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Wheats developed for high yield on stored soil moisture have deep vigorous root systems

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Abstract. Many rainfed wheat production systems are reliant on stored soil water for some or all of their water inputs. Selection and breeding for root traits could result in a yield benefit; however, breeding for root traits has traditionally been avoided due to the difficulty of phenotyping mature root systems, limited understanding of root system development and function, and the strong influence of environmental conditions on the phenotype of the mature root system. This paper outlines an international field selection program for beneficial root traits at maturity using soil coring in India and Australia. In the rainfed areas of India, wheat is sown at the end of the monsoon into hot soils with a quickly receding soil water profile; in season water inputs are minimal. We hypothesised that wheat selected and bred for high yield under these conditions would have deep, vigorous root systems, allowing them to access and utilise the stored soil water at depth around anthesis and grain-filling when surface layers were dry. The Indian trials resulted in 49 lines being sent to Australia for phenotyping. These lines were ranked against 41 high yielding Australian lines. Variation was observed for deep root traits e.g. in eastern Australia in 2012, maximum depth ranged from 118.8 to 146.3 cm. There was significant variation for root traits between sites and years, however, several Indian genotypes were identified that consistently ranked highly across sites and years for deep rooting traits.

Additional keywords: deep roots, field phenotyping, soil coring, root penetration rate, maximum depth, total root length.

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Introduction

Wheat provides a significant amount of the daily total dietary calories worldwide (16% in the developing world and 26% in developed countries) and future productivity of wheat crops will play a significant role in global food security (Dixon *et al*. 2009). Around 70% of worldwide wheat production is rainfed (Rosegrant *et al*. 2002; Portmann *et al*. 2010). Unpredictable temperature and rainfall events worldwide and increased exploitation of water resources in developing countries is creating a need for wheat growers to maximise yield per unit land area per unit of rainfall received. This water use efficiency (WUE) can be gained through both improved agricultural practices (Kirkegaard and Hunt 2010; Hunt and Kirkegaard 2011) and the breeding and introduction of more efficient and adapted wheat genotypes (Richards *et al*. 2010).

Many rainfed wheat production systems are reliant on stored soil water for some or all of their water inputs (Passioura 1972; Lilley and Kirkegaard 2011). For example, in Australia most wheat is rainfed and grown over the winter. Rainfall is largely unpredictable in amount and distribution but as a rule in the south it is winter-dominant, whereas in the north it is summer-dominant. This out of season rain in the north builds up a reservoir of soil water for crops to use through to maturity. Like the north of Australia, rainfed Indian wheat is sown in the autumn at the end of the rainy season and crops are reliant on stored soil water throughout the season (Wasson *et al*. 2014).

Water stored deep in the soil profile is a vital resource to maximise yield as this water is mostly used towards the end of the season and is used for increasing carbohydrate supply to the growing grains (Condon *et al*. 1993). In a terminal drought, which is frequent, the soil dries gradually from the surface through evaporation and transpiration, resulting in greater water availability in deeper parts of the soil profile as the season progresses. In situations where rainfall occurs during the growing season, surface soils will periodically gain moisture; however, the penetration and durability of this water in the shallow soil profile is unpredictable whereas the water available at depth can be reliably measured at sowing (Kirkegaard *et al*. 2007).

There are numerous water use and water capture traits being trialled for integration into breeding programs with the aim of developing new genotypes with improved yields under water limited conditions (Richards *et al*. 2010). Direct assessment of root traits is typically avoided by plant breeders due to phenotyping difficulty, lack of understanding of root system development and function and the strong influence of environmental conditions on the mature root system phenotype (Manschadi *et al*. 2006; Wasson *et al*. 2012; Rich and Watt 2013). However, it is possible that selection and breeding for root traits could result in a significant yield benefit.

Modelling indicates that a more even distribution of wheat roots (i.e. more proliferation at depth) could in theory improve yield in winter wheats (King *et al*. 2003). Several modelling studies have used faster root descent (which would give deeper roots at anthesis and maturity) and shown benefits to water uptake (9 mm) and yield ($0.1\,\mathrm{t\,ha^{-1}}$) in a range of Australian wheat growing scenarios (Lilley and Kirkegaard 2011). However, Semenov *et al.* (2009) showed little benefit of faster root elongation for two sites in the UK, although slower root elongation rates did result in lower yields. Modelling also suggests that any additional water gained after anthesis will generate higher yields (Manschadi *et al*. 2006). This has been demonstrated in the field by Kirkegaard *et al.* (2007) who used direct root and soil water measurements in the field to show that a 30 cm increase in root system depth could capture an extra 10 mm of deep soil water at the time of grain development resulting in an extra $0.5\,\mathrm{t\,ha^{-1}}$. This deep, late-season water is valuable because it can prolong the grain-filling period (by delaying plant senescence), and therefore contributes directly to the allocation of carbohydrate to grain. The ability to access water after anthesis is most likely to come from deeper roots as demonstrated under controlled conditions by Manschadi *et al.* (2006) who showed greater root length in deeper soil layers in root boxes allowed for greater water extraction and Reynolds *et al.* (2007) who showed that increased partitioning of root mass to deeper soil profiles (between 60 and 120 cm) increased the ability of synthetic derived lines to extract moisture from those depths, improving their performance over recurrent parents.

