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REGULAR ARTICLE

Genotypic differences in root penetration ability of wheat through thin wax layers in contrasting water regimes and in the field

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Abstract A significant proportion of arable land in south-western Australia is highly susceptible to subsoil compaction, which limits access of roots of wheat to water and nutrients at depth. Genotypic variation in the ability of roots to penetrate a hardpan has been reported for other cereals, using a pot technique, where a thin wax-layer of paraffin wax and petroleum jelly is placed in a soil column to simulate a hardpan. Previously we have modified and validated this technique for measuring root penetration ability of wheat seedlings under contrasting water regimes. Here we report on a series of five experiments (runs), two in well-watered and three in drought stress conditions, which evaluated seminal and nodal root penetration ability through thin wax layers among 24 Australian wheat cultivars and breeding lines (entries). These results were compared with observations on their rooting depths in two contrasting soil types in field trials, including a sandy duplex that contained a hardpan and a red clay that increased in soil strength with depth. Nodal roots ceased growth early under soil water deficit, and water uptake was instead dependant on seminal roots under conditions imposed in the pots. Plants were then reliant on the ability of seminal roots to penetrate the wax layer. Eight entries had superior root penetration ability in both well-watered and drought stressed conditions. Roots of three other entries, which failed to penetrate the wax layers, died under drought stress conditions. In field trials, there was a significant interaction between site and entry for maximum root depth. Our results from the pot studies and field trials indicate that there exists genotypic variation in root traits that are required to penetrate uniformly hard soil, dry soil or soil containing a hardpan. As four of the eight superior entries also showed superior root penetration ability at both sites in the field, there was an overall consistency, but there were exceptions at individual field sites. Factors

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likely to result in such exceptions were discussed, and topics for further research identified.

Keywords Drought · Traffic pan · Nodal roots · Seminal roots · *Triticum aestivum* L.

Introduction

South-western Australia has a Mediterranean-type climate with a winter-dominant pattern of rainfall distribution. Around 18 million ha of annual crops, primarily wheat, followed by barley, canola and leguminous crops, are sown at the break of season in May. Physiological traits, such as greater early seedling vigour (i.e. leaf area development), may confer a grain yield advantage in this environment by minimising loss of soil water by evaporation while the vapour pressure deficit is relatively low (Richards 1991). Additional water transpired by the crop then contributes to improved grain yield, on uniform soil profiles that receive adequate winter rainfall (Asseng et al. 2003; Botwright et al. 2002). Greater seedling vigour in these environments is considered to require a shallow and extensive root system that is capable of maximising soil water extraction early (Manschadi et al. 2006), and a large root length density in wheat bred for greater seedling vigour has been shown to improve nutrient uptake compared with a less vigorous cultivar (Liao et al. 2004). However, soils in south-western Australia are highly weathered and nutritionally poor, and it is estimated that 24% of agricultural land is highly susceptible to subsoil compaction with 43% moderately susceptible (D. Van Gool, Department of Agriculture and Food Western Australia, personal communication).

Hardpans of compacted soil form at depths of 0.15 to 0.25 m in south-western Australia (Hamblin et al. 1982), in response to traffic from heavy farm machinery, intensive cropping, grazing stock and inappropriate soil management (Hamza and Anderson 2005). Hardpans have a high soil bulk density and small soil pores. Roots can penetrate soil pores that are narrower than their diameter (Bengough et al. 1997), but on encountering mechanical impedance, roots become distorted and thicken radially (Atwell 1990; Bengough and Mullins 1990). Radial thickening is observed to make roots more resistant to buckling in hard soil (Bengough et al. 1997). Restriction of root growth to soil above the hardpan rapidly depletes soil nitrogen and water in the surface soil, which can then promote the early onset of drought and reduced grain yield, which may be further exacerbated by greater early vigour (Barraclough and Weir 1988; Dracup et al. 1992). Management approaches to ameliorating subsoil compaction, such as deep ripping during cultivation and the application of gypsum to improve soil structure and aggregate stability, have been shown to promote root exploration at depth in the soil profile and to improve grain yield (Hamblin and Tennant 1979; Hamza and Anderson 2003). An added benefit would be to identify genotypic variation among the currently-available Australian wheat cultivars and breeding lines for deep roots that are capable of penetrating a hardpan to access water and nutrients below (Botwright Acuna and Wade 2005). An extra 10.5 mm of soil water used in the 1.35–1.85 m layer after anthesis increased grain yield by 0.62 + ha^{-1}, representing an efficiency of 59 kg ha . mm^{-1}, or 3 times that of seasonal water use (Kirkegaard et al. 2007). Such a root system may be similar to the uniform, compact and deep root system suggested as suited for crops grown on stored soil water, unlike the shallow and extensive root system suggested for wheat grown on current rainfall (Manschadi et al. 2006). Interestingly, vertical root growth in unploughed soil was fastest in a wheat line bred for greater early seedling vigour (Watt et al. 2005), although it is not known whether genetic diversity exists for root growth of wheat in soils specifically containing a hardpan.

Root growth is difficult to study, especially in the field. The ability of roots to penetrate hard soil has been simulated experimentally by growing plants in artificially-compacted soil or in pots containing a thin paraffin wax-petroleum jelly layer. An advantage of the thin wax-layer technique, first developed by Taylor and Gardner (1960), is the physical properties of wax are not affected by changes in water content that can change the strength of artificially-compacted soil (Yu et al. 1995). The thin wax-layer technique has been applied successfully in the identification of rice genotypes capable of penetrating hard soils (Babu et al. 2001; Clark et al. 2000, 2002; Price et al. 2000; Yu et al. 1995) and results confirmed in field trials (Cairns et al. 2004; Samson et al. 2002). Our previous research (Botwright Acuna and Wade 2005) modified and validated the thin-wax layer technique for evaluating root penetration of bread wheat under contrasting water regimes. However, no attempt has been made to relate cultivar differences in root penetration ability through thin wax layers to field performance in wheat.

Upland rice cultivars, with their deep and thick root systems, were capable of penetrating hard wax layers of around 1.5 MPa in strength (Babu et al. 2001). The root penetration ability of bread and durum wheat through thin wax-layers was around half that of rice (Botwright Acuna and Wade 2005; Kubo et al. 2004). Partitioning of the soil column by the wax layer made it possible to examine the interaction between hardpan strength and soil water stress. The distribution of seminal roots was less affected by water regime than nodal roots, which were severely reduced in number when drought was imposed at 14 DAS, compared with well-watered conditions (Botwright Acuna and Wade 2005). The number and depth of penetrating seminal root axes declined as wax-layer strength increased, and a significant proportion of total length and DM of main seminal root axes were instead restricted to the soil above the wax-layer (Botwright Acuna and Wade 2005).

The objectives of this paper were to use the thin wax-layer technique, as validated by Botwright Acuna and Wade (2005), to (1) evaluate the ability of roots of a range of Australian wheat cultivars and breeding lines to penetrate a thin wax layer when grown under drought and well-watered conditions in pots; (2) assess root depth in relation to soil properties in the 24 breeding lines and cultivars in the field on two contrasting soil types at Merredin, Western Australia; and (3) relate cultivar differences in root penetration ability through thin wax layers to their root depth in the field.

