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# Relationship between root morphology and grain yield of wheat in north-western NSW, Australia

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## Abstract

Roots are crucial for water up take and nutrient supply both under water limiting and non-limiting conditions, thus influencing crop water-use-efficiency (WUE) and grain yield. The field experiments were conducted in relatively dry conditions in 2009 to assess the impact of genotypic variation for root traits on WUE and yield. Fifteen wheat genotypes were planted in alpha-lattice designs with three replications and tested under high and low moisture regimes. Genetic variability in root length, root diameter and root length density was determined and the impact of these root traits on above ground traits, water use, water use efficiency and productivity assessed. Significant variation in all root traits was observed among wheat genotypes in both high and low moisture environments. A significant reduction in the overall expression of these root traits was observed in response to water stress. Maximum root trait values were observed near the surface (0-15 cm depth) and these decreased with depth with the exception of root diameter in non-water limiting conditions. Mean root length density in both environments was greater than 0.5 $cm^3$ $cm^{-3}$ which was considered sufficient to extract all the moisture from the soil. All root traits were highly heritable and the synthetic derived genotypes were generally superior for all root traits and produced higher grain yield and superior WUE. Stronger relationships between root and above ground traits were observed when water was not limiting. Root traits collectively contributed between 31 and 45% of total variance in improved WUE and grain yield, respectively under water stress and genotypes were identified that extracted water more efficiently under drought resulting in improved WUE and grain yield. It is concluded that under water stress, crop water extraction and adaptation depends on root distribution. The genotypes with superior root characteristics can be combined in crosses with sources of resistance to root diseases, such as crown rot, to improve the adaptation of wheat in north-western NSW.

**Key words:** water deficit, drought, genetic variation, root traits, water use efficiency, wheat.
**Abbreviations:** TRL_total root length (cm) in 0-60 cm soil depth; TRD_total root diameter (mm) in 0-60 cm soil depth; RLD_root length density (cm $cm^{-3}$) in 0-60 cm soil depth; $WU_a$_crop water use at anthesis; $WU_m$_crop water use at maturity; $WUE_{DM}$_Maturity_water use efficiency for dry matter production at maturity; $WUE_{Grain}$_water use efficiency for grain yield.

## Introduction

Roots comprise close to half total wheat plant biomass and are critically involved in water up take and nutrient supply. Despite their crucial role less attention has been given to roots compared to more easily assessable above ground traits. Drought is the most common crop stress globally and characters that improve water-use-efficiency (WUE) such as subsoil water extraction by roots can be enhanced through agronomic management or plant breeding to increase yield. However, the benefits depend on the seasonal pattern of water availability as influenced by rainfall distribution, soil type and management (Lilley and Kirkegaard, 2011). Under soil water deficit, crop water extraction depends on root distribution and depth (Dardanelli et al., 2004). Deeper roots can extract more water from depth thus avoiding water deficits at critical growth stages resulting in higher harvest indices and reduced water loss by deep drainage (Ludlow and Muchow, 1990). Increased early vigour leads to faster, deeper root growth and more adventitious roots in the top soil thus improving water and nutrient use and reducing evaporative losses from the top soil (Richards et al., 2001). Generally, 70% of the total root volume is found in the top 0-30 cm soil layer, where most nutrients are present in the majority of the agricultural soils (Manske and Vlek, 2002). The existing root length density of wheat is not sufficient to extract all the available water deep in the soil profile (Clarke and Townley-Smith, 1984). However, the improved performance of synthetic derived lines under drought stress compared to their adapted recurrent parents was found to be associated with increased partitioning of root mass deeper in the soil profile (between 60-120 cm) thus increasing water extraction from depth (Reynolds et al., 2007). Where soil water is replenished at depth between crops, greater rooting depth leads to improved stability in grain yield, but where the soil water is not replenished greater rooting depth has little advantage (Ludlow and Muchow, 1990). Several researchers have reported the importance of a deep root system for extracting moisture and improving performance under water limited environments in various crops (Sinclair, 1994 in Sorghum; Turner et al., 2001 in pulses; Kamoshita et al., 2002 in rice; Reynolds et al., 2007; Wasson et al., 2012 in wheat). Manske and Vlek (2002) reported that differences in total root volume in 0-100 cm of soil profile were not responsible for improved WUE. However, greater water extraction deep in the soil increases WUE because this water is used solely for transpiration, not lost through evaporation (Richards, 1991). Richards argued that the root mass (adventitious roots) in spring cereals is abundant in the top 30 cm of the soil, and can be reduced by reducing tillering. Passioura (1982) reported that root length density greater than 0.5 cm $cm^{-3}$ can be sufficient to extract all the water from the soil. He concluded that two root traits are important in conferring drought resistance of crop plants. These are; (i) root length density which determines the extent to which the roots can extract water; in wet soils large root length density (> 0.5 cm $cm^{-3}$) at depth is required for the complete extraction of available water, and (ii) longitudinal resistance to flow in the main xylem vessel in the seminal axes which can influence the rate at which water is transported to the shoot through a dry topsoil. Depending on the environment, Passioura suggested either decreasing the resistance (increased xylem) if the crops leave available water in the soil at maturity or increasing resistance (decreased xylem) if the roots leave an insufficient supply of stored water in the soil at flowering to support high harvest index. In the drying soil profile the roots send chemical signals to leaves thus reducing transpiration, decreasing growth rate and increasing WUE (Reynolds et al., 2005). Genes controlling root length and thickness may improve drought tolerance as deeper, more effective root systems avoid or delay the effects of drought (Ober, 2008). Manschadi et al. (2008) suggested that selection for root growth angle and number of seminal roots may result in better adaptation to drought conditions. Vigorous or large root systems contribute to adaptation in dry environments when crops rely on seasonal rainfall; however they are less valuable in environments where the crop growth is dependent on stored soil water due to the risk of soil water depletion during grain filling (Palta et al., 2011). Wasson et al. (2012) recently proposed that wheat varieties with a deep root system, increased root density at depth, decreased root density at the surface and greater radial hydraulic conductivity at depth (through an increase in root hairs and/or xylem diameter) would have higher yield in rainfed systems where crops rely on deep water for grain filling. The simplest way to increase root depth and distribution is to increase the duration of the vegetative period by sowing earlier or planting later-flowering genotypes (Richards et al., 2001). Genotypic variation in wheat root traits has been reported in both controlled environments and under field conditions. However, there is a risk that traits selected in the laboratory on young plants will not translate to superior performance in the field (Wasson et al., 2012). The root study reported here was conducted under natural field conditions in a dry year (2009) at Narrabri in north-western NSW. The objective of the study was to; (i) determine the extent of genotypic variation in root traits at various soil depths in genetically diverse wheat germplasm tested under high and low moisture conditions; (ii) examine the association of root traits with the above ground agronomic traits and their contribution to water use, water use efficiency and productivity, and (iii) estimate the heritability of root traits.

