Root architecture - Impacts on late season crop development to improve yield and yield stability under water-stress.

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Key words

late deep root development, growth, senescence, wheat varieties, phenotyping, drought tolerance, crop adaptation

Take home message

- Late season water-stress occurs in most seasons at most locations in Australian cropping regions
- Increased late, deep root development can be advantageous to yield where deep soil moisture is available
- Phenotyping in the field is inefficient for many reasons. Improving the efficiency and reliability of non-field phenotyping is required as differences observed in seedlings or small plants are often not replicated in the field
- A system of phenotyping in 1.5m tubes was developed to reliably study post-heading root development
- Differences between genotypes were observed in well-watered conditions, but differences were much greater when a moderate water-stress was applied post heading
- Differences in late, deep root development can be repeatably detected in this non-field system
- The growth and senesce pattern of roots after heading differ among genotypes
- Results obtained using this method can be used to identify candidate breeding parents for improved drought adaptation and to direct later field-based assessments.

Introduction

Late season water-stress occurs in the majority of seasons and at most cropping locations in Australian wheat cropping regions (Chenu *et al.,* 2013). Improved access to water late in the season is particularly beneficial for wheat crops under late season water-stress, as accessing a relatively small amount of subsoil water can have a major impact on grain yield (Kirkegaard *et al.*, 2007). Wheat crop simulations have shown that additional water used after anthesis can be converted to grain with an efficiency of up to 60 kg ha^{-1} mm^{-1} (Manschadi *et al.*, 2006). Similar results were observed in field experiments (Kirkegaard *et al.*, 2007). Thus, wheat genotypes with greater root length density at depth can improve the water availability for the crop, by improving the rate of water uptake or increasing the amount of water that can be extracted from the soil when water is available at depth (Manschadi *et al.*, 2006; Kirkegaard *et al.*, 2007).

The aim of this study was to characterise post-heading wheat root development over time, in well-watered and water-stressed conditions. The root system of two wheat cultivars were examined at key stages from heading to maturity in well-watered conditions and in a range of post anthesis water stress treatments. A system of 1.5 m polyvinyl chloride (PVC) tubes was developed using Mace and Scout as test genotypes. The system was later used to investigate differences between seven wheat genotypes both above and below-ground in response to water stress right through to physiological maturity.

Materials and methods

Plant material

Two wheat cultivars were chosen to test the phenotyping system. Firstly, Mace which is adapted to the western and southern cropping regions in areas reliant on small bouts of in-season rainfall and has a wide seedling seminal root angle. To contrast with Mace, the genotype Scout was chosen as it is adapted to southern regions often with deeper soils and has a narrower seedling seminal root angle.

Growing conditions

Experiments were conducted in 1.5m long PVC tubes of 90 mm diameter in an outdoor open area during the winter growing season at the Queensland Department of Agriculture and Forestry (DAFQ), Lesley Research Facility in Toowoomba, Queensland, Australia (latitude 27.5598° S, longitude 151.9507° E, altitude 691 m) (Figure 1). Plump seeds of uniform size of each genotype were selected for sowing. Seed was sourced from spaced increase rows with full irrigation grown at Warwick, Queensland in 2017 (28.21°S 152.10°E, 480 m). Three seeds of each genotype were sown in each tube at a depth of 2 cm. Plants were thinned to one vigorous seedling per tube following emergence.

Seeds were sown in a packed soil which had first been airdried and passed through a 2 mm sieve. To ensure non-limiting nutrient supply, 2 gm L^{-1} of Osmocote® fertilizer containing trace elements (N 15.3% P 1.96%, K 12.6%) was added to the soil mix. The soil was watered to field capacity at the start of each experiment.

