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Root System Architecture and Nitrogen Uptake Efficiency of Wheat Species

K.M.C. Fernando[1*,2], O.G. Ehoche[1], J.A. Atkinson[1] and D.L. Sparkes[1]

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ABSTRACT

Purpose : *Plant nitrogen use efficiency can be increased by improving N uptake efficiency (NUpE). Root system architecture of the plant plays a vital role in uptaking water and nutrients including N. The present study was conducted to compare root architectural traits of different wheat species at early seedling development and vegetative growth stage while plant nitrogen uptake at vegetative and mature stages under controlled environment.*

Research Method : *Ten genotypes belong to four wheat species; einkorn, emmer, spelt and modern bread wheat were compared. Growth pouches were used to develop root systems of seedlings and images were analysed by using RootNav software. Root charcteristics and above-graound growth at vegetative growth phase was studied using semi-hydrophonic system where WinRhizo software was used to analyse root images. Finally, glasshouse pot experiment was conducted to calculate NUpE at maturity.*

Findings : *The highest number of seminal roots, total root length and wider tip angle of seminal root were recorded in emmer. However, total root length was not significantly different from spelt cv. Oberkulmer. Genotypes with narrow and wide tip angles of seminal roots produced deep and shallow seedling root systems, respectively. Positive relationship between root traits and nitrogen uptake at the vegetative growth stage was found. Fertiliser NUpE of ancient wheat species varied significantly among genotypes where emmer recorded the highest value followed by spelt, bread wheat and einkorn.*

Originality/ Value : *Wheat species varied for seedling root system architecture. Emmer had wide and shallow seedling root systems while having high NUpE at maturity. It suggests the importance of a well-spred shallow root system to uptake N fertiliser rapidly before leached down. Further, emmer is an ancient wheat species with an ability to uptake N efficiently which suggests that when bread wheat was selected for high yield, favourable root traits related to NUpE might be neglected. Favourable root traits in emmer related to NUpE could be introduced to bread wheat through the direct crossing or creating synthetic wheat.*

Keywords: *nitrogen uptake efficiency, nitrogen use efficiency, root architecture, wheat*

INTRODUCTION

Nitrogen (N) is an essential element for crop growth and development (Kraiser *et al*., 2011). Globally, the application of N fertiliser in agriculture has increased significantly during the last five decades (Hirel *et al*., 2007), with half of the global food production dependent on synthetic N fertiliser (Dawson and Hilton, 2011). The average application of N fertiliser to winter wheat in the UK was 220 kg N ha^{-1} in 2011 (Defra, 2012) but only some portion of this is taken up by the crop (Barraclough *et al*., 2010). The unused N fertiliser in agriculture has many potential detrimental effects on the environment. It may be lost as surface runoff, leached as nitrate (NO_3^-), and through volatilisation and production of

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[1] *Division of Agriculture and Environmental Sciences, University of Nottingham, Sutton Bonnington Campus, Loughborough, Leicestershire, LE12 5RD, The United Kingdom*

menaka@crop.ruh.ac.lk

[2] *Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya 81100, Sri Lanka*

http://orcid.org/0000-0002-6130-7669

nitrous oxide gas due to microbial denitrification (Conley *et al*., 2009). Improving N use efficiency (NUE; grain yield per unit N availability) of cereal crops through N management strategies (Liao *et al*., 2006), traditional plant breeding methods and biotechnology, while maintaining sustainable yield, would be beneficial to farmers and the environment (Beatty *et al*., 2010). NUE can be achieved by improving N uptake efficiency (NUpE; plant N uptake per unit N availability in the soil) and/or N utilisation efficiency (NUtE; grain yield per unit plant N uptake) (Garnett *et al*., 2009; Ehdaie *et al*., 2010).

Based on the results of QTL studies for N uptake and root traits, it has been suggested that breeding crops with efficient root systems to maximise N uptake is an important goal in wheat breeding (Atkinson *et al*., 2015). Selection for root architectural traits in crop breeding programmes depends on understanding the various components of root architecture (Lynch and Brown, 2012). Bowman *et al*. (1998), experimenting on bentgrass, concluded that plants with large and deep root systems utilise more N fertiliser than those with small and shallow root systems. Deep and narrow root systems provide opportunities to uptake more N from deeper layers of the soil (Garnett *et al*., 2009). Rooting depth of wheat and barley, like other crops, depends on soil texture, structure, nutrition and depth of the groundwater table (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). To acquire sufficient N, plants increase root length density to explore a greater soil volume and increase N uptake (Liu *et al*., 2009) while deep rooting enables plants to take-up residual N from the subsoil (Barraclough *et al.,*2010). Root length and root biomass of wheat have shown to possess strong correlation with N uptake suggesting the contribution of those two traits towards the efficiency of N uptake (Brady *et al*., 1993; Bowman *et al*., 2002).

Nakomoto and Oyangi (1994), working with Japanese wheat germplasm, found that genotypes with narrower angles of seminal roots ended up with deep root systems while shallow root systems had the 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 angle 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.

Breeding for efficient use of N in crop plants requires adequate genetic variability for selection of useful traits. However, genetic diversity of modern bread wheat is narrowed due to random hybridisation events and selective breeding for high yield. Hence, it has become necessary to search for novel sources of genetic variation for NUpE. One of the sources could be the ancient wheat species; einkorn, emmer and splet (Trethowan and Mujeeb-Kazi, 2008; Sparkes, 2010). The aims of this study were to investigate the variability of seedling root system architecture and nitrogen uaptake at maturity of different wheat species.