## Results

### *Analysis of root traits*

Significant genotype, depth and genotype x depth interactions were detected for root traits among the fifteen wheat genotypes assessed under high moisture (E1) (Table 1). Root diameter did not differ with soil depth. Genotypes varied significantly for root length, root diameter and root length density at each depth. The genotypes with the greatest total root length and root length density at all depths and the highest root diameter were all synthetic derived. Genotypes 4 and 5 (both synthetic) were superior for all three root traits studied. All root traits had high heritability (H = 0.88-0.98) particularly in the water non-limiting environment. Significant genotypic variability was found for all root traits in the low moisture environment (E2) (Table 2). Significant depth and genotype x depth interactions were also observed. Significant genotypic differences were detected for all root parameters at each soil depth and when averaged over the total soil profile. Genotypes 4, 5, 6, 8 and 11 had the highest root length and root length density, while 4, 5, 6 and 7 had the greatest root diameter. The synthetic derived genotypes 4, 5 and 6 were superior for all three root traits in both environments. Similar to the water non-limiting environment the heritability was high (H = 0.88-0.97) for the assessed root parameters. Root parameters of fifteen genotypes at different soil depths up to 60 cm in E1 and E2 are presented in Figure 1. In E1 the synthetic derived genotypes 5, 6 and 10 had the greatest root length and root length density at all depths. The synthetic genotype 4 had greater root length, root diameter and root length density at 0-15 cm depth only, whereas genotype 11 was superior for root diameter at all depths. Similarly, genotype 6 had a higher root diameter at 30-60 cm depth. In E2 the synthetic derived genotypes 4, 5 and 6 had the greatest root length, root diameter and root length density at all the soil depths studied. Genotype 6 had high values for root traits in this environment. The combined analysis showed a reduced expression of all root traits in E2 and this reduction was observed for all genotypes at all depths.

### *Relationships among traits*

Root traits at different depths showed varying degrees of relationship in E1 (data not shown). Root length and root length density at different depths showed strong associations (r = 0.83-0.91) across depths. Root length and root length density did not correlate with root diameter, whereas both were significantly associated (r = 0.83-1.0) with each other.

The relationships among root and above ground traits, water use and water use efficiency in E1 are presented in Table 3. NDVI was significantly associated (r = 0.66) with root diameter (30-60 cm). Among leaf traits studied, leaf area and weight were significantly associated with root length (0-15 cm) and root length density (0-15 cm). Similarly, leaf length correlated with root length and root length density at all depths. Heading dates of individual genotypes did not affect the expression of root traits. Many spike traits were associated with the root parameters. Number of spikelets per spike and grain weight per spike were significantly associated with the total root diameter (r = 0.56). Significant associations were also detected between all three root traits assessed at different depths in the whole soil profile (0-60 cm) and number of grains per spike and number of kernels per spikelet. Thousand grain weight was not associated with greater root values. Biomass at anthesis was correlated with root diameter (30-60 cm) (r = 0.72) and total root diameter (0-60 cm) (r = 0.54). Increased water use was not associated with higher root trait values. However, root diameter at different depths contributed to higher $WUE_{DM}$-Maturity and grain yield. Root length and root length density showed a weak but positive association with WUE and grain yield. Relationships among root traits at different depths in E2 (data not shown) revealed highly significant associations among depths for root length (r = 0.75-0.96), root diameter (r = 0.65-0.81) and root length density (r = 0.75-0.96). Root length and root length density at different depths were significantly correlated with root diameter. A strong association was also detected between root length and root length density at all

