RESEARCH ARTICLE

GENOTYPIC DEPENDENCE OF WHEAT SPECIES IN NITROGEN UPTAKE DETERMINES BY ROOT MORPHOLOGY AT MATURITY

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ABSTRACT

The root system is essential for taking up nutrients while providing anchorage to the plant. The controlled environment experiment was conducted to study root morphological traits, plant growth, yield and nitrogen (N) uptake in different wheat species. Seven genotypes including cultivated emmer (*Triticum dicoccum*), spelt (*T. spelta* L.) and modern bread wheat (*T. aestivum*) under three N levels were examined. A split-plot design was used in the experiment where the main plot factor was N levels and the sub-plot factor was genotypes and replicated three times. Root samples at five depth layers were scanned and analysed using *WinRHIZO* software at anthesis and maturity. Total root length, root volume, root biomass, root diameter, root length density, rooting depth and root N uptake efficiency were recorded. Plant N uptake, N uptake efficiency, N utilisation efficiency and N use efficiency were calculated at maturity (excluding N in roots). Neither interaction effect between main factors nor N level were significant for all measured root traits except N uptake efficiency of roots. Similar results were observed for growth and yield traits together with plant N uptake, N uptake efficiency, N utilisation efficiency and N use efficiency. All measured parameters were significantly different among genotypes. Spelt genotypes recorded the highest total root length, root volume, root biomass and root length density at all depth layers while emmer genotypes recorded the greatest root N uptake efficiency. Plant N uptake was significantly different between genotypes where all spelt genotypes had high plant N uptake followed by bread wheat and emmer. Plant N uptake of the genotypes had a very strong positive correlation with total root length, root volume, root biomass and rooting depth of the genotypes. Therefore, it could be concluded that the high plant N uptake of spelt may be due to the robust and vigorous growth of root systems.

Keywords: N uptake, maturity, root traits, spelt genotypes, wheat species

INTRODUCTION

Wheat is the first cereal known to be domesticated about ten thousand years ago. For the past eight thousand years, wheat served as 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 (Braun *et al.* 2010). Wheat provides 35 to 60% of the daily calories for people in North Africa, Central and West Asia. 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). It is predicted that, 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 living in developing countries. However, at

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present, the estimated rate of yield increment of wheat is 0.9% per year (Ray *et al.* 2013). 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 is 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).

Wheat production in the UK relies on nitrogen fertilizer for high yield and protein content affecting significantly the cost of production. Due to the nature of mobility of nitrate, there is a huge potential of rapid leaching below root zones and subsequently, reach to the groundwater table. Irrigated spring wheat recorded 51% (Dhugga and Waines 1989) to 60% (Ehdaie *et al.* 2001) of nitrogen use efficiency (the proportion of supplied N in grains) suggesting that the rest of the supplied N may be lost to the environment through runoff, leaching, soil biological denitrification and volatilization creating several environmental issues. This usually happened when root systems of the plants are unable to uptake N efficiently (Ehdaie *et al.* 2010). Therefore, there is a direct relationship between root morphological characteristics and N uptake of the crops. Plants with large and deep root systems can reduce the fraction of supplied N leached to the bottom layers of the soil horizon compared to the plants having small and shallow root systems (Ehadaie *et al.* 2010). Further, due to greater root surface area, plants with dense root systems with numerous lateral roots and root hairs can uptake more nutrients than the plants with scattered root systems (Gahoonia *et al.* 2007). Robinson *et al.* (1994) find a weak relationship between root length density and N uptake efficiency of bread wheat. However, subsoil nitrate depletion is affected by root length density in corn (*Zea mays*) (Wiesler and Horst 1994). According to Liao *et al.* (2006), N uptake of wheat genotypes is affected by grater root biomass. Less nitrate leaching due to high nitrate uptake of Kentucky bluegrass (*Poa pratensis* L.) is associated with high root biomass production (Geron *et al.* 1993). Shallow rooted creeping bent grass (*Agrostis palustrrs* Huds.) report high nitrate leaching compared to

deep-rooted genotypes hence low N uptake (Bowman *et al.* 1998). Breeding could be successfully used to improve mineral nutrient uptake by introducing morphological and physiological traits. However, the selection of root traits is laborious under field conditions due to changing environmental and soil factors (Hamada *et al.* 2012).

Consequently, there is a need to breed wheat genotypes with effective root system architecture enabling efficient uptake of N from the soil. Traditionally, characteristics of aboveground organs and yield parameters have been considered for decades when breeding crops while root morphological traits have been largely neglected (Waines and Ehdaie 2007). There is a possibility to develop new wheat varieties with efficient root systems by studying appropriate germplasm through suitable field and laboratory experiments. Ancient wheat species could be used as genetic resources to improve modern bread wheat. However, very little is known on root morphological traits and N uptake efficiency of ancient wheat species. The objectives of this study were to investigate root morphological traits and N uptake of different wheat species while identifying the relationship between root morphological traits and plant N uptake at anthesis and maturity.

