Visual greeness score
Thermal time post- anthesis (°Cd)
0
2
4
6
8
10
0
200
400
600
800

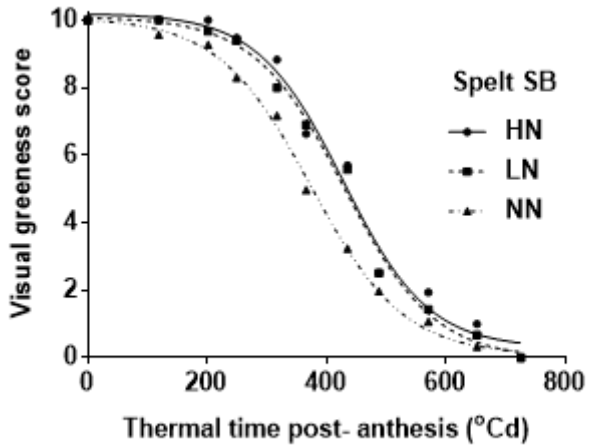
D


Emmer 2
HN
LN
NN
Visual greeness score
Thermal time post- anthesis (°Cd)
0
2
4
6
8
10
0
200
400
600
800

E



F



G

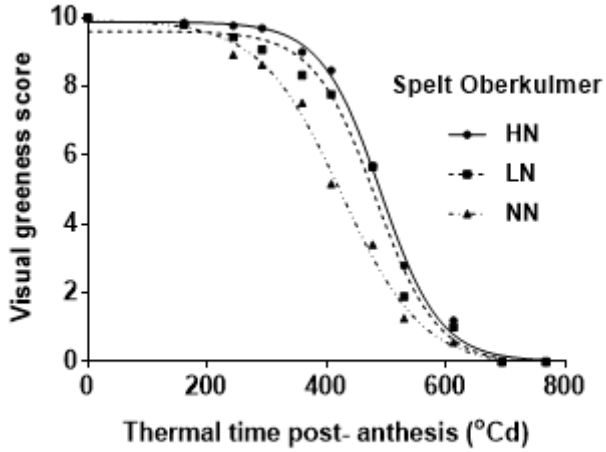


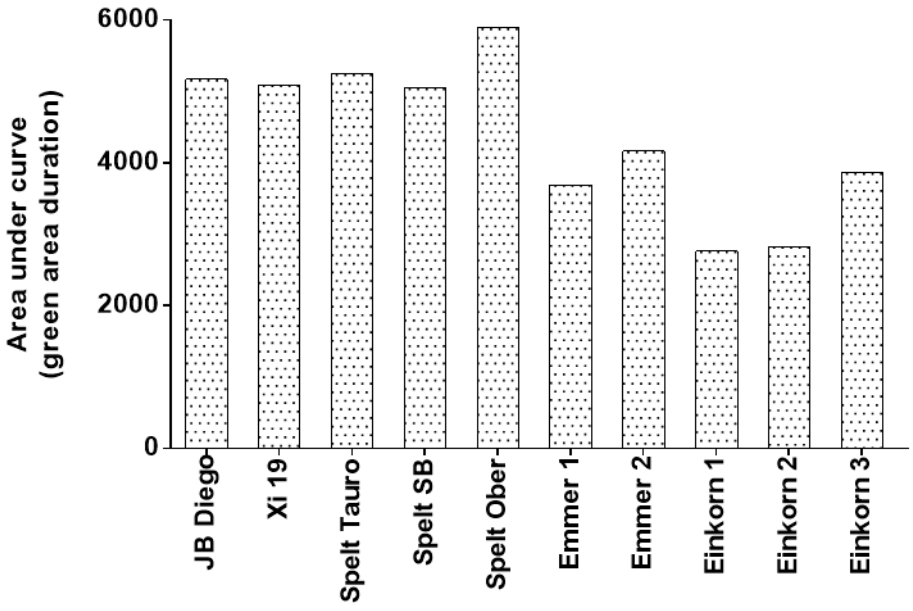
Figure 3.20 Observed values and fitted curves (estimated parameters are presented in Table 4.16) for post- anthesis flag leaf greenness in the mean of visual score for (A) JB Diego (B) Xi 19 (C) Emmer 1 (D) Emmer 2 (E) Spelt Tauro (F) Spelt SB and (G) Spelt Oberkulmer in 2014 experiment under NN; zero N fertiliser, LN; low N fertiliser, HN; high N fertiliser

Green area duration

The green area duration (GAD; green area ^{o}Cd) was calculated (area under the curve) by summing the areas calculated between each assessment point. In the 2012 experiment, GAD was significantly different between genotypes ($P < 0.001$). Spelt cv. Oberkulmer had the highest GAD followed by spelt cv. Tauro. GAD of spelt cv. Oberkulmer and Tauro was statistically difference between each other and higher than bread wheat cv. JB Diego and Xi 19. As a species, spelt had the highest GAD followed by bread wheat, emmer and einkorn though GAD of spelt and bread wheat was not statistically different (Figure 3.21A) in 2012 field experiment. In 2014, genotype, N level and GT x N was highly significant for GAD ($P < 0.001$) (Figure 3.21B).

There was a significant correlation between green area duration and AGB production at maturity in 2012 (r = 0.66, $P < 0.05$) while explaining more than 37% of the variation in AGB production (Figure 3.22). However, the relationship between green area duration and AGB production of the genotypes in 2014 experiment was not significant but a positive trend was identified under HN (r = 0.55, $P < 0.2$)

A



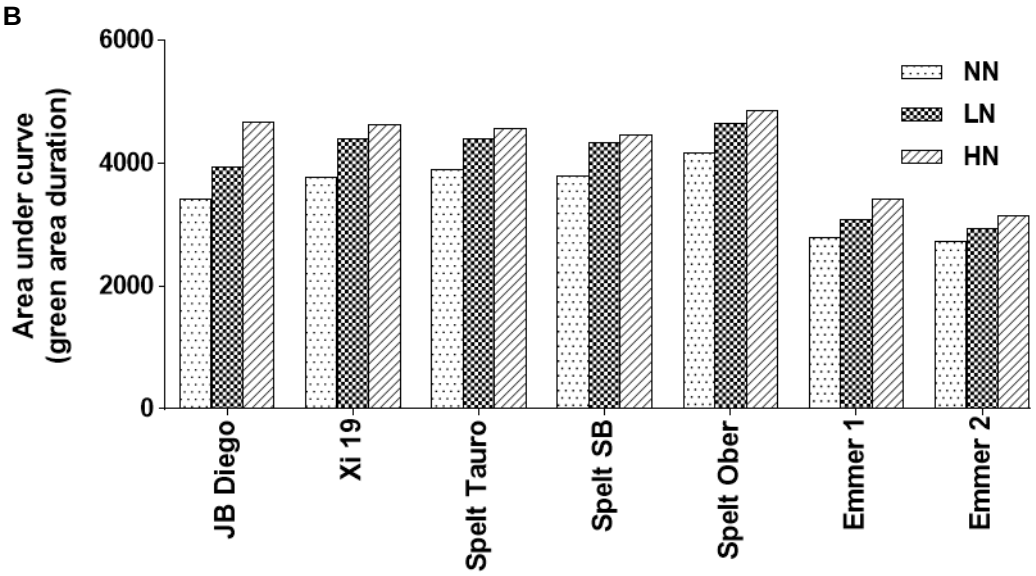


Figure 3.21 Area under greenness curve of genotypes (green area duration) (A) 2012 experiment (SED GT = 31.42, *df* = 27) and (B) 2014 experiment (SED GT x N = 80.55, *df* = 39.7)

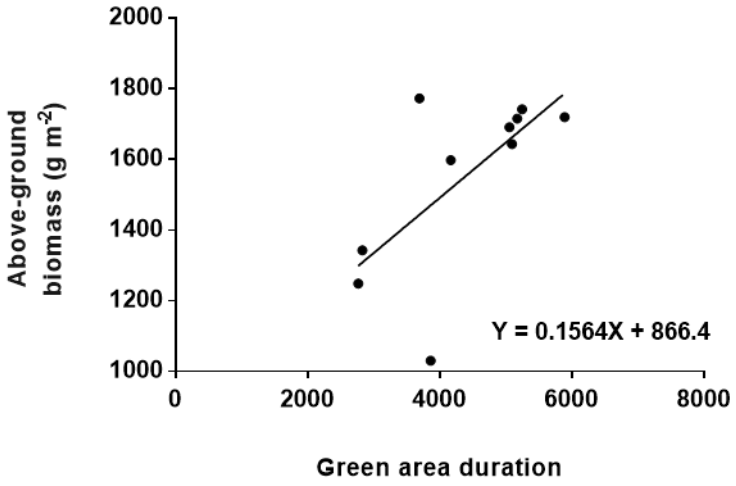


Figure 3.22 Linear regression between above-ground biomass production at maturity and green area duration for 10 genotypes at post-anthesis (area under curve) for 2012 field experiment

Relationship between SPAD and visual greenness score

A strong positive linear relationship was found between SPAD readings and visual greenness score during the post-anthesis grain filling period for all ten genotypes in 2012. Figure 3.23 shows comparison of regressions between visual greenness score and SPAD readings post-anthesis for bread wheat, spelt, emmer and einkorn (R^2 = 0.93, $P < 0.001$). When the SPAD value increased by one, visual greenness score

increased by 0.26, 0.64, 0.46 and 0.48 for bread wheat, spelt, emmer and einkorn, respectively.

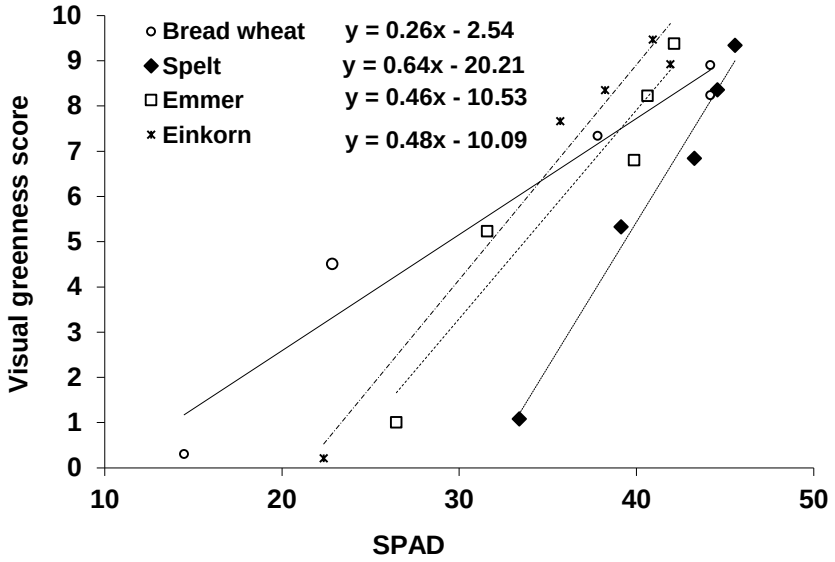
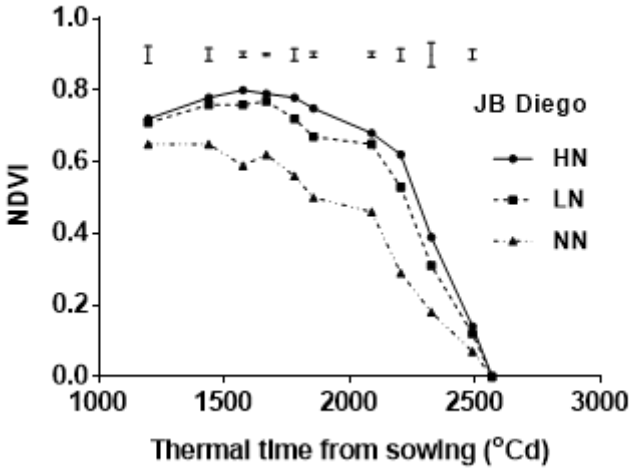


Figure 3.23 Comparison of regressions between visual greenness score and SPAD values of four wheat species (R^2 = 0.93, P < 0.001)

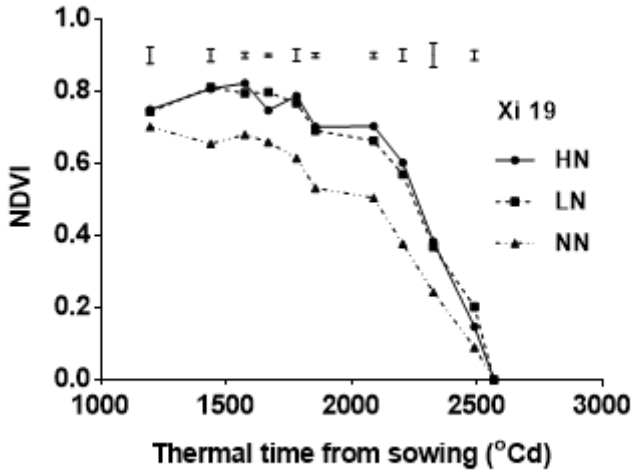
3.2.12.3 NDVI

Canopy NDVI was taken from GS31 until flag leaf fully senesced in 2014. At GS31 and GS61, only genotype and species were significant for NDVI (P < 0.001) (Figure 3.24 and 3.25). However, GT, N and GT x N effects were significantl for post-anthesis NDVI. Table 3.18 shows NDVI in 2014 field experiment under HN condition. According to that, NDVI of all spelt genotypes were significantly higher than bread wheat and emmer during late-grain filling period, especially spelt cv. Oberkulmer. A strong linear relationship was found between SPAD readings and NDVI. The relationship between visual greenness score of flag leaf and post-anthesis NDVI for all species under any N level was also significant.

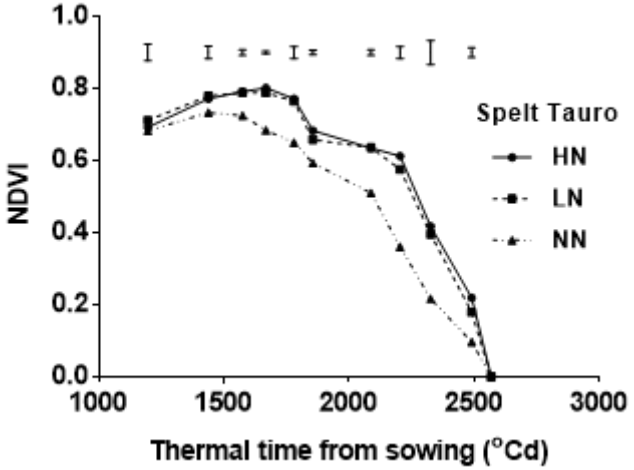
A


JB Diego
HN
LN
NN
NDVI
1.0
0.8
0.6
0.4
0.2
0.0
1000
1500
2000
2500
3000
Thermal time from sowing (°Cd)

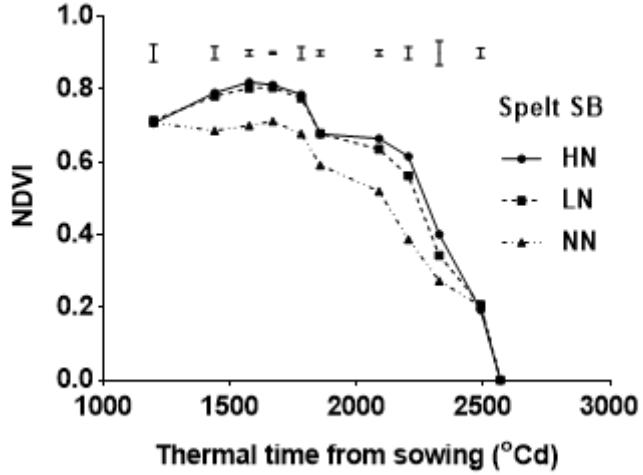
B

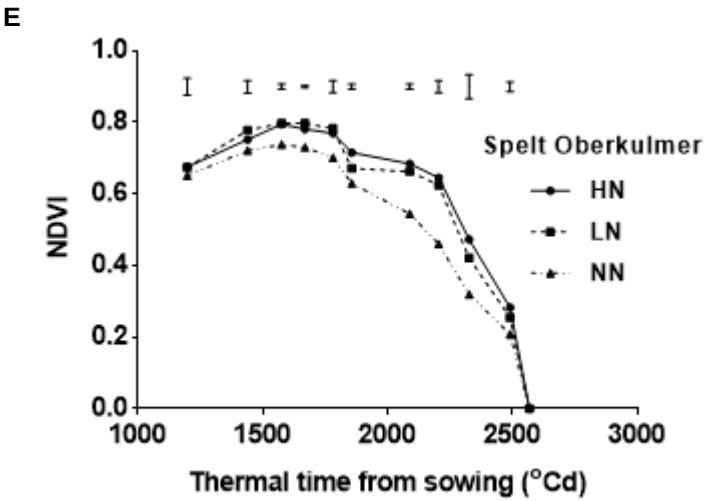

Xi 19
HN
LN
NN
NDVI
1.0
0.8
0.6
0.4
0.2
0.0
1000
1500
2000
2500
3000
Thermal time from sowing (°Cd)

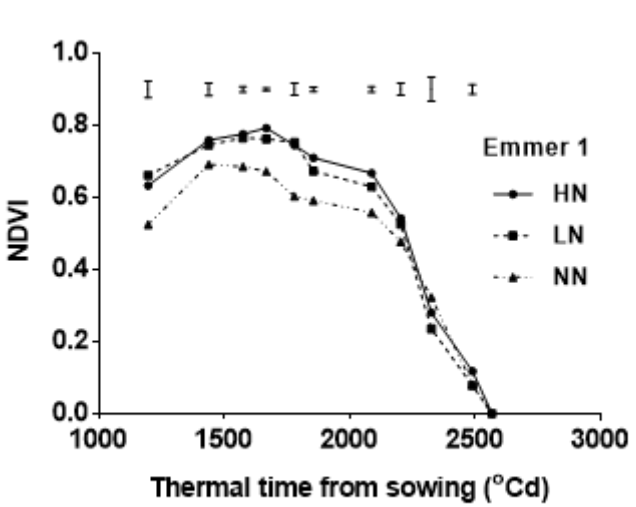
C


Spelt Tauro
HN
LN
NN
NDVI
1.0
0.8
0.6
0.4
0.2
0.0
1000
1500
2000
2500
3000
Thermal time from sowing (°Cd)

D


Spelt SB
HN
LN
NN
NDVI
1.0
0.8
0.6
0.4
0.2
0.0
1000
1500
2000
2500
3000
Thermal time from sowing (°Cd)





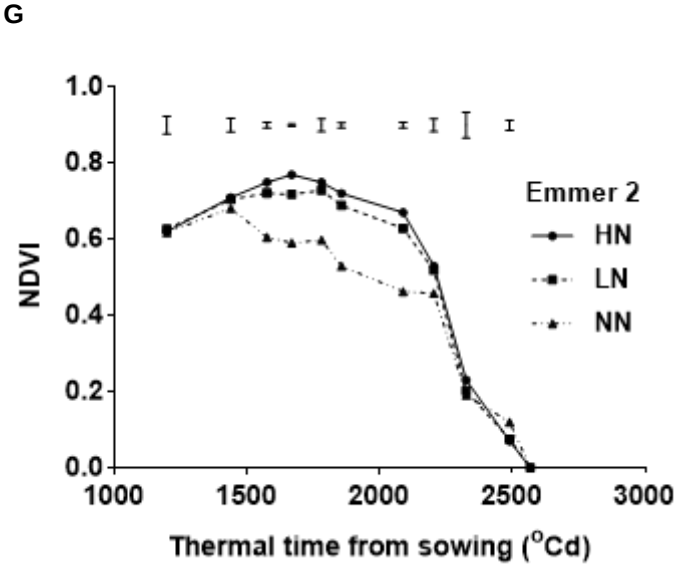


Figure 3.24 NDVI at three N levels (zer, low and high) in 2014 experiment for (A) JB Diego (B) Xi 19 (C) Spelt Tauro (D) Spelt SB (E) Spelt Oberkulmer (F) Emmer 1 and (G) Emmer 2. Bars indicate SED N

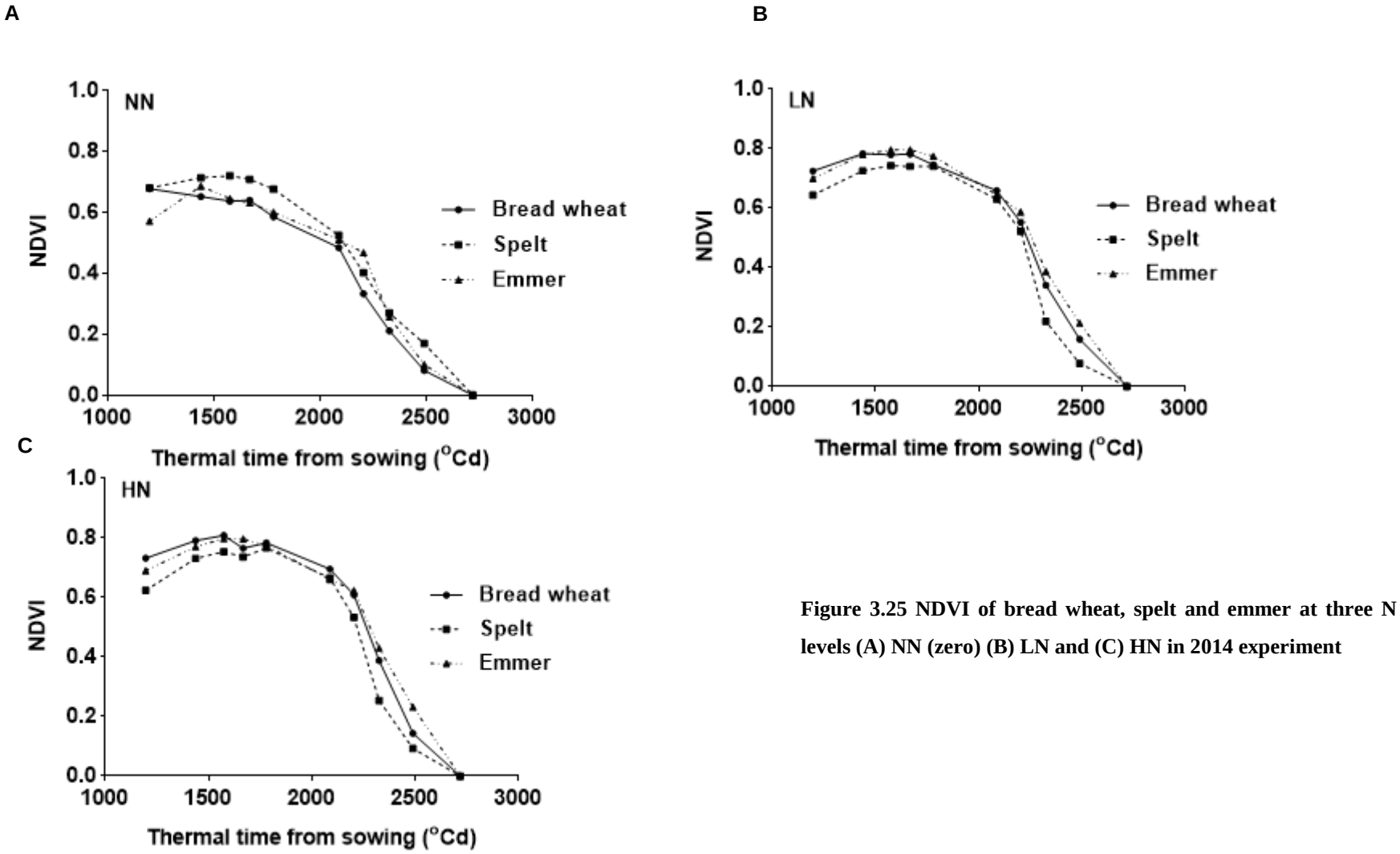


Figure 3.25 NDVI of bread wheat, spelt and emmer at three N levels (A) NN (zero) (B) LN and (C) HN in 2014 experiment

Table 3.18 NDVI of the genotypes in 2014 field experiment under HN treatment

GT	NDVI at 2014 field experiment under HN (150 kg N ha^{-1})									
	30th April	**19th May**	**26th May**	**06th June**	**13th June**	**18th June**	**3rd July**	**10th July**	**17th July**	**25th July**
JB Diego	0.72	0.78	0.80	0.79	0.78	0.75	0.68	0.62	0.39	0.14
Xi 19	0.75	0.81	0.82	0.75	0.79	0.70	0.70	0.60	0.38	0.15
Spelt Tauro	0.69	0.77	0.79	0.80	0.77	0.68	0.63	0.61	0.42	0.22
Spelt SB	0.71	0.79	0.82	0.81	0.79	0.68	0.66	0.62	0.40	0.19
Spelt Oberkulmer	0.68	0.75	0.79	0.78	0.77	0.72	0.68	0.65	0.47	0.28
Emmer 1	0.63	0.76	0.78	0.79	0.74	0.71	0.67	0.54	0.28	0.12
Emmer 2	0.62	0.71	0.75	0.77	0.75	0.72	0.67	0.53	0.23	0.07
SED (*df* = 30)	0.0133***	0.0186***	0.0083***	0.0119***	0.0089***	0.0122***	0.0166***	0.0191***	0.0215***	0.0133***

***** Significant at *P* < 0.001**

3.3 Discussion

General crop growth and development together with stay-green properties of three ancient hulled wheat species of einkorn, cultivated emmer and spelt, together with modern bread wheat are discussed in this results chapter. Currently, some of these ancient wheat species are not cultivated commercially (Salamini *et al.,* 2002). However, plant breeders and researchers use them as genetic resources to improve modern bread wheat. Contribution of *B* genome for modern wheat was from emmer but *A* and *D* genomes are not directly from einkorn and spelt, respectively. Therefore, studying einkorn, emmer and spelt through efficient, accurate and systematic evaluation, is useful since it provides valuable information about their growth, structure, resource capture/use and grain production, which can be used for further improvement of modern bread wheat.

3.3.1 Plant morphology, growth and development

3.3.1.1 Plant establishment

Plant establishments of ancient wheat species were poor compared with bread wheat cultivars in all seasons. However, in 2012 and 2014 general plant establishment was higher than 2013. Seeds of ancient and bread wheat were untreated which may be an important factor first to determine plant emergence. Seeds are usually treated with fungicide to prevent seed-borne disease and hence improve seed germination and plant establishment. Quality of the seed and the favourable weather conditions at seed sowing are also very important for early plant establishment. Poor plant establishment in 2013 may be due to the quality of the seed which were obtained from previous experiment where severe fusarium head blight was reported. Seeds were drilled without seed treatments and hence there was a possibility to have fusarium seedling blight (Paveley *et al.*, 2000). Additionally, damping-off often occurs when the disease is seed-born. *Fusarium graminearum* is associated with the cereal damping-off complex which results poor germination hence reduce plant density.

Mean air temperature was relatively low and the prolonged winter in 2013 may have had an adverse effect on plant establishment. Sowing density is one of the most important factors which affects plant establishment for winter sown cereals. Sowing density of 2013 experiment was 16% and 30% less than that of 2012 and 2014

experiments, respectively. This was due to limited availability of seeds from the previous crop. Threshability of ancient wheat species are very low (2 to 40%) hence seeds were threshed 2-3 times using drum thresher just after harvest. It may cause seed damage and resulting in broken seeds. Further, einkorn drilled with glumes which may affect significantly on seed germination especially under harsh weather conditions. However, Ruegger and Winzeler (1993) suggested that, if the weather is unfavourable, it is better to sow spelt as spikelets, as glumes protect grains from unfavourable weather conditions such as waterlogging and fungal colonisations.

Sowing date is one of the key factors affecting plant establishment. Based on the results of three experiments, Spink *et al*. (2000) concluded that, economic optimum plant density for late-September, mid-October and mid-November sowings was 62, 93 and 139 plants m^{-2}. Further, increased shoot number per plant and grain number per ear can be achieved as a result of compensation for reduced population.

3.3.1.2 Plant development

Bread wheat cvs. developed faster than ancient wheat genotypes in all experiments. Development of einkorn genotypes was slower than other genotypes. However, the gap between bread wheat and einkorn to reach to particular growth stage narrowed gradually with plant development (Parmar, 2014). Bread wheat cvs. matured 3 to 4 days earlier than others. Maturity date of bread wheat and emmer were similar for all three experiments while spelt took 3 to 4 more days to be fully senesced.

3.3.1.3 Plant growth

Most of the physiological parameters discussed in this chapter were assessed comparatively however the developmental stages of different genotypes varied significantly. This is due to sampling date which was decided by 50% of the genotypes of the experiment. Above-ground biomass (AGB) was calculated based on quadrat samples collected on calendar dates when 50% of the genotypes reached a particular growth stage. Therefore, AGB of einkorn species was always less than that of other species in all experiments under any N supply. To avoid the misunderstanding of the relationship between different genotypes at different growth stages, important physiological data are presented against thermal time not on calendar date.

3.3.1.4 Above-ground biomass production

AGB production of bread wheat, spelt and emmer was not significantly different in 2012 season though einkorn had less AGB at maturity. This was partially consistent with previous findings that the biomass production and yield of spelt and bread wheat were either similar or that bread wheat was higher under favourable conditions (Ruegger and Winzeler 1993). In 2013, at NN, bread wheat cvs, spelt cv. Oberkulmer and emmer 2 had higher AGB than other genotypes. AGB increased with N supply. The difference of AGB at HN and NN was high in spelt cv. Oberkulmer while low in emmer 2 and einkorn 1. Difference in AGB between LN and HN was not significant for all genotypes. Spelt performed well under N limited conditions. Moudry and Dvoracek (1999) suggested that spelt is able to utilise nutrients more efficiently when grown in a low input conditions. Remarkably, bread wheat, spelt and emmer were not significantly different in AGB production at maturity in 2014. However, N supply increased AGB of all genotypes. Nevertheless, emmer genotypes did not produce significantly high AGB at HN when compared to NN. This may be due to poor plant growth in non-lodged areas where quadrat samples were taken. Emmer has long and weak culms which easily lodge under windy (Marino, *et al*., 2009) and wet conditions, especially under high N. Further, emmer needs very low N addition due to its susceptibility to lodging. Giunta *et al*. (2007) reported positive correlation between N fertiliser application and seasonal trends on lodging of durum wheat. Similarly, in 2012, lodging rate of emmer was more than 90% and influenced by above average summer rainfall.

3.3.1.5 Above-ground biomass partitioning

Even though AGB at maturity was not significantly different between bread wheat, spelt and emmer, grain yield was different. When we consider dry matter partitioning, it was clear that bread wheat allocated a higher proportion of dry mater toward ear production at maturity while spelt, emmer and einkorn toward true stem plus leaf sheath at any level of N supply. In addition, bread wheat allocated more than 70% of ear biomass to produce grains while spelt, emmer and einkorn used 65%, 55%, and 40% of ear biomass for grain production. These results are in agreement with Laghetti *et al*. (1999), who reported that, husk values of ancient wheat are significantly higher,

as a mean of applied treatment. Further the percentage of chaff was apparently influenced by N application (Mariani *et al*., 2009).

3.3.1.6 Grain yield and HI

Grain yield of bread wheat cv. JB Diego was higher in 2012 though cv. Xi 19, spelt SB and spelt Oberkulmer were not significantly different. Increased grain yield of bread wheat is due to improved harvest index. The number of grains per ear was higher in bread wheat than spelt. However, thousand grain weight (TGW) of spelt was higher than bread wheat (Ruegger and Winzeler, 1993).

In 2013, grain yield increased with N supply though the difference in grain yield at HN and LN was not significant for genotypes while HN had more grain yield than NN. Grain yield of bread wheat was higher than other species despite the N supply. In 2014, a similar trend was observed for grain yield under different N supply except emmer genotypes, which reported less grain yield at HN than LN. As mentioned earlier, low grain yield at HN was possibly due to poor plant growth and plant density in non-lodged areas where quadrat samples were taken. However, no increase grain yield with N application for emmer was found by Castagna *et al*. (1992).

In general, grain yield of genotypes in 2012 was lower than that of 2013 and 2014. The possible reasons for low yield in 2012 would be low solar radiation, high rainfall and fusarium head blight disease during post-anthesis grain filling. There was a negative relationship between disease severity and grain yield. In 2013, despite poor plant establishment, all HN and LN plots received N calculated based on plot area where the number of plants per unit area reduced due to poor establishment, hence remaining plants received more nutrients, space and solar radiation. Therefore, the high grain yield in 2013 may be due to less competition between individual plants in a given plot. Further, incident solar radiation from sowing to harvest was higher in 2013 while lowest in 2012.

HI of the genotypes in 2012 and 2014 experiments were significantly smaller than 2013 despite N treatment. In 2013, highest HI was observed in NN than LN and HN. HI of bread wheat in 2012 and 2014 experiments were less than 0.4, which was bit lower than the value for elite cultivars of winter wheat. Gaju *et al*. (2011) reported HI of 16 UK and French bread wheat and feed wheat ranged from 0.44 to 0.60 depending on the site and season. The lower HI in 2012 and 2014 may be related to slower rate

of biomass remobilisation efficiency. In 2012, wet weather during post-anthesis grain filling may have an adverse effect on biomass remobilisation efficiency. Further, senescence rate of the genotypes were much lower in 2012 than 2014. N remaining in straw was significantly higher in 2012 than 2013 and 2014 (Chapter 4) which may be associated with delayed senescence and lower rate of biomass remobilisation.

3.3.1.7 Green area index

On average, green area index (GAI) increased gradually with plant development. In 2012, all bread wheat cvs., spelt cvs. and emmer 2 increased GAI until GS39 while emmer 1 and einkorn until GS61. However, bread wheat cv. JB Diego had the highest GAI throughout the experiment. GAI of JB Diego at GS39 and GS59 was more than 10. This can be explained by favourable weather conditions due to mild winter weather in early months of 2012 and high rainfall during stem elongation to anthesis together with N fertiliser application and high soil mineralisation (HGCA, 2008). On the other hand, plant establishment and seed rate of the experiment were relatively high in 2012. Large canopies have more leaf area than necessary to intercept all available sunlight hence extra cost to the plant. Also, they are highly susceptible to lodging and foliar diseases. Optimum canopy size to capture maximum sunlight at anthesis is about 6 (HGCA, 2008).

Overall GAI nearly doubled between NN and HN treatments for almost all of the genotypes in 2013 and 2014. As expected in the N limited treatment, N shortage was expressed through reduced GAI through restricted canopy expansion (Grindlay, 1997). This was clearer among bread wheat genotypes than ancient wheat species. Interestingly, at NN, spelt and emmer genotypes recorded the highest GAI at GS61 when compared with bread wheat and einkorn. However, it was clear that the plant establishment of spelt and emmer was less than bread wheat in 2013. The difference of GAI at NN in 2013 and 2014 season reflects the difference of soil mineral N availability in two experiment sites.

3.3.1.8 Plant height

Plant height is a valuable parameter when studying NUE due to significant contribution of N for plant structure. Ancient wheat genotypes were taller than bread wheat cvs. at maturity. Bread wheat contributed more accumulated biomass toward

ear growth while ancient genotypes used them for true stem plus leaf sheath (Toyota *et al.,* 2010). One of the most important factors contributing to increased wheat yields has been the introduction of semi-dwarfing genes *Rht1* and *Rht2* which are associated with reduced plant height as well as reduced lodging risks (Miralles and Slafer, 1997) together with biomass partitioning more towards grain production hence high HI.

In the experiments, particularly in 2012, high percentage of lodging occurred with tall ancient wheat species of emmer, einkorn and spelt. This was mainly due to plant height but accelerated by adverse weather conditions at anthesis and continued until end of the season. Therefore photosynthetic ability of the canopy drastically reduced depressing grain yield. Further, the lodging has a negative impact on the harvestable yields collected by combine harvester.

In 2013 and 2014, unfertilised plants were significantly shorter than fertilised plants. Reduced GAI consequently limited the C assimilates of the plant hence low yield (Ercoli *et al.,* 2008). However, plant height of all genotypes increased with N supply.

3.3.1.9 Flag leaf properties

Studying flag leaf properties is important since flag leaf is the last leaf developed before ear emergence hence last leaf to be senesced. Thef lag leaf is at the top of the plant and therefore has the ability to intercept more light than the lower leaves of the plant. Lupton (1972) based on modelling approaches estimated that at least 45% of the grain carbohydrates are derived from the flag leaf. Flag leaf length, width, green area, SLA and SPAD values were significantly different between genotypes. The greatest green area, SPAD value and maximum leaf width were recorded in bread wheat compared to ancient wheat species. However, longest flag leaves can be seen in spelt and emmer in 2012 while SLA was high in einkorn followed by emmer, spelt and bread wheat. There was a positive influence on flag leaf properties by N supply in 2013 but not in 2014. It seems that bread wheat used more assimilates to produce a strong flag leaf with high chlorophyll content, which had greater photosynthetic capacity than ancient wheat species. Further, it can be suggested that tall ancient wheat species invest more assimilates for structural materials of true stems and use less carbohydrate to develop flag leaf hence low SLA.

3.3.2 Canopy persistence and flag leaf green area

3.3.2.1 Visual greenness score

Two major breeding targets of modern bread wheat programmes are high yield and grain protein concentration. There is a negative relationship between grain yield and grain protein content (Slafer *et al*., 1990; Simmonds, 1995; Oury *et al*., 2003) hence, to increase grain yield without decreasing grain protein is one of the key challenges faced by wheat breeders. Physiological traits which have a positive influence on post-anthesis N uptake can be used to increase grain protein content without affecting grain yield (Bogard *et al*., 2010). Post-anthesis flag leaf senescence was identified as one of the potential physiological traits which could be used to improve both grain yield and grain protein concentration of bread wheat (Bogard *et al*., 2010).

Flag leaf senescence is a complex phenomenon. Both genetic (Christopher *et al*., 2008) and environmental conditions have significant effects on senescence dynamics by allowing recycling of nutrients from vegetative parts towards reproductive organs (Lim *et al*., 2007; Guiboileau *et al*., 2010). In wheat, leaf senescence has a strong relationship with remobilisation of N from leaf to grain resulting 40-90% of grain N from leaf (Van Sanford and Mackown, 1986; Kichey *et al*., 2007). Post-anthesis flag leaf senescence was affected by the balance between N supply and N demand (van Oosterom *et al*., 2010). Accelerated or delayed senescence may be a result of an imbalance of N supply and demand (Rajcan and Tollenaar, 1999). The increased temperature hastens the onset of senescence (Spiertz, 1977) while water and nutrient deficiencies, especially N, may have detrimental effect on flag leaf green area duration (Gregory *et al*., 1981). N uptake efficiency is significantly influenced by water availability post-anthesis. Limited water supply may disturb the balance of N supply and demand; hence accelerated leaf senescence can be seen (Gan and Amasino, 1977).

According to our experiments, in 2012, none of the genotypes started to senesce of flag leaf before anthesis. However, initial senescence rate of bread wheat, emmer and einkorn was higher than spelt genotypes. Further, senescence rate of spelt genotypes was much slower than others. Especially, timing of rapid flag leaf senescence, which describes the time (^{o}Cd) when absolute rate of flag leaf senescence is maximum, was higher in spelt cv. Oberkulmer (606 oCd). Spelt cv. Oberkulmer had a slow senescence rate until later–grain filling period. Statistical differences between spelt cv.

Oberkulmer and bread wheat cv. JB Diego was significant though spelt cv. SB and cv. Tauro was not different from either bread wheat cv. JB Diego or Xi 19 in 2012 field experiment (Table 3.16). Similarly, post-anthesis green area duration (GAD) was higher in spelt cv. Oberkulmer. GAD of spelt cv. Oberkulmer and Tauro were statistically difference between each other and higher than bread wheat cv. JB Diego and Xi 19 (Table 3.16). Further, SPAD values of spelt genotypes were higher than all other genotypes in the 2012 field experiment during late-grain filling period (Table 3.15). However, in the 2014 field experiment, timing of rapid flag leaf senescence is high in spelt cv. Oberkulmer than other spelt genotypes (Table. 3.17).

Stay-green genotypes usually have a delayed onset of senescence (Harris *et al*., 2007) and slow senescence rate. Bread wheat, emmer and einkorn completed flag leaf senescence four to five days earlier than spelt genotypes. In 2014, emmer genotypes together with bread wheat cv. JB Diego started flag leaf senescence before anthesis at NN. Flag leaf senescence completed six to seven days earlier at NN than HN, with little difference between LN and HN. Lack of N accelerates flag leaf senescence due to remobilisation of N and other nutrients from flag leaf to grain (Sinclaire and Amir, 1992). Green area duration of the genotypes increased with additional N supply in 2014.

A logistic model can be used to simulate the flag leaf senescence area with thermal time from flag leaf emergence (Carberry *et al*., 1993). Gooding *et al*. (2000) proposed that more variance can be accounted for by fitting a modified Gomperz model to the green area of flag leaf over time from flag leaf emergence than a logistic model. However, in our study, a standard logistic model fitted to observed values of senescence score accounted for more than 99% of variance for seven out of ten genotypes in the 2012 field experiment. Previous studies on senescence used a monomolecular-logistic equation describing leaf senescence kinetics during post-anthesis as a two phase process (Ge´nard *et al*., 1999; Bogard *et al*., 2011; Gaju *et al.,* 2011).

The date of onset and the rate of senescence determine C assimilation during post-anthesis grain filling (Thomas and Howarth, 2000). Hirel *et al*. (2007) suggested that C is as important as N since the plant needs energy and a favourable structure (shoot and root) for N uptake post-anthesis (Dreccer, 2005). Hence C and N metabolism is equally important for the senescence process (Spano *et al*., 2003). Delaying senescence

provides an opportunity to produce more C assimilates by prolonging photosynthesis. However, delayed senescence is not always beneficial, in terms of yield, if straw has more unused carbohydrate when the season terminates (Yang *et al*., 1997). Therefore, remobilisation of C and N is crucial in the senescence process.

A genotype with the ability to retain leaf green area for longer than an ordinary genotype is often called a "stay-green" phenotype (Thomas and Smart, 1993). The role of stay-green traits in increasing or maintaining high grain yield was discussed in previous studies (Borrell *et al*., 2001, Spano *et al*., 2003; Harris *et al*., 2007). However, some research evidenced that there is a negative relationship between stay-green traits and grain yield in rice (Jiang *et al*., 2004). Similarly in wheat, Gong *et al*. (2005) reported that delayed senescence of hybrid winter wheat cv. XN901 due to strong hybrid vigour which left more C in straw at maturity. Further, as a result of long post-anthesis grain filling period of cv. XN901 grain yield increased significantly but not HI when compared with ordinary cv. Shaan 229.

There were two main "stay-green" types called "functional stay-green" and "non-functional stay-green". In functional stay green, plants produce more assimilated C though active photosynthesis during post anthesis grain filling while in non-functional stay green, plant leaves remain green due to lesions of chlorophyll catabolism, which lack photosynthetic capacity (Jiang *et al*., 2004). Previous studies have identified wheat lines with functional stay-green though physiological processes underlying the results were not extensively studied (Foulkes *et al*., 2007). However, Christopher *et al*. (2008) working in Australia found that the stay-green property of spring wheat SeriM82 was due to extraction of deep soil water.

According to the results of our experiment in 2012, there is a positive relationship between above-ground biomass production at maturity and green area duration of the genotypes. Spelt cv. Oberkulmer and Tauro had greatest green area duration when compared to bread wheat, emmer and einkorn genotypes though spelt SB had low GAD. Stay-green properties of spelt are functioning (Parmar, 2014) hence green leaves are active in assimilating C during the grain filling period.

3.3.2.2 Chlorophyll concentration, SPAD and N status

SPAD values can be used as an indirect measurement of chlorophyll content since it corresponds to the amount of chlorophyll present in the leaf (Minolta, 1989). Rate of chlorophyll loss can be successfully assessed by using SPAD (Harris *et al*., 2007). The majority of leaf N is contained in chlorophyll molecules, and therefore the relationship between chlorophyll content and leaf N is reliable (Yoder and Pettigrew- Crosby, 1995). The relationship may vary from species to species depending on chlorophyll content and N status. Wood *et al*. (1993) suggested that, the relationship between chlorophyll concentration [chl] and leaf N may be nonlinear due to non-chlorophyll N accumulated in the leaf as nitrate (NO_3^-).

Previous studies have found that SPAD readings explained more than 93% of the variation of [chl] in rice, wheat and soybean (Monje and Bugbeee, 1992) while extractable chlorophyll for 11 food crop species were highly correlated with SPAD values (Marquad and Tipton, 1987). Under greenhouse conditions the relationship between [chl] of St. Augustine grass and SPAD values were significant ($R^2 = 0.79$) but correlation in the field study was not as strong as in the greenhouse (Rodrigue and Miller, 2000). In sorghum, the linear relationship was found between [chl] and SPAD values of the genotypes (Xu *et al*., 2000; Yamamoto *et al*., 2002).

According to the results of our studies, we have found that the strength of the relationship between actual [chl] and SPAD values varied depending on the species, under field condition. Yamamoto *et al*. (2002) reported a linear relationship between [chl] and SPAD for sorghum (*Sorghum bicolor*) and pigeon pea (*Cajanus cajan*). However, Uddling *et al*. (2007) observed non-linear relationship between [chl] and SPAD for birch (*Betula pendula*), wheat (*Triticum aestivum*) and potato (*Solanum tuberosum*) possibly due to non-uniform distribution of chlorophyll in leaf lamina.

In our experiment, we used only ten leaf samples to extract chlorophyll due to limited time availability. By increasing the number of samples, a stronger relationship between SPAD and actual [chl] might be achieved. Also, specific leaf area (SLA) (Broge and Mortensen, 2002) significantly affected this relationship. Peng *et al*. (1993) reported that the relationship between N concentration and SPAD readings in rice was highly affected by leaf thickness as reflected in specific leaf weight (SLW). Hoel *et al* (1998) found that, the irradiance has a significant effect on SPAD values by changing the

orientation of the chloroplasts within the cell. According to their results, the highest SPAD value was observed at low irradiance under glasshouse conditions while the lowest were measured at the high irradiance in the middle of the day. Most of the SPAD readings in our study, under field conditions, have been taken in the middle of the day. Therefore irradiance should be considered when the SPAD reading is to be used to estimate crop N status.

3.3.2.3 NDVI

Normalised vegetation index (NDVI) was significantly affected by increased plant density, N treatment and growth stage. Increase in greenness may result in low reflectance of red waves due to increase absorption due to high density of pigments per unit area while the opposite effect was observed for near-infrared reflectance (Hinzman *et al*., 1986). NDVI was higher under HN treated plots than LN and NN plots for almost all of the genotypes in our study and had a strong relationship between SPAD readings and visual score of flag leaf senescence. NDVI can be used to identify the stay-green genotypes successfully (Lopes and Reynolds 2012; Christopher *et al*., 2014). The genotypes which can maintain maximum greenness close to maturity had strong relationship with grain yield under stress environments (Lopes and Reynolds 2012).

3.4 Conclusions

- Plant growth and development were significantly different between genotypes used in the study. Above-ground biomass of bread wheat, spelt and emmer was either similar or bread wheat was slightly higher at maturity.
- High grain yield of bread wheat was due to improved harvest index when compared to tall ancient wheat species. Bread wheat allocated more C assimilates towards ear production than ancient wheat genotypes. Also, the highest ear partitioning (grain: chaff; 7: 3) was recorded in bread wheat.
- Green area partitioning was significantly different among bread wheat and ancient wheat species where green leaf lamina contributes more towards GAI of the canopy at anthesis in bread wheat.
- Delayed onset of flag leaf senescence, slow senescence rate and prolonged leaf greenness were observed in spelt genotypes, especially in cv. Oberkulmer when compared to the other genotypes.
- SPAD declining, visual senescence score and NDVI during post-anthesis grain filling period have a strong relationship with above-ground biomass production at maturity.

Chapter 4

Nitrogen use efficiency and its components

4.1 Introduction

Nitrogen use efficiency (NUE) and its components, plant N uptake (N_{off}), N harvest index (NHI) and apparent fertiliser N recovery rate of ancient wheat species together with bread wheat are presented and discussed in detail in this chapter. The relationships between grain yield, above-ground biomass, green area, stay-green duration with NUE and its components are also discussed. Three field experiments (discussed in Chapter 3) and two glasshouse experiments were conducted to calculate NUE and related components. Above-ground biomass and grain yield from the glasshouse experiments are set out in this chapter with associated microclimate parameters.

NUE was calculated for four species under three N regimes in the field and two and three levels in glasshouse experiments in 2013 and 2014, respectively. The effect of genotype and N levels on N uptake efficiency (NUpE), N utilisation efficiency (NUtE) and consequently N use efficiency (NUE) are examined.

Objective

The main objective of this chapter is to quantify NUE and its components of ancient wheat species and bread wheat under field and controlled environment conditions. Four hypotheses are tested in this chapter.

Specific hypotheses

5. NUE and related components are different between genotypes used in the study and decrease with N supply.
6. NUpE of ancient wheat species are higher than bread wheat and associated with plant height and root system architecture.
7. NUtE of bread wheat is higher than ancient wheat species and closely related to harvest index.
8. NUpE is closely related to NUE at low N supply while NUtE is closely related to NUE at high N supply.

The relationship between NUpE and below ground physiological traits (root system architecture) of ancient wheat species are discussed in detail in Chapter 5.

4.2 Results

Results of three field experiments are presented. In 2012, only genotypic differences in NUE and related components are discussed. In 2013 and 2014, effect of genotype and N supply are considered and presented side by side to show the consistency of measured parameters.

Two glasshouse experiments were carried out to find out fertiliser NUE of the genotypes and root development in relation to N supply for ancient wheat species together with modern bread wheat.

4.2.1 2012 field experiment

A field experiment was conducted to determine the genotypic differences of NUE and related components in three ancient wheat species and bread wheat grown at the recommended N fertiliser level (total 220 kg N ha^{-1}). Altogether ten genotypes were used in this experiment (Chapter 2; section 2.2).

4.2.1.1 N uptake

4.2.1.1.1 Early seedling N content (above-ground)

N uptake of the genotypes at GS26 was calculated in February 2012, parallel to soil mineral N analysis. Seedling N% varied from 4.6 to 5.6% among genotypes ($P < 0.001$) (Figure 4.1). However, seedling N uptake (N_{off}; total N in the above-ground biomass) was higher in bread wheat cv. JB Diego, emmer 2 and spelt Tauro (Figure 4.2) while einkorn 2 and 3 had the lowest N_{off} ($P < 0.001$).

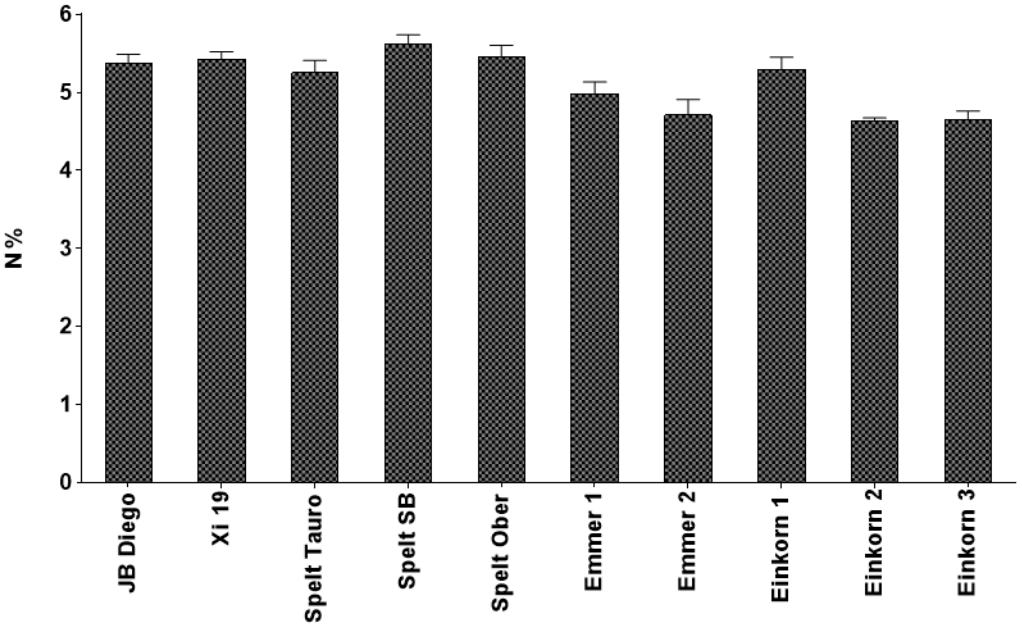


Figure 4.1 N% of the genotypes at GS26 in 2012 experiment. Error bars represent SE of the means of genotype (*df* = 27)

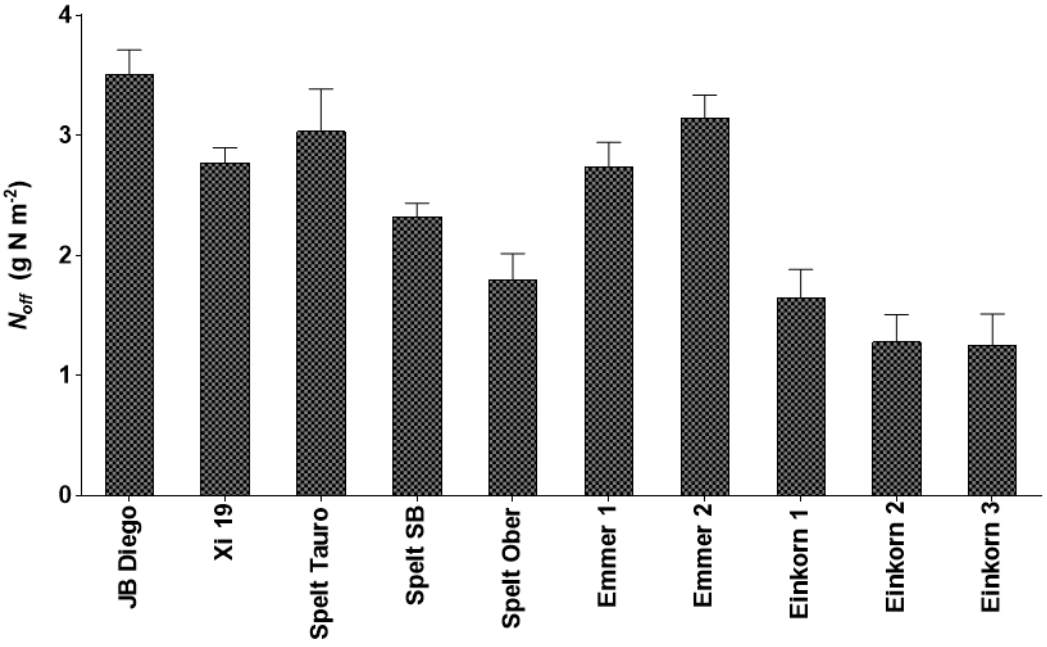


Figure 4.2 Seedling N_{off} of the genotypes at GS26 in 2012 experiment. Error bars represent SE of the means of genotype (*df* = 27)

4.2.1.2 N% of plant components at maturity

In 2012, N% of straw, chaff and grain varied significantly between genotypes ($P < 0.001$) and species ($P < 0.001$). Emmer 2 had the highest straw N% of 1.75 and grain N% of 4.28 while chaff N% was 1.53. The lowest straw, chaff and grain N% was

recorded in bread wheat cv. JB Diego and cv. Xi 19 though straw and chaff N% of spelt Oberkulmer was not significantly different. However, grain N % of spelt Oberkulmer was higher than bread wheat cvs (Figure 4.3). As a species, einkorn had the highest straw and chaff N% (1.68 and 1.89, respectively) while the lowest values were recorded in bread wheat (1.19%). Emmer had the highest grain N% followed by einkorn, spelt and bread wheat (Table 4.1).

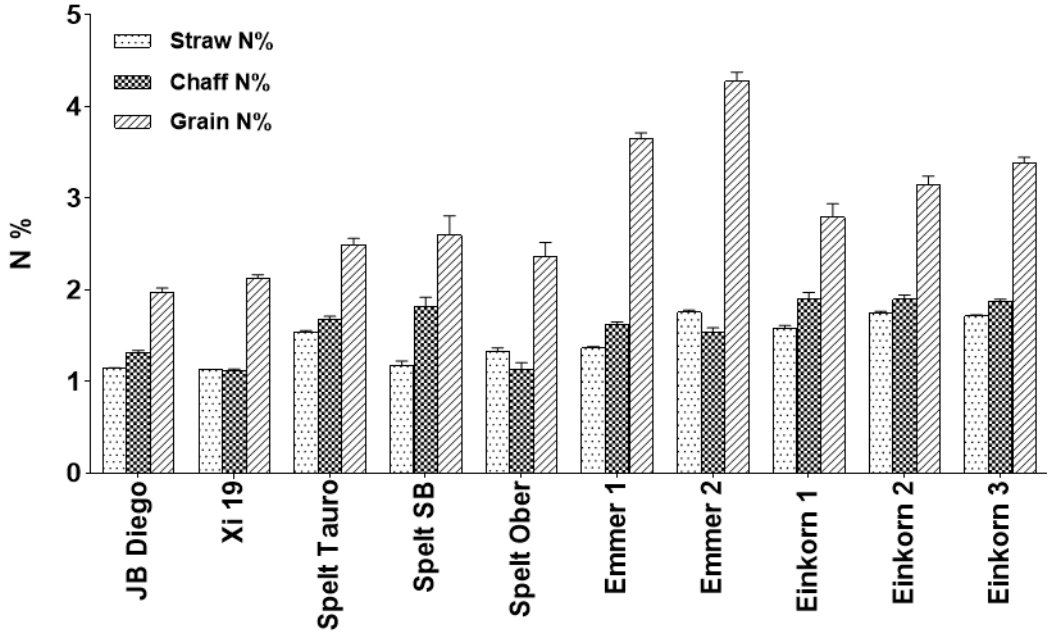


Figure 4.3 N % of straw, chaff and grain at maturity in 2012 experiment. Error bars represent SE of the mean of genotype (*df* = 27)

Table 4.1 N% of different crop components (straw, chaff and grain), remaining N in straw and chaff, and grain N (as a percentage of total N_{off}) and N_{off} of bread wheat, spelt, emmer and einkorn in 2012 experiment at maturity. Different letters indicate significant differences (Duncan's multiple range test, $P < 0.05$)

Species	N %			N_{off} (%)			N_{off} (kg N ha^{-1})
	Straw	Chaff	Grain	Straw	Chaff	Grain	
Bread wheat	1.19^{c}	1.19^{c}	2.15^{d}	40.92^{b}	11.58^{b}	47.51^{a}	255.31^{bc}
Spelt	1.35^{bc}	1.74^{ab}	2.54^{c}	47.14^{b}	14.44^{ab}	38.42^{a}	293.74^{b}
Emmer	1.56^{ab}	1.57^{b}	3.96^{a}	48.06^{b}	12.88^{b}	39.07^{a}	358.42^{a}
Einkorn	1.68^{a}	1.88^{a}	3.11^{b}	61.72^{a}	17.52^{a}	20.77^{b}	230.46^{c}

4.2.1.3 Total plant N uptake (N_{off})

N_{off} at maturity varied significantly between genotypes where emmer and spelt genotypes had high N_{off} while bread wheat and einkorn genotypes recorded the low values ($P < 0.001$). Additionally, emmer 2, as a genotype, had the highest $N_{off,}$ 48% higher than einkorn 3. When averaged across the species, the highest N_{off} was observed in emmer followed by spelt, bread wheat and einkorn (Table 4.1).

Contribution of different plant components (straw, chaff and grain) for N_{off} was significantly affected by genotype ($P < 0.01$). Einkorn 3 had the highest contribution of 66.8% from straw compared with 36% for bread wheat cv. Xi 19. N remaining in chaff as a proportion of N_{off} varied from 9% (emmer 2) to 19% (einkorn 2). As expected, bread wheat cv. Xi 19 and cv. JB Diego contributed highest proportion (> 50%) of N_{off} to the grain (NHI) while proportion of N_{off} allocated to grains of einkorn 2 and 3 was less than 16% (Figure 4.4). Nevertheless, on average, contribution of remaining N in straw, chaff and grain N for N_{off} was not significantly different between bread wheat, spelt and emmer but it was significantly high for straw and chaff while low for grain in einkorn (Table 4.1). The increased proportions of straw and chaff N represent the remaining N of the plant which may be associated with lower rate of N remobilisation. On the other hand, in ancient wheat species, due to plant height, plant structural N may be higher than bread wheat hence more N remained in straw.

4.2.1.4 N uptake efficiency (NUpE)

NUpE was calculated by dividing N_{off} by N availability for plants from soil and fertiliser (N_{ava}). Two methods were used to calculate N_{ava} hence two values for NUpE were recorded only in 2012 field experiment. According to the method 1, N_{ava} was a sum of SMN in February plus fertiliser N application throughout the growth season. In second method, SMN and plant N_{off} in February plus fertiliser N application were used as sources of N supply to the plant. However, ranking of the genotypes for NUpE was remained unchanged from 1 to 6 in both methods (Table 4.2). NUpE of emmer 2 was higher than all genotypes (method 1, Figure 4.5) ($P < 0.01$). The lowest NUpE was observed in einkorn 3. Nine out of ten genotypes had NUpE larger than one which was due to residual N in the soil and N supply through mineralisation during the season. For NUpE, the effect of error accounted for a larger percentage (66%) of the variation than genotype (34%). As a species, emmer had the highest NUpE followed

by spelt, bread wheat and einkorn. Therefore NUpE of emmer was 18, 29 and 36% higher than spelt, bread wheat and einkorn, correspondingly (Table 4.3).