**Table 1.** Combined analysis (mean squares) of mean root traits for different depths and total depth in high moisture environment (E1) during 2009.

| SOV | d.f. | Root length | Root diameter | Root length density |
|---|---|---|---|---|
| Genotype | 14 | 17398.8*** | 0.00140*** | 0.1423*** |
| *Residual* | *14* | *265.1* | *0.00017* | *0.00228* |
| Depth | 2 | 274687.6*** | $0.00004^{ns}$ | 7.81872*** |
| Genotype.Depth | 28 | 2004.0*** | 0.00046*** | 0.02800*** |
| *Residual* | *30* | *270.5* | *0.00010* | *0.00264* |
| Total | 89 | | | |
| Genotype (Individual depth) | | | | |
| 0-15 cm | 14 | 13772.7*** | 0.00062*** | 0.15826*** |
| 15-30 cm | 14 | 2096.3*** | 0.00083*** | 0.02408*** |
| 30-60 cm | 14 | 5537.7*** | 0.00087*** | 0.01596*** |
| Genotype (Total depth, 0- 60 cm) | | | | |
| Genotype | 14 | 52196.3*** | 0.00046*** | 0.04743*** |
| 1 | | 1034.3 d | 0.1827 e | 1.01 cd |
| 2 | | 907.1 f | 0.2110 ab | 0.89 e |
| 3 | | 1051.4 cd | 0.1949 bcde | 1.03 c |
| 4 | | 1099.1 bc | 0.2175 a | 1.09 b |
| 5 | | 1143.4 ab | 0.1883 cde | 1.12 ab |
| 6 | | 1149.1 ab | 0.2040 abc | 1.11 ab |
| 7 | | 1042.3 cd | 0.1903 cde | 1.02 c |
| 8 | | 894.5 f | 0.1994 bcd | 0.87 ef |
| 9 | | 884.6 f | 0.1807 e | 0.86 ef |
| 10 | | 1186.2 a | 0.1627 f | 1.15 a |
| 11 | | 852.4 f | 0.2201 a | 0.83 fg |
| 12 | | 970.3 e | 0.1832 e | 0.95 d |
| 13 | | 775.4 g | 0.1906 cde | 0.78 gh |
| 14 | | 749.8 g | 0.1832 e | 0.74 h |
| 15 | | 640.4 h | 0.1850 de | 0.63 i |
| CV (%) | | 2.9 | 3.9 | 2.9 |
| Heritability | | 0.98 | 0.88 | 0.98 |