MATERIALS AND METHODS

A controlled environment column experiment was conducted to study root morphological traits and plant nitrogen uptake of different wheat species at Sutton Bonington Campus, University of Nottingham, UK. Seven genotypes including cultivated emmer (*Triticum dicoccum*), spelt (*T. spelta* L.) and modern bread wheat (*T. aestivum*) were used as genetic materials. Two genotypes of emmer, three cultivars (cv) of spelt (SB, Oberkulmer and Tauro) and two cultivars of bread wheat (Xi19 and Forno) were compared. A split-plot design was used in the experiment where the main plot factor was N levels and the sub-plot factor was genotypes and replicated three times. Three levels of N equivalent to 50, 100 and 200 kg N ha^{-1} were applied. A single seed per hole was sown in modular trays filled with compost medium. Seedlings were subjected to
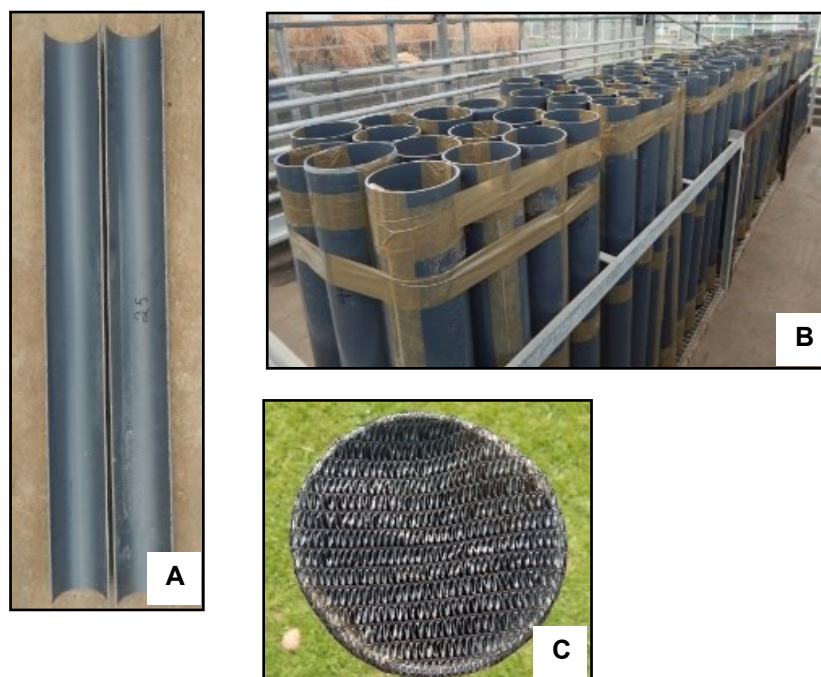
Figure 1: (A) Split column of one meter length (B) two halves of the column tapped together and (C) bottom of the column covered by a piece of shading net

a vernalization period of 10 weeks in the glasshouse at 4°C under artificial light. After vernalization, seedlings were transplanted into the columns with 10cm in diameter and 100 cm in height, keeping one plant per column and drip irrigation was installed. All columns were split into two halves longitudinally (Fig. 1A), which were then taped together with brown parcel tape (Fig. 1B) and the bottom was closed by using a piece of black shading net gage two (20 cm x 20 cm) to allow drainage (Fig. 1C). All the columns were filled with hydroleca clay pellets and washed thoroughly to remove soil and broken pieces as much as possible. Plants were provided with plant supports from growth stage 39 (GS

39; flag leaf emergence) (Zadoks *et al.* 1974) to avoid lodging. The experiment was conducted under natural daylight with maximum day and night temperatures of 18 and 13°C for the first two weeks and then increased up to 25 to 18°C, respectively. The maximum temperature of the glasshouse was regulated by opening roof vents automatically.

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 fertilizer 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
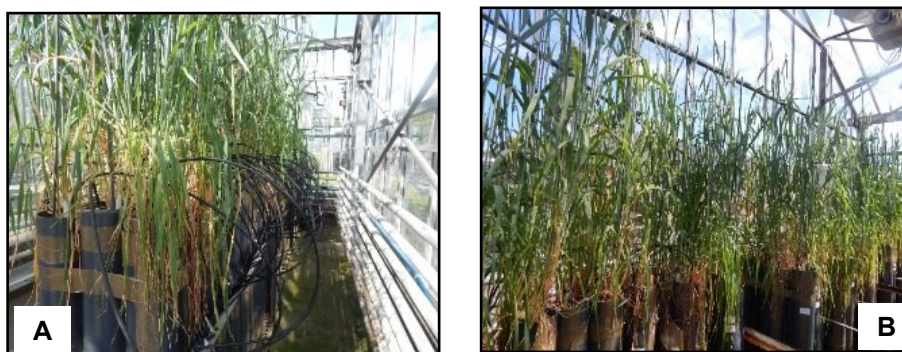
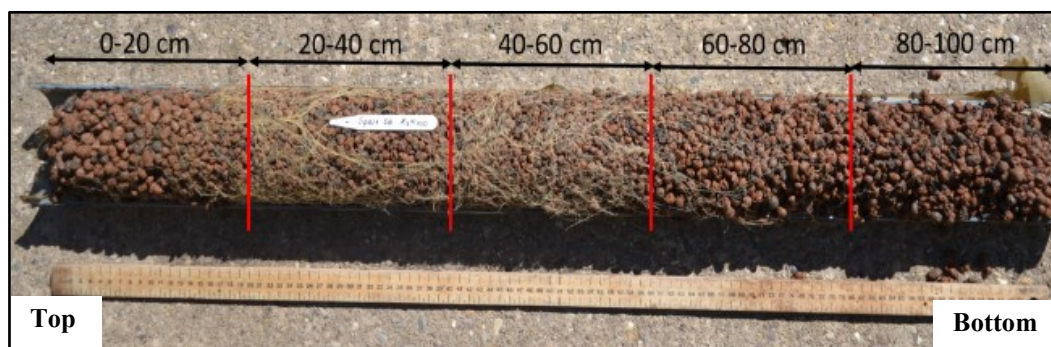**Figure 2: (A) Drip irrigation (B) plants having supports at anthesis**