MATERIALS AND METHODS

Three ancient hulled wheat species (einkorn, emmer, spelt) and bread wheat were used as genetic materials for experiments conducted in 2013, under controlled environment conditions at Sutton Bonington Campus, University of Nottingham, UK. Three einkorn (*Triticum monococcum* L.) genotypes (einkorn 1, einkorn 2 and einkorn 3), two genotypes of emmer (*T. dicoccum*) (emmer 1 and emmer 2), three cultivars (cvs) of spelt (*T. spelta* L.) (SB, Oberkulmer and Tauro) and two cvs of modern bread wheat (Xi 19 and JB Diego) were compared. Uniform seeds of the same diameter were selected using a set of sieves. The seed size of bread wheat cvs, spelt cvs, emmer 1, emmer 2 and einkorn 2 ranged from 2.8 to 3.35 mm while einkorn 1 and einkorn 3 were between 2 to 2.8 mm.

Experiment 1: 2D root phenotyping in growth pouches

The experiment was set up as a completely randomised design with 27 replicates. There were 27 plastic containers with ten place holders and each place had a unique number. Each place holder contains one genotype. Seeds were surface sterilised by submerging in 70 % (v/v) ethanol for 60 seconds and then in 5 % (v/v) sodium hypochlorite (NaOCl) solution for 10 minutes. Seeds were then rinsed thoroughly with sterilised distilled water and germinated on moist blotting paper under dark conditions at 4°C.

Growth pouches consisting of black polythene sheet and blue germination blotting paper were used. The black polythene and blue blotting paper (24.1 x 30 cm, Anchor Paper, St. Paul, MN, USA) were attached to a plastic rod with two 19 mm foldback clips, one on each side of the upper edge of the pouch (Xie *et al.,* 2017). Two days after germination, seeds were placed on the moistened blotting paper with the embryo facing downwards in the growth pouches. Then growth pouches were hung into the plastic containers with 2 cm of the lower end immersed in the nutrient solution (1/4 strength of Hoagland's No. 2, Basal Salt Mixture, Sigma). 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 $Fe_2(C_4H_4O_6)_3$ (5 mg l^{-1}).

The pouches were kept in the growth room under a 12 hour light/dark cycle at 18°C during light and 13°C during dark photoperiod and a photon flux density of 400 µmol m^{-2} s^{-1} for two weeks before capturing images. Images of root systems were obtained using a digital SLR camera (Nikon D5100 DSLR) mounted on a modified copy stand. The images were analysed using *RootNav* software to quantify the number of seminal and lateral roots, total root length, seminal and lateral root length, maximum width, maximum depth convex hull (the area covered by all root materials), tip angles of seminal roots and emergence angles of seminal roots (Pound *et al*., 2013).

Experiment 2: Root morphology at vegetative stage in semi-hydrophonic system

The second experiment was set up according to randomised complete block design with three replicates to account for the variation of photosynthetically active radiation (µmol m^{-2} s^{-1}) in the glasshouse. The seeds were sown in sand compost medium (Levington F2+S) consisting N 150 g m^{-3}, P 200g m^{-3}, K 350 g m^{-3} and pH 5.3-5.7. Ten day old healthy seedlings were transplanted into 3 litre pots filled with hydroleca clay pellets. 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 daylight and the maximum temperature of 30°C.

Plants were harvested at nine day intervals at 14, 23, 32 and 41 days after transplanting (DAT). At each sampling date, a leaf area meter (LI-3100 Area Meter, LI-COR inc, Lincoln, Nebraska, USA) was used to measure the green area of the plant (cm^2) before being dried at 80°C for 48 hours to establish biomass. Soil Plant Analysis Development (SPAD meter) (Minolta, Osaka, Japan) was used to measure chlorophyll concentration index, an indicator of chlorophyll content of the green leaves, avoiding leaf midrib due to thickness and paleness which could affect readings. SPAD measurements were taken on the fully developed, newest emerged leaf.

Roots were extracted from the hydroleca and thoroughly washed with tap water. Cleaned root samples were digitised at 400 dpi resolution and 256 grey contrasts (tiff format) with a scanner with a transparency adapter (*WinRHIZO* STD 1600+, Regent Instruments Inc., Quebec; Canada). The scanned images of the root systems were analysed using *WinRHIZO* regular V.2002c software (*WinRHIZO* STD 1600 +, Regent Instruments Inc., Quebec; Canada). Total root length, mean diameter, root volume and root length in different diameter classes were measured. Following scanning, roots were oven-dried at 80°C for 48 hours before determining their dry weight. Specific root length (length to mass ratio), root length density (length to volume ratio), root elongation rate (root length difference at two sampling points divided by the number of days between sampling points) and root N uptake efficiency ($NUpE_R$; plant N content to unit root biomass or root length) were calculated. The specific absorption rate of N ($S_{AB}R_N$) was calculated based on the relationship described by Hackett (1969) and Liao *et al*. (2004). It was calculated based on root biomass and total root length for the period between the first sampling (14 DAT) and the last sampling (41 DAT), using Equation 1.

$$S_{AB}R_N = (PN_2\text{-}PN_1/T_2\text{-}T_1) \text{ x } (\ln R_2\text{-}\ln R_1/R_2\text{-}R_1)$$

Equation (1)

Where, PN= total plant N (mg), T= time in days, R= root biomass (mg) or total root length (m).