The capacity of wheat root systems to extract water from the soil decreases with depth, due to reduced residency time and root length density, increased clumping, confinement of roots to pores and structural features of the soil, and reduced root-soil contact (White and Kirkegaard 2010). Effective root length density, sufficient to extract all the water from the soil, has been predicted as being $1–5\,\mathrm{cm\,cm^{-3}}$ (Noordwijk 1983), and $>0.5\,\mathrm{cm\,cm^{-3}}$ (Passioura 1983), and measured at $1.0\,\mathrm{cm\,cm^{-3}}$ for barley and $0.4\,\mathrm{cm\,cm^{-3}}$ for chickpea (Gregory and Brown 1989). Root length densities as little as $0.1\,\mathrm{cm\,cm^{-3}}$ in wheat, and $0.2\,\mathrm{cm\,cm^{-3}}$ in sorghum, can have an impact on water uptake (Robertson *et al*. 1993; Kirkegaard *et al*. 2007). There are numerous examples across various crops where water at depth does not appear to have been extracted even in the presence of theoretically sufficient root density (e.g. Hurd 1974; Robertson *et al*. 1993; Kirkegaard *et al*. 2007; Christopher *et al*. 2008).

In the rainfed areas of India, wheat is sown at the end of monsoon into very hot soils with a quickly receding water profile: in season water inputs are minimal. Our hypothesis was that wheat selected and bred for high yield under these conditions would also have deep, vigorous root systems.

There have been limited studies which correlate controlled environment screen to wheat roots in the field, while correlations to earlier stages of plant growth have been found, screens in controlled environments and even young plants in the field relate poorly to mature root systems (Oyanagi *et al*. 1993; Watt *et al*. 2013). Our hypothesis was tested by measuring the mature root systems of Indian wheats in the field in both India and Australia. The hypothesis was tested in multi-environment, multi-year field trials, rather than in screens of seedlings or young plants in a controlled environment, because it is the mature root system as influenced by edaphic factors that are responsible for capturing subsoil moisture during grain-filling (Wasson *et al.* 2012, 2014; Rich and Watt 2013). Contrasts selected were: Indian wheats bred for rainfed conditions vs Indian wheats bred for irrigated conditions; Indian wheats (from rainfed or irrigated backgrounds) grown in Australian wheat growing regions vs Australian wheats from the Southern and Western (which experience in-season rainfall) and Northern (which are reliant on soil moisture).

Materials and methods

Germplasm

The germplasm selected for these experiments (89 lines; see Table S1, available as Supplementary Material to this paper) consisted of 49 classic high yielding lines from various regions of India. These lines were benchmarked at three sites (see details below) in India before being transferred to Australia. In Australia, they were trialled alongside 26 of the highest yielding cultivars from both the northern and southern/western growing regions of Australia (selected from the Australian National Variety Trials). Fourteen other lines (primarily novel breeding lines) were included, as they had shown root traits of interest in an earlier phase of the project.

Experimental sites and design

In India, three sites were used covering the peninsular (the Agharkar Research Institute (ARI) in Pune), central (Indian Agricultural Research Institute (IARI) in Indore) and north-western (Directorate of Wheat Research (DWR) in Karnal) wheat growing areas. In Australia, trials were conducted in the south-eastern (Yanco Agricultural Institute, NSW) and northern (Gatton Research Station, Qld) wheat growing areas. The sites covered a range of soil and climatic conditions capturing some

Table 1. Trial site characteristics

Site	Australia		India		
	South-eastern	Northern	Peninsular	Central	North-west
Location	34.60°S, 146.40°E	27.32°S, 152.20°E	18.52°N, 73.85°E	22.72°N, 75.86°E	29.0°N, 76.0°E
Average wheat growing season (timely sown)	180 days	150 days	100 –110 days	100 –110 days	140 days
Average in season or annual rainfall^2	205 mm^A	260 mm^A	550 mm^B	800 mm^B	199 mm^B
Soil type	Grey Vertosol^C	Black Vertosol^C	Black Vertosol^C	Black Vertosol^C	Sandy loam to clay loam
Temperature range over growing season	5−29°C	6−30°C	7−32°C	9−35°C	0−37°C

^{A}Australian Bureau of meteorology data; rainfall means south-eastern 1957–2013, northern 1968–2013; temperature means south-eastern 1999–2013, northern 1968–2007.

^{B}Pre-sowing monsoonal rains recharging stored soil moisture occur at the Indian sites.

^{C}Isbell (2002).

Fig. 1. Site locations for the trials. (*a*) North-west India (Karnal); (*b*) Central India (Indore); (*c*) Indian Peninsular region (Pune); (*d*) Northern Australia (Gatton); (*e*) South-east Australia (Leeton).

of the variation experienced by farmers in the two countries (Table 1; Fig. 1).

Experiments started with hill-plot trials (see below) of the 47 Indian lines (7–11 lines cored; 2–3 replicates) at the three Indian sites over the 2011 season, these were followed by hill-plot trials of 90 Australian and Indian lines at the two Australian sites in the 2012 season (31 and 90 lines cored; eight replicates) and at one Australian site in 2013 with both hill-plot trials (29 lines;

eight replicates cored) and plots (20 lines; five replicates cored). The lines used in the 2013 trials were selected as high root trait performers from rankings in the two 2012 Australian trials, along with four low performing checks (HW2045, Kennedy, Janz and Westonia), plus some extra Indian checks in the 2013 hill-plots. See Table 2 for a full description of sowing, experimental design and seasonal weather across the three years and five sites.

As we focus on screening for root traits in our experiments, we chose to use a hill-plot sowing configuration similar to that followed by Wasson *et al*. (2014). This provided controlled competition from neighbouring root systems, economy of sowing area (thereby reducing spatial variation) and it provided a high root system density for ease of root phenotyping at depth.

The hill-plot planting design was achieved by hand sowing into machine or hand pulled rows (depth 20–40 mm). At measured intervals down the row, 30 seeds were sown in a tuft in a 12.57 cm^2 area (termed hill-plot) and lightly covered with soil, sowing was facilitated by a funnel topped PVC tube (1.2 m long, 40 mm diameter) that seeds were poured down. Each experimental line was sown into the centre of a square formed by sowing a control winter wheat hill (30 seeds) creating a uniform competitive environment around the lines. Spacing between lines and their control hills varied slightly from sites to site due to variation in row spacing (Table 2). Trials were bird netted and surrounded by buffer crops to decrease any edge effects. Mini-plots were sown in 2013. The plots were sown into 10 rows spaced 180 mm apart in plots 2 m long and 1.8 m wide. Establishment counts were taken at about the two-leaf stage indicating an average of 210 plants m^{-2}.