Materials and methods

Root penetration of thin wax layers under contrasting water regimes in controlled conditions

Our previous results indicated that a 35:65 ratio of wax to petroleum jelly would provide sufficient resistance to root growth to quantify root penetration ability of wheat (Botwright Acuna and Wade 2005). Wax-layers (WV, 35:65 paraffin wax to petroleum jelly, Sigma-Aldrich, equivalent to a strength of 0.45 MPa), 100 mm in diameter and 3 mm thick, were prepared and placed at a depth of 0.24 m in split soil columns, 0.1 m in diameter and 0.5 m tall, as reported in a previous paper (Botwright Acuna and Wade 2005). The soil was a commercial mix of loam, river sand and sawdust (50:40:10), pH 5.5–6.5, amended with the appropriate micro- and macronutrients. Seed of 24 Australian bread wheat cultivars and breeding lines (entries) (Table 1), were pregerminated at 4°C overnight in a Petri dish lined with moist filter paper, and planted at a depth of 20 mm with two replicates in a randomised complete block design (RCBD). Plants were grown in a controlled environment chamber at a 21/16°C day/night temperature, with a 10-h day length and 70% RH. Five experiments (runs) were undertaken. Runs 1 and 2 were well-watered and harvested at 28 and 36 days after sowing (DAS), respectively. Runs 3, 4 and 5 were well-watered until 14 DAS, when water was withheld until harvest at 38, 49 and 52 DAS, respectively. Soil columns were weighed in the drought treatments every 2 to 3 days to measure plant water-use. At the same time, plant development was scored by counting the number of main stem leaves. For all runs, shoots were cut at the soil surface and leaf stage and tiller number recorded. Pots were split in half and roots were washed from the soil column at depths of 0.0–0.12 and 0.12–0.24 m above the wax-layer; and below the wax-layer. The numbers of seminal and nodal root axes were counted in each section. Root and shoot dry mass was measured after drying in an oven at 70°C for 24 h. Data for run, replicate, depth, entry and their interaction were analysed using the generalised linear model procedure GLM in SAS V9.1 (SAS 1990).

Field trial

Field experiments were conducted at Merredin (31° 29′ S.: 118° 12′ E.; altitude 315 m above sea level) in Western Australia in 2005 at two sites with contrasting soil properties. Site 1 was a loamy sand overlying a mottled sandy clay with ferruginous nodules ('sandy duplex'), and site 2 a red sandy loam overlying a clay loam to clay ('red clay'). The sandy duplex is classified as a Calcic Lixisol and the red clay a Calcic Solonetz (Isbell 1996). The sandy duplex had a neutral to moderately acid pH (Tang et al. 2002) and was well-drained, with an upper limit of plant available water of 6.9% and a lower limit of 3.8%

Table 1 Details of wheat breeding lines and entries

Entry	Abbreviation	Release	Maturity	Number of leaves	Number of tillers	Number of seminal roots
Ajana	AJA	WA	S	6.7	8	3.9
Amery	AMY	WA	S	6.5	6	3.8
Brookton	BRK	WA	L	5.7	8	3.8
Camm	CAM	WA	L	5.9	9	4.1
Carmanah	CAR	WA	M	6.2	8	3.2
Cascades	CAS	WA	M	6.7	10	3.5
Chuan Mai 18	C18	China	M	7.3	4	2.7
Cranbrook	CBK	WA	M	6.3	11	3.9
Cunderdin	CUN	WA	M	6.4	9	4.1
EGA Bonnie Rock	BR	WA	M	6.4	9	4.0
EGA Castle Rock	CR	WA	M	5.8	7	3.5
Gamenya	GAM	NSW	M	6.1	9	3.3
Halberd	HAL	SA	L	6.4	9	4.3
Janz	JAN	QLD	L	6.3	6	3.7
Kalannie	KAL	WA	S	5.9	8	3.8
Karlgarin	KAR	WA	M	6.0	10	3.6
Machete	MAC	SA	L	5.7	9	3.7
Perenjori	PER	WA	M	6.1	8	3.0
Spear	SPR	SA	L	5.8	9	3.6
Stiletto	STL	SA	L	5.9	7	4.0
Vigour 18	V18	BL	M	6.4	5	3.8
Westonia	WST	WA	S	6.5	6	3.9
Wilgoyne	WIL	Mexico	S	6.7	6	3.8
Wyalkatchem	WYK	WA	M	6.1	11	3.9
Tukey P=0.05				1.0	3	1.3

Data are main effect of entry on number of leaves and tillers, and number of seminal roots at harvest above the wax layer in controlled conditions.

BL CSIRO Plant Industry breeding line, *NSW* Sydney University, *QLD* Queensland Department of Primary Industry, *SA* Roseworthy College of Agriculture, *WA* Department of Agriculture and Food Western Australia. Maturity classes: *S* short, *M* mid, *L* late.

(Rickert et al. 1987). The red clay had a neutral to acidic pH (Hamza and Anderson 2002) and poorly drained, with an upper limit of plant available water of 9.7% and a lower limit of 5.0% (Rickert et al. 1987). The sandy duplex contained a hardpan at a depth of about 0.2 m, whereas the red clay did not contain a distinct hardpan but soil strength instead gradually increased with depth. Seed of 24 wheat entries were sown 20 mm apart in 1 m long rows with a 0.5 m row spacing on June 15 2005 at the two sites, with two replicates in a RCBD. Plots were fertilised with 90 kg ha^{-1} of urea at seeding and top-dressed with 40 kg ha^{-1} at 21 and 70 DAS. Plots were kept free of weeds, pests and diseases. Soil strength was measured at around anthesis at 75 and 89 DAS on the red clay and sandy duplex sites, respectively, using a Rimik Cone Penetrometer to a depth of 0.6 m, and root depth measured by visually examining soil cores sampled using a 67 mm diameter dormer auger within the row. Soil was sampled at the soil surface and at depths of 0.15–0.25, 0.35–0.45 and 0.55–0.65 m in three plots for measurement of gravimetric soil water content. Shoots of five plants were harvested for each plot at anthesis and maturity and leaf and tiller number recorded. Plants were then dried at 70°C for 24 h and above-ground dry weight recorded. Data for site, genotype, depth and their interaction were analysed using the generalised linear model procedure GLM in SAS V9.1 (SAS 1990).

Results

Do entries differ in seminal root distribution above and below the wax layer?

For seminal root number, there was a significant main effect of entry when averaged across runs and depth (Tables 1 and 2). Entries Camm, Cunderdin, Bonnie

Table 2 Root penetration of thin wax layers in controlled conditions

Source	Shoot components			Root components				
	df	Number of MS leaves	Number of tillers	*df*	Seminal root		Nodal root	
					Number of main axes	Dry matter (mg)	Number of main axes	Dry matter (mg)
Run	4	51.63***	343.53***	4	24.17***	329690.41***	4343.71***	380825.21***
Within-run error	5	0.71	12.85*	5	1.62	9617.15	1.85	1376.71
Depth	–	–	–	2	1180.28***	1670533.00***	6639.00***	740027.13***
Entry	23	4.76***	32.00***	23	3.86**	11191.37*	16.56***	4848.60***
Depth × entry	–	–	–	46	1.21	11629.95**	9.30***	2671.90**
Run × depth	–	–	–	8	9.47*	85473.41***	1365.01***	106514.80***
Run × entry	92	0.49**	6.53**	92	1.82	12201.55***	9.97***	2617.23***
Run × depth × entry	–	–	–	184	1.03	8623.05	5.44*	1599.78
Residual error	114	0.30	4.11	350	1.51	7079.73	4.18	1473.62
Contrasts								
DS v WW		0.19***	0.08***	1	0.014***	0.0001	0.29***	0.23***

Mean squares for run, depth, entry and their interactions, and treatment contrasts for shoot and root components.