Figure 3: Root distribution of one-meter long column split into two halves and divided further to five sections

sulphur, water-soluble B (0.025%), Cu (0.01%), Fe (0.07%), Mn (0.04%), Zn (0.025%) and Mo (0.004%) and all micronutrients were chelated by EDTA, except Mo. Calcium (Ca) is not in this mixture. The fertigation system had been shut down for 10 days to reduce N supply and was reopened with water. Leachate of the column did not analyse for NO^{3-} or other elements. Fig. 2A and B show plants at anthesis having drip irrigation system and plant supports.

Two destructive samplings were done at anthesis (flowering) and maturity. At anthesis, the number of tillers per plant, above-ground biomass, green leaf area, plant N % and plant N uptake (except root) were recorded. At maturity, ear, leaf and stem dry weight were established by oven drying them at $80^{o}C$ for 48 hours. Ears were hand threshed and grains were separated carefully from the chaff. The weight of re-dried grains was subtracted from ear weight to obtain the chaff weight of the particular genotype.

Root screening

Columns were split into two halves (Fig. 3) using a sharp blade along the length through the taped area to collect root samples. The root system was divided into five segments from 0-100 into 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 (80 to 100 cm depth) thus deeper root was identified by a higher score. Roots were kept in water until the 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. Root samples were spread in an acrylic box (A4 size) (Fig. 4A) with tap water to minimize the overlaps. A scanner (Fig. 4B) with a transparency adapter (*WinRHIZO* STD
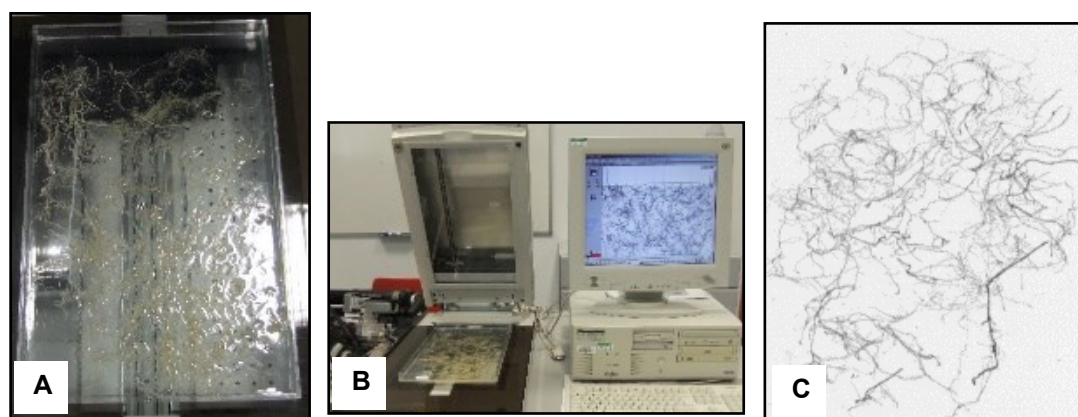

Figure 4: (A) Submerged root sample in acrylic box (B) *WinRHIZO* root scanner (C) scanned image

1600+, Regent Instruments Inc., Quebec; Canada) was used to digitized root samples at 400 dpi resolution and 256 grey contrasts (Tiff format). After that, *WinRHIZO* regular V.2002c software was used to analyse all scanned root images (Fig. 4C) (Fernando *et al*. 2021). Following scanning, roots together with shoots were oven-dried at 80°C for 48 hours before determining their dry weight.

Total root length, root volume and average root diameter were recorded. Root N uptake efficiency (NUpER, equation 1) and root length density (RLD, equation 2) were calculated based on the original data of the root scan.