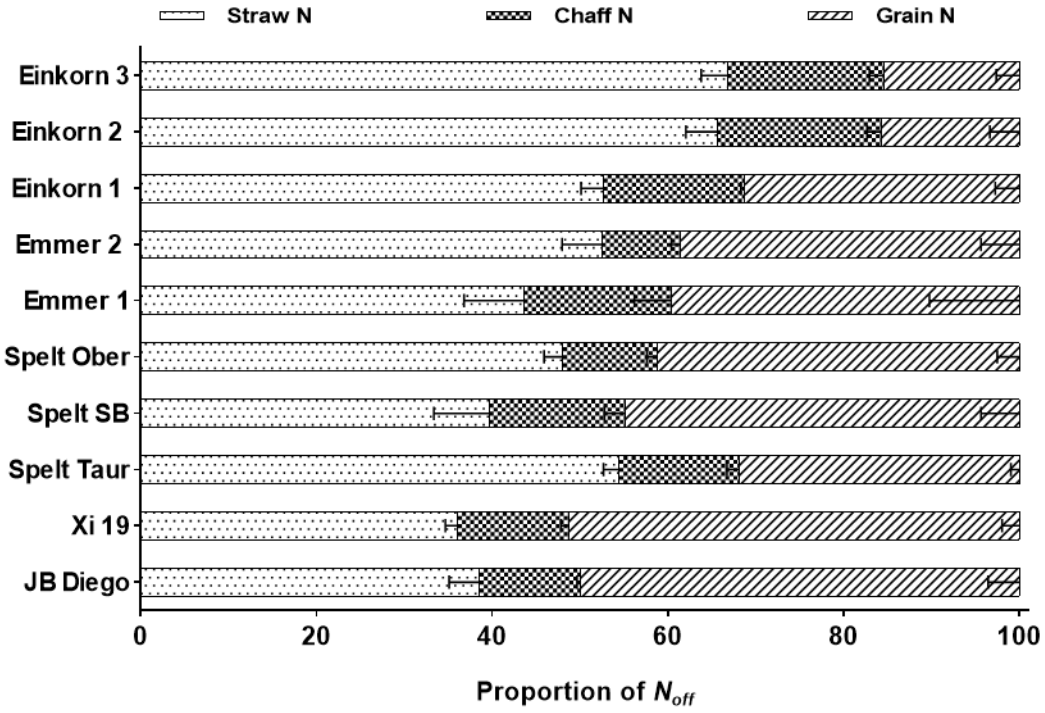


Figure 4.4 Remaining N in straw and chaff, and grain N as a proportion of N_{off} at maturity in 2012 experiment. Error bars represent SE of the mean of genotype (*df* = 27)

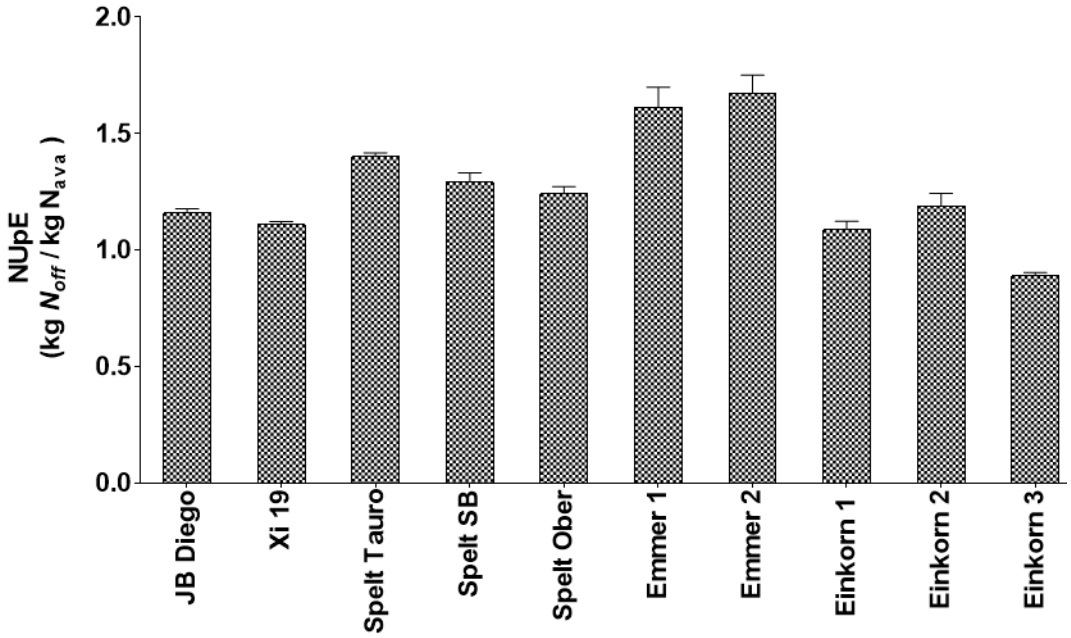


Figure 4.5 NUpE of ten genotypes at maturity in 2012 experiment. Error bars represent SE of the mean of genotype (*df* = 27)

Table 4.2 Comparing NUpE and rankings of genotypes for NUpE in 2012 field experiment. Two different methods were used to calculate NUpE at maturity. (In method 1: N_{ava} for the crop = SMN availability in February + fertiliser N; method 2: N_{ava} for the crop = SMN availability in February + crop N_{ava} in February + fertiliser N)

GT	Method 1		Method 2	
	NUpE (N_{off}/N_{ava})	Rank	NUpE (N_{off}/N_{ava})	Rank
JB Diego	1.16	7	1.00	8
Xi 19	1.11	8	0.99	9
Spelt Tauro	1.40	3	1.23	3
Spelt SB	1.29	4	1.17	4
Spelt Oberkulmer	1.24	5	1.15	5
Emmer 1	1.61	2	1.43	2
Emmer 2	1.67	1	1.47	1
Einkorn 1	1.09	9	1.01	7
Einkorn 2	1.19	6	1.12	6
Einkorn 3	0.89	10	0.84	10
SED ; GT (*df*)	0.209 (27)*		0.186 (27)*	

4.2.1.5 N utilisation efficiency (NUtE)

NUtE was significantly affected by genotype ($P < 0.001$) where bread wheat cvs had the highest NUtE (26.18 for cv. JB Diego and 24.12 for cv. Xi 19) while einkorn 2 (5.24) and einkorn 3 (4.61) had lowest values (Figure 4.6). The contribution from genotype for the total variance was 90% hence NUtE was mainly controlled by genetics. As a species, bread wheat had the highest NUtE followed by spelt, emmer and einkorn (Table 4.3).

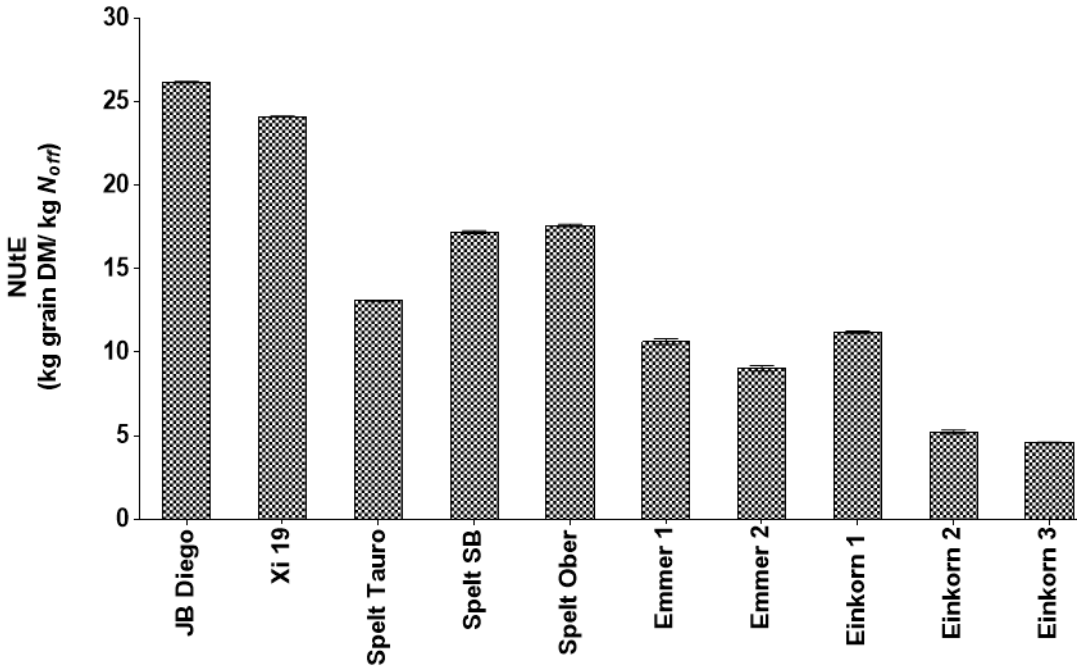


Figure 4.6 NUtE of ten genotypes at maturity in 2012 experiment. Error bars represent SE of the mean of genotype (*df* = 27)

4.2.1.6 N use efficiency (NUE)

The highest NUE was recorded in bread wheat cv. JB Diego (29.95) while the lowest value was observed for einkorn 3 (4.14) ($P < 0.001$). Nevertheless, NUE of spelt SB and spelt Oberkulmer was not significantly different from bread wheat cv. JB Diego and cv. Xi 19 (Figure 4.7). The effect of genotype accounted for 66% of the total variance. As a species, as expected, NUE of bread wheat was higher than three ancient wheat species where NUE of bread wheat was 22, 33 and 71% greater than spelt, emmer and einkorn, respectively. NUE was not significantly different between spelt and emmer (Table 4.3).

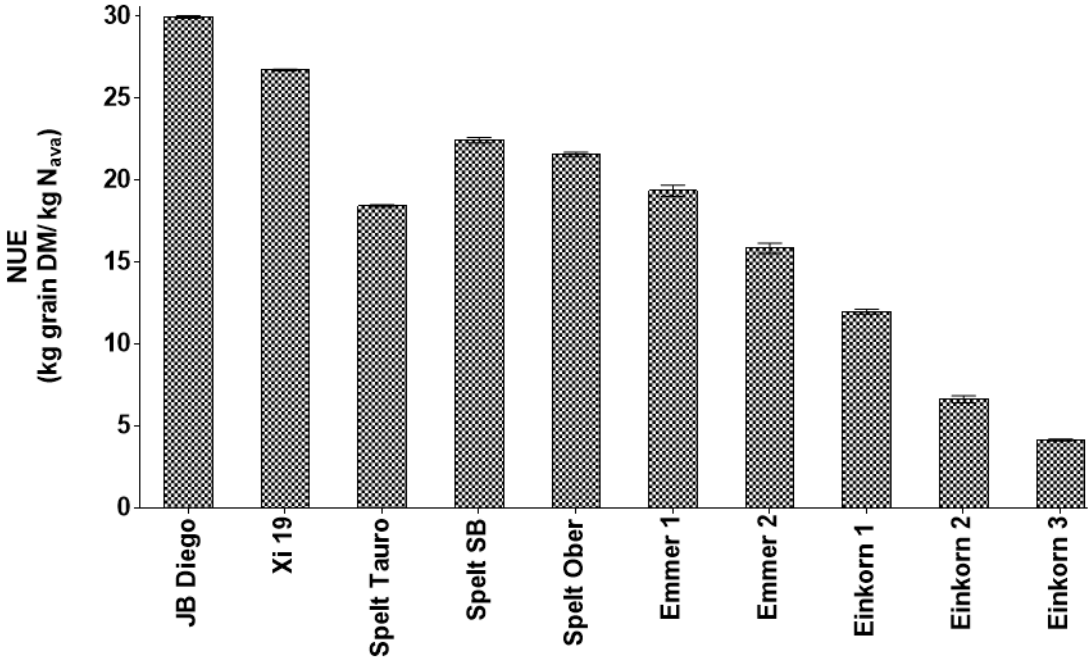


Figure 4.7 NUE of ten genotypes at maturity in 2012 experiment. Error bars represent SE of the mean of genotype (*df* = 27)

Table 4.3 N uptake efficiency (NUpE; kg N_{off} /kg N_{ava})), N utilisation efficiency (NUtE; kg grain DM/kg N_{off}), N use efficiency (NUE; kg grain DM/ kg N_{ava}) and N harvest index (NHI) at maturity in 2012 experiment. Different letters indicate significant differences (Duncan's multiple range test, $P < 0.05$)

Species	NUpE (kg N_{off} /kg N_{ava})	NUtE (kg grain DM/kg N_{off})	NUE (kg grain DM/ kg N_{ava})	NHI
Bread wheat	1.17^{bc}	22.63^{a}	26.08^{a}	0.47^{a}
Spelt	1.35^{b}	15.13^{b}	20.42^{b}	0.38^{a}
Emmer	1.64^{a}	9.84^{c}	17.59^{b}	0.39^{a}
Einkorn	1.06^{c}	7.01^{c}	7.58^{c}	0.21^{b}

4.2.1.7 N harvest index (NHI)

Effect of genotype and species for NHI was significant ($P < 0.001$) where bread wheat cv. Xi 19 had the highest NHI of 0.51 and the einkorn 3 had the least of 0.15. 71% of the total variance was explained by genotypes for NHI. When averaged across the species, bread wheat, spelt and emmer had higher NHI than einkorn (Table 4.3).

4.2.2 2013 and 2014 field experiments

4.2.2.1 N% of plant components at maturity

Straw, chaff and grain N% were significantly affected by genotype ($P < 0.05$), N treatment ($P < 0.01$) and GT x N ($P < 0.001$) for both experiments. N% increased significantly with N supply for all components (Table 4.4 and 4.5). In 2013, the lowest grain N% was recorded in bread wheat cultivars for all N levels while highest value was recorded in emmer 2, einkorn 1 and 2 at HN. Straw N% was significantly higher in ancient wheat species than bread wheat under LN and HN but einkorn 2 recorded low straw N% at NN.

In 2014, emmer 2 had the highest grain N% at HN followed by emmer 1. Grain N% of bread wheat cultivars was significantly lower than all other genotypes at all N levels. Also, straw N% of bread wheat cultivars at HN was significantly lower than ancient wheat species.

Table 4.4 N% of straw, chaff and grain in 2013 experiments for three N levels (NN; zero fertiliser applied, LN; 100 kg N ha^{-1} and HN; 200 kg N ha^{-1})

GT	Straw N%			Chaff N%			Grain N%		
	NN	LN	HN	NN	LN	HN	NN	LN	HN
JB Diego	0.48	0.53	0.65	0.74	0.68	0.85	1.60	1.93	2.26
Xi 19	0.48	0.50	0.68	0.73	0.76	0.91	1.63	2.06	2.31
Spelt Tauro	0.54	0.54	0.76	0.88	0.91	0.98	2.48	2.75	3.01
Spelt SB	0.46	0.55	0.82	0.80	0.81	0.83	2.35	2.76	2.88
Spelt Oberkulmer	0.44	0.64	0.74	0.82	0.94	0.88	2.56	2.87	2.90
Emmer 2	0.44	0.51	0.72	0.46	0.61	0.73	2.15	3.03	3.44
Einkorn 1	0.49	0.87	0.79	0.57	0.98	0.87	2.85	3.65	3.31
Einkorn 2	0.34	0.65	0.79	0.54	0.63	0.74	2.56	3.13	3.05
SED; GT (*df*)	0.022 (42)***			0.031 (42)***			0.046 (42)***		
N (*df*)	0.015 (4)***			0.024 (4)**			0.037 (4)***		
GT x N (*df*)	0.039 (43.5)***			0.056 (40.6)***			0.083 (39.9)***		

***** Significant at $P < 0.001$, **significant at $P < 0.01$**

Table 4.5 N% of straw, chaff and grain in 2014 experiments for three N levels (NN; zero fertiliser applied, LN; 100 kg N ha^{-1} and HN; 150 kg N ha^{-1})

GT	Straw N%			Chaff N%			Grain N%		
	NN	LN	HN	NN	LN	HN	NN	LN	HN
JB Diego	0.36	0.45	0.67	0.50	0.54	0.71	1.33	1.66	2.02
Xi 19	0.41	0.53	0.68	0.49	0.53	0.68	1.60	1.86	2.21
Spelt Tauro	0.34	0.45	0.94	0.40	0.51	0.46	2.10	2.80	2.98
Spelt SB	0.28	0.44	0.89	0.37	0.58	0.58	1.97	2.50	2.77
Spelt Oberkulmer	0.31	0.52	0.79	0.38	0.56	0.63	2.14	2.38	2.98
Emmer 1	0.36	0.49	0.85	0.34	0.39	0.90	2.00	2.54	3.89
Emmer 2	0.30	0.40	0.91	0.41	0.78	0.86	1.77	2.61	4.11
SED ; GT (*df*)		0.024 (36)*			0.021 (36)***			0.037 (36)***	
N (*df*)		0.009 (4)***			0.017 (4)**			0.050 (4)***	
GT x N (*df*)		0.039 (39.1)***			0.039 (36.3)***			0.079 (18.7)***	

***** Significant at *P* < 0.001, **significant at *P* < 0.01, *significant at *P* < 0.05**

4.2.2.2 Total plant N uptake (N_{off})

In 2013, spelt Tauro had the highest N_{off} at NN and HN while einkorn 2 recorded the lowest value at NN and einkorn 1 at HN (Table 4.6). When averaged across the species at NN, the highest N_{off} was observed in spelt followed by emmer, bread wheat and einkorn while at HN, emmer took up most N hence N_{off} was higher than other species. N_{off} differed significantly between genotypes ($P < 0.05$) and N treatment ($P < 0.001$) in 2014. Spelt Oberkulmer had the most N_{off} at NN while emmer 2 uptake most N at HN (Table 4.6). N treatment ($P < 0.001$) was highly significant for N_{off} of the species ($P < 0.05$) where spelt and emmer had higher N_{off} than bread wheat regardless of the N treatment.

In general, in 2013, about 17% of the N_{off} remained in straw for all genotypes at maturity and this value increased with N supply ($P < 0.05$). However in 2014, genotypes ($P < 0.001$) differed in their N_{off} in straw and there was a GT x N ($P < 0.01$). N supply had a positive relationship with remaining N in straw (19.78%). Therefore, it can be said that the increased supply of N fertiliser may increase N remaining in straw.

In both experiments, percentage of N_{off} remaining in chaff at maturity was significantly affected by genotype ($P < 0.001$) and GT x N ($P < 0.01$) where 8.5% and 13.5% of the N_{off} was due to remaining N in chaff in 2013 and 2014, respectively. There was no effect of main factors and the interaction for the contribution of grain N for N_{off} (NHI) in 2013 though genotype ($P < 0.05$), N treatment ($P < 0.01$) and GT x N ($P < 0.01$) was significant in 2014. About 74% and 67% of N_{off} was found in grains in 2013 and 2014 experiments, respectively (Figure 4.8 A and B).

Effect of species on the contribution of remaining N in straw for N_{off} was significant for both experiments ($P < 0.05$) where N treatment and species x N interaction was also significant in 2014 ($P < 0.001$). However, contributions of remaining N in chaff and grain N for N_{off} were not affected by species in 2013 but highly significant in 2014 ($P < 0.001$ for chaff and $P < 0.01$ for grain). Nevertheless, N treatment was significant for the contribution of remaining N in chaff and grain N on N_{off} in 2013 ($P < 0.001$ and $P < 0.05$) while only significant for grain N in 2014 ($P < 0.01$).

Table 4.6 Total plant N uptake of genotypes at maturity in 2013 and 2014 experiments for three N levels (NN; zero fertiliser applied, LN; 100 kg N ha^{-1} and HN; 200 kg N ha^{-1} in 2013 and LN; 100 kg N ha^{-1} and HN; 150 kg N ha^{-1} in 2014)

GT	N_{off} (kg N ha^{-1})					
	2013			2014		
	NN	LN	HN	NN	LN	HN
JB Diego	119.92	197.95	231.37	124.51	218.84	297.52
Xi 19	121.33	191.36	240.03	130.14	197.87	235.67
Spelt Tauro	141.42	196.35	257.41	144.40	222.04	320.22
Spelt SB	124.06	218.87	250.35	150.16	239.21	289.07
Spelt Oberkulmer	124.92	243.73	242.72	136.11	209.44	298.24
Emmer 1	-	-	-	135.82	211.31	302.72
Emmer 2	127.91	210.81	283.44	134.95	221.27	345.85
Einkorn 1	101.43	176.65	173.62	-	-	-
Einkorn 2	98.42	160.21	206.10	-	-	-
SED ; GT (*df*)	15.071 (42)***			13.022 (36)*		
N (*df*)	13.433 (4)**			5.164 (4)***		
GT x N (*df*)	27.871 (43.5)^{NS}			21.514 (39.2)^{NS}		

***** Significant at *P* < 0.001, **significant at *P* < 0.01, *significant at *P* < 0.05, *NS* - Not significant**

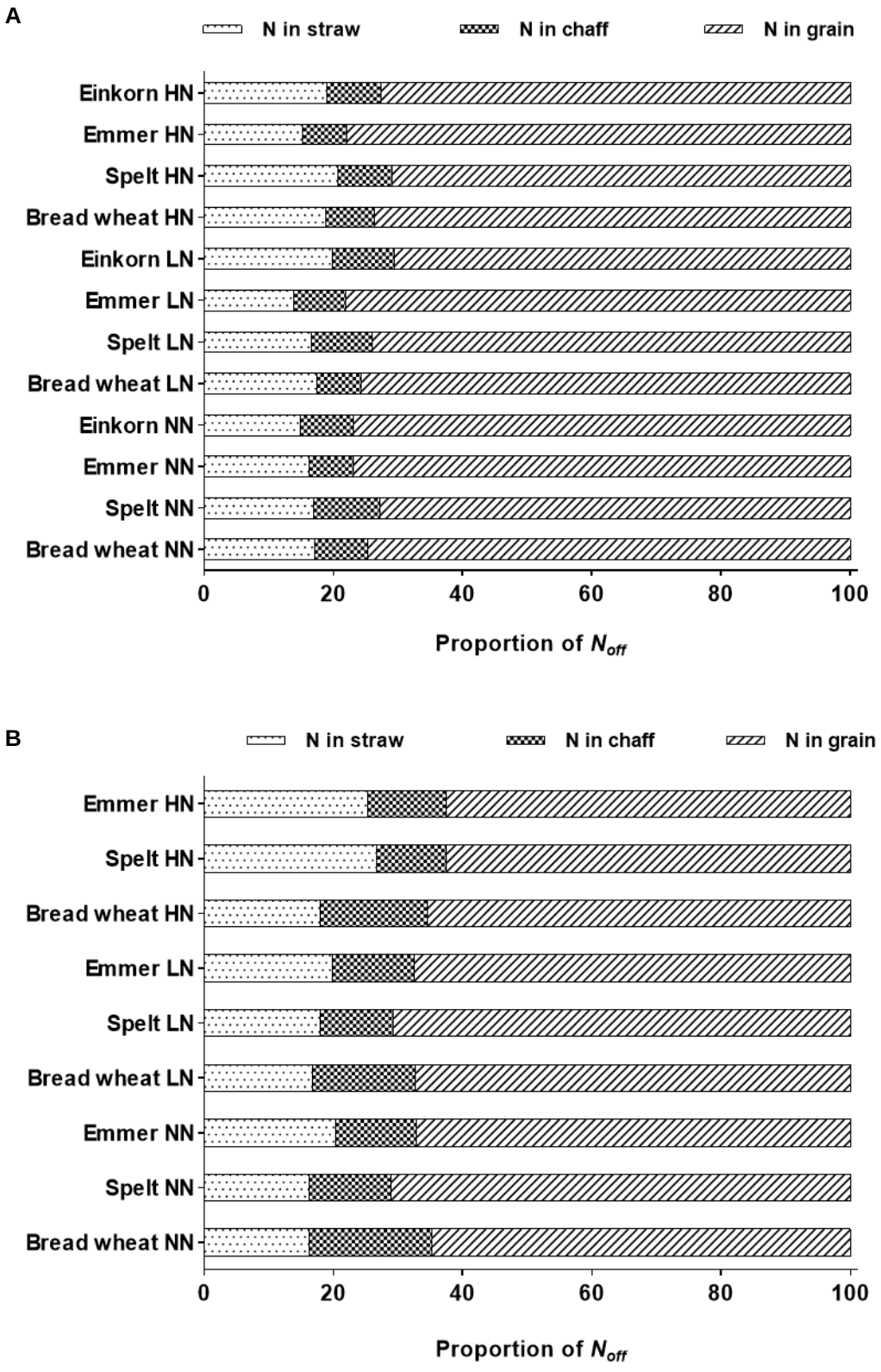


Figure 4.8 Remaining N in straw and chaff, and grain N as a proportion of N_{off} at maturity in (A) 2013 and (B) 2014 experiment

4.2.2.3 N uptake efficiency

NUpE of the genotypes varied significantly ($P < 0.001$) and decreased with N supply ($P < 0.01$) in 2013. Spelt Tauro and emmer 2 had the highest NUpE at NN and HN, respectively while einkorn 2 and einkorn 1 recorded the lowest NUpE at NN and HN, correspondingly. However, NUpE of bread wheat, spelt and emmer genotypes were not statisticaly different at HN but statisticaly different at NN in 2013 field experiment.

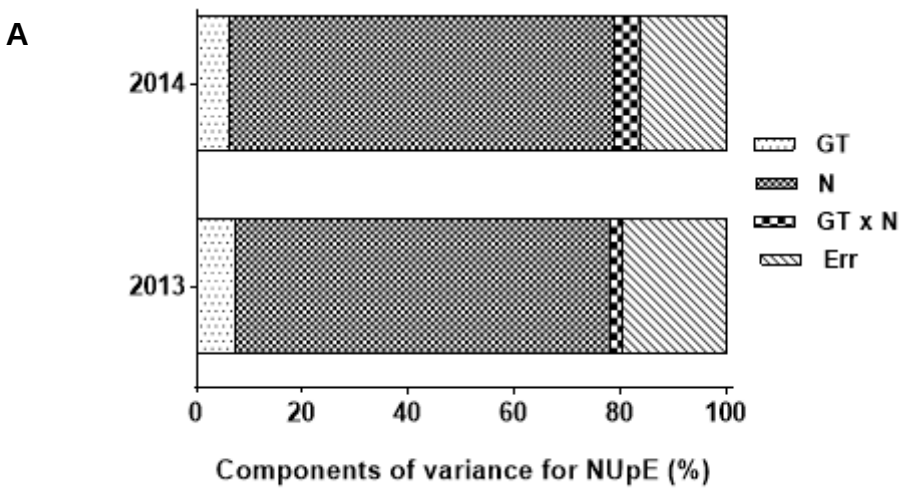
In 2014, genotypes ($P < 0.05$) and N treatment ($P < 0.001$) had a significant effect on plant NUpE. At NN and HN, spelt SB and emmer 2 recorded the highest NUpE, respectively (Table 4.7). At HN, NUpE of emmer 2 and spelt Tauro was significantly higher than all other genotypes but at NN, statistical differences between getypes of emmer, spelt and bread wheat were not significant.

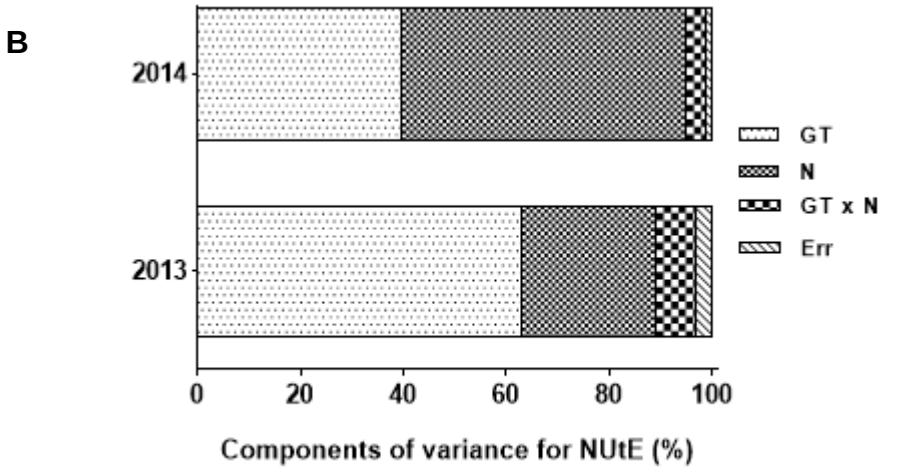
Effect of the species and N treatment ($P < 0.01$) was significant for NUpE in 2013 where the highest NUpE was observed in spelt followed by emmer, bread wheat and einkorn at NN. However, emmer had a greatest NUpE than spelt at HN. In 2014, NUpE was more strongly affected by N treatment ($P < 0.001$) than species ($P < 0.05$). The 2013 and 2014 experiments showed a similar contribution of genotype, N and GT x N on total variance of NUpE (Figure 4.10A). Total variance of NUpE varied mainly due to N treatment rather than genotypic effect for both years.

Table 4.7 NUpE of the genotypes at maturity in 2013 and 2014 experiments for three N levels (NN; zero fertiliser applied, LN; 100 kg N ha^{-1} and HN; 200 kg N ha^{-1} in 2013 and LN; 100 kg N ha^{-1} and HN; 150 kg N ha^{-1} in 2014)

GT	NUpE (kg N_{off} / kg N_{ava})					
	2013			2014		
	NN	LN	HN	NN	LN	HN
JB Diego	1.99	1.28	0.91	1.74	1.28	1.34
Xi 19	2.00	1.23	0.94	1.82	1.15	1.06
Spelt Tauro	2.55	1.28	1.01	2.03	1.30	1.45
Spelt SB	2.26	1.40	0.98	2.11	1.39	1.31
Spelt Oberkulmer	2.31	1.57	0.95	1.90	1.22	1.35
Emmer 1	-	-	-	1.90	1.23	1.37
Emmer 2	2.31	1.37	1.11	1.88	1.29	1.56
Einkorn 1	1.85	1.13	0.68	-	-	-
Einkorn 2	1.73	1.04	0.81	-	-	-
SED ; GT (*df*)		0.116 (42)***			0.082 (36)*	
N (*df*)		0.170(4)**			0.025 (4)***	
GT x N (*df*)		0.254 (17.2)^{NS}			0.135(38.3)^{NS}	

***** Significant at *P* < 0.001, **significant at *P* < 0.01, *significant at *P* < 0.05, *NS* - Not significant**





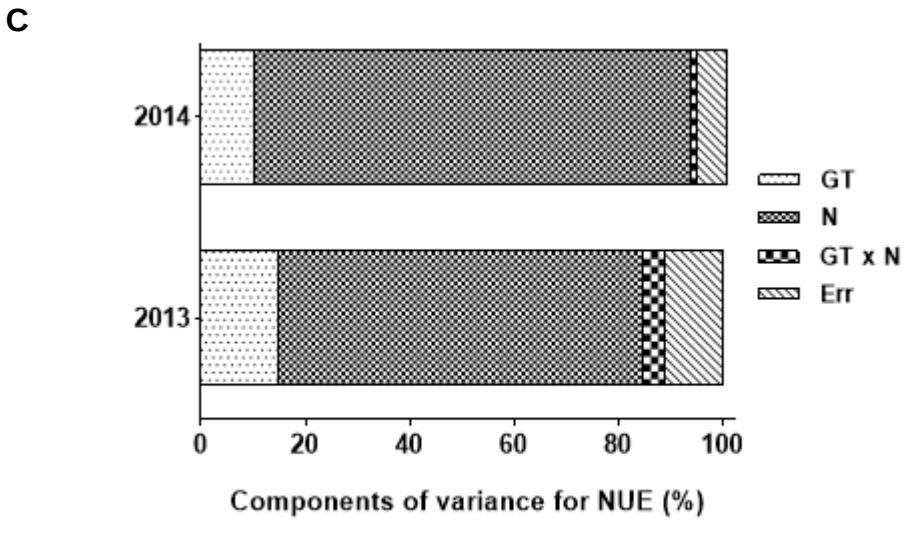


Figure 4.9 Contribution of components of variance on total variance of (A) NUpE (B) NUtE and (C) NUE at maturity in 2013 and 2014 experiment. (GT; genotype, N; N supply, GT x N; genotype by N level interaction and Err; error)

4.2.2.4 N utilisation efficiency

In both experiments, genotype, species, N treatment, GT x N interaction and species x N interaction significantly affected NUtE where bread wheat cv. JB Diego had the highest NUtE at any level of N and decreased with N supply. Einkorn 1 recorded the lowest NUtE for all N regimes in 2013 and spelt Oberkulmer at NN and emmer 2 at HN in 2014. NUtE of bread wheat was 40, 38 and 25% higher than einkorn, spelt and emmer at NN and 29, 25 and 30% at HN in 2013 experiment (Table 4.8).

Interestingly, the genotypic contribution for total variance was high for NUtE reporting 63% and 40% in 2013 and 2014, respectively where variance due to N was also high (26% and 55% in 2013 and 2014, respectively) (Figure 4.9B).

Table 4.8 NUtE of the genotypes at maturity in 2013 and 2014 experiments for three N levels (NN; zero fertiliser applied, LN; 100 kg N ha^{-1} and HN; 200 kg N ha^{-1} in 2013 and LN; 100 kg N ha^{-1} and HN; 150 kg N ha^{-1} in 2014)

GT	NUtE (kg grain DM/ kg N_{off})					
	2013			2014		
	NN	LN	HN	NN	LN	HN
JB Diego	49.87	39.63	32.32	49.18	40.56	31.97
Xi 19	45.63	36.28	32.23	39.98	36.24	30.06
Spelt Tauro	29.83	25.95	23.92	33.45	26.33	20.81
Spelt SB	31.27	27.48	25.12	36.68	28.69	22.33
Spelt Oberkulmer	27.83	26.03	23.53	33.12	28.10	21.46
Emmer 1	-	-	-	34.71	26.70	16.13
Emmer 2	35.79	25.78	22.7	36.54	25.81	15.17
Einkorn 1	26.60	19.35	22.06	-	-	-
Einkorn 2	30.74	22.55	23.66	-	-	-
SED; GT (*df*)		0.779 (42)***			0.497 (36)***	
N (*df*)		0.422(4)***			0.628(4)***	
GT x N (*df*)		1.331 (45.9)***			1.015(38.3)***	

***** Significant at $P < 0.001$**

4.2.2.5 N use efficiency

In 2013, as expected, bread wheat (JB Diego and Xi 19) recorded the highest NUE at all N treatments while einkorn genotypes had lowest NUE (Table 4.9). However, N

supply significantly decreased NUE of all the genotypes. In general, NUE at NN was 67% higher than HN. When averaged across species, bread wheat had the highest NUE followed by emmer, spelt and einkorn although spelt and emmer was not significantly different from each other (Table 4.10).

NUE in 2014 was significantly different between genotype ($P < 0.001$) and N levels ($P < 0.001$) while no GT x N interaction was found. The same trend of NUE among different N regimes was found as in 2013 where bread wheat cv. JB Diego had the most NUE at all N treatments (Table 4.9). Nevertheless, NUE of the genotypes was 58% higher in NN than in HN treatment. Overall, NUE of the genotypes in 2014 experiment was less than that of 2013 experiment. This may be due to more N supply through soil mineralisation in 2014 than in 2013. The contribution of the variance due to N treatment of total variance was high in 2014 (Figure 4.9C).

Table 4.9 NUE of the genotypes at maturity in 2013 and 2014 experiments for three N levels (NN; zero fertiliser applied, LN; 100 kg N ha^{-1} and HN; 200 kg N ha^{-1} in 2013 and LN; 100 kg N ha^{-1} and HN; 150 kg N ha^{-1} in 2014)

GT	NUE (kg grain DM/ kg N_{ava})					
	2013			2014		
	NN	LN	HN	NN	LN	HN
JB Diego	96.61	50.31	29.25	85.26	51.59	42.94
Xi 19	89.06	44.64	30.28	72.91	41.70	31.89
Spelt Tauro	76.11	33.25	24.36	67.83	34.11	30.15
Spelt SB	70.23	38.56	24.68	77.10	40.02	29.25
Spelt Oberkulmer	65.05	40.75	22.35	62.90	34.37	28.85
Emmer 1	-	-	-	65.84	32.92	22.15
Emmer 2	83.05	35.29	24.99	68.88	33.42	23.68
Einkorn 1	49.05	21.87	15.12	-	-	-
Einkorn 2	53.06	23.84	19.19	-	-	-
SED; GT (*df*)		3.572 (42)***			2.653 (36)***	
N (*df*)		5.487 (4)**			1.213 (4)***	
GT x N (*df*)		7.975 (16)*			4.424 (39.7)^{NS}	

***** Significant at $P < 0.001$, **significant at $P < 0.01$, *significant at $P < 0.05$, *NS* - not significant**

Table 4.10 NUE of the species (SP) at maturity in 2013 and 2014 experiments for three N levels (NN; zero fertiliser applied, LN; 100 kg N ha^{-1} and HN; 200 kg N ha^{-1} in 2013 and LN; 100 kg N ha^{-1} and HN; 150 kg N ha^{-1} in 2014)

Species	2013			2014		
	NN	LN	HN	NN	LN	HN
Bread wheat	92.84	47.48	29.77	79.09	46.64	37.41
Spelt	70.46	37.52	23.80	69.28	36.17	29.41
Emmer	83.05	35.29	24.99	67.36	33.17	22.92
Einkorn	51.05	22.86	17.16	-	-	-
SED; SP(*df*)		4.212 (18)***			1.793 (12)***	
N (*df*)		6.941 (4)**			1.213 (4)***	
SP x N (*df*)		12.211 (12.2)*			15.692 (16)^{NS}	

***** Significant at *P* < 0.001, **significant at *P* < 0.01, *significant at *P* < 0.05, NS - not significant**

4.2.2.6 N harvest index

There were no significant effects of treatments on NHI in 2013 but genotype ($P < 0.05$), N treatment ($P < 0.01$) and GT x N ($P < 0.01$) were significant in 2014. NHI decreased with N supply for both experiments. However, when averaged across species and N treatments, emmer had the highest NHI followed by bread wheat, spelt and einkorn in 2013, while in 2014 spelt had the highest NHI.

4.2.2.7 Apparent fertiliser N recovery rate

The high N_{off} under the field condition is due to N availability from soil and fertiliser. Therefore, it is worth calculating N_{off} only due to fertiliser N, the apparent fertiliser N recovery. Fertiliser N recovery rate of the genotypes ($P < 0.001$) varied significantly from 36% (einkorn 1) to 78% (emmer 2) in 2013 (Figure 4.10).

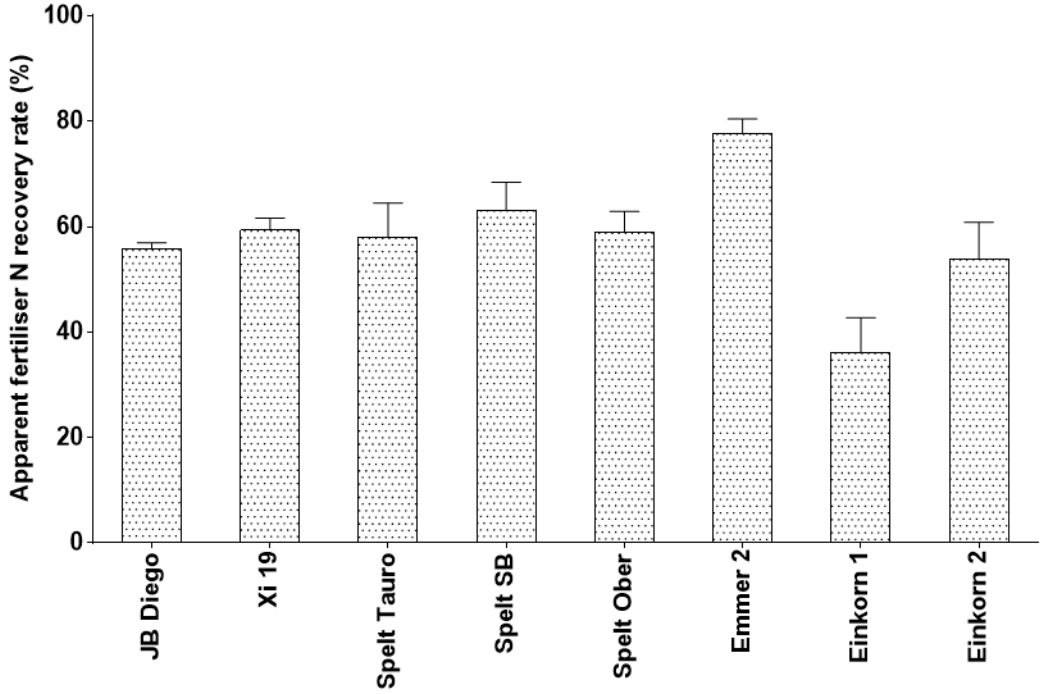


Figure 4.10 Apparent fertiliser N recovery rate of the genotypes in 2013 experiment. Error bars represent SE (*df* = 23)

4.2.3 Glasshouse experiments

Microclimatic conditions of the glasshouse

Total irradiance during experiment period was 1854 and 1821 MJ m^{-2} in 2013 and 2014, respectively. Average air temperature of the glasshouse in 2013 was 21.9 ^{o}C and 21.3 ^{o}C in 2014 (Figure 4.11). Duration of the experiment from transplantation to maturity in 2013 was 104 days and 113 days in 2014.

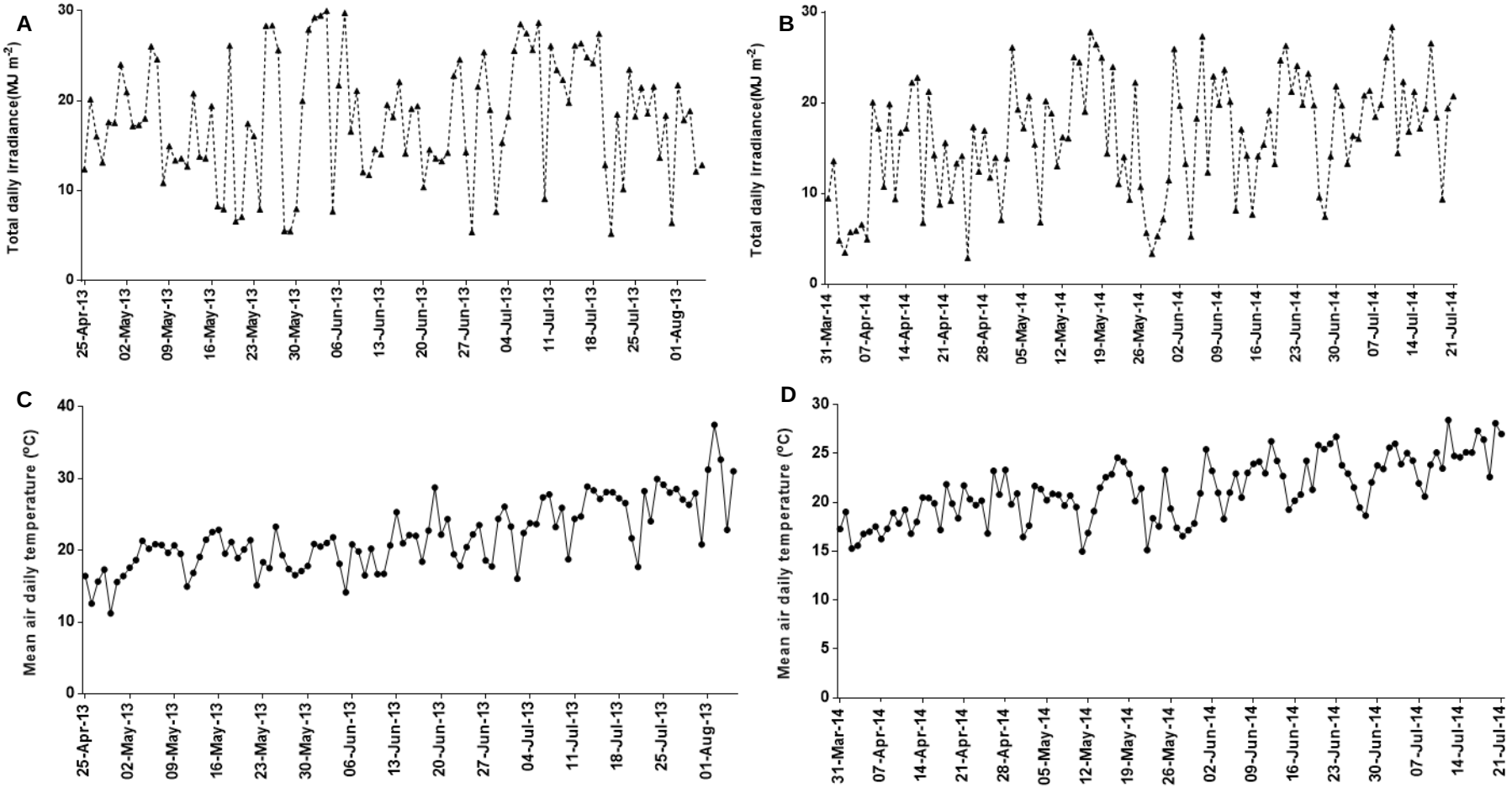


Figure 4.11 Total daily irradiance (MJ m^{-2}) in (A) 2013 (B) 2014 and mean air daily temperature (ºC) in (C) 2013 (D) 2014 experiments. 2013 experiment was conducted during 25 April to 5 August while for 2014 experiment was carried out from 31 March to 21 July

4.2.4 Glasshouse pot experiment

Two N levels equivalent to 50 kg N ha^{-1} (LN) and 200 kg N ha^{-1} (HN) were used as N treatments for the experiment with ten genotypes (Chapter 2; section 2.2). The experiment was conducted from February to August 2013.

4.2.4.1 Above-ground biomass production and grain yield

AGB and grain yield were significantly different between genotypes ($P < 0.001$) and N level ($P < 0.01$). Emmer 2 had the highest AGB at both N levels and einkorn the lowest. The most grain yield was observed in bread wheat cv. JB Diego for LN and HN conditions (Table. 4.11).

Table 4.11 Above-ground biomass and grain yield of the genotypes at LN (50 kg N ha^{-1}) and HN (200 kg N ha^{-1}) in glasshouse experiment in 2013

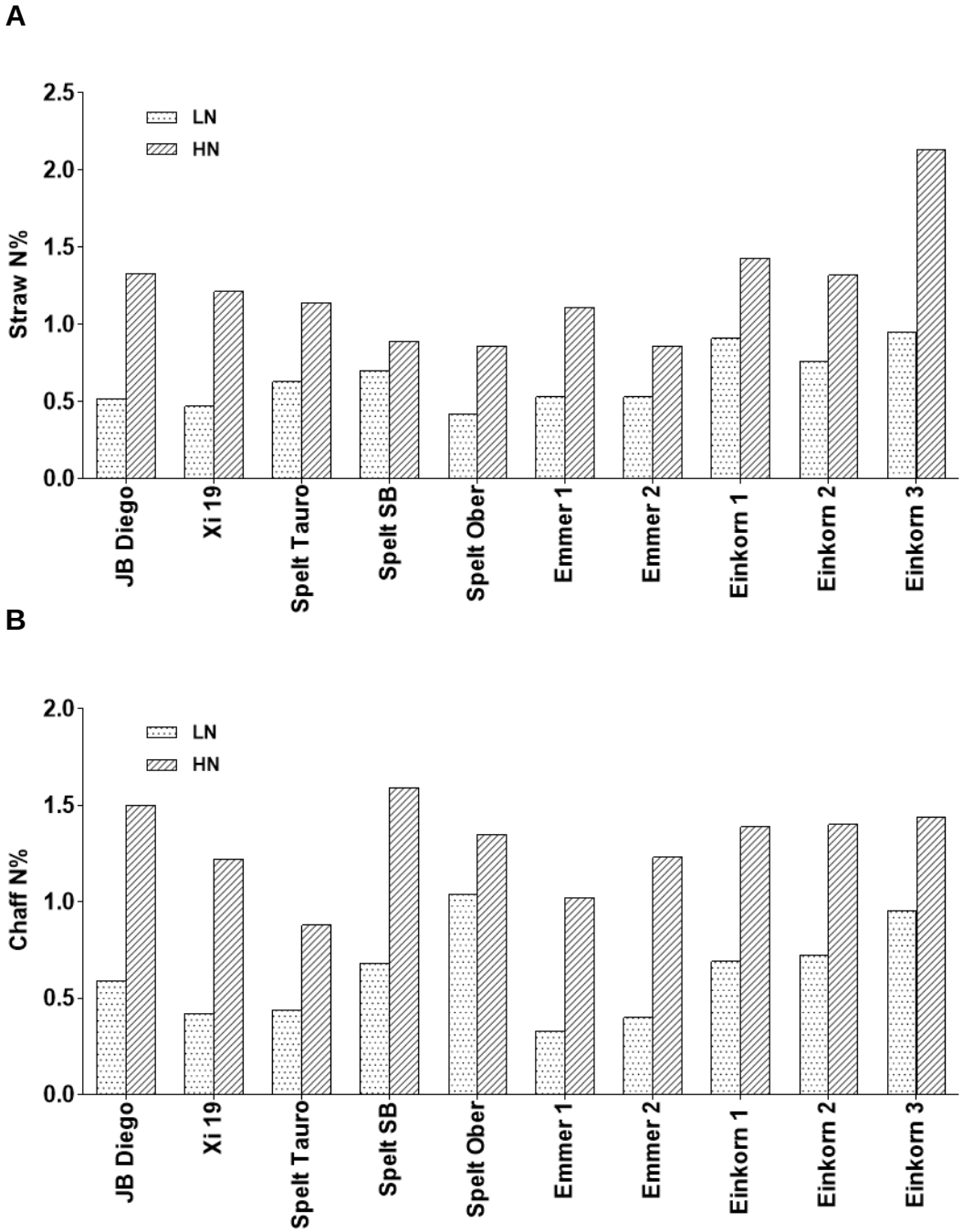
GT	Above-ground biomass (g $plant^{-1}$)		Grain yield (g $plant^{-1}$)	
	LN	HN	LN	HN
JB Diego	36.03	50.98	12.47	20.59
Xi 19	32.27	50.91	11.71	18.89
Spelt Tauro	30.37	44.83	8.94	12.60
Spelt SB	32.53	60.42	8.26	16.03
Spelt Oberkulmer	33.57	49.43	5.42	12.35
Emmer 1	37.75	56.40	8.28	14.04
Emmer 2	41.99	61.81	10.10	13.26
Einkorn 1	17.55	39.38	3.36	5.55
Einkorn 2	12.56	28.63	1.46	3.27
Einkorn 3	18.34	25.44	2.18	3.24
SED ; GT (*df*)		3.541 (36)***		1.957 (36)***
N (*df*)		0.835 (2)**		0.326 (2)**
GT x N (*df*)		4.823 (37.6)^{NS}		2.646 (36.9)^{NS}

***** Significant at $P < 0.001$, **significant at $P < 0.01$, *significant at $P < 0.05$, *NS* - not significant**

4.2.4.2 N% of plant components and N_{off} at maturity

Straw N% ranged from 0.42% (spelt Oberkulmer) to 0.95% (einkorn 3) at LN and 0.86% (spelt Oberkulmer and emmer 2) to 2.13% (einkorn 3) at HN. Effect of

genotype ($P < 0.001$) and N treatment ($P < 0.05$) was significant and there was no interaction between genotype and N (Figure 4.12A). The same effect of main factors and the interaction was found for chaff N% and grain N%. The highest chaff N% was observed in spelt Oberkulmer and spelt SB at LN and HN, respectively while emmer 1 and spelt Tauro had lowest chaff N% at LN and HN (Figure 4.12B). Grain N% was high among einkorn genotypes regardless of the N supply (Figure 4.12C).



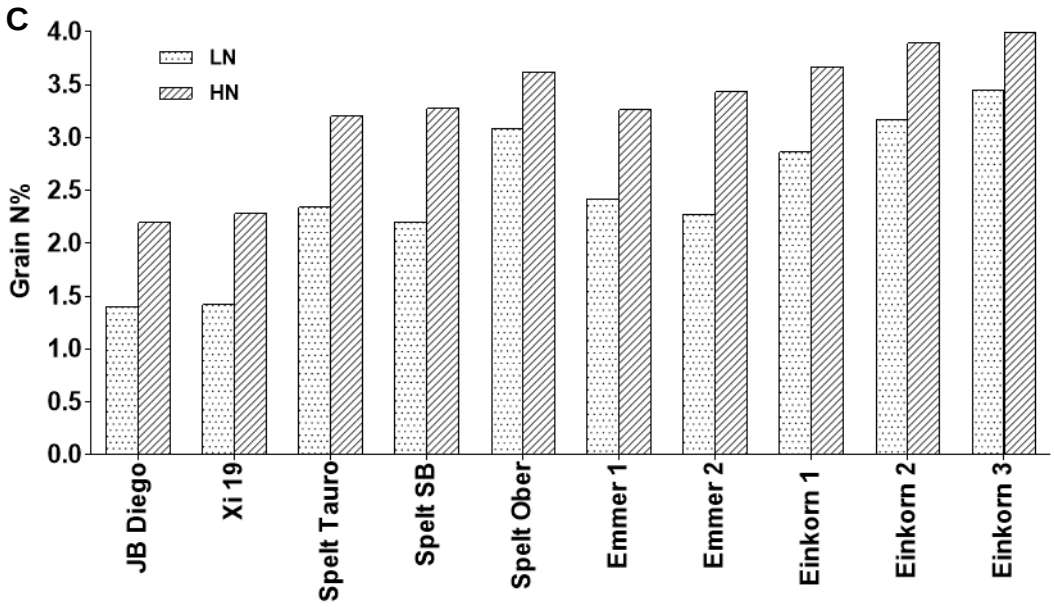
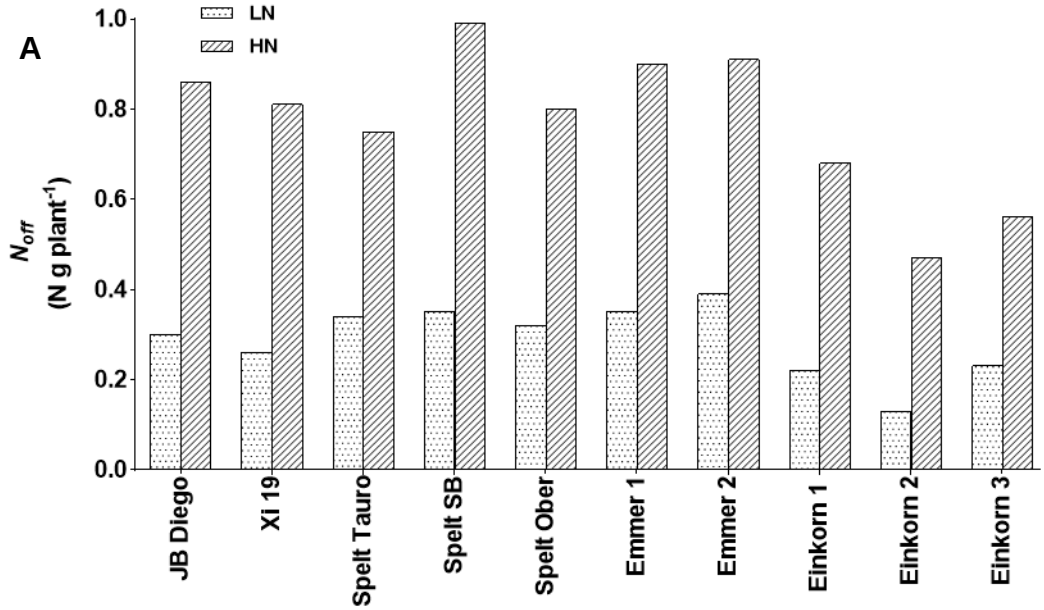


Figure 4.12 (A) Straw N% (B) chaff N% and (C) grain N% of the genotypes at LN (50 kg N ha^{-1}) and HN (200 kg N ha^{-1}) in glasshouse experiment in 2013

For N_{off}, the highest contribution for total variance was from N treatment (>70%) (P < 0.01). Emmer 2 and spelt SB took up most N_{off} at maturity (P < 0.001) at LN and HN, respectively while einkorn 1 had lowest N_{off} at both N levels (Figure 4.13A). Contribution of remaining N in straw and N in grain for N_{off} was only significant for genotype (P < 0.001) although percentage of N_{off} remaining in chaff was significantly affected by genotype (P < 0.01), N treatment (P < 0.05) and GT x N (P < 0.01) (Figure 4.13B and C).



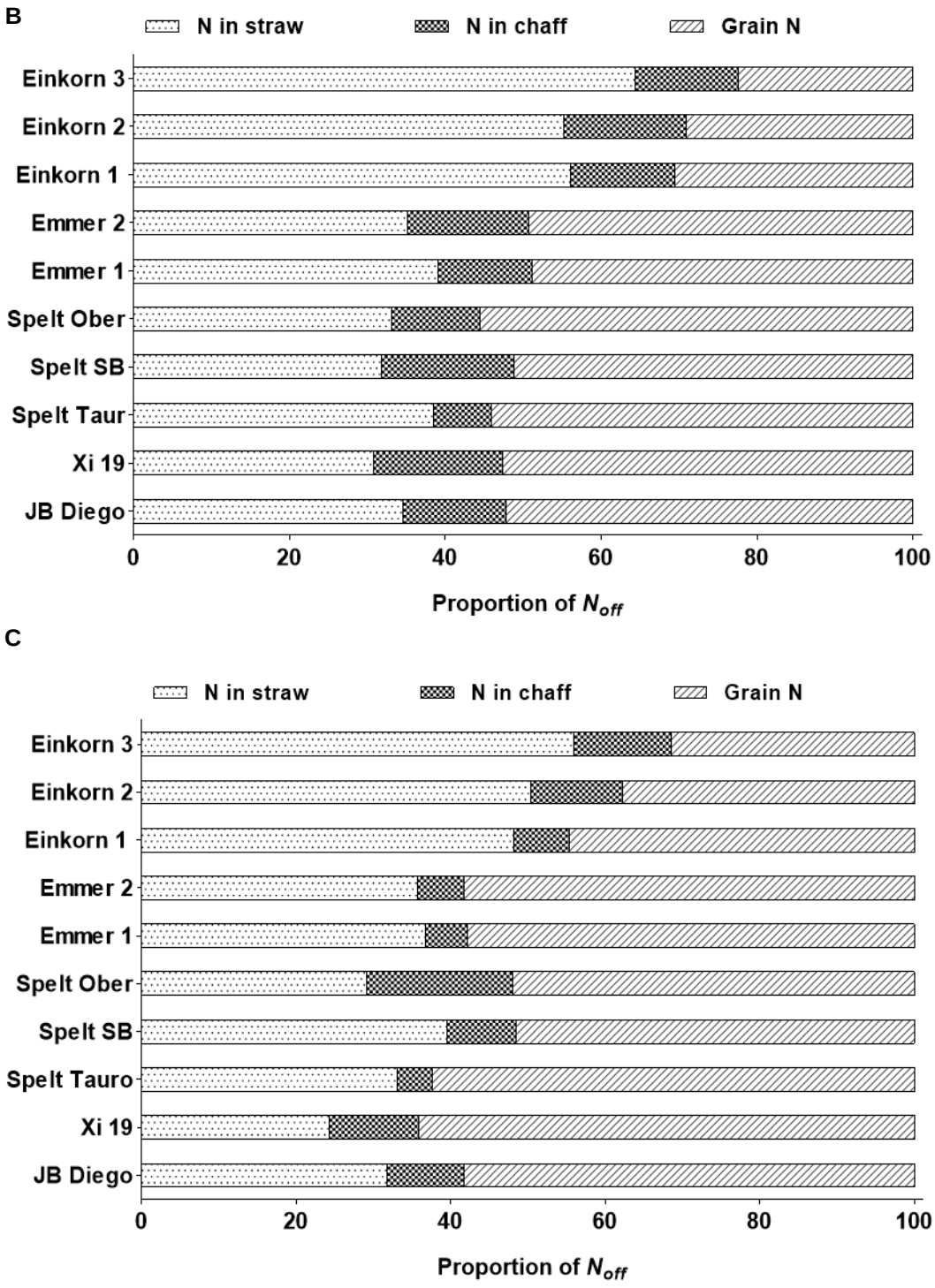


Figure 4.13 (A) N_{off} at maturity (N g plant^{-1}) and (B) Straw, chaff and grain N as a proportion of N_{off} at maturity at LN (50 kg N ha^{-1}) and (C) at HN (200 kg N ha^{-1}) in glasshouse experiment in 2013 (SED; GT x N for N_{off} = 0.099 (*df* = 36) and straw (7.14, *df* = 37.7), chaff (7.46, *df* = 37.1) and grain (3.02, *df* = 36.9)

4.2.4.3 N uptake efficiency

NUpE was highest in emmer 2 and lowest in einkorn 2 at LN while spelt SB and einkorn 2 at HN, respectively ($P < 0.001$). At LN, NUpE of emmer 2 was statistically different from all spelt, bread wheat and einkorn genotypes. However, NUpE of spelt and bread wheat was not significantly different at LN. Only einkorn genotypes had significantly low NUpE at HN when compared to the other genotypes. NUpE decreased significantly with additional N ($P < 0.05$) (Table 4.12). The only way to supply N for the experiment was N fertiliser. Therefore, NUpE under glasshouse is same as apparent fertiliser N recovery rate of the genotype. On average, NUpE of emmer was 48, 24 and 9% higher than einkorn, bread wheat and spelt at LN while 37% higher than einkorn and 8% higher than bread wheat and spelt at HN. However, as a species, emmer had the highest NUpE.

4.2.4.4 N utilisation efficiency

NUtE was significantly affected by genotype ($P < 0.001$), N treatment ($P < 0.01$) and GT x N interaction ($P < 0.001$). Similar to the field experiments, bread wheat (cv. JB Diego and cv. Xi 19) had the highest NUtE and einkorn genotypes had the least. N application significantly reduced NUtE of all genotypes (Table 4.12). Bread wheat recorded 72, 49 and 43% of NUtE than einkorn, spelt and emmer at LN. The reduction of NUtE at HN was reduced significantly in spelt and emmer (32%) when compared to bread wheat though einkorn remained unchanged (71%).

4.2.4.5 N use efficiency

There were significant effects of genotype ($P < 0.001$), N treatment ($P < 0.001$) and GT x N ($P < 0.001$) on NUE. Overall, NUE of all genotypes were very low in the glasshouse when compared to the field and decreased further due to N supply. Bread wheat (cv. JB Diego and cv. Xi 19) had the highest NUE at LN and HN while einkorn genotypes had low NUE (Table 4.12). When averaged across the species and N levels, the highest NUE was observed in bread wheat followed by emmer, spelt and einkorn though NUE of spelt and emmer was not significantly different.

Table 4.12 N uptake efficiency (NUpE, g N_{off} /g N_{ava}), N utilisation efficiency (NUtE, g grain DM/ g N_{off}) and N use efficiency (NUE, g grain DM/ g N_{ava}) at maturity in 2013 glasshouse experiment

GT	NUpE (g N_{off} / g N_{ava})		NUtE (g grain DM/ g N_{off})		NUE (g grain DM/ g N_{ava})	
	LN	**HN**	**LN**	**HN**	**LN**	**HN**
JB Diego	0.41	0.29	41.58	23.78	17.21	7.13
Xi 19	0.36	0.28	45.14	23.11	16.16	6.51
Spelt Tauro	0.46	0.26	26.64	16.92	12.33	4.35
Spelt SB	0.48	0.34	23.45	15.58	11.42	5.53
Spelt Oberkulmer	0.44	0.28	16.92	15.35	7.47	4.26
Emmer 1	0.48	0.31	23.94	16.64	11.43	4.84
Emmer 2	0.54	0.31	25.81	14.86	13.94	4.57
Einkorn 1	0.30	0.23	15.64	8.36	4.63	1.91
Einkorn 2	0.18	0.16	10.92	7.31	2.02	1.13
Einkorn 3	0.31	0.19	10.01	5.27	3.01	1.12
SED; GT (*df*)	0.034 (36)***		2.097 (36)***		0.937 (36)***	
N (*df*)	0.016(2)*		1.019 (2)**		0.160 (2)***	
GT x N (*df*)	0.048 (35.6)^{NS}		2.992 (35.1)***		1.267 (37)***	

***** Significant at *P* < 0.001, **significant at *P* < 0.01, *significant at *P* < 0.05, *NS* - not significant**

4.2.5 Glasshouse column experiment

Three spelt, two emmer and two bread wheat genotypes including cv. Forno (a bread wheat cultivar) were used in this experiment with three N levels equivalent to 50 kg N ha^{-1}, 100 kg N ha^{-1} and 200 kg N ha^{-1}. Bread wheat cv. JB Diego and three einkorn genotypes were excluded from the experiment. Table 4.13 shows the dates which genotypes reached important growth stages during the experiment. It is important to note that spelt genotypes reached anthesis later than other genotypes. Since plants were harvested at anthesis (not calendar date) to study root systems, spelt genotypes received more liquid nutrients through the fertigation system than other genotypes. The fertigation system was disconnected at anthesis for all genotypes and only water was supplied until maturity. The experiment was conducted in 2014 from January to August.