Trials were sown into a full soil water profile (pre-irrigated if needed). In Australia, in season rainfall and temperature were logged on site (RG3 data logging rain gauge, Onset Computer Corporation, Bourne, MA, USA). Gravimetric soil water was collected at the beginning and end of the season via 2 m cores. During crop growth, plots were maintained weed free by both hand weeding and the application of post emergent broadleaf herbicides as needed.

In season and maturity shoot measures

Plots were scored for emergence at the two-leaf stage and then their growth monitored through the season. From head emergence of the earliest maturing line until to the end of flowering, all plots were scored at least weekly using the Zadoks decimal system (Zadoks *et al*. 1974) allowing for the accurate measurement of flowering time (Z65) for each plot.

After anthesis, plots were left to fully mature in the field (~4 weeks) before hand-harvesting at the base of the shoot. The entire hill-plot was weighed and threshed to ascertain harvest index (HI) and yield. In the mini-plots in 2013 a random cut from the centre of each plot was hand harvested (0.3 m^2). This sample was used for tiller counts as well as yield and HI scoring.

Soil coring, core-break root counting and measurement of root length density

At all three Indian sites, a manual method of soil coring was used (Fig. 2*a*). Steel sampling tubes with an internal diameter of 76.2 mm were placed over the harvested hill-plot and driven in using a mallet (Fig. 2*b*). The tubes are marked in 100 mm interval and are removed at each interval and the 100 mm core section gently tapped out into a sieve for washing on the spot (Fig. 2*c*) or into a bag for storage at 4°C to be washed at a later date. Washing was completed manually by gently agitating the core section under running water, so the soil is lifted off the roots and washed away through the sieve. Washed samples were stored in 50% ethanol until analysis.

In Australia, a hydraulic tractor-mounted soil corer was used to drive sample tubes (42 mm internal diameter) to a depth of 1.8–2.0 m. Soil cores were taken over the centre of a harvested hill-plot or the centre of the row in standard plots. These soil cores were carefully removed from the sample tubes on site and cut into 100 mm segments; the top 100 mm was discarded, and because the reached depth was inconsistent, any core segments deeper than 1.8 m were also discarded. In 2012, the remaining 17 core segments were broken at 100 mm depth intervals and the number of exposed live wheat roots on each face counted (core break method; Van-Noordwijk *et al*. 2010). In 2013, each 100 mm section was broken in two places and root counts made as in 2012. A subset of cores (six in 2012 and 10 in 2013) were sealed in plastic bags and stored at 4°C for washing to separate the roots and soil. Core sections were washed using an automated hydropneumatic elutriation system (Smucker *et al*. 1982) with 0.078 mm sieves, washed samples were stored in 50% ethanol until analysis.

In both countries, root samples were rinsed in water and then floated in trays on a flatbed scanner fitted with a transparency unit to eliminate any shading caused by the floating roots (Epson Perfection V700 Photo or Epson Expression 10 000 XL, custom transparency unit fitted by Regent Instruments, Quebec, Canada). Roots were scanned at 400 dpi and analysed for total root length with WINRhizo software (v2013d; Regent Instruments). In India, as all cores were washed and scanned, these data were converted to root length density in the core sections and used directly in analysis. In Australia, only a subset of cores were collected and scanned, and this information was used to estimate root length densities for the remaining cores (see below).

Statistical analysis

Core break counts differ from site to site and year to year due to soil property differences. Therefore, comparisons were made by converting counts to root length density. At each site a subset of cores were collected, washed and the root material scanned (see above) so that the exact length of root material was ascertained. These lengths were used to create a correlation with counts recorded for these cores for each counter in the field and these individual correlations were used to convert the core break counts from the field to root length density. To avoid the variation between different people counting, for each counter in the field, 102 core segments per counter in 2012 and 170 core segments per counter in 2013 were collected and scanned to create individual core break count to root length density correlations. In 2012, the coefficient of determination for all counters ranged from $r^2 = 0.71–0.72$, and in 2013 $r^2 = 0.74–0.75$

To analyse trials for genotypic variation we generated summary statistics on four traits; maximum depth (the deepest

Table 2. Field experiments management details and sampling configurations

Trial	Harvest year	Site	Rotation	In-season rainfall	GDD	Number genotypes cored	Sowing configuration	Density or number of seeds	Replicates	Coring depth	Coring method	Root measures	Shoot measures
1	2011	India-peninsular	Soybean	32 mm	1671	10	Hill-plot 50 cm gap	30 seeds	3	0.5 m	Hand cored, core diameter 7.62 cm	Core sections hand washed, scanned for length and weighed for dry mass	Entire hill plot, air-dried, weighed, threshed, seed weighed
2	2011	India- central	Soybean	82.5 mm	812	11	Hill-plot 50 cm gap	30 seeds	3	0.5 m	As above	As above	As above
3	2011	India-north-west	NA	11.6 mm	1450	7	Hill-plot 50 cm gap	30 seeds	2	1.2 m	As above	As above	As above
4	2012	Australia-south-east	Canola	103.8 mm	914	90	Hill-plot 72 cm gap	30 seeds	8	2 m	Hydraulic tractor mounted corer, diameter 4.02 cm	Core-break counting; lengths correlated with 10% washed out cores (automated core washer)	As above
5	2012	Australia-north	Sorghum	212.9 mm	1206	31	Hill-plot 50 cm gap	30 seeds	8	2 m	As above	As above	As above
6	2013	Australia-south-east	Canola	206.2 mm	854	29	Hill-plot 72 cm gap	30 ± 3 seeds	8	2 m	As above	As above	As above
7	2013	Australia-south-east	Canola	206.2 mm	816	20	Mini-Plot 3.7 m^2	210 plants m^{-2}	5	2 m	As above	As above	Quadrat cut (0.25 m^2), air-dried, weighed, threshed, seed weighed

0.1 m long core segment roots were detected), maximum depth of 90% of the roots (to remove bias of single roots that have descended deeply through a pore or crack), total root length in the core (the sum of root length as calculated from the root length density for each 0.1 m core segment) and root penetration rate (calculated as maximum depth over the growing degree days to anthesis). The effect of genotype for these traits was analysed in a linear mixed model.