Plant N content (%) was measured using the Dumas method (Fernando and Sparkes 2020). N uptake (N_{off}; Equation 3) was calculated based on the nitrogen content of the plant dry matter. N_{off} does not include root N in this calculation. Nitrogen uptake efficiency (NUpE; Equation 4) (Ehdaie, *et al*. 2010), nitrogen utilisation efficiency (NUtE; Equation 5), nitrogen use efficiency (NUE; Equation 6) and nitrogen harvest index (NHI; Equation 7) were calculated at maturity.

Statistical analysis
Data were analysed using GenStat 15th edition. ANOVA appropriate to the experiment design was performed. Mean separation was done according to LSD at 5% probability level. The correlation and regression analysis were used to find the relationship between N uptake and root morphological traits at anthesis and maturity.

RESULTS AND DISCUSSION
Modern bread wheat is the product of series of naturally crossbreed events of different ancient wheat species (Harris 1990). Varietal selection and domestication of wheat occurred in many regions of the world for a long period. At present, modern bread wheat is one of the commercially important wheat species grown worldwide. However, ancient wheat species still provide valuable genetic materials for breeding programmes (Talbert *et al*. 1991). The number of chromosomes found in the vegetative cells is used to classify wheat

$$NU_pE_R = \frac{N_{off}\ (\text{g N plant}^{-1})}{RB\ (\text{g plant}^{-1})} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textbf{Equation 1}$$

$$RLD\ (\text{cm cm}^{-3}) = \frac{TRL\ (\text{cm})}{RV\ (\text{cm}^3)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.. \textbf{Equation 2}$$

Where; TRL; total root length, RB; root biomass, *N$_{off}$*; total plant N uptake (except roots), RV; root volume

$$\text{N uptake (g N)} = \text{Plant DW (g)} \times \text{N content of the plant (\%)} \ldots\ldots\ldots\ldots\ldots.. \textbf{Equation 3}$$

$$NUpE = \frac{N_{off}\ (\text{g N plant}^{-1})}{N_{ava}\ (\text{g N Plant}^{-1})} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. \textbf{Equation 4}$$

N$_{ava}$= fertiliser N amount (g N)

$$NUtE = \frac{\text{Grain yield (g Plant}^{-1})}{N_{off}\ (\text{g N Plant}^{-1})} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textbf{Equation 5}$$

$$NUE = \frac{\text{Grain yield (g Plant}^{-1})}{N_{ava}\ (\text{g N Plant}^{-1})} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. \textbf{Equation 6}$$

$$NHI = \frac{\text{Grain N (g N Plant}^{-1})}{N_{off}\ (\text{g N Plant}^{-1})} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textbf{Equation 7}$$

species. According to that three wheat groups can be identified as wheat species with 14 chromosomes (the diploid or einkorn), 28 chromosomes (the tetraploid or emmer) and 42 chromosomes (the hexaploid or spelt and modern bread wheat (Gill *et al*. 1991). In the present study, above-ground characteristics and root morphological traits of three wheat species of emmer, spelt and modern bread wheat were compared under three nitrogen fertilizer levels under a controlled environment.

Root morphology at anthesis and maturity
Total root length, root volume, root diameter, root biomass and root length density were not significantly influenced neither by genotype and N level (GT x N) interaction nor the main factor of N at anthesis and maturity. The interaction effect between the two main factors was significant only for N uptake efficiency of roots (NUpER) at anthesis.

Total root length
Total root length was significantly different among genotypes at all depths at anthesis (P<0.01) and maturity (P<0.001) (Fig. 5). Spelt cv. Oberkulmer consistently had the