Table 4.13 The date genotypes reached stem elongation (GS31), flag leaf emergence (GS39), anthesis (GS61) and maturity in glasshouse column experiment in 2014

GT	GS31	GS39	GS61	Maturity
Xi 19	25-Apr-14	07-May-14	21-May-14	08-Jul-14
Forno	28-Apr-14	12-May-14	27-May-14	08-Jul-14
Spelt Tauro	05-May-14	15-May-14	28-May-14	10-Jul-14
Spelt SB	12-May-14	22-May-14	09-Jun-14	17-Jul-14
Spelt Oberkulmer	12-May-14	22-May-14	04-Jun-14	10-Jul-14
Emmer 1	25-Apr-14	12-May-14	27-May-14	30-Jul-14
Emmer 2	14-Apr-15	24-May-14	21-May-14	30-Jul-14

4.2.5.1 At anthesis (GS61)

Plant N% was significantly different between genotypes ($P < 0.001$) and N treatments ($P < 0.05$). Total number of tillers, total green area per plant, AGB and N_{off} was significantly affected only by genotype ($P < 0.001$). Table 4.14 shows plant N%, number of tillers, green area, AGB and N_{off} of the genotypes when averaged across N treatments. There was no effect of N level or GT x N interaction for above mentioned parameters.

Table 4.14 Number of tillers, green area, above ground biomass and total plant N uptake per plant (above ground) in ten genotypes when averaged across N treatments at GS61 for column experiment in 2014 at anthesis

GT	Plant N%	Number of tillers plant^{-1}	Green area (cm^2 plant^{-1})	Above-ground biomass (g plant^{-1})	N_{off} (g N plant^{-1})
Xi 19	2.55	5.22	414.22	5.48	0.16
Forno	2.25	4.00	465.14	5.90	0.15
Spelt Tauro	1.80	7.33	749.07	16.14	0.28
Spelt SB	2.04	8.78	624.43	12.87	0.26
Spelt Oberkulmer	1.85	11.11	846.28	16.43	0.30
Emmer 1	2.39	4.56	392.56	5.62	0.17
Emmer 2	2.50	5.78	416.82	5.05	0.20
SED (*df* = 36); GT	0.127***	1.231 ***	106.6***	1.411***	0.022***

***** Significant at $P < 0.001$**

4.2.5.2 At maturity

AGB and grain yield of the genotypes ($P < 0.001$) at maturity were significantly different but there was no effect of N or interaction between GT x N (Figure 4.14). AGB of spelt genotypes was significantly higher than bread wheat and emmer genotypes while grain yield was higher in spelt and bread wheat genotypes than emmer.

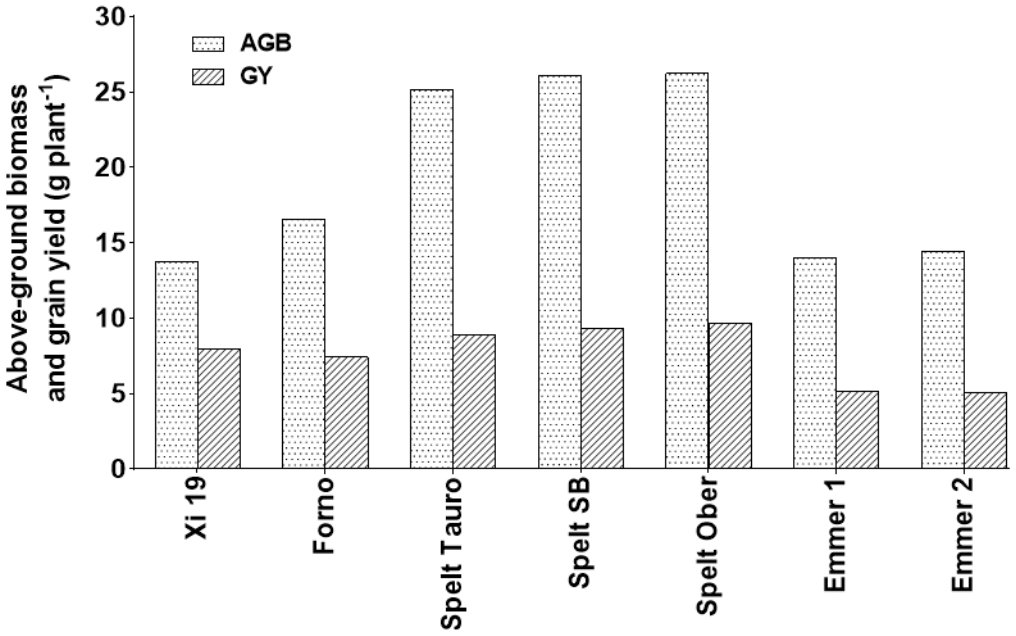


Figure 4.14 Above-ground biomass (AGB) and grain yield (GY) per plant at maturity when averaged across N treatments in glasshouse column experiment in 2014. SED GT (*df* = 36) for AGB and GY are 2.411 1.043, respectively

4.2.5.3 N% of plant components and N_{off}

Straw, chaff and grain N% at maturity was significantly different between genotypes ($P < 0.001$). There were no significant effects of N and GT x N on N% of plant components. The highest straw N% was recorded in emmer 1 (0.85%) and lowest was in spelt Oberkulmer (0.61%). Chaff N% of spelt genotypes and emmer 1 was about 0.5% while bread wheat had 0.7%. As in previous experiments, grain N% of bread wheat cultivars was significantly lower than other genotypes. All spelt genotypes had highest N_{off} followed by bread wheat and emmer at maturity.

4.2.5.4 N uptake, utilisation and use efficiency

NUpE, NUtE, NUE and NHI were significantly different between genotypes but the effect of N treatment and GT x N interaction was not significant. The highest NUpE was observed in spelt followed by bread wheat and emmer. The highest NUtE was recorded in bread wheat cv. Xi 19 while NUtE of emmer genotypes were less than others. NUE of spelt and bread wheat was not significantly different though emmer had a lower NUE (Table 4.15).

Table 4.15 N uptake efficiency (NUpE, g N_{off} / g N_{ava}), N utilisation efficiency (NUtE, g grain DM/ g N_{off}) and N use efficiency (NUE, g grain DM / g N_{ava}) at maturity in 2014 glasshouse experiment

GT	NUpE (g N_{off} / g N_{ava})	NUtE (g grain DM/ g N_{off})	NUE (g grain DM/ g N_{ava})
Xi 19	0.48	32.79	15.92
Forno	0.46	26.81	12.39
Spelt Tauro	0.63	22.53	14.28
Spelt SB	0.49	23.48	11.85
Spelt Oberkulmer	0.56	24.29	13.6
Emmer 1	0.37	22.88	8.76
Emmer 2	0.41	22.28	9.78
SED (*df* = 36) GT	0.055***	1.399***	1.762**

***** Significant at *P* < 0.001; ** Significant at *P* < 0.01**

Summary of NUpE, NUtE and NUE for genotypes with relative rankings in 2012, 2013 and 2014 field experiments and 2013 and 2014 glasshouse experiments are shown in Table 4.16, 4.17 and 4.18, respectively.

Table 4.16 Summary of NUpE and rankings of genotypes for NUpE in 2012, 2013 and 2014 field experiments together with 2013 and 2014 glasshouse experiments. N availability for the plants in field experiments is the sum of soil mineral and residual soil N in February and fertiliser N. Only fertiliser N was applied to 2013 and 2014 glasshouse experiments. NUpE of HN fertiliser N treated plants are shown in 2013 and 2014 field experiments (2013 – 200 kg N ha^{-1}; 2014- 150 kg N ha^{-1}. NUpE of 2014 glasshouse experiment is the average across N treatments. NUpE calculation only based on method 1 is shown in 2012 field experiment

GT	NUpE (N_{off} / N_{ava})									
	2012 Field		2013 Field		2014 Field		2013 Glasshouse		2014 Glasshouse	
	Recom.	Rank	HN	Rank	HN	Rank	HN	Rank	Average	Rank
JB Diego	1.16	7	0.91	6	1.34	5	0.29	3	-	-
Forno	-	-	-	-	-	-	-	-	0.46	5
Xi 19	1.11	8	0.94	5	1.06	7	0.28	4	0.48	4
Spelt Tauro	1.40	3	1.01	2	1.45	2	0.26	5	0.63	1
Spelt SB	1.29	4	0.98	3	1.31	6	0.34	1	0.49	3
Spelt Oberkulmer	1.24	5	0.95	4	1.35	4	0.28	4	0.56	2
Emmer 1	1.61	2	-	-	1.37	3	0.31	2	0.37	7
Emmer 2	1.67	1	1.11	1	1.56	1	0.31	2	0.41	6
Einkorn 1	1.09	9	0.68	8	-	-	0.23	6	-	-
Einkorn 2	1.19	6	0.81	7	-	-	0.16	8	-	-
Einkorn 3	0.89	10	-	-	-	-	0.19	7	-	-
SED ; GT (*df*)	0.209 (27)*		0.116 (42)***		0.082 (36)*		0.034 (36)***		0.055 (36)***	

Table 4.17 Summary of NUtE and rankings of genotypes for NUtE in 2012, 2013 and 2014 field experiments together with 2013 and 2014 glasshouse experiments.

GT	NUtE (grain DM/ N_{off})									
	2012 Field		2013 Field		2014 Field		2013 Glasshouse		2014 Glasshouse	
	Recom.	Rank	HN	Rank	HN	Rank	HN	Rank	Average	Rank
JB Diego	26.18	1	32.32	1	31.97	1	23.78	1	-	-
Forno	-	-	-	-	-	-	-	-	32.79	1
Xi 19	24.12	2	32.23	2	30.06	2	23.11	2	26.81	2
Spelt Tauro	13.08	5	23.92	4	20.81	5	16.92	3	22.53	6
Spelt SB	17.19	4	25.12	3	22.33	3	15.58	5	23.48	4
Spelt Oberkulmer	17.59	3	23.53	6	21.46	4	15.35	6	24.29	3
Emmer 1	10.64	7	-	-	16.13	6	16.64	4	22.88	5
Emmer 2	9.04	8	22.70	7	15.17	7	14.86	7	22.28	7
Einkorn 1	11.21	6	22.06	8	-	-	8.36	8	-	-
Einkorn 2	5.24	9	23.66	5	-	-	7.31	9	-	-
Einkorn 3	4.6	10	-	-	-	-	5.27	10	-	-
SED ; GT (*df*)	2.031 (27)***		0.779 (42)***		0.497 (36)*		2.097(36)***		1.399 (36)***	

Table 4.18 Summary of NUE and rankings of genotypes for NUE in 2012, 2013 and 2014 field experiments together with 2013 and 2014 glasshouse experiments.

GT	NUE (grain DM/ N_{ava})									
	2012 Field		2013 Field		2014 Field		2013 Glasshouse		2014 Glasshouse	
	Recom.	Rank	HN	Rank	HN	Rank	HN	Rank	Average	Rank
JB Diego	29.95	1	29.25	2	42.94	1	7.13	1	-	-
Forno	-	-	-	-	-	-	-	-	15.92	1
Xi 19	26.72	2	30.28	1	31.89	2	6.51	2	12.39	4
Spelt Tauro	18.42	6	24.36	5	30.15	3	4.35	6	14.28	2
Spelt SB	22.43	3	24.68	4	29.25	4	5.53	3	11.85	5
Spelt Oberkulmer	21.57	4	22.35	6	28.85	5	4.26	7	13.6	3
Emmer 1	19.34	5	-	-	22.15	7	4.84	4	8.76	7
Emmer 2	15.84	7	24.99	3	23.68	6	4.57	5	9.78	6
Einkorn 1	11.97	8	15.12	8	-	-	1.91	8	-	-
Einkorn 2	6.63	9	19.19	7	-	-	1.13	9	-	-
Einkorn 3	4.14	10	-	-	-	-	1.12	10	-	-
SED ; GT (*df*)	4.052 (27)***		3.572 (42)***		2.653 (36)*		2.097(36)***		1.399 (36)***	

4.2.6 Relationship between NUE and its components

NUE of the genotypes was regressed against NUpE and NUtE. According to the results of 2012 field experiment, NUpE and NUtE explained 25.5% and 72.2% of the variation in NUE, respectively ($P < 0.001$). Therefore, NUtE was more important to determine NUE than NUpE in 2012 field experiment (Figure 4.15 A and B).

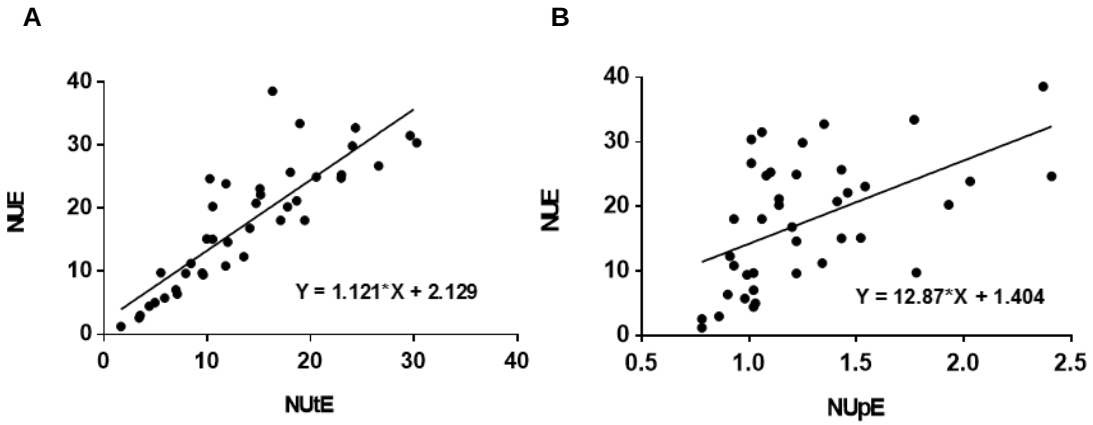


Figure 4.15 Linear regression between (A) NUE and NUpE ($R^2 = 0.25$, $P < 0.001$) (B) NUE and NUtE ($R^2 = 0.72$, $P < 0.001$) in 2012 field experiment

However, this was different in 2013 and 2014 field experiments. In 2013, NUpE explained 60% of the variation in NUE at HN and 50% at NN (Figure 4.16A and B) while NUtE explained 47% and 40% of the variation in NUE at HN and NN respectively (Figure 4.16C and D) ($P < 0.001$). It seems that when NUpE increased by 1, NUE increased 24.97 and 25.95 at HN and NN, respectively. Remarkably in the 2014 experiment, there was no significant relationship between NUE and NUpE both at NN and HN conditions but NUtE explained 70% of the variation in NUE at HN and 54% at NN (Figure 4.17 A and B).

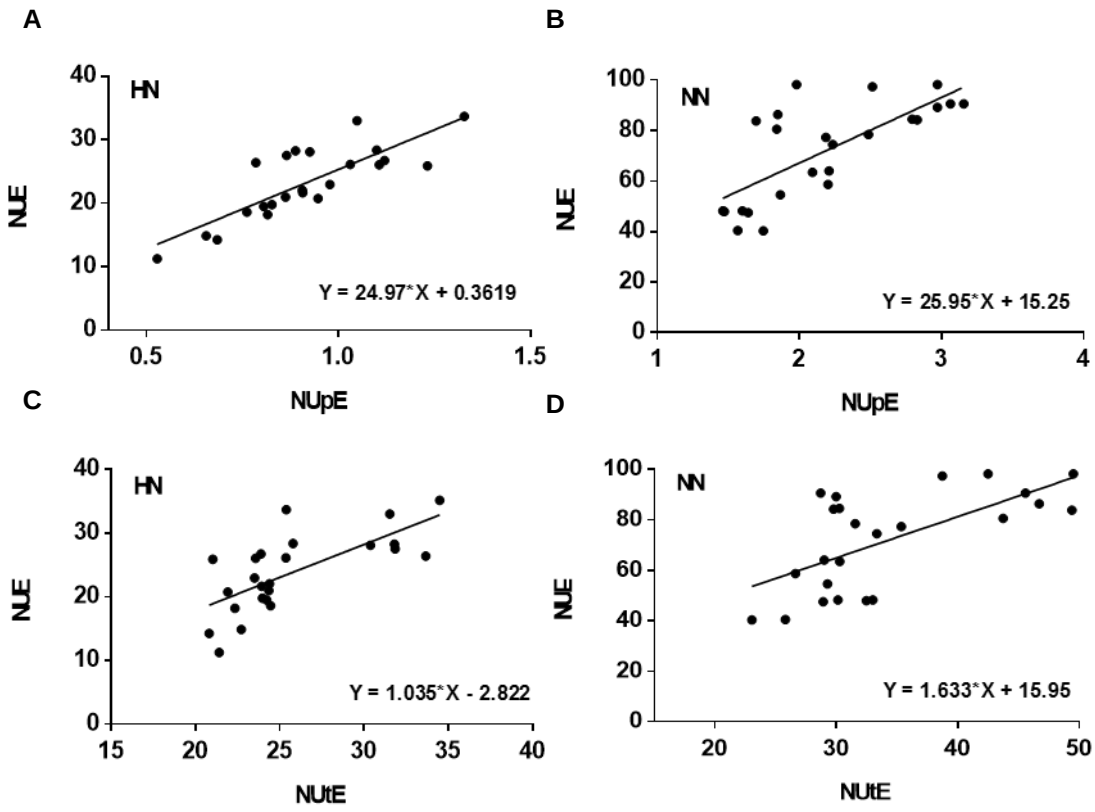


Figure 4.16 Linear regression between NUE and NUpE at (A) HN (R^2 = 0.6, *P* < 0.001) (B) NN (R^2 = 0.5, *P* < 0.001) and NUtE at (C) HN (R^2 = 0.47, *P* < 0.001) (D) NN (R^2 = 0.4, *P* < 0.001) in 2013 field experiment

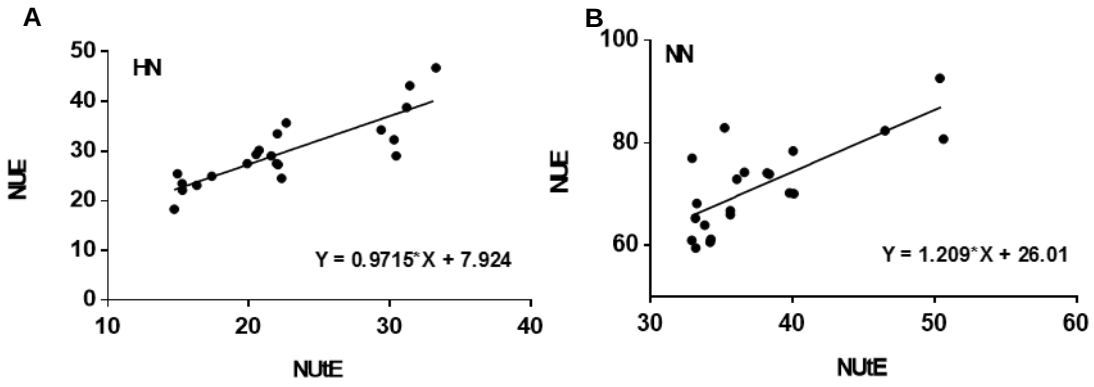


Figure 4.17 Linear regression between NUE and NUtE at (A) HN (R^2 = 0.7, *P* < 0.001) (B) NN (R^2 = 0.54, *P* < 0.001) in 2014 field experiment

Fertiliser NUE of the genotypes in 2013 glasshouse experiment showed positive and linear relationships with NUpE and NUtE at LN and HN. NUE had a positive linear relationship with NUpE at HN ($R^2 = 0.50$, $P < 0.001$) and LN ($R^2 = 0.42$, $P < 0.001$) (Figure 4.18 A and B). NUtE explained 83.1% and 81.9% ($P < 0.001$) of the variation in NUE at HN and LN, respectively (Figure 4.18 C and D).

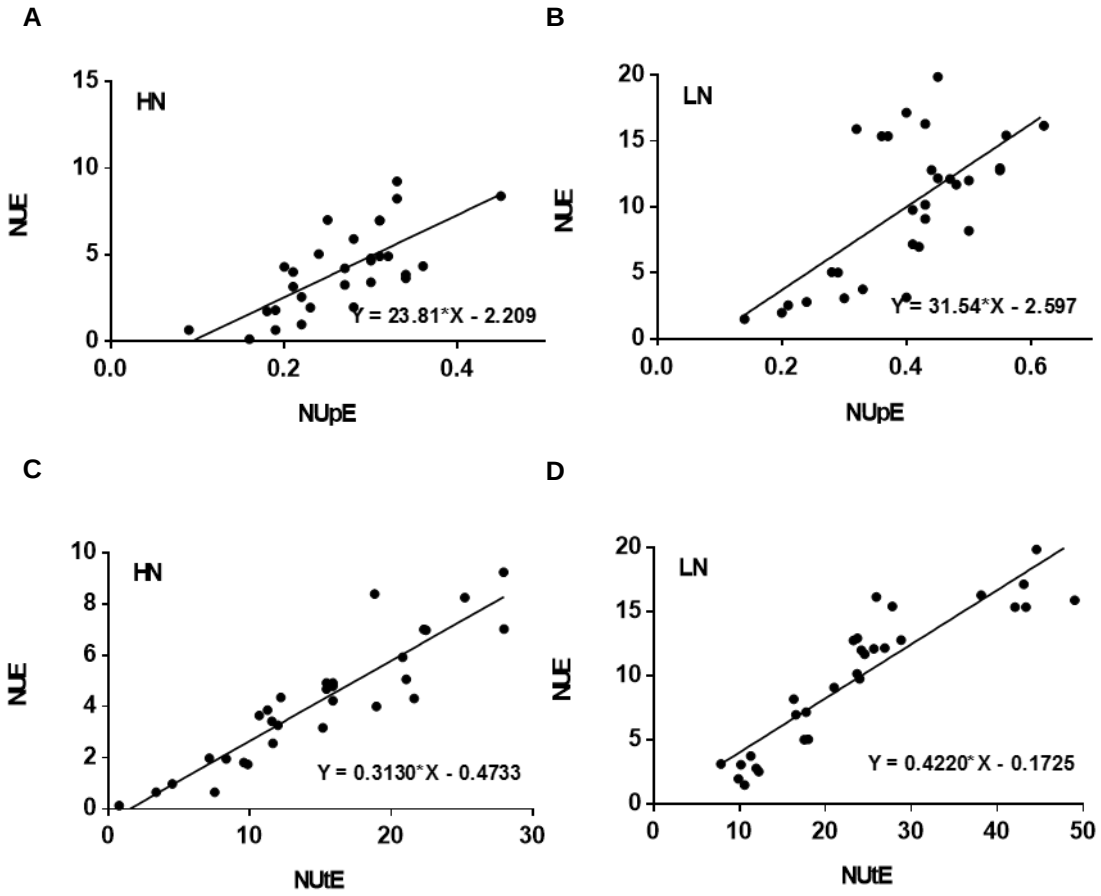


Figure 4.18 Linear regression between (A) NUE and NUpE at HN ($R^2 = 0.50$, $P < 0.001$) and (B) LN ($R^2 = 0.42$, $P < 0.001$). NUE and NUtE at (C) HN ($R^2 = 0.83$, $P < 0.001$) (D) LN ($R^2 = 0.82$, $P < 0.001$) in 2013 glasshouse experiment

When NUE was averaged across N treatment, NUpE was more associated with NUE of the genotypes used in glasshouse column experiment 2014. The regression between NUE and NUpE was strong ($R^2 = 0.77$, $P < 0.001$). It appeared that the NUtE was less related to NUE ($R^2 = 0.51$, $P < 0.001$) (Figure 4.19A and B).

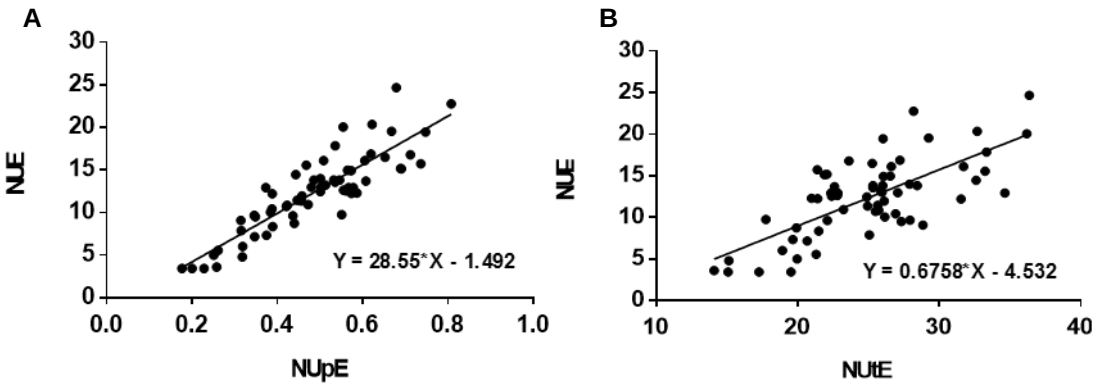


Figure 4.19 Linear regression between (A) NUE and NUpE (R^2 = 0.77, P < 0.001) and (B) NUE and NUtE (R^2 = 0.51, P < 0.001) in 2014 glasshouse experiment

4.2.6.1 Relationship between green area duration and NUE

Averaged green area duration (area under curve of greenness) of the genotypes regressed against NUE in the 2012 field experiment revealed a positive relationship (R^2 = 0.5, P < 0.05) (Figure 4.20). However, einkorn separated from rest of the genotypes and formed two distinct groups. Nither NUpE nor NUtE had a significant relationship with green area duration of the genotypes.

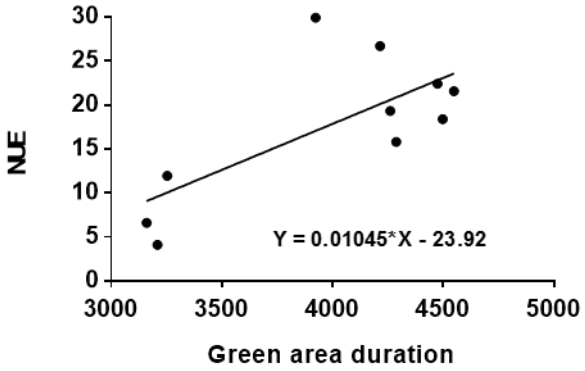


Figure 4.20 Linear regression between NUE and green area duration of the genotypes in 2012 filed experiment (R^2 = 0.51, P < 0.05)

4.2.6.2 Relationship between grain yield, NUpE and NUtE

Grain yield of the genotypes had a strong relationship with NUpE and NUtE. NUtE explained more variation in Grain yield than NUpE under optimum N conditions in 2012 field experiment (Figure 4.21 A and B). In 2013 field experiment, at HN, NUpE of the genotypes approximately equally explained by grain yield (R^2 = 0.60, P < 0.001)

and NUtE ($R^2 = 0.56$, $P < 0.001$). However, at NN, NUtE was more closely associated with grain yield ($R^2 = 0.55$, $P < 0.001$) than NUpE ($R^2 = 0.31$, $P < 0.001$).

There was no significant relationship between grain yield and NUpE at HN and NN in the 2014 experiment although NUtE at HN and NN explained 71% and 51% of the variation in grain yield, respectively. The inconsistency of the relationships among grain yield and the components of NUE observed may be due to different genotypes used in the experiments and difference of experiment site/season.

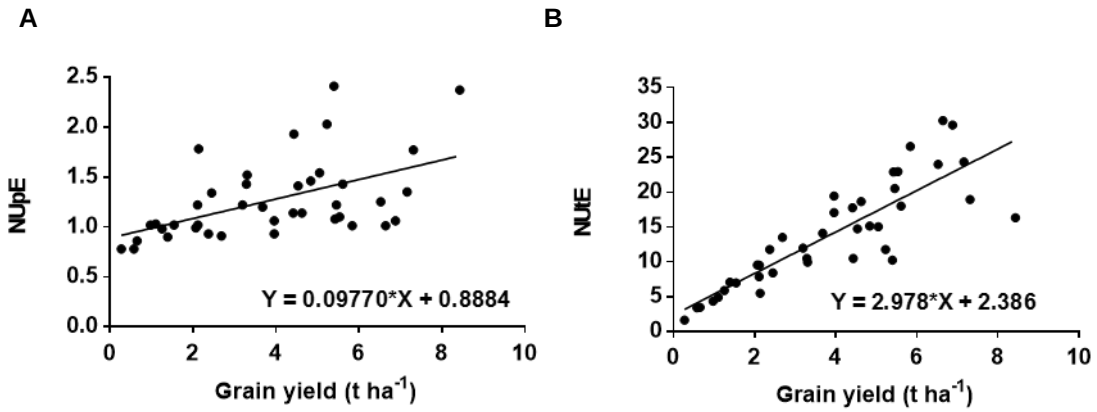


Figure 4.21 Linear regression between (A) grain yield and NUpE ($R^2 = 0.27$, $P < 0.001$) (B) grain yield and NUtE ($R^2 = 0.73$, $P < 0.001$) in 2012 field experiment

4.2.6.3 Relationship between grain yield and N% of plant components

Grain, straw and chaff N% had negative correlation with grain yield of the genotypes in 2012 field experiment. When grain yield increased, the N% of the grain decreased significantly. This phenomena is commonly identified as the N dilution effect (r = 0.42. $P < 0.01$). Similarly, high N% in straw (r = 0.57. $P < 0.001$) and chaff (r = 0.47. $P < 0.01$) may reduce grain yield of the genotype (Figure 4.22).

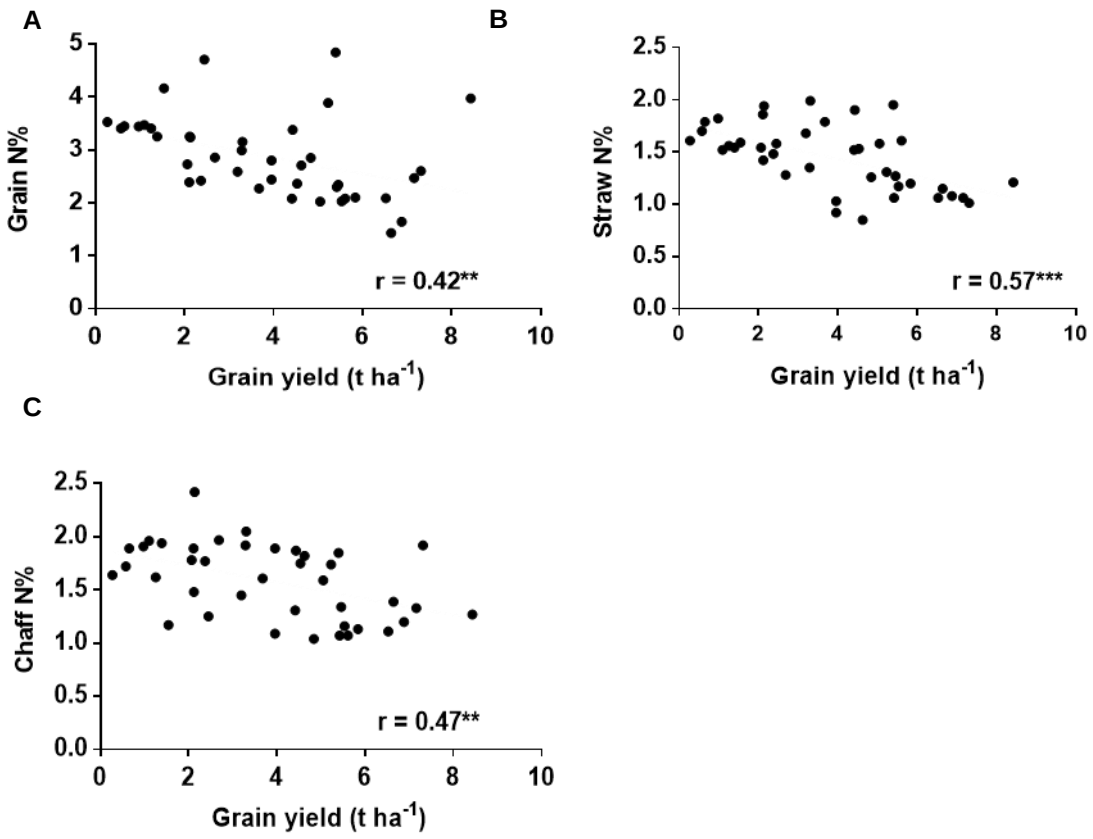


Figure 4.22 Correlation between grain yield and (A) grain N% (r = 0.42. *P* < 0.01) (B) straw N% (r = 0.57. *P* < 0.001) and (C) chaff N% (r = 0.47. *P* < 0.01) of the genotypes in 2012 field experiment. Each value is the mean of 4 replicates

Correlation between N% of grain, straw and chaff in 2013 and 2014 experiment at NN, LN and HN was shown in Table 4.19. According to that, grain N% had a negative relationship with grain yield regardless of the N supply.

Table 4.19 Correlation coefficients between grain, straw and chaff N% and grain yield in 2013 and 2014 field experiments at NN, LN and HN

N%	2013- Grain yield			2014- Grain yield		
	NN	LN	HN	NN	LN	HN
Grain	-0.73***	-0.78***	-0.56**	-0.63**	-0.67***	-0.81***
Straw	*NS*	0.55**	*NS*	*NS*	*NS*	0.56**
Chaff	*NS*	*NS*	*NS*	0.47*	*NS*	*NS*

***** Significant at *P* < 0.001, **significant at *P* < 0.01, *significant at *P* < 0.05, *NS* - not significant**

4.2.6.4 Relationship between remaining N in straw, plant height and NUE

Response of remaining N in straw to plant height of the genotypes was positive (R^2 = 0.24, $P < 0.001$) (Figure 4.23A). A negative regression was found between NUE and plant height where bread wheat and ancient wheat genotypes formed two distinct groups (R^2 = 0.23, $P < 0.01$) in 2012 field experiment (Figure 4.23B).

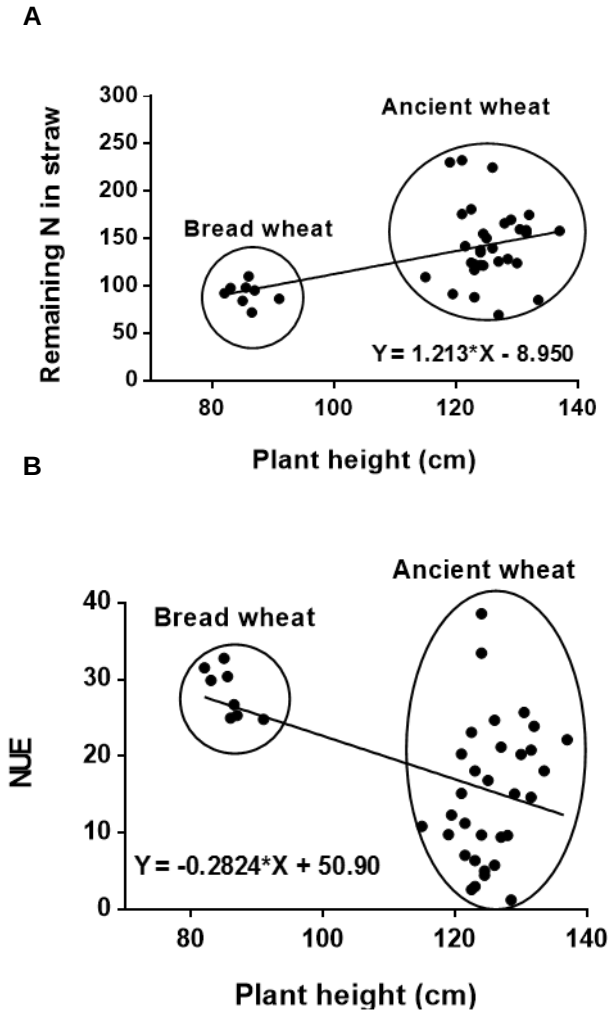


Figure 4.23 Linear regression between (A) straw N content at maturity (kg N ha^{-1}) and plant height (cm) (R^2 = 0.24, $P < 0.001$) (B) NUE and plant height (cm) (R^2 = 0.23, $P < 0.001$) at maturity in 2012 field experiment. Each value is the mean of 4 replicates

4.3 Discussion

Grain dry matter yield per unit availability of N from soil and/ or fertiliser is defined as nitrogen use efficiency (NUE) (Moll *et al*., 1982). N efficient plants provide economic benefits through increased grain yield and reduced cost of production due to less N fertiliser application (Gaju *et al*., 2011). Additionally, more than 70% of greenhouse gas emission in wheat is associated with fertiliser N application (Mortimer *et al*., 2004). NUE can be achieved by improving its components of nitrogen uptake efficiency (NUpE; total plant N uptake (N_{off})) per unit N availability from soil and/or fertiliser) and nitrogen utilisation efficiency (NUtE; grain dry matter yield per unit plant N uptake (N_{off})). Studying ancient wheat species for their NUE and related components is important to improve bread wheat since additional variation, if available in ancient wheat species, can be introduced through breeding programmes.

4.3.1 Genetic diversity of NUE and its components in ancient wheat

In the present study, NUE among genotypes varied significantly with highest NUE for bread wheat followed by spelt, emmer and einkorn. However, in general, N_{off} and NUpE were high in emmer and spelt compared to bread wheat. NUtE of bread wheat was always higher than ancient wheat genotypes resulting in high NUE. NUE of bread wheat genotypes in field experiments varied from 20-43 (kg grain DM $kg^{-1}N$) while spelt was 20-30 (kg grain DM $kg^{-1}N$), emmer 18-25 (kg grain DM $kg^{-1}N$) and einkorn 8-19 (kg grain DM $kg^{-1}N$). Gaju *et al*. (2011) reported a significant variation of NUE (18-38 kg grain DM $kg^{-1}N$) among 16 UK and French wheat genotypes under optimum N availability in the field. However, it is difficult to compare NUE data between experiments since people use different methods to calculate N availability for the plant from soil. In the 2013 glasshouse experiment, NUE together with NUpE were significantly lower than field experiments. This may be due to N fertiliser losses during sprinkler irrigation. Similarly, the glasshouse column experiment, where drip irrigation was used to supply nutrients and water, recorded low NUE due to leaching issues related to the irrigation system together with media (hydroleca clay pellets).

The genetic variation of NUpE of the genotypes can be explained partialy by senescence kinetics of the genotypes and plant height (Chapter 3). Delayed senescence of spelt genotypes, especially spelt cv. Oberkulmer together with deep and vigorous

root system may result in high N_{off} at maturity. Deep root systems allowed plant to extract N from deep soil layers where more leached N accumulated. However, for emmer 2, it can be suggested that, the high N_{off} and NUpE in all field experiments may be associated with high fertiliser N recovery rate. It can be suggested that, at early growth stage, emmer 2 has the potential to take up more fertiliser N from top layers of the soil before it is transferred to deeper layers of the horizon. King *et al*. (2003), based on modelling, and suggested that high NUpE can be achieved by optimising root system architecture. Due to the nature of mobilisation of NO_3^-, it is vital to absorb N rapidly within the active root zone (Liao *et al*., 2004). However, NUpE of emmer and spelt genotypes or spelt and bread genotypes were not statistically difference between each other while emmer had significantly high NUpE than bread wheat in 2012 field experiment (Table 4.2). In 2013 field experiment at HN, NUpE of emmer, sepelt and bread wheat was not statistically different while in the 2014 field experiment, NUpE of emmer 2 and spelt Tauro was significantly higher than all other genotypes at HN but at NN, statistical differences between getypes of emmer, spelt and bread wheat were not significant (Table 4.7).

In general, under optimum condition, wheat crops are little limited by assimilate supply during grain filling (Dreccer et al., 1977; Calderini et al., 2006) while more limited under low to moderate N supply. This is affected by early remobilisation of N from vegetative parts of the plant hence the start senescence. Early senescence decreases the capacity of the plant to be active in photosynthesis. Therefore, stay-green properties are useful to produce more assimilates during the grain filling period. In our study, delayed onset of senescence of spelt cv. Oberkulmer may be due to significant content of storage N in true stem which facilitate grain N demand without significant loss of photosynthetic tissues on the flag leaf at early phase of grain filling. However, at NN stored N is limited and not enough to fulfil the demand of grain N hence accelerated flag leaf senescence was observed. Detailed studies have been carried out previously to understand the mechanism of stay-green. In sorghum, N dynamics play a major role in balancing N demand and supply during grain filling which reduce the process of N remobilisation (Borrell and Hammer 2000). Stay-green mutants have also been identified in durum wheat (*Triticum turgidum* spp. durum) (Spano *et al*., 2003). In emmer (*Triticum turgidum* spp. dicoccodes), a transcription factor (*NAM- B1*) accelerates senescence hence rapid N remobilisation occurs during grain filling

whereas durum wheat varieties carry the non-functional *NAM-B1* alleles (Uauy *et al*., 2006). Christopher *et al*. (2008) found that the stay-green properties of SeriM82, spring wheat genotype, are associated with deep root system which is able to extract water from the deep layers of the soil in Australia.

With regards to the positive and strong relationship with plant height and N_{off} of ancient wheat genotype in our study, it can be suggested tall plants have more storage reserves of N in stems and investment of N in stem structural material hence high N_{off} (but einkorn had lower N_{off} and NUpE throughout the study). Generally in cereals, maximum plant N accumulation occurs at anthesis and subsequently decreases through maturity (Martre *et al*., 2003). At anthesis about 35% of the total N in the wheat canopy is in the leaf lamina and rest of the N is partitioned between leaf sheath, true stem and ear. Among them 30% of canopy N can be found in true stems (Critchley, 2001). Stem N can be located as structural, transport, storage and metabolic functions (Pask, 2009). However, majority of stem N allocated between structural and reserve pools which, during grain filling, remobilise to fulfil grain N demand. Pask (2009) working with four UK feed winter wheat cultivars, indicated that 60% of the N in the stem belongs to reserve N pool under optimum conditions. Foulkes *et al*. (2009) suggested that a high capacity to absorb N in the true stem together with storage capacity subsequently enhance N_{off} hence high NUpE. True stem N absorption has therefore be identified as a favourable trait to improve NUpE especially under high soil N mineralisation. In the present study, N_{off} increased with additional N supply though NUpE decreased massively. High NUpE of the genotypes at NN was observed in 2013 than in 2014.

Post-anthesis assimilates directly transferred to grains and remobilisation of stored assimilates in vegetative parts of the plant takes place in grain crops (Gebbing *et al*., 1999). When abiotic stress is severe during the grain filling period, pre-anthesis stored reserves play a major role for grain filling (Tahir and Nakata 2005). On the other hand, the importance of pre-anthesis assimilate translocation was also reported under biotic stress (Katsantonis *et al*., 2008). Under optimum conditions, stored carbohydrates in stems contribute 10-12% of the final grain yield in wheat while it increased up to 40% under drought (Bidinger *et al*., 1977) may due to high biomass remobilisation efficiency hence N remobilisation efficiency. The present results indicated a strong relationship between NUtE and grain yield of the genotypes. Improved harvest index

and introduction of semi-dwarf genes are associated with high NUtE of bread wheat than ancient wheat genotypes.

4.3.2 Effect of N% and N retained in straw on NUE

N% of the straw, chaff and grain varied significantly among genotypes and interacted with N supply. It was clear that the N% of straw in bread wheat under HN was lower than other genotypes indicating its ability to remobilise more N towards grain production hence increase grain yield. Supporting this argument, further we found a strong association between NUE and NUtE of the genotypes at HN by reporting higher NUtE in bread wheat cultivars throughout the study. Grain N% was significantly lower in bread wheat cultivars throughout the study while recording highest grain yield, this phenomenon is commonly identified as the N dilution effect. This is one of the main challenges faced by wheat breeders; increase grain yield without decreasing grain protein concentration. Einkorn and emmer genotypes had less grain yield hence more grain N%. Moudry *et al*. (2011) reported that crude protein yield per hectare of emmer and spelt was higher than bread wheat.

In the 2012 field experiment, under optimum N application, N retained in straw and chaff together with grain N for N_{off} at maturity differed among species. The majority of N_{off} of bread wheat at maturity was in the grain (47%) though einkorn only partitioned 21% of N_{off} towards the grain. On the other hand the most N in straw was observed in einkorn (62%) while bread wheat had the lowest (40%). The proportion of N_{off} partitioned to grain and straw was not significantly affected by the species hence it was not different between bread wheat, spelt and emmer. A small amount of N expected to be in root systems (10- 20%; Anderson *et al*., 2006) though was not accounted for in this study.

In 2013 and 2014 experiment, in general, grain N uptake as a proportion of N_{off} (NHI) at maturity was 74 to 67% which was higher than 2012. In general, grain N represents approximately 75% of N_{off} for cereals (Vaidyanathan, 1984). Further, 18 to 19% of N_{off} was observed in straw as remaining N and 9 to 13% in chaff as remaining N. The lower percentage of N_{off} in grains at maturity in 2012 experiment than in 2013 and 2014 experiments may be related to lower rate of N remobilisation hence more N remained in straw and chaff. This can be partially explained by wet and warm weather during 2012 growth season when compared to 2013 and 2014 which result in soil N

mineralisation hence high N_{off}. Lodging of emmer and einkorn may have had an adverse effect on stem N remobilisation to grains in all experiments. Further, low biomass remobilising efficiency of genotypes together with delayed senescence may have had an adverse effect on grain N uptake in 2012. Supporting this argument, genotypes reported lower HI in 2012 than in 2013 and 2014.

Partitioning N_{off} to straw, chaff and grain N gradually increased with N supply. Previous study reported positive response of all straw components toward N uptake partitioning at high N level (Cox *et al*., 1985). Genotypic differences in N partitioning at maturity in two out of three field experiments were significant. This may be due to vast genetic background of the genotypes used in the study which belong to four species of wheat. Pask (2009) suggested that the possible reason for the varietal difference in N partitioning for straw at harvest may be associated with true stem length. NHI of bread wheat cultivars in 2012 was 0.5 while einkorn genotypes were 0.15. All spelt and emmer had NHI in between 0.5 and 0.15. Overall, in 2013 and 2014 NHI of bread wheat was broadly similar at 0.74 and 0.66 respectively. Usually for winter wheat NHI is around 0.70 (Austin and Jones, 1975). In agreement with Bloom *et al*. (1988) NHI appeared to be stable across the range of N treatments but has been observed to decrease with N supply (Blacklow and Incoll, 1981). Relatively low NHI in 2012 may be related to low NUtE hence more N remains in straw and chaff. Also, low total irradiance and high rainfall in post-anthesis grain filling (June and July) had an adverse effect on NHI. Further, grain filling was significantly affected by fusarium head blight recorded in 2012 field experiment.

4.3.3 NUE in relation to its components

According to the results of the 2012 field experiment, genetic variation in NUE was more associated with NUtE than NUpE. This may be due to high N_{off} of all genotypes despite their grain yield. In contrast, experimenting on 25 varieties of wheat in USA illustrated significant variation for NUE with NUpE accounting for 54% of the variation (Van Sanford and MacKnow, 1986). However, in the 2013 field experiment a stronger relationship was found between NUE and NUpE than NUtE under HN and NN conditions.

Similarly, Le-Gouis *et al*. (2000) experimenting on 20 winter wheat cultivars in France found that NUpE accounted for more of the genetic variation in NUE at LN than HN.

Interestingly, in the 2014 field experiment, there was no significant relationship between NUE and NUpE either at HN or NN. This may be due to less variation of NUpE among genotypes in the 2014 experiment. However, NUtE explained more variation in NUE at HN than NN. Gaju *et al*. (2011) reported that the genetic variation in NUE was more closely associated with NUtE than NUpE under both HN and LN conditions. In barley, NUtE explained 60% of the variation in NUE while NUpE explained only 40% (Bingham *et al*., 2012).

Results of 2013 glasshouse experiment showed that NUpE explained more variation of NUE than NUtE. Though NUE in the 2014 glasshouse experiment was more associated with NUpE than NUtE suggesting that there is a huge impact of growing media and environment on NUpE than NUtE. In general our data showed that the contribution of NUpE and NUtE were dependent on N level. NUtE explained more variation in NUE in three out of five experiments conducted to quantify. Effective management strategies of N have large potential to increase NUE by improving NUpE (Foulkes *et al*., 2009).

4.4 Conclusions

- NUE was higher in bread wheat followed by spelt, emmer and einkorn.
- Emmer 2 and and spelt cv. Oberkulmer had higher NUpE when compared to the other genotypes.
- NUtE of bread wheat was higher than ancient wheat species and closely related to harvest index and reduced plant height.
- High NUpE of emmer 2 may be related to N capturing capacity at early plant growth and high fertiliser N recovery rate
- High NUpE of spelt cv. Oberkulmer may be related to delayed senescence hence extended green area duration.
- N supply has a negative relationship with NUpE and its components.
- NUpE and NUtE explained more of the variation in NUE at HN than LN/NN.
- Relationship between NUE and its components was not consistent between experiments. NUtE explained more of the variation in NUE than NUpE in three of five experiments. Further, NUpE and NUtE explained more of the variation in NUE at HN than NN.

Chapter 5

Root system architecture and nitrogen uptake

5.1 Introduction

In the present chapter, the root system architecture of ancient wheat species of einkorn, emmer and spelt, together with modern bread wheat, is explored with special reference to NUpE at maturity.Differences in root architecture of ancient wheat throughout plant growth are described in detail in this chapter. Root morphological parameters of length, biomass, volume, diameter and root length distribution, together with number of seminal roots, lateral roots, tip angles and emergence angles of seminal roots, maximum width, depth and width to depth ratio and convex hull of the seedling root system are discussed.

Different materials such as germination papers, sand, compost and hydroleca clay pellets were used as growth media of the experiments while *RootNav* and *WinRHIZO* software were used to analyse images. Four experiments were conducted under controlled environment conditions at Sutton Bonington Campus, University of Nottingham.

Objective

The main objective of this chapter is to identify and quantify root architectural traits of ancient wheat species and develop relationship with NUpE at maturity.

Specific hypotheses

9. Root system architecture of ancient wheat species is significantly different from modern bread wheat.
10. Early seedling root architecture has a significant influence on mature root system.
11. A large root biomass would enhance N uptake, which contributes to the greater green area and above-ground biomass production.
12. Seedling root traits could be used as selection criteria for NUpE at maturity.

5.2 Results

5.2.1 2D root phenotyping experiment

5.2.1.1 Number of seminal roots and lateral roots

The mean number of seminal and lateral roots was significantly different between genotypes at 14 days after transferral to germination paper (DAT). The greatest number of seminal roots (5.27, $P < 0.001$) and lateral roots (1.43, $P < 0.01$) was recorded in emmer 2, while the lowest number of seminal roots (3.76) was recorded in bread wheat cv. JB Diego (Figure 5.1A). However, the number of seminal roots produced by emmer 1 and 2 was not significantly different. Einkorn 1 and einkorn 2 did not produce any lateral roots by 14 DAT.

According to the average of the species, emmer produced the most seminal roots followed by einkorn, spelt and bread wheat (Table 5.1). Emmer produced 17, 20 and 22% more seminal roots than einkorn, spelt and bread wheat, respectively.

5.2.1.2 Average length of seminal root

Spelt Oberkulmer produced the longest average seminal root length of 141.6 mm ($P < 0.001$) (Figure 5.1B). However, all spelt genotypes; emmer 1, bread wheat cv. JB Diego also had long seminal roots at 14 DAT. Average length of seminal roots of the species is shown in Table 5.1. According to that, the longest seminal roots were recorded in spelt followed by bread wheat, emmer and einkorn. Spelt had 29, 11 and 7% longer seminal roots than einkorn, emmer and bread wheat, respectively.

5.2.1.3 Total root length

Total root length was higher in emmer 1 (639.21 mm, $P < 0.001$) but not significantly different from emmer 2 and spelt Oberkulmer (Figure 5.1C). The shortest total root length was shown in einkorn 1 which was 46% shorter than emmer 1. When averaged across species, emmer produced 35, 20 and 11% greater total root length than einkorn, bread wheat and spelt, respectively (Table 5.1).

5.2.1.4 Maximum width

Emmer 2, emmer 1 and bread wheat cv. Xi 19 produced root systems with a maximum width of 142.2 mm ($P < 0.001$) (Figure 5.1D). Overall, emmer and bread wheat had the widest root system while spelt and einkorn species had narrow seedling root systems. Root systems of einkorn, spelt and bread wheat were 56, 47 and 12% narrower than that of emmer.

5.2.1.5 Maximum depth

It was observed that bread wheat cv. JB Diego had a deep root system (Figure 5.1E) recording maximum root depth of 202 mm ($P < 0.001$) while a shallower root system was observed in einkorn 1 (127.1 mm). On average, modern bread wheat had the deepest root systems followed by spelt then emmer and einkorn. Root systems of einkorn species were narrow and shallower than all other genotypes at 14 DAT. Depth of the root system of bread wheat was 9, 24 and 32% deeper than spelt, emmer and einkorn, respectively.

5.2.1.6 Width to depth ratio

The maximum width to depth ratio was recorded in emmer 2 (1.05, $P < 0.001$) while the lowest value was recorded in einkorn 3 (0.36). Average width to depth ratio for emmer, bread wheat, einkorn and spelt was 0.96, 0.63, 0.46 and 0.40, respectively. However, it was not significantly different between spelt and einkorn species.

5.2.1.7 Convex hull

Convex hull of the root system of bread wheat cv. Xi 19 was 14850 mm^2 while 4675 mm^2 in einkorn 3 (Figure 5.1F). Average convex hull of bread wheat and emmer was not significantly different but spelt and einkorn species were significantly lower than both bread wheat and emmer. Convex hull of the bread wheat was 4% higher than emmer but it was 39% and 63% higher than spelt and einkorn.

5.2.1.8 Tip angle of seminal root

Average tip angle of seminal roots was significantly different between genotypes in the range of 16.7^o to 35.8^o ($P < 0.001$) (Figure 5.1G). Tip angles were calculated

relative to the Y-axis of the image for a given root. Emmer 2 recorded largest tip angle of seminal roots while narrow tip angles of seminal roots were observed in spelt SB. On average, the largest tip angle was recorded in emmer (31.9^{o}) followed by bread wheat (27.6^{o}) then einkorn (23.2^{o}) and spelt (18.7^{o}). Emmer had 42, 27 and 14% wider tip angle than spelt, einkorn and bread wheat, respectively.

5.2.1.9 Emergence of seminal root

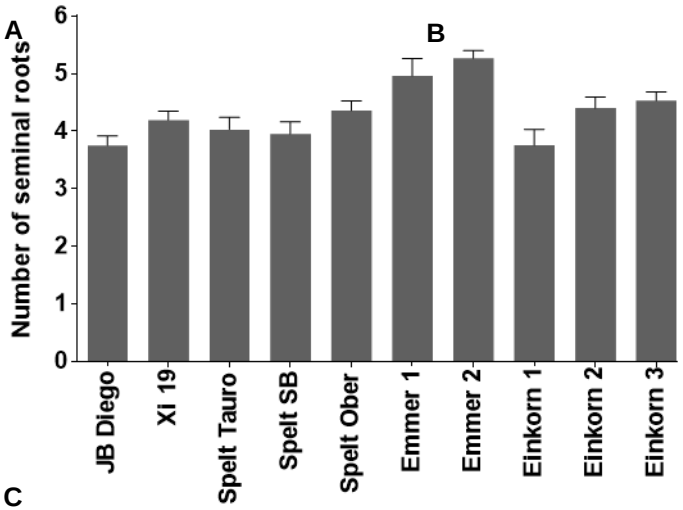
Average emergence angle of seminal root was 21.6^{o} to 35.1^{o} between genotypes ($P <$ 0.01) (Figure 5.1H). Einkorn 1 showed widest emergence angles at 14 DAT while spelt Oberkulmer had the narrowest emergence angle which was 61% narrower than einkorn 1. Nevertheless, average emergence angle of einkorn was not significantly different from emmer species. Also, emergence angle of emmer was not significantly different from bread wheat while spelt had the narrowest emergence angle at 14 DAT.

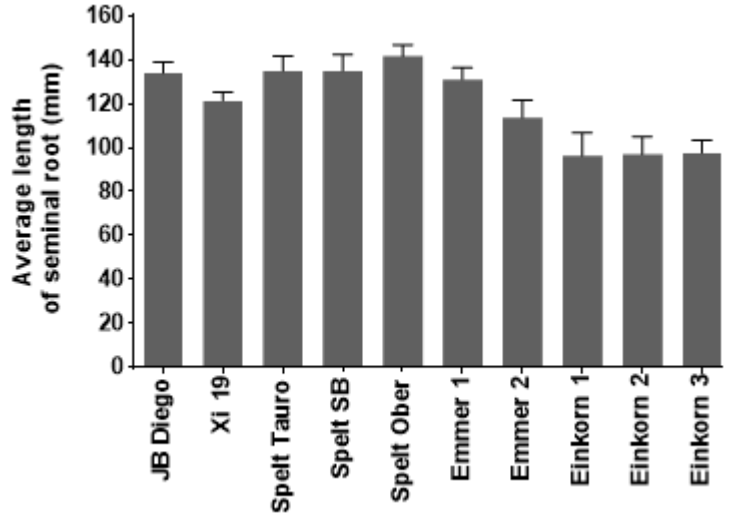
Figure 5.2 shows representative images of *RootNav* illustrating root system architecture (RSA) of 10 genotypes while Figure 5.3 shows overlays of RSA images of all replicates of genotypes at 14 DAT.