Finely ground plant material (particle size of < 200 µm. 45 to 50 mg) was used to analyse N%. Dumas method was applied by using a Fisons NA-2000 elemental analyser (Fisons, Ipswich, UK) calibrated against Methyl-N standard (N content = 9.28%). N uptake of the plant (except roots) was calculated using Equation 2.

Plant N uptake (g N) = Plant dry weight (g) x N content of the plant (%)

Equation (2)

Experiment 3: Glasshouse pot experiment

One seed, per hole, was sown in modular trays filled with sand compost media (Levington F2+S) and kept in a growth room at 4°C for eight weeks to fulfil their vernalisation requirement. Eight weeks after germination, selected healthy plants were transplanted into two litre black plastic pots with one plant per pot, containing low N compost with P 125 gm^{-3} and K 300 gm^{-3}. The experiment was set up as split-plot design where N treatment was randomised on the main plots, genotypes were randomised on the sub-plots and each treatment was replicated three times. Two levels of N fertiliser were imposed at an equivalent rate of 50 kgN ha^{-1} (LN) and 200 kgN ha^{-1} (HN). Ammonium nitrate was applied in liquid form and 50 kgN ha^{-1} was applied 3 days after transplanting for all plants. Two more applications of 50 kgN ha^{-1} and100 kgN ha^{-1} were used for HN treatment at stem elongation and flag leaf emergence. The experiment was conducted under natural daylight and the maximum temperature of 30°C. Destructive sampling was done at maturity and grain yield and above ground biomass were recorded. Total plant nitrogen uptake (except N in roots) was calculated based on N content of the plant. N % of the straw, chaff and grain were analysed according to Dumas method. N uptake efficiency of the plant was calculated using Equation 3.

NUpE = [Total plant N uptake (g)/total N availability (fertiliser) (g)] x 100

Equation (3)

Statistical analysis

Data were analysed using GenStat 15th edition. ANOVA appropriate to the design of each experiment was performed. Mean differences were tested using LSD at 5% probability level. Pearson correlation analysis was used to examine the relationships between different root architectural traits and above ground growth parameters.

RESULTS

Three experiments were conducted to study seedling root systems architecture on growth pouches, root morphology and N uptake at vegetative stage and NUpE at maturity for ten genotypes of four wheat species. Seedling root architectural traits were measured using images under undisturbed conditions in experiment 1. The mean number of seminal and lateral roots were significantly different between genotypes at 14 days after transferral (DAT) to growth pouch. The greatest number of seminal roots (5.25, *P*< 0.001) and lateral roots (1.42, *P*< 0.01) were recorded in emmer 2, while the lowest number of seminal roots (3.72) was recorded in bread wheat cultivar (cv.) JB Diego (Figure 01A). Einkorn 1 and einkorn 2 did not produce any lateral roots within 14 days. Spelt cv. Oberkulmer produced the longest seminal root (141.6 mm, *P*< 0.001) (Figure 01B). However, all spelt genotypes, emmer 1, bread wheat cv. JB Diego also had long seminal roots at 14 DAT.