DS drought stress, *MS* main-stem, *WW* well-watered.

*,**,***Indicates statistical significance at P=0.05, 0.01 and 0.001, respectively.

Rock, Halberd and Stiletto tended to produce an average of four or more seminal roots, while C18 produced the fewest seminal roots (Table 1). Although the depth × entry interaction was not significant for seminal number, Cranbrook and Halberd had the most roots below the wax layer in WW and DS conditions, respectively (Fig. 1a). No roots of entries C18, Cranbrook or Karlgarin penetrated the wax layer under DS conditions (Fig. 1a).

The depth × entry interaction was highly significant for seminal DM (Table 2), and a test of the effect of depth within the depth × entry interaction revealed significant differences in DM below (P=0.0001), but not above, the wax layer. Figure 1b shows the relationship between seminal DM in WW and DS conditions (contrast significant at P=0.0001). Around twice the seminal root DM was produced in DS than in WW conditions (Fig. 1b). Roots of entries C18, Karlgarin and Cranbrook did not penetrate the wax layer (Fig. 1b). Machete and Camm produced the most seminal DM below the wax layer under DS, while Bonnie Rock, Halberd, Stiletto and Wilgoyne produced more seminal DM below the wax layer in both environments.

Do entries differ in nodal root distribution above and below the wax layer?

The depth × entry interaction was highly significant for the number and DM of nodal roots (Table 2), and a test of the effect of depth within the depth × entry interaction revealed significant differences in number and DM above (P=0.0001), but not below, the wax layer. The number and DM of nodal roots across entries in the 0–0.12 m depth was strongly correlated with that at 0.12–0.24 m (r=0.66, P=0.004; r=0.60, P=0.0019, respectively), so only data for the 0–0.12 m depth are presented in Fig. 2. Drought stress conditions restricted the appearance and hence DM production of nodal roots compared with WW (Fig. 2). Superior entries included C18, Wyalkatchem and Cascades under WW conditions. Wyalkatchem produced the most nodal roots across environments and entries (Fig. 2a), and its ranking for nodal root DM rose from 11 in WW to 1 in DS conditions (Fig. 2b). In comparison, C18 was ranked highly for nodal root DM across environments, and its ranking for number of nodal roots rose from 12 in WW to 1 in DS conditions. Gamenya was ranked 24 for both nodal root number and DM (Fig. 2).

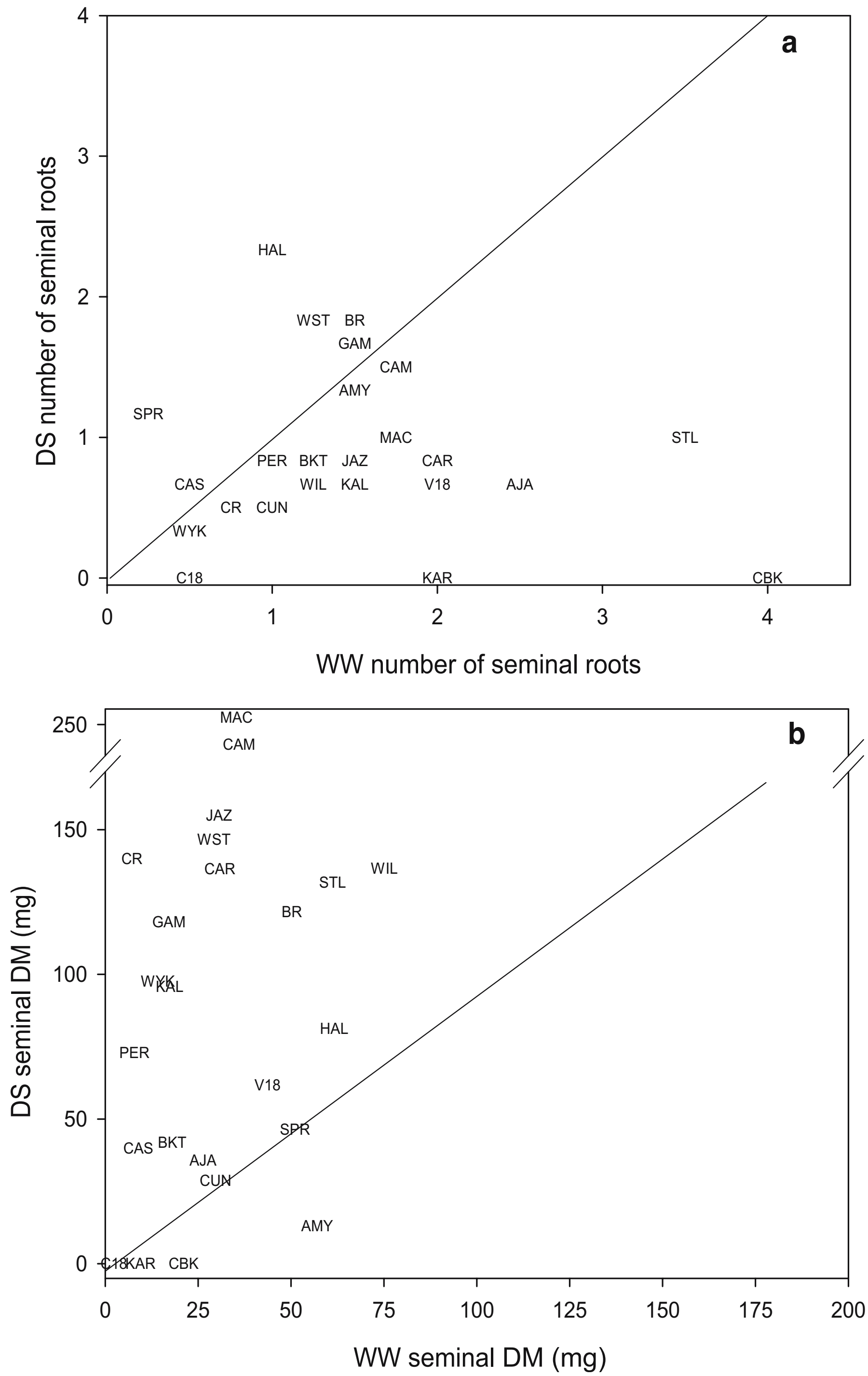

Fig. 1 Effect of water regime on seminal root **a** number; and **b** dry matter below the wax layer for 24 wheat entries. Refer to Table 1 for cultivar abbreviations. *DS*, drought stress; *WW*, well-watered. The line represents the 1:1 ratio. LSDs: **a** DS=1.5, WW=3.0; **b** DS=190, WW = n.s.