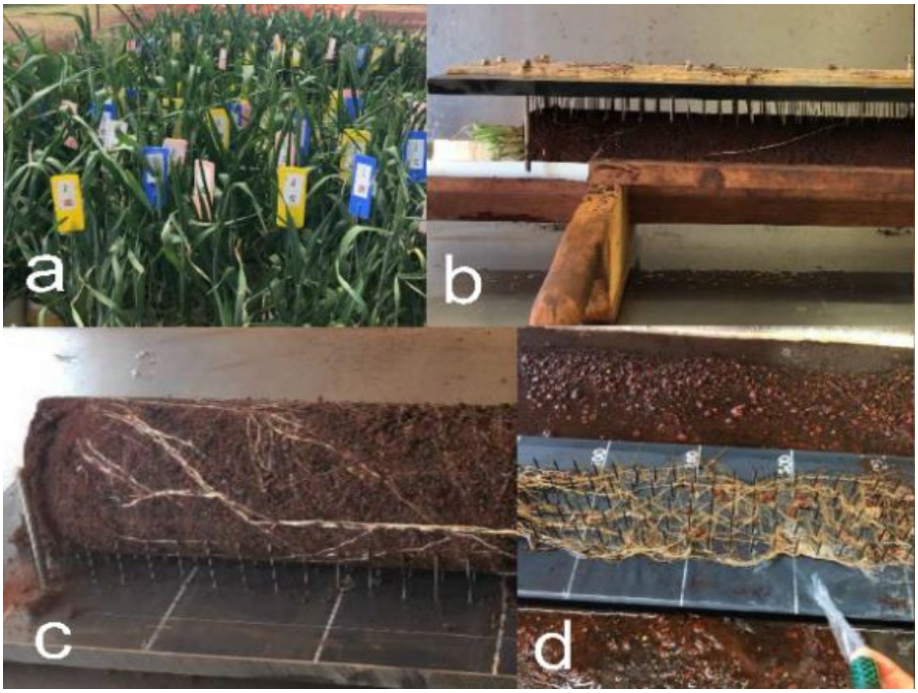

Figure 1. Plants growing in the PVC tube system in an open area in Toowoomba in southern Queensland (a); soil removed from split tubes mounted on the nail board prior to root washing (b) and (c); removal of soil from the nail board by washing (d).

Experimental treatments (TMT) were denominated by the experiment number followed by 'WW' for well-watered, 'MDE' for moderate drought early in grain filling, 'MDM' for moderate drought mid-grain filling, or 'SD' for severe drought from head emergence to maturity (Table 1). Growth stages of individual replicate plants were monitored, and watering withheld between the required

developmental periods. Water withholding periods were determined according to the Zadoks decimal growth stage for individual tubes (Zadoks et al., 1974) (Table 1).

Table 1. Experiment characteristics, including the experiment identifier (ID), treatment name (Tmt), the developmental stages (Zadoks decimal growth stages) between which irrigation was withheld (water deficit period; WD period), average number of days from sowing to anthesis and maturity, and yield per plant (g) at maturity (Z92) for Mace and Scout. Values for days to anthesis and maturity as well as grain yield per plant are the mean and standard error for eight replicates.

ID	Tmt*	Water withholding period**	Genotype	Sowing	Anthesis		Maturity		Yield	
					(Z65)	Difference	(Z92)	Difference	(Z92)	Difference
					(days)	(days)***	(days)	(days)	(g plt-1)	(g)
Experiment 1 2020	E1- WW	none	Mace	4/08/2020	77± 0.9	6	129±0.8	2	16±1.5	-2
			Scout		83± 0.8		131±0.9		14±1.5	
	E1-MDE	60-71	Mace	4/08/2020	75±0.9	5	117±0.8	16	7±1.6	9
			Scout		80±0.8		133±0.9		16±1.8	
	E1-MDM	71-81	Mace	4/08/2020	78±0.9	7	117±0.8	14	8±1.8	4
			Scout		85±0.8		131±0.9		12±1.5	
Experiment 2 2021	E2-WW	none	Mace	24/08/2021	68±1.9	6	106 ±1.9	4	12±0.4	1
			Scout		74±2.2		110 ±2.2		13±0.5	
	E2-MDE	60-71	Mace	24/08/2021	65±1.9	10	96±1.9	23	8±0.4	6
			Scout		75±2.2		119±1.9		14±0.4	
Experiment 3 2019	E3-SD	50-92	Mace	4/07/2019	79±0.8	7	115±0.78	5	2.5±0.19	0.5
			Scout		86±0.8		120±0.78		3±0.19	

*Experimental treatments (Tmt) were denominated by the experiment number followed by 'WW' for well-watered, 'MDE' or 'MDM' for moderate drought early- and mid-grain filling, 'SD' for severe drought from head emergence to maturity.

** Period of withholding watering is indicated by the Zadoks decimal growth stage from when watering was discontinued followed by the stage when watering was recommenced.

***Differences were calculated as the mean value for Mace subtracted from that of Scout.

Plant measurements

Phenological Zadok's decimal growth stages were recorded regularly throughout the experiments (Zadoks *et al.,* 1974). Plants were harvested at heading (Z50), early grain filling (Z75) and at maturity (Z92). Shoots were dried for 72 hr at 70˚C before recording dry biomass. For each harvest, to maintain the root distribution, roots were washed and recovered on a nail board, with nails spaced every 30 mm (Figure 1 b, c, d). The soil was washed from between the nails using a jet of water. Root sections were excised at 10 cm intervals for measurement of dry root biomass. The root biomass was measured following drying as per the shoot samples. Root length density was measured using a WinRhizo Regular 2019 image analysis system.