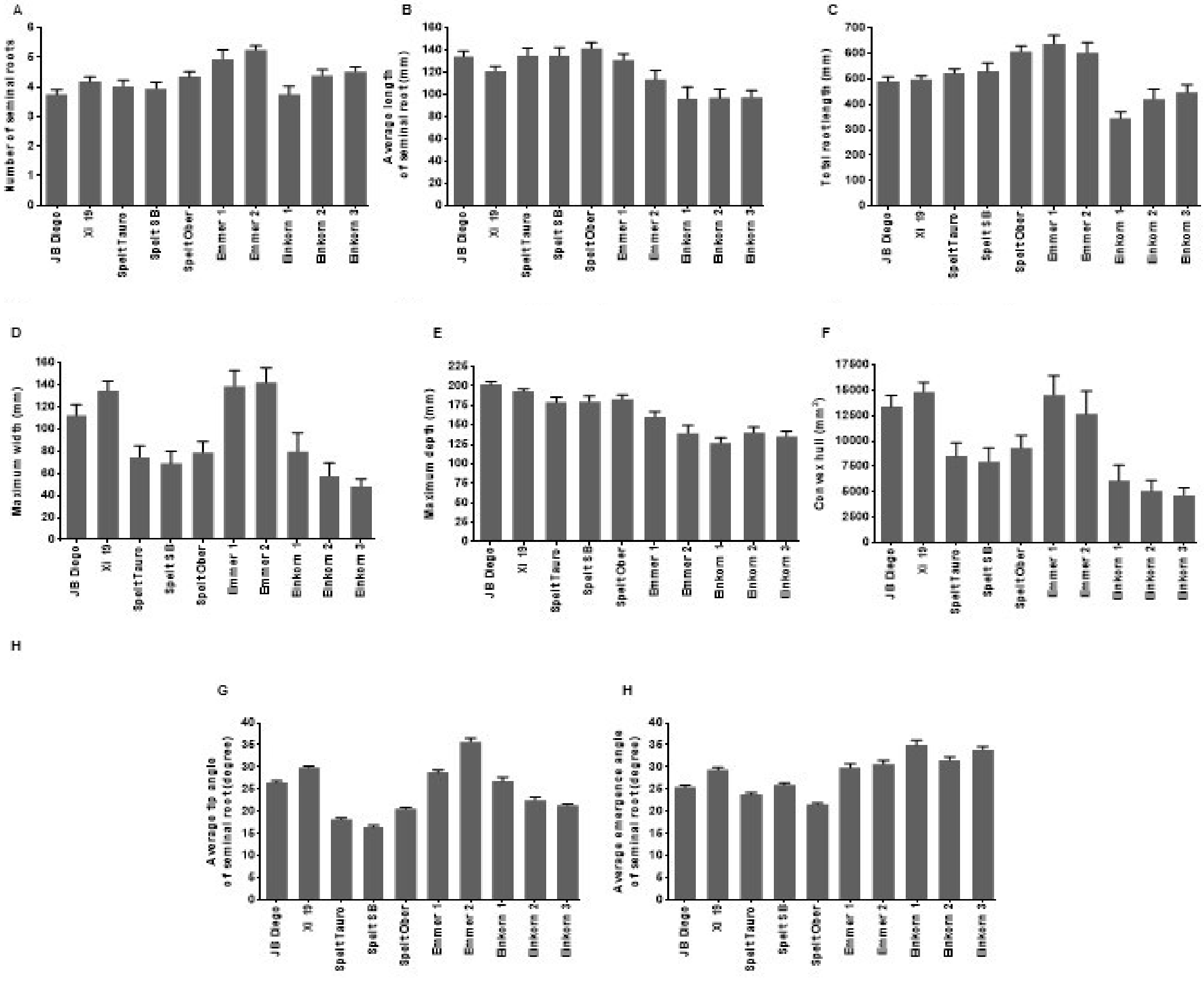

Figure 01: **Phenotypic variation of root system architectural traits. (A) Number of seminal roots (B) average length of a seminal root (mm) (C) total root length (mm) (D) maximum width (E) maximum depth (F) convex hull (mm^2) (G) average tip angle of seminal root (degree) (H) average emergence angle of seminal root (degree), of the root systems grown vertically on growth pouches at 14 DAT. Error bars represent SE of the means of genotypes.**

Total root length was significantly longer in emmer 1 (614.20 mm, $P < 0.001$) when compared to all other genotypes (Figure 01C). The average length of lateral roots was not significantly different between genotypes. Emmer 2 produced root systems with maximum width of 143.74 mm ($P < 0.001$) (Figure 01D) and width to depth ratio (1.06, $P < 0.001$). Root systems of einkorn genotypes were narrow and shallower than all other genotypes. It was observed that bread wheat cv. JB Diego had a deep root system (Figure 01E) recording the maximum root depth of 201.56 mm ($P < 0.001$). Average width to depth ratio for emmer, bread wheat, einkorn and spelt was 0.97, 0.63, 0.48 and 0.42, respectively. The convex hull of the root system was significantly different between genotypes; highest in Xi 19 and lowest in einkorn 3 (Figure 01F). Tip angle of the seminal roots was significantly different among genotypes in the range of 16.73^0 to 35.78^0 (Figure 01G). Emmer 2 recorded the widest tip angle of seminal roots while narrow angles of seminal roots were observed in all spelt genotypes. Average emergence angle of the seminal root was 21.6^o to 35.1^o between genotypes ($P < 0.01$) (Figure 01H). Einkorn 1 showed the widest emergence angles at 14 DAT while spelt Oberkulmer had the narrowest emergence angle which was 61% narrower than einkorn 1. Nevertheless, the average emergence angle of einkorn was not significantly different from emmer species. Also, the emergence angle of emmer was not significantly different from bread wheat while spelt had the narrowest emergence angle at 14 DAT. Figure 02 and 3 show root system architecture (RSA) of 10 genotypes at 14 DAT.

There was a positive correlation between the tip angle of the seminal roots and the maximum width of the root system (r = 0.84; $P < 0.001$). Also, the ratio between width to depth was strongly correlated with the tip angle of the seminal roots (r = 0.92, $P < 0.001$). A strong relationship was observed between the average length of seminal root and the maximum depth of the root system (r = 0.85; $P < 0.01$).

At every sampling date, genotypes differed in the number of tillers per plant ($P < 0.001$) in Experiment 2. Spelt SB had the most tillers while einkorn 2 produced the least. When averaged across species, emmer and spelt showed vigorous tiller production at 41 DAT (Table 01). Green area was the highest in spelt at most of the sampling points followed by emmer, then bread wheat and least in einkorn ($P < 0.001$)(Table 01). Spelt and emmer produced the most shoot biomass when compared to bread wheat and einkorn ($P < 0.001$) (Table 02).

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 (data not shown). The spelt and emmer genotypes had low SPAD values representing less N in their leaves but had higher N uptake than cv. Xi 19. It may therefore, be presumed that the genotypes utilise N differently. While the spelt and emmer used it to produce more shoot biomass with the lower leaf N concentration, modern bread wheat seemed to produce less shoot biomass but with a higher leaf N concentration.