Data was collated and plotted in the R environment using the ‘ggplot2’ (Wickham 2009), ‘reshape2’ (Wickham 2007) and ‘dplyr’ (Wickham and Francois 2014) packages. Mixed linear models were fitted with ASReml-R (Butler *et al*. 2009). Unless otherwise specified replicate was fitted as a random factor and genotype as a fixed factor in the initial analysis of genotype means. The fitted means and variance-covariance matrix were used in *a priori* orthogonal contrasts for specific comparisons of interest. Unless otherwise stated statistical significance was at $P = 0.05$.

Results

Indian coring

Lines cored in India showed significantly lower root length density than those in Australia (Fig. 3; Fig. S2, available as Supplementary Material to this paper). Indian lines showed proliferation at very shallow depths but root mass dropped off quickly. The difficult nature of hand coring in these hard dry clay soils made data collection deeper than 50 cm very difficult, so maximum root depths for some genotypes exceeded the measured depth, although root length densities tended to be very low by depths of 50 cm (see Fig. S1*a*, available as Supplementary Material to this paper). All lines in central and peninsular India were cored to 1.2 m and all lines had roots present at this depth, although root density was very low; due to low replication ($n = 2$) in this trial, preliminary statistical analyses did not reveal reliable genetic differences between lines.

The root data was plotted in Fig. S1*b*, *c*. Although the maximum depth of the wheats could not be distinguished at in the peninsular region (Pune), the wheats with a rainfed background were significantly deeper than the irrigated background wheats in central India (Indore). It appears that wheats bred for rainfed management had prioritised depth in central India as they had had lower total root length as compared with those bred for irrigated areas; this differed from the peninsular region where rainfed background genotypes had longer total root lengths.

Fig. 2. A manual method of soil coring was developed at the Indian sites. (*a*) The team and equipment used at the Karnal site, including steel sampling tubes with an internal diameter of 762 mm and inserted handle rods, sledge hammers and wooden dollies, plungers, and buckets. (*b*) The sampling tube being hammered in with the sledge hammer. (*c*) A 100 mm sample being tapped out of the tube into a sieve for washing. In Australia a combination of hydraulic soil coring and core-break-counting was used. (*d*) A 1 tonne hydraulic push-press mounted on a 3tonne tractor. The operator is driving a 2 m long molybdenum-steel sampling tube into a harvested hill-plot with the system. (*e*) The soil cores are then emptied into a cradle and cut into 100 mm segments. (*f*) Each segment is then broken (once in 2012, twice in 2013) and the number of live roots exposed on each broken face is recorded.