Design and analysis

For each experiment, a randomized complete block design was used with eight replicates per cultivar for each treatment, a replicate being a single plant in a tube.

Analysis of variance (ANOVA) was performed using a linear mixed model approach in the R platform (v3.2.5; R Core Team 2019). A Student–Newman–Keuls (SNK) test was used to compare means for genotypes and treatments, with a significance level of 0.05.

Results

Moderate water stress increased differences in time to maturity as well as grain yield per plant

Moderate water-stress imposed by withholding water for approximately seven days increased differences between genotypes in the time between sowing and maturity (E1-MDE, E1-MDM and E2-MDE, compared to E1-WW and E2-WW respectively, Table 1). This appeared largely due to water-stress induced senescence in Mace resulting in shortening of the period to maturity of Mace while Scout was little changed. This differential change in the period to maturity was also evident in the differences for grain yield per plant (Table 1). In contrast, a severe stress imposed from heading to maturity (over 40 days) adversely affected both genotypes similarly (E3-SD, Table 1). Severe water-stress tended to greatly reduce the period to maturity for both genotypes as well as the yield per plant.

Differences in shoot and root biomass, were highlighted under moderate water-stress

Genotypic differences in total plant biomass at maturity also tended to be greatest for moderate water-stress treatments (E1-MDE, E1-MDM and E2-MDE, Fig. 2). Differences were larger for shoot biomass, but the smaller values for total root biomass tended to follow a similar trend. With severe water stress, values of all three traits were greatly reduced compared to well-watered conditions, and they differed little between genotypes (E3-SD, Fig. 2)

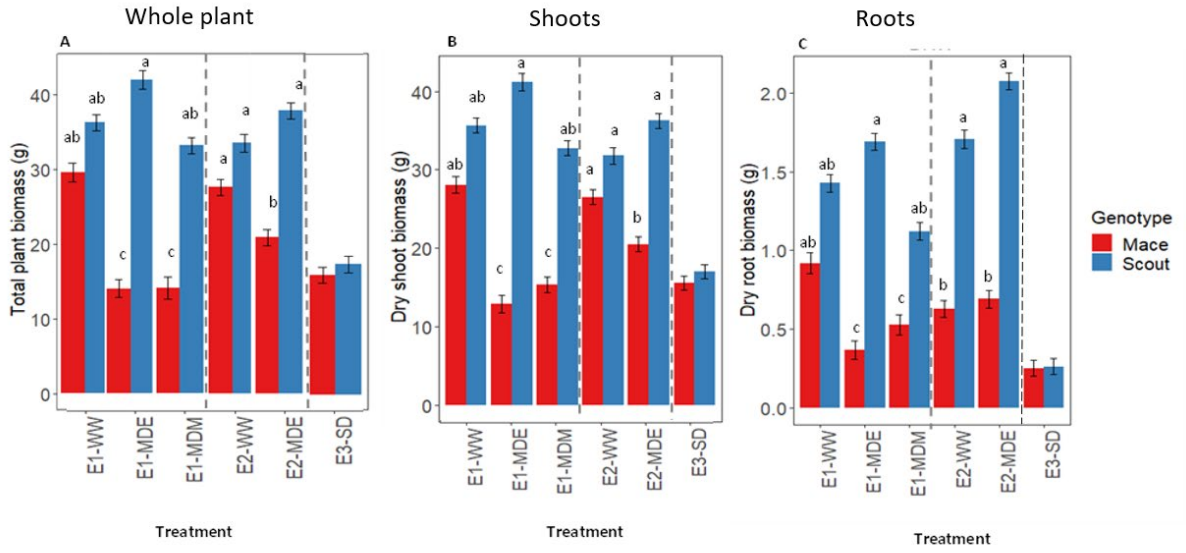

Figure 2. (A) Dry biomass at maturity (Z92) of wheat genotypes Mace and Scout for the whole plant, (B) shoots and (C) roots in different soil water status treatments in experiments E1, E2 and E3 (Table 1). Means that are significantly different (P<0.05) between Scout and Mace within each experiment are shown by different letters above the bars. Error bars represent the standard error of the mean (n=8). The dotted lines separate the three different experiments for which analysis of variance was performed separately.