*** indicate significant at P=<0.001; ns = Non-significant.

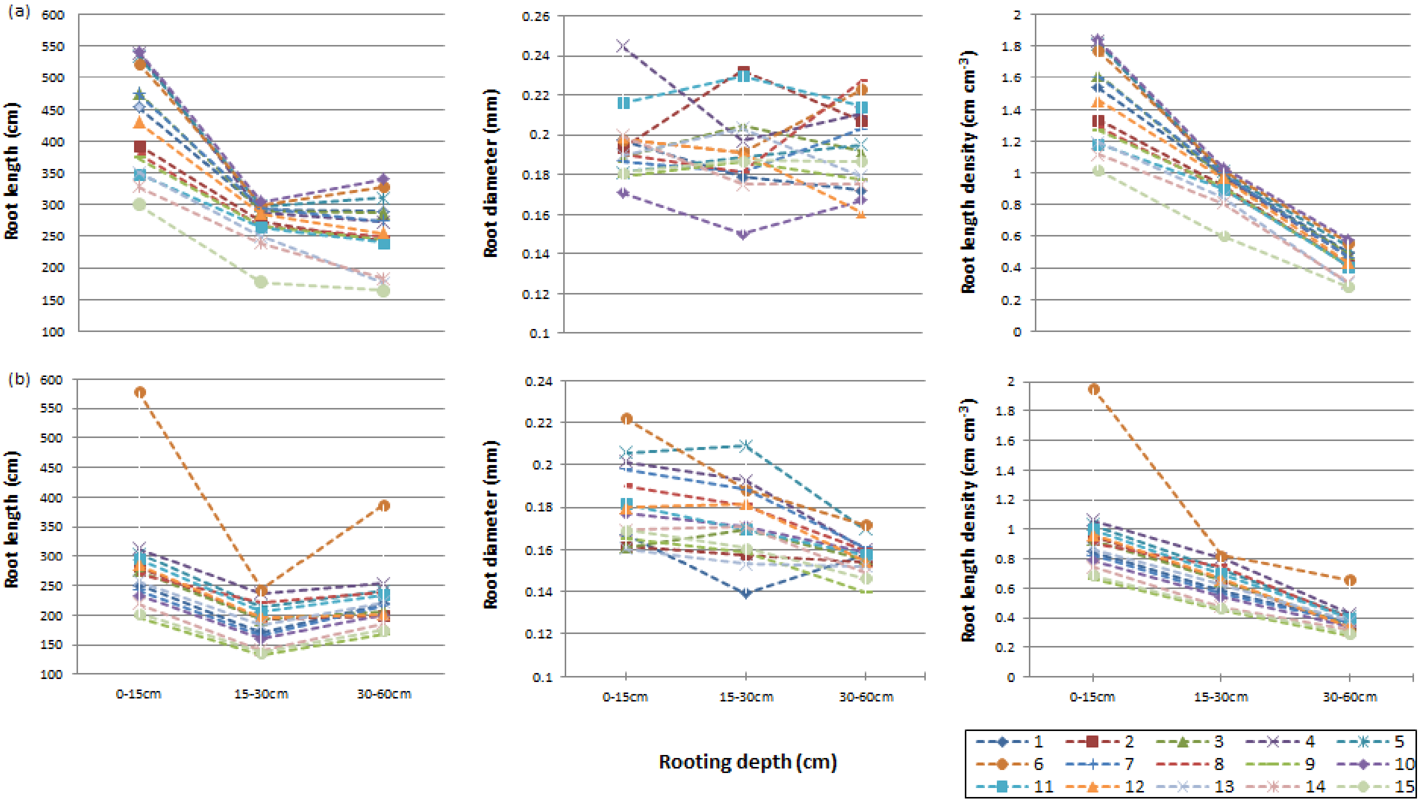

**Fig 1.** Root length (cm), root diameter (mm) and root length density (cm cm$^{-3}$) distribution in different soil layers up to 60 cm depth for 15 wheat genotypes in high (a, upper E1) and low (b, lower E2) moisture environments during 2009.

**Table 2.** Combined analysis (mean squares) of root traits for different depths and total depth in low moisture environment (E2) during 2009.

| SOV | d.f. | Root length | Root diameter | Root length density |
|---|---|---|---|---|
| Genotype | 14 | 18899.7*** | 0.00115*** | 0.15701*** |
| *Residual* | *14* | *536.2* | *0.00016* | *0.00410* |
| Depth | 2 | 67229.9*** | 0.00459*** | 2.45130*** |
| Genotype.Depth | 28 | 2498.9*** | 0.00016* | 0.03575*** |
| *Residual* | *30* | *203.9* | *0.00008* | *0.00184* |
| Total | 89 | | | |
| Genotype (Individual depth) | | | | |
| 0-15 cm | 14 | 16103.2*** | 0.00071*** | 0.18504*** |
| 15-30 cm | 14 | 2440.1*** | 0.00063* | 0.02803*** |
| 30-60 cm | 14 | 5354.1*** | 0.00013* | 0.01543*** |
| Genotype (Total depth, 0- 60 cm) | | | | |
| Genotype | 14 | 56699*** | 0.00038*** | 0.05233*** |
| 1 | | 641.7 efg | 0.1545 f | 0.60 ef |
| 2 | | 674.7 cdefg | 0.1578 ef | 0.65 cde |
| 3 | | 673.3 cdefg | 0.1620 def | 0.64 cde |
| 4 | | 803.5 b | 0.1847 ab | 0.76 b |
| 5 | | 758.0 bc | 0.1947 a | 0.72 bc |
| 6 | | 1208.9 a | 0.1941 a | 1.15 a |
| 7 | | 624.3 fgh | 0.1824 abc | 0.58 efg |
| 8 | | 727.0 bcde | 0.1769 bcd | 0.69 bcd |
| 9 | | 495.6 j | 0.1545 f | 0.47 i |
| 10 | | 593.0 ghi | 0.1688 cdef | 0.56 fgh |
| 11 | | 734.5 bcd | 0.1696 bcdef | 0.70 bcd |
| 12 | | 682.0 cdef | 0.1720 bcde | 0.66 cde |
| 13 | | 662.4 defg | 0.1551 f | 0.62 def |
| 14 | | 545.9 hij | 0.1630 def | 0.51 ghi |
| 15 | | 514.3 ij | 0.1588 ef | 0.48 hi |
| CV (%) | | 5.8 | 4.3 | 5.7 |
| Heritability | | 0.97 | 0.86 | 0.97 |

*, *** indicate significant at P=<0.05 and P=<0.001.

depths. The data for many above ground traits were not available for E2 and several of the assessed traits did not show any association with root traits, hence these relationships are not presented. Early heading genotypes tended to have slightly higher values for root traits (Table 4). Roots in this environment were important in the extraction of soil moisture at anthesis and maturity as shown by generally positive and sometimes significant associations. Root length and root length density at 15-30 cm and root diameter at 30-60 cm showed significant association with $\text{WUE}_{\text{Grain}}$ (r = 0.66 and 0.52, respectively). The other root depths also contributed positively to $\text{WUE}_{\text{Grain}}$ though not significantly. Grain yield was significantly correlated with root diameter (r = 0.51-0.66), root length (15-30 cm) (r = 0.74) and root length density (15-30 cm) (r = 0.74). Total root length and density and total average diameter (0-60 cm) also contributed significantly to increased grain yield.