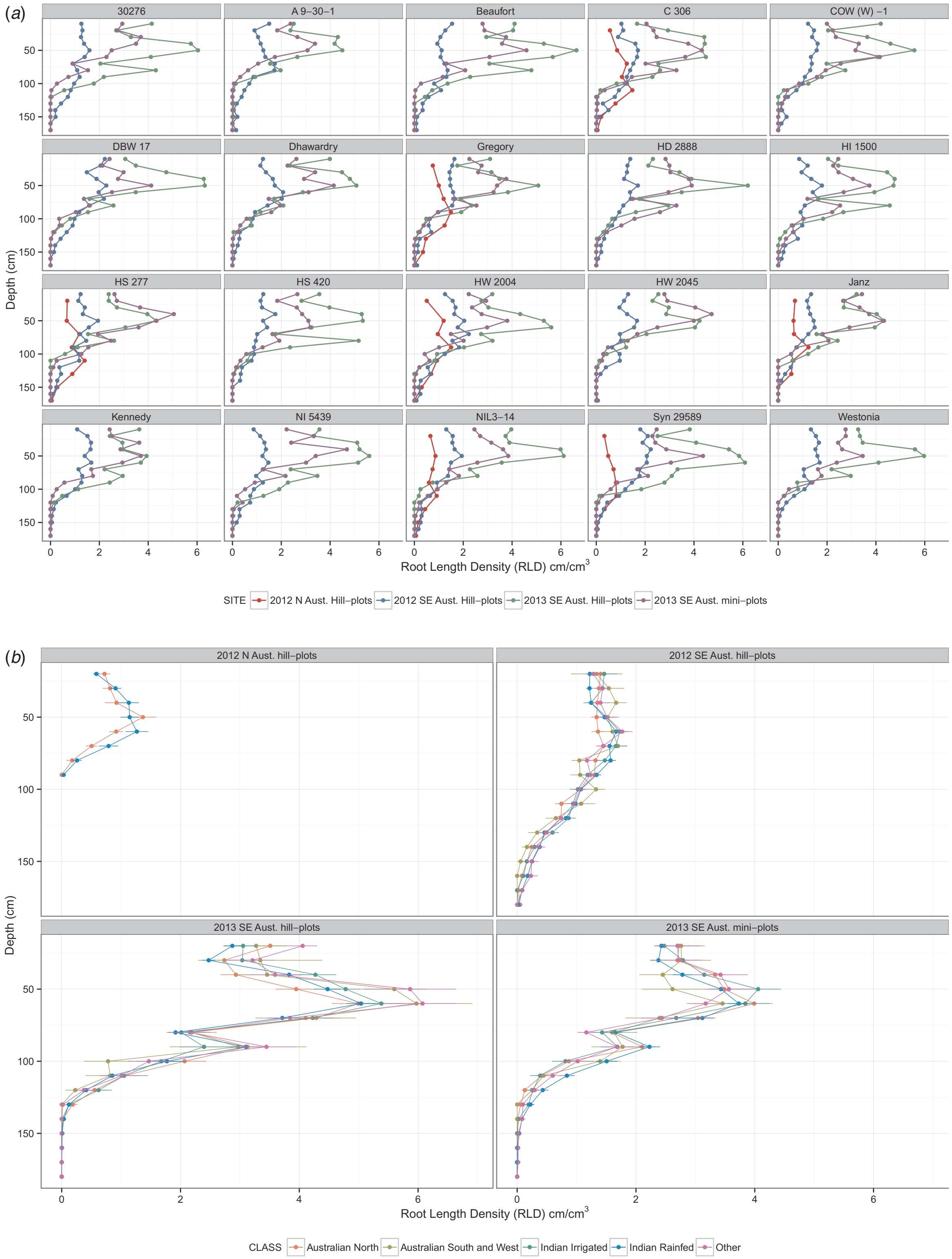

Fig. 3. Root length density (cm cm^{-3}) plotted against depth (cm – reverse axis). (*a*) The plot is faceted by genotype and the lines show mean data from four trials at three sites and two seasons. Hill-plot data is shown from the South-east Australia in 2012 (red) and 2013 (blue) and from Northern Australia in 2012 (purple). Mini-plot data is shown from South-east Australia in 2013 (green). (*b*) The plot is faceted by trial and lines show group means of genotypes by breeding background (geographic for Australian lines and management for Indian lines). Genotypes were classed as Australian North (red), Australian South and West (green), Indian irrigated (aqua), Indian rainfed (blue) or other (pink). Error bars show the standard error of the mean from eight replicated cores in the hill-plots and five replicated mini-plots with two cores/plot.

Australian coring

Large variation in root distribution patterns was found in the 90 lines in the four Australian trials (Fig. 3, Fig. S2). Some of the broad distribution patterns evident in these root distribution graphs can be attributed to temporal and spatial effects. Weather patterns in the two years were very different with 2013 receiving twice as much in season rainfall as in 2012 (206.2 mm and 103.8 mm respectively); there was a significant storm which dropped ~40 mm three months after sowing. In contrast, in 2012, a third of the seasons rainfall occurred a week before harvest when the crop had essentially senesced and the only other significant rainfall event (24 mm) occurred less than a month after sowing. Both trials run in 2013 show extensive proliferation at mid-range depths (~0.5 m) and the maximum depth achieved in these trials tended to be less deep than in 2012. It is likely that there was little value in mid-range proliferation in 2012 as the water profile was retreating throughout the season. Maximum depth in eastern Australia in 2012 ranged from 118.75 ± 6.99 cm to 146.25 ± 6.99 cm while the eastern Australian trials in 2013 reached depths of 144 ± 8.07 cm and 122.5 ± 6.37 cm for the mini-plots and hill-plots respectively. The Queensland trial tended to have lower root length density than those trials run in New South Wales (Fig. 3, Fig. S2).

As a factor, genotype showed strong significance for total root length in three of the trials, genotype was also significant for both measures of maximum depth in three of the trials and for root penetration rate in both 2013 trials (Table 3).

Planned pairwise comparisons of physiological groups (Table S1; geographic breeding for Australian lines, 'northern' vs 'south and western'; management background for the Indian lines, 'irrigated' vs 'rainfed') and root traits were performed (Table 4). The Indian lines had longer total root length than the northern Australian lines in 2012. In 2013, the Indian lines had faster root penetration rates than the Australian lines in the hill-plots, and the northern Australian lines in the mini-plots. The rainfed Indian lines were also generally deeper than the Australian lines in both 2013 trials (Table 4). We noted that the two groups of Australian lines only showed any difference in root traits for total root length in the 2012 NSW hill-plots, and likewise the Indian rainfed and irrigated lines only could be separated on depth in the 2013 mini-plots.

C306 is a high yielding genotype that was released in India in 1965 and has been used extensively as a parent in breeding programs. C306 was a high performer for all root traits in 2013 (Fig. S3) and was therefore a line of interest. We performed planned pairwise comparisons between the same physiological groups as above and our lines with C306 backgrounds (Table 5). In 2012, lines with the C306 background performed significantly better for all traits than almost all other groups. In 2013, however, the C306 derived lines only performed consistently better than

Table 3. Significant genotype effects for root traits. Root traits were modelled with traits as dependant variables in a mixed linear model with genotype as a fixed factor and replicate as a random factor