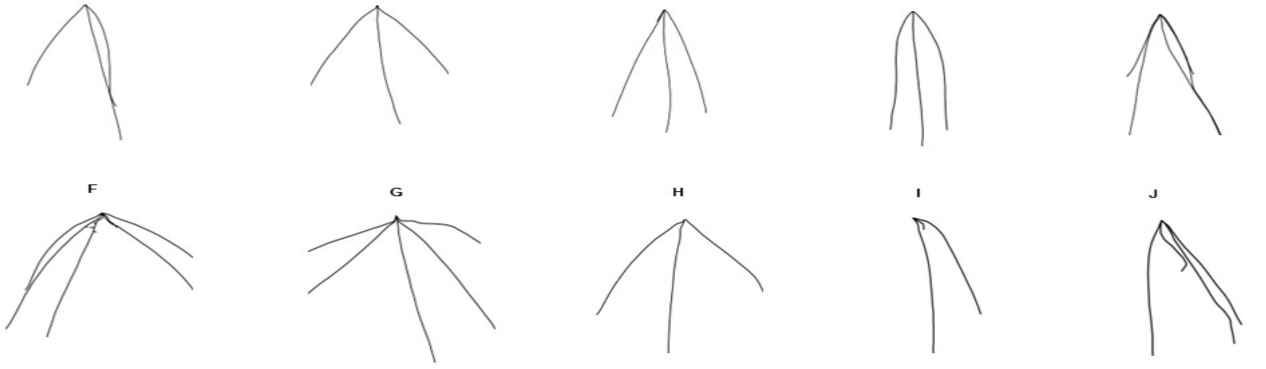

Figure 02: Representative root system architectural images of bread wheat (A) JB Diego (B) Xi 19 (C) Spelt Tauro (D) Spelt SB (E) Spelt Oberkulmer (F) Emmer 1 (G) Emmer 2 (H) Einkorn 1 (I) Einkorn 2 and (J) Einkorn 3 at 14 DAT in experiment 1

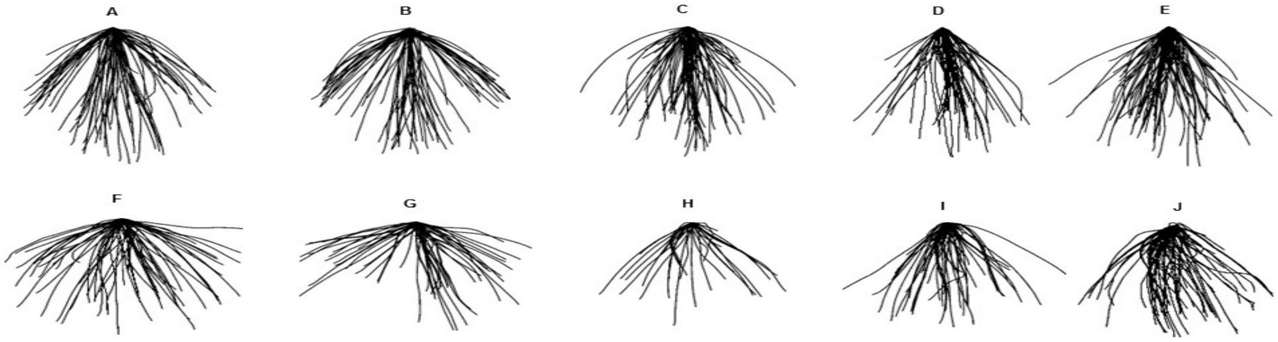

Figure 03: Root system architecture of bread wheat (A) JB Diego (B) Xi 19 (C) Spelt Tauro (D) Spelt SB (E) Spelt Oberkulmer (F) Emmer 1 (G) Emmer 2 (H) Einkorn 1 (I) Einkorn 2 and (J) Einkorn 3 at 14 DAT in experiment 1. These images are produced by overlaying all RSA images of the replicates of 10 genotypes using *RootNav* software (n = 10 to 25)

Table 01: Number of tillers per plant, green area (cm^2 $plant^{-1}$) and biomass production (g $plant^{-1}$) of the plant at 14, 23, 32 and 41 days after transplanting (DAT) in Experiment 2

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)	0.71	2.71	5.66	8.70	33.722	59.504	67.001	269.707
P value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 02: Shoot biomass production (g $plant^{-1}$) and nitrogen uptake (gN $shoot^{-1}$) of the plant at 14, 23, 32 and 41 days after transplanting (DAT) in Experiment 2.

GT	Shoot biomass (g $plant^{-1}$)				N_{off}(gN $shoot^{-1}$)			
	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

GT	Shoot biomass (g plant^{-1})				N_{off}(gN shoot^{-1})			
	14 DAT	23 DAT	32 DAT	41 DAT	14 DAT	23 DAT	32 DAT	41 DAT
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
P value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

The highest shoot N% was recorded in bread wheat cv. JB Diego; 5.06 and 4.14 at 23 and 41 DAT, respectively ($P < 0.001$). Einkorn 2 recorded the lowest shoot N% during the experiment. Table 2 shows shoot N uptake of the ten genotypes throughout the experimental period. The ranking of the genotypes forN uptake at 41 DAT was spelt cv. SB > spelt cv. Oberkulmer>bread wheat cv. Xi 19 and emmer 2 > emmer 1 > bread wheat cv. JB Diego > spelt cv. Tauro>> einkorn 3> einkorn 1> einkorn 2.

The heighest total root length was recorded in spelt cv. SB and Oberkulmer than other genotypes throughout the experiment 2 ($P< 0.001$) (Figure 04A). All einkorn genotypes showed very weak root growth and produced fewer roots than the other genotypes. The total root length of emmer and bread wheat was in between spelt and einkorn. The highest root biomass (Figure 04B) of the genotypes was recorded in spelt cv. SB at 32 and 41 DAT (87% and 81% higher than the lowest value produced by einkorn 2, respectively). The ranking of the genotypes for root biomass production at 41 DAT was spelt cv. SB > spelt cv. Oberkulmer > bread wheat cv. Xi 19 > emmer 2 > bread wheat cv. JB Diego > spelt cv. Tauro > emmer 1 > einkorn 1 > einkorn 3 > einkorn 2.

Similar to the results of total root length, the highest root volume (Figure 05) was observed in spelt genotypes and the lowest values in einkorn. Average root diameter was high in emmer genotypes and lower in einkorn, suggesting that emmer has thicker roots than all other genotypes. Specific root length (SRL) was high in einkorn 2 and low in emmer genotypes. SRL of all genotypes decreased over time, indicating younger plants had thinner roots.