Water-stress treatment affected the root distribution differently between genotypes

In well-watered treatments, Scout had slightly more roots at most depths than Mace but differences tended to be small (E1-WW, E2-WW, Figure 3 A). For Mace under moderate water-stress conditions, root length density at all depths below 40 cm tended to be less than that for well-watered plants (E1-MDE, E1-MDM and E2-MDE, Figure 3 A). In contrast, for Scout root biomass of plants exposed to moderate water-stress tended to be similar to, or greater than, that of well-

watered plants (E1-MDE, E1-MDM and E2-MDE, Figure 3 A). However, both genotypes had similarly low biomass at all depths when exposed to severe water-stress (E3-SD, Figure 3)

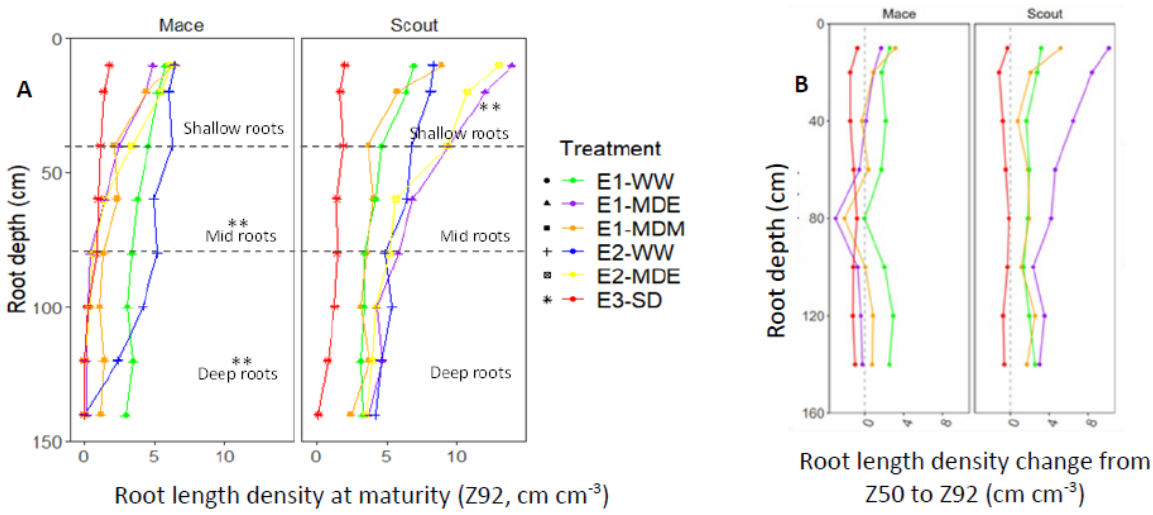

Figure 3. (A) Root length density (cm cm-3) at maturity of Mace and Scout for different depths (0 - 150 cm). The horizontal dotted lines represent partitions between shallow (0 to 40 cm), mid (60 to 80 cm), and deep root (100 to 140 cm) layers. Asterisks indicate differences between treatments for total shallow, mid or total deep layer root length density (P<0.01). (B) Differences in the root length density between heading (Z50) and maturity (Z92) for different depths for Experiment 1 only. The vertical dotted line highlights a value of zero representing no change between stages.

The differences between genotypes in root length density at maturity are likely due to the changes from heading (Z50) to maturity (Z92) (Figure 3 B). For Mace, the root length density increased between Z50 and Z92 at most depths only for the well-watered plants (E1-WW, Figure 3 B). For the moderately water-stressed treatments, there were only small changes from Z50 to Z92 (E1-MDE and E1-MDM, Figure 3 B). In contrast for Scout, root length density tended to increase to a similar degree, or for the E1-MDE possibly even a greater degree, for moderately stressed treatments compared to the well-watered (Figure 3 B). For the severely water-stressed plants, the root length density tended to decrease for both genotypes for most depths between Z50 and Z92 (E3-SD, Figure 3 B).

Differences at individual depths were pooled for three larger soil layers, shallow, mid, and deep layers of 50cm each, as shown in Figure 3 A. Examination of the differences in root biomass in these larger layers indicates similar differences to those observed for other traits. The differences between genotypes are most clearly expressed in the moderately water-stressed treatments (E1-MDE, E1-MDM and E2-MDE, Figure 4). In contrast, there was little difference between genotypes in either the well-watered, or severely stressed treatments (E1-WW, E2-WW, E3-SD, Figure 4). Thus, it appears important to impose a moderate, but not severe water-stress treatment in order to enhance differences between genotypes in root adaptation to water-stress.