highest root length at all depths whilst two emmer genotypes had the lowest root length at all depths and did not extend below 60 cm of the column. The highest root length was reported in 0-20 cm layer for all genotypes at anthesis and maturity while decreased gradually with depth. The total root length of the genotypes was longer at maturity than anthesis for most of the genotypes. However, total root length of spelt cv. Oberkulmer and bread wheat cv. Xi 19 was longer at anthesis. At maturity, spelt produced 59% and 67% of greater root length than bread wheat and emmer, respectively. Fernando *et al*. (2021) found that two weeks old seedlings of emmer had wider emergence angles and tip angles in seminal roots than spelt, bread wheat and einkorn. According to the results of the present study, emmer genotypes developed shallow root systems at maturity. Nakomoto and Oyangi (1994) working on Japanese wheat, revealed that the angular spread of seminal roots determined the depth of the root system. Furthermore, they found that deep root systems were formed by seminal roots with narrow angels and shallow root systems were developed by horizontally distributed seminal roots with wider angles. Therefore, emmer
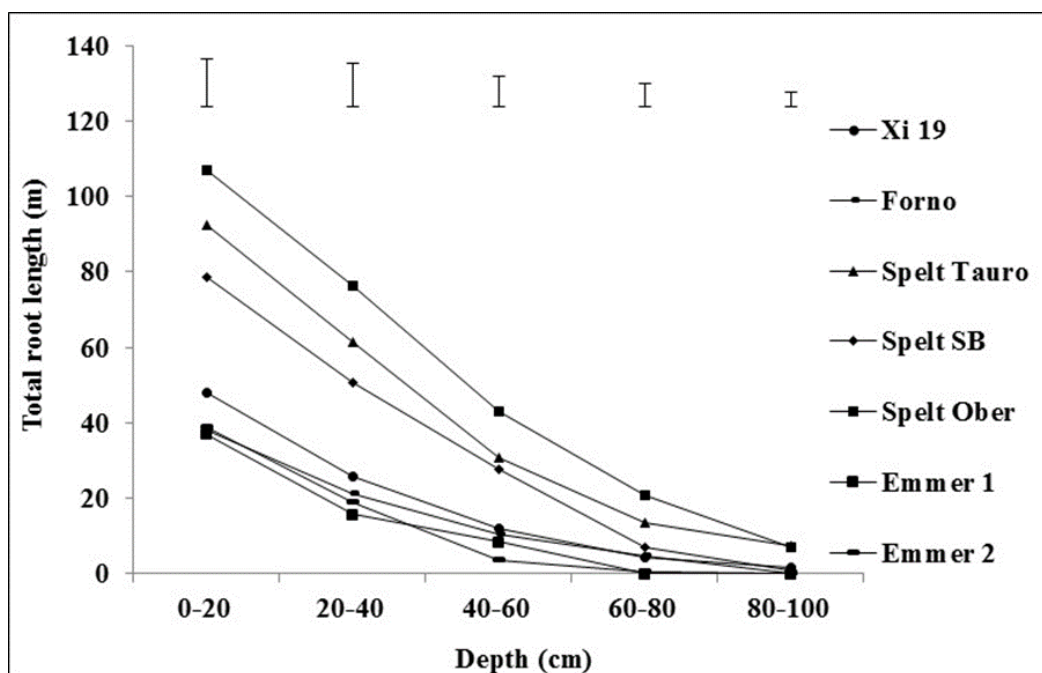

Figure 5: Root length distribution of genotypes at anthesis. Error bars represent the SED of genotypes in different depth layers (*df* = 29.16)

genotypes produced shallow and wide root systems than spelt and bread wheat at maturity.

Root volume

Root volume was significantly different between genotypes at all depths at anthesis (P<0.05) and maturity (P<0.001). Further, a significant difference was found between genotypes (P<0.001) for total root volume per plant at anthesis and maturity. Spelt had higher total root volume under any depth level when compared to bread wheat and emmer. Total root volume per plant at anthesis was significantly high in spelt (22.23 cm^3) followed by bread wheat (12.3 cm^3) and emmer (8.4 cm^3). At maturity, on average, spelt produced the highest root volume followed by bread wheat and emmer. Root volume of spelt was 59% and 68% greater than bread wheat and emmer, respectively.

Root diameter

At anthesis, the average root diameter at 0-20 cm was significantly different between genotypes. 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 among genotype at 20-40 cm depth for average root diameter.

Root biomass

Genotypes recorded significant difference for root biomass at different root depths (P<0.001) except 80-100 cm layer. Spelt genotypes had very high root biomass production when compared to all other species. Spelt cv. Tauro (1.99 g), spelt cv. Oberkulmer (1.96 g) and spelt cv. SB (1.94 g) recorded high root biomass production at maturity which was greater than the other genotypes. Interestingly, all species produced higher root biomass at maturity than anthesis. Further, the increase in biomass production from anthesis to maturity was high in emmer (23.5%) than bread wheat (13.1%) and spelt (5.7%).

Root length density

Root length density (RLD) at anthesis and maturity showed the same pattern of distribution with depth as root length since roots were extracted from the same volume of the sections of the column. According to that, higher RLD
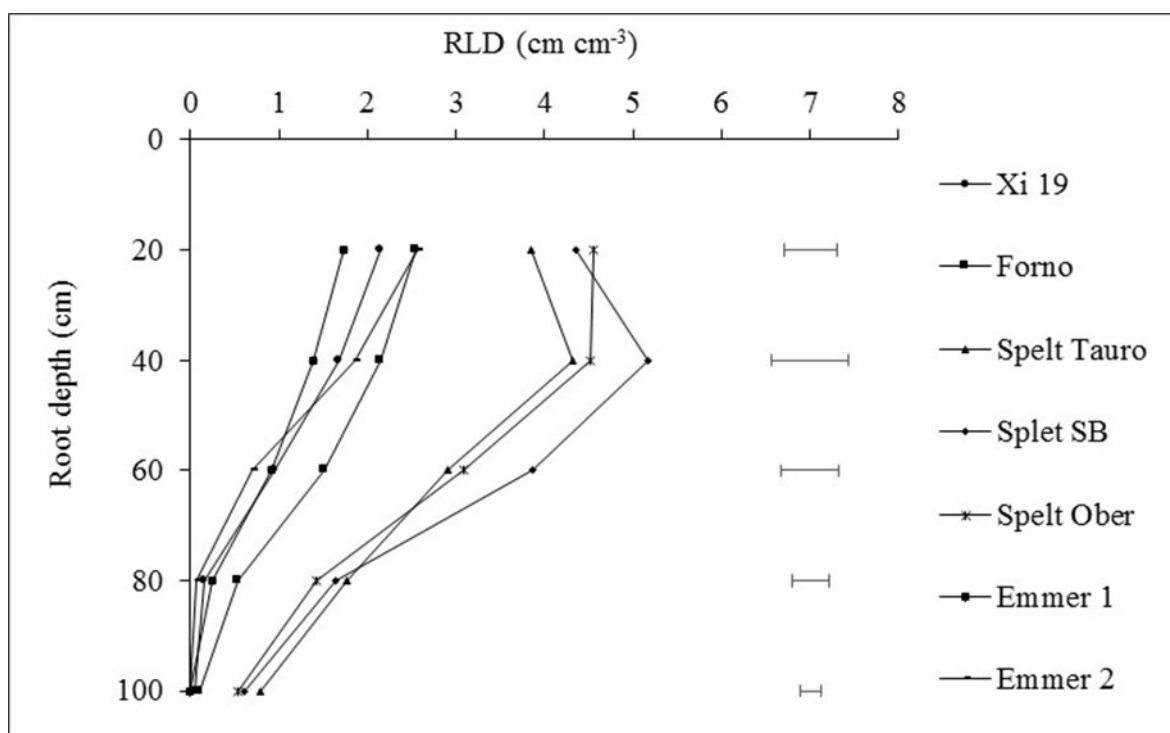