Root length was recorded in different diameter (mm) classes where lower diameters represent more lateral roots and the higher diameter classes represent more seminal and nodal roots. Length of very fine roots (LVFR; < 0.5 mm diameter) represented 78% to 85% of the total root length at 41 DAT, depending on the species.

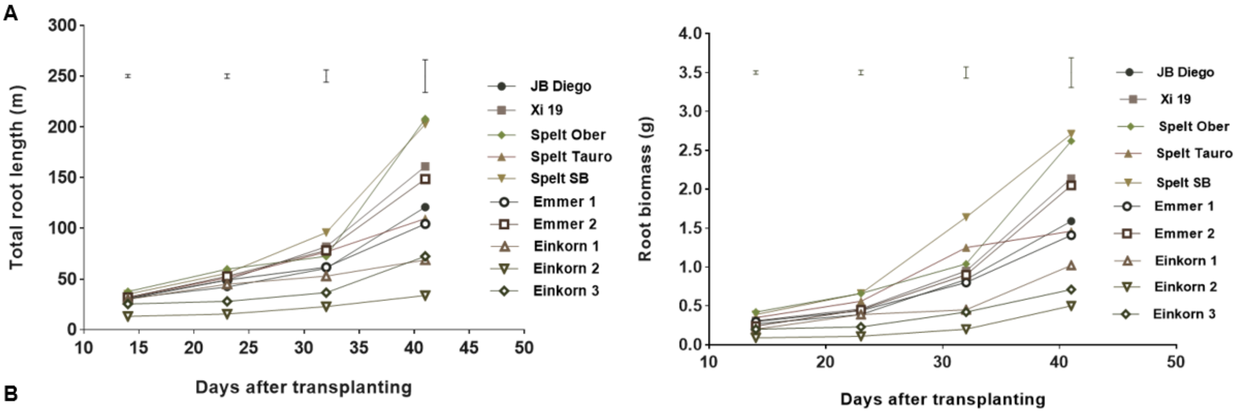

Figure 04: **(A) Total root length (B) root biomass of the genotypes at 14 DAT in Experiment 2. Error bars represent the SED of the genotypes at *P< 0.001* (*df* =18).**

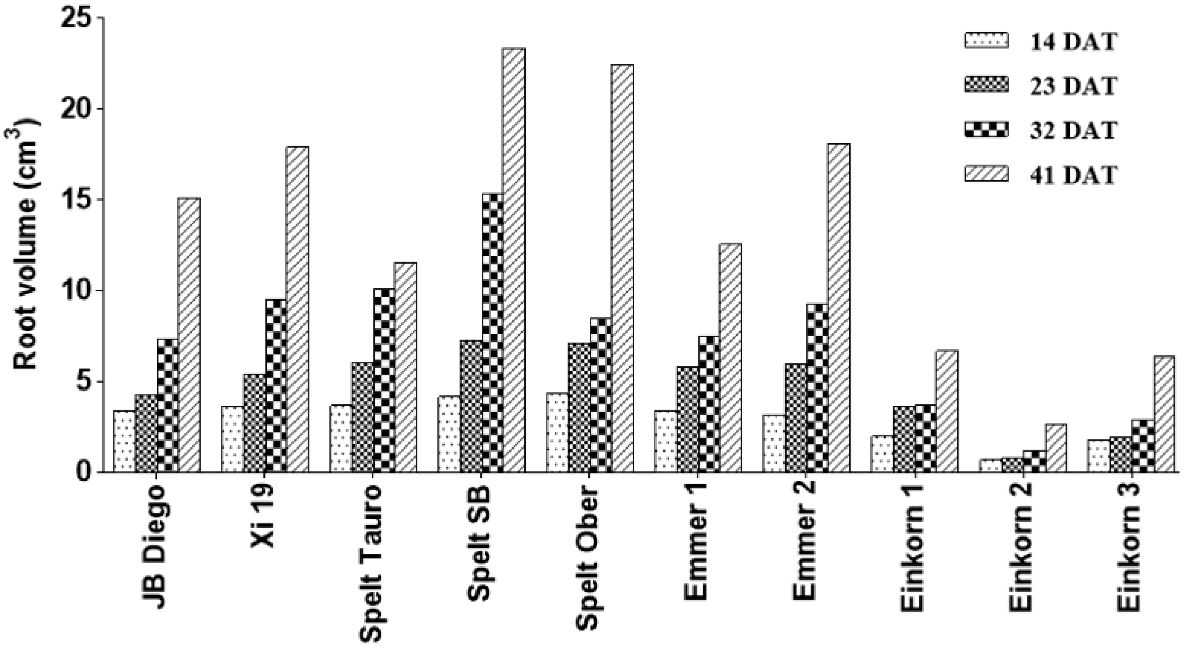

Figure 05: **Root volume of the genotypes at 14, 23, 32 and 41DAT in Experiment 2. SED for GT at 14, 23, 32 and 41 DAT was 0.49, 0.56, 1.69 and 3.68, respectively (*df* =18).**

Spelt genotypes had the highest root elongation rate (RER) and einkorn the lowest. 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. Nitrogen uptake efficiency of roots ($NUpE_R$) was significantly different among genotypes throughout the experiment and mean across all sampling dates, the highest value was recorded in emmer and lowest in einkorn ($P< 0.001$) (Table 03). The average $NUpE_R$ of emmer at 41 DAT was 25%, 35% and 166% higher than bread wheat, spelt and einkorn, correspondingly. The highest specific absorption rate of roots ($S_{AB}R_N$) was recorded in emmer 1 both for root biomass and total root length but there is no significant difference between Spelt cv. SB and emmer 1 ($P< 0.001$) (Table 03).