Role of root penetration through thin wax layers in water deficit

For simplicity, root penetration of only four of the 24 entries are presented in Fig. 3. Main stem leaf appearance and plant water use were similar until 15 days after stress imposition (DASI, Fig. 3). Camm and Machete mostly maintained rate of leaf appearance during the experiment and an average of three seminal roots penetrated the wax layer. Dry weight of

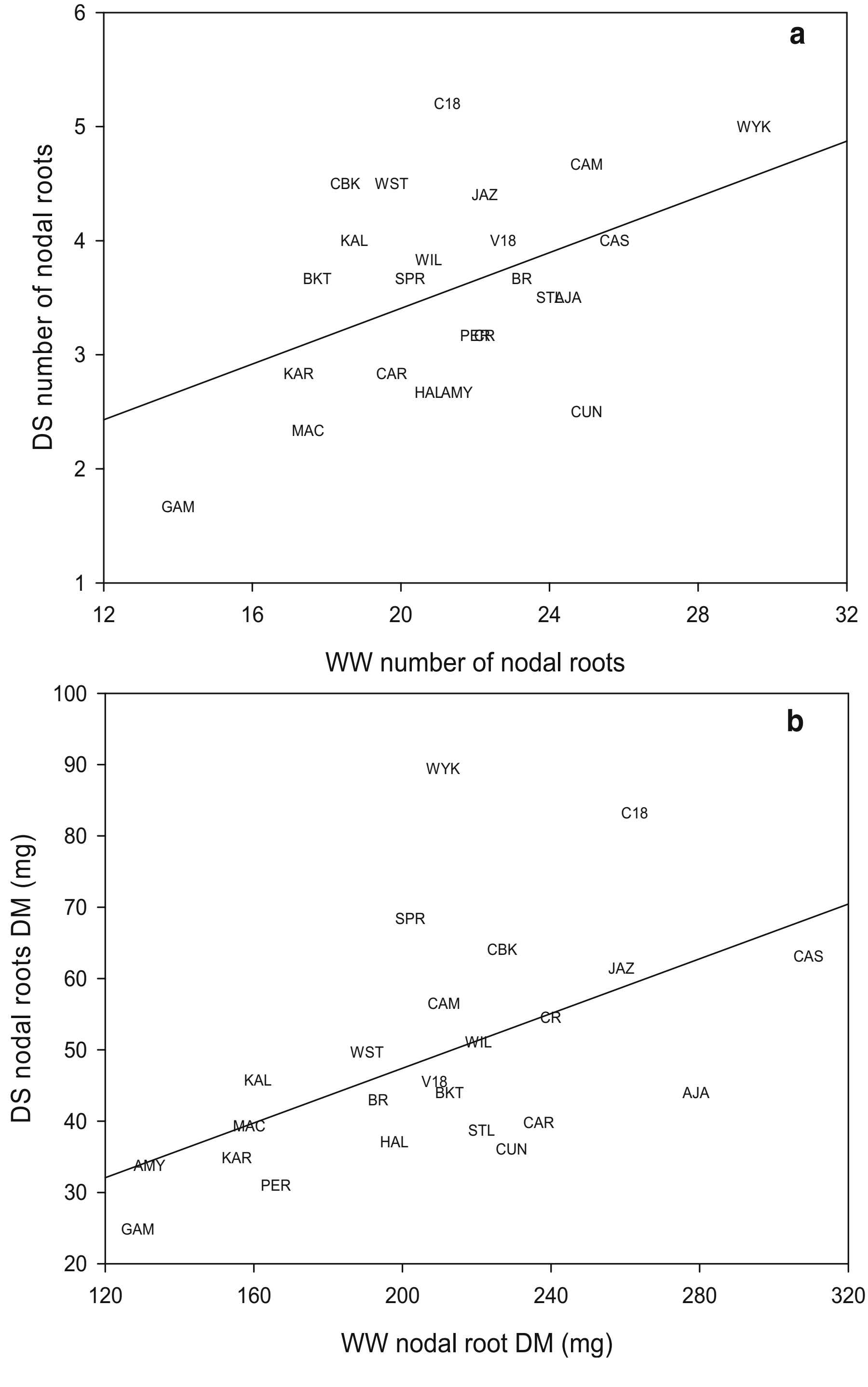

Fig. 2 Effect of water regime on nodal root **a** number; and **b** dry matter, in the soil surface 0–0.12 m for 24 wheat entries. Refer to Table 1 for cultivar abbreviations. *DS*, drought stress; *WW*, well-watered. The line represents the 1:1 ratio. LSDs: **a** DS=2, WW=12; **b** DS=44 mg, WW=135 mg

roots below the wax layer for Camm and Machete was 37 and 70 mg, respectively. Leaf appearance of C18 was faster than Camm, at 0.20 leaves day^{-1}, while Cranbrook and Machete reached an average maximum of 6.5 leaves at 24 DASI (Fig. 3a). Water use of C18 and Cranbrook declined relative to that of Camm and Machete from 17 DASI onwards (Fig. 3b). No roots of C18 or Cranbrook penetrated the wax layer (Fig. 1).

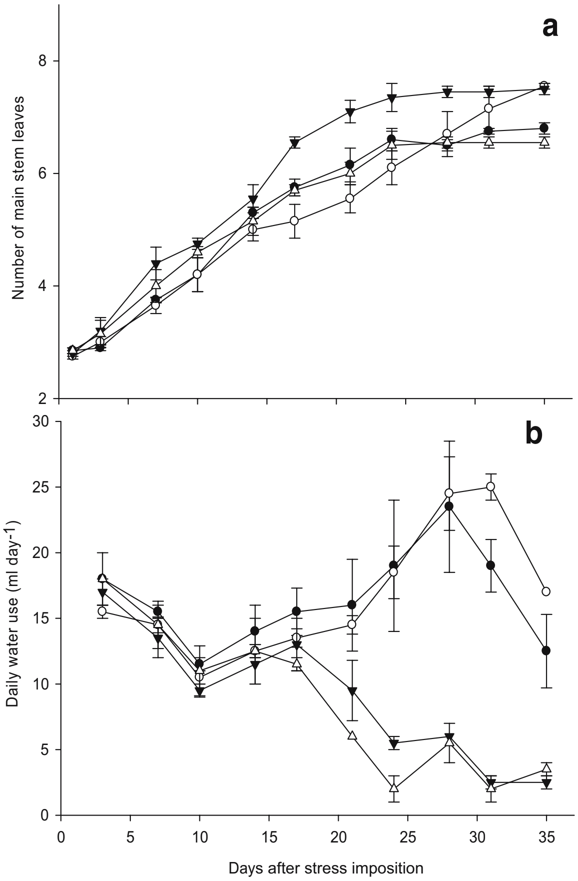

Fig. 3 Number of main stem leaves and daily water use over time of selected entries for run 5 under drought-stress in controlled conditions. *Filled circle*, Camm; *open circle*, Machete; *filled triangle*, C18; and *open triangle*, Cranbrook

Effect of wax layer and water regime on shoot components

There were significant main effects on number of main stem leaves and tillers (Table 2). In general, C18 produced the greatest number of leaves across runs, but had the fewest tillers (Table 1). V18 similarly produced few tillers, while Cascades, Cunderdin, Machete and Wyalkatchem tillered profusely. Brookton, Perenjori and Stiletto produced the fewest leaves (Table 1). Maturity class affected shoot, but not root components (data not shown). Short season wheats produced more main-stem leaves but fewer tillers in total than long season wheats (6.6 *vs*. 6.1 leaves and 6.6 *vs*. 8.7 tillers, respectively).