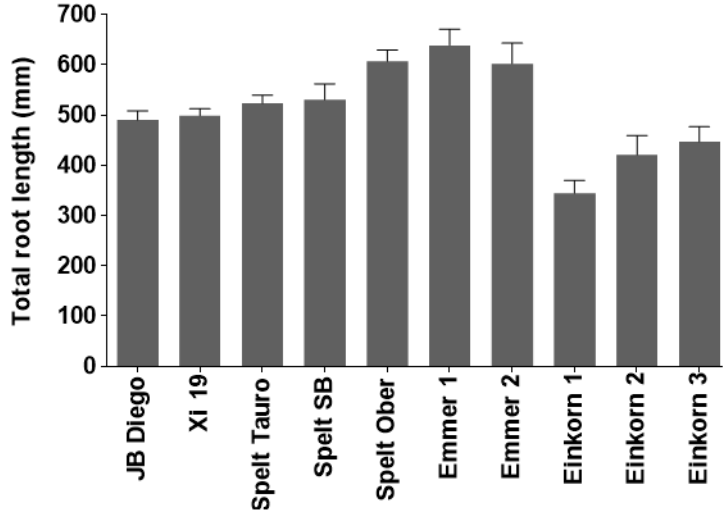
Table 5.1 Number of seminal roots, total root length, average length of a seminal roots, maximum width, depth, convex hull and width to depth ratio of the species of modern bread wheat, spelt, emmer and einkorn at 14 DAT. In each section, mean values followed by the same letter within columns are not significantly different ($P < 0.05$) according to Duncan's multiple range test

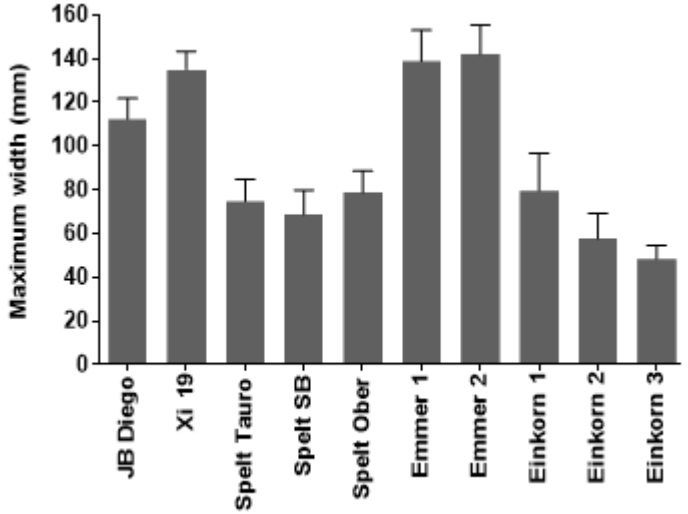
Species	Number of seminal roots	Total root length (mm)	Average length of seminal roots (mm)	Maximum width (mm)	Maximum depth (mm)	Convex hull (mm^2)	Width : depth ratio
Bread wheat	3.98^{c}	494.84^{c}	127.64^{b}	124.21^{a}	197.71^{a}	14150^{a}	0.63^{b}
Spelt	4.15^{bc}	558.81^{b}	137.51^{a}	75.09^{b}	180.82^{b}	8700^{b}	0.41^{c}
Emmer	5.14^{a}	628.12^{a}	123.53^{b}	140.18^{a}	151.42^{c}	13759^{a}	0.95^{a}
Einkorn	4.34^{b}	417.75^{d}	97.20^{c}	59.12^{b}	135.40^{d}	5180^{c}	0.44^{c}
SED	0.168	22.091	5.114	8.773	4.986	1023.4	0.050
(*df* = 157)* **							

***** Significant at $P < 0.001$**


A
Number of seminal roots
0
1
2
3
4
5
6
JB Diego
Xi 19
Spelt Tauro
Spelt SB
Spelt Ober
Emmer 1
Emmer 2
Einkorn 1
Einkorn 2
Einkorn 3


B
Average length of seminal root (mm)
0
20
40
60
80
100
120
140
160
JB Diego
Xi 19
Spelt Tauro
Spelt SB
Spelt Ober
Emmer 1
Emmer 2
Einkorn 1
Einkorn 2
Einkorn 3


C
Total root length (mm)
0
100
200
300
400
500
600
700
JB Diego
Xi 19
Spelt Tauro
Spelt SB
Spelt Ober
Emmer 1
Emmer 2
Einkorn 1
Einkorn 2
Einkorn 3


D
Maximum width (mm)
0
20
40
60
80
100
120
140
160
JB Diego
Xi 19
Spelt Tauro
Spelt SB
Spelt Ober
Emmer 1
Emmer 2
Einkorn 1
Einkorn 2
Einkorn 3

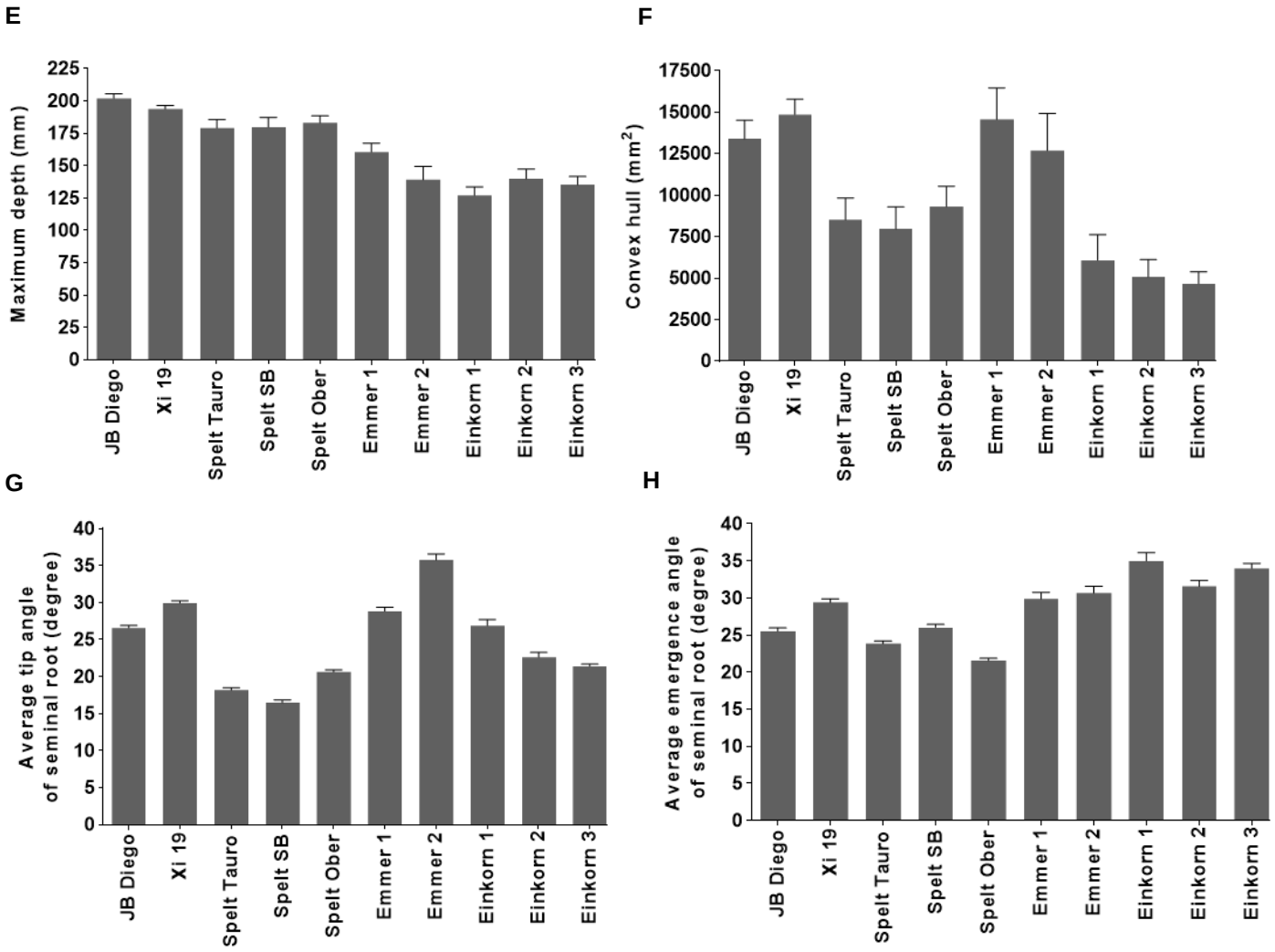


Figure 5.1 Phenotypic variation of RSA traits. (A) Number of seminal roots (B) average length of a seminal root (mm) (C) total root length (mm) (D) maximum width (E) maximum depth (F) convex hull (mm^2) (G) average tip angle of seminal root (degree) (H) average emergence angle of seminal root (degree), of the root systems grown vertically on germination paper at 14 DAT. Error bars represent SE of the means of genotypes

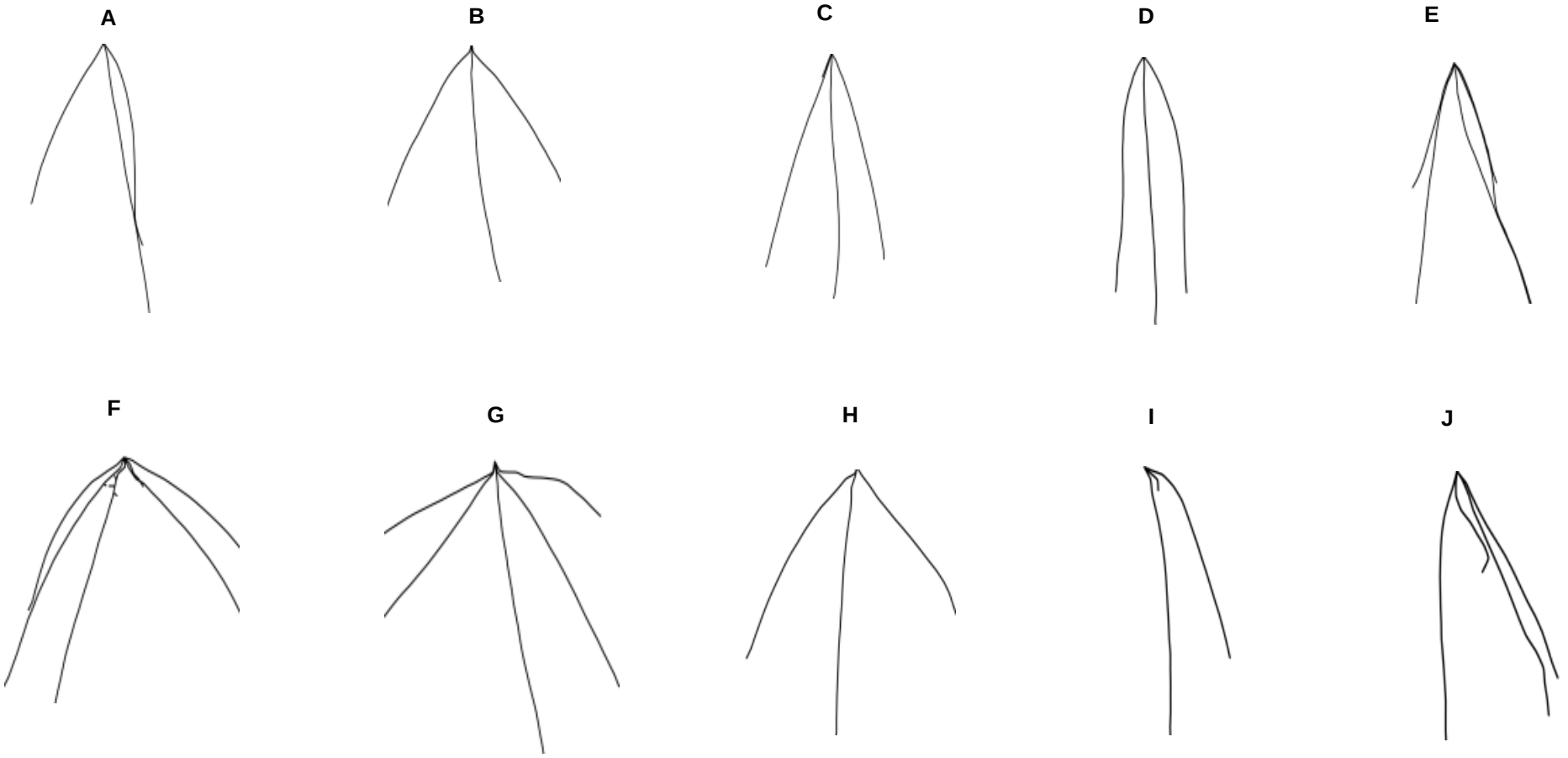


Figure 5.2 Representative RSA images of bread wheat (A) JB Diego (B) Xi 19 (C) Spelt Tauro (D) Spelt SB (E) Spelt Oberkulmer (F) Emmer 1 (G) Emmer 2 (H) Einkorn 1 (I) Einkorn 2 and (J) Einkorn 3 at 14 DAT in 2D root phenotyping experiment

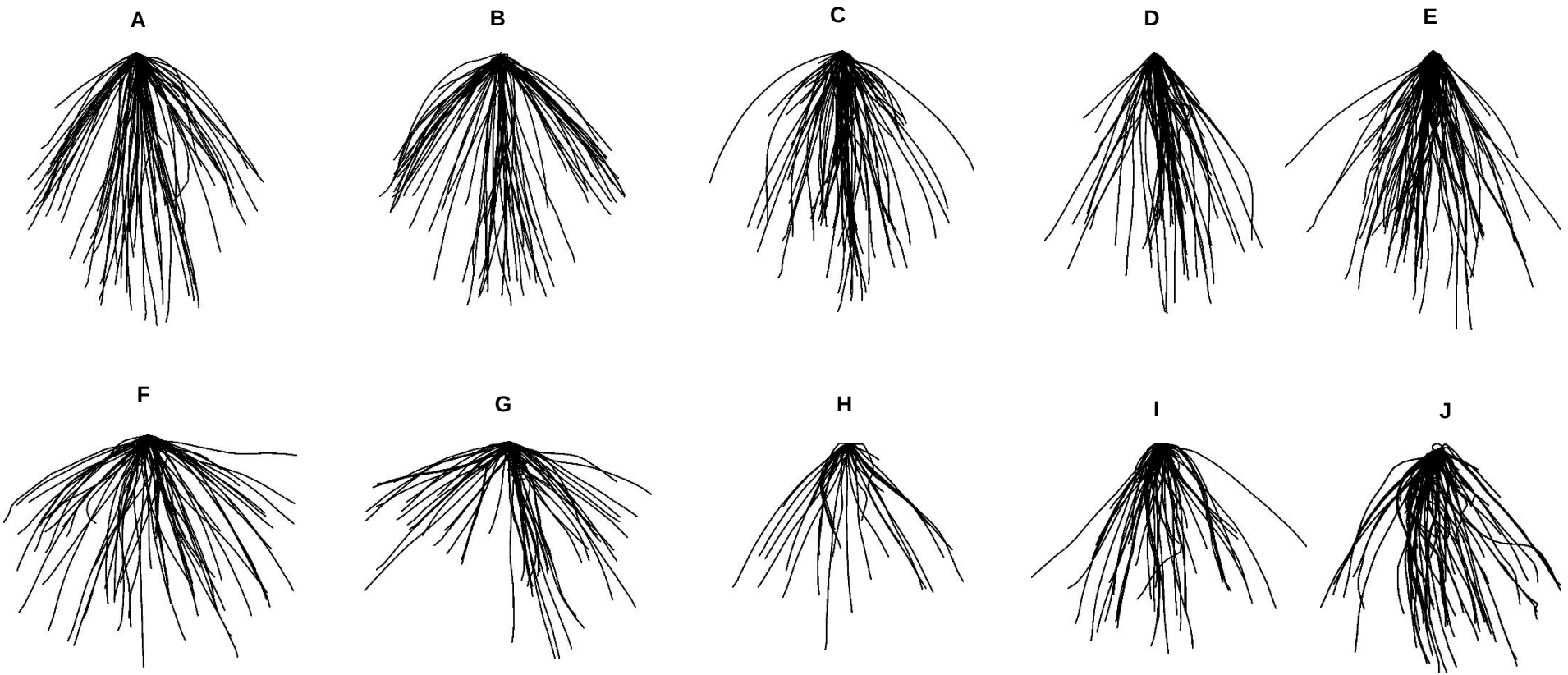


Figure 5.3 Root system architecture of bread wheat (A) JB Diego (B) Xi 19 (C) Spelt Tauro (D) Spelt SB (E) Spelt Oberkulmer (F) Emmer 1 (G) Emmer 2 (H) Einkorn 1 (I) Einkorn 2 and (J) Einkorn 3 at 14 DAT in 2D root phenotyping experiment. These images are produced by overlaying all RSA images of the replicates of 10 genotypes using *RootNav* software (n = 10 to 25)

5.2.1.10 Relationship between root traits

There was a positive correlation between total root length and the average length of a seminal root (r = 0.62; *P* < 0.001) (Figure 5.4A). The correlation between maximum width of the root system and tip angle of the seminal roots was significant (r = 0.54; *P* < 0.001) (Figure 5.4B). A strong positive relationship was observed between maximum depth of the root system and the average length of a seminal root (r = 0.74; *P* < 0.01) (Figure 5.4C). Also, the ratio between width to depth was strongly correlated with the tip angle of the seminal roots (r = 0.93, *P* < 0.001). Total root length had a positive correlation with number of seminal roots (r = 0.43, *P* < 0.001) (Figure 5.4D).

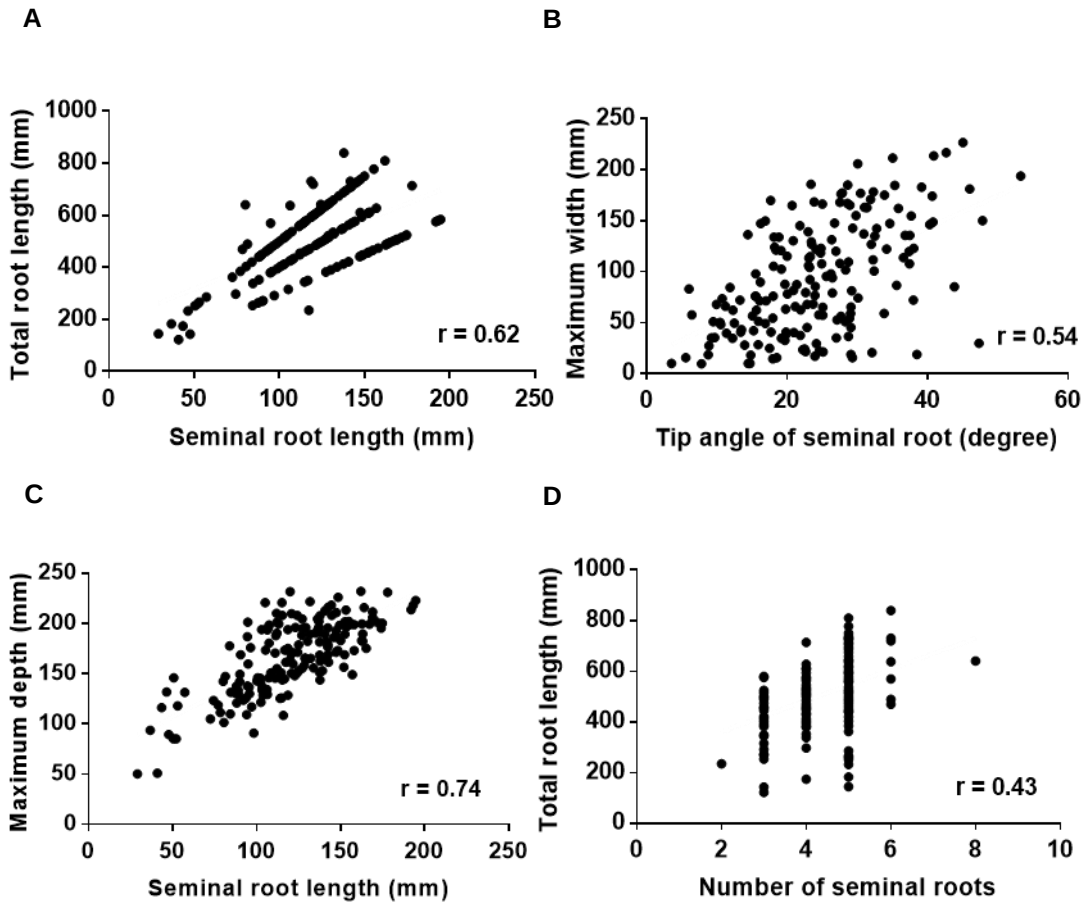


Figure 5.4 Correlation between (A) total root length and average length of the seminal root (r = 0.62, *P* < 0.001) (B) maximum width of the root system and the tip angle of the seminal root (r = 0.54, *P* < 0.001) (C) maximum depth of the root system and the average length of the seminal root (r = 0.74, *P* < 0.01) (D) total root length and the number of seminal roots (r = 0.43, *P* < 0.001) at 14 DAT

5.2.2 Glasshouse modular tray experiment

The effect of genotype, medium and the interaction were highly significant ($P < 0.001$) for number of seminal roots, total root length, root volume, root diameter, root biomass, root to shoot ratio and SRL at 14 DAT.

5.2.2.1 Number of seminal roots

The greatest number of seminal roots were observed in emmer 2 (7.30 in compost and 7.35 in sand), while lowest value was recorded in einkorn 1 (4.85) and bread wheat cv. JB Diego (4.64) in compost and sand, respectively (Figure 5.5). All of the ancient wheat genotypes produced more seminal roots in sand than in compost.

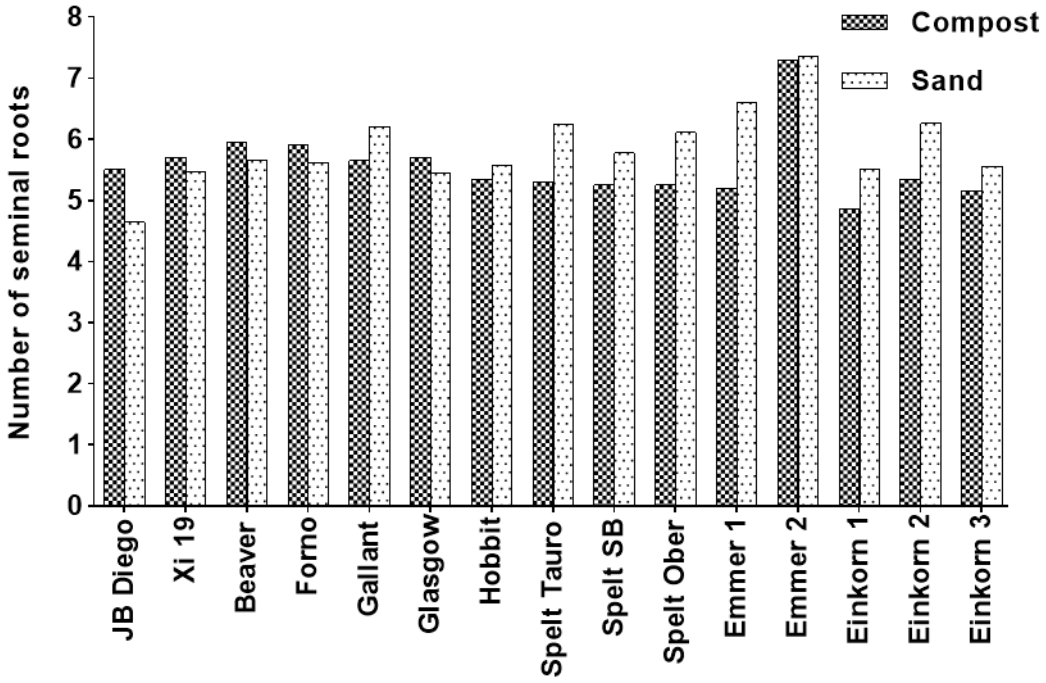


Figure 5.5 Number of seminal roots in sand and compost at 14 DAT (SED GT x M = 0.21, *df* = 545)

5.2.2.2 Total root length

The longest root length was recorded in emmer 2 both in sand and compost (Figure 5.6). All genotypes, except bread wheat cv. JB Diego, recorded longer roots in sand than in compost. Averaged across species, total root length was higher in emmer followed by spelt then bread wheat and einkorn in both media. Emmer, spelt, bread wheat and einkorn produced 18.8, 20.7, 14.5 and 5.7% longer roots in sand than compost, respectively. It was observed that the number of lateral roots were very high

in sand, though it was not possible to count them all manually and measure the length separately.

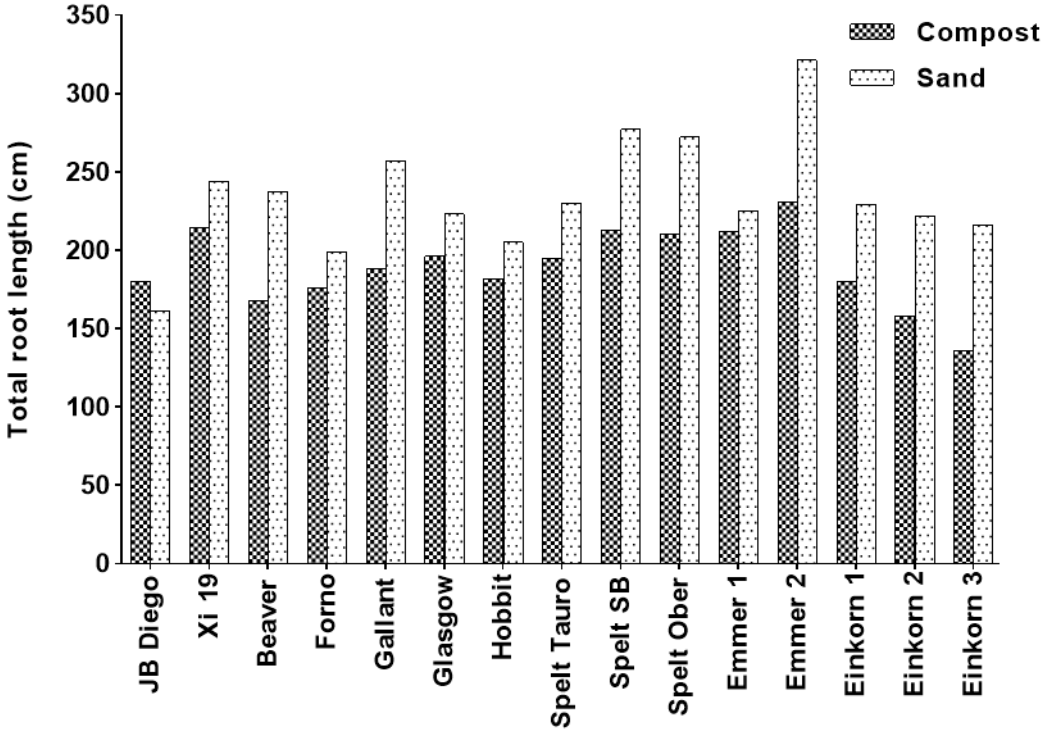


Figure 5.6 Total root length of 15 genotypes in sand and compost at 14 DAT (SED GT x M = 15.78, *df* = 545)

5.2.2.3 Root diameter

The average root diameter of the genotypes was between 0.22 to 0.32 mm in compost and 0.27 to 0.33 mm in sand at 14 DAT. Spelt Tauro recorded the highest average root diameter (0.32, $P < 0.001$) in compost while bread wheat cv. JB Diego showed the highest value in sand (0.33, $P < 0.001$). It was noticed that emmer 2, spelt Tauro, einkorn 2 and einkorn 3 had same root diameter in both growth media. Overall, the thickest roots were observed in sand.

5.2.2.4 Root volume

Genotypes grown in sand produced the highest root volume. The highest value was recorded in emmer 2 for both media. Lowest root volume was observed in einkorn 3 for compost and einkorn 1 for sand (Table 5.2). Overall, root volume of two week old

seedlings was higher in emmer and spelt than bread wheat and einkorn in both growing media (Figure 5.7).



Figure 5.7 Average root volume of bread wheat, spelt, emmer and einkorn in compost and sand media (SED Species x M = 0.008, *df* = 567)

5.2.2.5 Root and shoot biomass

Root biomass was approximately twofold higher in sand than compost ($P < 0.001$). Emmer 2 had the heaviest root systems in compost and spelt SB had the heaviest roots in sand (Table 5.2). Spelt produced 4, 16 and 41% heavier root systems than emmer, bread wheat and einkorn in sand, correspondingly. Figure 5.8 shows root and shoot biomass of the seedlings under sand and compost. Emmer and spelt genotypes had highest shoot biomass production in sand while bread wheat cvs showed higher shoot biomass in compost.

5.2.2.6 Root to shoot ratio (R: S)

At 14 DAT, R: S was significantly different between genotypes (Table 5.2). The lowest R: S value was observed in einkorn 1 and 3 in compost and all einkorn cvs in sand. R: S was higher in sand than in compost. It was noticed that bread wheat cv. Gallant had a higher R: S value of 0.79 in sand. On average, bread wheat had the highest R: S values followed by emmer, spelt and einkorn. R: S of bread wheat, spelt, emmer and einkorn in sand was 69, 53, 52 and 56% higher than it was in compost, respectively. Also, R: S of bread wheat in sand was 26, 25 and 38% higher than spelt, emmer and einkorn, respectively.

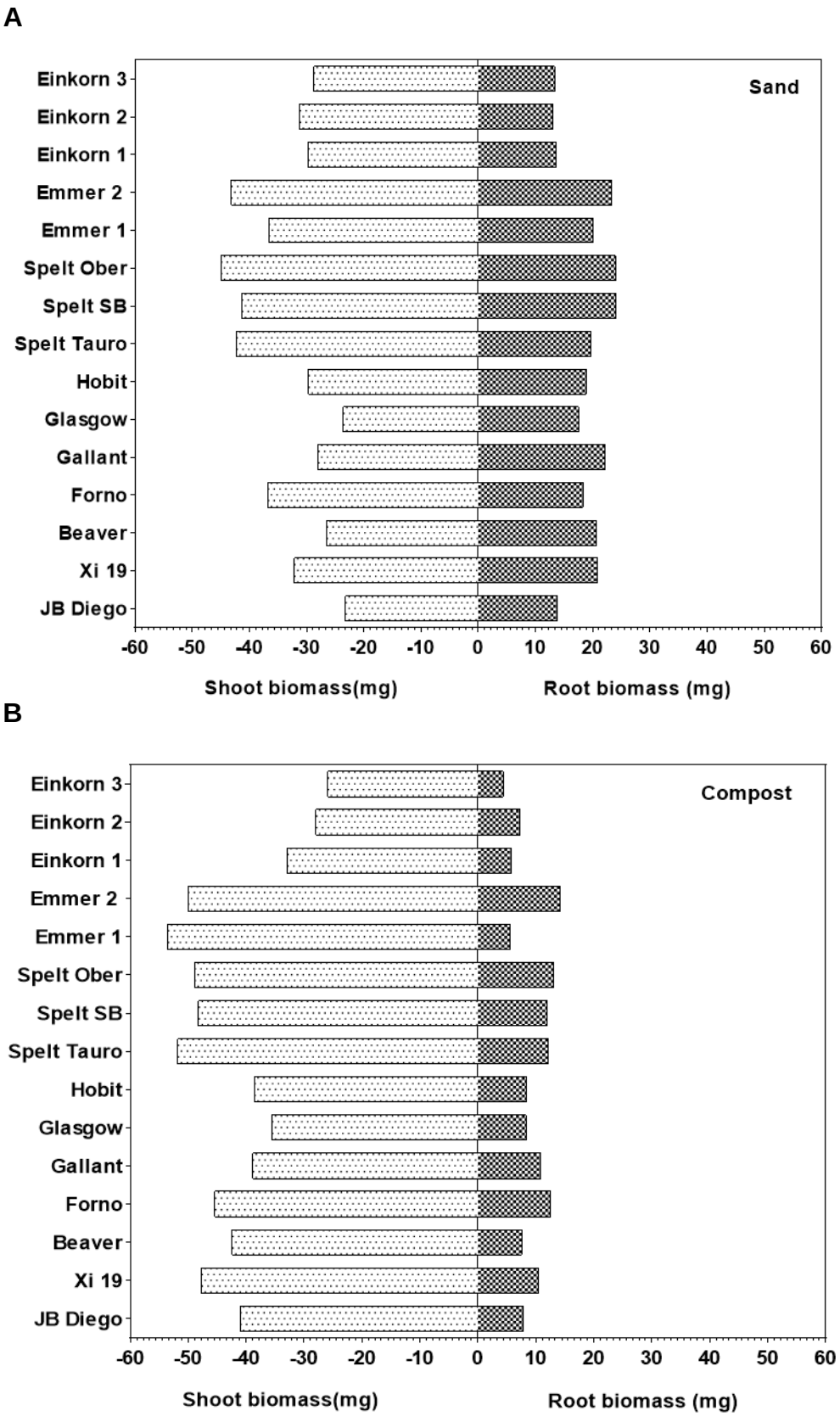


Figure 5.8 Shoot and root biomass production of seedlings in (A) sand and (B) compost at 14 DAT (SED GT x M for shoot biomass and root biomass are 4.514 and 0.001, respectively; *df* = 545)

5.2.2.7 Specific root length

SRL was significantly different between genotypes and the highest value was recorded in einkorn species which had the thinnest roots, in both compost and sand. The second highest SRL was recorded in bread wheat followed by emmer and spelt in both growth media (Table 5.2).

Table 5.2 Root volume (cm^3), root biomass (mg), root to shoot ratio and specific root length (cm mg^{-1}) of fifteen genotypes at 14 DAT in compost and sand

GT	Root volume (cm^3)		Root biomass (mg)		R:S		SRL (cm mg^{-1})	
	Compost	Sand	Compost	Sand	Compost	Sand	Compost	Sand
JB Diego	0.11	0.13	7.85	13.84	0.19	0.59	23.35	11.53
Xi 19	0.14	0.18	10.48	20.88	0.22	0.66	20.71	11.69
Beaver	0.11	0.19	7.52	20.60	0.18	0.78	22.58	11.67
Forno	0.13	0.16	12.51	18.29	0.27	0.50	14.47	10.90
Gallant	0.14	0.18	10.80	22.20	0.28	0.79	17.59	11.65
Glasgow	0.13	0.15	8.27	17.54	0.23	0.72	24.28	13.06
Hobbit	0.12	0.16	8.38	18.85	0.22	0.63	21.95	10.88
Spelt Tauro	0.15	0.18	12.18	19.74	0.23	0.47	16.12	11.75
Spelt SB	0.16	0.20	11.85	23.99	0.25	0.59	18.17	11.56
Spelt Ober	0.16	0.19	13.05	23.97	0.27	0.55	16.23	11.37
Emmer 1	0.15	0.17	10.63	20.12	0.24	0.54	19.98	11.23
Emmer 2	0.16	0.22	14.16	23.29	0.28	0.54	16.33	13.79
Einkorn 1	0.08	0.11	5.69	13.66	0.17	0.47	31.79	16.93
Einkorn 2	0.09	0.13	7.25	13.09	0.26	0.42	22.00	16.70
Einkorn 3	0.05	0.12	4.36	13.33	0.17	0.46	31.73	15.93
SED								
GT (*df* = 545)	0.007***		0.724***		0.015***		0.562***	
M (*df* = 545)	0.002***		0.265***		0.006***		0.205***	
GT x M (*df* = 545)	0.009***		1.025***		0.022***		0.795***	

***** Significant at $P < 0.001$**

5.2.2.8 Correlation between two media for root traits

There were positive relationships between most of the root traits in sand and compost. For example, total root length (r = 0.62, $P < 0.05$) (Figure 5.9A), root volume (r = 0.84 $P < 0.001$) (Figure 5.9B), root biomass (Figure 5.9C) (r = 0.81, $P < 0.001$) and root diameter (r = 0.66, $P < 0.01$) (Figure 5.9D) had strong correlation in the two media.

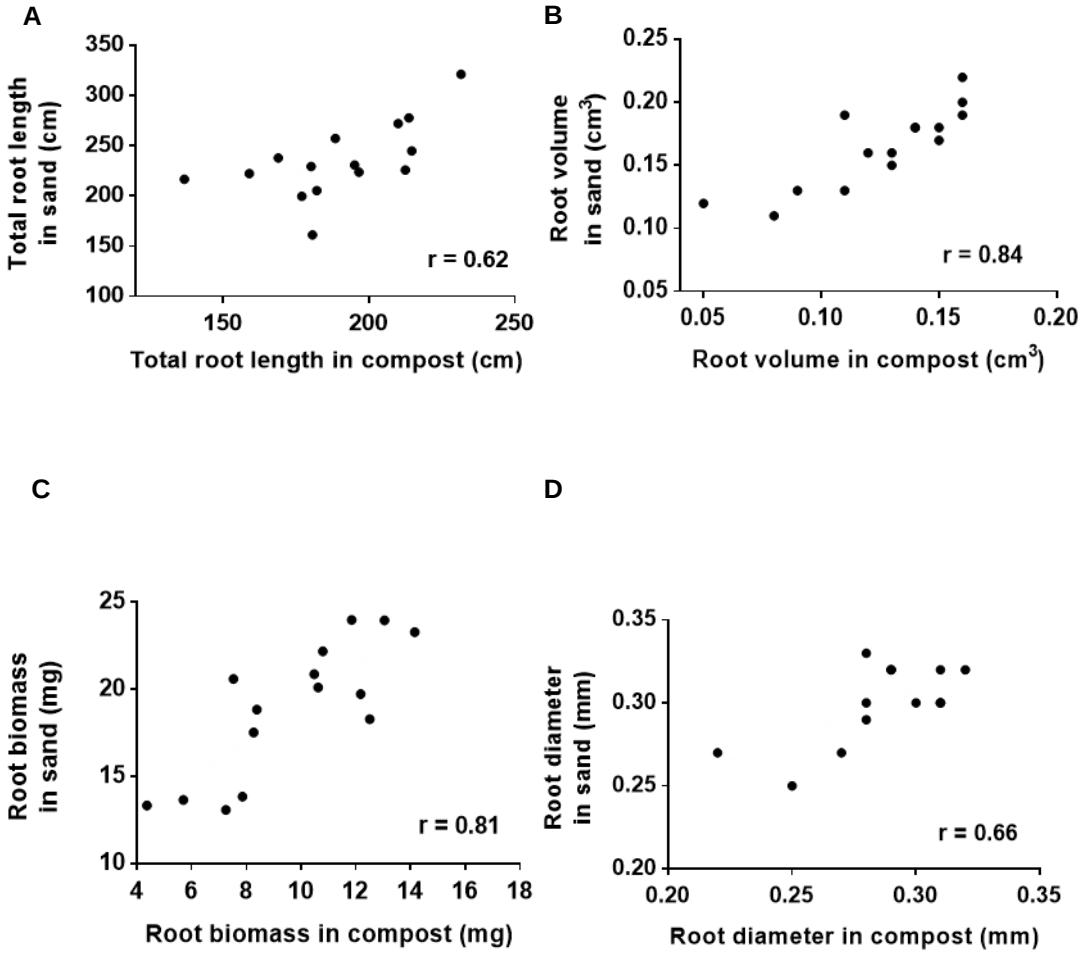


Figure 5.9 Relationship between (A) total root length (r = 0.62, $P < 0.05$) (B) root volume (r = 0.84, $P < 0.001$) (C) root biomass (r = 0.81, $P < 0.001$) (D) root diameter (r = 0.66, $P < 0.01$) in sand and compost at 14 DAT

5.2.2.9 Relationship between different root traits

Total root length and root volume of the genotypes had a positive correlation with number of seminal roots in sand (r = 0.81, $P < 0.001$ and r = 0.61, $P < 0.05$, respectively) (Figure 5.10 A, B). The relationship between root volume and total root length was very strong and positive in compost (r = 0.89, $P < 0.001$) (Figure 5.10C) but accounted for less variation in sand (r = 0.74, $P < 0.01$) (Figure 5.10D).

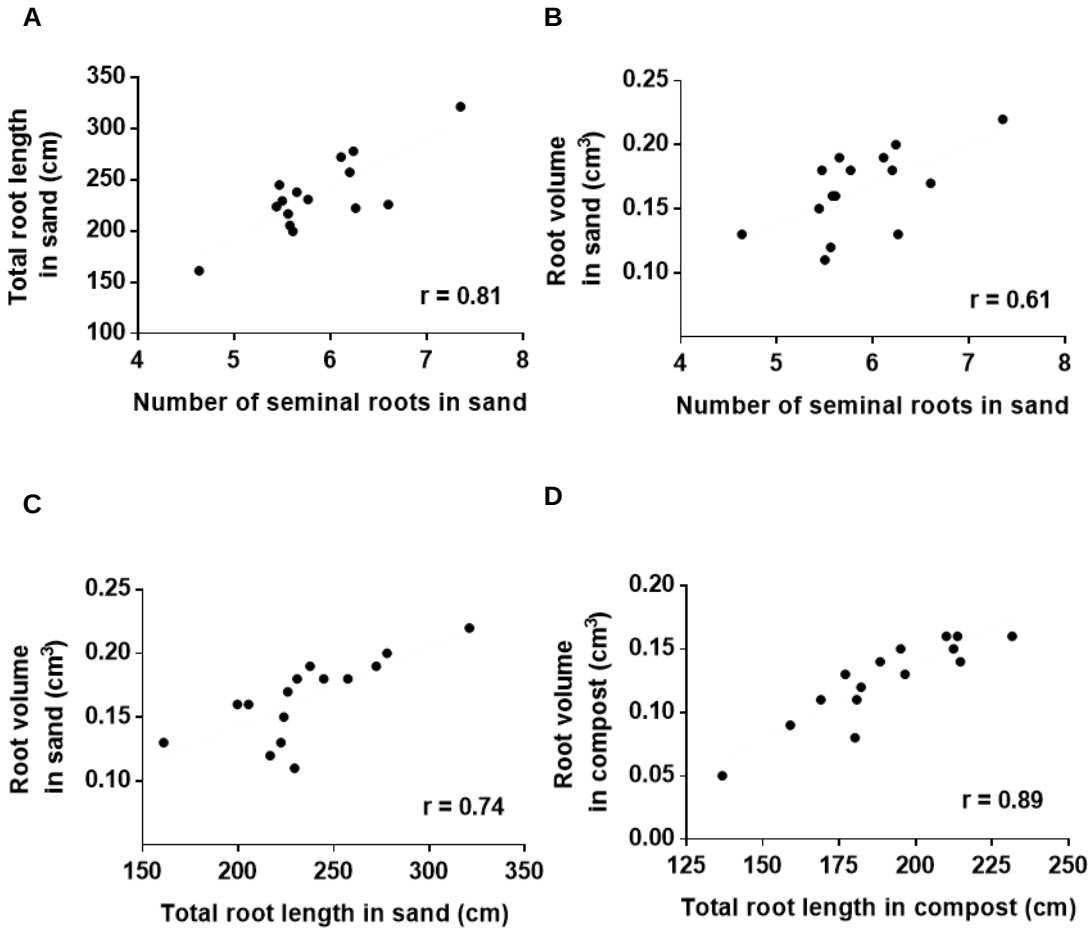


Figure 5.10 Relationship between (A) total root length and number of seminal root (r = 0.81, *P* < 0.001) and (B) root volume and number of seminal roots (r = 0.61, *P* < 0.05) in sand media, (C) root volume and total root length in compost (r = 0.89, *P* < 0.001) and (D) root volume and total root length in sand (r = 0.74, *P* < 0.01) at 14 DAT

5.2.2.10 Relationship between root traits of 14 day old seedlings in two experiments

Root length in compost had a strong, positive relationship with total root length in the 2D root phenotyping experiment (r = 0.70, $P < 0.05$) (Figure 5.11A). Number of seminal roots in sand had a strong relationship with the number of seminal roots of bread wheat, spelt, emmer and einkorn genotypes in 2D experiment ($R^2 = 0.66$, $P < 0.01$) (Figure 5.11B).

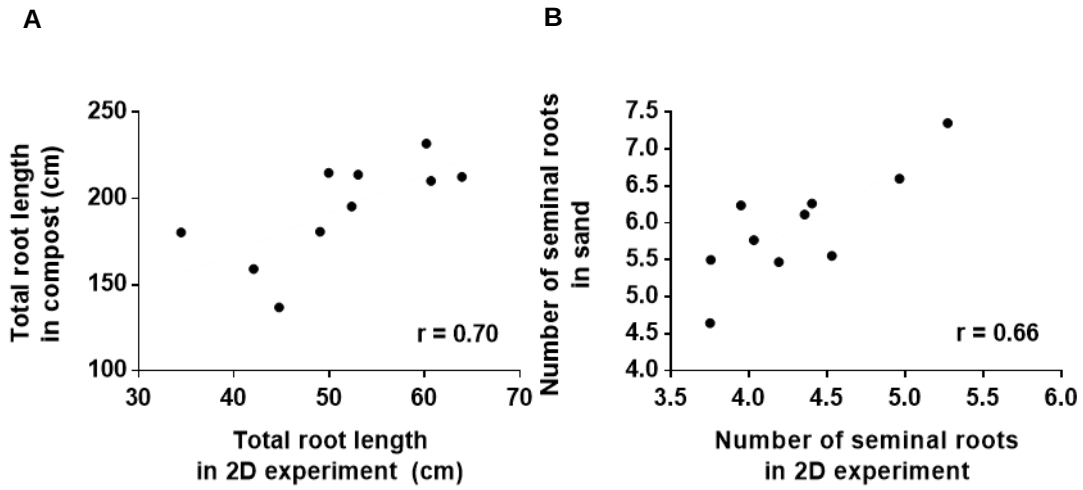


Figure 5.11 Relationship between (A) total root length in compost and 2D phenotyping experiment (r = 0.70, $P < 0.05$) and (B) number of seminal roots in sand and 2D root phenotyping experiment (r = 0.66, $P < 0.01$) at 14 day old seedlings

5.2.3 Root phenotyping pot experiment

Root measurements

5.2.3.1 Total root length

The longest root length was observed in spelt Oberkulmer throughout the experiment, except 32 DAT where spelt SB had longest root length ($P < 0.001$). All einkorn genotypes showed very weak root growth and produced a smaller root length than the other genotypes. At 23 DAT, total root length of emmer, bread wheat and einkorn was 9, 18 and 47% shorter than that of spelt. At 32 DAT, on average, total root length of spelt, bread wheat and emmer was 118, 90 and 87% longer than einkorn, respectively (Figure 5.12). Total root length of emmer and bread wheat was in between spelt and einkorn.

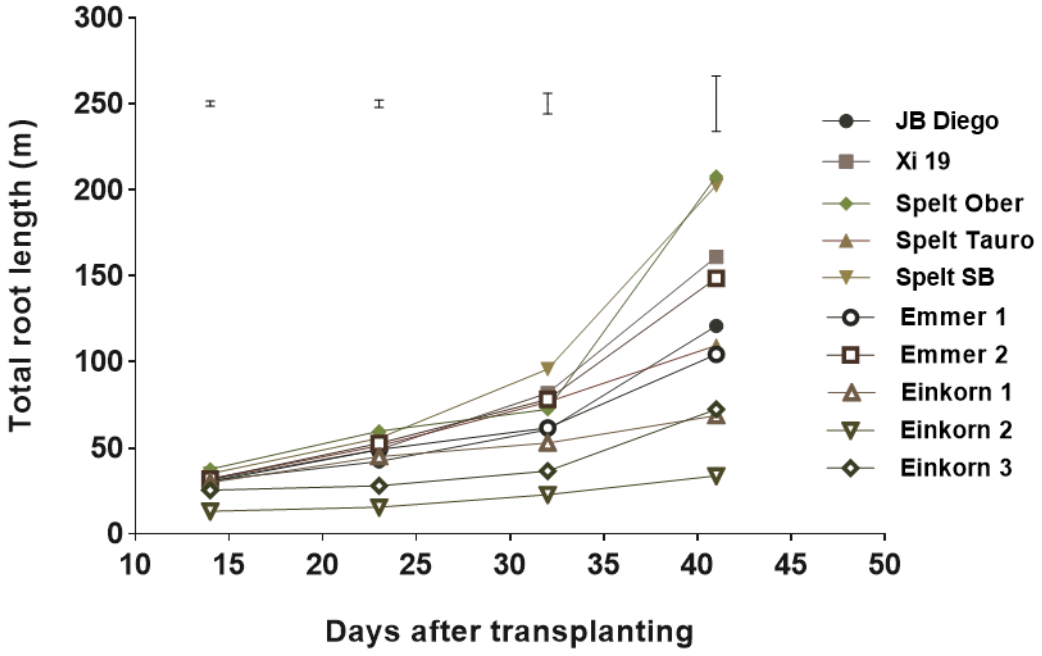


Figure 5.12 Total root length of the genotypes at 14, 23, 32 and 41 DAT. Ear bars represent the SED for GT (*df* =18)

5.2.3.2 Root diameter

Average root diameter of emmer, spelt and bread wheat was higher than einkorn at 14, 23, 32 and 41 DAT ($P < 0001$). Average root diameter of the root systems at 14 DAT was between 0.26 mm (einkorn 2) to 0.39 mm (Xi 19, spelt SB, spelt Oberkulmer and emmer 1). It was 0.26 mm (einkorn 2) to 0.40 mm (spelt SB) at 23 DAT. Spelt SB recorded the highest average root diameter (0.45 mm) at 32 DAT and einkorn the lowest (0.26 mm). 41 days after transplanting, bread wheat cv. JB Diego and emmer 2 (0.4 mm) had the highest average root diameter while einkorn 2 (0.31 mm) still recorded the lowest value.

5.2.3.3 Root volume

Root volume was significantly different between genotypes at all harvest dates (Figure 5.13) with spelt SB recording the largest values at each harvest ($P < 0.001$). Similar to the results of total root length, the highest values were observed in spelt genotypes and the lowest values for einkorn.

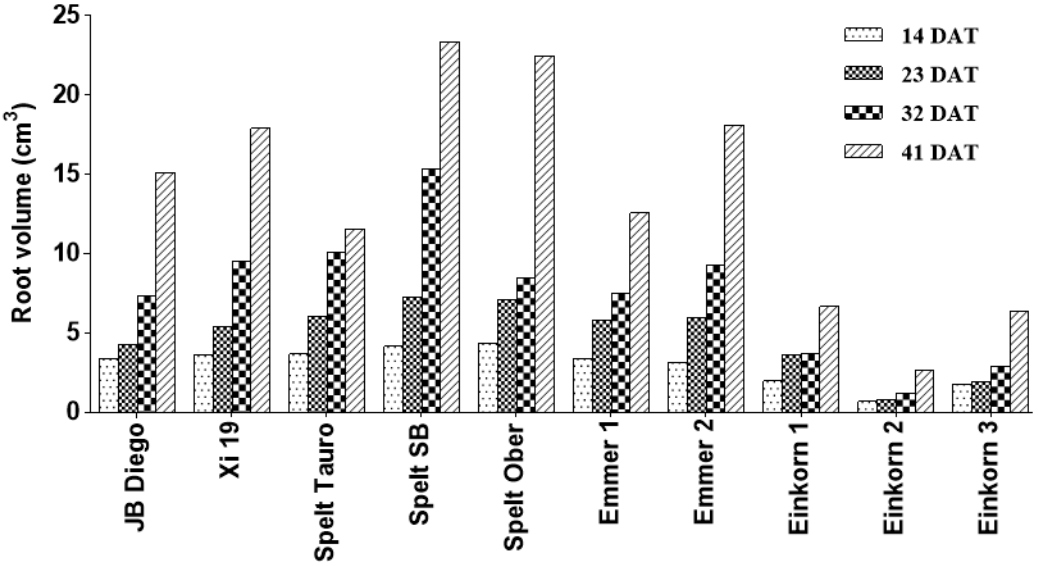


Figure 5.13 Average root volume of the genotypes at 14, 23, 32 and 41 DAT. SED for GT at 14, 23, 32 and 41 DAT was 0.49, 0.56, 1.69 and 3.68, respectively (*df*=18)

5.2.3.4 Root biomass

The heaviest root biomass was recorded in spelt genotypes at 14 and 32 DAT (0.42 g) except spelt SB. Spelt SB had high biomass only at 32 and 41 DAT. However, it was not significantly different from spelt Oberkulmer, emmer 2 and Xi 19 at 41DAT. The lowest root biomass was observed in einkorn 2 throughout the experiment (Figure 5.14).

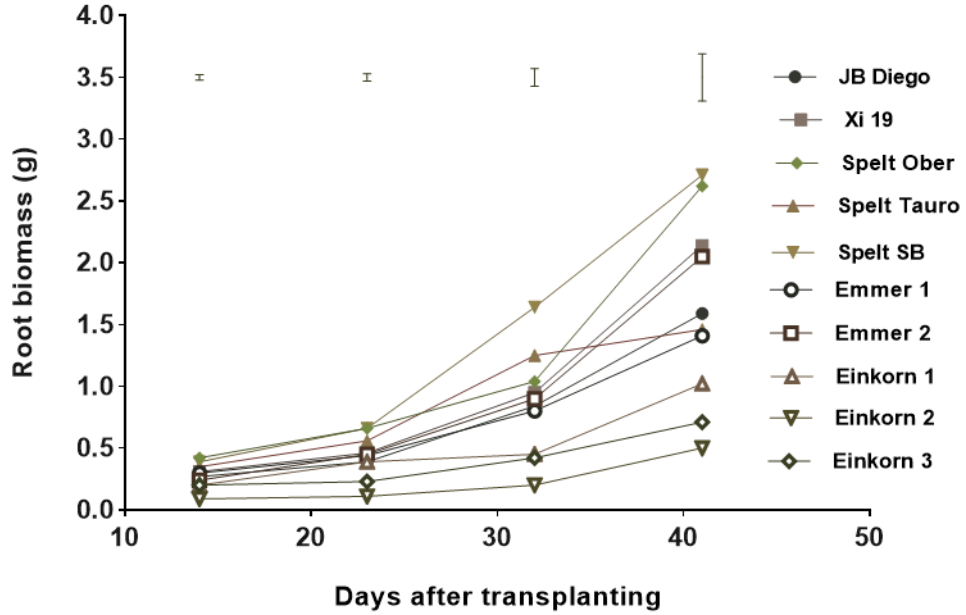


Figure 5.14 Root biomass at 14, 23, 32 and 41 DAT. Error bars represent the SED for GT (*df*=18)

5.2.3.5 Root elongation rate (root length per day)

As a species, spelt had the highest root elongation rate (RER) and einkorn the least ($P < 0.001$). RER of spelt was higher than bread wheat by 23%, while emmer and einkorn genotypes had lower RER than bread wheat by 11% and 142%, respectively.

5.2.3.6 Specific root length

SRL was higher in einkorn 2 and lower in emmer genotypes ($P < 0.001$). SRL of all genotypes decreased over time, indicating younger plants had thinner roots and as plant development occurred, thicker roots were produced.

Shoot measurements and N_{off}

At every sampling, significant differences existed between the numbers of tillers possessed by each genotype ($P < 0.001$). Spelt SB had the highest number of tillers while einkorn 2 produced the least. However, when averaged across species, emmer showed vigorous tiller production at 41 DAT but not significantly different from spelt. Spelt produced the most shoot biomass during the early sampling points followed by emmer, but later emmer recorded more shoot biomass than spelt ($P < 0.001$). Shoot biomass production of bread wheat and einkorn were always less than emmer and spelt (Table 5.3). Green area production was highest in spelt at most of the sampling points followed by emmer, then bread wheat and least in einkorn ($P < 0.001$). Shoot biomass and green area production of the genotypes at different sampling points are shown in Table 5.3.

Chlorophyll concentration index of the leaf (as SPAD value) was very high in bread wheat cv. Xi 19 throughout the experiment, despite the fact that all genotypes were supplied with the same amount of nutrients. The spelt and emmer genotypes had low SPAD values representing less N in their leaves, but had higher N_{off} than cv. Xi 19. It may therefore be presumed that the genotypes utilise N differently. The spelt and emmer produced more shoot biomass with the lower leaf N concentration while bread wheat seemed to produce less shoot biomass but with a higher leaf N concentration.

N_{off} of the plant was significantly different between genotypes at all sampling points ($P < 0.001$) (Table 5.3) where spelt SB had the highest N_{off} at 32 and 41 DAT of 0.31 g N plant^{-1} and 0.70 g N plant^{-1}, respectively. Bread wheat cv. JB Diego and cv. Xi 19 had 0.36 g N plant^{-1} and 0.54 g N plant^{-1} at 41 DAT. N_{off} of einkorn genotypes were

significantly lower than other genotypes throughout the experiment. Averaged across genotypes, N_{off} of emmer and spelt were higher than bread wheat and einkorn (Figure 5.15).

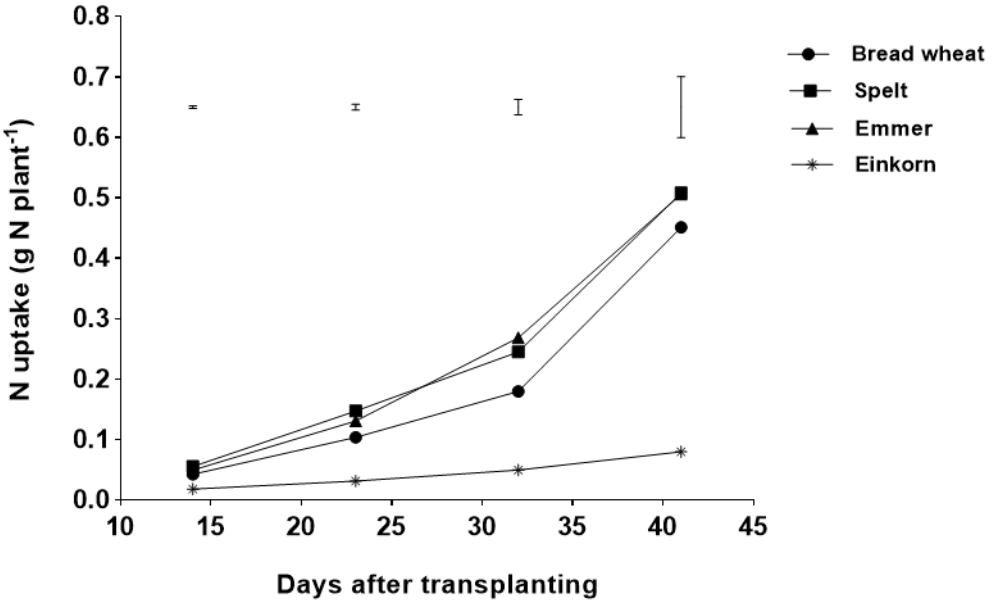


Figure 5.15 Plant N_{off} (g N plant^{-1}) for bread wheat, spelt, emmer and einkorn at 14, 23, 32 and 41 DAT. Error bars represents SED of the species

Table 5.3 Number of tillers and green area (cm^2 plant^{-1}) at 14, 23, 32 and 41 days after transplanting (DAT). (All measured parameters are significant at $P < 0.001$)

GT	Number of tillers (plant^{-1})				Green area (cm^2 plant^{-1})			
	14 DAT	23 DAT	32 DAT	41 DAT	14 DAT	23 DAT	32 DAT	41 DAT
JB Diego	9	23	31	59	151.57	365.37	471.59	1070.83
Xi 19	6	13	26	56	174.33	478.75	590.32	1252.82
Spelt Tauro	7	19	22	35	247.85	646.67	647.62	740.53
Spelt SB	8	26	88	87	242.59	694.02	1117.31	2277.46
Spelt Oberkulmer	10	27	55	79	230.87	693.54	832.45	1922.62
Emmer 1	7	13	30	72	201.81	570.47	959.94	1427.81
Emmer 2	6	16	27	67	228.68	647.91	925.90	1408.47
Einkorn 1	7	14	22	36	88.86	196.96	200.52	453.73
Einkorn 2	5	9	16	26	35.85	54.21	91.82	159.61
Einkorn 3	7	7	17	37	88.41	146.69	230.75	359.47
SED (*df=18*)	0.711	2.712	5.663	8.702	33.722	59.504	67.001	269.707

Table 5.4 Shoot biomass production (g plant^{-1}) and N_{off} (g N plant^{-1}) of the plant at 14, 23, 32 and 41 day after transplanting (DAT). (All measured parameters are significant at $P < 0.001$)

GT	Shoot biomass (g plant^{-1})				N_{off} (g N plant^{-1})			
	14 DAT	23 DAT	32 DAT	41 DAT	14 DAT	23 DAT	32 DAT	41 DAT
JB Diego	0.79	2.35	3.56	8.61	0.04	0.09	0.14	0.36
Xi 19	0.87	2.94	5.69	13.53	0.04	0.12	0.22	0.54
Spelt Tauro	1.19	3.78	6.11	6.01	0.06	0.14	0.19	0.20
Spelt SB	1.11	3.81	7.74	18.53	0.05	0.15	0.31	0.70
Spelt Oberkulmer	1.10	3.71	6.63	16.16	0.06	0.15	0.23	0.63
Emmer 1	0.95	3.10	7.26	13.03	0.05	0.12	0.26	0.47
Emmer 2	1.08	3.81	7.45	15.77	0.05	0.14	0.28	0.54
Einkorn 1	0.50	1.27	1.90	4.60	0.02	0.04	0.06	0.09
Einkorn 2	0.20	0.40	0.83	1.41	0.01	0.01	0.02	0.04
Einkorn 3	0.49	1.29	2.38	3.58	0.02	0.04	0.07	0.10
SED (*df=18*)	0.13	0.29	0.61	2.72	0.01	0.01	0.03	0.11

Green area of the plant at all sampling points had a positive and strong relationship with N uptake ($R^2 = 0.90$, $P < 0.001$ at 41 DAT; Fig 5.16A). A similar relationship was seen between N uptake of the plant and shoot biomass ($R^2 = 0.96$, $P < 0.001$ at 14 DAT; $R^2 = 0.92$, $P < 0.001$ at 23 DAT; $R^2 = 0.96$, $P < 0.001$ at 32 DAT; $R^2 = 0.96$, $P < 0.001$ at 41DAT). $NUpE_R$ explained green area production by 66% and shoot biomass production by 74% at 41 DAT. Figure 5.16 (B, C and D) shows linear regression of root length against total number of tillers, green area against number of tillers and total root length against green area at 41 DAT. Number of tillers explained 70% of the variation in total root length ($P < 0.001$) and observed variation of green area production per plant is associated with tiller production ($R^2 = 0.81$, $P < 0.001$) therefore, total root length of the plant had a strong relationship with green area production ($R^2 = 0.75$, $P < 0.001$) or number of leaves/ or number of tillers per plant at 41 DAT.

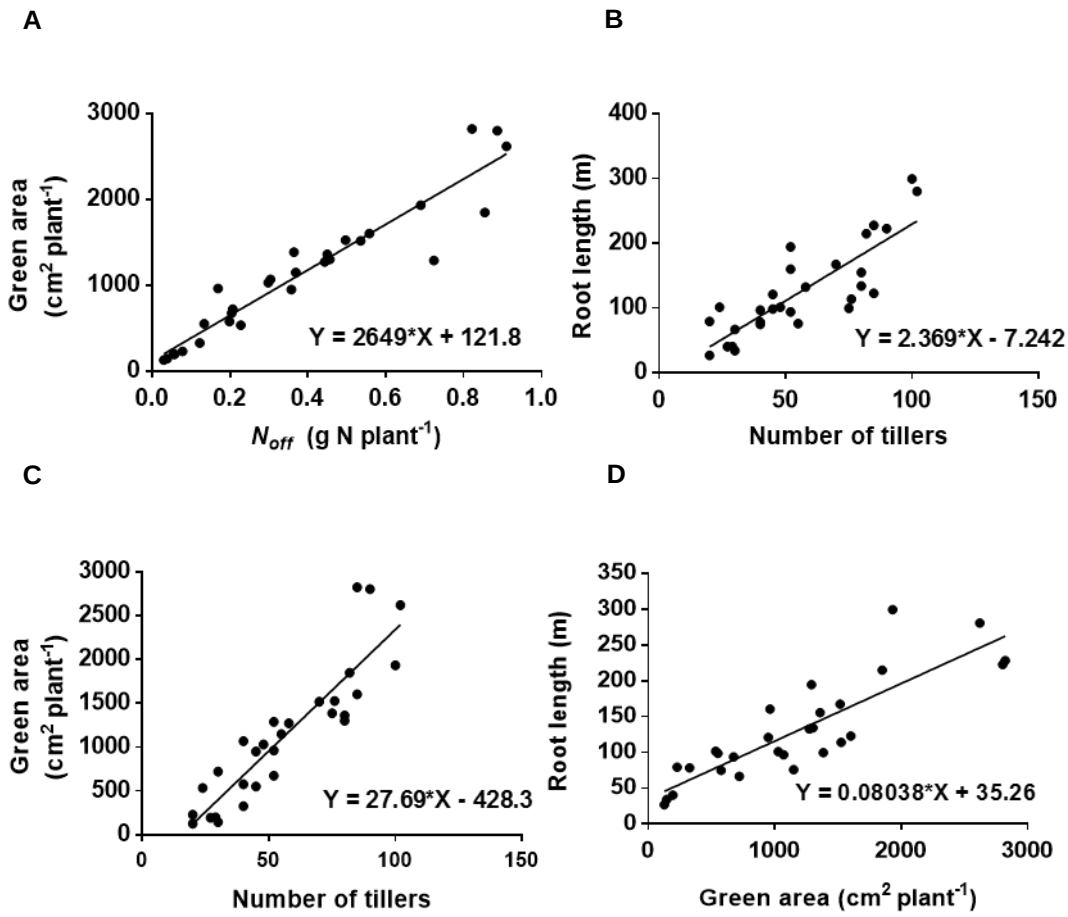


Figure 5.16 Linear regression analysis between (A) green area production and plant N_{off} (R^2 = 0.90, P < 0.001) (B) root length and number of tillers per plant (R^2 = 0.70, P < 0.001) (C) green area production and number of tillers per plant (R^2 = 0.81, P < 0.001) (D) root length and green area production (R^2 = 0.75, P < 0.001) per plant and at 41 DAT

5.2.3.7 Root nitrogen uptake efficiency ($NUpE_R$)

$NUpE_R$ was significantly different between genotypes throughout the experiment and on average the highest value was recorded in emmer and lowest in einkorn (P < 0.001) (Table 5.5). When averaged across species, it was not significantly different between spelt and bread wheat at all harvesting points. The average $NUpE_R$ of emmer at 41 DAT was 25%, 35% and 166% higher than bread wheat, spelt and einkorn, correspondingly.

5.2.3.8 Specific absorption rate of N

Emmer absorbed more N per unit length and unit biomass of root (Table 5.5). $S_{AB}R_N$

of emmer was 23%, 45% and 253% higher than bread wheat, spelt and einkorn, respectively for root biomass and 23%, 36% and 331% for total root length.

Table 5.5 Root N uptake efficiency and the specific absorption rate of N based on root biomass (mg g^{-1} $rootday^{-1}$) and root length (mg m^{-1} $rootday^{-1}$) at 14, 23, 32 and 41 DAT. (All measured parameters are significant at $P < 0.001$).

GT	Root N uptake efficiency (%)				Specific absorption rate ($S_{AB}R_N$)	
	14 DAT	23 DAT	32 DAT	41 DAT	mg g^{-1} root day^{-1}	mg m^{-1} root day^{-1}
JB Diego	15.33	22.27	16.59	22.17	15.58	0.18
Xi 19	14.34	26.40	23.09	24.87	18.88	0.23
Spelt Tauro	16.56	26.14	15.30	15.26	7.43	0.09
Spelt SB	14.04	23.18	19.34	25.94	19.37	0.24
Spelt Oberkulmer	13.17	22.29	22.89	23.99	16.94	0.21
Emmer 1	15.65	27.41	32.94	33.54	22.05	0.27
Emmer 2	21.58	32.43	30.87	25.37	20.25	0.23
Einkorn 1	11.83	10.60	13.29	8.81	5.38	0.05
Einkorn 2	9.06	10.47	11.65	9.60	5.32	0.05
Einkorn 3	12.35	17.91	16.87	14.79	7.27	0.06
SED (*df=18*)	1.83	3.09	2.35	3.46	2.72	0.04

5.2.3.9 Root traits and plant N uptake at 14, 23, 32 and 41 DAT

The correlation coefficients between N_{off} and selected root traits at 14, 23, 32 and 41 DAT are shown in Table 5.6. Total root length, length of the roots in < 0.5 mm diameter and root biomass had a strong correlation with N_{off} per plant at all measurement dates.