Trial		Maximum depth (cm)	Maximum depth (90th percentile, cm)	Root penetration rate (cm$^{-{}^{\circ}\mathrm{C\ d}}$)	Total root Length (m)
Hillplots 2012	Genotype *P*-value	ns	ns	<0.001	ns
	Australian North	134.24 ± 7.33	105.49 ± 6.37	15.5 ± 1.56	0.18 ± 0.01
	Australian South and West	132.52 ± 7.15	104.06 ± 6.29	14.3 ± 1.59	0.18 ± 0.01
	Indian irrigated	133.04 ± 7.28	103.43 ± 6.38	14.11 ± 1.61	0.19 ± 0.01
	Indian rainfed	134.23 ± 7.24	105.14 ± 6.38	14.56 ± 1.59	0.19 ± 0.01
	Other	132.29 ± 7.36	102.99 ± 6.49	14.3 ± 1.63	0.18 ± 0.01
	Site mean	133.36 ± 7.14	104.24 ± 6.28	14.5 ± 1.53	0.19 ± 0.01
Qld Hillplots 2013	Genotype *P*-value	0.024	0.086	<0.001	NA
	Australian North	67.71 ± 4.02	54.17 ± 3.63	4.94 ± 0.86	NA
	Australian South and West	71 ± 4.04	56.75 ± 3.64	4.79 ± 0.79	NA
	Indian irrigated	66.25 ± 3.99	51.88 ± 3.49	4.59 ± 1.24	NA
	Indian rainfed	69.53 ± 4.16	53.94 ± 3.8	5.42 ± 0.85	NA
	Other	64.56 ± 6.46	51.51 ± 5.03	4.24 ± 1.78	NA
	Site mean	68.49 ± 3.94	54.11 ± 3.57	4.95 ± 0.8	NA
Hillplots 2013	Genotype *P*-value	0.061	0.09	<0.001	<0.001
	Australian North	109.06 ± 6.7	83.75 ± 5.53	27.49 ± 4.05	0.14 ± 0.01
	Australian South and West	101.25	77.5	28.14	0.14
	Indian irrigated	108.59 ± 6.62	81.25 ± 5.36	27.6 ± 4.21	0.15 ± 0.01
	Indian rainfed	110.24 ± 6.68	79.6 ± 5.27	27.75 ± 3.71	0.15 ± 0.01
	Other	107.08 ± 6.7	80 ± 6.26	32.7 ± 4	NA
	Site mean	108.99 ± 6.48	80.6 ± 5.19	28.2 ± 3.63	0.15 ± 0.01
Mini-plots 2013	Genotype *P*-value	<0.001	<0.001	ns	<0.001
	Australian North	111.5 ± 9.87	101.5 ± 7.92	43.86 ± 5.82	0.14 ± 0.01
	Australian South and West	110	108	38.69	0.15
	Indian irrigated	112 ± 10.32	98.67 ± 9.98	40.81 ± 6.26	0.15 ± 0.01
	Indian rainfed	122.22 ± 9.23	113.11 ± 8.65	42.24 ± 6.1	0.16 ± 0.01
	Other	119.33 ± 9.04	108.67 ± 8.7	38.7 ± 5.81	0.16 ± 0.01
	Site mean	117.5 ± 8.5	107.7 ± 8.06	41.64 ± 5.66	0.16 ± 0.01

Table 4. Planned comparisons of wheat genotypes grouped into geographical and management classes, sown and cored in the four Australian trials

Trial	Group 1	Group 2	Maximum depth (cm)	Maximum depth (90th percentile, cm)	Root penetration rate (cm$^{-°C\ d}$)	Total root length (m)
			P-value			
South-east Australian Hillplots 2012	Australian North	Australian South and West	ns	ns	ns	0.042
	Indian irrigated	Australian North	ns	ns	ns	0.0081
	Indian irrigated	Australian. South and West	ns	ns	ns	ns
	Indian rainfed	Australian North	ns	ns	ns	0.065
	Indian rainfed	Australian South and West	ns	ns	ns	ns
	Indian rainfed	Indian irrigated	ns	ns	ns	ns
Northern Australian Hillplots 2012	Australian North	Australian South and West	ns	ns	NA	ns
	Indian irrigated	Australian North	ns	ns	NA	ns
	Indian irrigated	Australian South and West	ns	0.081	NA	ns
	Indian rainfed	Australian North	ns	ns	NA	ns
	Indian rainfed	Australian South and West	ns	ns	NA	ns
	Indian rainfed	Indian irrigated	ns	ns	NA	ns
South-east Australian Hillplots 2013	Australian North	Australian South and West	ns	ns	ns	ns
	Indian irrigated	Australian North	ns	ns	0.027	ns
	Indian irrigated	Australian South and West	ns	ns	0.059	ns
	Indian rainfed	Australian North	ns	0.054	0.015	ns
	Indian rainfed	Australian South and West	0.065	ns	0.049	ns
	Indian rainfed	Indian irrigated	ns	ns	ns	ns
South-east Australian Mini-plots 2013	Australian North	Australian South and West	ns	ns	ns	ns
	Indian irrigated	Australian North	ns	ns	0.028	ns
	Indian irrigated	Australian South and West	ns	ns	ns	ns
	Indian rainfed	Australian North	0.0066	0.0032	<0.001	ns
	Indian rainfed	Australian South and West	0.074	ns	ns	ns
	Indian rainfed	Indian irrigated	0.019	<0.001	ns	ns

other lines in root penetration rate, although they did outperform some Australian lines in maximum depth (Table 5; Fig. S3*a*).

Pairwise comparisons helped to highlight our high performing groups but the trial-to-trial variation made discerning overall 'best' performers difficult. Comparisons of traits at the different sites were useful in identifying lines performing above or below average for each trait across two sites. Fig. 4 shows performance of lines in the 2012 hill-plots and the 2013 mini-plots for predicted maximum depth of 90% of the root system (Fig. 4*a*) and root elongation rate (Fig. 4*b*). Lines such as C306, HI 1500 and COW (W)-1 all appeared in the upper right quadrant on both graphs and as such had above average performance in both trials for both traits. The results of the hill-plots in 2013 were less correlated with the other two trials, however, the comparative performance of root elongation between the hill-plots and plots in 2013 (Fig. 4*c*) and total root length in 2012 and 2013 hill-plots Fig. 4*c*) still show these lines performing above average. Pairwise comparisons were used alongside post-hoc comparisons of root traits (Table 6). Lines were classed as the best and worst for each trait and trial (Table 7) for use in *post-hoc* testing and while these groups showed variation due to environmental interactions, there were still lines such as HI1500, COW (W)-1 and those with a C306 background which continually appeared in our top class. With few exceptions these classes could be differentiated from the different physiological groups (Table 6).