How consistent are the data: the effect of run

Run had a large and highly significant effect ($P < 0.001$) on the number of main stem leaves and tillers (Table 2). Differences in leaf and tiller number among runs were correlated with the duration of growth (Table 2). For example, run 2 was harvested 8 days later than run 1 and produced an additional two leaves and six tillers on average (Table 2). Run, depth and the run × depth interaction were highly significant for all root components and dominated the mean squares computed by ANOVA (Table 2). The within-run error was not significant, however, indicating that the data for root components were consistent within runs (Table 2).

An average of five seminal roots was produced across runs in the soil surface (Fig. 4a,b). Around 25 and 50% of seminal roots penetrated the wax layer under WW conditions in runs 1 and 2, respectively. Plants were harvested 8 days later in run 2, which may have contributed to more roots penetrating the wax layer than in run 1. Drought stress conditions in runs 3, 4 and 5 resulted in fewer roots penetrating the wax layer than in runs 1 and 2 (Fig. 1b).

Seminal DM was largest in all runs above the wax layer at a depth of 0.12–0.24 m. Seminal DM among entries varied for runs 1 and 2 at 0.12–0.24 m and for runs 3 to 5 at all depth intervals (Fig. 4a,b). For runs 3 to 5, the difference in seminal DM is consistent with increasing plant age (Fig. 4b).

Many nodal roots were produced in the soil surface layer at 0.0–0.12 m, particularly in runs 1 and 2 under well-watered conditions (Fig. 5c). Plants in run 2 at harvest were 8 days older than run 1, and had more nodal roots and DM above the wax layer (Fig. 5c,d). The numbers of nodal roots were relatively consistent among runs 3 to 5 and were not correlated with plant age (Fig. 5c). Nodal DM, in comparison, was smaller in run 3 than either runs 4 or 5 under DS conditions, which was correlated with plant age at harvest (Fig. 5d).

Field trial

Rainfall from March to September at Merredin exceeded the long-term mean in all months, except for July, which was particularly dry (Table 3). Maximum air temperatures ranged from 32.1°C in March, to 16.1°C in June, with July and August experiencing the coolest minimum temperatures of around 4°C.

Soil strength of the red clay increased gradually with depth, reaching a maximum of 4 MPa at 0.6 m

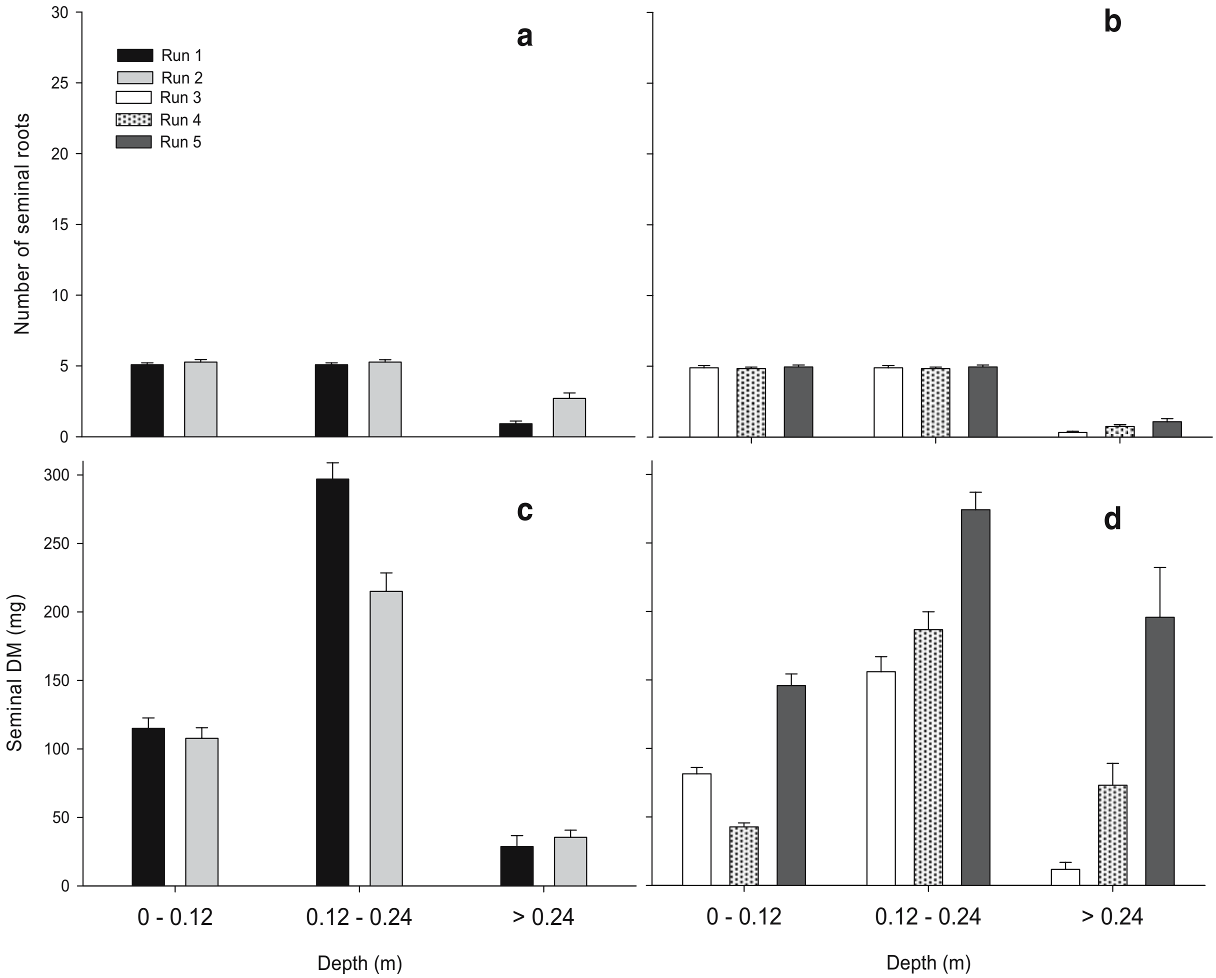

Fig. 4 Effect of run, depth and water regime on seminal root number in **a** WW and **b** DS; and seminal root DM in **c** WW and **d** DS conditions

(Fig. 6a). In contrast, soil on the sandy duplex site contained a distinctive hardpan of 4 MPa at a depth of around 0.2 m, with a subsequent gradual decline in soil strength with increasing depth (Fig. 6a). Gravimetric soil water content was approximately threefold less at all depths on the sandy duplex compared with the red clay (Fig. 6b). Soil water availability on the sandy duplex was sufficient for plant growth at all depths, except in the surface 0.10 m, which was less than the lower limit of 3.8% for this soil type. In comparison, gravimetric soil water on the red clay exceeded field capacity of 9.7% at all depths, which was consistent with the above-average rainfall at the time of soil sampling in August 2005 (Table 2).

Plants on the sandy duplex site were harvested during late vegetative growth 2 weeks later than on the red clay, and hence produced more main stem leaves (7.6 and 6.7 leaves, respectively, P=0.07) and greater stem DM (0.53 and 0.35 g/plant, respectively, P=0.07). Poor soil fertility on the sandy duplex likely contributed to the production of fewer tillers compared with plants grown on the red clay (2.3 and 3.3, respectively, P=0.07). Leaf DM and above-ground DM did not vary significantly across sites at anthesis (data not shown). However, at the sandy duplex site there was a significant relationship between root depth at anthesis and above-ground DM (P=0.02) (Fig. 7), and tiller number (P=0.04, r^2=−0.32, data not shown) at maturity.