The green area of the plant at all sampling dates had a positive and strong relationship with respective shoot N uptake ($r = 0.97$, $P < 0.001$). A similar relationship was observed between shoot biomass and N uptake of the shoot ($r = 0.96$, $P < 0.001$). Nitrogen uptake efficiency of roots ($NUpE_R$) explained green area (50% to 66%) and shoot biomass production (53% to 74%) in all genotypes throughout the experiment. The 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 ($r = 0.90$, $P < 0.001$) and therefore, total root length of the plant had a strong relationship with green area production ($r = 0.87$, $P < 0.001$) or number of leaves/ number of tillers per plant at 41 DAT.

Total root length and root length density explained 90% of N uptake of the shoot at 41 DAT. The relationship between root volume and N uptake of the shoot was strong ($r = 0.94$, $P < 0.001$). Root biomass explained 93% of N uptake of the shoot ($P < 0.001$). A close relationship was found between N uptake and length of very fine roots, and more than 88% of the variation in N uptake was explained by this diameter class ($P < 0.001$).

In experiment 3, above-ground biomass (AGB) and grain yield were significantly different between genotypes ($P < 0.001$) and N level ($P < 0.01$) at maturity. 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 04).

Plants treated with HN had greater N% in the straw, chaff and grain than LN plants ($P < 0.05$). Genotypes differed significantly ($P < 0.001$) although there was no significant interaction between genotype and N level. Spelt cv. Oberkulmer recorded the lowest straw N% at 0.42 and 0.86 for LN and HN treatments, respectively, while einkorn 3 recorded the highest values for both N levels (Table 05). Chaff N% varied between 0.33 to 1.04 for LN and 0.88 to 1.59 for HN plants. Highest grain N% was recorded in einkorn 2 in both LN (3.45) and HN (3.99). Bread wheat cv. JB Diego recorded the lowest N% of the grain of 1.40 and 2.19 for LN and HN, respectively.

Table 03: **Root N uptake efficiency (%) at 14, 23, 32 and 41 days after transplanting (DAT) and the specific absorption rate of N based on root biomass (mg g^{-1} root day^{-1}) and root length (mg m^{-1} root day^{-1}) of the plants during 14 DAT to 41 DAT in Experiment 2**

GT	Root N uptake efficiency (%)				Specific absorption rate	
	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
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 04: **Above-ground biomass (g $plant^{-1}$) and grain yield (g plant-1) of the plant at maturity in Experiment 3 at LN (an equivalent rate of 50 kg N ha^{-1}) and HN (an equivalent rate of 200 kg N ha^{-1})**

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*

Table 05: **Straw N%, chaff N% and grain N% of the genotypes at maturity in Experiment 3 at LN (an equivalent rate of 50 kg N ha^{-1}) and HN (an equivalent rate of 200 kg N ha^{-1})**

GT	Straw N%		Chaff N%		Grain N%	
	LN	HN	LN	HN	LN	HN
JB Diego	0.52	1.33	0.59	1.50	1.40	2.19
Xi 19	0.47	1.21	0.42	1.22	1.42	2.28
Spelt Tauro	0.63	1.14	0.44	0.88	2.34	3.20
Spelt SB	0.70	0.89	0.68	1.59	2.20	3.27
Spelt Ober	0.42	0.86	1.04	1.35	3.08	3.62
Emmer 1	0.53	1.11	0.33	1.02	2.42	3.26
Emmer 2	0.53	0.86	0.40	1.23	2.27	3.43
Einkorn 1	0.91	1.43	0.69	1.39	2.86	3.67
Einkorn 2	0.76	1.32	0.72	1.40	3.17	3.89
Einkorn 3	0.95	2.13	0.95	1.44	3.45	3.99
SED ; GT (*df*)	0.190 (36)**		0.135(36)***		0.176(36)***	
N (*df*)	0.81 (2)*		0.104 (2)*		0.098 (2)*	
GT x N (*df*)	0.268 (36.9)^{NS}		0.201(21.6)^{NS}		0.256 (32.1)^{NS}	

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

NUpE of the genotypes was between 0.20 to 0.54 for LN and 0.16 to 0.34 for HN plants. The highest NUpE was recorded in emmer species followed by spelt, bread wheat and then einkorn (*P*< 0.001) (Figure 06) and always higher at LN. However, no interaction was observed between genotype and N level.

DISCUSSION

The development of root systems which promote N uptake is important (Delmer, 2005; Foulkes *et al*., 2009; Gaju *et al.,* 2011) considering the significant impact of artificial N fertiliser on cost of production and its detrimental effects on the environment (Conley *et al*., 2009; Vitousek *et al*., 2009; Dourado-Neto *et al*., 2010).

In Experiment 1 the ancient wheat genotypes, together with bread wheat, exhibited substantial variation in the number of seminal roots which ranged from 3.76 to 5.27, compared to Gregory *et al*. (1978) who reported that, on average, the winter wheat grown in temperate weather conditions produced six seminal roots. The greatest number of seminal roots was recorded in emmer 2, suggesting that emmer has the potential to develop a strong root system at the early stages of crop growth. The total root length of the seedling was also high in emmer when compared to all other genotypes. Therefore, it can be proposed that, mature emmer will develop a horizontally grown root system enabling it to uptake more fertiliser N from the top layers of the soil before it is transferred to deeper layers of the soil horizon.