### *Contribution of root traits*

In E1, regression analysis showed that total root diameter contributed 38.3% of the variance in $\text{WUE}_{\text{DM}}$-Maturity (Table 5). Similarly, total root length and total root diameter were responsible for 37.8% of the variance in $\text{WUE}_{\text{Grain}}$ and 37.9% in grain yield. In E2, all three root traits were important and contributed 30.7% of the variance in $\text{WUE}_{\text{Grain}}$ and 45.3% in grain yield.

## Discussion

Water limitation reduces crop yield in rainfed agriculture worldwide (Manschadi et al., 2006). Maximizing soil moisture capture for transpiration is the main target for yield improvement under drought stress. This can be achieved by improved partitioning of available water for transpiration which is influenced by root depth (Blum, 2009). In Mediterranean environments greater root length in intermediate soil depths (0.5 to 0.6 m) is probably more important than deeper roots for higher water uptake (Gregory et al., 2009). Root diameter is also important as small diameters may limit the rate of water and solute transport to the shoot (Nagesh, 2006). Synthetic hexaploid wheat developed by crossing durum wheat and *Aegilops tauschii* has introduced new genetic diversity for stress tolerance (Trethowan and Mahmood, 2011) and these stress adaptive traits, once characterized, can be utilized in breeding and have contributed significantly to the improved drought adaptation of CIMMYT wheat germplasm (Reynolds et al., 2005). The yield advantage of synthetic derived lines over their parents is due to greater partitioning of root mass at depth (60-120 cm) and increased ability to extract water, not an overall increase in dry root mass (Reynolds et al., 2007).

In the current study significant variation in root traits was observed among genotypes in both high and low moisture environments. A significant reduction in the overall expression of these root traits was observed in response to water stress. Reductions in root length (Asseng et al., 1998), root diameter (Munoz-Romero et al., 2010) and root length density (Schweiger et al., 2009) in wheat were reported earlier under water deficit. Under non-stressed conditions maximum root length and root length density was observed at 0-15 cm depth and this decreased with the depth, whereas root diameter did not reduce significantly with soil depth. Under water deficit there was an observed reduction of all root traits with the highest mean values recorded near the

**Table 3.** Relationship (correlation coefficients) of root traits[1] with above ground traits, water use and water use efficiency in the high moisture environment (E1) during 2009.

| Trait[2] | RL cm (0-15cm) | RL cm (15-30cm) | RL cm (30-60cm) | TRL cm (0-60cm) | RD mm (0-15cm) | RD mm (15-30cm) | RD mm (30-60cm) | TRD mm (0-60cm) | RLD cm cm$^{-3}$ (0-15cm) | RLD cm cm$^{-3}$ (15-30cm) | RLD cm cm$^{-3}$ (30-60cm) | RLD cm cm$^{-3}$ (0-60cm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NDVI (grainfill) | 0.27 | 0.08 | 0.24 | 0.23 | 0.04 | 0.18 | 0.66** | 0.40 | 0.27 | 0.08 | 0.24 | 0.23 |
| LA | 0.61* | 0.24 | 0.38 | 0.49 | 0.16 | -0.17 | -0.11 | -0.06 | 0.61* | 0.24 | 0.38 | 0.50 |
| LL | 0.55* | 0.45 | 0.56* | 0.55* | -0.36 | -0.02 | -0.10 | -0.19 | 0.55* | 0.45 | 0.56* | 0.55* |
| LW | 0.48 | 0.21 | 0.36 | 0.41 | -0.03 | 0.22 | 0.33 | 0.23 | 0.48 | 0.21 | 0.36 | 0.41 |
| LWT | 0.55* | 0.25 | 0.38 | 0.46 | 0.17 | -0.08 | -0.10 | -0.02 | 0.55* | 0.25 | 0.38 | 0.47 |
| DH | -0.37 | -0.08 | -0.20 | -0.27 | -0.18 | -0.19 | 0.18 | -0.07 | -0.37 | -0.08 | -0.20 | -0.28 |
| SL | 0.29 | 0.21 | 0.33 | 0.30 | -0.02 | 0.35 | 0.12 | 0.20 | 0.29 | 0.21 | 0.33 | 0.29 |
| NSPS | 0.05 | 0.23 | 0.17 | 0.13 | 0.10 | 0.54* | 0.63* | 0.56* | 0.05 | 0.23 | 0.17 | 0.12 |
| NGPS | 0.52* | 0.56* | 0.56* | 0.56* | 0.35 | 0.49 | 0.69** | 0.66** | 0.52* | 0.56* | 0.56* | 0.56* |
| GWPS | 0.32 | 0.35 | 0.33 | 0.34 | 0.31 | 0.48 | 0.50 | 0.56* | 0.32 | 0.35 | 0.33 | 0.34 |
| NKPS | 0.71** | 0.64** | 0.69** | 0.71** | 0.40 | 0.30 | 0.54* | 0.54* | 0.71** | 0.64** | 0.69** | 0.71** |
| TGW | -0.17 | -0.45 | -0.35 | -0.29 | 0.00 | -0.27 | -0.50 | -0.35 | -0.17 | -0.45 | -0.35 | -0.28 |
| BIM | 0.26 | 0.09 | 0.16 | 0.21 | 0.23 | 0.29 | 0.72** | 0.54* | 0.26 | 0.09 | 0.16 | 0.21 |
| WU$_a$ | 0.24 | 0.13 | 0.07 | 0.17 | -0.27 | -0.22 | -0.17 | -0.28 | 0.24 | 0.13 | 0.07 | 0.19 |
| WU$_m$ | 0.21 | 0.27 | 0.18 | 0.22 | -0.26 | -0.02 | 0.13 | -0.05 | 0.21 | 0.27 | 0.18 | 0.23 |
| WUE$_{DM}$-Maturity | 0.14 | -0.10 | 0.04 | 0.07 | 0.45 | 0.34 | 0.72** | 0.65** | 0.14 | -0.10 | 0.04 | 0.07 |
| WUE$_{Grain}$ | 0.34 | 0.28 | 0.33 | 0.34 | 0.32 | 0.26 | 0.74** | 0.58* | 0.34 | 0.28 | 0.33 | 0.34 |
| GRY | 0.41 | 0.38 | 0.39 | 0.42 | 0.19 | 0.24 | 0.76** | 0.53* | 0.41 | 0.38 | 0.39 | 0.42 |