There was a significant genotype × site interaction for rooting depth, which exceeded 0.6 m in genotypes Brookton, Cunderdin, Halberd, Vigour 18 and Wil-

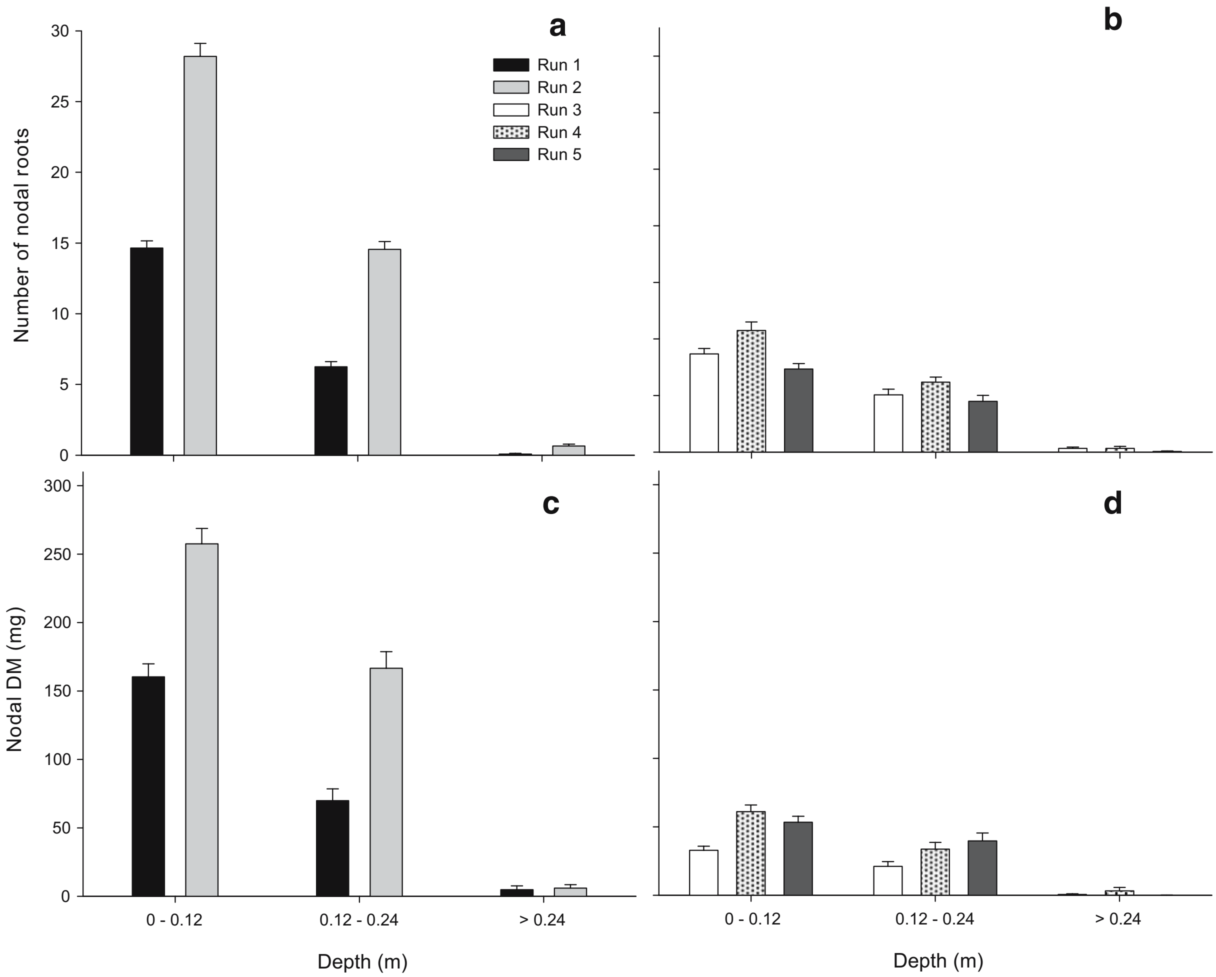

Fig. 5 Effect of run, depth and water regime on nodal root number in **a** WW and **b** DS; and nodal root DM in **c** WW and **d** DS conditions

goyne on the red clay (Fig. 7). Of these genotypes, Cunderdin had somewhat deeper roots, while those of Wilgoyne were much shallower at the sandy duplex site. Perenjori and Spear also had deep roots on the sandy duplex, while rooting depth of Cranbrook and Janz was relatively shallow (Fig. 8).

Discussion

The effect of wax layers and water regime

Drought stress has well-established effects on reducing root and shoot biomass production of wheat

Table 3 Climate data from March to September at Merredin in 2005

	Mar	Apr	May	June	July	Aug	Sept	Total
Rainfall (mm)	30	25	62	70	12	53	30	269
20 year mean	13	19	41	39	43	35	22	212
Temperature (°C)								
Maximum	32.1	26.2	21.8	16.1	16.8	17.4	19.7	
Minimum	19.3	13.0	12.5	6.9	4.2	4.5	5.7	

Trials were sown on 15 June and harvested on 30 August (red clay) and 13 September (sandy duplex).

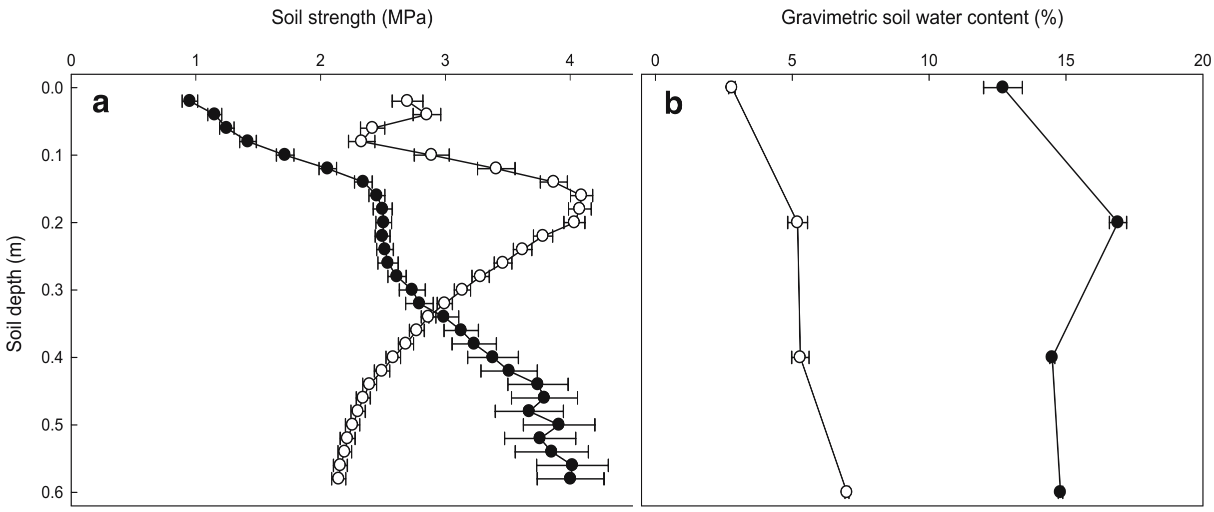

Fig. 6 Change in **a** soil strength and **b** gravimetric soil water content with soil depth on contrasting soil types at Merredin in 2005. *Filled circle*, red clay; *empty circle*, sandy duplex. Soil sampled at 75 and 89 DAS, respectively. Field capacity and the permanent wilting point for the sandy duplex were 6.9 and 3.8%, respectively; and for the red clay 9.7 and 5.0%, respectively (Rickert et al. 1987). *Bars* represent the SE