Figure 6: Root length density of the genotypes throughout the root depth at maturity. Error bars represent SED for genotypes at 0-20, 20-40, 40-60, 60-80 and 80-100 cm layers (*df* = 36)

at the bottom layers of the root system was recorded in spelt Oberkulmer. The root length density of the root system at anthesis and maturity was significantly different between genotypes (P<0.001). Further, RLD at different depths of the root system was also highly significant at maturity. The 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. Fig. 6 shows the distribution of RLD throughout the root depth at maturity.

Nitrogen uptake efficiency of roots

Nitrogen uptake efficiency of roots was significantly affected by the interaction between N x GT (P<0.05) at anthesis. On average, emmer showed higher NUpER followed by bread wheat and spelt (Fig. 7). However, the highest NUpER at maturity was recorded in bread wheat cv. Xi 19 (38.5%) but it was not significantly different either from emmer 1 (33.5%) or emmer 2 (38.3%). All spelt genotypes recorded significantly lower values for NUpER.

Plant growth, yield and nitrogen dynamics

The interaction between GT x N and main factor N was not significantly different for the total number of tillers, total green area per plant, above-ground biomass, plant nitrogen content (%) and plant nitrogen uptake (N_{off}) at anthesis. However, total number of tillers (P<0.001), total green area per plant (P<0.001), above-ground biomass (P<0.001), plant nitrogen content (%) (P<0.001) and N_{off} (P<0.001) were significantly different among genotype at anthesis. Table 1 shows the number of tillers, green leaf area, above-ground biomass, plant N% and N_{off} of the genotypes at anthesis. Above-ground biomass and grain yield (P< 0.001) at maturity were also significantly different only among genotypes (Fig. 8). Above-ground biomass 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.

According to the results of the present study, remarkable differences have existed among wheat species for plant above-ground parameters and root morphological traits. Spelt genotypes produced a high number of tillers, most of the green leaf area and the above-ground biomass in the present study with vigorous root growth. Spelt is a suitable crop for marginal lands with low nutrient availability and adapted to harsh ecological conditions. Therefore, the requirement for pesticides and other mineral fertilizer inputs are very low (Bonafaccia *et al.* 2000). Fernando and Sparkes (2020) reported that the high N uptake and above-ground biomass production of
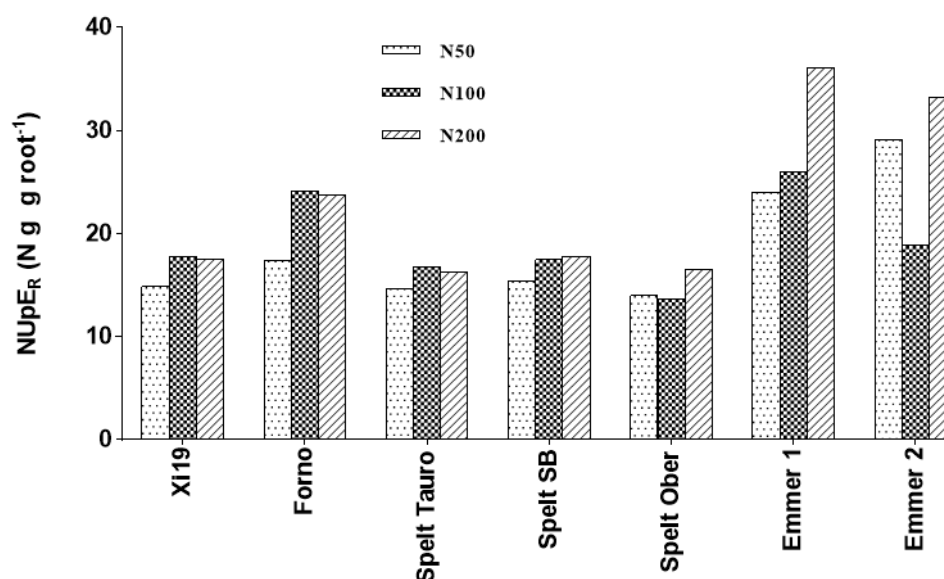