The seminal root tip angle of emmer 2 was wider than the other ancient wheat genotypes suggesting the development of a wider root system. Supporting the above suggestion, emmer 2 recorded the maximum width of the root system within 14 DAT. Spelt and bread wheat genotypes had a narrow tip angle of seminal roots and deeper root systems when compared to emmer and einkorn. The current results showed some consistency with previous investigations by Nakomoto 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. Liao *et al*. (2004) suggested that wheat crops with a large root biomass and a deeper root system might be more efficient in recovering soil N. In this study, emmer species recorded the highest uptake efficiency of fertiliser N at maturity although bread wheat seedlings produced the deepest roots. This is in partial agreement with previous investigations that the soil N uptake of modern, high yielding bread wheat varieties, are considerably lesser efficient than their ancestors (Foulkes *et al*., 1998), though einkorn recorded the lowest NUpE in present study.

The highest average length of the seminal roots was produced by spelt cv. Oberkulmer. The average length of the seminal roots had a promising relationship with the 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 the mature root systems of emmer and einkorn will be shallower than those of spelt and bread wheat. Lateral root production was significantly different between genotypes. 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 a poor seedling growth. 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). Large root biomass, high root length density and seminal roots with shallow growth angles are among the root traits for a proposed N efficient ideotype capable of efficient N acquisition with early root vigour (Lynch, 2013). These traits are well suited to capture N at deeper soil layers since NO_3^- moves rapidly to deeper layers of the soil profile during the growing season (Lynch, 2013).

According to the results of experiment 2, spelt cv. SB and Oberkulmer recorded the highest total root length, root volume, root length density, root diameter, root biomass and root elongation rate. However, there is an effect of nodal roots on the size of the root systems. Spelt produced a very large number of tillers in the vegetative phase when compared to all other genotypes and had a strong positive correlation between root length. Previous findings suggested that the number of nodal roots of wheat has a 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.

Liao *et al*. (2004) reported that early uptake of N in wheat is controlled by fast and early crop growth. In agreement, in the current study, the highest plant biomass production was recorded in spelt species at early harvesting points suggesting its ability to capture N at early plant development. Regression of shoot biomass and root biomass revealed a strong positive linear relationship, thus it was presumed that higher root biomass translated into higher shoot biomass because bigger root systems were able to acquire more nutrients and support more shoot biomass. However, Gallais and Coque (2005) were of the opinion that a large root system would compete for assimilates, resulting in less assimilate for grain production. Similarly, Passioura (1983) also argued that smaller root systems would increase resource use efficiency by making more assimilate available for shoots. 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). In contrast, our results showed that, while emmer and spelt had large root systems, einkorn produced the least amount of biomass, owing to the small root size that drastically reduced resource capturing capacity.

Nielsen & Schjorring (1983) and Egle *et al*. (1999) observed a significant variation between cereal genotypes on specific root length and root length density. In our study, einkorn genotypes obtained the highest specific root length, indicating it had the thinnest roots, and in fact, 84% of the total root length was $\leq$ 0.5mm in diameter. Bread wheat cv. Xi 19 and emmer genotypes had the lowest specific root length and thus, thicker roots compared to the other species. Root biomass was negatively correlated to specific root length, 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 thicker diameters.

There was a negative relationship between the specific root length and N uptake. Specific root length was found to be highest in einkorn 2 which had the least N. Additionally, total root length of einkorn 2 was significantly lower than all other genotypes. The specific root length 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 Specific root length generally produce higher root length density and therefore, capture resources more efficiently. However, in the current 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 uptake. King *et al*. (2003) affirmed that decreasing 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 hada 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 uptake 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 nitrate leaching, especially in sandy soils (Gastal and Lemaire, 2002). The highest N uptake was recorded in emmer and spelt throughout the experiment and a 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 experiment 3, there was a negative relationship between grain N % and the grain yield, similar to the findings of Oury *et al*. (2003) and Kade *et al*. (2005) who found a negative relationship between grain protein content and grain yield in wheat. This phenomenonis commonly identified as the N dilution effect. NUpE of the mature plant was found to be higher under LN conditions than HN conditions. Ortiz-Monasterio *et al*. (1997), LeGouis *et al*. (2000) and Gaju *et al*. (2011) also reported that NUpE in LN was greater than in HN treated plants. The overall result of these three experiments emphasises the importance of variation in root architectural traits in ancient wheat species and the relationships between early root architectural traits and NUpE at maturity.

CONCLUSIONS

Root architecture of wheat species used in the study varied significantly at the seedling stage. Total root length, root volume, root biomass, root length density and root diameter had strong relationships with shoot N uptake of the plants at vegetative growth phase hence improved green area, shoot biomass production and N uptake. At maturity, NUpE showed a negative relationship with N supply. A significant difference for NUpE was observed between ploidy levels, with a higher value for tetraploid wheat than hexaploid or diploid wheat. These findings suggest that as bread wheat was selected for high yield, favourable root traits related to NUpE were neglected. Emmer has more favourable root traits that are related to NUpE despite genetic background as a tetraploid plant. Further studies are needed to identify QTLs associated with NUpE and root architectural traits of emmer. However, emmer has already been used in wheat breeding programmes since it serves as a gene reservoir for disease resistance and drought tolerance traits. Likewise, favourable root traits in emmer related to NUpE could be introduced to bread wheat through the direct crossing or creating synthetic wheat.

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