(Barraclough et al. 1989; Blum 1996; Chaves et al. 2003). For instance, plants grown under drought stress had fewer tillers, and less leaf area and above-ground dry matter compared with well-watered conditions. The imposition of drought stress from 14 DAS coincided with nodal root and tiller initiation at the 2-leaf stage (Klepper et al. 1984), which limited nodal root production. This was consistent with our previous findings that nodal roots of wheat ceased growth under early soil water deficit, and water uptake was instead dependant on seminal roots (Botwright Acuna and Wade 2005). Plants were then reliant on the ability of seminal roots to penetrate the wax layer. In the present study, seminal root number and dry matter were greater in the 0.12–0.24 m layer of soil immediately above the wax, as was observed by (Barraclough and Weir 1988) in field trials in soil that contained a hardpan. Fewer seminal roots penetrated the wax layer under drought stress than well-watered conditions, but those that did were considerably longer and had much greater dry matter. Extensive exploration by seminal roots of the soil

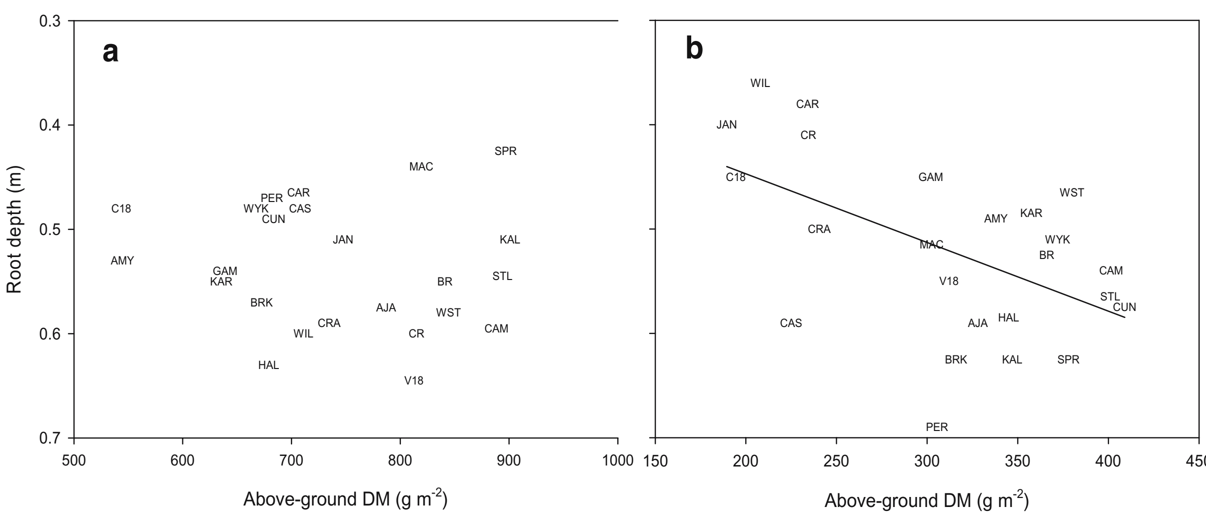

Fig. 7 Relationship between root depth and above-ground DM at maturity for **a** red clay (n.s.), and **b** sandy duplex (P=0.02; r^2=0.28) at Merredin in 2005. *Filled circle*, red clay; *empty circle*, sandy duplex. Soil sampled for root depth at 75 and 89 DAS, respectively. Refer to Table 1 for entry abbreviations

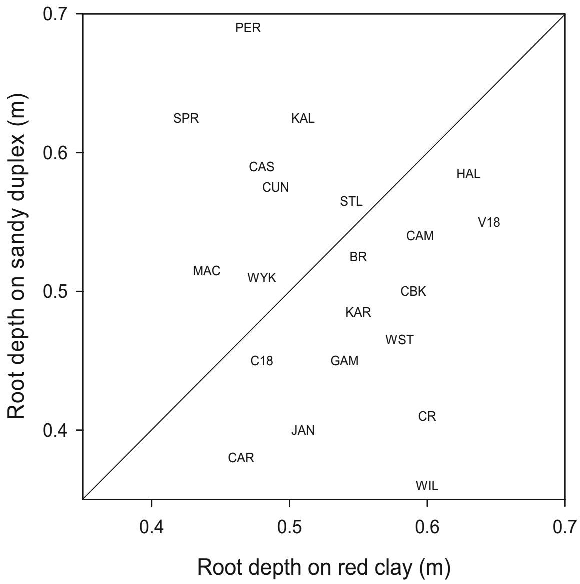

Fig. 8 Site × entry interaction for root depth at two sites in Merredin in 2005. *Bar* represents the site × entry. LSD=0.15 at P=0.05. The *line* represents the 1:1 ratio

below the wax layer contributed to greater daily water use, which maintained plant growth beyond 24 days after water deficit was induced. A compact, uniform and deep root system in wheat is considered to confer drought-tolerance in environments where wheat is grown on stored soil water (Manschadi et al. 2006). In contrast, ready access to both water and nutrients in well-watered conditions supported growth of an extensive root system above the wax layers of five seminal roots and 28 nodal roots, consistent with our previous experiments (Botwright Acuna and Wade 2005) and the observations of (Richards and Passioura 1989) in moist soil. Likewise, in field conditions, root growth has been shown to be mostly confined to soil above a hardpan when water and nutrients are not limiting (Barraclough and Weir 1988).

Genotypic variation in root penetration and the effect of water regime

Genotypic variation in the ability of roots to penetrate wax layers has been shown in rice (Babu et al. 2001; Clark et al. 2000, 2002; Price et al. 2000; Yu et al. 1995) and durum wheat (Kubo et al. 2004), but has not been related to root ontogeny nor plant response to water regime. Here, genotypic differences were observed in the ability of seminal roots of bread wheat to penetrate a thin wax layer, with genotypic differences being modified by water availability as drought stress progressed. Roots of eight of the 24 entries, including Camm, Carnamah, Bonnie Rock, Halberd, Janz, Machete, Stiletto and Wilgoyne had the greatest seminal DM below the wax layer, across water regimes. Five of these were late-maturing entries, four of which were either bred in South Australia or shared common parents. Extended vegetative growth of late-maturing plants may have permitted continued root growth when there was access to water and nutrients at depth. For example, extensive exploration by seminal roots of the soil below the wax layer by the long-season wheat cultivars Machete and Camm contributed to greater daily water use, which maintained plant growth beyond 24 days after water deficits were induced (Fig. 3). In contrast, roots of C18 and Cranbrook failed to penetrate the wax layer and water extraction declined from 17 days after stress imposition (Fig. 3). Shoot growth slowed within another 2 days and plants died prematurely. However, there were no particular plant characteristics among those measured linking these two to the exclusion of the other 21 entries.