Figure 7: Nitrogen uptake efficiency of roots at anthesis (SED N x GT = 3.86, *df* = 10.45)

Table 1: Number of tillers, green area, above-ground biomass and total plant N uptake per plant (above ground) in seven genotypes at anthesis

GT	Plant N%	Number of tillers plant⁻¹	Green leaf area (cm² 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; GT (*df* = 36)	0.127***	1.231 ***	106.6***	1.411***	0.022***

*** Significant at *P*< 0.001

spelt may be associated with the late start of flag leaf senescence, slow flag leaf senescence rate and long-lasting flag leaf greenness, based on the results of their field experiments. On the other hand, the results of the present study suggest the possible relationship between high NUpE of spelt cv. Tauro and Oberkulmer and root morphological traits such as total root length, root volume, root biomass and rooting depth. The commonly used semi-dwarfing alleles of *Rht-B1b* and *Rht-D1b* in modern bread wheat may be associated with reduced NUpE of bread wheat (Austin *et al*. 1977) and rooting under field conditions. It may be due to severe dwarfing (Wojciechowski *et al*. 2009). However, ancient wheat species do not carry semi-dwarfing alleles.

Straw N%, chaff N%, grain N%, NUpE, NUtE, NUE and NHI calculated at maturity were not significantly affected by GT x N interaction or N level. However, the parameters measured in this study were significantly different among genotypes (P< 0.001) (Table 2 and 3). The highest straw N% was recorded in emmer 1 (0.85%) and the 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% of N. The grain N% of bread wheat cultivars was significantly lower than other genotypes. This phenomenon is recognised as the N dilution effect and happened commonly when increasing grain yield. Grain crude protein percentage is high in spelt and emmer compared to bread wheat hence greater in grain N% (Moudry *et al*. 2011). Wheat and spelt showed low straw N% com-

Table 2: Grain N%, chaff N %, straw N% and total plant N uptake per plant (above ground) in seven genotypes at maturity.

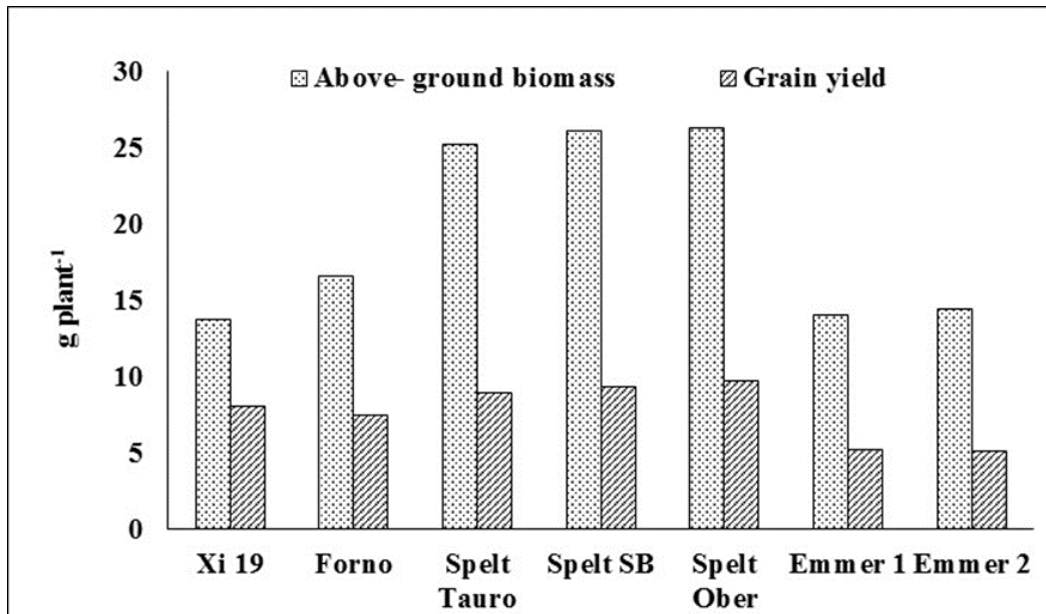| GT	Grain N%	Chaff N%	Straw N%	N*off* (g N plant⁻¹)
Xi19	2.53	0.70	0.75	0.24
Forno	2.96	0.72	0.68	0.28
Spelt Tauro	3.33	0.52	0.64	0.39
Spelt SB	3.09	0.55	0.72	0.39
Spelt Oberkulmer	3.12	0.57	0.61	0.40
Emmer 1	3.03	0.57	0.85	0.22
Emmer 2	3.24	0.71	0.67	0.21
SED; GT (*df* =36)	0.160(36)***	0.057(36)**	0.044(36)***	0.033(36)***