Table 5.6 Correlation coefficient of total root length, root length in < 0.5 mm diameter class and root biomass with their respective N_{off} of the plants at 14, 23, 32 and 41 DAT

Root traits	N_{off} (mg N $plant^{-1}$)			
	14 DAT	23 DAT	32 DAT	41 DAT
Total root length (m)	0.79	0.86	0.85	0.90
Root length < 0.5 mm (m)	0.68	0.81	0.80	0.88
Root biomass (mg)	0.86	0.86	0.84	0.93

5.2.4 Column experiment

Root screening at anthesis and at maturity

5.2.4.1 Root length

Root length differed between genotypes at all depths at anthesis ($P < 0.01$) but there was no effect of N or interaction between N and genotype (N x GT) (Figure 5.17). However, the interaction between N and species (N x S) was significant ($P < 0.05$). Generally at all depths, root length of spelt was decreased at N100 (100 kg N ha^{-1}) from N50 (50 kg N ha^{-1}) and again increased at N200 (200 kg N ha^{-1}) but emmer and bread wheat increased their root length from N50 (50 kg N ha^{-1}) up to N100 and then decreased at N200. Spelt Oberkulmer consistently had the longest root length at all depths whilst both emmer genotypes had lowest root length at all depths and did not extend below 60 cm. Root length of all genotypes decreased gradually with depth. Spelt genotypes had significantly greater root length at all depths than emmer and bread wheat (Figure 5.18).

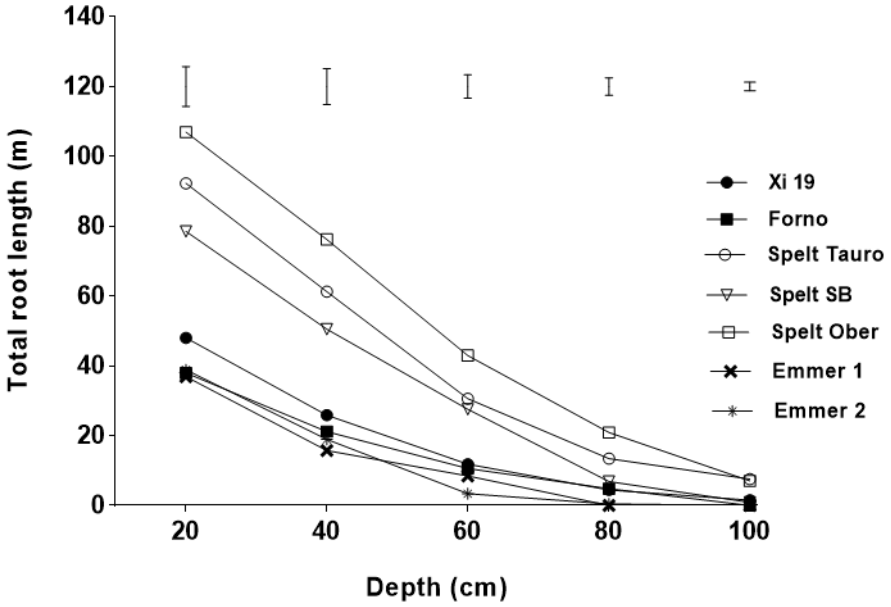


Figure 5.17 Root length distribution of genotypes when averaged across N treatments at anthesis. Error bars represent the SED of genotypes in different depth layers at anthesis (*df* = 29.16)

Total root length at maturity in the different depths was only significant between genotypes and species ($P < 0.001$). There was no effect of N or interaction with genotypes or species. All spelt genotypes had longer root length than bread wheat and

emmer. Total root length of the genotypes were longer at maturity than at anthesis for most of the genotypes, but total root length of spelt Oberkulmer and bread wheat cv. Xi 19 was longer at anthesis. Spelt produced 59% longer root length than bread wheat and 67% than emmer at maturity.

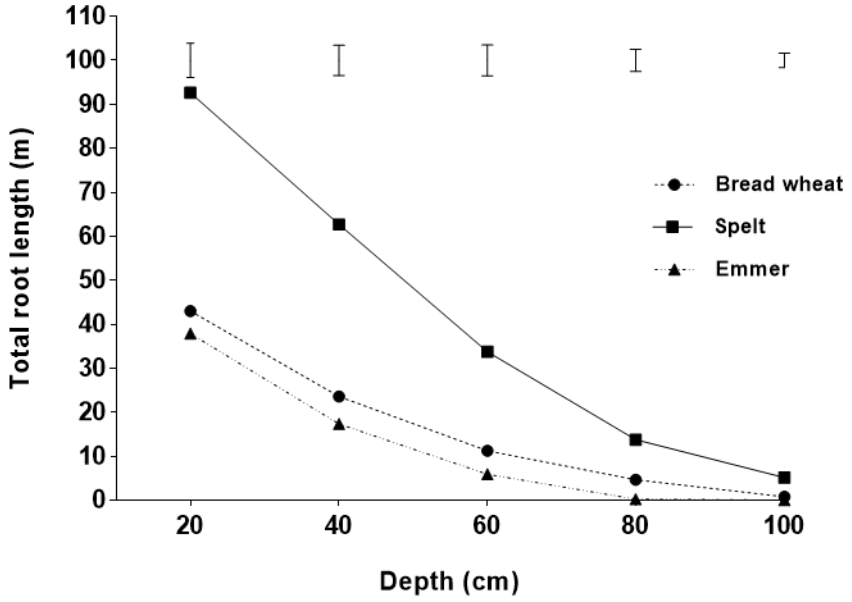


Figure 5.18 Total root length distribution of bread wheat, spelt and emmer at anthesis. Error bars represent the SED x species in different depths at anthesis (*df* = 12)

5.2.4.2 Root diameter

Effect of N supply was not significant either on genotype or species for any depth at anthesis and at maturity for root diameter. At anthesis, average root diameter at 0-20 cm was significantly different between genotypes and species. On average, the thickest roots were observed in bread wheat cv. Xi 19 (0.46 mm) while the thinnest root was observed in spelt Tauro (0.40 mm). There was no significant difference between genotype and species at 20-40 cm depth for average root diameter. No significant interaction was observed between genotypes or species and N supply for rest of the soil depth but average root diameter of the species at 40-60 cm and average root diameter of the genotypes at 60-80 and 80-100 cm was highly significant.

5.2.4.3 Root volume

Root volume was significantly different between genotypes at all depths at anthesis ($P < 0.05$). There was no direct effect of N on root volume at any depth, where N x S interaction was significant for only the top three depths ($P < 0.01$).

For total root volume per plant, significant differences were also found between genotypes ($P < 0.001$), species ($P < 0.001$) and N x S ($P < 0.01$). Spelt had a higher total root volume under all N levels and at any depth when compared to bread wheat and emmer. Total root volume per plant at anthesis was significantly higher in spelt (22.23 cm^3) followed by bread wheat (12.3 cm^3) and emmer (8.4 cm^3). Figure 5.19 shows the effect of N x S on root volume at different depths at anthesis.

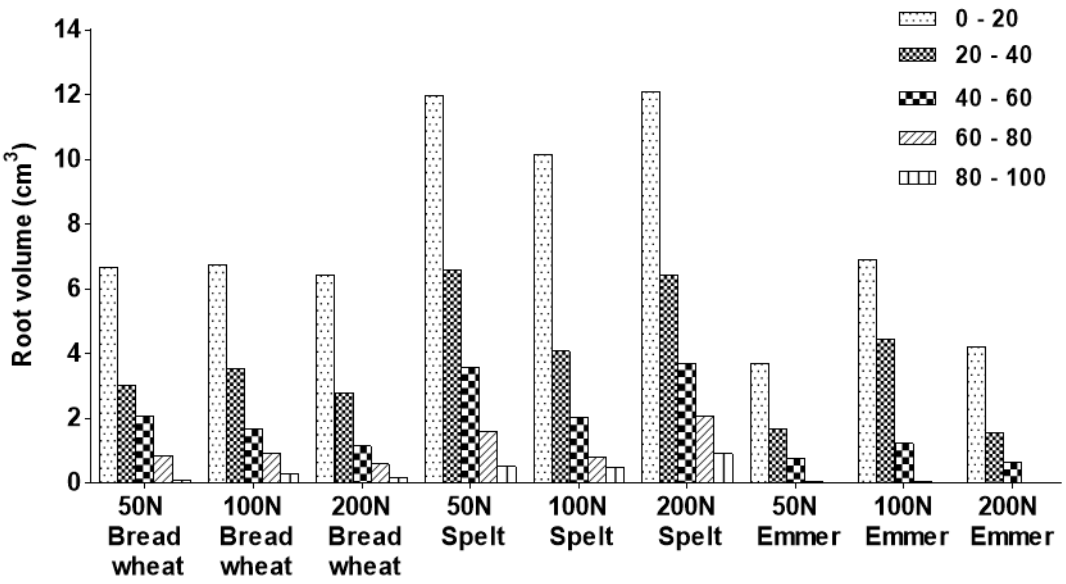


Figure 5.19 N x S interaction of root volume at different depth at anthesis. SED N x S at 0-20, 20-40, 40-60, 60-80 and 80-100 cm depth was 1.36, 1.21, 0.82, 0.42 and 0.21, respectively (*df* = 7.8)

Total root volume per plant at maturity was significantly different between genotypes and species but spelt genotypes were not significantly different from each other. On average, spelt produced the highest root volume followed by bread wheat and emmer. Root volume of spelt was 59% higher than bread wheat and 68% higher than that of emmer.

5.2.4.4 Root biomass

All genotypes and species recorded significant differences for root biomass at different root depth ($P < 0.001$ for GT except 80-100 cm; $P < 0.01$ for species). However, N supply had no effect on root biomass at anthesis. Further, the interaction between N and species was significant for 0-20 and 20-40 cm layers ($P < 0.001$) but not for the rest of the layers. Also, spelt had a very high root biomass production when compared to all other species under the different levels of N supply at anthesis (Figure 5.20).

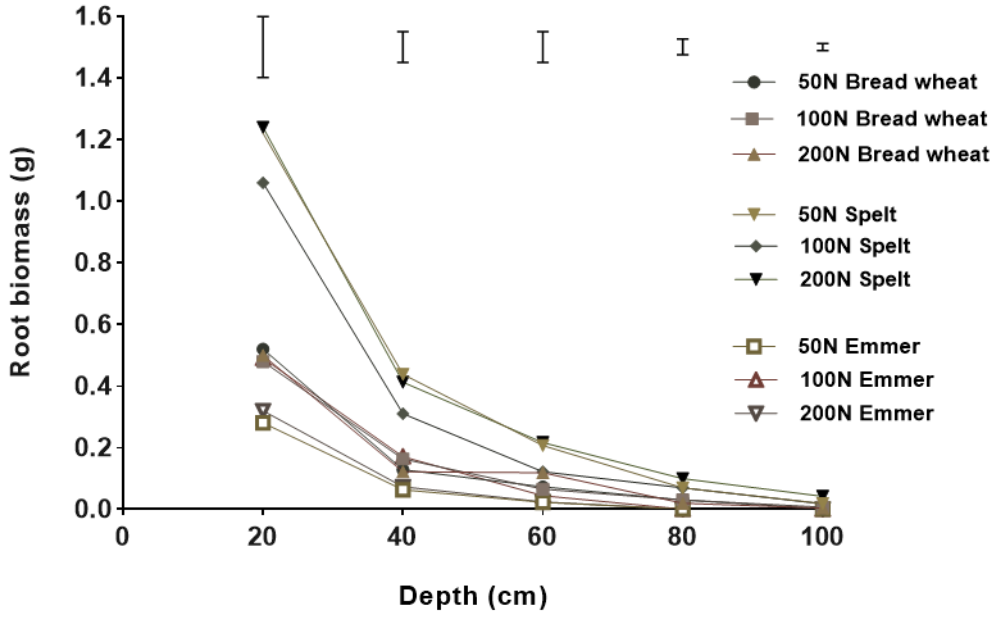


Figure 5.20 N x S interaction for root biomass at different root depths at anthesis. Error bars represents SED for N x S for five layers (*df* = 40)

Spelt Tauro (1.99 g), spelt Oberkulmer (1.96 g) and spelt SB (1.94 g) recorded higher root biomass production at maturity than other genotypes. Genotypes and species had significant effects on root biomass ($P < 0.001$) but the effect of N supply or interaction was not significant at maturity.

Average root biomass production of spelt was 1.97 g and it was 1.1 g and 1.3 g higher than that of bread wheat and emmer, respectively. Interestingly, all species produced higher root biomass at maturity than at anthesis. Further, the increase in biomass from anthesis to maturity was higher in emmer (23.5%) than bread wheat (13.1%) and spelt (5.7%).

5.2.4.5 Root length density

Root length density (RLD) at anthesis had the same pattern of distribution with depth as root length since roots were extracted from the same volume of the column. According to that, higher RLD at the bottom layers of the root system was recorded in spelt Oberkulmer for all N levels but the effect of N or N x S on RLD at 60-80 and 80-100 cm depth was not significant.

RLD of the root system at maturity was significantly different between genotypes and species. Further, RLD at different depths of the root system was also highly significant at maturity. However, there was no impact of N application or GT xN interaction either on plant RLD or RLD at different root depths. Highest RLD at different layers and total RLD of the plant were recorded in spelt genotypes. RLD decreased gradually with the depth of the column. Figure 5.21 shows the distribution of RLD throughout the root depth.

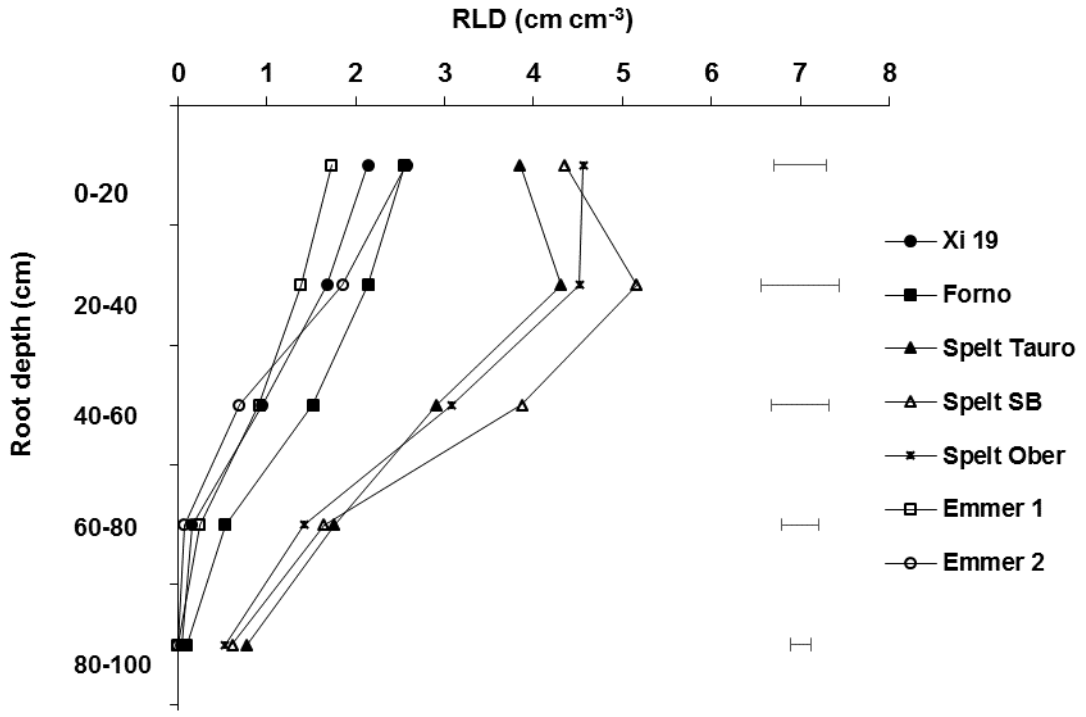


Figure 5.21 RLD of the genotypes when averaged over N treatments throughout the root depth at maturity. Error bars represent SED for genotypes at 0-20, 20-40, 40-60, 60-80 and 80-100 cm layers (*df* = 36)

5.2.4.6 Root length in different diameter classes

More than 75 % of the total root length belonged to < 0.5 mm diameter class at all depths at anthesis. However, there was no effect of N supply on root distribution in different diameter classes of the genotypes but a significant interaction between species and N level was found at 0-20 and 20-40 cm layers.

Root length in < 0.5 mm, 0.5-1.0 mm and > 1.00 mm diameter class of the whole root system was significantly different between genotypes ($P < 0.001$) and species ($P < 0.001$) while the interaction of N x S was also significant for 3 different diameter classes ($P < 0.001$, $P < 0.01$ and $P < 0.01$, respectively). The highest contribution of 85% was observed in spelt Oberkulmer at N100 for < 0.5 mm diameter class and the lowest value was recorded in bread wheat at N200 (77%). Emmer at N50 contributed 16% of the total root length from 0.5-1.0 mm diameter class where, contribution of spelt at N100 was 11%. On average, the contribution of the roots in the >1.00 mm diameter class to total root length was not more than 8%. Figure 5.22 shows the distribution of different root diameters at different N levels. Regardless of the N supply, spelt had proportionately thinner roots than other species.

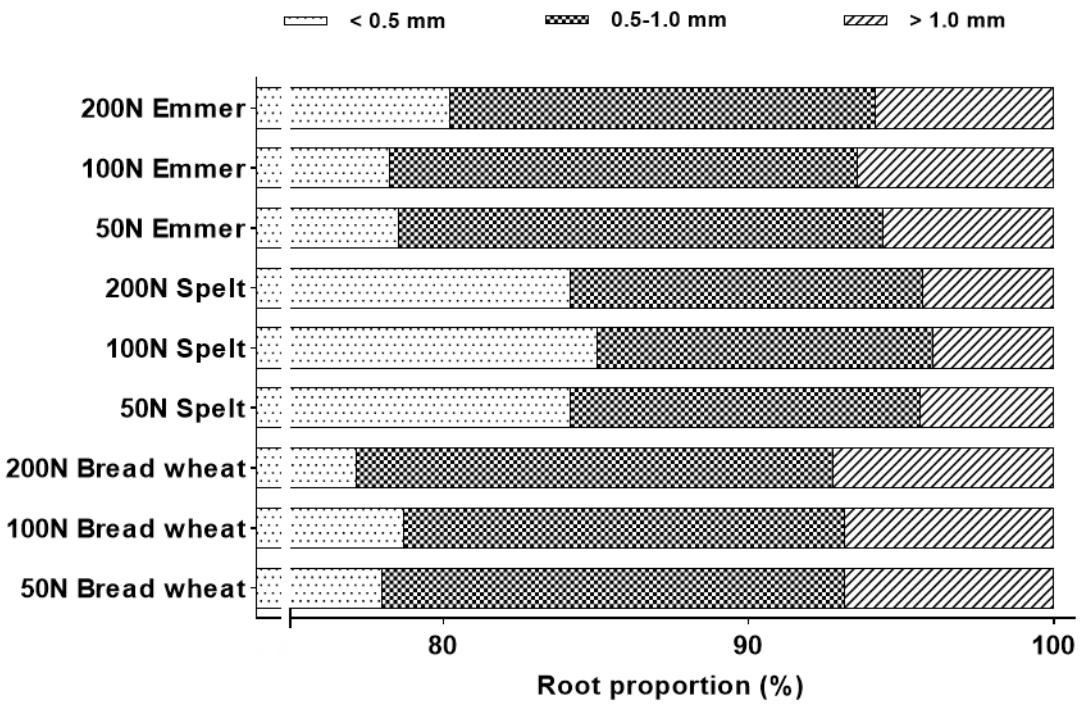


Figure 5.22 N x S of root length distribution of different diameter classes at anthesis for whole root system. SED for 0- 0.5 mm, 0.5-1.0 mm and > 1.00 mm diameter class was 1.32, 0.28 and 0.13 respectively (*df* = 40)

At maturity, more than 90% of the total root length of the plant belongs to < 0.5 mm diameter class while roots in 0.5 - 1.0 mm class were 4%. Roots having diameter of > 1.00 mm was 1% of the total root length.

5.2.4.7 Root shoot ratio (R: S)

There was a significant difference in R: S between genotype and species ($P < 0.001$) but no significant impact of N at anthesis. The highest value (0.16) was recorded in bread wheat cv. Xi 19 while the lowest value was shown in emmer 1 (0.09).

At maturity, the highest R: S was recorded in spelt Tauro (0.082) while the lowest value was observed in emmer 2 (0.04) at maturity ($P < 0.001$). Nevertheless, it was not significantly different between spelt genotypes but low in emmer and bread wheat genotypes.

5.2.4.8 Nitrogen uptake efficiency of roots

Genotypes differed in the $NUpE_R$ ($P < 0.001$) and in their response to N level ($P < 0.05$) at anthesis. However, there was no direct effect of N supply on $NUpE_R$. The highest $NUpE_R$ was recorded in emmer at N200 and the lowest value was observed in spelt Oberkulmer at N100. There was a trend to increase $NUpE_R$ with N supply and significantly different between species ($P < 0.001$). However, no significant interaction between species and N level was found. On average emmer showed higher $NUpE_R$ followed by bread wheat and spelt (Figure 5.23).

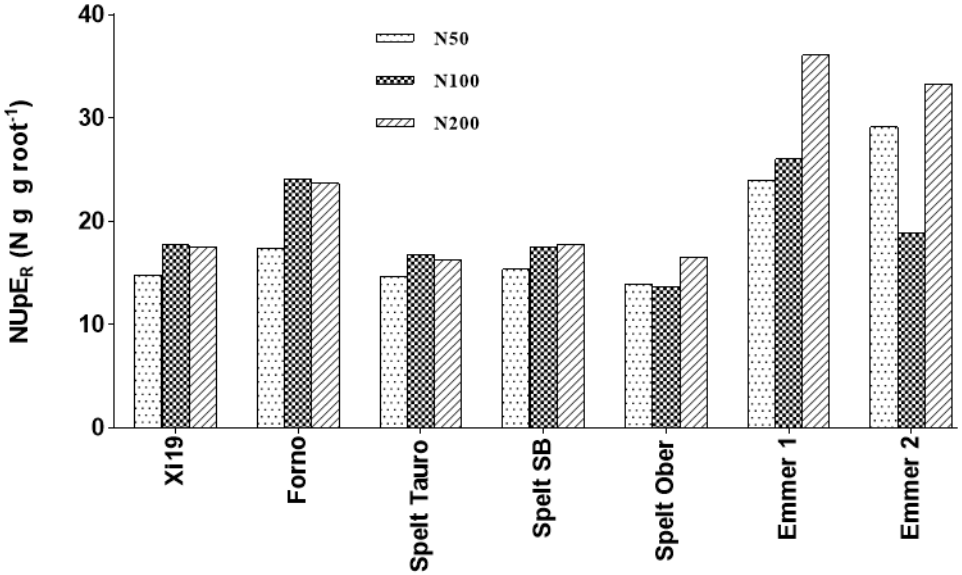


Figure 5.23 Nitrogen uptake efficiency of roots at anthesis (SED N x GT = 3.86, *df* = 10.45)

The highest $NUpE_R$ at maturity was recorded in bread wheat cv. Xi 19 (38.5%) but it was not different either from emmer 1 (33.5%) or emmer 2 (38.3%). It was significantly low for all spelt genotypes. As a species, emmer had highest $NUpE_R$ though it was not significantly different from bread wheat. However, spelt had a lower $NUpE_R$ at maturity and it was 43 and 38% lower than emmer and bread wheat, respectively.

5.2.4.9 Regression analysis between shoot parameters and root traits at anthesis

Green area of the plant at anthesis had a positive and strong relationship with N_{off} (R^2 =0. 83, P< 0.001). A similar relationship was noticed between shoot biomass and N_{off} of the plant (R^2 = 0. 90, P < 0.001) (data not shown). Figure 5.24A and B shows linear regression of root length against number of tillers (Table 4.13 for tiller data) and total root length against green area at anthesis. Number of tillers explained 61% of the variation in total root length (P < 0.001) and observed variation of green area production per plant is mainly due to tiller production (R^2 = 0.74, P < 0.001). Therefore, total root length of the plant had a strong relationship with green area production/ or number of leaves/ or number of tillers per plant at anthesis.

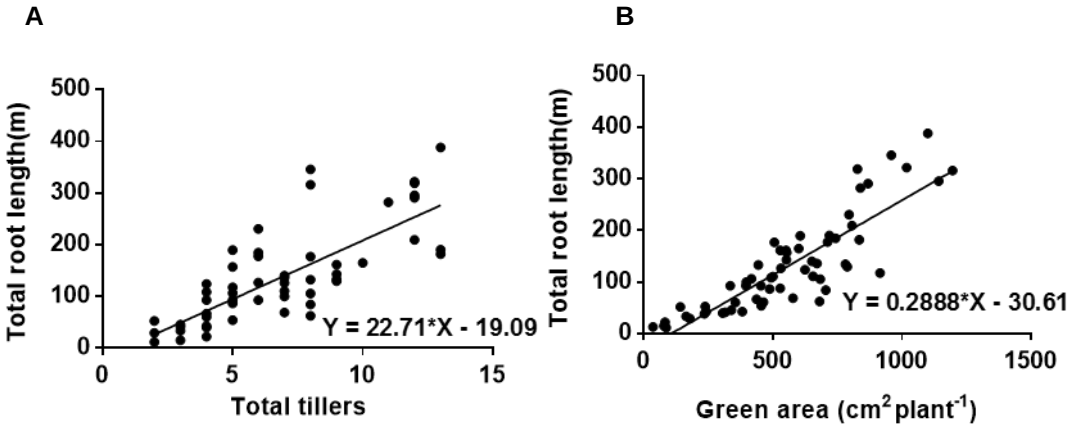


Figure 5.24 Linear regression analysis between (A) total root length per plant and number of tillers (R^2 = 0.61, P < 0.001) (B) total root length per plant and green area production per plant at anthesis (R^2 = 0.74, P < 0.001). Each value is the mean of 3 replicates.

5.2.4.10 Relationship between grain yield and root traits at maturity

Grain yield had a positive relationship with total root length (r = 0.81, P < 0.001), root volume (r = 0.75, P < 0.001) and root biomass production (r = 0.75, P < 0.001) of the

plant at maturity (Fig 5.25 A, B and C). Each value of grain yield and root traits is the mean of 3 replicates for all genotypes under all N treatments in 2014 glasshouse experiment.

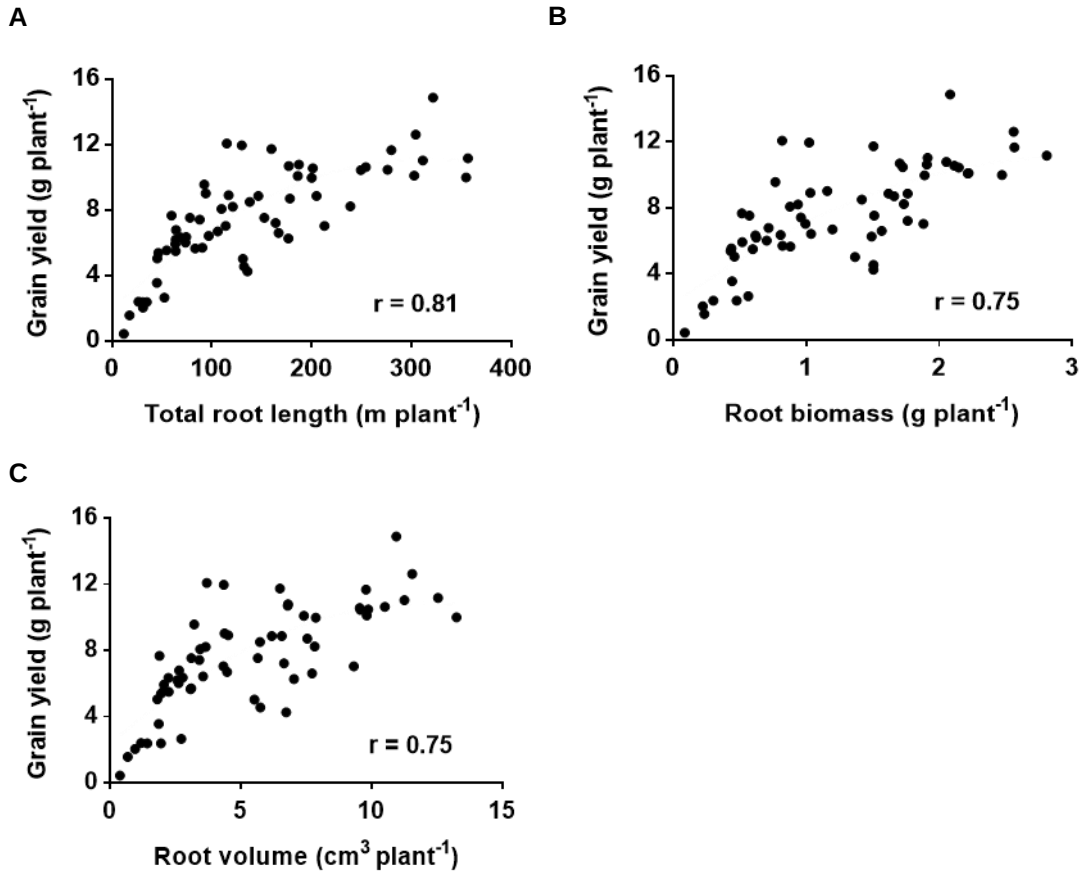


Figure 5.25 Correlation analysis between (A) grain yield and total root length (r = 0.81, *P* < 0.001) (B) grain yield and root biomass (r = 0.75, *P* < 0.001) (C) grain yield and root volume at maturity (r = 0.75, *P* < 0.001). Grain yield and root traits represent the mean value of 3 replicates of all genotypes under all N treatments in 2014 glasshouse experiment.

Comparison of regressions was performed to test whether species differed their relationships between grain yield and root traits. Total root length explained more than 65% of the variation in grain yield for bread wheat, spelt and emmer at maturity ($P < 0.001$). Similarly, root volume and root biomass of three species explained 58% and 64% of the variation in grain yield, respectively ($P < 0.001$) (Figure 5.26A, B and C). Bread wheat and emmer produced more grain yield per unit root length than spelt. However, grain yield per unit availability of root volume and root biomass was higher in emmer followed by spelt and bread wheat.

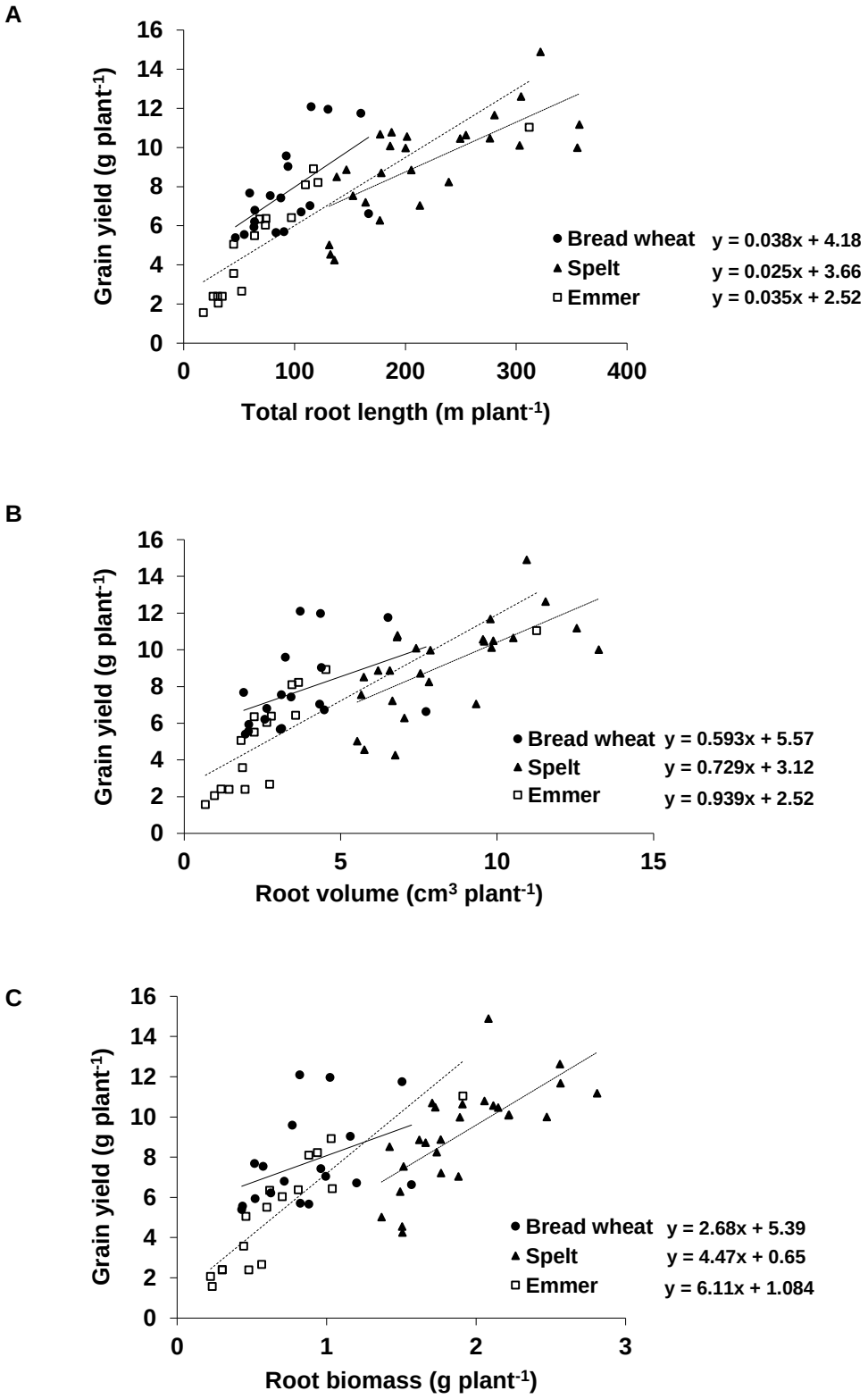


Figure 5.26 Comparison of regressions (linear regression in groups/ species) between grain and (A) total root length (R^2 = 0.65, P < 0.001) (B) root volume (R^2 = 0.58, P < 0.001) and (C) root biomass at maturity (R^2 = 0.64, P < 0.001). Each value is the mean of 3 replicates. Pooled data from different genotypes within a species and replicates are presented

5.2.4.11 N_{off} and root traits at anthesis and maturity

N_{off} of the genotypes at anthesis and maturity had a very strong relationship with total root length of the plant ($R^2 = 0.80$, $P < 0.001$). N_{off} at anthesis and at maturity regressed against the root volume of the respective plant showed strong relationship. A similar trend was observed between N_{off} at anthesis and at maturity ($R^2 = 0.81$, $P < 0.001$) for root biomass production. Also, rooting depth (depth score) of the plant at anthesis and at maturity had a strong positive linear relationship with N_{off} ($R^2 = 0.62$). Estimated parameters of comparison regression are shown in Table 5.7.

Table 5.7 Estimated parameters for the relationship between N_{off} and root traits at anthesis and maturity (X = root trait, Y = N uptake, M = slope and C = constant). N_{off} and root traits data represent the mean value of 3 replicates of all genotypes under all N treatments in 2014 glasshouse experiment

Root traits	Y = MX + C				R^2
	Anthesis		Maturity		
	M	C	M	C	
Total root length (m)	0.0009	0.0736	0.0011	0.1500	0.80
Root volume (cm^3)	0.0078	0.0788	0.0285	0.1477	0.73
Root biomass (g)	0.1121	0.0704	0.01421	0.1238	0.81
Rooting depth (depth score)	0.0526	0.0153	0.0831	-0.0831	0.62

5.2.4.12 N_{off} and root traits in different wheat species at anthesis and maturity

Comparison of regression revealed a strong and positive relationship between N_{off} and root traits of different wheat species at anthesis and at maturity. Table 5.8 shows estimated parameters for Y = MX +C.

Table 5.8 Estimated parameters for the relationship between N_{off} and root traits at anthesis and maturity for bread wheat, spelt and emmer (X = root trait, Y = N uptake, M = slope and C = constant). N_{off} and root traits data represent the mean value of 3 replicates of genotypes under all N treatments in 2014 glasshouse experiment. Pooled data from different genotypes within a species and replicates were used to develop the relationship

	Root traits	Y = MX + C						R^2
		Bread wheat		Spelt		Emmer		
		M	C	M	C	M	C	
Anthesis	Total root length (m)	0.0013	0.0281	0.0007	0.1403	0.0015	0.0421	0.86
	Root volume (cm^3)	0.0067	0.0571	0.0047	0.1768	0.0081	0.0672	0.77
	Root biomass (g)	0.1336	0.0391	0.0847	0.1248	0.1661	0.0509	0.80
	Rooting depth (depth score)	0.0367	0.0069	0.2720	0.1699	0.0441	0.0271	0.65
Maturity	Total root length (m)	0.0009	0.1760	0.0007	0.2233	0.0011	0.1217	0.74
	Root volume (cm^3)	0.0165	0.2011	0.0218	0.2099	0.0315	0.1205	0.70
	Root biomass (g)	0.0893	0.1835	0.1377	0.1248	0.2024	0.0761	0.77
	Rooting depth (depth score)	0.0555	0.0571	0.0561	0.1332	0.0727	0.0009	0.70

5.2.5 Seedling root architectural traits and N_{off}

N_{off} measured in the pot experiment at 41 DAT regressed against total root length and average length of the seminal root measured in 2D phenotyping experiment explained 67% and 56% variation in N_{off} ($P < 0.01$).

Comparison of regressions showed that the relationship between NUpE of the plants in 2013 glasshouse experiment and total root length of 2D phenotyping experiment was significant ($R^2 = 0.72$, $P < 0.001$) (Figure 5.27A). Further, it was observed that NUpE of the mature plants had positive and strong relationship with seminal root length ($R^2 = 0.66$, $P < 0.001$) (Figure 5.27B) and convex hull of the 2D phenotyping experiment ($R^2 = 0.45$, $P < 0.05$).

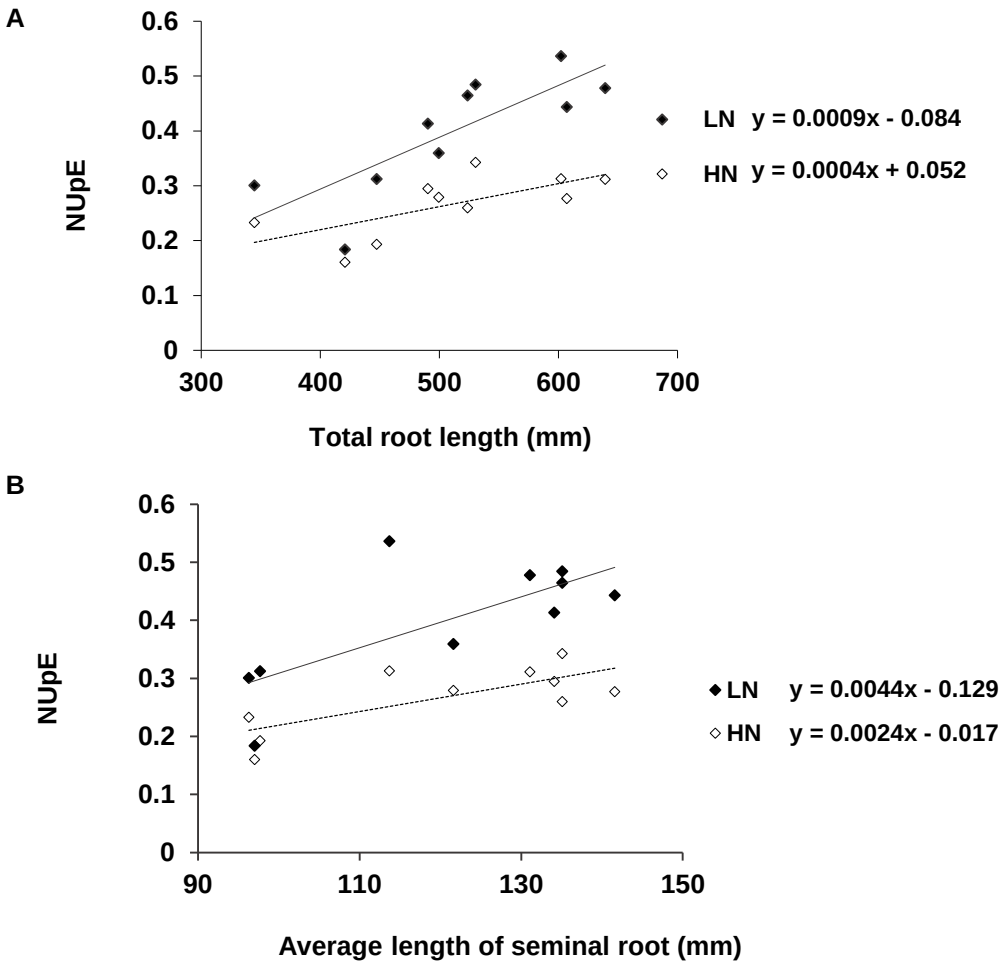


Figure 5.27 Comparison of regressions between NUpE at maturity at 2013 glasshouse experiment under HN and LN with (A) total root length (R^2 = 0.72, P < 0.001) and (B) average length of the seminal root (R^2 = 0.66, P < 0.001) of 2D phenotyping experiment. Each value of NUpE is the mean of 3 replicates while for root traits, the number of replicates varied from 10 to 25, depending on the genotype

N_{off} of the plants in the column experiment at anthesis and at maturity had a positive and strong relationship with the average length of the seminal root in 2D phenotyping experiment (R^2 = 0.71, P < 0.001) (Fig 5.28A). In addition, N_{off} at anthesis and at maturity had a strong negative relationship with tip angle of the seminal root (R^2 = 0.87, P < 0.01) (Figure 5.28B). As a result of wider tip angles, plants had low N_{off} at maturity and anthesis.

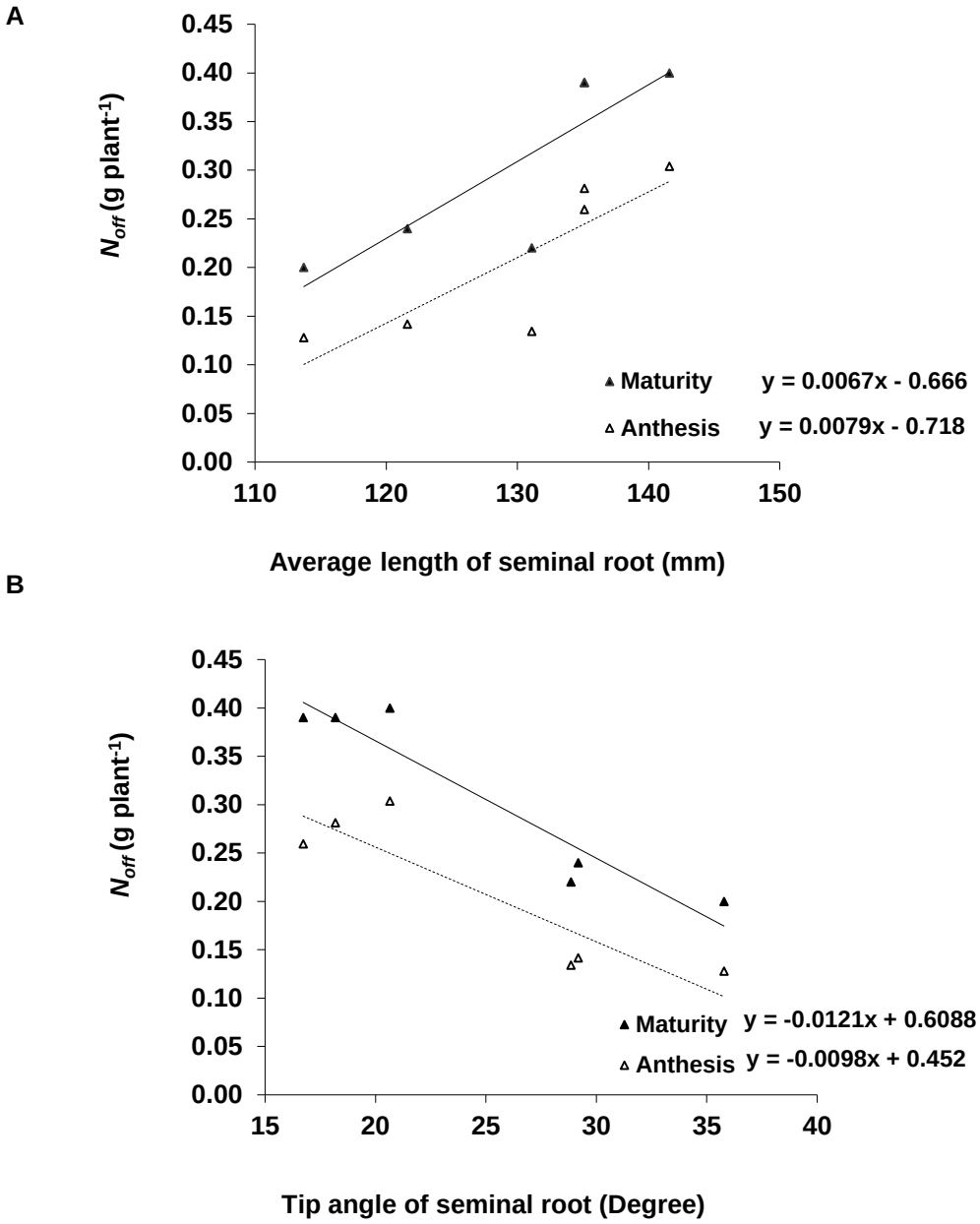


Figure 5.28 Comparison of regressions between N_{off} at anthesis and at maturity of column experiment (A) average length of the seminal root (R^2 = 0.71, P < 0.001) (B) tip angle of seminal root (R^2 = 0.87, P < 0.001) of 2D phenotyping experiment. Each value of NUpE is the mean of 3 replicates while for root traits, the number of replicates varied from 10 to 25, depending on the genotype

5.2.6 Seedling root traits and NUpE in the field

NUpE of the genotypes in 2012 field experiment had a positive linear relationship with total root length of the seedlings of 2D phenotyping experiment (R^2 = 0.54, P < 0.01). Under NN, LN and HN in 2013 experiment, total root length of the seedlings of 2D phenotyping experiment, explained 95% of the variation in NUpE at maturity (R^2 =

0.95, $P < 0.001$(Figure 5.29A). At NN, NUpE of the plant per unit availability of the total root length was higher than LN and HN. Similar relationship was found between NUpE and average length of seminal root of the seedlings of 2D phenotyping experiment ($R^2 = 0.93$, $P < 0.001$) (Figure 5.29B). However, there was no significant relationship between NUpE in 2014 field experiment and total root length/ average length of seminal root in 2D phenotyping experiment.

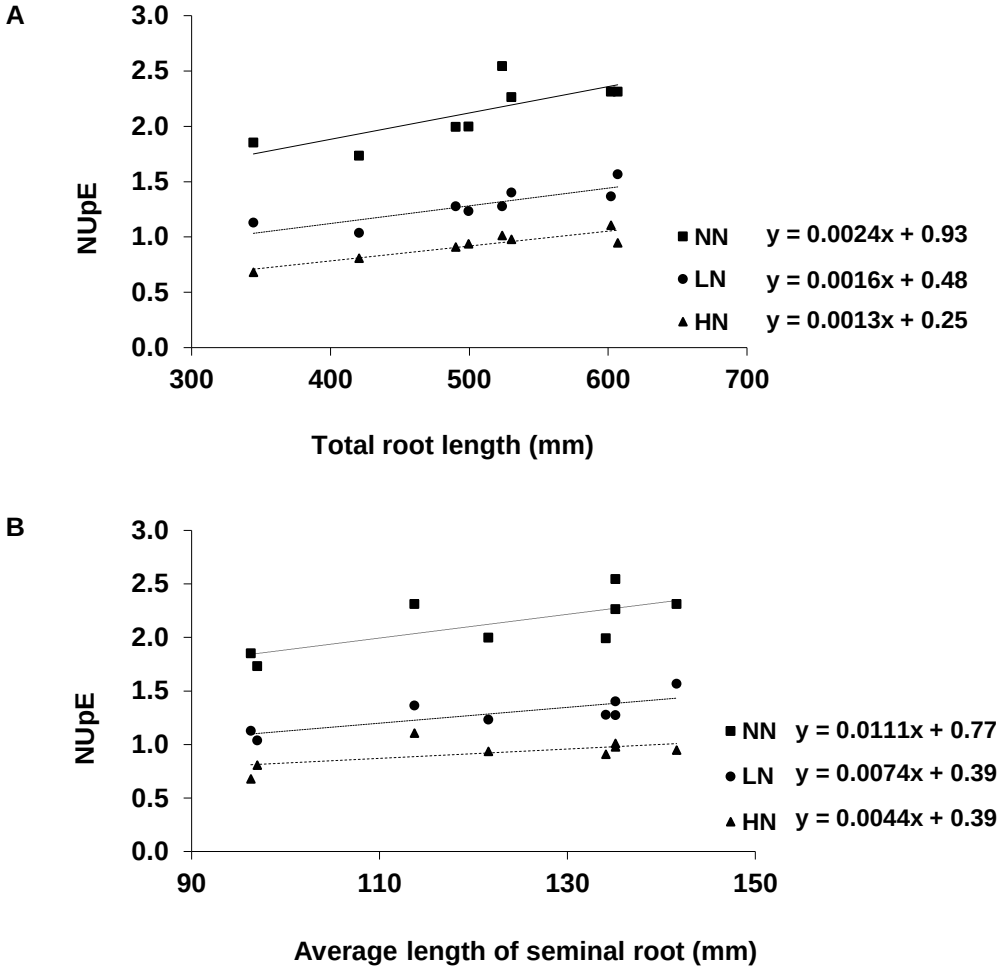


Figure 5.29 Comparisons of regression between NUpE in 2013 field experiment under NN, LN and HN with (A) total root length ($R^2 = 0.95$, $P < 0.001$) and (B) average length of seminal root ($R^2 = 0.93$, $P < 0.001$) of 2D root phenotyping experiment. Each value of NUpE is the mean of 3 replicates while for root traits, the number of replicates varied from 10 to 25, depending on the genotype

5.3 Discussion

Recently, there has been a growing interest in exploration of root architectural traits relevant to uptake of water, nitrogen and phosphorus. One of the major concerns of present wheat breeding programmes is to develop genotypes with efficient root systems to uptake more water and nutrients under resource limited conditions. Therefore targeted integration of root traits into breeding programmes needs adequate diversity of the existing genotypes. Ancient wheat species are widely used as a genetic resource to improve modern bread wheat.

5.3.1 Relationship between seedling root architecture and mature root system

In this study, germination papers were used where seedlings grew vertically followed by digital imaging and analysed using *RootNav* software (2D phenotyping). This technique is rapid, simple and cost effective way to analyse seedling root traits, especially seminal root characteristics. Further, different growth materials and N levels were used to examine root growth of ancient wheat genotypes in different growth stages by analysing scanned images using *WinRHIZO* software.

According to the results of 2D phenotyping experiment, the significant genetic variation among ancient wheat genotypes was examined for seedling root traits. However, these results might not encompass the full extent of genetic variation for the seedling root traits of ancient wheat since a limited number of genotypes were used. The ancient wheat genotypes together with bread wheat exhibited substantial variation in the number of seminal roots where emmer had the greatest number of seminal roots. The range of seminal root number among genotypes tested was 3.76 to 5.27 in 2D phenotyping experiment, compared to Gregory *et al.* (1978) who reported that, on average, the winter wheat grown in temperate weather conditions produced six seminal roots. Although, number of seminal roots in compost media of the genotypes was 4.85 to 7.3 and 4.6 to 7.3 in sand in modular tray experiment. Most of the bread wheat cultivars produced more than six seminal roots in both media.

In the present study, the total root length of the seedling was also high in emmer genotypes when compared to all other genotypes in 2D phenotyping experiment and modular tray experiment in sand and compost. In addition, emmer recorded wider tip

angle of the seminal roots than the other ancient wheat genotypes and bread wheat cultivars at 14 DAT suggesting that emmer has the potential to develop a wide root system at early stages of crop growth. Seedlings grown in sand and compost media had a similar trend of producing more seminal roots and total root length for emmer suggests that despite the growth media, growth and development of early seedling root systems of emmer was similar. In sand and compost, emmer had vigorous early seedling growth with high shoot and root biomass therefore it can be proposed that, mature emmer will develop horizontally grown root system enabling it to uptake more fertiliser N from the top layers of the soil before being transferred to deeper layers of the soil horizon.

Similar to the results of previous study on Australian spring wheat (O'Brien, 1979), there was no relationship between number and the tip angle of the seminal root of the seedlings in 2D phenotyping experiment. Also, Christopher *et al*. (2013) reported no relationship between number and the angle of the seminal roots in SeriM82 x Hartog DH population. However, there was a strong relationship between tip angle of the seminal root and the maximum width of the root system. Spelt and bread wheat genotypes had a narrow tip angle of seminal roots and deeper root systems when compared to emmer and einkorn. The present results showed consistency with previous investigations by Nakomota and Oyangi (1994) working on Japanese wheat germplasm, found that genotypes with narrower angles of seminal roots developed deep root systems while shallow root systems had much wider angle and horizontally grown seminal roots. The nutrient uptake of many plant species has a strong relationship between root angles of the seminal roots, since this trait plays a major role in determining root system architecture of the mature plant (Manschadi, *et al*., 2008). In 2D phenotyping experiment, average length of the seminal root had a promising relationship with maximum depth of the root system, where bread wheat and spelt had deeper seedling root systems than emmer and einkorn. Therefore, it can be predicted that mature root systems of the emmer and einkorn will be shallower than that of spelt and bread wheat.

In the column experiment, root system of emmer at anthesis and at maturity did not exceed 60 cm depth but root systems of spelt and bread wheat reached to the bottom of the column of 1 m. Further, a strong linear relationship was found between rooting depth of the mature plant and the tip angle of the seminal root of the seedling. This

was in agreement with the results of 2D root phenotyping experiment where spelt and bread wheat had narrow seminal root tip angles and developed deeper root systems at maturity than emmer and einkorn. These findings suggest that the seminal root characteristics of the seedling are closely linked with the root architecture of the mature plant. Hence, structure of the mature root systems is heavily influenced by tip angle of the seminal root, number and the average length of the seminal root of the seedling. Also, root angle and the length of the seminal root control maximum width and depth of the seedling root system. However, total root length seems to be the major root trait which controls function of the root system at maturity.

Generally, emmer is identified as a drought tolerant species having special adaptations to water limited conditions. Seedling and mature root systems of emmer were much wider and shallower than all other genotypes. This would enable emmer to extract available water and nutrients from the top layers of the soil more efficiently and reduce water losses due to evaporation. This is in partial agreement with Southern and Western Australia, where climatic conditions resemble the Mediterranean, and wheat cultivars with wider growth angles with fewer seminal roots are usually used. However, uptake of N from deeper layers of the soil is limited in emmer due to its root architecture. According to the results of 2012 and 2014 field experiments, it was revealed that NUpE of emmer 2 was greater than all other genotypes under optimum N fertiliser supply. Further, fertiliser N recovery rate of emmer 2 in 2013 field experiment and 2013 glasshouse experiment at LN was higher than other genotypes . Ehdaie *et al*. (2010) suggested that early uptake of N is crucial to reduce loss of N fertiliser through leaching during early plant growth; therefore, genotypes with early vigour would be an appropriate option.

Genotypes of einkorn did not produce any lateral roots at 14 DAT, illustrating their inability to develop a strong root system at early growth stages, leading to poor seedling growth in the present study. Plants possessing dense root systems with many lateral branches and root hairs are better adapted to acquire nutrients in the soil due to their higher root surface area (Gahoonia *et al.,* 2007).

Wheat genotypes having superior performances in deep clay soil in Australia had narrower angles of seminal roots, suggesting that spelt has an ability to grow successfully in hard and compacted soil and extract soil mineral N from deeper layers of the soil horizon (Manschadi, *et al*., 2008). In modular tray experiment, spelt had

highest root biomass production suggesting its ability to grow vigorously at early seedling stages. Spelt seedlings grown in hydroleca also had very high root length, volume and root and shoot biomass production. In addition to the number of seminal roots, there is an effect of nodal roots on the size of the root systems. It was observed that, spelt produced very high number of tillers during post-anthsis plant growth when compared to all other genotypes and had strong positive correlation between root lengths. Previous findings suggested that, the number of nodal roots of wheat have strong linear relationship with the number of leaves on the culm (Gregory *et al*., 1978), so that genotypes with vigorous early growth and tillering capacity may develop more leaves, hence more nodal roots. However, growing depth of nodal roots is not very deep due to less sensitivity to gravitropism than seminal roots (Klepper, 1992). Consequently, nodal roots are only useful to uptake water and nutrient from top soil layers in the soil. Lynch (2013) suggested that, based on the results of maize, narrow root angles of the seminal roots helped to develop deep root systems while crown roots have potential to develop more superficial roots, hence can uptake N from both topsoil layers and deep soil layers efficiently. Root system of spelt has above mentioned characteristics and therefore utilisation of resource from top soil layers as well as deeper layers are efficient.

Overall, it can be suggested that seedling root architecture of ancient wheat and bread wheat cultivars examined in the experiment was closely linked to the function of the mature root systems. Therefore, seedling root traits, which determine the structure of the mature root system, may be used as selection criteria for crop breeding for root systems (Oyangi 1994; Manschadi, *et al*., 2008). In a previous study, it was found that the characteristics of the seedling root architecture shows high degree of phenotypic plasticity in response to rooting environment (Poorter and Nagel 2000) hence this may complicate the identification of genotypic variation in root traits (Price *et al*., 2002). However, in our study early seedling root traits such as number of seminal roots, tip angles of the seminal root, total root length, maximum width, depth and convex hull together with root volume, root diameter and root biomass had strong relationship with root traits of matured root systems.

5.3.2 Phenotypic variation of seedling root traits under different growth media

Large phenotypic variation of roots in response to various growth conditions were found among ancient wheat species together with bread wheat. Root systems in 2D phenotyping experiment where roots grow vertically on the germination paper in nutrient media, was much smaller than those were in sand and compost at 14 days after germination. Nutrient availability was significantly higher in compost than sand. Root growth of the seedlings in sand was very strong for all species, suggesting that root growth was encouraged by limited supply of nutrient. On the other hand, foraging capacity of the roots is very high under resource limited conditions. Root length in < 0.5 mm diameter class was greater in sand suggesting that, all genotypes produced thinner roots, therefore increase surface area. In addition to that, allocation of carbon for root growth was limited under resource limiting conditions. Species variation for number of seminal roots, total root length, root volume, root biomass production and average diameter of the roots were very high among ancient wheat species. Emmer 2 had highest number of seminal roots, total root length and root volume, under any growth conditions at early seedling stage.

In contrast, spelt had very strong root systems in hydroleca at vegetative stage after 14 days. Vigorous root/shoot growth of spelt was seen at 14, 23, 32 and 41 DAT in phenotyping pot experiment and at anthesis and at maturity in column experiment, while recording high N_{off}. Water holding capacity of hydroleca is comparatively low and therefore frequent watering is needed. The excess water eventually transport N to the deeper layers hence less availability to the plants to be taken at upper layers of the root system, resulting in poor root growth in the top layers while having strong and heavy root biomass production in lower layers. Spelt and bread wheat have longest seminal roots and therefore uptake more N from the deep layers than emmer and einkorn therefore, root growth of emmer and einkorn was very poor in hyrdoleca.

5.3.3 Root morphology changes in response to depth of the soil horizon

Root length of the genotypes was relatively higher in the top 20 cm of the soil profile and gradually decreased with the depth. Total root length, root biomass, root volume and root length in < 0.5 mm diameter class, together with RLD, decreased constantly

with the depth. However, the rate of reduction of root length, root volume, root biomass and RLD was considerably low in spelt genotypes when compared to other genotypes in column experiment. This means spelt had comparatively longer roots, heavier root biomass, high root volume and RLD at the bottom layers of the soil profile when compared to emmer and bread wheat. The highest values of root length were found at anthesis with values slightly decreasing at harvest for most genotypes. Some genotypes of spelt (spelt SB and Oberkulmer) took 7-14 more days to reached anthesis than bread wheat and emmer genotypes in column experiment (Table 4.12). This suggests that deep and large root systems of spelt genotypes may be related to long pre-anthesis growth phase. Therefore, delayed senescence of spelt genotype may be associated with post-anthesis N uptake due to deep root system. Similarly, early senescence of emmer may be associated with shallow root systems which do not help to uptake soil mineral N from depth. Christopher *et al*. (2008) suggested that delayed senescence of SeriM82 was associated with deep root system.

5.3.4 Root traits and N_{off}

In the root phenotyping pot experiment, the highest above-ground biomass production was recorded in spelt species at early harvesting points suggesting its ability to capture N at early plant development. Regression of above-ground biomass and root biomass revealed a high positive linear relationship, thus it was presumed that higher root biomass translated into higher above-ground biomass because bigger root systems were able to acquire more nutrients and support more growth. Bloom *et al*. (1988) concluded that the large plants of wheat helped to recover more fertiliser N than small plants. This may be due to growth rate of the plant or efficient uptake of the N from the root system. Later, Ehdaie *et al*. (2001) confirmed that, both reasons; plant size and the efficient root systems actively engaged in recovering fertiliser N. The highest N_{off} was in spelt followed by bread wheat, emmer and einkorn species, respectively. Liao *et al*. (2004) reported that early uptake of N in wheat is controlled by fast and early crop growth.

Similarly, in the column experiment which used hydroleca as a growth substrate, the highest fertiliser N uptake and NUpE was recorded in spelt at anthesis and at maturity. As mentioned earlier, due to the nature of the hydroleca, it can be assumed that most of the N supplied as a liquid fertiliser was leached down rapidly to the bottom of the

column and therefore time to uptake N from the top layers was limited. This might be the reason why emmer had a low NUpE in hydroleca at anthesis and maturity since emmer has a shallow root system. Root systems of all spelt genotypes at anthesis and maturity were considerably larger than bread wheat and emmer genotypes, although there was no direct effect of N and GT x N on root growth. Ehdaie *et al*. (2010) found that bread wheat 'Pavon 76' and its three near-isogenic translocation lines, under low N supply resulted in more reduction of root production in top layers than bottom layers of the column, hence plants recovered relatively small fraction of applied fertiliser. Additionally, greater shallow root production was observed as a result of optimum level of N supply. Spelt genotypes had longer and heavier root systems while having highest root volume and average root diameter.