The line V18, bred for greater early seedling vigour, is perhaps notable for its absence in the list of eight wheat entries that consistently penetrated the wax layers across runs. Closer examination of our data revealed that V18 in particular did not perform well under drought. Greater early seedling vigour has been shown to increase grain yield in environments with adequate rainfall on uniform soils, presumably by improved water-use efficiency (Asseng et al. 2003; Botwright et al. 2002). In low rainfall environments, a larger root system associated with greater early seedling vigour can accelerate terminal drought if all of the available soil water is used too early. Root length density of V18 measured in soil columns was larger than Janz to a depth of 0.6 m (Liao et al. 2004). Presumably, fast root growth of V18 may have quickly depleted soil water above the wax layer, leading to premature plant death under drought stress.

Root depth in the field

At Merredin, there was a site × cultivar interaction for root depth at anthesis and for above-ground DM at maturity. Soil strengths of 2.5 MPa in the hardpan zone of a loamy sand caused a severe reduction in root growth of wheat (Hamblin et al. 1982), but nevertheless there was a significant relationship between root depth at anthesis and above-ground DM at harvest. The red clay became progressively harder with depth, reaching 4 MPa at 0.6 m, but unlike the sandy duplex, there was no relationship between root depth at anthesis and above-ground DM at harvest. Entries V18 and Halberd had the deepest roots on the red clay, while Janz and Carnamah had relatively shallow roots at both sites. These observations compare favourably with Watt et al. (2005), where roots of V18 reportedly grew faster vertically in unploughed soil than the less vigorous cultivar, Janz. On the duplex site, entries Perenjori, Spear and Kalannie had the deepest roots. The duplex soil contained a hardpan of 4 MPa at a depth of approximately 0.2 m and was approximately threefold drier at all depths compared with the red clay. Roots growing in a drying soil would experience an increase in soil hardness (Barley and Greacen 1967), which slows root growth (Belford et al. 1987). Genotypic variation between sites may then relate to differences in root traits, should these confer an ability to penetrate a sudden versus gradual increase in soil hardness or adaptation to drought. For example, roots of V18 were deeper on the red clay than on the sandy duplex that contained a hardpan. The response of V18 on the red clay may relate to a faster root front velocity that allowed roots to grow to depth before soil hardened with drying. This result was also consistent with our finding that roots of V18 did not penetrate the wax layer under drought stress in controlled conditions, nor on the sandy duplex in the field, where the hardpan was more severe. Further field trials are required to confirm these observations, in particular early in the season when roots first enter the critical soil depths, and encounter the hardpan as soil dries.

Comparison of root growth between controlled conditions and the field

In wheat, there have been no attempts to relate cultivar differences in root penetration ability through thin wax layers to field performance. In eight rice cultivars grown in three environments containing a hardpan in Bangladesh, Samson et al. (2002) reported that roots of one cultivar were restricted to soil above the hardpan, while another cultivar consistently had a large root length density below the hardpan, and this was confirmed with wax layers by Babu et al. (2001). Others, such as (Clark et al. 2002), reported that root growth of rice cultivars in controlled conditions through a weighted column of sand was unrelated to penetration ability through wax layers, although differences in root diameter were consistent among cultivars and experiments.

In this study, four of the eight wheat entries identified in pot trials as having a greater seminal root DM below the wax layer, Camm, Halberd, Bonnie Rock and Stiletto, had deep roots at both field sites. Conversely, C18 had relatively shallow roots in the field at both sites, and failed to penetrate the wax layer in controlled conditions. Consequently, much of the overall relationship between pots and field was consistent, but there were exceptions. At the sandy duplex site, the three entries with deepest roots, Kalannie, Perenjori and Spear, were intermediate in root growth below the wax layer in pots, even though we expected this site with a hardpan to be similar to the wax layer in pots. Such exceptions are a reminder that a number of factors are not controlled in field experiments, so results at a particular site may differ from a controlled test for a number of reasons. Hence it is important to conduct validations at more than one site, and to monitor soil conditions and plant growth carefully.

A number of soil and plant factors could influence the results at a field site. Firstly, a number of other soil factors such as soil pH were not controlled, so ranking of cultivars may be influenced by adaptation to specific soil conditions, such as subsoil acidity or sodicity or toxic ion effects. The water regime was not controlled, so the timing and rate of water deficit may influence the outcome. A cultivar with a greater root front velocity may be able to grow roots to depth before penetration resistance rises on subsequent soil drying. Further, a more vigorous cultivar may have more DM to allocate to roots, and greater dry matter allocation may be important where thicker roots were observed to be beneficial for root penetration to depth. Such relationships are complex, however, as an ability to get roots to depth may then permit greater access to subsoil reserves of water below a hardpan, thereby increasing above-ground dry matter. All of these relationships will in turn be modified by crop phenology, the timing of water deficit, and the ability to get roots to depth, either by root front velocity or penetration ability. Such issues can only be resolved by careful study of the dynamics of penetration resistance, root growth and water extraction through time, in relation to soil attributes and crop development. Nevertheless, effort to validate the responses in the field is essential, so promising cultivars can be adopted by growers or used with confidence in crop improvement programs.

Repeatability

Root growth is inherently variable in response to small changes in environment (Bingham and Bengough 2003), which can make quantification of significant treatment differences challenging, particularly in the field. All of the published evidence supporting the repeatability of the thin wax-layer technique is for rice. The ability of roots of rice entries Bala and Azucena to penetrate wax layers is largely consistent (Clark et al. 2000, 2002; Price et al. 2000). The issue of replication was further investigated by (MacMillan et al. 2006) in their paper on genotype × environment interactions of inherited root traits in rice, reporting that variation in root traits due to replicate screens was less than between environments or genotypes. We found no within-run variation in root traits, although there were large differences between runs. The run effect could be explained by treatment effects or differences in plant size and age at harvest. Therefore, if runs were to be used as replicates, it would be important to ensure consistency in treatment and plant age.

Field trials were small in scale and replicated across and within sites. Even with relatively few (two) within-site replications, there was a significant site × entry interaction for root depth. Future field experiments are planned to build on the field data reported here, by utilising additional replicates, more sites and fewer genotypes to improve the reliability of the predictive analysis to evaluate the relationship between root penetration of wax layers and root growth in realistic field conditions.

Conclusions

This was the first attempt in wheat to relate cultivar differences in wheat in root penetration ability through thin wax layers to field performance. Wheat entries were identified that differed in their ability to penetrate the wax layer under contrasting water regimes, and in rooting depth in the field. Four of the eight superior entries from the pot trials all tended to have deep roots across sites, while another entry with relatively shallow roots in the field failed to penetrate the wax layer in controlled conditions. Research is in progress to understand the mechanisms of root penetration and their genetics, by assessing root penetration ability of doubled haploid lines of wheat through thin wax layers. Further research is needed, however, to validate the response in the field. This requires closer monitoring of depth versus soil strength and water content through time for a valid test. While there is evidence of soil water being available below a hardpan in field environments, and that deep roots may efficiently extract this water to increase grain yield, it remains to be demonstrated whether deep roots reliant on soil water below the hardpan alone are fully effective in extracting soil water and maintaining transpiration under terminal drought, especially when vapour pressure deficit and evaporative demand vary. Research is proceeding to examine these important questions.

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