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

Figure 8: Above-ground biomass (AGB) and grain yield (GY) per plant of different genotypes at maturity. SED GT (*df* = 36) for AGB and GY are 2.411 and 1.043, respectively

pared to emmer suggesting their ability to translocate more N towards the production of grains and remain less in straw. However, all spelt genotypes had the highest N_{off} followed by bread wheat and emmer at maturity. High nitrogen uptake of spelt may be due to late anthesis, allowing plants to uptake more N until anthesis. Further, spelt genotypes have more number of tillers and leaf green area where more N accumulated as storage N. The ability of the plant to remove N from the soil as nitrate and ammonium ions is defined as NUpE (Hirel *et al*. 2007). Inorganic N in the soil is from the natural mineralisation of organic matter and N fertiliser. However, plant N uptake may be affected by fertiliser application rate and time, soil type, soil moisture content, type of N fertiliser and soil microbial activities (Petrovic 1990). It is well known that 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 can 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). Furthermore, Wheat

yield and harvest index improved with the increase in chromosome number in evaluation (Evans and Dunstone 1970).

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 3).

Bread wheat recorded the highest NUtE among genotypes due to its greater harvest index. However, NUE of spelt and bread wheat genotypes are higher than emmer genotypes. NUE (the grain dry matter yield divided by available N for the plant through fertilizer and/or soil minerals) is derived by multiplying NUpE and NUtE (Molle *et al*., 1982). Huang *et al*. (2007) found that NUE of wheat increase with ploidy level. 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 a 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 the average

Table 3: 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}) in seven genotypes at maturity.

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; GT ($df = 36$)	0.055***	1.399***	1.762**

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

fertiliser N recovery efficiency of cereals was about 50% (Ladha *et al*. 2005).

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 the growth rate of the plant or efficient uptake of the N from the root system. Later, Ehdaie *et al*. (2001) confirmed for both reasons; plant size and the efficient root systems actively engaged in recovering fertilizer N. Liao *et al*. (2004) reported that early uptake of N in wheat is controlled by fast and early crop growth. 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). N treatment and the interaction effect of N x GT were not significant for any parameter taken in this experiment. This may be due to the rapid downward movement of the nutrients in the column due to hydroleca clay pellets used in the system as a rooting media. However, it suggested that the uptake of N should have done rapidly before leached down to the bottom of the column. Liao *et al*. (2004) suggested that a wheat crop with large root biomass and a deeper root system might be more efficient in recovering soil N. Waines and Ehdaie (2007) revealed that the root systems of the ancient genotypes are large when compared to modern bread wheat cultivars. This may be due to the effect of *rht* genes on root growth. Robinson *et al*. (1994) found a weak relationship between root length density and N uptake efficiency of bread wheat. Similarly, root length density was not significantly correlated with N uptake and anthesis and maturity in our study.

Relationship between nitrogen uptake (N_{off}) and root traits 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 maturity. Nitrogen uptake of the genotypes at anthesis and maturity had a very strong relationship with the total root length of the plant ($R^2=0.80$, $P<0.001$). A strong positive relationship was observed when nitrogen uptake was regressed at anthesis and maturity against the root volume of the respective plant. A similar trend was observed between nitrogen uptake and root biomass at anthesis and maturity ($R^2=0.81$, $P<0.001$). Furthermore, rooting depth (depth score) of the plant at anthesis and maturity had a strong positive linear relationship with N_{off} ($R^2=0.62$). Estimated parameters of comparison regression are shown in Table 4.

Relationship between nitrogen uptake (N_{off}) of wheat species and root traits

Table 4: 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.

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

Nitrogen uptake of different wheat species at anthesis and maturity had a very strong relationship with total root length of the plant (R^2=0.86, P<0.001). A positive relationship between N_{off} with root volume at anthesis and at maturity was found (R^2=0.77, P<0.001). A similar trend was observed between N_{off} at anthesis and maturity for root biomass production (R^2=0.80, R^2=0.77; P<0.001). Furthermore, N_{off} and rooting depth of the plant at anthesis (R^2=0.65, P<0.001) and maturity (R^2=0.70; P<0.001) showed positive relationship (Table 5).

CONCLUSION

Spelt genotypes had the highest N uptake over rest of the genotypes tested. Total root length, root volume, root biomass and RLD were high in spelt genotypes. Total root length, root volume, root biomass and rooting depth were strongly associated with plant N uptake. Our results inferred that high plant N uptake of spelt genotypes may link to the robust and vigorous root systems at anthesis and maturity. Favourable root traits found in spelt, related to N uptake, could be introduced to improve the N uptake efficiency of bread wheat through the direct crossing or creating synthetic wheat.

Table 5: 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. 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

Author contribution
KMCF conceptualized and designed the experiment. KMCF and CW performed the experiment. KMCF analyzed the data. KMCF, CW and DLS wrote the paper with input from all authors. All authors discussed the results and commented on the manuscript.

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