According to our results, root biomass had a significant effect on N_{off} at anthesis while both root biomass and root volume influenced N_{off} at maturity. This is in partial agreement with the previous findings that large root biomass, high RLD and seminal roots with shallow growth angles are among the root traits for a proposed ideotype capable of efficient N acquisition (Dunbabin *et al*., 2004; Lynch, 2013). These traits are pertinent to capture N at deeper soil layers since NO_3^- moves rapidly to lower sections of the soil profile during the growing season especially under wet conditions (Lynch, 2013). Liao *et al*. (2004) suggested that a wheat crop with large root biomass and deeper root system might be more efficient in recovering soil N. Our results indicated that rooting depth of the plant had a positive and linear relationship with N_{off} at anthesis and at maturity, where spelt produced larger and deeper root systems than bread wheat. Not only that, due to high tillering capacity, spelt produced more nodal roots and therefore had a strong shallow root system. Waines and Ehdaie (2007) found that the root systems of the ancient genotypes are larger than modern bread wheat cultivars. This may be due to the effect of *rht* genes on root growth (Wojciechowski *et al*., 2009). Our results showed that the einkorn species produced the least amount of biomass, owing to the small root size that drastically reduced resource capturing capacity but spelt and emmer produced more roots than bread wheat.

Interestingly, in the column experiment, most of the root traits including total root length, root biomass and root volume of spelt were significantly lower at 100 than 50 and 200 kg N ha^{-1}. It can be suggested that, the limited supply of N encouraged root growth but decreased with further N supply up to 100 kg N ha^{-1} and then increased

again at 200 kg N ha^{-1}. Although bread wheat and emmer recorded highest total root length, root biomass and root volume at 100 than at 50 and 200 kg N ha^{-1}, suggesting that 100 kg N ha^{-1} will be the optimum N level for both species. N_{off} and total root length increased up to some extent and then a plateau with N fertiliser application. This may be due to trade-off between shoot assimilates and root growth at high level of N fertiliser application where root used more shoot assimilates to grow strong root systems than above ground biomass production. Similar relationship was found between total root length/root biomass and grain dry matter production at harvest for genotypes. Supporting this argument, Gallais and Coque (2005) were of the opinion that a big root system would use more C assimilates resulting in less assimilate to produce grain. Similarly, Passioura (1983) also argued that smaller root systems would increase resource use efficiency by making more assimilate available for shoots.

Nielson & Schjorring (1983) and Egle *et al*. (1999) observed significant variation between cereal genotypes on SRL and RLD. In the current study, einkorn genotypes obtained the highest SRL, indicating it had the finest roots, and in fact 84% of the total root length was < 0.5mm in diameter at 41 DAT. Xi 19 and emmer genotypes had the lowest SRL and thus, thicker roots compared to the other species. Root biomass was negatively correlated to SRL, plants with higher root biomass tended to have thicker roots compared to plants with smaller root biomass. As root biomass increased, greater quantities of the root length were partitioned to roots with higher diameters. There was a negative relationship between SRL and N_{off}. SRL was found to be highest in einkorn 2 which had the least N_{off}. Additionally, TRL of einkorn 2 was significantly lower than all other genotypes. A similar trend was observed in the column experiment where emmer had a lower SRL followed by bread wheat and spelt at anthesis and at maturity (einkorn was not used in this experiment). SRL is not only dependent on finer roots but also root tissue density and root biomass. In contrast, a study by Eissenstat (1992) concluded that plants with high values of SRL generally produce higher RLD and therefore capture resources more efficiently. However, in this study, thicker roots increased the capacity of the root system to capture N. This can partly be explained by the fact that thicker roots may have a larger surface area due to root hairs for absorption of nutrients, hence higher levels of N_{off}. King *et al*. (2003) confirmed that decreasing specific root weight hence increasing SRL would confer greater N capture and yield under low N availability.

Spelt species had the highest root elongation rate, 23% more than bread wheat while emmer had lower root elongation rate than bread wheat. Burgos *et al.* (2001) reported that spelt species have higher growth rates and better early seedling hardiness compared to modern bread wheat. Since, the seedling stage is a critical period for young plants, vigorous plants with fast growing roots enable the plant to cope with abiotic and biotic stress. Root elongation rate was positively correlated with N_{off} indicating that fast growing roots ensure optimum surface area and rooting volume for nutrient capture. This trait is particularly desirable for wheat crops which have been sown in winter, a period with a high risk of soil N leaching, especially in sandy soils (Gastal and Lemaire, 2002). The highest N_{off} was recorded in emmer and spelt followed by bread wheat then einkorn throughout the experiment. Similar trend was observed in terms of $NUpE_R$. However, $NUpE_R$ of emmer and spelt, on average across all harvesting points, was not significantly different. In the column experiment, $NUpE_R$ was much higher in emmer and bread wheat than in spelt. Emmer and bread wheat allocate less photosynthetic assimilates to develop root system than spelt. On the other hand, in order to uptake unit N from the soil, spelt needs relatively long and heavy root biomass than emmer and bread wheat.

In 2013 glasshouse pot experiment, emmer species recorded the highest fertiliser NUpE/apparent fertiliser N recovery rate at maturity but not significantly different from spelt, where growth media was peat compost and different N rates were applied as liquid fertiliser. NUpE of bread wheat was between spelt and einkorn. This is in partial agreement of the investigations that the N_{off} of modern high yielding bread wheat varieties are considerably less efficient than their ancestors (Foulkes *et al*., 1998), since einkorn recorded the lowest NUpE. NUpE of the mature plant was found to be higher under LN conditions than HN conditions, suggesting that plant takes up N more efficiently under N deficit conditions. Supporting our results, Ortiz-Monasterio *et al*. (1997), Le-Gouis *et al*. (2000) and Gaju *et al*. (2011) reported that NUpE in LN was greater than in HN treated plants.

5.3.5 Predicting NUpE at maturity using seedling root traits

In the present study, it is obvious that tip angle of the seminal root of seedling had a significant influence on the maximum width of the root systems at maturity. Also, narrow tip angles of the seminal roots led to develop deep root systems at maturity.

Deep root systems have the ability to uptake more N from the bottom layers of the soil profile, especially in the later stages of the growth season when nitrate leached down to the deeper layers of the soil horizon. Our results showed strong negative linear relationship between tip angle of the seminal root at 14 DAT and rooting depth of the mature root system in column experiment, where, rooting depth has strong positive relationship with N_{off} at anthesis and at maturity. Previous studies have found that genotypes with shallow tip angles are well adapted for topsoil foraging (Zhu *et al*., 2005; Lynch, 2011). N_{off} at anthesis and at maturity in column experiment had a positive linear relationship with average length of the seminal roots and tip angle of the seminal root in 2D phenotyping experiment. Tip angle of the seminal root explained the variation of N_{off} at maturity more strongly than at anthesis. This suggests that the characteristics of the seminal root at seedling stage have massive influence on N_{off} at anthesis and at maturity. However, in column experiments leaching issues of NO_3^- may have occurred due to the nature of the media of hydroleca clay pellets. Therefore, this relationship may only hold for situations where N is leached down the profile and available mainly in the deep layers of the soil horizon and not for normal field conditions in the UK for winter wheat.

Total root length and average length of seminal roots of the seedlings in 2D phenotyping experiment explained variation in NUpE at maturity in 2013 glasshouse experiment significantly. Therefore, selected root architectural traits of early seedlings such as total root length, tip angle of the seminal root, average length of a seminal root and root biomass could be used to predict NUpE at maturity. However, this relationship was massively influenced by einkorn genotypes which recorded poor growth and NUpE at maturity in 2013 glasshouse experiment.

NUpE of the genotypes in 2012 and 2013 field experiment had a strong relationship with total root length of the seedling in 2D phenotyping experiment. However, as in the 2013 glasshouse experiment einkorn genotypes may have had a significant effect on this relationship both in 2012 and 2013 field experiment. Further, NUpE of the field is associated with several other factors including weather conditions, soil type and soil N mineralisation. Hence, the relationship between seedling root traits and NUpE at matuarity is complex and easily be modified by external factors.

5.4 Conclusions

- Root architecture of ancient wheat and bread wheat genotypes used in the study varied significantly at seedling stage and at maturity.
- Seminal root characters of the seedling such as tip angle, number of seminal roots and seminal root length are closely related to mature root systems.
- There is a possibility of using you seedling root traits, which determine the function of the mature root system, as selection criteria for efficient roots for N uptake.
- There is a high degree of phenotypic variation in response to temporal and spatial variation in rooting environment.
- Spelt had long seminal roots with narrow tip angles hence develop deep root systems while having well developed superficial root system due to nodal roots. Therefore, both top soil foraging and deep soil foraging occurs efficiently.
- Wider tip angles of the seminal roots and the greatest number of seminal roots help emmer to develop superficial root system architecture, especially in early stage of the plant growth.
- Seedling root traits such as total root length, average length of the seminal root and tip angle of the seminal root might be used to forecast NUpE at maturity.
- Emmer had more favourable seedling root traits related to NUpE while spelt showed more favourable root traits at maturity to uptake N more efficiently than modern bread wheat and einkorn.
- Further studies are needed to identify QTLs associated with NUpE and root architectural traits of spelt and emmer. Favourable root traits in emmer and spelt related to NUpE could be introduced to bread wheat through direct crossing or creating synthetic wheat.
- The introgression of spelt into modern bread wheat will be useful for molecular study to develop marker assisted breeding for high NUpE and thus may alleviate the constraint of root phenotyping in breeding programmes in the future.
- Additionally, identification of root QTLs in a wheat x spelt population related to NUpE could be an interesting objective for future research.

Chapter 6

Genetic basis of physiological traits of root architecture in a wheat x spelt population

6.1 Introduction

This chapter describes the results of the phenotypic evaluation and genetic analysis of recombinant inbred lines (RILs) produced from the cross between Swiss winter wheat variety Forno (*Triticum aestivum*) and Swiss winter spelt variety Oberkulmer (*T. spelta*) (Messmer *et al*., 1999). Two experiments were carried out in controlled environmental conditions by using modified growth pouches. Thirteen day old seedling root systems were imaged and those images were analysed using the *RootNav* software (Pound *et al*., 2013).

The tall male parent spelt Oberkulmer, developed deep root system with longest total root length, heaviest root biomass and high RLDs when compared with female wheat parent Forno at maturity, while the measured nitrogen uptake efficiency (NUpE) of spelt was significantly higher than Forno. Furthermore, a strong relationship was found between seedling root architectural traits and NUpE at maturity suggesting that seedling root architectural traits could be used as selection criteria for selecting genotypes for high NUpE at maturity.

Objective

The aim of the present chapter is to explore the phenotypic variation present in the wheat x spelt population and to identify the genetic locations of genes controlling seedling root architectural traits.

Specific hypotheses

13. Two parents of the population are significantly different for seedling root traits.
14. Seedling root system architecture varies significantly between RILs of a wheat x spelt population. Significant segregation of root traits among RILs of this population is observed.
15. QTLs can be identified for root architectural traits.
16. The introgression of spelt into modern bread wheat could provide novel genetic lines with improved NUpE.

6.2 Phenotypic results

A total of 202 RILs of the wheat x spelt population together with parents were arranged in a completely randomised design with 10 replicates. However, due to complexity of the experiment, it was conducted as four runs accommodating three replicates on each line on run one, two and three, and one replicate in run four. This experiment was repeated while maintaining same growth room conditions and number of replicates. However, phenotypic data presented here and used for the QTL analysis were the pooled mean values of two experiments.

Number of seminal roots, average seminal root length, total root length, maximum width, maximum depth, width to depth ratio, tip angle and emergence angle of seminal root were measured in this experiment (Figure 6.1). Seed germination from the different lines was not synchronised and thus differences in root length may result from differences in both germination time and rate of root extension. However, it was assumed that emergence angle, tip angle and number of seminal roots wouldn't affected by germination date.

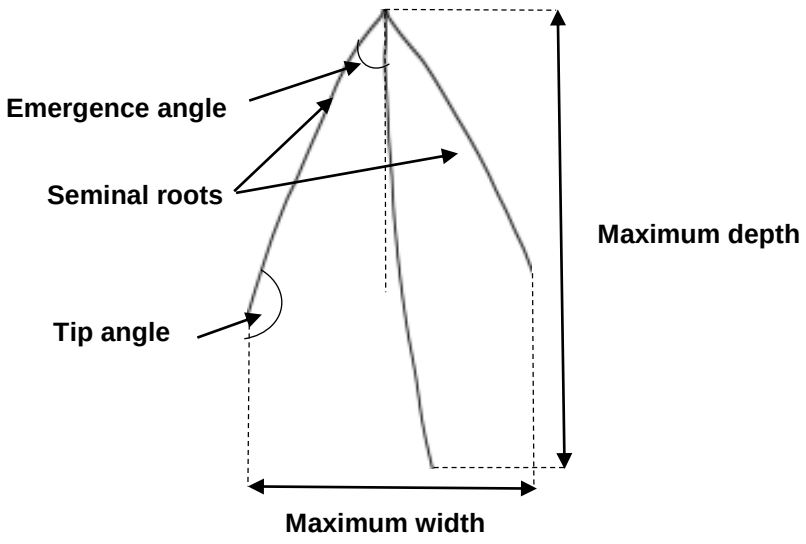


Figure 6.1 Schematic diagram of seedling root traits measured at 13 day after seeds placed on the germination paper (DAS)

6.2.1 Number of seminal roots

The highest number of seminal roots was recorded in F_5-230 (4.72 $\pm$ 0.9) while the lowest number was recorded in F_5-21 (3.02 $\pm$ 0.9). Figure 6.2 shows the distribution of the average number of seminal roots of RILs within the population. The average number of seminal roots of the parental lines Forno and Oberkulmer was 3.58 and 4.23, respectively. Among RILs of the population, 26% had fewer seminal roots than Forno and 20% had more seminal roots than Oberkulmer.

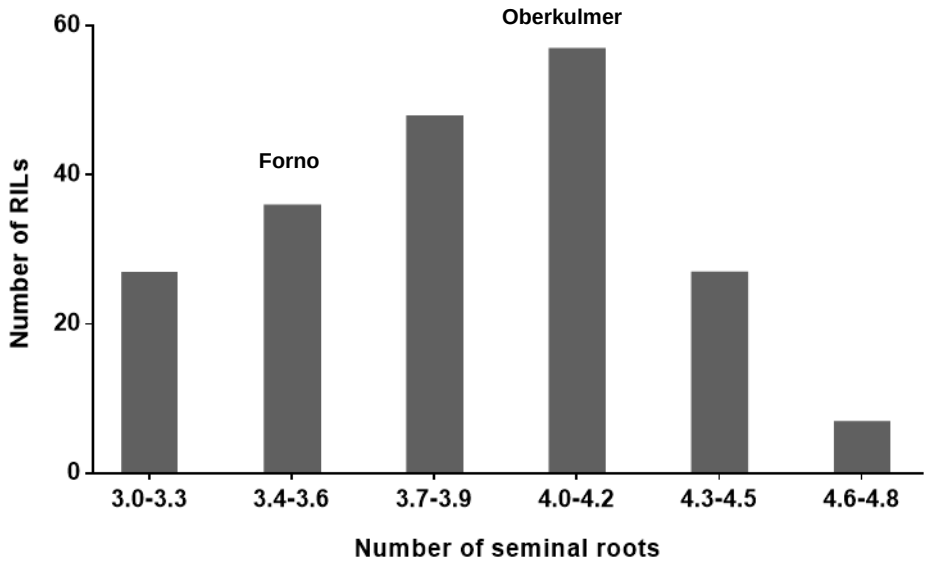


Figure 6.2 Frequency distribution of average number of seminal roots of 202 RILs of wheat x spelt population (*SD* = 0.9, *df* = 3502) *sk*; coefficient of skewness = -0.131

6.2.2 Seminal root length

Average length of the seminal roots of the RILs in the population was between 119.2 to 186.9 ($\pm$ 40.3) mm while the grand mean of the population was 149.6 mm. More than 67% of the population had longer seminal roots than the spelt Oberkulmer. The average length of the seminal root of 27% of the RILs was between the two parental values, while only 6% had shorter seminal roots than Forno. Frequency distribution of the average seminal root length of the RILs is shown in Figure 6.3.

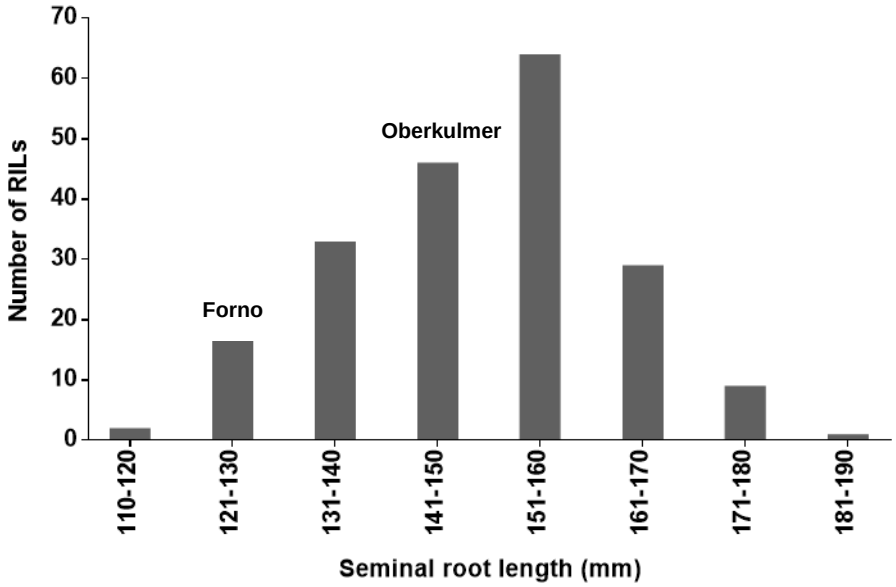


Figure 6.3 Frequency distribution of the average length of seminal roots of the 202 RILs of wheat x spelt population (*SD* = 40.3, *df* = 3502). *sk*; coefficient of skewness = -0.061

6.2.3 Total root length

The total root length of the root system was significantly different between the RILs ($P < 0.001$) (Figure 6.4). The longest total root length was found in F_5-248 (771.12 mm $\pm$ 186.3) and the shortest was observed in F_5-132 (408.52 mm $\pm$ 186.3). Total root length of wheat parent Forno and spelt parent Oberkulmer were 502.3 mm and 603.69 mm, respectively. Total root length of 16% of the RILs was less than that of Forno while 27% of the RILs was higher than Oberkulmer.

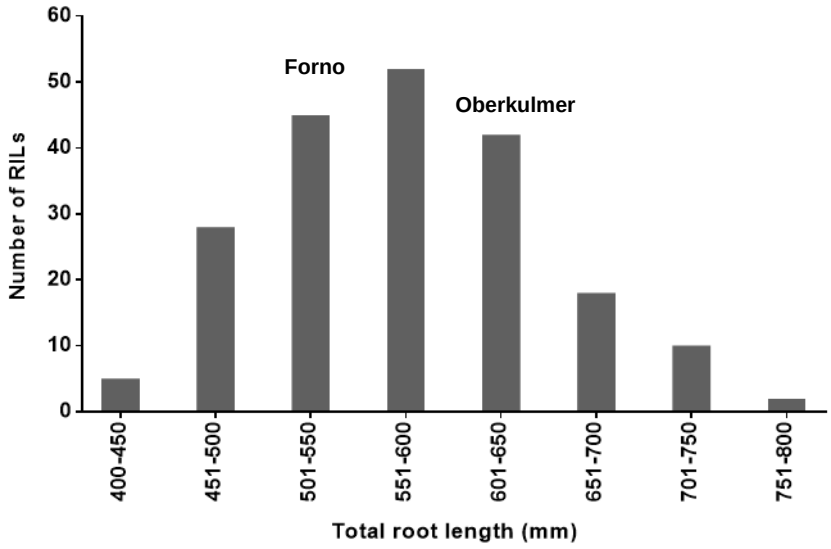


Figure 6.4 Frequency distribution of mean total root length of 202 RILs of wheat x spelt population (*SD* = 186.3, *df* = 3502) *sk*; coefficient of skewness = 0.214

6.2.4 Maximum width

The widest root system was observed in F_5-12 (127.24 mm ± 52.9) and narrowest root system was recorded in F_5-67 (49.21 mm ± 52.9) (Figure 6.5). Width of the root system of Forno and Oberkulmer was 94.1 mm and 72 mm, respectively. 20.3% of the population had narrower root systems than Oberkulmer while 31% had wider root systems than Forno.

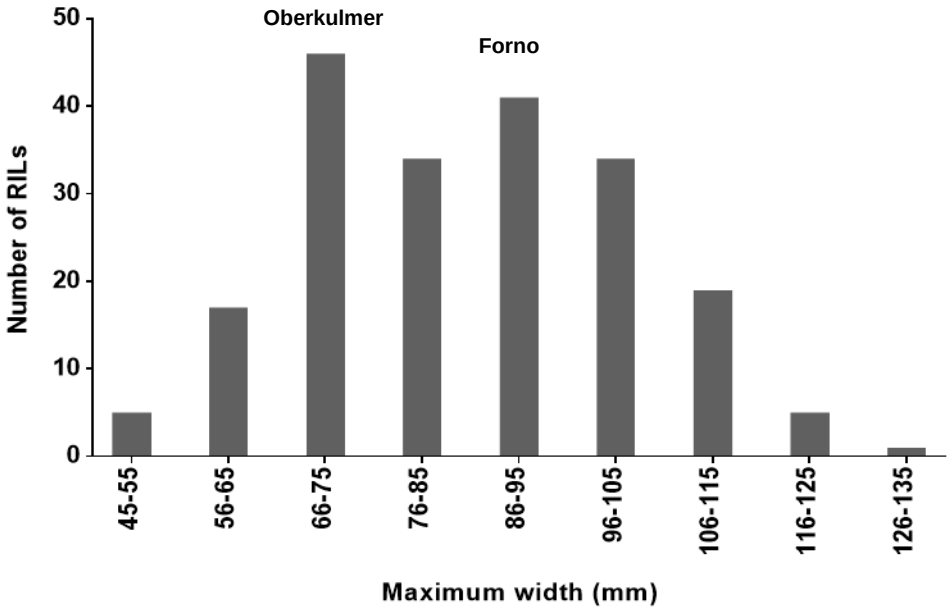


Figure 6.5 Frequency distribution of maximum width of the root system of 202 RILs of wheat x spelt population (*SD* = 52.9, *df* = 3502) *sk*; coefficient of skewness = 0.119

6.2.5 Maximum depth

The maximum depth of the root system differed among the RILs ($P < 0.001$) but Forno and Oberkulmer were not significantly different ($P = 0.121$). The deepest root system was observed for F_5-164 (215.71 mm ± 44.8) while the shallowest root system was observed for F_5-5 (125.22 mm ± 44.8). The maximum depth of the root system of wheat parent Forno (150.4 mm) and spelt parent Oberkulmer (165.21 mm) was not significantly different. About 7% of the RILs had shallow root systems than Forno but 73% of the RILs had deeper root systems than Oberkulmer (Figure 6.6).

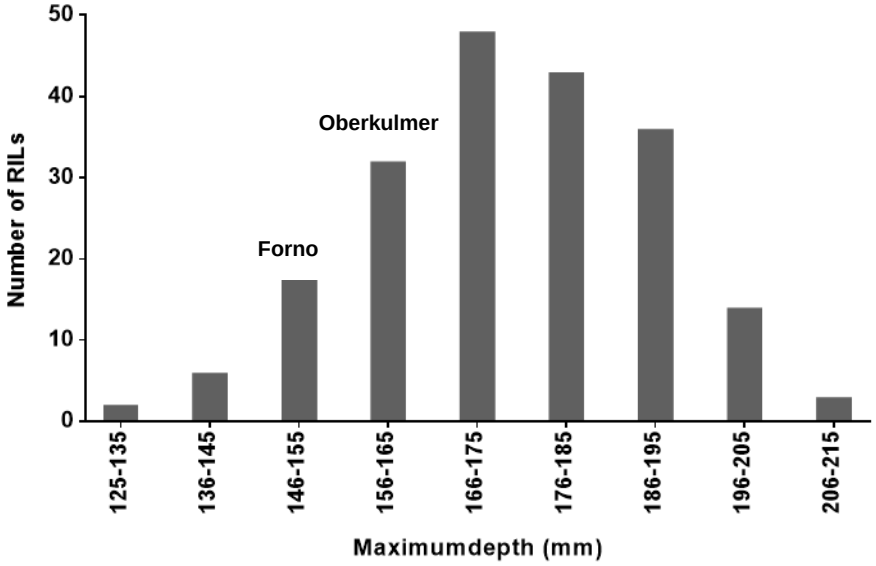


Figure 6.6 Frequency distribution of maximum depth of the root system of 202 RILs of wheat x spelt population (*SD* = 44.8, *df* = 3502). *sk*; coefficient of skewness = -0.273

6.2.6 Width to depth ratio

The width to depth ratio of the root system of the RILs was between 0.28- 0.80 (± 0.3). The highest ratio was recorded in F_5-12 and the lowest ratio was recorded in F_5-249 ($P < 0.001$). Width to depth ratio of Forno and Oberkulmer was 0.66 and 0.44, respectively ($P < 0.001$). Figure 6.7 shows frequency distribution of RILs for width to depth ratio. Width to depth ratio of 26% of the population was less than that of Oberkulmer while only 5% of the RILs had higher values than Forno.

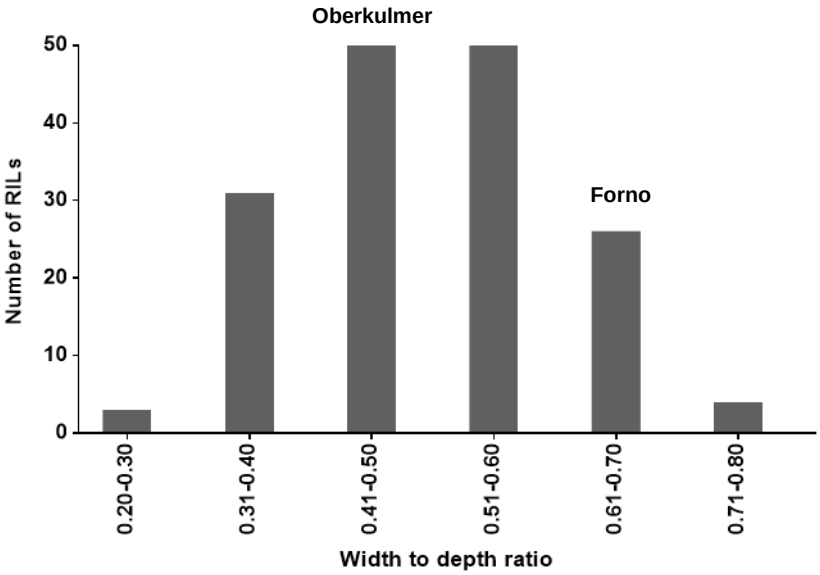


Figure 6.7 Frequency distribution of width to depth ratio of the root system of 202 RILs of wheat x spelt population (*SD* = 0.3, *df* = 3502). *sk*; coefficient of skewness = 0.15

6.2.7 Tip angle of seminal root

Tip angle of the seminal root of RILs was significantly different between lines, where the widest tip angle was recorded in F_5-168 (41.04^o $\pm$ 11.8 ^{o}) and the narrowest tip angle was observed in F_5-231 (18.08^o $\pm$ 11.8 ^{o}) ($P < 0.001$). Tip angle of the seminal root of Forno and Oberkulmer was 32.3^o and 22.2^o, respectively ($P < 0.001$). Tip angle of the seminal root of Oberkulmer was 36% narrower than Forno (Figure 6.8).

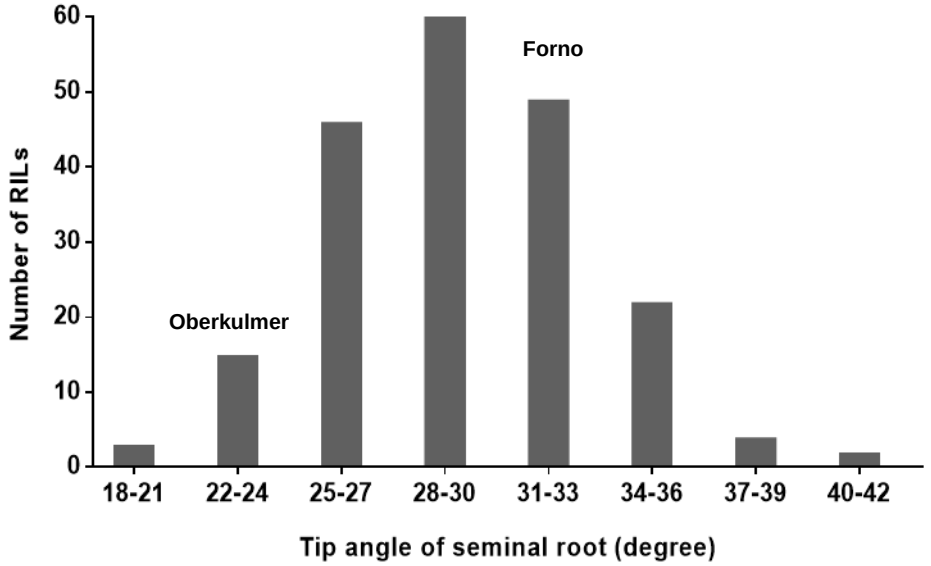


Figure 6.8 Frequency distribution of tip angle of the seminal root of the root system of 202 RILs of wheat x spelt population (*SD* = 11.8, *df* = 3502). *sk*; coefficient of skewness = 0.217

6.2.8 Emergence angle of seminal root

The emergence angle of the root system was between 16.5^o to 35.4^o ($\pm$ 10 ^{o}) where F_5-121 having the widest emergence angle and F_5-28 having the narrowest emergence angle at 13 DAT. Forno and Oberkulmer recorded 25.6^o and 21.2^o emergence angle respectively ($P < 0.01$). Figure 6.9 shows frequency distribution of RILs for emergence angle of the seminal root.

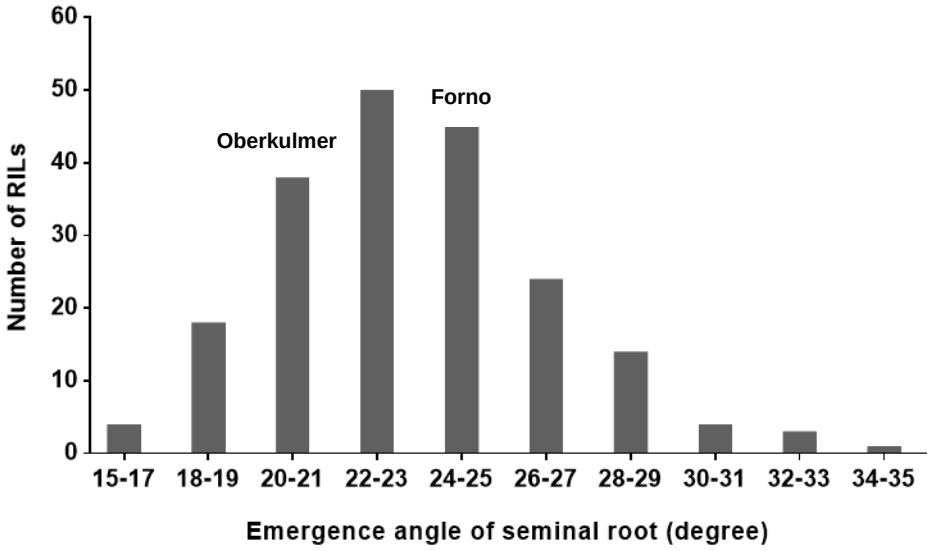


Figure 6.9 Frequency distribution of emergence angle of the seminal root of the root system of 202 RILs of wheat x spelt population (SD = 10, *df* = 3502). *sk*; coefficient of skewness = 0.399

Figure 6.10 shows thumbnail images produced by *RootNav* software for Forno and Oberkulmer. Table 6.1 shows descriptive statistics on root traits of parent lines and RILs.

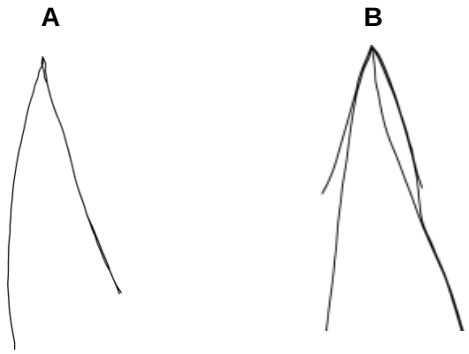


Figure 6.10 Representative thumbnail images of (A) bread wheat Forno and (B) Spelt Oberkulmer at 13 DAS in 2D root phenotyping experiment

Table 6.1 Descriptive statistics on the root traits of the parents and recombinant inbred lines (RILs) of the mapping population. (NSR; number of seminal roots, SRL; seminal root length, TRL; total root length, MW; maximum width, MD; maximum depth, W/D; width to depth ratio, TA; tip angle of seminal root, EA; emergence angle of seminal root, SD; standard deviation, *NS;* not significant at $P < 0.05$)

Trait	Parental lines			RILs		
	Forno	**Oberkulmer**	***P*- value**	**Mean (min; max)**	***P*-value**	**SD**
NSR	3.58	4.23	< 0.01	3.89 (3.02; 4.72)	< 0.001	0.9
SRL	127.71	143.34	< 0.01	149.60 (109.21; 186.92)	< 0.001	40.3
TRL	502.32	603.69	< 0.01	576.62 (408.51; 771.11)	< 0.001	186.3
MW	94.11	72.03	< 0.001	85.96 (49.21; 127.24)	< 0.001	52.9
MD	150.42	165.21	*NS*	174.77 (125.20; 215.74)	< 0.001	44.8
W/D	0.66	0.44	< 0.001	0.504 (0.28; 0.80)	< 0.001	0.3
TA	32.30	22.25	< 0.001	29.83 (18.08; 41.04)	< 0.001	11.8
EA	25.63	21.24	< 0.001	23.86 (16.55; 35.37)	< 0.001	10.0

Ten RILs having the highest and lowest values for root traits are shown in Table 6.2. RILs F_5-134 F_5-146 and F_5-230 had a high number of seminal roots and hence high total root length. Similarly, F_5-140 and F_5-132 had fewer seminal roots and hence lower total root length. F_5-36 had a high number of seminal roots, total root length and maximum width. F_5-242 and F_5-256 had lowest number of seminal roots but the highest average length of seminal root. Among the 202 RILs, F_5-10, F_5-36, F_5-134, F_5-146, F_5-230 and F_5-234 could be identified as top lines for selected root traits such as number of seminal roots, seminal root length, total root length and maximum depth of the root system (Chapter 5).

Table 6.2 Ten RILs of mapping population having the highest mean values and lowest mean values of each root trait. (NSR; number of seminal roots, SRL; seminal root length, TRL; total root length, MW; maximum width, MD; maximum depth, W/D; width to depth ratio, TA; tip angle of seminal root, EA; emergence angle of seminal root)

Traits	Top ten RILs (F_5)	Bottom Ten RILs (F_5)
NSR	31, **36**, 101, **134**, **146**, **170**, 214, 224, **230**, **267**	**21**, 71, 95, 127, **132**, **140**, 210, **240**, **242**, 256
SRL	**10**, 85, 133, **164**, 204, 231, **234**, 235, 242, 256	**5**, **62**, 83, **124**, **137**, **168**, 170, 196, 262, 267
TRL	20, **36**, 50, 52, **134**, **146**, 154, 203, **230**, **248**	**124**, 126, **132**, **137**, 139, **140**, 166, **168**, **186**, 245
MW	**12**, 30, **36**, **78**, **135**, 151, 163, **175**, **202**, **248**	**7**, 25, **67**, **140**, **231**, 237, **240**, **249**, 253, **258**
MD	**10**, 22, 37, **78**, 113, 125, **164**, 167, **234**, 265	**5**, **62**, 122, 124, 132, 137, **168**, **186**, 189, **244**
W/D	**5**, **12**, 33, 98, 145, **146**, **175**, 184, **248**, **267**	**7**, **10**, 42, **67**, 172, **231**, **244**, **249**, 253, **258**
TA	**5**, 83, 118, **135**, 142, **146**, 161, 168, 189, 250	**10**, **28**, 37, 44, 110, 153, 202, 210, **231**, 243
EA	62, **78**, 79, 121, **134**, **170**, 180, 194, 199, **202**	**10**, **28**, 109, 181, 187, 216, 224, **231**, 241, 247

Highlighted RILs in red appear two or more times/root traits as top line and highlighted in blue are common lines appearing as bottom lines

6.2.9 Phenotypic correlation between root traits

As in the previous 2D root phenotyping experiment, significant correlation was found between most of the root traits. Figure 6.11A shows the relationship between total root length and average length of seminal root (r = 0.55, $P < 0.001$). There was a strong correlation between total root length and number of seminal root (r = 0.71, $P < 0.001$) (Figure 6.11B). Maximum depth of the root system had strong and positive relationship with average length of the seminal root (r = 0.85, $P < 0.001$) (Figure 6.11C). Tip angle of the seminal roots had a negative relationship with maximum depth of the root system (r = 0.36, $P < 0.001$) (Figure 6.11D). Table 6.3 shows all possible correlations between measured root parameters of the population.

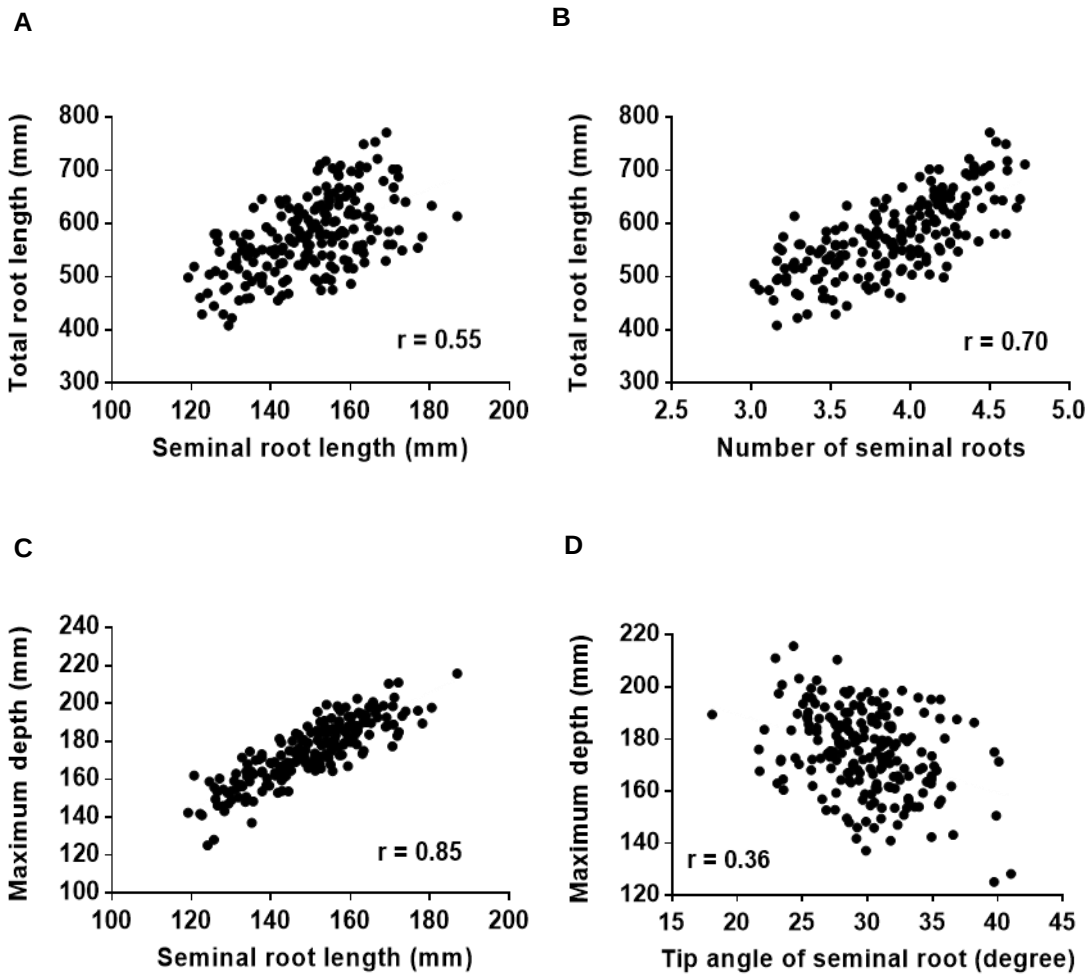


Figure 6.11 Correlation between (A) total root length and average length of seminal root (r = 0.55, *P* < 0.001) (B) total root length and number of seminal roots (r = 0.70, *P* < 0.001) (C) maximum depth and seminal root length (r = 0.85, *P* < 0.001) and (D) maximum depth and tip angle of seminal root (r = 0.36, *P* < 0.001) of 202 RILs of the wheat x spelt population

Table 6.3 Phenotypic correlation analysis of root traits in the mapping population. (NSR; number of seminal roots, SRL; seminal root length, TRL; total root length, MW; maximum width, MD; maximum depth, W/D; width to depth ratio, TA; tip angle of seminal root, EA; emergence angle of seminal root)

	NSR	SRL	TRL	MW	MD	W/D	Tip ang
NSR							
SRL	-0.19**						
TRL	0.71***	0.55***					
MW	0.43***	0.17*	0.51***				
MD	0.1^{NS}	0.85***	0.69***	0.26***			
W/D	0.38***	-0.23***	0.18*	0.85***	-0.21*		
TA	0.15*	0.26***	-0.05^{NS}	0.35***	-0.36***	0.53***	
EA	0.21**	-0.13^{NS}	0.13^{NS}	0.48***	-0.1NS	0.54***	0.37***

***** Significant at *P* < 0.001, ** significant at *P* < 0.01, * Significant at *P* < 0.05, NS - Not significant**

6.3 QTL results

A total of 26 significant QTLs (above the genome wide threshold as determined by a permutation test for each root trait) for root traits were identified using interval mapping. These QTLs were located on ten different chromosomes (1BS, 2A, 2D, 3A, 3B, 4A, 5A, 5B, 7AL and 7D) (Table 6.4 and 6.5). The majority of the QTLs were clustered on chromosome 2A (Figure 6.12). The threshold for QTLs detection was determined with 1000 permutations at $\alpha = 0.05$ (Churchill and Doerge, 1994). The significant LOD scores ranged from 3.15 to 8.96 and the proportion of phenotypic variation explained by individual QTL varied from 6.9 to 18.5%. Forno provided 61.5% of the trait increasing alleles and Oberkulmer 38.5%. Table 6.4 shows a summary of significant QTLs detected. Among 26 significant QTLs, 57% came from *A* genome of wheat, 27% from *B* genome and only 16% from *D* genome, indicating that *A* genome is more important for root traits, in this cross.

6.3.1 Number of seminal roots

Six significant QTLs (*QNsr*) were detected and mapped on chromosomes 1BS, 2A, 4A, 5A, 7AL and 7D for number of seminal roots while the threshold LOD was 3.1. The QTLs had LOD score ranging from 3.78 to 8.96, explaining 8.3 to 18.5% of the phenotypic variation. More than 66% of the trait increasing alleles for these QTLs are

from Forno. The QTL with largest effect for number of seminal roots, *QNsr-(F)* had an additive effect of 0.19 which explained 18.5% of the number of seminal roots range of 1.8 (LOD = 8.96). The QTL with the least effect, *QNsr-(O)*, had an additive effect of -0.13 which represents 8.3% of the range in NSR (LOD = 3.8).

6.3.2 Seminal root length

For average length of seminal roots, three significant QTLs (*QSrl*) were detected on chromosomes 2D, 3A and 3B while three indicative QTLs were detected on chromosomes 2A, 5A and 7B. The significant LOD threshold for seminal root length was 3 and LOD values of significant QTLs ranged from 3.3 to 4.48. All the QTLs, except one on chromosome 3B, had trait increasing alleles from Oberkulmer. For seminal root length, the largest QTL explained 10.5% of the range of 77.7 mm, the smallest explaining 7.3% of the range.

6.3.3 Total root length

Eight significant QTLs were detected for total root length (*QTrl*). Significant QTLs were located on chromosomes 1BS, 2A, 2D, 3A, 3B, 4A, 5A and 7AL while indicative QTLs were mapped on chromosome 2B and 7D. Five out of eight QTLs had trait increasing allele from Forno. LOD scores for total root length ranged from 3.30 to 8.48 while the significance threshold value was 3. Individual QTLs for total root length explained 7% to 17.6% of phenotypic variation.

6.3.4 Maximum width

There were two significant QTLs for maximum width (*QMw*) of the root system. They located on chromosomes 1BS and 2A. The significance LOD threshold for maximum width was 3 while QTLs had LOD score ranging from 3.22 to 5.36. Significant QTLs had an additive effect of 4.47 and 5.4% was from Forno. Phenotypic variation explained by these QTLs was between 7.7-11.5%.

6.3.5 Maximum depth

For maximum depth of the root system, six significant QTLs (*QMd*) located on chromosomes 2A, 2D, 3A, 3B, 5A and 5B were identified. Half of the QTLs had trait increasing alleles from Forno. Individual QTLs explained 6.9 to 9.1% of the total

phenotypic variation present in the cross. The significance threshold LOD value for the QTLs for maximum depth was 3.1 while the LOD range observed was 3.5 to 4.15.

6.3.6 Width to depth ratio

A single significant QTL (*QWd*) was detected on chromosome 2A for width to depth ratio of the root system. Phenotypic variation explained by the QTL was 10.4% with a LOD value 5.01.

6.3.7 Tip angle of seminal roots

Two indicative QTLs were detected for tip angle of seminal roots (*QTa*) (LOD $\geq$ 2) on chromosome 4DL and 7S.

6.3.8 Emergence angle of seminal roots

Two indicative QTLs were detected for emergence angle of seminal roots (*QEa*) on chromosome 4DL and 7S. Interestingly, QTLs on chromosome 4DL for tip angle and emergence angle were co-located while explained 4.3 to 5.2% of the variation. Favourable alleles for all QTLs were from Oberkulmer.

Table 6.4 Summary of significant QTLs detected in the Forno x Oberkulmer mapping population. (NSR; number of seminal roots, SRL; seminal root length, TRL; total root length, MW; maximum width, MD; maximum depth, W/D; width to depth ratio)

Trait	Number of QTLs	Chromosomes	Trait increasing alleles	
			Forno	Oberkulmer
NSR	6	1BS, 2A, 4A, 5A, 7AL, 7D	4	2
SRL	3	2D, 3A, 3B	1	2
TRL	8	1BS, 2A, 2D, 3A, 3B, 4A, 5A, 7AL	5	3
MW	2	1BS, 2A	2	0
MD	6	2A, 2D, 3A, 3B, 5A, 5B	3	3
W/D	1	2A	1	0
Total	26		16	10

Table 6.5 Summary of significant QTL by chromosome

Chr[a]	Trait	Position[b]	Confidence interval[c]	AE[d]	Marker interval[e]	R^2 (%)
1BS	Number of seminal roots	12.12	11.12 - 15-72	0.21	*Xpsr593a-1BS - Xglk301a-1BS*	17.2
	Total root length	13.12	12.12 - 16.72	30.86	*Xpsr593a-1BS - Xglk301a-1BS*	14.9
	Maximum width	13.72	12.12 - 19.72	4.47	*Xpsr593a-1BS - Xglk301a-1BS*	7.1
2A	Number of seminal roots	24.18	19.18 - 28.55	0.19	*Xpsr908-2A - Xglk197-2AS*	18.5
	Total root length	25.55	18.18 - 30.55	22.05	*Xpsr908-2A - Xglk197-2AS*	8.9
	Maximum width	96.89	95.89 - 97.89	8.23	*Xpsr919b-2A - Xglk293b-2AL*	11.5
	Maximum depth	156.02	154.48 - 162.02	-4.62	*Xglk278b-2AL - Xpsr630-2A*	8.0
	Width to depth ratio	97.89	96.88 - 98.88	0.04	*Xpsr919b-2A - Xglk293b-2AL*	10.8
2D	Seminal root length	94.83	94.37 - 95.83	-3.72	*Xpsr335-2D - Xglk529a-2D*	7.3
	Total root length	94.83	94.37 - 96.83	-19.63	*Xpsr335-2D - Xglk529a-2D*	7.0
	Maximum depth	94.83	94.37 - 95.83	-4.57	*Xpsr335-2D - Xglk529a-2D*	7.7
3A	Seminal root length	66.94	58.94 - 73.32	-4.29	*Xpsr598-3A - Xprs570-3A*	7.7
	Total root length	64.94	57.94 - 70.34	-29.57	*Xpsr598-3A - Xprs570-3A*	11.9
	Maximum depth	65.94	58.94 - 73.32	-5.68	*Xpsr598-3A - Xprs570-3A*	9.1

Continued……

Chr[a]	Trait	Position[b]	Confidence interval[c]	AE[d]	Marker interval[e]	R^2 (%)[f]
3B	Seminal root length	49.19	47.69 - 53.94	4.69	*Xpsr919a-3B* - *Xpsr1101b-3B*	10.5
	Total root length	51.94	48.19 - 54.94	20.66	*Xpsr919a-3B* - *Xpsr1101b-3B*	7.3
	Maximum depth	40.94	32.72 - 44.33	5.22	*Xglk538-3BS* - *Xpsr902-3B*	9.0
4A	Number of seminal roots	41.11	34.18 - 48.11	0.17	*Xpsr914-4A* - *CD16.2-4A*	13.6
	Total root length	42.11	32.18 - 48.11	35.39	*Xpsr914-4A* - *CD16.2-4A*	17.6
5A	Number of seminal roots	97.25	94.93 - 98.25	0.13	*Xglk317c-5AL* - *Xglk614a-5AL*	9.2
	Total root length	96.93	93.93 - 98.25	29.94	*Xpsr912-5A* - *Xglk317c-5AL*	15.1
	Maximum depth	94.93	90.93 - 100.25	5.43	*Xpsr912-5A* - *Xglk317c-5AL*	7.5
5B	Maximum depth	131.63	117.63 - 165.25	5.05	*Xpsr370-5B* - *Xpsr935-5B*	6.9
7AL	Number of seminal roots	28.9	24.15 - 45.99	-0.14	*pwir232a-7AL* - *Xpsr121-7AL*	8.8
	Total root length	28.9	25.15 - 37.99	-24.42	*pwir232a-7AL* - *Xpsr121-7AL*	8.9
7D	Number of seminal roots	7	1.12 - 26.46	-0.13	*Xglk184a-7DS* - *Xpsr618-7D*	8.3

[a] Chromosome name

[b] Position in the chromosome (cM)

[a] Confidence intervals (cM) - calculated as a 1LOD drop from the peak LOD score using the IM profile

[n] Additive effect; A positive sign means trait increasing alleles from bread wheat parent Forno while negative sign means that the trait increasing alleles from spelt parent Oberkulmer

[e]Nearest markers to the QTL, [f] % of variation explained by an individual QTL

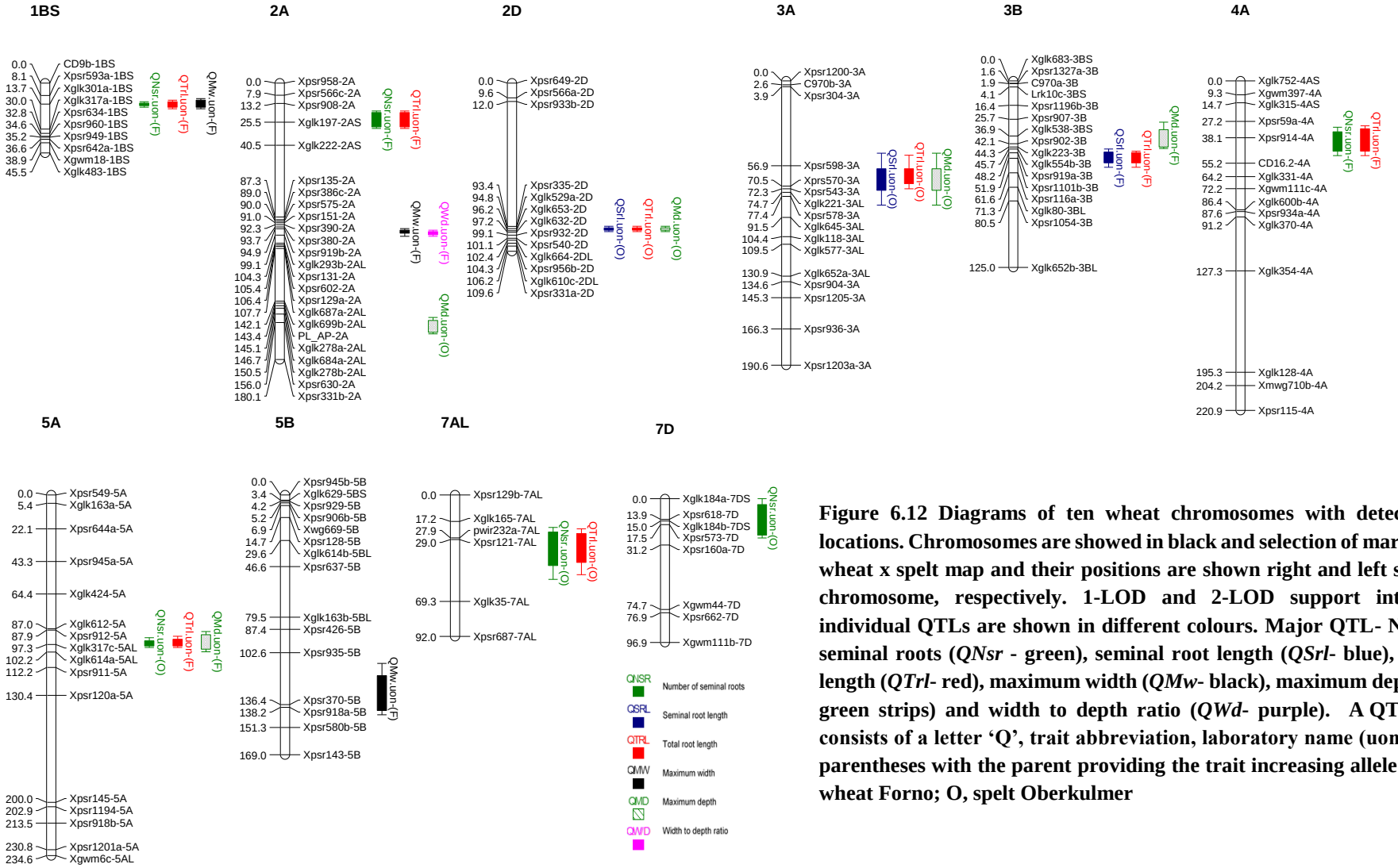


Figure 6.12 Diagrams of ten wheat chromosomes with detected QTL locations. Chromosomes are showed in black and selection of markers from wheat x spelt map and their positions are shown right and left side of the chromosome, respectively. 1-LOD and 2-LOD support intervals of individual QTLs are shown in different colours. Major QTL- Number of seminal roots (*QNsr* - green), seminal root length (*QSrl*- blue), total root length (*QTrl*- red), maximum width (*QMw*- black), maximum depth (*QMd*- green strips) and width to depth ratio (*QWd*- purple). A QTL symbol consists of a letter 'Q', trait abbreviation, laboratory name (uon), and the parentheses with the parent providing the trait increasing allele: F, bread wheat Forno; O, spelt Oberkulmer

6.3.9 QTL coincidence and linkage among different root traits

QTL coincidence was observed among root traits. QTLs for number of seminal roots were coincident with these for total root length and maximum width on two chromosomes (1BS and 2A). QTLs for total root length were coincident with those for number of seminal roots, seminal root length and maximum depth. These are in line with their strong phenotypic correlation (Table 6.3). Figure 6.13A to C shows identified QTLs for number of seminal roots, total root length and maximum width of the root system, respectively.

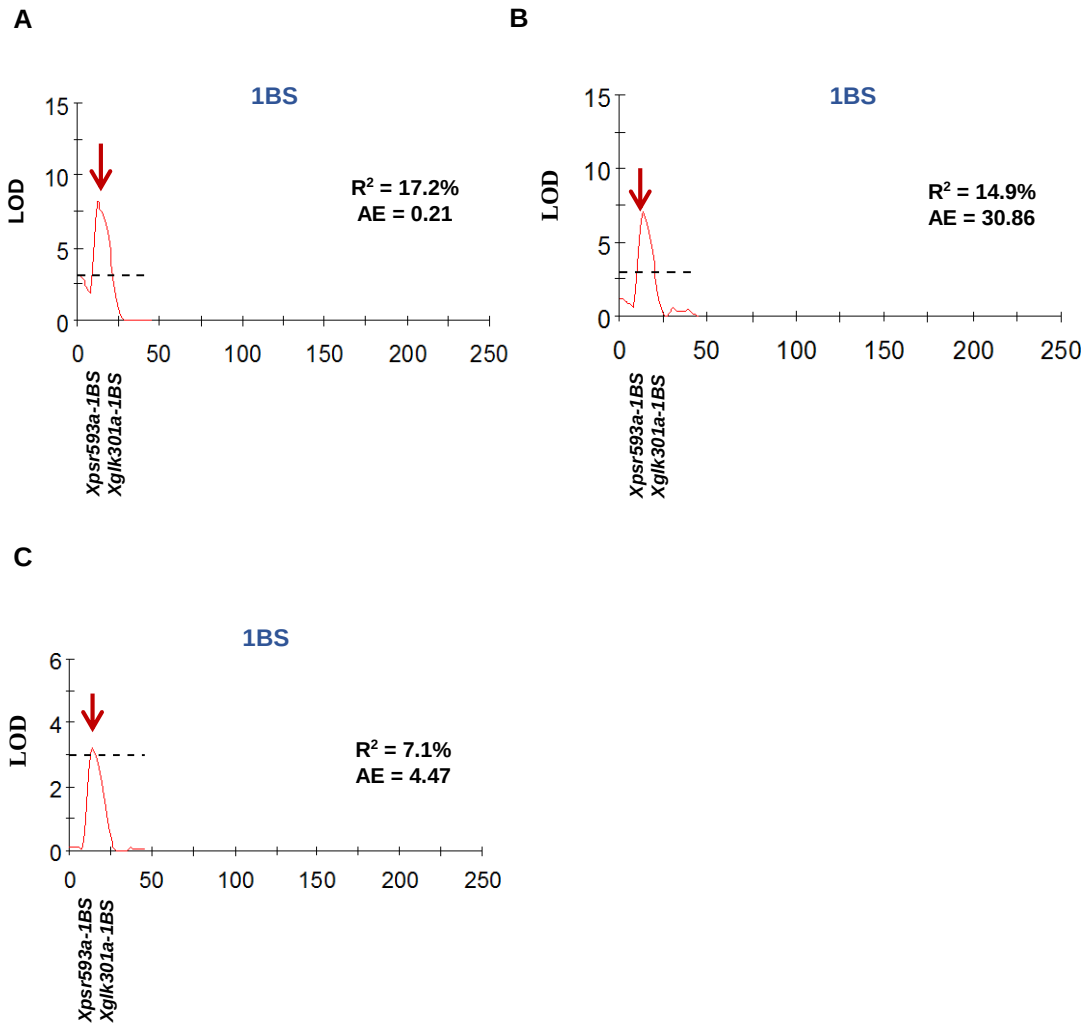


Figure 6.13 Detected QTLs for (A) number of seminal roots (B) total root length (C) maximum width. R^2; phenotypic variation explained by individual QTL (%), AE; Additive effects (positive alleles from bread wheat Forno and negative alleles form spelt Oberkulmer. Red arrow; QTL locations (LOD peaks above the threshold - dashed horizontal line)

6.4 Discussion

Seedling root system architecture (RSA) is important since it is a major influence on the structure of the mature root system. Also, RSA of seedlings had a significant relationship with NUpE at maturity (section 5.2.5). Therefore, studying seedling RSA is vital to find out root traits which are associated with NUpE at maturity. Such traits could be used as selection criteria to select genotypes for efficient N uptake. However, roots grow below the soil surface and are therefore very difficult to study *in-situ* (Nagel *et al*., 2009). Availability of high-throughput root phenotyping techniques, which can be used to identify QTLs for root traits, has provided novel information for a new era of wheat breading programmes (Bai *et al*., 2013). Hence the use of germination paper pouches to grow seedlings and analysing roots imaged using the *RootNav* software provided one easy access approach to studying seedling RSA in *ex-situ* conditions. This method is a rapid and cost effective way to analyse seedling root systems. In a single run of the experiment, it was possible to allocate 720 seedlings and the length of a single run was thirteen days. Use of germination paper based techniques has been reported in the literature. Hund *et al*. (2009) used germination paper pouches to grow maize seedlings followed by image acquisition using a flat-bed scanner while Bai *et al*. (2013) used germination papers in a 'cigar roll system' for wheat.

In this study, we quantified the phenotypic variation of root architectural traits and tried to identify their genetic basis in bread wheat (Forno) x spelt wheat (Oberkulmer) mapping population. Spelt was chosen as a male parent because of the higher level of genetic and trait diversity expected to be generated by making a cross between wheat and spelt, than within wheat only cross (Liu *et al*., 2009; Sidler *et al*., 1994). The wheat x spelt cross was originally developed to increase genetic diversity for lodging resistance and disease resistance (Schmid and Winzeler 1990; Schmid *et al*., 1994).

6.4.1 Phenotypical variation of root traits

In our study, the seedling root system of wheat 'Forno' had a wider tip angle (TA) of seminal roots, hence maximum width (MW), while the highest number of seminal roots (NSR), average length of seminal roots (SRL) and total root length (TRL) was higher in the spelt 'Oberkulmer'. The relationship between TA of the seminal root and the MW of the root system has been reported previously (Manschadi *et al*., 2008;

Nakamoto *et al*., 1991; Oyangi *et al*., 2001). In glasshouse modular tray experiment, Oberkulmer recorded the longest total root length, heaviest root biomass and highest root volume in both sand and compost media for two week old seedlings, while the number of seminal roots and seminal root length was not significantly different (Chapter 5, section 5.2.2). However, according to QTL analysis, more than 66% and 62% of the trait increasing alleles for NSR and TRL were from Forno. Interestingly, in glasshouse column experiment, the root systems of Oberkulmer at maturity were bigger than Forno (section 5.2.4.1) where total root length, root volume, root biomass and RLDs in the deeper layers of the column were significantly greater than Forno. This may be due to lateral branching of seminal roots and nodal roots which are related to the high capacity of tillering in spelt. Root systems of Forno and Oberkulmer reached the bottom of the 1m column suggesting their ability to develop deep root systems. The average plant heights of Forno and Oberkulmer were 80 cm and 113 cm, respectively. This suggests that Forno is a semi-dwarf winter wheat variety while Oberkulmer is a tall winter spelt variety having *rht* genes.