Shoot traits

Post anthesis terminal drought can shorten the grain filling period due to earlier senescence, however, may increase assimilate remobilisation to the grain, the balance between these two processes will influence the final yield and harvest index (Palta *et al*. 1994; Ercoli *et al*. 2008). Given the hypothesis that deeper roots would provide more water during grain-filling, there is basis to test for a correlation between harvest index (HI) and root traits. There was a significant correlation between HI and total root length in hill plots ($r_s = 0.23$), and between HI and maximum depth in mini-plots ($r_s = 0.20$). Biomass and yield correlated with total root length in hill plots ($r_s = 0.44$ and 0.49 respectively), but not maximum depth. In mini-plots, biomass was correlated with maximum depth and total root length ($r_s = 0.30$ and 0.28 respectively), but yield was only correlated with maximum depth ($r_s = 0.28$). A summary of shoot trait means can be found in Table S2. Trial, genotype, and their interaction were significant factors in a mixed model of biomass, yield and harvest index (Table S2).

Root depth and water interactions

In the 2013 mini-plots, six lines were chosen to have cores kept for gravimetric water analysis, these lines were chosen from the 2012 data and were classed as either deep rooting (C306,

Table 5. Planned comparison of wheats with C306 background (Group 1) against wheat genotypes grouped into geographical and management classes (Group 2)

Trial	C306 background (Group 1) mean ± s.e.		Group 2	Maximum depth (cm)	Maximum depth (90th percentile, cm)	Root penetration rate (cm$^{-°\mathrm{C}\ \mathrm{day}}$)	Total root length (m)
				Contrast P-value (Z score)			
South-east Australian Hillplots 2012	Maximum depth (cm)	139.84 ± 7.2	Australian North	ns (1.64)	0.047 (1.98)	0.017 (2.38)	ns (1.34)
	Maximum depth (90th percentile, cm)	111.52 ± 6.49	Australian South and West	0.031 (2.16)	0.014 (2.46)	0.0095 (2.59)	0.0032 (2.94)
	Root penetration rate (cm$^{-°\mathrm{C}\ \mathrm{day}}$)	0.20 ± 0.01	Indian irrigated	0.032 (2.14)	0.0042 (2.86)	0.016 (2.41)	<0.001 (3.43)
	Total root length (m)	16.51 ± 1.7	Indian rainfed	0.023 (2.27)	0.0038 (2.89)	0.0035 (2.92)	<0.001 (3.58)
South-east Australian Hillplots 2013	Maximum depth (cm)	111.97 ± 7.77	Australian North	ns (0.87)	ns (–1.38)	0.0032 (2.95)	ns (0.94)
	Maximum depth (90th percentile, cm)	80.05 ± 5.19	Australian South and West	0.042 (2.04)	ns (0.61)	0.014 (2.45)	ns (0.41)
	Root penetration rate (cm$^{-°\mathrm{C}\ \mathrm{day}}$)	0.15 ± 0.01	Indian irrigated	ns (1.16)	ns (–0.51)	ns (1.21)	ns (1.03)
	Total root length (m)	29.53 ± 3.73	Indian rainfed	ns (0.87)	ns (0.29)	0.094 (1.67)	ns (1.39)
South-east Australian Mini-plots 2013	Maximum depth (cm)	121.33 ± 8.42	Australian North	0.047 (1.98)	ns (1.93)	<0.001 (4.07)	ns (0.33)
	Maximum depth (90th percentile, cm)	110.67 ± 9	Australian South and West	ns (1.51)	ns (0.37)	ns (1.58)	ns (1.04)
	Root penetration rate (cm$^{-°\mathrm{C}\ \mathrm{day}}$)	0.17 ± 0.01	Indian irrigated	ns (1.76)	0.024 (2.37)	0.08 (1.75)	ns (1.0)
	Total root length (m)	45.21 ± 6.53	Indian rainfed	ns (–0.29)	ns (–0.83)	ns (1.09)	ns (1.17)

COW(W)-1, HW2004, NIL3–14) or shallow rooting (Westonia and Kennedy, Fig. 5). The deep rooting lines appear to have utilised more water deeper than 140 cm; however, this relationship is only weakly significant ($P = 0.0504$).

Discussion

This study sought to contrast the root systems of wheats sown in India for rainfed conditions with those in Australia; the hypothesis being that, due to the reliance of these Indian rainfed wheats on stored soil moisture, they would have been indirectly selected to possess root architectures with superior access to deeper layers of soil to maximise soil water capture and uptake.

Genetics as a driver of root architecture

The high-throughput soil coring methodologies employed in this project allow the detection of genotypic variation in root traits, but have also revealed a high degree of variation driven by field environment and plot-to-plot variation between replicated genotypes (Wasson *et al*. 2014). The genetic signal can be difficult to detect in this noise. Genotype was a significant driver of maximum depth (90th percentile) in the 2013 mini-plot trial, weakly significant in the two 2013 hill-plot trials, and insignificant in the 2012 hill-plot trials (Table 3). Similarly, genotype was a strongly significant driver of root penetration rate in the 2013 hill and mini-plot trials, but not in the 2012 trial. In contrast, genotype was a significant driver of total root length in the three hill-plot trials, but not the mini-plot trial. Variation in the significance of the effect of different traits at different sites may reflect the strong influence of environmental and edaphic factors on root traits, or in the case of the mini to hill-plot contrast, management. Overall, the genetic influence on root traits is sufficiently detectable to allow the comparison of Indian rainfed, irrigated, and Australian genotypes.

Do the root systems of Indian rainfed and irrigated wheat differ in India or Australia?