[1] The details of root traits are presented in Table 5. [2] NDVI, normalized difference vegetation index; LA, leaf area (cm$^2$); LL, leaf length (cm); LW, leaf width (cm); LWT, leaf weight (mg); DH, days to heading; SL, spike length (cm); NSPS, number of spikelets per spike; NGPS, number of grains per spike; GWPS, grain weight per spike (g); NKPS, number of kernels per spikelet; TGW, thousand grain weight (g); BIM, biomass at maturity (kg ha$^{-1}$); WU$_a$, crop water use at anthesis (mm); WU$_m$, crop water use at maturity (mm); WUE$_{DM}$-Maturity, water use efficiency for dry matter production at maturity (kg/ha/mm); WUE$_{Grain,}$ water use efficiency for grain yield (kg/ha/mm); GRY, grain yield (kg ha$^{-1}$). *, ** indicates significant at P=<0.05 and P=<0.01.

**Table 4.** Relationship (correlation coefficients) of root traits[1] with days to heading, water use and water use efficiency in low moisture environment (E2) during 2009.

| Trait[2] | RL cm (0-15cm) | RL cm (15-30cm) | RL cm (30-60cm) | TRL cm (0-60cm) | RD mm (0-15cm) | RD mm (15-30cm) | RD mm (30-60cm) | TRD mm (0-60cm) | RLD cm cm$^{-3}$ (0-15cm) | RLD cm cm$^{-3}$ (15-30cm) | RLD cm cm$^{-3}$ (30-60cm) | RLD cm cm$^{-3}$ (0-60cm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DH | -0.31 | -0.43 | -0.22 | -0.32 | -0.24 | -0.38 | -0.28 | -0.33 | -0.31 | -0.43 | -0.22 | -0.34 |
| WU$_a$ | 0.51** | 0.35 | 0.49 | 0.49 | 0.53* | 0.48 | 0.39 | 0.52* | 0.51* | 0.35 | 0.49 | 0.49 |
| WU$_m$ | 0.44 | 0.38 | 0.45 | 0.45 | 0.48 | 0.45 | 0.39 | 0.49 | 0.44 | 0.38 | 0.45 | 0.45 |
| WUE$_{Grain}$ | 0.40 | 0.66** | 0.37 | 0.46 | 0.41 | 0.44 | 0.52* | 0.47 | 0.40 | 0.66** | 0.37 | 0.48 |
| GRY | 0.49 | 0.74** | 0.46 | 0.56* | 0.51* | 0.53* | 0.61* | 0.58* | 0.49 | 0.74** | 0.46 | 0.57* |

[1] The details of root traits are presented in Table 5. [2] DH, days to heading; SL, spike length (cm); WU$_a$, crop water use at anthesis (mm); WU$_m$, crop water use at maturity (mm); WUE$_{Grain,}$ water use efficiency for grain yield (kg/ha/mm); GRY, grain yield (kg ha$^{-1}$). *, ** indicates significant at P=<0.05 and P=<0.01.

**Table 5.** Multiple regression analysis using grain yield, $WUE_{DM}$-Maturity, and $WUE_{Grain}$ as the response (dependent) variables.

| Explanatory variables[1] | $WUE_{DM}$-Maturity | $WUE_{Grain}$ | Grain yield |
|---|---|---|---|
| Environment 1 | | | |
| 1. | TRD | TRL | TRL |
| 2. | | TRD | TRD |
| Variance (%) | 38.3 | 37.8 | 37.9 |
| | | | |
| Environment 2 | | | |
| 1. | − | TRL | TRL |
| 2. | − | TRD | TRD |
| 3. | − | RLD | RLD |
| Variance (%) | − | 30.7 | 45.3 |

[1] TRL, total root length (cm) in 0-60 cm soil depth; TRD, total root diameter (mm) in 0-60 cm soil depth; RLD, root length density (cm $cm^{-3}$) in 0-60 cm soil depth; $WUE_{DM}$-Maturity, water use efficiency for dry matter production at maturity (kg/ha/mm); $WUE_{Grain,}$ water use efficiency for grain yield (kg/ha/mm); GRY, grain yield (kg $ha^{-1}$).