At present, more than 70% of the modern bread wheat cultivars contain major dwarfing genes (Evans, 1998). In northwest Europe, the semi-dwarfing alleles *Rht-B1b* (formerly *Rht1*) and *Rht-D1d* (formerly *Rht2*) are widely used; these have functionally similar mutations in two homeologous genes on chromosomes 4B and 4D in the hexaploid wheat genome (Börner, *et al*., 1997). However, the effect of the *Rht* alleles on root systems is less clear. The results reported under different environmental conditions appear contradictory to each other. According to the results of a field experiment, Subbiah *et al*. (1968) reported that semi-dwarf wheat cultivars had shorter root systems than tall cultivars. Further, they argued that small genotypes with small root systems might be less adapted to dry and resource limited environments due to decreased water and nutrient uptake. Similarly Siddique *et al*. (1990) reported that semi-dwarf wheat cultivars invested comparatively less dry matter into their root system than tall cultivars, resulting in a lower root: shoot mass ratio.

McKey (1973), based on the results of the progeny of wheat crosses between cultivars containing *Rht-B1b* and *Rht-D1b*, alleles found that root systems of genotypes having *Rht* alleles are smaller than tall plants. In contrast, Lupton *et al*. (1974) reported that root length, root distribution and phosphorus uptake between tall and semi-dwarfing

wheat cultivars under field condition were not significantly different. Similarly, Cholick *et al*. (1977) and Richard and Passioura (1981) found that there was no effect of *Rht* alleles on root growth of wheat. Furthermore, there was no relationship between cultivar height and rooting depth under dryland field conditions for winter wheat (Cholick *et al*., 1977). Nevertheless, Bai *et al.* (2013) found that TRL, laterals root length, lateral root surface area and lateral root volume were significantly reduced in dwarf and semi-dwarf lines compared to Mercia, *rht* plant, in cigar roll paper experiment.

As expected, there was significant variation among RILs for all measured root traits. It was observed that some RILs showed better performance than others. However, due to lack of synchronisation of seeds, total root length, seminal root length, width and depth of seedling root systems may be affected by actual germination time. They had two or more seedling root traits which could be used to predict the characteristics of the mature root system and, hence, potentially N uptake at maturity. The number of seminal root and total root length were strongly related. Four RILs F_5-36, F_5-134, F_5-146 and F_5-230 had both high number of seminal roots and total root length. On the other hand, F_5-132 and F_5-140 had the least seminal roots and lowest total root length. This indicates that there are common genes, or at least closely related genes, contributing to both traits.

6.4.2 Root QTLs

In total 26 significant QTLs were revealed for root traits. Four out of six QTLs for NSR and five out of eight QTLs for TRL had trait increasing alleles from Forno leading to a larger root system which is inconsistent with the observation that the Oberkulmer had seedling root system with high NST and TRL (Figure 6.9) and strong and large root systems recorded at maturity in column experiment (section 5.2.4.1). However, as a result of transgressive effect and some degree of interaction between alleles, unexpected values compared to the parental lines can be possible. There were three significant QTLs detected for SRL and two of these had trait increasing alleles from Oberkulmer. Trait increasing alleles from both parents equally contributed to QTLs for MD of the root system as parents were not significantly different for this trait. Two

and one QTL for MW and width to depth ratio, respectively, were detected and trait increasing alleles for all of them from Forno.

Studies on the number of seminal roots and root angle have been reported in previous studies for wheat (Christopher *et al*., 2013; Ren *et al*., 2012). Four major QTLs were detected for seminal root angle located on 2A, 3D, 6A and 6B chromosomes while two significant QTLs for number of seminal roots were detected on 4A and 6A in SeriM82 x Hartog DH population (Christopher *et al*., 2013). Ren *et al*. (2012) identified two QTLs for seminal root number of chromosome 3A and 6A while Sharma *et al*. (2011) detected one on 1A. Nodal root angle QTLs were detected in sorghum (Singh *et al*., 2012; Mace *et al*., 2012) and maize (Omori and Mano 2007). QTLs for seminal root characters have been identified in rice (Norton and Price, 2009) together with deep: shallow root QTLs (Uga *et al*., 2012) and other for vertical root distribution (Yadav *et al*., 1977).

Strong phenotypic correlations between NSR, SRL, TRL and maximum width to depth ratio may be due the coincidence of QTL or through traits which share mechanisms. QTLs for NSR and TRL were identified in chromosome 1BS, 2A, 4A, 5A and 7AL. On each chromosome, QTLs for the two traits overlapped, as shown by overlapping confidence intervals. However, three more QTLs for TRL were found on chromosome 2D, 3A and 3B where QTLs for SRL overlapped. Independent QTLs for NSR, TRL and SRL were not identified. Interestingly, trait increasing alleles for those co-located QTLs are from same parent.

6.4.3 Constraints of the experiment

The lack of synchronisation of germination and surface sterilisation of seeds due to large number of RILs, perhaps contributed significantly on actual date of germination. Further, application of deionised water without nutrient may have detrimental effect of early seedling development.

RootNav is generally user–friendly software which produced an accurate representation of each root system being recorded. However, there is a possibility to have inaccurate measurements for tip angles and emergence angle of seminal roots and seminal root length. Also, some RILs produced root systems which clumped together

and grew the same path (Figure 6.14). In such cases, accurate measurements of tip angle, maximum width, depth and width to depth ration could not be possible.

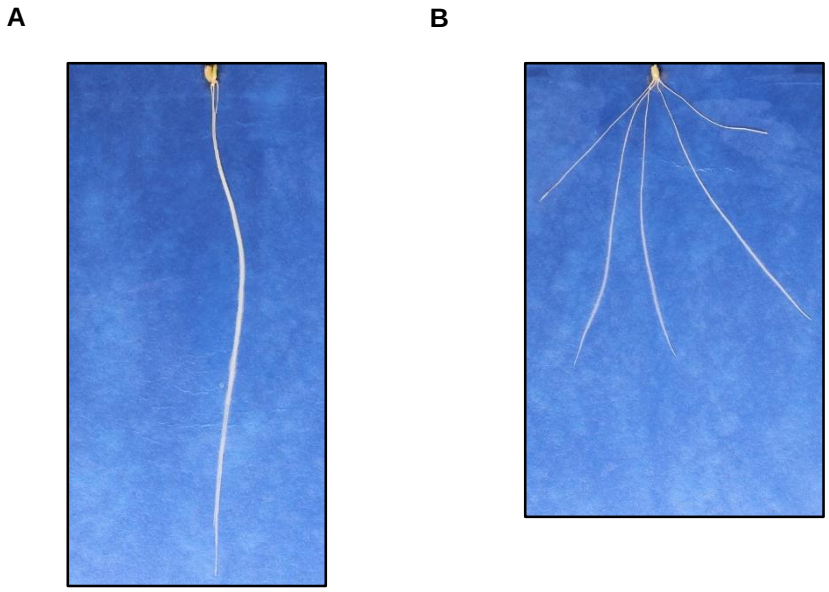


Figure 6.14 Seedling root system on germination paper at 13 days after seeding (A) seminal roots clumped together and grew the same path (B) well-distributed seminal roots with clear tip angles and emergence angles

6.5 Conclusions

- Two parents of the population were significantly different for a number of traits including: number of seminal roots, average length of seminal roots, total root length, maximum width, width to depth ratio and tip angle of seminal root.
- All measured root traits of seedlings of the RILs of a wheat x spelt population varied significantly.
- A total of 26 significant QTLs were identified; six for number of seminal roots, three for seminal root length, eight for total root length, two for maximum width, six for maximum depth and one for width to depth ratio.
- QTL coincidence was found for total root length either with number of seminal roots or seminal root length of the seedlings which showed strong phenotypic relationship.
- The number of seminal roots and total root length were mainly controlled by the same QTLs detected on chromosome 1BS, 2A, 4A and 7AL.
- QTLs for seminal root length always co-located with QTLs for total root length and mapped on chromosome 2D, 3A and 3B.
- Variation for depth of the root system was mainly controlled by QTLs on 2A, 2D, 3A, 3B, 5A and 5B while the two parents gave equal contributions for the favourable alleles.
- RILs F_5-10, F_5-36, F_5-134, F_5-146, F_5-230 and F_5-234, could be efficient genotypes for N uptake at maturity.
- The contribution of the *A* genome to the phenotypic variation observed within this cross for the development of the seedling root system is more important than the *B* and *D* genomes, as assessed by QTL analysis.

Chapter 7

General discussion and conclusions

Chapter 7

7.1 Introduction

The main aim of this study is to characterise above-ground and below-ground traits of ancient wheat, which are associated with NUE, and compare them with bread wheat. In the first results chapter, detailed analysis of plant establishment, plant development, plant morphology, above-ground biomass, grain yield and stay-green properties were examined for ten genotypes in the field. The second results chapter focused on NUE and its components of ancient wheat species and bread wheat in the field and control environments. Root system architecture of four wheat species was studied in the third results chapter. Identification of root QTLs of the wheat x spelt population was a primarily interest of fourth results chapter.

The objective of general discussion and conclusions chapter is to address major hypotheses of the study and to compare current results with published literature. The potential for future research on wheat breeding for efficient NUE, based on current findings, are discussed in this chapter. Final conclusions are made based on the results of three field experiments, four glasshouse experiments and three growth room experiments.

7.2 How do ancient wheat species differ from bread wheat?

General plant growth and development of ancient wheat genotypes used in the study was different from bread wheat. Plant establishment of bread wheat was always higher than ancient wheat species under field conditions and plant development more rapid. Fast developmental rate of bread wheat may be associated with early vigour hence rapid canopy expansion. GAI of bread wheat was higher than ancient wheat species in field experiments. However, AGB production was significantly higher in spelt and emmer genotypes or similar to bread wheat under optimum growing conditions. Plant morphology, green area duration and root system architecture of wheat species may have an effect of observed variations in AGB production. Ancient wheat genotypes were 30 to 33% taller than bread wheat cultivars used in the study. Stay-green properties are identified in spelt genotypes.

NUE of bread wheat was significantly higher than ancient wheat species but NUpE of spelt and emmer was higher than bread wheat under field and control environment.

High NUtE of bread wheat was associated with improved HI. Seedling root system architecture was significantly different between species. Emmer had greater number of seminal roots and wider tip angles hence maximum width of the root system. Root systems of spelt and bread wheat was narrower and deeper than emmer. Einkorn had small root system, as resulting from poor seedling and mature growth in the present study.

7.3 Is it possible to use ancient wheat species as a genetic resource to improve NUE in bread wheat?

There is substantial variation for NUE and its components among elite winter wheat cultivars (Van Sanford and MacKown, 1987; May *et al*., 1991, Le Gouis *et al*., 2000). However, additional variation can be introduced to wheat breeding programmes from ancient wheat species. Generally, genetic gain in NUE in wheat is associated with both NUpE and NUtE (Hirel *et al*., 2007). High NUpE is a desirable trait under N abundant conditions due to reduce risk of pollution by minimizing NO_3^- leaching.

The current study showed that high NUE of bread wheat was associated with NUtE rather than NUpE. Ancient wheat species of spelt and emmer had higher NUpE than bread wheat but NUtE was lower. High NUtE of bread wheat only related to HI but not to above-ground biomass production. However, in the UK, HI of winter wheat varieties is reported to have reached the theoretical optimum of 0.62 (Foulkes *et al*., 2007; Austin, 1980). Therefore, there is a limited possibility to improve NUE by improving NUtE in winter wheat. Biomass production efficiency (above-ground biomass production per unit N supply) could be an important trait to improve modern bread wheat in the future. Varieties which increase biomass production, under low or moderate N supply, while maintaining present values of HI can be selected as efficient genotypes for NUE (Calderini *et al*., 1999; Foulkes *et al.*, 2007).

Under field conditions, at zero N fertiliser application, all species had higher NUE than at high N application due to improve NUpE and NUtE. NUpE and NUtE explained NUE more efficiently at HN than NN in 2013 field experiment where einkorn, emmer, spelt and bread wheat were examined. However, there was no relationship between NUpE and NUE at any level of N supply but NUtE explained NUE strongly under optimum N supply in 2014 field experiment. This may be due to high NUpE of all

wheat species used in 2014 field experiment and less variation between genotypes used in the experiment for NUpE.

In Mexico, for spring wheat, NUpE explained NUE more efficiently than NUtE under high N supply (Ortiz-Monasterio *et al*., 1997). Genotype and environment have significant contribution in the expression of either NUpE or NUtE as a major component of NUE (Coque and Galliais, 2006). According to the results of present study, it can be suggested that spelt and emmer could be used to improve NUpE of the bread wheat especially under limited N supply. However, einkorn did not show any favourable trait associated with NUpE and/or NUtE. Therefore, einkorn may not be a useful resource in wheat breading programme for efficient NUE.

7.4 What are the mechanisms behind the variation observed in ancient wheat species for NUE and its components?

Stay-green traits were identified in some spelt genotypes such as spelt cv. Oberkulmer under all N levels in the present study. Increased NUpE of spelt may be associated with post-anthesis N uptake hence increased green area duration. According to Foulkes *et al*. (2007), low N remobilisation efficiency (associated with stay-green properties), high capacity of N accumulation in the stem and leaves, increased root length density (RLD) in depth and decreased specific root weight are some of the short-term strategies which could be used to improve N economy of the wheat. However, in bread-making wheat it is important to maintain acceptable grain N concentration of approximately 2.3% while having profitable yield. Except above mentioned traits, high accumulation of N in vegetative organs during pre-anthesis, which enables efficient remobilisation during post-anthesis grain filling, is one of the desirable traits in winter wheat breading. Delayed post-anthesis flag leaf senescence was identified as one of the potential physiological traits which could be used to improve both grain yield and grain protein concentration of bread wheat (Bogard *et al*., 2010). A link between high yield and stay-green properties in wheat and sorghum genotypes under drought stress were found in previous studies. Therefore, stay-green could be an important trait under water limited environments (Christopher *et al*., 2008; Lopes and Reynolds 2012).

7.4.1 Flag leaf senescence

Flag leaf greenness score was fitted against the thermal time by using a standard logistic model. The observed values of greenness score explained more than 99% of variance for seven out of ten genotypes. Previous studies on senescence used monomolecular-logistic equation describing leaf senescence kinetics during post-anthesis as two phase process (Ge´nard *et al*., 1999; Bogard *et al*., 2011; Gaju *et al.,* 2011). Onset of senescence was earlier in bread wheat than other genotype while senescence rate of spelt genotypes especially spelt cv. Oberkulmer, were comparatively slow in 2012 season. As a result of delayed onset of senescence in spelt, length of the growth season expands leading to greater post-anthesis AGB accumulation. Bread wheat, emmer and einkorn completed flag leaf senescence four to five days earlier than spelt genotypes. In 2013 and 2014, flag leaf senescence completed six to seven days earlier at NN than HN, with little difference between LN and HN. However, senescence rate of spelt cv. Oberkulmer at NN was comparatively slower than the other wheat species. The high post-anthesis green area duration observed in all spelt genotypes investigated, especially in spelt Oberkulmer, under NN condition suggests its ability to cope with resource limited conditions. Limited availability of N in the soil accelerates flag leaf senescence due to remobilisation of N and other nutrients from flag leaf to grain (Sinclaire and Amir, 1992). Post-anthesis N uptake usually delays flag leaf senescence due to continued supply of N from the soil.

Plants are assimilated more C due to delaying senescence by active photosynthesis during grain filling period. However, delayed senescence is not always beneficial, in terms of yield, if straw has more unused carbohydrate when season terminated (Yang *et al*., 1997). Furthermore, Jiang *et al*. (2004) found a negative relationship between stay-green traits and grain yield in rice. In the current study, green area duration due to delayed senescence had a positive relationship with above-ground biomass production at maturity.

7.4.2 Plant morphology

Storage capacity of the vegetative organs of the plant has strong relationship with N uptake. Stem and leaves act as storage organs of the plant and store N optimally at anthesis for wheat. Canopy of the well fertilised wheat crop contain more N than is required for maximum growth at anthesis (Pask, 2009). Excess N may act as a reservoir that can be used during post-anthesis grain filling period (Thornton and Millard, 1996). However, that which remains in straw at maturity should be minimised in the genotype which is efficient in NUE. In the present study, improved N uptake at maturity was observed with N supply.

7.4.3 Root system architecture

Ancient wheat genotypes and bread wheat cultivars varied highly for seedling root system architecture. Emmer developed shallow root systems with wider tip angles of seminal roots while spelt and bread wheat had narrow and deep seedling root systems. There was a positive relationship between narrower tip angle of the seminal root and maximum depth of the root system (Manschadi *et al*., 2008; Oyangi *et al*., 2001; Nakamoto *et al*., 1991). Usually seminal roots reached deeper soil layers hence important for deep soil foraging. Previous investigations by Nakamota and Oyangi (1994) who, based on the variation of the angular spread of seminal roots of Japanese wheat germplasm, found that genotypes with narrower angles of seminal roots developed deep root systems while shallow root systems had much wider angle and horizontally grown seminal roots. Similarly, lateral distribution of the mature root systems of CIMMYT genotype SeriM82 was narrow due to sharp seminal root angles (Manschadi *et al*., 2006). Manschadi *et al*. (2008) suggested that selection for growth angle and number of seminal roots may help to identify genotypes with a root system architecture that is better adapted to drought conditions due to greater root length density at depth. As expected, a strong relationship was found between seedling root system architecture and mature root systems. Tip angle of the seminal root of the seedling had a strong and negative relationship with the depth of the root system at maturity hence emmer produced shallow root systems at maturity when compared to spelt and bread wheat. Further, emmer had the highest number of seminal roots and total root length at seedling stage which may be useful to develop a well spread surface

root system with high topsoil foraging capacity. Two types of roots occur in cereals; seminal roots and nodal roots. Seminal roots are directly formed from the embryo while lower nodes of the tillers produce nodal roots (Manske and Vlek 2002). Apparent fertiliser N recovery rate of the emmer under field conditions was higher than other wheat species may be due to well-distributed root systems of the top soil layers of the soil horizons which enable emmer to uptake fertiliser N before being transferred to deeper layers. Similar results were obtained under glasshouse experiment where low N peat compost was used as a growth media.

Spelt and bread wheat developed deep root systems at maturity than emmer. Total root length of the spelt was higher than bread wheat and emmer. Also, due to its high tillering capacity, spelt produced more nodal roots which spread relatively shallow in the soil. Therefore, spelt may be able to uptake N from the top soil layers as well as deep soil layers of the horizon. Deep root system architecture may be related to stay-green traits observed in spelt due to deep soil foraging for N and water, as was reported by Christopher *et al*. (2008). Root length density of all spelt genotypes was higher than 1 cm cm^{-3} until 80 cm of the depth in column experiment but both emmer genotypes and bread wheat cv. Xi 19 only to 40 cm at maturity. Similarly, Hoad *et al*. (2004) found < 1 cm cm^{-3} of root length density in winter wheat below 40 cm depth but in the plough layer, it was between 3-6 cm cm^{-3}.

Total root length and seminal root length in 2D phenotyping experiment explained the variation in NUpE at maturity at 2013 glasshouse experiment, 2012 and 2013 field experiments. This suggests that the characteristics of the seminal root at seedling stage have massive influence on NUpE at maturity. Therefore, selected root architectural traits of early seedlings such as total root length, tip angle of the seminal root, average length of a seminal root and root biomass could be used to predict NUpE at maturity.

7.5 Can useful traits of ancient wheat be introgressed into bread wheat?

Genetic analysis of recombinant inbred lines (RILs) produced from the cross between Swiss winter wheat variety Forno (*Triticum aestivum*) and Swiss winter spelt variety Oberkulmer (*T. spelta*) was conducted to find out possible segregation of seedling root traits which can be used to forecast NUpE at maturity. More than 20% of the population had greater number of seminal roots than the male parent of Oberkulmer. Seminal root length and total root length of more than 66% of the RILs were higher than the parents. 20.3% of the population had narrower root systems than Oberkulmer while 31% had wider root systems than Forno. Interestingly, more than 50% of the population had deeper root systems than both parents. However, only four RILs had narrower tip angle of seminal root than Oberkulmer. Among 202 RILs of the population, only six lines; F_5-10, F_5-36, F_5-134, F_5-146, F_5-230 and F_5-234 have been identified as superior lines for selected root traits such as number of seminal roots, seminal root length, total root length and maximum depth of the root system. These RILs may be more efficient in NUpE than parent Oberkulmer. This is one of the examples to show that the introgression of ancient wheat into modern bread wheat could provide novel genetic lines with improved traits for modern bread wheat.

A total of 26 significant QTLs for root traits were identified and located on ten different chromosomes; 1BS, 2A, 2D, 3A, 3B, 4A, 5A, 5B, 7AL and 7D. QTLs for number of seminal roots and seminal root length were co-located while either number of seminal roots or seminal root length was co-located with total root length. Therefore, it can be suggested that those root traits may be controlled by same gene or closely related genes.

7.6 General conclusions

Addressing the specific hypotheses stated in the thesis at the end of the Chapter 1, final conclusions are made.

1. Plant establishment of bread wheat was higher than ancient wheat genotypes while bread wheat reached a particular growth stage faster than ancient wheat species.
2. Above-ground biomass of bread wheat, spelt and emmer was either similar or bread wheat was slightly higher at maturity. Higher grain yield was recorded in bread wheat than ancient wheat species due to improved HI at any level of N supply. Additional supply of N fertiliser increased grain yield and above-ground biomass production at maturity for all wheat species.
3. Spelt genotypes, especially spelt cv. Oberkulmer consistently displayed stay-green traits, lengthening growth season, leading to high above-ground biomass accumulation. Delayed senescence of spelt may be associated with root system architecture hence improved post-anthesis N uptake.
4. Flag leaf length, width, green area, specific leaf area and SPAD values were significantly different between genotypes. The greatest green area, SPAD value and maximum width were recorded in bread wheat, suggests that bread wheat used more assimilates to produce a strong flag leaf with high chlorophyll concentration, which would have greater photosynthetic capacity than ancient wheat species.
5. NUE and its components; NUpE and NUtE were different among wheat species. Bread wheat had high NUE and NUtE while spelt and emmer had high NUpE at maturity. NUE and its components decreased gradually with N supply.
6. Improved NUpE of spelt and emmer may be related to plant height and root system architecture.
7. Highest NUtE was observed in bread wheat than ancient wheat species and associated with improved HI.
8. Relationship between NUE and its components was not consistent between experiments. NUtE explained more variation in NUE than NUpE in three of

five experiments. Further, NUpE and NUtE explained more variation in NUE at HN than NN.

9. Root system architecture of ancient wheat and bread wheat genotypes used in the study varied significantly at seedling stage and at maturity. Seedling root system of emmer was shallower and wider than other wheat species ideal for scavenging N in top soil. Spelt and bread wheat had narrow and deep root systems although root system of einkorn was smaller than all other species.
10. Seminal root characters of the seedling such as tip angle, number of seminal roots and seminal root length were closely related to mature root systems. Therefore, early root system architecture can be used to forecast the nature of mature root system.
11. Seedling root traits such as seminal root length, total root length and root biomass had a relationship with NUpE at maturity. Therefore, early seedling root traits might be used as selection criteria for NUpE at maturity.
12. A large root biomass and total root length recorded in spelt enhanced N uptake hence increased biomass and green area production. Ancient wheat species had high root N uptake efficiency and specific absorption rate of N when compared to bread wheat. Therefore, ancient wheat used relatively less assimilates for root growth.
13. Two parents of the population (wheat Forno x spelt Oberkulmer) were significantly different for a number of traits, including: number of seminal roots, average length of seminal roots, total root length, maximum width, width to depth ratio, tip angle and emergence angle of seminal root.
14. The RILs of the mapping population exhibited significant variation for root traits at seedling stage.
15. A total of 26 significant QTLs were detected for seedling root traits. Bread wheat parent contributed more trait increasing alleles for root traits than spelt parent. The contribution of the *A* genome to the phenotypic variation observed within this population for the development of the seedling root system is more important than the *B* and *D* genomes, as assessed by QTL analysis.
16. The introgression of spelt into bread wheat provides novel resources to improve bread wheat for high NUpE at maturity. RILs of F_5-10, F_5-36, F_5-134, F_5-146, F_5-230 and F_5-234 could be efficient for N uptake at maturity.

7.7 Limitations of the study

While this study aimed to compare ancient wheat species with bread wheat, these results might not encompass the full extent of genetic variation for identified traits since I used limited number of genotypes. In general, plant growth and development varied significantly between species where bread wheat reached a particular growth stage faster than ancient wheat species. However, all genotypes were sampled on the same calendar date for growth stages in order to facilitate sampling and statistical analysis, except in column experiment. Therefore, significant variation in growth related parameters may be due to different rate of plant growth. However, growth parameters were presented against thermal time rather than calendar date in the study to avoid misunderstanding. Uneven establishment of the plants in 2013 and 2014 field experiments may have an adverse effect on growth parameters. However, when sampling, necessary actions were taken as much as possible to avoid such errors.

The main aim of glasshouse column experiment was to find out variation of root system architecture, NUE and its components of genotypes under different N levels, but only response to genotype was observed. Therefore, this objective was only partially achieved. Because there were no responses to N application and GT X N on root traits may be due to the N availability of the fertigation system which disturbed N treatments. Further, growth media, hydroleca clay pellets, have very small water holding capacity hence all nutrients together with water leached down rapidly.

7.8 Future work

Stay-green traits identified in spelt genotypes may be not only due to post-anthesis N uptake, associated with deep root systems, but also water availability. Therefore, conducting experiments under drought conditions may be useful to further confirm the present results since all experiments in this study were carried out either under rain fed or controlled environment condition with irrigation. Analyse biomass remobilisation efficiency and N remobilisation efficiency seem to be useful when discuss N_{off} of the genotypes at maturity. Therefore, N analysis at anthesis is essential for field experiments.

QTL analysis should be carried out by using sub-set of RILs of the wheat x spelt population. This helps to confirm identified root QTLs of the present study. Also, synchronisation of germination is more convenient for less number of RILs than whole population. Application of nutrients to deionised water would be beneficial for early seedling growth of the RILs. Further, field experiments will be conducted to collect data of GAI, AGB production, grain yield and NUE and its components of the above population and develop physiological and genetic relationship between different traits and based on the results; N efficient lines could be identified.

Chapter 8

References

ADDISCOTT, T.M., WHITMORE, A.P. & POWLSON, D.S. (1991). Farming, fertilisers and the nitrate problem. Pp. 170. CAB International, Wallingford.

AHMAD, I.S., REID, J.F., NOGUCHI, N. & HANSEN, A.C. (1999). Nitrogen Sensing for precision agriculture using chlorophyll maps, Written for presentation at the 1999ASAE/CSAE-SCGR Annual International Meeting Sheraton Centre, Toronto, Canada.

AHMED, T.A., TSUJIMOTO, S. & SASAKUMA, T. (2000). QTLs associated with plant height and related characters in haploid wheat. *Breeding Science*. **50**, 267-273.

AL-HAKIMI, A. (1998). Primitive tetraploid wheat species to improve drought resistance in durum wheat (*Triticum durum* Desf.). In: Jaradat AA (ed) Triticeae III symposium. Science Publisher Inc., USA, pp 305–312.

AN, D., SU, J., LIU, Q., ZHU, Y., TONG, Y., LI, J., JING, R., LI, B. & LI, Z. (2006). Mapping QTLs for nitrogen uptake in relation to the early growth of wheat (*Triticum aestivum* L.). *Plant and Soil.* **284**, 73–84.

ANDERSON, A. & JOHANSSON, E. (2006). Nitrogen partitioning in entire plants of different spring wheat cultivars. *Journal of Agronomy and Crop Science*. **192**, 121-131.

ARAI-SANOH, Y., TAKAI, T., YOSHINAGA, S., NAKANO, H., KOJIMA, M., SAKAKIBARA, H., KONDO, M. & UGA, Y. (2014). Deep rooting conferred by DEEPER ROOTING 1 enhances rice yield in paddy fields. *Nature Scientific Reports*. **4**, 5563.

ATKINSON, D. (2000). Root Characteristics: Why & What to Measure. In Root Methods. A Handbook Eds A. L. Smit, A. G. Bengough, C. Engels, N. v. Noordwijk, S. Pellerin & S. C. Geijn), pp. 1-32. Berlin: Springer-Verlag. ATWELL.

ATKINSON, J.A., WINGEN, L.U., GRIFFITHS M., POUND M.P., GAJU O., FOULKES M.J., LE GOUIS J., GRIFFITHS S., BENNETT M.J., KING J. & WELLS, D.M. (2015). Phenotyping pipeline reveals major seedling root growth QTL in hexaploid wheat. *Journal of Experimental Botany.* **66**, 2283-2292.

ATTA, B.M., MAHMOOD, T. & TRETHOWAN, R.M. (2013). Relationship between root morphology and grain yield of wheat in north-western NSW, Australia. *Australian Journal of Crop Science.* **7**, 2108-2115.

AUSTIN, R.B. & JONES, H.G. (1975). The physiology of wheat. Pp. 327-355. Annual Report Plant Breeding Institute, Cambridge, UK.

AUSTIN, R.B. (1980). Physiological limitations to cereals yields and ways of reducing them by breeding. In: Opportunities for increasing crop yields, pp. 3– 19 (Eds. Hurd, R.G. et al.), Association of Applied Biology, Pitman, Boston.

AUSTIN, R.B. (1999). Yield of wheat in the United Kingdom: Recent advances and prospects. *Crop Science.* **39**, 1604-1610.

AUSTIN, R.B., FORD, M.A., EDRICH, J.A. & BLACKWELL, R.D. (1977). The nitrogen economy of winter wheat. *Journal of Agricultural Science.* **88**, 159167.

BAI, C., LIANG, Y. & HAWKESFORD, M.J. (2013). Identification of QTLs associated with seedling root traits and their correlation with plant height in wheat. *Journal of Experimental Botany.* **64**, 1745–1753.

BANGAL, B. B., BIARI, B. M. & PATIL, K. G. (1988). Root characters – the important criteria for drought resistance in wheat. *J. Maharashtra Agric. Univ*. **13**, 242–243.

BARRACLOUGH, P. B. (1989). Root-growth, macro-nutrient uptake dynamics and soil fertility requirements of a high-yielding winter oilseed rape crop. *Plant and Soil*. **119**, 59-70.

BARRACLOUGH, P. B., WEIR, A. H. & KUHLMANN, H. (1991). Factors affecting the growth and distribution of winter wheat roots under UK field conditions. *Developments in agricultural and managed-forest ecology*. **24**, 410-441.

BARRACLOUGH P.B., HOWARTH J.R., JONES J., LOPEZ-BELLIDO R., PARMAR S., SHEPHERD C. E. & HAWKESFORD M.J. (2010). Nitrogen efficiency of wheat: genotypic and environmental variation and prospects for improvement. *European Journal of Agronomy*. **33**, 1-11.

BAR-YOSEF, O. (1998). The Natufian culture in the Levant, threshold of the origin of agriculture, *Evolutionary Anthropology*. **6**, 159-177.

BASANTA, M. V., DOURADO-NETO, D., REICHARDT, K., BACCHI, O. O., OLIVEIRA, J. C., TRIVELIN, P. C., TIMM, L. C., TOMINAGA, T. T., CORRECHEL, V., & CASSARO, F. A. (2003). Management effects on nitrogen recovery in a sugarcane crop grown in Brazil. *GEODERMA*. **116**, 235-248.

BASF. (2004). The encyclopaedia of cereal diseases. www.agricentre.basf.co.uk/.../BASF_Disease_Encyclopedia.pdf

BENGOUGH, A.G., GORDON, D.C., AL-MENAIE, H., ELLIS, R.P., ALLAN, D., KEITH, R., THOMAS, W.T.B. & FORSTER, B.P. (2004). Gel observation chamber for rapid screening of root traits in cereal seedlings. *Plant and soil*. **262**, 63-70.

BENGOUGH, A.G., BRANSBY, M.F., HANS, J., MCKENNA, S.J., ROBERTS, T.J. & VALENTINE, T.A. (2006). Root response to soil physical conditions; growth dynamics from field to cell. *Journal of Experimental Botany*. **57**, 437–447.

BIDINGER, F., MUSGRAVE, R. B. & FISCHER, R. A. (1977). Contribution of stored pre-anthesis assimilate to grain yield in wheat and barley. *Nature*. 270, 431-433.

BINGHAM, I.J., KARLEY, A.J., WHITE, P.J., THOMAS, W.T.B. & RUSSELL, J.R. (2012). Analysis of improvements in nitrogen use efficiency associated with 75 years of spring barley breeding. *European Journal of Agronomy*. **42**, 49-58.

BLACKLOW, W.M. & INCOLL, L.D. (1981). Nitrogen stress of winter wheat changed the determinants of yield and the distribution of nitrogen and total dry matter during grain filling. *Australian Journal of Plant Physiology*, **8**, 191—200.

BLOOM, T.N., SYLVESTER-BRADELU, R., VAIDYANATHAN, L.V. & MURRAY, A.W.A. (1988). Apparent recovery of fertiliser nitrogen by winter wheat. In: Nitrogen efficiency in Agricultural soils, pp. 27-37 (Eds. Jenkinson, D.S & Smith, K.A.), Elsevier Applied Science, London, UK.

BOGARD, M., ALLARD, V., BRANCOURT-HULMEL, M., HEUMEZ, E., MACHET, J., JEUFFROY, M., GATE, P., MARTRE, P. & LE GOUIS, J. (2010). Deviation from the grain protein concentration–grain yield negative relationship is highly correlated to post-anthesis N uptake in winter wheat. *Journal of Experimental Botany*. **61**, 4303-4312.

BOGARD, M., JOURDAN, M., ALLARD, V., MARTRE, P., PERRETANT, M.R., RAVEL, C., HEUMEZ, E., ORFORD, S., SNAPE, J., GRIFITHS, S., GAJU, O., FOULKES, J. & LE GOUIS, J. (2011). Anthesis date mainly explained correlations between post-anthesis leaf senescence, grain yield, and grain protein concentration in a winter wheat

population segregating for flowering time QTLs. *Journal of Experimental Botany.* **62**, 3621-3636.

BÖRNER, A., RÖDER, M., & KORZUN, V. (1997). Comparative molecular mapping of GA insensitive *Rht* loci on chromosomes 4B and 4D of common wheat (*Triticum aestivum L.*). *Theoretical and Applied Genetics.* 95, 1133-1137.

BORRELL, A.K., GARSIDE, A.L., FUKAI, S. & REID, D.J. (1998). Season, nitrogen rate, and plant type affect nitrogen uptake and nitrogen use efficiency in rice. *Australian Journal of Agriculture Research*. **49**, 829-843.

BORRELL, A.K. & HAMMER, G.L. (2000). Nitrogen dynamics and the physiological basis of stay-green in sorghum. *Crop Science.* **40**, 1295-1307.

BORRELL, A.K., HAMMER, G.L. & OOSTEROM, E. (2001). Stay-green: a consequence of the balance between supply and demand for nitrogen during grain filling? *Annals of Applied Biology,* **138**, 91–95.

BOWMAN, D.C., DEVITT, D.A, ENGELKE, M.C. & RUFTY, T.W. (1998). Root Architecture Affects Nitrate Leaching from Bent grass Turf. *Crop Science.* **38**, 1633-1639.

BOWMAN, D.C., CHERNEY, C.T. & RUFTY, T.W. JR. (2002). Fate and transport of nitrogen applied to six warm-season turf grasses. *Crop Science.* **42**, 833-841.

BRADY, D.J., GREGORY, P.J. & FILLERY, I.R.P. (1993). The contribution of different regions of the seminal roots of wheat to uptake of nitrate from soil. *Developments in Plant Soil Science.* **54**, 169-172.

BRANDOLINI, A., HIDALGO, A. & MOSCARITOLO, S. (2008). Chemical composition and pasting properties of einkorn (*Triticum monococcum* L. subsp. *monococcum*) wholemeal flour. *Journal of Cereal Science.* **47**, 599-609.

BRAUN, H.J., ATLIN, G. & PAYNE, T. (2010). Multi-location testing as a tool to identify plant response to global climate change. In: REYNOLDS, C.R.P. (eds.). Climate change and crop production, CABI, London, UK.

BROGE, N.H. & MORTENSEN, J.V. (2002). Deriving green crop area index and canopy chlorophyll density of winter wheat from spectral reflectance data. *Remote Sensing of Environment.* **81**, 45-57.

BURGOS, S.M., MESSMER, M.M., STAMP, P. & SCHMID J.E. (2001). Flooding tolerance of spelt (*Triticum spelta* L.) compared to wheat (*Triticum aestivum* L.) – A physiological and genetic approach. *Euphytica.* **122**, 287-295.

CALDERINI, D.F. & SLAFER, G.A. (1998). Changes in yield and yield stability in wheat during the 20th century. *Field Crops Research.* **57**, 335-347.

CALDERINI, D.F., ABELEDO, L.G., SAVIN, R. & SLAFER, G.A. (1999). Effect of temperature and carpel size during pre-anthesis on potential grain weight in wheat. *Journal of Agricultural Science*. **132**, 453-459.

CALDERINI, D.F., REYNOLDS, M.P. & SLAFER, G.A. (2006). Source–sink effects on grain weight of bread wheat, durum wheat, and triticale at different locations. *Australia Journal of Agricultural Research*. **57**, 227–233.

CAMPBELL, C.A., MYERS, R.J.K. & CURTN, D. (1995). Managing nitrogen for sustainable crop production. *Fertiliser Research*. **42**, 277-296.

CAMPBELL, J. E. LOBELL, D. B. GENOVA, R. C. & FIELD, C. B. (2008). The Global Potential of Bioenergy on Abandoned Agriculture Lands. *Environmental Science & Technology*. **42**, 5791-5794.

CARBERRY, P.S., HAMMER, G.L. & MUCHOW, R.C. (1993). Modelling genotypic and environmental control of leaf dynamics in grain sorghum. III. Senescence and prediction of green leaf area. *Field Crops Research.* **33**, 329-351.

CARVALHO, PM. (2009). Optimising root growth to improve uptake and utilization of water and nitrogen in wheat and barley. PhD thesis, The University of Nottingham

CASSMAN, K. G., BRYANT, D. C., FULTON, A. E., & JACKSON, L. F. (1992). Nitrogen Supply Effects on Partitioning of Dry Matter and Nitrogen to Grain of Irrigated Wheat. *Crop Science*. **32**, 1251-1258.

CASTAGNA, R., MINOIA, C., PORFIRI, O. & ROCCHETI, G. (1996). Nitrogen Level and Seeding Rate Effects on the Performance of Hulled Wheats (*Triticum monococcum* L., *T. dicoccum* Schubler and *T. spelta* L.) Evaluated in Contrasting Agronomic Environments. *Journal of Agronomy and Crop Science.* **176**, 173-181.

CHAPMAN, S.C., MATHEWS, K.L., TRETHOWAN, R.M. & SINGH, R.P. (2007). Relationships between heights and yield in near-isogenic spring wheats that contrast for major reduced height genes. *Euphytica*. **157**, 391-397.

CHAMORRO, A.M., TAMAGNO, L.N., BEZUS, R. & SARANDON, S.J. (2002). Nitrogen accumulation, partition, and nitrogen-use efficiency in canola under different nitrogen availabilities. *Communications in Soil Science and Plant Analysis*. **33**, 493-504.

CHOLICK, F.A., WELSH, J.R. & COLE, V.C. (1977). Rooting pattern of semi-dwarf and tall winter wheat cultivars under dryland field conditions. *Crop Science*. 17, 637-639.

CHRISTOPHER, J.T., MASCHADI, A.M., HAMMER, G.L. & BORRELL, A.K. (2008). Developmental and physiological traits associated with high yield and stay-green phenotype in wheat. *Australian Journal of Agricultural Research.* **59**, 354-364.

CHRISTOPHER, J., CHRISTOPHER, M., JENNINGS, R., JONES, S., FLETCHER, S., BORRELL, A., MANSCHADI, A.M., JORDAN, D., MACE, E. & HAMMER, G. (2013). QTL for root angle and number in a population developed from bread wheats (*Triticum aestivum*) with contrasting adaptation to water-limited environments. *Theoretical and Applied Genetics.* **126**, 1563–1574.

CHRISTOPHER, J.T., VEYRADIER, M., BORREL, A.K., HARVEY, S.F. & CHENU, K. (2014). Phenotyping novel stay-green traits to capture genetic variation in senescence dynamics. *Functionl Plant Biology*. **41**, 1035-1048.

CHURCHILL, G. & DOERGE, R. (1994). Empirical threshold values for quantitative trait mapping. *Genetics.* 1**38**, 963-971.

CIMMYT (2011). Wheat: Global alliance for improving food security and the livelihoods of the resource-poor in the developing world. Proposal submitted by CIMMYT and ICARDA to the CGIAR consortium board.

CLARKE, S., SYLVESTER-BRADLEY, R., FOULKES, J., GINSBURG, D., GAJU, O., WERNER, P., JACK, P., FLATMAN, E. & SMITH-REEVE, L. (2012). Adapting wheat to global warming or 'ERYCC'- Earliness and Resilience for Yield in a Changing Climate. HGCA Project Report No. 496.

COQUE, M. & GALLAIS A. (2006). Genomic regions involved in response to grain yield selection at high and low nitrogen fertilization in maize. *Theoretical and Applied Genetics*. **112**, 1205–1220.

COQUE, M., MARTIN, A., VEYRIERAS, J.B., HIREL, B. & GALLAIS A. (2008). Genetic variation for N-remobilization and postsilking N-uptake in a set of maize recombinant inbred lines, 3. QTL detection and coincidences. *Theoretical and Applied Genetics*. **117**, 729-747.

COX, M.C., QUALSET, C.O. & RAINS, D.W. (1985). Genetic variation for nitrogen assimilation and translocation in wheat. 1. Dry matter and nitrogen accumulation. *Crop Science*. **25**, 430-435.

CRITCHLEY, C.S. (2001) A physiological explanation for the canopy nitrogen requirement of winter wheat. Ph.D. Thesis. University of Nottingham, UK.

CURTIS, B.C. (2002). Wheat in the world. In: Bread Wheat: Improvement and Production, pp. 1-17 (Eds. Curtis, B.E., Rajaram, S. and Gomez Macpherson, H.), FAO, Rome, Italy.

DAMANIA, A.B., HAKIM, S. & MOUALLA, M.Y. (1992). Evaluation of variation in T. dicoccum for wheat improvement in stress environment. *Hereditas*. **116**, 163–166.

DAMANIA, A.B. (1998). Domestication of cereal crop plant and in situ conservation of their genetic resources in the Fertile Crescent. In: Damania AB, Valkoun J, Willcox G, Qualset CO (eds) The origin of agriculture and crop domestication. ICARDA, Aleppo, pp 307–316.

DAWSON, C.J. & HILTON, J. (2011). Fertiliser availability in a resource-limited world: Production and recycling of nitrogen and phosphorus. *Food Policy*. **36**, S14-S22.

DEPARTMENT FOR ENVIRONMENT, FOOD & RURAL AFFAIRS (2012). The British Survey of Fertiliser Practice: Fertiliser use on farm crops for crop year 2011. Crop Statistics.

DERKE, A.P. (2013). Improving nitrogen used and yield with stay-green phenotypes in wheat. PhD thesis, University of Nottingham, UK.

DEVIENNE-BARRET, F., RICHARD-MOLARD, C., CHELLE, M., MAURY, O. & NEY, B. (2006). Ara-Rhizotron: an effective culture system to study simultaneously root and shoot development of Arabidopsis. *Plant and Soil: An International Journal on Plant-Soil Relationships.* **280**, 253-266.

DHUGGA, K.S., & J.G. WAINES. (1989). Analysis of nitrogen accumulation and use in bread and durum wheat. *Crop Science*. **29**, 1232-1239.

DODD, I.C, WHALLEY, W.R., OBER, E.S. & PARRY, M.A.J. (2011). Genetic and management approaches to boost UK wheat yields by ameliorating water deficits. *Journal of Experimental Botany*. **62**, 5241-5248.

DRECCER, M.F., GRASHOFF, C. & RABBINGE, R. (1997). Source-sink ratio in barley (*Hordeum vulgare* L.) during grain filling: effects on senescence and grain protein concentration. *Field Crops Research.* **49**, 269-277.

DRECCER, M.F. (2005). Nitrogen use at the leaf and canopy level: a framework to improve NUE. *Journal of Crop Improvement.* **15**, 97-128.

DUNBABIN, V., DIGGLE, A. & RENGEL Z. (2003). Is there an optimal root architecture for nitrate capture in leaching environments? *Plant Cell Environ*. **26**, 835-844.

DUNBABIN, V., RENGEL, Z. & DIGGLE, A. J. (2004). Simulating form and function of root systems: efficiency of nitrate uptake is dependent on root system architecture and the spatial and temporal variability of nitrate supply. *Functional Ecology*, **18**, 204-211.

DVORAK, J., DITERLIZZI, P., ZHANG, H.B. & RESTA, P. (1993). The evolution of polyploidy wheats: identification of the A genome donor species. *Genome.* **36**, 21-31.

DVORAK, J., LUO, M.C. & YANG, Z.L. (1998). Genetic evidence on the origin of Triticum aestivum L. In: Damania AB, Valkoun J, Willcox G, Qualset CO (eds) The origins of agriculture and crop domestication. Proceedings of the Harlan symposium. ICARDA, Aleppo, pp 235-251

DVORAK, J. & AKHUNOV, E. (2005). Tempos of gene locus deletions and duplications and their relationship to recombination rate during diploid and polyploid evolution in the Aegilops-Triticum alliance. *Genetics.* **17**, 323-332.

DVORAK, J., AKHUNOV, E.D., AKHUNOV, A.R., DEAL, K.R. & LUO, M.C. (2006). Molecular characterization of a diagnostic DNA marker for domesticated tetraploid wheat provides evidence for gene flow from wild tetraploid wheat to hexaploid wheat. *Molecular Biology and Evolution*. **23**, 1386-96.

DVORAK, J., LUO, M.C. & AKHUNOV, E.D. (2011). N.I. Vavilov's theory of centers of diversity in light of current understanding of wheat diversity, domestication and evolution. *Czech Journal of Genetics and Plant Breeding.* **47**, S20-S27.

EARL, C.D. & AUSUBEL, F.M. (1983). The genetic engineering of nitrogen fixation. *Nutrition reviews*. **41**, 1-6.

EASTERLING, W., AGGERWAL, P., BATIMA, P., BRANDER, K., ERDA, L., HOWDEN, S., KIRILENKO, A., MORTON, J., SOUSSANA, J.F. & SCHMIDHUBER, J. (2007). Food fibre and forest products. In: Parry M, Canziani O, Palutikof J, Linden PVD, Hanson C, eds. Climate change 2007: impacts, adaptation and vulnerability. Contribution of Working Group II to the 4th Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, UK: Cambridge University Press, 273–313.

ECHARTE, L., ROTHSTEIN, S. & TOLLENAAR, M. (2008). The response of leaf photosynthesis and dry matter accumulation to nitrogen supply in an older and a newer maize hybrid. *Crop Science*. **48**, 656-665.

EHDAIE, B., HALL, A.E., FARQUHAR, G.D., NGUYEN, H.T. & WAINES, J.G. (1991). Water-use efficiency and carbon isotope discrimination in wheat. *Crop Science*. **31**, 1282-1288.

EHDAIE, B., SHAKIBA, M. R. & WAINES, J. G. (2001). Sowing date and nitrogen input influence nitrogen-use efficiency in spring bread wheat and durum wheat genotypes. *Journal of plant Nutrition*. **24**, 899–919.

EHDAIE, B., MERHAUT, D.J., AHMADIAN, S., HOOPS, A.C., KHUONG, T., LAYNE, A.P. & WAINES, J.G. (2010). Root system size influences Water-Nutrient uptake and Nitrate leaching potential in wheat. *J Agro* Crop Science. **196**, 455-466.

EISSENSTAT, D.M. (1992). Costs and benefits of constructing roots of small diameter. *Journal of plant Nutrition.* **15**, 763-782.

EGLE, K., MANSKE, G., R¨OMER, W. & VLEK, P.L.G. (1999). Improved phosphorus efficiency of three new wheat genotypes from CIMMYT in comparison with an older Mexican variety. *Journal of Plant Nutrition and Soil Science.* **162**, 353–358.

EPSTEIN, E. & BLOOM, A. (2005). Mineral nutrition of plants: principles and perspectives, 2nd edn. Sunderland, MA: Sinauer Associates, Inc.

ERCOLI, L., LULLI, L., MARIOTTI, M., MASONI, A. & ARDUINI, I. (2008). Post-anthesis dry matter and nitrogen dynamics in durum wheat as affected by nitrogen supply and soil water availability. *European Journal of Agronomy*. **28**, 138-147.

EVANS, L.T. (1998). Feeding the Ten Billion: Plants and Population Growth. Cambridge University Press, Cambridge, UK.

EVENSON, R.E. & GOLLIN, D. (2003). Assessing the impact of the Green Revolution 1960 to 2000. *Science*. **300**, 758-762.

FAOSTAT (2015). Food and Agricultural Organization of the United Nations. 1961-2012. FAO: Rome.

FELDMAN, M. (2001). The world wheat book A history of wheat Breeding, *Eds* Bonjean, AP. And Angus, W.J, Tec. & Doc. Editions, London.

FISCHER, R.A. (1981). Optimizing the use of water and nitrogen through breeding of crops. *Plant and Soil*. **58,** 249-278.

FOULKES, M.J., SYLVESTER-BRADLEY, R. & SCOTT, R.K. (1998). Evidence for differences between winter wheat cultivars in acquisition of soil mineral nitrogen and uptake and utilization of applied fertilizer nitrogen. *Journal of Agricultural Science.* **130**, 29–44.

FOULKES, M. J., SCOTT, R. K. & SYLVESTER-BRADLEY, R. (2002). The ability of wheat cultivars to withstand drought in UK conditions: formation of grain yield. *Journal of Agricultural Science*. **138**, 153-169.

FOULKES, M.J., SYLVESTER-BRADLEY, R., WEIGHTMAN, R. & SNAPE, J.W. (2007). Identifying physiological traits associated with improved drought resistance in winter wheat. *Field Crops Research*, **103**, 11-24.

FOULKES, M.J., HAWKESFORD, M.J., BARRACLOUGH, P.B., HOLDSWORTH, M.J., KERR, S., KIGHTLEY, S. & SHEWRY, P.R. (2009). Identifying traits to improve the nitrogen economy of wheat: recent advances and future prospects. *Field Crops Research*, **114**, 329-342.

FU, J. & LEE, B. (2008). Changes in photosynthetic characteristics during grain filling of a functional stay-green rice SNU-SG1 and its F1 hybrids. *Journal of Crop Science and Biotechnology.* **11**, 75-82.

GAHOONIA, T.S., ALI, R., MALHOTRA, S.R., JAHOOR, A. & RAHMAN, M.M. (2007). Variation in root morphological and physiological traits and nutrient uptake of chickpea genotypes. *Journal of Plant Nutrition*, **30**, 829-841.

GAHUKAR, R. T. (2009). Food security: The challenges of climate change and bioenergy. *Current Science.* **96**, 26-28.

GAJU, O., ALLARD, V., MARTRE, P., SNAPE, J.W., HEUMEZ, E., LE GOUIS, J., MOREAU, D., BOGARD, M., GRIFITHS, S., ORFORD, S., HUBBART, S. & FOULKES, M.J. (2011). Identification of traits to improve the nitrogen use efficiency of wheat genotypes. *Field Crops Research*. **152**, 123-139.

GALE, M.R. & GRIGAL, D.F. (1987.). Vertical root distributions of northern tree species in relation to successional status. *Canadian Journal Forest Research.* **17**, 829-834.

GALLAIS, A. & COQUE, M. (2005). Genetic variation and selection for nitrogen use efficiency in maize: a synthesis. *Maydica*. **50**, 531–537.

GALLOWAY, J.N., SCHLESINGER, W.H., LEVY, I.I.V., MICHAELS, A. & SCHNOOR, J.L. (1995). Nitrogen fixation: anthropogenic enhancement environmental response. *Global Biogeochemical Cycles*. **9**, 235-252.

GALLOWAY, J.N. & COWLING, E.B. (2002). Reactive nitrogen and the world: 200 years of change. *Ambio*. **31**, 64-71.

GAN, S. & AMASINO, R.M. (1997). Making sense of senescence. *Plant Physiology*. **113**, 313-319.

GARNETT, T., VANESSA, C. & KAISER, B.N. (2009). Root based approaches to improving nitrogen use efficiency in plants. *Plant, Cell & Environment.* **32**, 1272-1283.

GASTAL, F. & LEMAIRE, G. (2002). N uptake and distribution in crops: an agronomical and ecophysiological perspective. *Journal of Experimental Botany*. **53**, 789-799.

GEBBING, T. & SCHNYDER, H. (1999). Pre-anthesis reserve utilization for protein and carbohydrate synthesis in grains of wheat. *Plant Physiology*. **121**, 871-878.

GE´NARD, M., REICH, M., LOBIT, P. & BESSET, J. (1999). Correlations between sugar and acid content and peach growth. *Journal of Horticultural Science and Biotechnology*. **74**, 772–776.

GHOLIZADEH, A., AMIN, A.S.M, ANUAR, A. R. & AIMRUN, W. (2009). Evaluation of Leaf Total Nitrogen Content for Nitrogen Management in a Malaysian Paddy Field by Using Soil Plant Analysis Development Chlorophyll Meter. *American Journal of Agricultural and Biological Sciences*. **4**, 278-282.

GILL, B.S., LI, W., SOOD, S., KURAPARTHY, V., FRIEBE SIMONS, K.J., ZHANG, Z. & FARIS, J.D. (2007). Genetics and genomics of wheat domestication-driven evolution. *Israel journal of plant Science*. **55**, 223-229.

GIUNTA, F., MOTZO, R. & PRUNEDDU, G. (2007). Trends since 1900 in the yield potential of Italian bread wheat cultivars. *European Journal of Agronomy*. **27**, 12-24.

GOLENBERG E.M. (1987). Estimation of gene flow and genetic neighbourhood size by indirect methods in selfing annual, *Triticum dicoccoides*. Evolution 41, 1326–1334.

GOODING, M.J., DIMMOCK, J.P.R.E., FRANCE, J. and JONES, S.A. (2000) Green leaf area decline of wheat flag leaves: the influence of fungicides and relationships with mean grain weight and grain yield. *Annals of Applied Biology*. **136**, 77-84.

GOPHER, A., ABBO, S. & LEV-YADUN, S. (2002). The 'when', the 'where', and the 'why' of the Neolithic revolution in the Levant. *Documenta Praehistorica.* **28**, 49-62.

GONG, Y.H., ZHANG, J., GAO, J.F., LU, J.Y. & WANG, J.R. (2005). Slow Export of Photoassimilate from Stay-green Leaves during Late Grain-Filling Stage in Hybrid Winter Wheat (*Triticum aestivum* L.). *Journal of Agronomy and Crop Science.***191**, 292-299.

GREGERSEN, P.L., HOLM, P.B. & KRUPINSKA, K. (2008). Leaf senescence and nutrient remobilization in barley and wheat. *Plant Biology*. **10**: 37-49.

GREGORY, P. J. MCGOWAN, M. BISCOE, P. V. & HUNTER, B. (1978). Water relations of winter wheat .1. Growth of root system. *Journal of Agricultural Science.* **91**, 91-102.

GREGORY, P.J, MARSHALL, B. & BISCOE, P.V. (1981). Nutrient relations of winter wheat. 3. Nitrogen uptake, photosynthesis of flag leaves and translocation of nitrogen to grain. *Journal of Agricultural Science*. **96**, 539-547.

GREGORY, P. J. & BROWN, S. C. (1989). Root growth, water use and yield of crops in dry environments: what characteristics are desirable? *Aspects of Applied Biology*. **22**, 235-243.

GRINDLAY, D.J.C. (1997). Towards an explanation of crop nitrogen demand based on the optimization of leaf nitrogen per unit leaf area. *Journal of Agricultural Science*. **128**, 377-396

GUIBOILEAU, A., SORMANI, R., MEYER, C. & MASCLAUX-DAUBRESSE, C. (2010). Senescence and death of plant organs: nutrient recycling and developmental regulation. *Comptes Rendus Biologies.* **333**, 382-391.

GUINGO, E., HERBERT, Y. & CHARCOSSET, A. (1998). Genetic analysis of root traits in maize. *Agronomie.* **18**, 225-235.

HACKETT, C. (1969). A study of the root system of barley II. Relationships between root dimensions and nutrient uptake. *New phytologist.* **68**, 1023-1030.

HALLORAN, G.M. & LEE, J.W. (1979). Plant nitrogen distribution in wheat cultivars. *Australian Journal of Agricultural Research*. **30**, 779-789.

HARLAN, J.R. & ZOHARY, D. (1966). Distribution of wild wheat and barley. *Science*. **153**, 1074-1080.

HARRIS, K., SUBUDHI, P., BORRELL, A., JORDAN, D., ROSENOW, D., NGUYEN, H., KLEIN, P., R. & MULLET, J. (2007). Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. *Journal of Experimental Botany*. **58**, 327-338.

HARTEMINK, A. E., JOHNSTON, M., O'SULLIVAN, J., & POLOMA, S. (2000). Nitrogen use efficiency of taro and sweet potato in the humid lowlands of Papua New Guinea. *Agriculture, Ecosystems & Environment*. **79**, 271-280.

HGCA (Home Grown Cereal Authority). (2008). The wheat growth guide. Second edition.

HEFFER, P. (2013). Assessment of fertilizer use by crop at global level 2010- 2010/2011, International Fertiliser Industry Association.

HEFFER, P. & PRUD'HOMME, M. (2014). Fertiliser Outlook. 82nd Annual Conference, Sydney, Australia, 26-28 May, International Fertiliser Industry Association.

HINZMAN, L.D., BAUER, M.E. & DAUGHTRY, C.C.T (1986). Effects of nitrogen fertilization on growth and reflectance characteristics of winter wheat. *Remote Sensing of Environment*. **19**, 47-61.

HIREL, B., LE- GOUIS, J., NEY, B. & GALLAIS A. (2007). The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *Journal of Experimental Botany.* **58**, 2369-2387.

HOAD, S.P., RUSSELL, G., KETTLEWELL, P.S. & BELSHAW, M. (2004). Root system management in winter wheat: practices to increase water and nitrogen use. Home Grown Cereals Authority (HGCA) Project Report No. 351.

HOEL, B.O. & SOLHAUG, K.A. (1998). Effect of Irradiance on Chlorophyll Estimation with the Minolta SPAD-502 Leaf Chlorophyll Meter. *Annals of Botany*. **82**, 389-392.

HUANG, S., SIRIKHACHORNKIT, A., SU, X., FARIS, J., GILL, B., HASELKORN, R. & GORNICKI, P. (2002). Genes encoding plastid acetyl-CoA carboxylase and 3-phosphoglycerate kinase of the *Triticum/Aegilops* complex and the evolutionary history of polyploid wheat. *Proceedings of the National Academy of Science of the United States*. **99**, 8133-8138.

HUBERT, B., ROSEGRANT, M., VAN BOEKEL, M.A.J.S. & ORTIZ, R. (2010). The future of food: scenarios for 2050. *Crop Science*. **50**, 33-50.

HUND, A., FRACHEBOUD, Y., SOLDATI, A. & STAMP, P. (2008). Cold tolerance of maize seedlings as determined by root morphology and photosynthetic traits. *European Journal of Agronomy*. **28**, 178-185.

HUND, A., TRACHSEL, S. & STAMP, P. (2009). Growth of axile and lateral roots of maize: I development of a phenotyping platform. *Plant and Soil*. **325**, 335-349.

INTERNATIONAL FERTILISER INDUSTRY ASSOCIATION (IFA) database. (2015)

JENKINS, G.J., PERRY, M.C. & PRIOR, M.J. (2008). The climate of the United Kingdom and recent trends. Met Office Hadley Centre, Exeter, UK.

JIANG, G.H., HE, Y.Q., XU, C.G., LI, X.H. & ZHANG, Q. (2004). The genetic basis of stay-green in rice analysed in a population of doubled haploid lines derived from an *indica* by *japonica* cross. *Theoretical and Applied Genetics*. **108**, 688-698.

KAMARA, A.Y, KLING, J.G, MENKIR, A. & IBIKUNLE, G. (2003). Agronomic performance of maize (*Zea mays* L.) breeding lines derived from a low nitrogen maize population. *Journal of Agricultural Science.* **141**, 221-230.

KARADAVUT, U., PALTA, C., OKUR O. & KAYIS, S.A. (2008). A growth curve application to compare plant heights and dry weights of some wheat varieties. *American-Eurasian Journal of Agricultural and Environmental Sciences.* **3**, 888-892.

KATO, Y., ABE, J., KAMOSHITA, A. & YAMAGISHI, J. (2006). Genotypic variation in root growth angle in rice (*Oryza sativa* L.) and its association with deep root development in upland fields with different water regimes. *Plant and Soil*. **287**,117-129.

KATSANTONIS, D., NTANOS, D.A., KOUTROUBAS S.D. & LUPOTTO, E. (2008). Effect of blast disease on nitrogen accumulation and remobilization to rice grain. *Journal of Plant Pathology*. **90**, 263-272.

KICHEY, T., HIREL, B., HEUMEZ, E., DUBOIS, F. & LEGOUIS, J. (2007). In winter wheat (*Triticum aestivum* L.), post-anthesis nitrogen uptake and remobilisation to the grain correlates with agronomic traits and nitrogen physiological markers. *Field Crops Research*. **102**, 22–32.

KILIAN, B., ÖZKAN, H., WALTHER, A., KOHL, J., DAGAN, T., SALAMINI, F. & MARTIN, W. (2007). Molecular diversity at 18 loci in 321 wild and 92 domesticated lines reveal no reduction of nucleotide diversity during *Triticum monococcum* (einkorn) domestication: Implication for the origin of agriculture. *Molecular Biology and Evolution*. **24**, 2657-2668.

KING, J., GAY, A., SYLVESTER-BRADLEY, R., BINGHAM, I., FOULKES, J., GREGORY, P. & ROBINSON, D. (2003). Modelling cereal root systems for water and nitrogen capture: Towards an economic optimum. *Annals of Botany*. **91**, 383-393.

KLEPPER, B. (1992). Development and growth of crop root systems. In: Hatfield JL, Stewart BA (eds) Limitations to plant root growth. Adv Soil Sci 19, pp 1-25.

KONDO, M., PABLICO, P.P., ARAGONES, D.V., AGBISIT, R., MORITA, S. & COURTOIS, B. (2003). Genotypic and environmental variations in root morphology in rice genotypes under upland field conditions. *Plant and Soil*. **255**, 189-200.

KRAISER, T., GRAS, D.E., GUTIERREZ, A.G., GONZALEZ, B. & GUTIERREZ, R.A. (2011). A holistic view of nitrogen acquisition in plants. *Journal of Experimental Botany*. **62**, 1455-1466.

KRAMER, P. J. & BOYER, J. S. (1995). Water relations of plants and soils. San Diego: Academic Press.