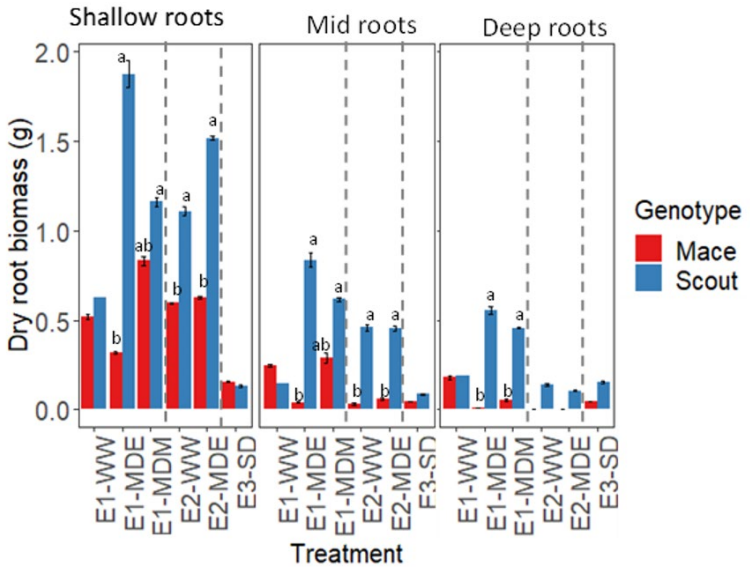

Figure 4. Partitioning of dry root biomass from mature plants (Z92) of Mace and Scout for shallow (0 to 50 cm), mid (60 to 100 cm), and deep (100 to 150 cm) soil layers. Different letters indicate significant differences between means for genotypes (P<0.05). The dotted lines the three different experiments for which analysis of variance was performed separately for each experiment.

Major differences in root development are occurring post heading

Having determined that the genotypic differences in root adaptation to water-stress were highlighted when a moderate post-flowering water-stress is applied, another experiment was conducted where all plants were moderately stressed and measurements taken at three different growth stages. In this instance seven genotypes were examined (Figure 5).

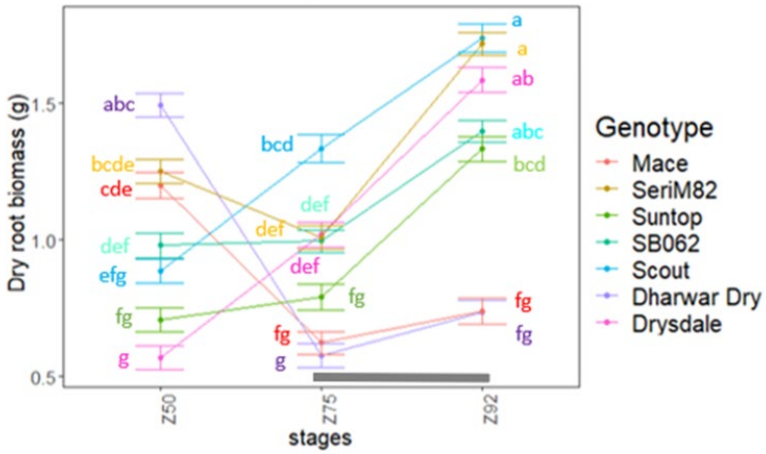

Figure 5. Total dry root biomass (g) of seven wheat genotypes under well-watered conditions at heading (Z50), mid grain-filling (Z75) and at maturity (Z92) after a late moderate water-stress. The horizontal bar indicates the period when watering was withheld to impose a mild water stress. Means that are significantly different (P<0.05) between genotypes within each developmental stage are shown by different letters above the bars. Error bars represent the standard error of the mean (n=8). Analysis of variance was performed together between each stage.

When the dynamics for total root biomass from heading (Z50) to early grain filling (Z75) and then to maturity (Z92) were examined, three groups of genotypes were elucidated (Figure 5). Scout and Drysdale formed a first group which tend to start with a relatively lower root biomass than other

genotypes at Z50 but then exhibited increased biomass at both Z75 and Z92. Suntop$^{\phi}$, SB062 and SeriM82 formed a second group which tended to have intermediate root biomass at Z50, change little by Z75 but then increase rapidly between Z75 and Z92. In contrast to both of these groups, the group containing Mace$^{\phi}$ and Dharwar Dry tended to have higher root biomass at Z50 but this then decreased sharply up to Z75 and remained low between Z75 and Z92 (Figure 5).

The tendency to increase root biomass post-flowering, is likely linked to improved adaptation to water-limitation in environments where water is available deep in the soil late in the season. The tendency for roots to senesce post-flowering may reflect an adaptation to reduce the growth period of roots and conserve photosynthate for grain filling in environments where deep water is not usually present.

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