− = data not available

**Table 6.** Wheat genotypes used for root studies during 2009.

| Code | Genotype | Type |
|---|---|---|
| 1 | MILAN/KAUZ/5/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/6/TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ | Synthetic (CIMMYT) |
| 2 | CROC_1/AE.SQUARROSA (224)//OPATA/3/PASTOR | Synthetic (CIMMYT) |
| 3 | CROC_1/AE.SQUARROSA (224)//2*OPATA/3/2*RAC655 | Synthetic (CIMMYT) |
| 4 | CETA/AE.SQUARROSA (327)//2*JANZ | Synthetic (CIMMYT) |
| 5 | QT6581/4/PASTOR//SITE/MO/3/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN | Synthetic (CIMMYT) |
| 6 | D67.2/P66.270//AE.SQUARROSA (320)/3/CUNNINGHAM | Synthetic (CIMMYT) |
| 7 | Janz | Cultivar |
| 8 | Giles | Cultivar |
| 9 | Cunningham | Cultivar |
| 10 | Sokoll | Cultivar |
| 11 | Crusader | Cultivar |
| 12 | LPB05-2271 | LongReach advance line |
| 13 | Scout | Cultivar |
| 14 | Envoy | Cultivar |
| 15 | Spitfire | Cultivar |

surface at 0-15cm. Root length density is an important plant trait that changes with water availability; it increases in the top soil layers in water non-limiting conditions but can increase deeper in the soil profile if the upper layers are dry for long periods (Blum, 2005). The mean root length density in the total soil profile (0-60 cm) of the current study was 0.94 and 0.65 $cm^3$ $cm^{-3}$ in high and low moisture environments, respectively, which was sufficient to extract all the moisture from the soil as root length densities greater than 0.5 $cm^3$ $cm^{-3}$ are considered sufficient to extract all soil moisture (Passioura, 1982). Significant genotype × environment interactions were detected for most traits. Overall the synthetic derived genotypes 4, 5 and 6 were superior for all root traits and this was reflected in their greater grain yield and superior WUE. Genotypes 4 and 5 were also among the most drought tolerant group. Although genotype 6 had exceptionally high values for root traits in the water limited environment compared to other genotypes, this may have been influenced by its late maturity. CIMMYT and Australian researchers suggest that the success of synthetic wheats may be due to their deeper and thicker roots which provide better access to soil water (Ginkel and Ogbonnaya, 2007). Deeper root penetration is potentially an important component of drought resistance (O'Toole, 1982; Fukai and Cooper, 1995; Reynolds et al., 2007; Wasson et al., 2012). Richards et al. 2001 suggested that the root depth and distribution can be increased by sowing earlier or planting later-flowering genotypes but in the current study the root traits were not significantly associated with the days to anthesis. This contradiction was resulted as the root traits in the current study were measured comparatively at shallow depth (60 cm). In low moisture environments all root traits at different soil depths were significantly associated. A similar observation was made for root length and root length density in high moisture conditions. More favourable relationships between the above ground traits (such as NDVI, leaf traits, spike traits, biomass at anthesis, WUE and grain yield) and root traits were observed in the non-limiting moisture conditions. Clearly, these easier to measure above ground characters can be used as indirect selection criteria, particularly in wetter conditions where heritabilities are higher. Germplasm selected in this way can be expected to perform well when moisture is limiting as these root traits were also linked to better drought tolerance. Healthy root systems improve water up-take during water stress thus improving plant water status and reducing injury (Cattivelli et al., 2008). Cattivelli aimed to identify non-disease related traits that improve WUE and grain yield that can be combined with improved resistance to disease. In the current study, genotypes were identified that extracted water more efficiently under drought resulting in higher WUE and grain yield. Root traits contributed between 31 and 45% of total variance in improved WUE and grain yield, respectively. Root disease was not a limitation in any of these experiments and those genotypes with superior root characteristics offer prospects to reconstitute plant architecture for superior performance; a combination in crosses with sources of root disease resistance to root diseases, such as crown rot, offers opportunity to improve wheat and the adaptation of wheat in north-western NSW.