LADHA, J.K., PATHAK, H., KRUPNIK, T.J., SIX, J. & VAN KESSEL, C. (2005). Efficiency of fertiliser nitrogen in cereal production: Retrospects and prospects. *Advances in Agronomy*. **87**, 85-156.

LAGHETTI, G., PIERGIOVANNI, A.R., VOLPE, N. & PERRINO, P. (1999). Agronomic performance of *Triticum dicoccon* (Shrank) and *T. spelta* (L.) accessions under Southern Italy conditions. *Agricoltura Mediterranea*. **129**, 199-211.

LAPERCHE, A., DEVIENNE-BARET, F., MAURY, O., LE- GOUIS, J. & NEY, B. (2007). A simplified conceptual model of carbon and nitrogen functioning for QTL analysis of winter wheat adaptation to nitrogen deficiency. *Theoretical and Applied Genetics*. **113**, 1131-1146.

LE- GOUIS, J., BÉGHIN, D., HEUMEZ, E. & PLUCHARD, P. (2000). Genetic differences for nitrogen uptake and nitrogen utilisation efficiencies in winter wheat. *European Journal of Agronomy*. **12**, 163-173.

LEV-YADUN, S., GOPHER, A. & ABBO, S. (2000). The cradle of agriculture. *Science*. **288**, 1602-1603.

LIAO, M.T., FILLERY, I.R.P. & PALTA, J.A. (2004). Early vigorous growth is a major factor influencing nitrogen uptake in wheat. *Functional Plant Biology*. **31**, 121-129.

LIAO, M., PALTA, J.A. & FILLERY, I.R.P. (2006). Root characteristics of vigorous wheat improve early nitrogen uptake. *Australian Journal of Agricultural Research*. **57**, 1097-1107.

LIM, P.O., KIM, H.J. & NAM, H.G. (2007). Leaf senescence. *Annual Review of Plant Biology*. **58**, 115-136.

LIU, Y.G., MORI, N. & TSUNEWAKI, K. (1990). Restriction fragment length polymorphism (RFLP) analysis in wheat. I. Genomic DNA library construction and RFLP analysis in common wheat. *Idengaku Zasshi*. **65**, 367-380.

LIU, X.M., SMITH, C.M., FRIEBE, B.R. & GILL, B.S. (2005). Molecular mapping and allelic relationships of Russian wheat aphid– resistance genes. *Crop Science*. **45**, 2273-2280.

LIU, J., LI, J., CHEN, F., ZHAN, F., REN, T., ZHUANG, Z. & MI, G. (2009). Mapping QTLs for root traits under different nitrate levels at the seedling stage in maize (*Zea mays* L.). *Plant Soil*. **305**, 253-265.

LOPES, M.S. & REYNOLDS, M.P. (2012). Stay-green in spring wheat can be determined by spectral reflectance measurements (normalized difference vegetation index) independently from phenology. *Journal of Experimental Botany*. **63**, 3789-3798.

LUCAS, M.E., HOAD, S.P., RUSSELL, G. & BINGHAM, I.J. (2000). Management of cereal root systems. HGCA Research Review 43. London: Home Grown Cereals Authority.

LUPTON, F.G.H. (1972). Further experiments on photosynthesis and translocation in wheat. *Annals of Applied Biology*. **71**, 69-71.

LYNCH, J. & VAN BEEM, J.J. (1993). Growth and architecture of seedling roots of common bean genotypes. Crop Science. **33**, 1253-1257.

LYNCH, J.P. (2007). Roots of the second green revolution. *Australian Journal of Botany*. **55**, 493-512.

LYNCH, J.P. (2013). Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. *Annals of Botany.* **112**, 347-357.

MACE, E.S., SINGH, V., VAN OOSTEROM E.J., HAMMER, G.L., HUNT, C.H. & JORDAN, D.R. (2012). QTLs for nodal root angle in sorghum (Sorghum bicolour L. Moench) co-locate with QTL for traits associated with drought adaptation. *Theoretical and Applied Genetics.* **124**, 97-109.

MACKEY, J. (1973). The wheat root. In: Sears ER, Sears LMS, editors. Proceedings 4th International Wheat Genetics Symposium. Columbia, Missouri, USA; 197, 827-842.

MAIZLICH, N.A, FRITTON, D.D. & KENDALL, W.A. (1980). Root morphology and early development of maize at varying levels of nitrogen. *Agronomy Journal.* **72**, 25-31.

MANSCHADI, A.M., CHRISTOPHER, J., deVOIL, P. & HAMMER, G. (2006). The role of root architectural traits in adaptation of wheat to water-limited environments. *Functional Plant Biology*. **33**, 823-837.

MANSCHADI, A.M., HAMMER, G.L., CHRISTOPHER, J.T. & deVOIL, P. (2008). Genotypic variation in seedling root architectural traits and implications for drought adaptation in wheat (*Triticum aestivum* L.). *Plant and Soil*. **303**, 115-129.

MANSKE, G.G.B. & VLEK, P.L.G. (2010). Root architecture- wheat as a model plant. In: Waisel, Y., Eshel, A., Kafkafi, U. (eds) Plant roots: the hidden half. Dekker, New York, PP 249-259.

MARIANI, B.M., D'EGIDIO, M.G. & NOVARO, P. (1995). Durum Wheat quality evaluation: influence of genotype and environment. *Cereal Chemistry*. **72**, 194-197.

MARINO, S., TOGNETTI, R. & ALVINO, A. (2009). Crop yield and grain quality of emmer populations grown in central Italy, as affected by nitrogen fertilization. *European Journal of Agronomy*. **31**, 233-240.

MARSCHNER, H. (1995). Mineral nutrition of higher plants. London: Academic Press, 889 pp.

MARTRE, P., PORTER, J.R., JAMIESON, P.D. & TRIBOÏ, E. (2003). Modelling grain nitrogen accumulation and protein composition to understand the sink/source regulations of nitrogen remobilization for wheat. *Plant Physiology*. **133**, 1959-1967.

MARTRE, P., SEMENOV, M.A. & JAMIESON, P.D. (2007). Simulation analysis of Physiological traits to improve yield, nitrogen use efficiency and grain protein concentration in wheat. *In* J.H.J. Spiertz, P.C. Struik and H.H. van Laar (eds.), Scale and Complexity in Plant Systems Research: Gene-Plant-Crop Relations, 181-201.

MARQUARD, R.D. & TIPTON, J.L. (1987). Relationship between extractable chlorophyll and an in situ method to estimate leaf greenness. *Hortscience.* **22**, 1327.

MATSUOKA, Y. & NASUDA, S. (2004). Durum wheat as a candidate for the unknown female progenitor of bread wheat: an empirical study with a high fertile F1 hybrid with *Aegilops tauschii* Coss. *Theoretical and Applied Genetics.* **109**, 1710-1717.

MAY, L., VAN SANFORD, D.A., MACKOWN, C.T. & CORNELIUS, P.L. (1991). Genetic variation for nitrogen use in soft red x hard red wheat populations. *Crop Science*. **31**, 626-630.

McCAIG, T.N. & MORGAN, J.A. (1993). Root and shoot dry matter partitioning in near-isogenic wheat lines differing in height. *Canadian Journal of Plant Science.* **73**, 679-689.

McINTOSH, R.A., YAMAZAKI, Y., DUBCOVSKY, J., ROGERS, J., MORRIS, C., SOMERS, D.J., APPELS, R. & DEVOS, K.M. (2008). Catalogue of gene symbols of wheat. 11th International wheat genetics symposium. 24-29 August 2008, Australia.

MESSMER, M.M., KELLER, M., ZANETTI, S. & KELLER, B. (1999). Genetic linkage map of a wheat x spelt cross. *Theoretical Applied Genetics*. **98**, 1163-1170.

MIAN, M.A.R., NAFZIGER, E. D., KOLB, F. L. & TEYKER, R. H. (1993). Root growth of wheat genotypes in hydroponic culture and in greenhouse under different moisture regimes. *Crop Science*. **33**, 283-286.

MI, G., TANG, L., ZHANG, F. & ZHANG, J. (2000). Is nitrogen uptake after anthesis in wheat regulated by sink size? *Field Crops Research.* **68**, 183-190.

MINOLTA (1989). Chlorophyll meter SPAD-502. Instruction manual. Minolta Co., Ltd., Radiometric Instruments Operations, Osaka, Japan

MIRALLES, D.J. & SLAFER, G.A. (1997). Radiation interception and radiation use efficiency of near-isogenic wheat lines with different height. *Euphytica*. 97, 201-208.

MIRALLES, D.J., SLAFER, G.A. & LYNCH, V. (1997). Rooting patterns in near-isogenic lines of spring wheat for dwarfism. *Plant and soil.* **197**, 79-86.

MIRALLES, D.J. & SLAFER, G.A. (2007). Sink limitations to yield in wheat: How could it be reduced? *Journal of Agricultural Science.* **145**, 139-149.

MOLL, R.H., KAMPRATH, E.J. & JACKSON, W.A. (1982). Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. *Agronomy Journal*. **74**, 562-564.

MOORE, A.M.T., HILLMAN, G.C. & LEGGE, A.J. (2000). Village on the Euphrates, from Foraging to farming at Abu Hureyra, Oxford University Press, Oxford.

MONJE, O.A. & BUGBEE, B. (1992). Inherent limitations of non-destructive chlorophyll meters – a comparison of 2 types of meters. *Hortscience.* **27**, 69-71.

MORTIMER, N.D., ELSAYED, M.A. & HORNE, R.E. (2004). Energy and greenhouse gas emission for bioethanol production from wheat grain and sugar beet. York, UK: NNFC: Report for British Sugar. No 23/1.

MOUDRY, J. & DVORACEK, V. (1999). Chemical composition of grain of different spelt varieties (*Triticum spelta* L.). *Rostlinna Vyroba.* **45**, 533-538.

MOUDRY, J., KONVALINA, P., STEHNO, Z., CAPOUCHOVA, I. & MOUDRY, J. (JR.). (2011). Ancient Wheat Species Can Extend Biodiversity of Cultivated Crops. *Scientific Research and Essay.* 6, 4273-4280.

MUJEEB-KAZI, A., SKOVMAND, B., HENRY, M., DELGADO, R. & CANO, S. (2000). New synthetic hexaploids (*Triticum dicoccum/ Aegilops tauschii*): Their production, cytology, and utilization as a source for Russian Wheat Aphid Resistance. *Annual Wheat Newsletter*. **46**, 79-81.

MURASE, K., HIRANO, Y., SUN, T.P. & AKOSHIMA, T. (2008). Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. *Nature.* **456**, 459-463.

MURCHIE, E., PINTO, M. & HORTON, P. (2009). Agriculture and the new challenges for photosynthesis research. *New Phytologist.* **181**, 532-552.

MUURINEN, S., SLAFER, G.A. & PELTONEN-SAINIO, P. (2006). Breeding effects on nitrogen use efficiency of spring cereals under northern conditions. *Crop Science*. **46**, 561-568.

NAGEL K.A., KASTENHOLZ, B., JAHNKE, S., VAN DUSSCHOTEN, D., MÜHLICH, M., TRUHN, D., SCHARR, H., TERJUNG, S., WALTER, A. & SCHARR, U. (2009). Temperature responses of roots: Impact on growth, root system architecture and implications for phenotyping. *Functional Plant Biology.* 36, 947-959.

NAKAMOTO, T. & OYANAGI, A. (1994). The direction of growth of seminal roots of *Triticum aestivum* L. and experimental modification thereof. *Annals of Botany*. **73**, 363-367.

NEETESON, J. J. (1989). Effect of reduced fertiliser nitrogen application rates on yield and nitrogen recovery of sugar beet and potato. *Netherlands Journal of Agricultural Science*. **37,** 227-236.

NESBITT, M. & SAMUEL, D. (1996). Promoting the Conservation and use of underutilised and neglected crops. In: Padulosi, S., Hammer, K., Heller, J. (eds) Hulled Wheats. International Plant Genetic Resources Institute, Rome.

NIELSEN, N.E. & SCHJØRRING, J.K. (1983). Efficiency and kinetics of phosphorus uptake from soil by various barley genotypes. *Plant Soil*. **72**, 225-230.

NORTON, G.J. & PRICE, A.H. (2009). Mapping of quantitative trait loci for seminal root morphology and gramitropic response in rice. *Euphytica.* **166**, 229-237.

OAKS, A. (1992). A re-evaluation of nitrogen assimilation in roots. *Bioscience.* **42**, 103-110.

O'BRIEN, L. (1979). Genetic variability of root growth in wheat (*Triticum aestivum* L.). *Australian Journal of Agricultural Research*. **30**, 587-595.

OLIVER, R.E., CAI, X., FRIESEN, T.L., HALLEY, S., STACK, R.W. & XU, S.S. (2008). Evaluation of Fusarium head blight resistance in tetraploid wheat (*Triticum turgidum* L.). *Crop Science*. **48**, 213-222.

OMORI, F., & MANO, Y. (2007). QTL mapping of root angle in F2 populations from maize 'B73' x teosinte '*Zea luxurians'*. *Plant Root.* 1, 57-65.

ORTIZ-MONASTERIO, J.I., SAYRE, K.D., RAJARAM, S. & MCMAHON, M. (1997). Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. *Crop Science.* **37**, 898-904.

OURY, F.X., BERARD, P., BRANCOURT-HULMEL, M., HEUMEZ, E., PLUCHARD, P., ROUSSET, M., DOUSSINAULT, G. ROLLAND, B. & TROTTET, M. (2003). Yield and grain protein concentration in bread wheat: a review and a study of multi-annual data from a French breeding program. *Journal of Genetics and Breeding.* **57**, 59-68.

OYANAGI, A. (1994). Gravitropic response growth angle and vertical distribution of roots of wheat (*Triticum aestivum* L.). *Plant Soil*. **165**, 323-326.

PALME, K., LI, X. & TEALE, W.D. (2014). Towards Second Green Revolution: Engineering nitrogen use efficiency. *Journal of Genetics and Genomics.* **41**, 315-316.

PAPONOV, F., AUFHAMMER, W., KAUL, H.P. & EHMELE, F.P. (1996). Nitrogen efficiency components of winter cereals. *European Journal of Agronomy*. **5**, 115-124.

PARMAR, A. (2014). Exploiting resource use efficiency and resilience in ancient wheat species. PhD thesis, University of Nottingham.UK.

PASK, A.J.D., SYLVESTER-BRADLEY, R., JAMIESON, P.D. & FOULKES, M.J. (2012). Quantifying how winter wheat crops accumulate and use nitrogen reserves during growth. *Field Crops Research.* **126**, 104-118.

PASK, A.J.D. (2009). Optimising nitrogen storage in wheat canopies for genetic reduction in fertiliser nitrogen inputs. PhD thesis, University of Nottingham, UK.

PASSIOURA, J.B. (1983). Root and drought resistance. *Agricultural Water Management.* **7**, 265-280.

PAVELEY, N., LOCKLEY, D., VAUGHAN, T., THOMAS, J. & SCHMIDT, K. (2000). Predicting effective fungicide doses through observation of leaf emergence. *Plant Pathology*. **49**, 748-766.

PELEG, Z., FAHIMA, T., ABBO, S., KRUGMAN, T., NEVO, E., YAKIR, D. & SARANGA, Y. (2005). Genetic diversity for drought resistance in wild emmer wheat and its ecogeographical associations. *Plant, cell and Environment.* **28**, 176-191.

PENG, J.H., SUN, D. & NEVO, E. (2011). Domestication evolution, genetics and genomics in wheat. *Molecular Breeding: New Strategies in Plant Improvement*. **28**, 281-301.

PERRINO, P., LAGHETTI, G., D'ANTUONO, L.F., AL AJLOUNI, M., KANBERTAY, M., SZABO, A.T., & HAMMER, K. (1996). Ecogeographical distribution of hulled wheat species. In: Padulosi S., Hammer, K., Heller, J (eds) Hulled Wheats. International Plant Genetic Resources Institute, Rome.

PILLINGER, C., PAVELEY, N., FOULKES, M.J. & SPINK, J. (2005). Explaining variation in the effects of take-all (*Gaeumannomyces graminis* var. tritici) on nitrogen and water uptake by winter wheat. *Plant Pathology.* **54**, 491-501.

POORTER, H. & NAGEL, O. (2000). The role of biomass allocation in the growth response of plants to different levels of light, CO2, nutrients and water: a quantitative review. *Australian Journal of Plant Physiology.* **27**, 595-607.

PORRA, R. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica Et Biophysica Acta.* 975, 384-394.

POUND, M.P., FRENCH, A.P., ATKINSON, J., WELLS, D.M., BENNETT, M.J. & PRIDMORE, T. (2013). RootNav: Navigating images of root architecture. *Plant Physiol*ogy. **162**, 1802-1814.

PRICE, A.H., STEELE, K.A., GORHAM, J., BRIDGES, J.M., MOORE, B.J., EVANS, J.L., RICHARDSON, P. & JONES, R.G.W. (2002). Upland rice grown in soil-filled chambers and exposed to contrasting water-deficit regimes: I. Root distribution, water use and plant water status. *Field Crops Research.* **76**, 11-24.

RAJCAN, I. & TOLLENAAR, M. (1999). Source-sink ratio and leaf senescence in maize. II. Nitrogen metabolism during grain filling. *Field Crops Research*. **60**, 255-265.

RAUN, W. R. & JOHNSON, G. V. (1999). Improving Nitrogen Use Efficiency for Cereal Production. *Agronomy Journal.* **91**, 357-363.

RAY DK, MUELLER ND, WEST PC, FOLEY JA. (2013). Yield trends are insufficient to double global crop production by 2050. PLoS One 8, E66428.

REN, Y., HE, X., LIU, D., LI, J., ZHAO, X., LI, B., TONG, Y., ZHANG, A. & LI, Z. (2012). Major quantitative trait loci for seminal root morphology of wheat seedlings. *Molecular Breeding.* 30, 139-148.

RODRIGUE, I.R. & MILLER, L.G. (2000). Using a Chlorophyll Meter to Determine the Chlorophyll Concentration, Nitrogen Concentration, and Visual Quality of St. Augustine grass. *Hortscience*. **35** 751-754.

ROSENGRANT, M., AGCAOILI-SOMBILLA, M. & PEREZ. N. (1995). Global food projections to 2020: implications for investment. Washington, DC, USA: IFPRI.

RUEGGER, A. & WINZELER, H. (1993). Performance of spelt (*Triticum spelta* L.) and wheat (*Triticum aestivum* L.) at two different seedling rates and nitrogen levels under contrasting environmental conditions. Journal of *Agronomy and Crop Science*. **170**, 289-295.

RYSER, P. & LAMBERS, H. (1995). Root and leaf attributes accounting for the performance of fast- and slow-growing grasses at different nutrient supply. *Plant and Soil*. **170**, 251-265.

RYSER, P. (1998). Intra- and interspecific variation in root length, root turnover and the underlying parameters. In Inherent variation in plant growth. Physiological mechanisms and ecological consequences Eds H. Lambers, H. Poorter & M. M. I. van Vuuren), Leiden: Backhuys Publishers.

SAGAN, M., NEY, B. & DUC, G. (1993). Plant symbiotic mutants as a tool to analyse nitrogen nutrition and yield relationship in field-grown peas (*Pisum sativum* L.). *Plant and Soil*. **153**, 33-45.

SALAMINI, F., ÖZKAN, H., BRANDOLINI, A., SCHÄFER-PREGL, R. & MARTIN, W. (2002). Genetics and geography of wild cereal domestication in the Near East. *Nature Reviews Genetics*. **3**, 429-441.

SAMUEL, D. (1994). An archaeological study of baking and bread in New Kingdom Egypt. PhD thesis, University of Cambridge.

SCHMID, J.E. & WINZELER, H. (1990). Genetic studies of crosses between common wheat (*Triticum aestivum* L.) and spelt (*Triticum spelta* L.). J*ournal of Genetics & Breeding*. **44**, 75-80.

SCHMID, J.E., WINZELER, M. & WINZELER, H. (1994). Analysis of disease resistance and quality characters of F1 hybrids of crosses between wheat (*Triticum aestivum*) and spelt (*Triticum spelta*). *Euphytica* **75**,105-110.

SHANAHAN, J.F., KITCHEN, N.R., RAUN, W.R. & SCHEPERS, J.S. (2008). Responsive in-season nitrogen management for cereals. *Computers and Electronics in Agriculture*. **61**, 51-62.

SHARMA, S., XU S., EHDAIE, B., CLOSE, T.J., LUKASZEWSKI, A.J. & WAINES, J.G. (2011). Dissection of QTL effects for root traits using a chromosome arm-specific mapping population in bread wheat. *Theoretical and Applied Genetics.* **122**, 759-769.

SHEARMAN, V.J., SYLVESTER-BRADLEY, R., SCOTT, R.K. & FOULKES, M.J. (2005). Physiological processes associated with wheat yield progress in the UK. *Crop Science*. **45**, 175-185.

SIDDIQUE, K.H.M., BELFORD, R.K. & TENNANT, D. (1990). Root: shoot ratios of old and modern, tall and semi-dwarf wheats in a Mediterranean environment. *Plant and Soil*. **121**, 89-98.

SIEDLER, H., MESSMER, M.M., SCHACHERMAYR, G.M, WINZELER, H., WINZELER, M. & KELLER, B. (1994). Genetic diversity in European wheat and spelt breeding material based on RFLP data. *Theoretical and Applied Genetics*. **88**, 994-1003.

SIMMONDS, N. (1995). The relation between yield and protein in cereal grain. *Journal of the Science of Food and Agriculture.* **67**, 309-315.

SINCLAIR, T.R. & AMIR, J. (1992). A model to assess nitrogen limitations on the growth and yield of spring wheat. *Field Crops Research.* **30**, 63-78.

SINGH, M., CECCARELLI, S. & HAMBLIN, J. (1993). Estimation of heritability of varietal trails data. *Theoretical and Applied Genetics*. **86**, 437-441.

SINGH, V., VAN OOSTEROM, E. J., JORDAN, D. R. & HAMMER, G. L. (2012). Genetic control of nodal root angle in sorghum and its implications on water extraction. *European Journal of Agronomy.* 42, 3-10.

SLAFER, G.A., ANDRADE, F.H. & FEINGOLD, S.E. (1990). Genetic improvement of bread wheat (Triticum aestivum L.) in Argentina: relationships between nitrogen and dry matter. *Euphytica*. **50**, 63-71.

SMITH, S. & DE SMET I. (2012). Root system architecture: insights from Arabidopsis and cereal crops. *Philos Trans R Soc*. **367**, 1441-1452.

SPANO, G., DI FONZO, N., PERROTTA, C., PLATANI, C., RONGA, G., LAWLOR, D.W., NAPIER, J.A. & SHEWRY, P.R. (2003). Physiological characterization of 'stay green' mutants in durum wheat. *Journal of Experimental Botany.* **54**, 1415-1420.

SPARKES, D.L. (2010). Are 'ancient wheat species' more adapted to hostile environments than modern bread wheat? *South African Journal of Plant and Soil.* **27**, 331-333.

SPIERTZ, J.H.J. & ELLEN, J. (1978). Effects of nitrogen on crop development and grain growth in winter wheat in relation to assimilation and utilisation of assimilates and nutrients. *Netherlands Journal of Agricultural Science*. *16*, 210-231.

SPIERTZ, J.H.J. (1977). The influence of temperature and light intensity on grain growth in relation to the carbohydrate and nitrogen economy of the wheat plant. *Netherlands Journal of Agricultural Science*. **25**, 182-197.

SPINK, J.H., SEMERE, T., SPARKES, D.L., WHALEY, J.M., FOULKES, M.J., CLARE, R.W. & SCOTT, R.K. (2000). Effect of sowing date on the optimum plant density of winter wheat. *Annals of Applied Biology.* **137**, 179-188.

STEELE, K.A, PRICE, A.H., WITCOMBE, J.R., SHRESTHA, R., SINGH, B.N., GIBBONS, J.M. & VIRK, D.S. (2013). QTLs associated with root traits increase yield in upland rice when transferred through marker-assisted selection. *Theoretical and Applied Genetics.* **126**, 101-108.

STALLKNECHT, G.F., GILBERTSON, K.M. & RANNEY, J.E. (1996). Alternative wheat cereals as food grains: einkorn, emmer, spelt, kamut, and triticale. In: Janick J (ed) Progress in new crops. ASHS Press, Alexandria, pp 156-170.

SUBBIAH, B., KATYAL, J., NARASIMHAM, R. & DAKSHINAMURTI, C. (1968). Preliminary investigations on root distribution of high yielding wheat varieties. *The International Journal of Applied Radiation and Isotopes.* 19, 385-390.

SUCHOWILSKA, E., KANDLER, W., SULYOK, M., WIWART, M. & KRSKA, R. (2009). Mycotoxins profiles in the grain of *Triticum monococcum, Triticum diccocum* and *Triticum spelta* after head infection with *Fusarium culmorum*. *Journal of the Science of Food and Agriculture*. **90**, 556-565.

SYLVESTER-BRADLEY, R., STOKES, D.T. & SCOTT, R.K. (2001). Dynamics of nitrogen capture without fertiliser: the baseline for fertilising winter wheat in the UK. *Journal of Agricultural Science.* **136**, 15-33.

SYLVESTER-BRADLEY, R. & KINDRED, D.R. (2009). Analysing nitrogen responses of cereals to prioritize routes to the improvement of nitrogen use efficiency. *Journal of Experimental Botany*. **60**, 1939-1951.

SYLVESTER-BRADLEY, R. (2010). Targets, traits and technologies to achieve sustainable productivity with arable crops in the UK. *Aspects of Applied Biology*. **105**, 107-114.

TAHIR, I.S.A. & NAKATA, N. (2005). Remobilisation of nitrogen and carbohydrate from stems of bread wheat in response to heat stress during grain filling. *Journal of Agronomy and Crop Science*. **191**, 106-115.

THOMAS, H. & SMART, C.M. (1993). Crops that stay-green. *Annals of Applied Biology*. **123**,193-201.

THOMAS, H. & HOWARTH, C.J. (2000). Five ways to stay green. *Journal of Experimental Botany*. **51**, 329-337.

THORNTON, B. & MILLARD, P. (1996). Effects of severity of defoliation on root functioning in grasses. *Journal of Range Management*. **49**, 443-447.

TOKER, C. (2004). Estimates of broad-sense heritability for seed yield and yield criteria in faba bean (*Vicia faba* L.). *Hereditas.* **140**, 222-5.

TOYOTA, M., SHIOTSU, F., BIAN, J., MOROKUMA, M. & KUSUTANI, A. (2010). Effects of reduction in plant height induced by chlormequat on radiation interception and radiation use efficiency in wheat in southwest Japan. *Plant Production Science*. **13**, 67-73.

TRÀPANI, N. & HALL, A.J. (1996). Effects of leaf position and nitrogen supply on the extension of leaves of field-grown sunflower (*Helianthus annuus* L.). *Plant and Soil*. **184**, 331-340.

TRETHOWAN, R.M. & MUJEEB-KAZI, A. (2008). Novel germplasm resources for improving environmental stress tolerance of hexaploid wheat. *Crop S*cience. **48**, 1255-1265.

TROCCOLI, A. & CODIANNI, P. (2005). Appropriate seeding rate for einkorn, emmer, and spelt grown under rainfed conditions in southern Italy. *European Journal of Agronomy*. **22**, 293-300.

TUBEROSA, R., SANGUINETI, M.C., LANDI, P., GIULIANI, M.M., SALVI, S. & CONTI, S. (2002). Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. *Plant Molecular Biology*. **48**, 697-712.

TZARFATI, R., SARANGA, Y., BARAK, V., GOPHER, A., KOROL, A. B. & ABBO, S. (2013). Threshing efficiency as an incentive for rapid domestication of emmer wheat. *Annals of Botany*. **112**, 829-37.

UAUY, C., DISTELFELD, A., FAHIMA, T., BLECHL, A. & DUBCOVSKY, J. (2006). A NAC Gene regulating senescence improves grain protein, zinc and iron content in wheat. *Science*. **314**, 1298-1301.

UDDLING, J., GELANG-ALFREDSSON, J., PIIKKI, K. & PLEIJEL, H. (2007). Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. *Photosynthesis research*. **91**, 37-46.

UGA Y, SUGIMOTO, K., OGAWA, S., RANE, J., ISHITANI, M., HARA, N., KITOMI, Y., INUKAI, Y., ONO, K., KANNO, N., INOUE, H., TAKEHISA, H., MOTOYAMA, R., NAGAMURA, Y., WU, J., MATSUMOTO, T., TAKAI, T., OKUNO, K. & YANO,

M. (2013). Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. *Nature Genetics.* **45**, 1097-1102.

UNITED NATIONS (2014). Probabilistic Population Projections based on the World Population Prospects: The 2012 Revision. Population Division, DESA. ST/ESA/SER.A/353. http://esa.un.org/unpd/ppp/

VAIDYANATHAN, L. (1984). Winter wheat yield variability. In: The Nitrogen Requirement of Cereals, pp. 69-77 (Eds. Needham, P., Archer, J.R., Sylvester Bradley, R. and Goodlass, G.), MAFF Reference book 385, HMSO, London, UK.

VAN OOIJEN JW. (2006). JoinMap® 4, software for the calculation of genetic linkage maps in experimental populations. The Netherlands: Kyazma BV.

VAN OOIJEN JW. (2009). MapQTL® 6, software for the mapping of quantitative traits loci in experimental populations of diploid species. The Netherlands: Kyazma BV.s.

VAN OOSTEROM, E., CHAPMAN, S., BORRELL, A., BROAD, I. & HAMMER, G. (2010). Functional dynamics of the nitrogen balance of sorghum. II. Grain filling period. *Field Crops Research.* **115**, 29-38.

VAN NOORDWIJK, M. (1983). Functional interpretation of root densities in the field for nutrient and water uptake. In Root Ecology and its practical application, Int. Symp. Gumpenstein 1982 Eds B. W, K. L & Lichtenegger), pp. 207–226. Bundesanstalt Gumpenstein: Irdning.

VAN SANFORD, D.A. & MACKOWN, C.T. (1986). Variation in nitrogen use efficiency among soft red winter wheat genotypes. *Theoretical and Applied Genetics.* **72**, 158-163.

VAN SANFORD, D.A. & MACKOWN, C.T. (1987). Cultivar differences in nitrogen remobilisation during grain filling in soft red winter wheat. *Crop Science*. **27**, 295-300.

VOORRIPS, R.E. (2002). MapChart: Software for the graphical presentation of linkage maps and QTLs. *The Journal of Heredity*. 93, 77-78.

WAINES, J. & EHDAIE, B. (2007). Domestication and crop physiology: roots of green-revolution wheat. *Annals of Botany.* **100**, 991-998.

WALCH-LIU, P., IVANOV, I.I., FILLEUR, S., GAN, Y., REMANS, A. & FORDE, B.G. (2006). Root structure and function, nitrogen regulation of root branching. *Annals of Botany*. **97**, 875-881.

WANG, Y., MI, G., CHEN, F., ZHANG, J. & ZHANG, F. (2004). Response of root morphology to nitrate supply and its contribution to nitrogen accumulation in maize. *Journal of Plant Nutrition*. **7**, 2189-2202.

WANG, H., MCCAIG, T.N., DEPAUW, R.M. & CLARKE, J.M. (2008). Flag leaf physiological traits in two high-yielding Canada Western Red Spring wheat cultivars. *Canadian Journal of Plant Science.* **88**, 35-42.

WHITE, C.A., SYLVESTER-BRADLET, R. & BERRY, P.M. (2015). Root length densities of UK wheat and oil seed rape crops with implications for water capture and yield. *Journal of Experimental Botany.* **66**, 2293-2303.

WU, L., MCGECHAN, M.B., WATSON, C.A. & BADDELEY, J.A. (2005). Developing existing plant root system architecture models to meet future agricultural challenges. *Advances in Agronomy*. **85**, 181-219.

WIESLER, F. & HORST, W.J. (1994). Root growth and nitrate utilization of maize cultivars under field conditions. *Plant and Soil.* **163**, 267-277.

WILHELM, W.W., MIELKE, L. N. & FENSTER, C. R. (1982). Root development of winter-wheat as related to tillage practice in western Nebraska. *Agronomy Journal.* **74**, 85-88.

WINZELER, H., SCHMID, J.E. & WINZELER, M. (1994). Analysis of the yield potential and yield components of F 1 and F 2 hybrids of crosses between wheat (*Triticum aestivum* L.) and spelt (*Triticum spelta* L.). *Euphytica.* **74**, 211-218.

WOJCIECHOWSKI, T., GOODING, M.J., RAMSAY, L. & GREGORY, P.J. (2009). The effects of dwarfing genes on seedling root growth of wheat. *Journal of Experimental Botany*. **60**, 2565-2573.

WOLLENWEBER, B., PORTER, J.R. & LUBBERSTEDT, T. (2005). Need for multidisciplinary research towards a second green revolution. *Current Opinion in Plant Biology*. **8**, 337-341.

WOOD, C.W., REEVES, D.W. & HIMELRICK, D.G. (1993). Relationships between chlorophyll meter readings and leaf chlorophyll concentration, N status, and crop yield: A review. *Proceedings Agronomy Society of New Zealand*. **23**, 1-9.

XU, W., ROSENOW, D.T. & NGUYEN, H.T. (2000). Stay green trait in grain sorghum: relationship between visual rating and leaf chlorophyll concentration. *Plant Breeding*. **119**, 365-367.

YADAV, R., COURTOIS, B., HUANG, N. & McLAREN, U. (1977). Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice. *Theoretical Applied Genetics.* **94**, 619-632.

YAMAMOTO, A., NAKAMURA, T., ADU-GYAMFI J.J. & SAIGUSA, M. (2002). Relationship between chlorophyll content in leaves of sorghum and pigeon pea determined by extraction method and by chlorophyll meter (SPAD-502). *Journal of Plant Nutrition*. **25**, 2295- 2301.

YANCHENKO, V.I. (1985). A study of interspecific hybrids of *Triticum durum* Desf. x *Triticum dicoccum* (Schrank) Schuebl. and ways of using them in durum wheat breeding. Naucho Tek Eyul Sibirskogo Old Vashknil (Scientific-Technical Bull Sibirsk Branch of Vashknil). **45**, 3-7.

YANG, J., WANG, Z. & ZHU, Q. (1997). Photosynthetic characteristics, dry matter accumulation and its translocation in inter-subspecies hybrid rice. Acta Agron. Sin. **23**, 82-88.

YANG, M., DING, G., SHI, L., FENG, J., XU, F. & MENG, J. (2010). Quantitative trait loci for root morphology in response to low phosphorous stress in *Brassica napus*. *Theoretical Applied Genetics*. **121**, 181-193.

YODER, B.J. & PETTIGREW-CROSBY, R.E. (1995). Predicting nitrogen and chlorophyll content and concentrations from reflectance spectra (400-2500 nm) at leaf and canopy scales. *Remote Sensing of Environment*. **53**, 199-211.

YOO, S.C., CHO, S.H., ZHANG, H., PAIK, H.C., LEE, C.H., LI, J., YOO, J.H., LEE, B.W., KOH, H.J., SEO, H.S. & PAEK, N.C. (2007). Quantitative trait loci associated with functional stay-green *SNU-SG1* in rice. *Molecules and Cells.* **24**, 83-94.

ZADOKS, J. C., CHANG, T. T. & KONZAK, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Research*. **14**, 415-421.

ZAHARIEVA, M., AYANA, N.G., AL HAKIMI, A., MISRA, S.C. & MONNEVEUX, P. (2010). Cultivated emmer wheat (*Triticum dicoccon* Schrank), an old crop with promising future: a review. *Genetic Resources and Crop Evolution.* **57**, 937-962.

ZHU, J., KAEPPLER, S. M. & LYNCH, J. P. (2005). Mapping of QTLs for lateral root branching and length in maize (*Zea mays* L.) under differential phosphorus supply. *Theoretical and Applied Genetics.* **111**, 688-695.

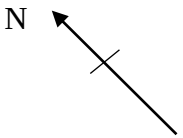
ZOHARY, D. & HOPF, M. (2000). Domestication of Plants in the Old World, 3rd edn, pp. 19-58. Oxford University Press, New York, USA.

Chapter 9

Appendices

Appendix 1A: 2011/ 2012 field layout

Experiment design: Randomised complete block design



Discard	Spelt SB	Einkorn 3	Spelt Oberk	Xi 19	Emmer 1	Spelt Tauro	Einkorn 2	JB Diego	Einkorn 1	Emmer 2	Discard	JB Diego	Spelt Oberk	Einkorn 1	Spelt Tauro	Xi 19	Emmer 2	Einkorn 2	Spelt SB	Emmer 1	Einkorn 3	Discard
	1	2	3	4	5	6	7	8	9	10		21	22	23	24	25	26	27	28	29	30	
Discard	Xi 19	Emmer 1	Einkorn 3	Spelt Tauro	Einkorn 1	JB Diego	Emmer 2	Spelt SB	Einkorn 2	Spelt Oberk	Discard	Emmer 2	Emmer 1	Einkorn 3	Einkorn 2	Spelt SB	Spelt Tauro	Spelt Oberk	JB Diego	Xi 19	Einkorn 1	Discard
	11	12	13	14	15	16	17	18	19	20		31	32	33	34	35	36	37	38	39	40	

Appendix 1B: Crop husbandry 2011/2012 field experiment

Fertiliser	24/02/2012	2.0 l/ha Headland Jet
	08/03/2012	116 kg/ha 34.5% Nitram (40kg/ha N)
	20/03/2012	Headland Jett @ 2l/ha
	11/04/2012	116 kg/ha 34.5% Nitram (40kg/ha N)
	30/04/2012	Manganese 15% @1.5l/ha
	10/05/2012	174 kg/ha 34.5% Nitram (60kg/ha N)
	23/05/2012	Magnor @ 1l/ha
	25/05/2012	Magnor @ 1l/ha
Herbicide	09/11/2011	Liberator @ 0.6l/ha
	20/03/2012	Lorate @ 25g/ha
	24/04/2012	Foxtrot @ 1l/ha + Toil @ 1l/ha
	23/05/2012	Spitfire @ 1l/ha
Fungicide	20/03/2012	Opus @ 0.75l/ha + Bravo @ 1l/ha + Instinct @ 0.4l/ha
	30/04/2012	Cortez @ 0.75l/ha + Phoenix @ 1.3l/ha
	23/05/2012	Opus @ 0.75l/ha + Phoenix @ 1.3l/ha
	25/06/2012	Orius @ 0.85l/ha + Vegas @ 0.15l/ha
Insecticide	09/11/2011	Permasect @ 0.25l/ha
	25/06/2012	Aphox @ 0.28kg/ha
PGR	20/03/2012	Moddus @ 0.2l/ha
	30/04/2012	Moddus @ 0.2l/ha + Chlormequat @ 1.5l/ha

Appendix 2A: 2012/ 2013 field layout

Experiment design: Split-plot design

HN = 200 kgN ha^{-1} | LN = 100 kgN ha^{-1} | NN = 0 kgN ha^{-1}

Discard	Spelt Ober 1	Emmer 2	Einkorn 2	X19	Spelt Tau	JB Diego	Einkorn 3	Spelt SB	Emmer 2	Einkorn 1	Discard	Discard	Emmer 1	JB Diego	Einkorn 3	Spelt SB	Einkorn 2	X19	Emmer 2	Einkorn 2	Spelt Tau	Spelt Ober	Discard	Discard	Spelt SB	Emmer 2	Spelt Ober	X19	Einkorn 1	Einkorn 3	JB Diego	Spelt Tau	Einkorn 2	Emmer 1	Discard
	1	2	3	4	5	6	7	8	9	10			11	12	13	14	15	16	17	18	19	20			21	22	23	24	25	26	27	28	29	30	
Discard	X19	Einkorn 3	Emmer 2	Spelt SB	Emmer 1	Einkorn 1	Spelt Ober	Spelt Tau	Einkorn 2	JB Diego	Discard	Discard	Spelt Tau	Einkorn 2	X19	JB Diego	Emmer 2	Spelt Ober	Einkorn 1	Emmer 1	Spelt SB	Einkorn 3	Discard	Discard	Spelt Ober	JB Diego	Spelt Tau	Einkorn 2	Einkorn 1	Emmer 1	Einkorn 3	Spelt SB	X19	Emmer 2	Discard
	31	32	33	34	35	36	37	38	39	40			41	42	43	44	45	46	47	48	49	50			51	52	53	54	55	56	57	58	59	60	
Discard	Emmer 1	Emmer 2	Spelt Tau	JB Diego	Einkorn 1	Spelt Ober	Spelt SB	X19	Einkorn 3	Einkorn 2	Discard	Discard	X19	Einkorn 2	Einkorn 3	Emmer 1	Emmer 2	Einkorn 1	Spelt SB	JB Diego	Spelt Ober	Spelt Tau	Discard	Discard	Einkorn 1	X19	Einkorn 3	Einkorn 2	Spelt SB	Spelt Ober	Spelt Tau	Emmer 1	Emmer 2	JB Diego	Discard
	61	62	63	64	65	66	67	68	69	70			71	72	73	74	75	76	77	78	79	80			81	82	83	84	85	86	87	88	89	90	

Appendix 2B: Crop husbandry 2012/2013 field experiment

Fertiliser	27/02/2013	Manganese 15% @ 1l/ha
	06/03/2013	116 kg/ha 34.5% Nitram (40kg/ha N) on both N+ treatments
	21/03/2013	Manganese 15% @ 2l/ha
	17/04/2013	174 kg/ha 34.5% Nitram (60kg/ha N) on 100kg/ha treatment
	17/04/2013	232 kg/ha 34.5% Nitram (80kg/ha N) on 200kg/ha treatment
	02/05/2013	Manganese 15% @ 2l/ha
	13/05/2013	Manganese 15% @ 1l/ha
	21/05/2013	232 kg/ha 34.5% Nitram (80kg/ha N) on 200kg/ha treatment
	03/06/2013	Magnor @ 1.0 l/ha
Herbicide	02/10/2012	Round-up @ 2.5 l/ha (Pre-drilling)
	10/04/2013	Hatra @ 1l/ha + Charge @ 2l/ha + Liberator @ 0.3l/ha + Zeal @ 0.3l/ha
	03/06/2013	Lorate @ 25g/ha
Fungicide	02/05/2013	Kestrel @ 1l/ha + Phoenix @ 1l/ha + Flexity @ 0.14l/ha
	03/06/2013	Brutus @ 1.25 l/ha + Amistar Opti @ 1.0 l/ha + Vegas @ 0.15 l/ha
	26/06/2013	Brutus @ 1l/ha + Bravo @ 1l/ha + Instinct @ 0.5l/ha
PGR	02/05/2013	Chlormequat @ 1.4l/ha
	13/05/2013	Moddus @ 0.2l/ha
	03/06/2013	Terpal @ 0.5 l/ha

Appendix 3A: 2013/2014 field Layout

Experiment design: Split-plot design

HN = 150 kgN ha^{-1} | LN = 100 kgN ha^{-1} | NN = 0 kgN ha^{-1}

Discard	Spelt Tauro	Xi 19	Spelt Ober	JB Diego	Spelt SB	Einkorn 2	Emmer 2	Einkorn 1	Emmer 1	Einkorn 3	Discard	Discard	JB Diego	Emmer 1	Xi 19	Spelt Tauro	Emmer 2	Einkorn 1	Einkorn 3	Einkorn 2	Spelt SB	Spelt Ober	Discard	Discard	Emmer 2	Einkorn 2	Spelt Ober	Xi 19	Spelt Tauro	Spelt SB	Einkorn 3	Einkorn 1	JB Diego	Emmer 1	Discard
	1	2	3	4	5	6	7	8	9	10			11	12	13	14	15	16	17	18	19	20			21	22	23	24	25	26	27	28	29	30	
Discard	JB Diego	Spelt Ober	Spelt Tauro	Xi 19	Einkorn 3	Einkorn 1	Emmer 2	Einkorn 2	Spelt SB	Emmer 1	Discard	Discard	Emmer 2	JB Diego	Spelt Ober	Einkorn 1	Einkorn 2	Spelt SB	Emmer 1	Spelt Tauro	Einkorn 3	Xi 19	Discard	Discard	JB Diego	Emmer 1	Spelt SB	Einkorn 2	Spelt Tauro	Emmer 2	Einkorn 1	Spelt Ober	Einkorn 3	Xi 19	Discard
	31	32	33	34	35	36	37	38	39	40			41	42	43	44	45	46	47	48	49	50			51	52	53	54	55	56	57	58	59	60	
Discard	JB Diego	Einkorn 1	Emmer 1	Spelt Tauro	Einkorn 2	Spelt SB	Einkorn 3	Spelt Ober	Xi 19	Emmer 2	Discard	Discard	Einkorn 3	Xi 19	Spelt SB	Emmer 1	Spelt Tauro	Einkorn 2	Einkorn 1	Emmer 2	Spelt Ober	JB Diego	Discard	Discard	Emmer 1	Einkorn 3	Spelt Tauro	Xi 19	Einkorn 2	Einkorn 1	Spelt SB	Emmer 2	Spelt Ober	JB Diego	Discard
	61	62	63	64	65	66	67	68	69	70			71	72	73	74	75	76	77	78	79	80			81	82	83	84	85	86	87	88	89	90	

Appendix 3B: Crop husbandry 2013/2014 field experiment

Fertiliser	12/03/2014	116 kg/ha 34.5% Nitram (40kg/ha N) on both N+ treatments
	14/03/2014	Human extra @ 1l/ha
	02/04/2014	Human extra @ 1l/ha
	28/04/2014	174 kg/ha 34.5% Nitram (60kg/ha N) on 100kg/ha plots
	28/04/2014	232 kg/ha 34.5% Nitram (80kg/ha N) on 150kg/ha plots
	28/04/2014	Human extra @ 1.1l/ha
	14/05/2014	87 kg/ha 34.5% Nitram (30kg/ha N) on 150kg/ha plots
	21/05/2014	Magnor @ 1l/ha
Herbicide	12/03/2014	Hatra @ 1.2l/ha + Headland charge @ 2l/ha + Zeal @ 0.3l/ha
	14/05/2014	Traton SX @ 45g/ha + Kantor @ 150ml/ha
	16/05/2014	Axial @ 0.39l/ha + Adigor @ 0.75l/ha
Fungicide	02/04/2014	Brutus @ 0.5l/ha + Bravo 500 @ 1l/ha + Vegas @ 0.13l/ha
	28/04/2014	Kestrel @ 0.75l/ha + Vegas @ 0.13l/ha + Phoenix @ 0.6l/ha
	21/05/2014	Opera @ 1l/ha + Phoenix @ 1l/ha + Brutus @ 1.2l/ha
	16/06/2014	Kestrel @ 0.75l/ha + Kantor @ 0.15l/ha
Insecticide	14/03/2014	Minuet @ 0.1l/ha
PGR	02/04/2014	Adjust @ 1l/ha
	28/04/2014	Adjust @ 0.8l/ha + Cleancrop Cutlass @ 0.2l/ha

Appendix 4: Glasshouse pot experiment 2013

Experiment design: Split-plot design

	R1	
	G4N1	G3N2
	G8N1	G5N2
	G5N1	G9N2
	G10N1	G1N2
	G1N1	G6N2
	G6N1	G8N2
	G9N1	G2N2
	G2N1	G4N2
	G7N1	G10N2
H1	G3N1	G7N2
	G5N1	G7N2
	G10N1	G1N2
	G1N1	G8N2
	G9N1	G10N2
	G2N1	G9N2
	G6N1	G5N2
	G4N1	G2N2
	G8N1	G4N2
	G3N1	G6N2
H2	G7N1	G3N2
	G6N1	G3N2
	G1N1	G1N2
	G9N1	G9N2
	G7N1	G2N2
	G2N1	G8N2
	G8N1	G4N2
	G5N1	G7N2
	G10N1	G5N2
	G3N1	G10N2
H3	G4N1	G6N2
	G1N1	G2N2
	G5N1	G9N2
	G10N1	G1N2
	G2N1	G3N2
	G6N1	G10N2
	G9N1	G4N2
	G4N1	G6N2
	G3N1	G8N2
	G8N1	G5N2
H4	G7N1	G7N2

R2	
G5N1	G2N2
G1N1	G5N2
G10N1	G10N2
G6N1	G8N2
G2N1	G1N2
G3N1	G4N2
G8N1	G7N2
G4N1	G9N2
G9N1	G6N2
G73N1	G3N2
G6N1	G4N2
G8N1	G2N2
G2N1	G9N2
G7N1	G7N2
G5N1	G1N2
G1N1	G8N2
G10N1	G3N2
G4N1	G6N2
G9N1	G10N2
G3N1	G5N2
G10N1	G2N2
G1N1	G8N2
G5N1	G1N2
G8N1	G7N2
G9N1	G4N2
G2N1	G10N2
G4N1	G3N2
G6N1	G9N2
G3N1	G6N2
G7N1	G5N2
G5N1	G2N2
G2N1	G1N2
G9N1	G10N2
G1N1	G4N2
G4N1	G5N2
G3N1	G8N2
G10N1	G3N2
G7N1	G9N2
G8N1	G7N2
G6N1	G6N2

R3	
G1N1	G3N2
G8N1	G5N2
G4N1	G9N2
G9N1	G1N2
G2N1	G6N2
G10N1	G8N2
G3N1	G2N2
G5N1	G4N2
G6N1	G10N2
G7N1	G7N2
G6N1	G2N2
G9N1	G7N2
G5N1	G8N2
G1N1	G1N2
G7N1	G10N2
G2N1	G9N2
G8N1	G3N2
G10N1	G5N2
G3N1	G4N2
G4N1	G6N2
G6N1	G6N2
G1N1	G8N2
G7N1	G1N2
G9N1	G4N2
G2N1	G9N2
G3N1	G2N2
G8N1	G5N2
G4N1	G3N2
G10N1	G7N2
G5N1	G10N2
G7N1	G9N2
G9N1	G1N2
G3N1	G2N2
G6N1	G8N2
G8N1	G10N2
G2N1	G3N2
G10N1	G7N2
G4N1	G4N2
G5N1	G6N2
G1N1	G5N2

G1 - JB Diego
G2 - Xi 19
G3 - Spelt Tauro
G4 - Spelt SB
G5 - Spelt Oberkulmer
G6 - Emmer 1
G7 - Emmer 2
G8 - Einkorn 1
G9 - Einkorn 2
G10 - Einkorn 3

N_1- 50 kg N ha^{-1}

N_2- 200 kg N ha^{-1}

Appendix 5: 2D root experiment 2013 (Ancient wheat)

Experiment design: Completely randomised design

1	Day 2	4	Day 2	7	Day 2	10	Day 2	13	Day 2	16	Day 1	19	Day 1	22	Day 1	25	Day 2
1	9	31	8	61	1	91	9	121	8	151	3	181	4	211	3	241	2
2	2	32	5	62	6	92	2	122	3	152	3	182	2	212	4	242	7
3	8	33	3	63	8	93	6	123	4	153	1	183	3	213	2	243	5
4	7	34	2	64	9	94	5	124	8	154	3	184	3	214	5	244	9
5	2	35	7	65	2	95	7	125	1	155	6	185	6	215	4	245	4
6	9	36	9	66	3	96	1	126	9	156	5	186	4	216	6	246	9
7	6	37	6	67	2	97	8	127	3	157	3	187	5	217	5	247	2
8	1	38	3	68	1	98	10	128	5	158	6	188	4	218	3	248	6
9	8	39	1	69	6	99	9	129	7	159	5	189	5	219	4	249	10
10	7	40	9	70	9	100	8	130	6	160	3	190	2	220	3	250	7
2	**Day 3**	**5**	**Day 1**	**8**	**Day 1**	**11**	**Day 2**	**14**	**Day 1**	**17**	**Day 3**	**20**	**Day 2**	**23**	**Day 2**	**26**	**Day 2**
11	10	41	2	71	2	101	10	131	2	161	0	191	6	221	1	251	3
12	10	42	4	72	3	102	9	132	4	162	0	192	2	222	8	252	8
13	10	43	5	73	1	103	7	133	5	163	10	193	8	223	1	253	7
14	10	44	1	74	5	104	1	134	1	164	0	194	5	224	5	254	5
15	10	45	4	75	4	105	8	135	6	165	10	195	1	225	9	255	3
16	10	46	3	76	2	106	9	136	1	166	10	196	7	226	1	256	7
17	10	47	1	77	6	107	10	137	5	167	0	197	8	227	7	257	0
18	10	48	6	78	5	108	8	138	6	168	10	198	6	228	2	258	1
19	10	49	5	79	4	109	1	139	4	169	10	199	7	229	6	259	10
20	10	50	3	80	3	110	7	140	6	170	10	200	9	230	8	260	7
3	**Day 2**	**6**	**Day 2**	**9**	**Day 2**	**12**	**Day 1**	**15**	**Day 2**	**18**	**Day 2**	**21**	**Day 3**	**24**	**Day 2**	**27**	**Day 2**
21	8	51	9	81	2	111	3	141	1	171	1	201	10	231	1	261	6
22	7	52	3	82	8	112	1	142	9	172	7	202	10	232	3	262	2
23	8	53	8	83	4	113	3	143	7	173	8	203	10	233	8	263	7
24	0	54	5	84	7	114	4	144	9	174	9	204	10	234	7	264	8
25	0	55	9	85	2	115	0	145	7	175	6	205	10	235	5	265	9
26	9	56	2	86	1	116	4	146	5	176	2	206	0	236	6	266	6
27	2	57	7	87	9	117	6	147	8	177	8	207	10	237	1	267	5
28	7	58	1	88	8	118	2	148	9	178	5	208	10	238	2	268	7
29	9	59	8	89	2	119	3	149	5	179	7	209	10	239	8	269	9
30	7	60	6	90	3	120	2	150	9	180	5	210	0	240	6	270	1

1 - JB Diego

2 - Xi 19

3 - Spelt Tauro

4 - Spelt SB

5 - Spelt Oberk

6 - Emmer 1

7 - Emmer 2

8 - Einkorn 1

9 - Einkorn 2

10 - Einkorn 3

Appendix 6: Glasshouse root experiment 2013

Experiment design: Randomised complete block design

	1	Emmer 1	9	Einkorn 1	17	Xi 19	25	Einkorn 3	33	Xi 19
	2	JB Diego	10	Spelt Ober	18	Spelt Ober	26	Einkorn SB	34	Einkorn 1
	3	Spelt Ober	11	Einkorn 3	19	Emmer 2	27	Spelt SB	35	Emmer 1
	4	Einkorn 3	12	Spelt Tau	20	Einkorn 1	28	JB Diego	36	Xi 19
	5	Spelt SB	13	JB Diego	21	Spelt Tau	29	Einkorn 2	37	Emmer 2
	6	Xi 19	14	Spelt Ober	22	Spelt SB	30	Emmer 1	38	Einkorn 2
	7	Spelt Tau	15	Emmer 1	23	Einkorn 2	31	Spelt Tau	39	Spelt SB
Rep 1	8	Emmer 2	16	Emmer 2	24	Einkorn 3	32	Einkorn 1	40	JB Diego
	41	Einkorn 3	49	Spelt SB	57	Spelt Ober	65	Einkorn 3	73	Emmer 2
	42	JB Diego	50	Xi 19	58	Einkorn 1	66	Spelt SB	74	Emmer 1
	43	Emmer 1	51	Einkorn 3	59	Xi 19	67	Emmer 2	75	Spelt Ober
	44	Einkorn 2	52	Spelt SB	60	Einkorn 3	68	JB Diego	76	Einkorn 1
	45	Spelt Ober	53	Emmer 1	61	JB Diego	69	Spelt Tau	77	Spelt Tau
	46	Emmer 1	54	Einkorn 1	62	Einkorn 2	70	Einkorn 2	78	JB Diego
	47	Spelt SB	55	Spelt Tau	63	Xi 19	71	Spelt Ober	79	Spelt Tau
Rep 2	48	Xi 19	56	Einkorn 2	64	Emmer 2	72	Einkorn 1	80	Emmer 2
	81	Xi 19	89	Spelt Ober	97	JB Diego	105	Einkorn 1	113	Einkorn 3
	82	Emmer 1	90	Spelt SB	98	Einkorn 3	106	Spelt Ober	114	Spelt Tau
	83	Einkorn 1	91	Xi 19	99	Emmer 2	107	Emmer 1	115	Einkorn 2
	84	Spelt Tau	92	Spelt Ober	100	Einkorn 3	108	Einkorn 2	116	Spelt Tau
	85	Einkorn 3	93	Einkorn 2	101	Spelt SB	109	Spelt Tau	117	JB Diego
	86	Emmer 2	94	Xi 19	102	Emmer 2	110	JB Diego	118	Spelt SB
	87	Einkorn 1	95	Emmer 1	103	Spelt Ober	111	Spelt SB	119	Einkorn 1
Rep 3	88	Emmer 2	96	JB Diego	104	Xi 19	112	Einkorn 2	120	Emmer 1

Appendix 7: Column experiment

Experiment design: Split-plot design

Block 1	**N1**	Emmer 1	Spelt SB	Spelt Ober	Forno	Spelt Tauro	Emmer 2	Xi 19
	N2	Spelt Ober	Spelt Tauro	Emmer 2	Xi 19	Emmer 1	Spelt SB	Forno
	N3	Emmer 2	Spelt SB	Emmer 1	Forno	Spelt Tauro	Xi 19	Spelt Ober
Block 2	**N1**	Xi 19	Emmer 1	Spelt Ober	Spelt SB	Emmer 2	Spelt Tauro	Forno
	N2	Spelt Ober	Spelt SB	Spelt Tauro	Xi 19	Forno	Emmer 2	Emmer 1
	N3	Xi 19	Emmer 2	Spelt Ober	Emmer 1	Spelt Tauro	Forno	Spelt SB
Block 3	**N1**	Emmer 1	Spelt Ober	Spelt SB	Forno	Spelt Tauro	Xi 19	Emmer 2
	N2	Xi 19	Emmer 1	Forno	Emmer 2	Spelt Ober	Spelt SB	Spelt Tauro
	N3	Spelt SB	Spelt Tauro	Emmer 2	Spelt Ober	Xi 19	Emmer 1	Forno

Appendix 8: Modular tray experiment (Compost/Sand)

Experiment design: Completely randomised design

JB Diego	Beaver	Spelt Ober	Forno	Emmer 1	Spelt Tauro	Gallant	Glasgow	Spelt Tauro	Einkorn 2	Hobbit	Einkorn 1	Gallant	Forno	Emmer 1
Spelt Ober	Emmer 2	Einkorn 1	Beaver	Einkorn 2	Einkorn 1	Xi 19	JB Diego	Gallant	JB Diego	Emmer 1	Forno	Xi 19	Emmer 2	Spelt Ober
Forno	Spelt SB	JB Diego	Emmer 2	Forno	Beaver	Spelt SB	Gallant	Xi 19	Hobbit	Emmer 2	Spelt SB	Spelt Ober	Spelt Tauro	Beaver
Spelt Tauro	Xi 19	Glasgow	Einkorn 1	Spelt Ober	JB Diego	Emmer 1	Spelt Tauro	Emmer 2	Spelt SB	Spelt Tauro	Hobbit	JB Diego	Xi 19	Xi 19
Glasgow	Emmer 1	Beaver	Xi 19	Hobbit	Einkorn 2	Spelt Ober	Gallant	Einkorn 2	Forno	Beaver	Glasgow	Einkorn 1	Einkorn 2	Gallant
Einkorn 1	Einkorn 2	Einkorn 3	Emmer 1	Spelt Tauro	Spelt SB	Forno	Beaver	Beaver	Einkorn 3	Xi 19	Emmer 1	Forno	Spelt SB	Spelt Tauro
Xi 19	Spelt Ober	Spelt SB	Glasgow	Einkorn 3	Xi 19	Glasgow	Emmer 1	JB Diego	Emmer 1	Spelt SB	Einkorn 3	Beaver	Hobbit	Hobbit
Hobbit	Emmer 2	Einkorn 1	JB Diego	Spelt SB	Glasgow	Emmer 2	Xi 19	Spelt SB	Einkorn 3	Einkorn 1	JB Diego	Spelt Tauro	Einkorn 3	Einkorn 3
Beaver	Einkorn 1	Hobbit	Gallant	Forno	Spelt Ober	Beaver	Einkorn 2	Forno	Emmer 2	Forno	Einkorn 2	Einkorn 3	Emmer 1	Emmer 2
Emmer 2	Gallant	Forno	Emmer 2	Gallant	Hobbit	JB Diego	Spelt Ober	Spelt Ober	Beaver	Glasgow	Emmer 2	Forno	Einkorn 2	Gallant
Spelt SB	Spelt Tauro	Einkorn 3	Glasgow	Beaver	Beaver	Spelt Tauro	Gallant	Einkorn 1	Xi 19	Einkorn 3	Beaver	Spelt SB	Spelt Ober	JB Diego
JB Diego	Xi 19	Einkorn 2	Emmer 1	Einkorn 1	Emmer 2	Einkorn 3	Forno	Einkorn 1	Gallant	JB Diego	Spelt Ober	Gallant	Beaver	Einkorn 2
Einkorn 2	Glasgow	Einkorn 1	Spelt Ober	Gallant	Einkorn 1	Einkorn 1	Spelt SB	Emmer 1	Spelt Tauro	Einkorn 3	Forno	JB Diego	Emmer 2	Spelt SB
Spelt Tauro	Hobbit	Spelt Ober	Spelt Tauro	Xi 19	Emmer 1	Xi 19	Einkorn 3	Glasgow	Glasgow	Gallant	Spelt Tauro	Hobbit	Glasgow	Forno
Einkorn 1	JB Diego	Einkorn 3	Spelt SB	Hobbit	Forno	Hobbit	Emmer 2	Einkorn 3	Hobbit	Spelt SB	Xi 19	Glasgow	Forno	Einkorn 1
Emmer 1	Einkorn 2	Spelt SB	Beaver	Glasgow	Spelt Ober	Gallant	Einkorn 2	Beaver	Spelt SB	Forno	Einkorn 3	Emmer 2	Gallant	Hobbit
Gallant	Beaver	Xi 19	Einkorn 2	JB Diego	Spelt Tauro	Glasgow	Emmer 1	Xi 19	Einkorn 2	Einkorn 2	Spelt SB	Einkorn 3	Xi 19	Spelt Ober
Spelt Ober	Einkorn 1	Spelt Tauro	Einkorn 1	Emmer 1	Einkorn 2	Spelt SB	JB Diego	Hobbit	Emmer 1	Einkorn 3	Gallant	Emmer 1	Einkorn 2	Glasgow
Forno	Glasgow	Emmer 2	Xi 19	Hobbit	JB Diego	Hobbit	Glasgow	Gallant	Spelt Ober	JB Diego	Hobbit	Einkorn 3	Spelt Tauro	Emmer 1
Emmer 2	Emmer 1	Glasgow	Spelt Ober	Spelt Ober	Einkorn 2	Emmer 2	Einkorn 1	Spelt Tauro	Einkorn 3	Hobbit	Spelt Tauro	Beaver	Emmer 2	JB Diego