The exploitation of genetic variation in root system traits will improve yield and adaptation. Selection for root traits has been limited compared to above ground characters. Stomatal aperture traits which are non-destructive, i.e. canopy temperature depression, stomatal conductance or carbon isotope discrimination, can be measured on the above ground plant quickly and effectively, and can be used as indirect measures of root depth and water use (Richards et al., 2008). The relationship between root traits recorded in 2009 and CTD measured on these genotypes during 2011 was calculated and root diameter was significantly associated (r = 0.66) with the CTD in E1, likewise root length and root length density was significantly associated (r = 0.52/0.54) with CTD in E2. It is clear that the varieties with higher root values capture and transpire more moisture resulting in cooler canopies and greater WUE and grain yield. Plant breeders can select QTLs for both drought related traits (e.g. root traits) and QTLs linked with yield potential to develop new genotypes with superior performance at all moisture levels using marker assisted selection (Cattivelli et al., 2008).

## Materials and Methods

The experiments were conducted during 2009 at the I.A. Watson Grains Research Centre, Narrabri, NSW, Australia. The 2009 winter growing season was relatively dry as it received only 119 mm of rainfall (June to November) compared to the eight year average of 278 mm.

### *Plant material and experimental design*

The fifteen wheat genotypes evaluated in the current study were previously selected on the basis of their superior performance in water deficit environments. These genotypes were tested under two moisture regimes; high moisture (E1) and low moisture (E2). In E1, two irrigations @ 25 mm each were applied 90 and 109 days after sowing to avoid water stress, whereas in E2 no supplementary irrigation was applied (rainfed). The tested material comprised synthetic derived genotypes, Australian released cultivars and an advanced breeding line (Table 6). Material was sown in alpha-lattice designs with three replications in 2 x 6 meter plots. Two irrigations of 25mm each were applied to the high moisture treatment at anthesis and milk stage using an overhead irrigator. The low moisture treatment was exposed to in season rainfall only. No fertilizer was applied before or after sowing in any of the environment. Standard agronomic practices were followed to control weeds and diseases as required.

### *Measurement of above ground traits*

Normalized difference vegetation index (NDVI) was recorded with GreenSeeker® (NTech Industries, Canada) at grainfill. Leaf parameters were recorded at anthesis on ten randomly selected leaves from each plot. Leaf area was recorded with the help of a leaf area meter (Delta-T Devices Ltd, England), leaf length and width with a ruler and fresh leaf weight on an electronic balance. Days to heading were recorded when 50% of plants in a plot were heading. Spike traits were recorded on ten random spikes harvested from each plot at maturity. Biomass was estimated at maturity by harvesting 1m$^2$ area per plot with subsequent drying in a dehydrator (Hurricane, WESSBERG & TULANDER, Australia). Soil water used by each genotype was estimated by placing an aluminium access tube in the centre of each plot to a depth of 60 cm and regular measurement using a neutron probe. Water use efficiency for dry matter and grain yield was calculated by dividing these parameters with the total crop water use. Grain yield was calculated from the machine harvested plot area and expressed as kg ha$^{-1}$.

### *Measurement of root traits*

Root sampling was conducted after harvesting the high (E1) and low (E2) moisture experiments in 2009. Soil cores of 44 mm width and 70 cm length were extracted from the middle of each plot using a tractor mounted hydraulic corer. The entire soil cores were then sectioned into 0-15, 15-30 and 30-60 cm lengths and each section was kept in resealable plastic bags to maintain the sample moisture. All samples were then stored at 5 ºC until washed. Each section was washed in tap water to separate roots from the soil and any debris using a 1 mm mesh sieve. Root data in each section were recorded using a digital image analysis system (WinRhizo Software, Colour Optical Scanner STD4800 with Special Lighting System and Roots Positioning System (Translucent Trays), Regent Instruments Inc., Canada). A transparent 10 x 15 cm tray was used to immerse the roots in water taking care to separate the roots to avoid any overlap and data were recorded using a simple scanning interface. Soil volume of each section was used to convert root length into root length density. Data recorded on root length, root diameter and root length density for each section was then used to estimate a value for the entire soil core.

### *Statistical analysis*

Analysis of variance was carried out for each trait separately and combined over environments using the general analysis of variance procedure of GenStat statistical software, version 14.1 (Payne et al., 2011). The treatment means were compared by Fisher's protected least significant difference test at $P < 0.05$. Relationships among root traits and other parameters were computed using Pearson’s simple correlation test of GenStat (Payne et al., 2011). Broad sense heritability (H) was calculated as described by Sanguineti et al. (2007) on a mean basis across three replications according to the following formula: $H = \sigma^2_G / (\sigma^2_G + \sigma^2_E/r)$
Where, $\sigma^2_G$ and $\sigma^2_E$ represent the genotypic and the environmental components of the phenotypic variance, respectively and r is the number of replications. Multiple regression was used to identify the percentage contribution of root traits to dry matter, grain WUE and grain yield. The General Linear Regression, Forward Selection Procedure of GenStat, version 14.1 was used for this purpose.

## Conclusion

It is concluded that under water stress, crop water extraction and adaptation is influenced by root distribution. Results suggest that the synthetic derived genotypes 4, 5 and 6 with superior root traits, improved WUE and higher grain yield in water deficit can be combined in crosses with sources of resistance to root diseases, such as crown rot, to improve the adaptation of wheat in north-western NSW. Clearly, several genotypes in the current study would be excellent parents for the development of mapping populations for QTL identification.

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