Although coring difficulties at the Indian sites (extremely hard clay soils and only manual coring methods available) lead to a limited number of lines in common being cored across sites, some general trends were evident. There were differences in shoot and root traits between the genotypes bred for irrigation and those bred for rainfed production Indian wheats at both Indian sites (Fig. S1). The Indore site produced significantly greater biomass and yield than the Pune site, and the rainfed genotypes produced more that the irrigated genotypes (under rainfed conditions). Although only measured to a depth of 50 cm, there was little evidence of truncation of the maximum depth results. As expected, the rainfed genotypes had significantly deeper roots in central India (Indore), but not in the peninsular region (Pune). The behaviour of the total root length trait varied with site; at Pune, the rainfed genotypes were longer, but not at Indore. The Indore site receives more pre-season rainfall (800 mm compared with 550 mm in Pune; Table 1) and during the study received more in-season rainfall (82.5 mm compared

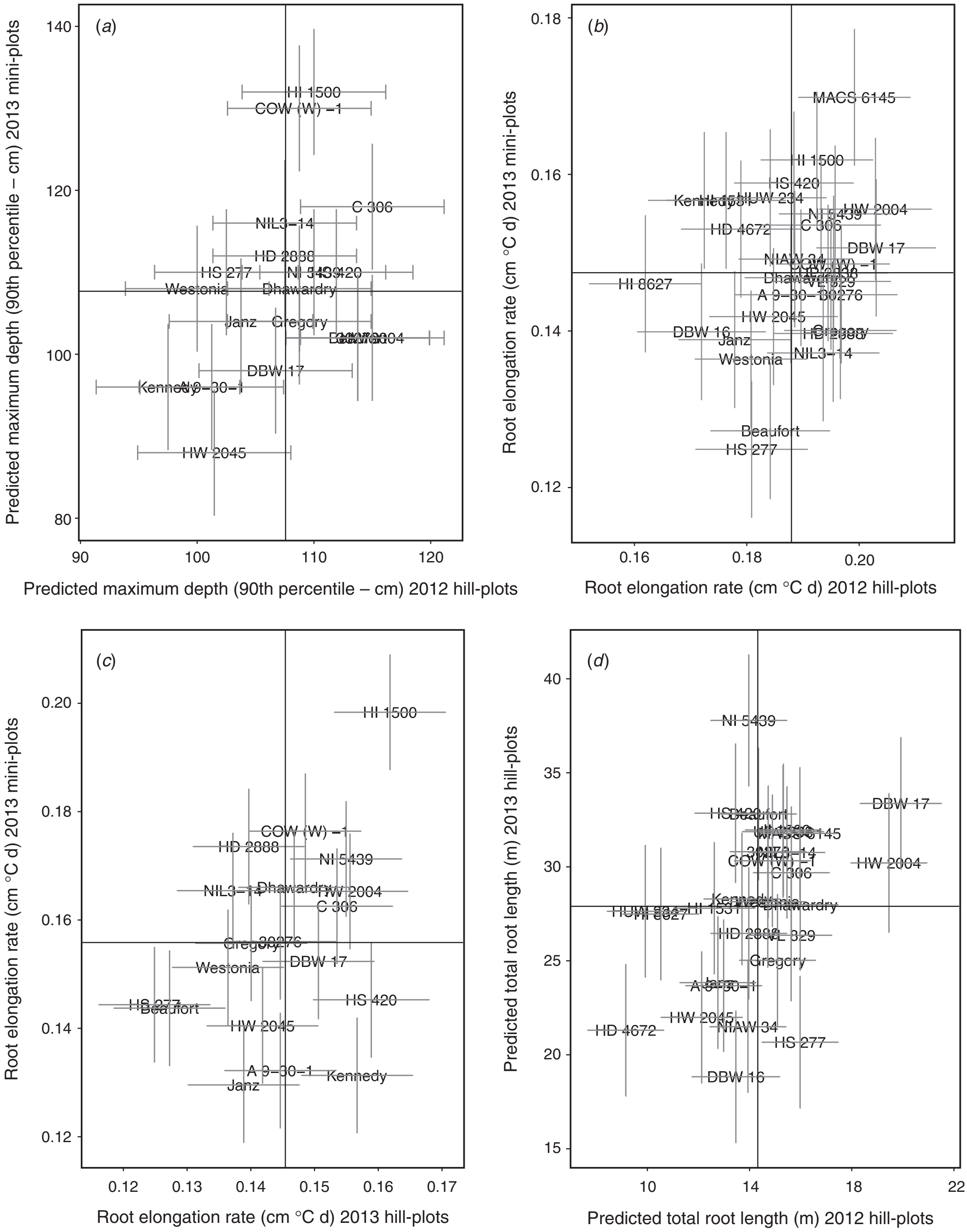

Fig. 4. Genotype means plotted against genotype means from different trials for various root traits. Means from hill-plots in 2012 plotted against genotype means from mini-plots in 2013 for maximum depth (*a*) and root elongation rate (*b*); means for the 2013 hillplots and mini-plot root elongation rates (*c*) and total root length of the 2012 and 2013 hill-plots (*d*). The means and standard errors were derived from a linear mixed model that treated genotype as a fixed effect and replicate as a random effect. Linear models were applied to assess the relationship between genotypes in the two datasets. There was no significant relationship (*a*–*c*, $P = >0.05$), however, there was a significant relationship between the total root length of the 2012 and 2013 hill-plots (*d*), $P = 0.034$.

Table 6. Post-hoc testing of wheat genotypes grouped into geographical and management background classes against the highs and lows (top and bottom 10th percentile of the ranked means) for various root traits, see Table 7 for genotypes in each class

Trial	Group 1	Group 2	Score	Maximum depth (cm)	Maximum depth (90th percentile, cm)	Root penetration rate (cm$^{-°\mathrm{C\ day}}$)	Total root length (m)
South-east Australian Hillplots 2012	High	Australian North	*P*-values	0.001	0.007	0.0014	<0.001
	High	Australian North	Z-scores	3.2	2.7	3.2	4.5
	High	Australian South and West	*P*-values	0.002	0.003	<0.001	<0.001
	High	Australian South and West	Z-scores	3.0	3.0	3.6	5.6
	High	Indian irrigated	*P*-values	<0.001	<0.001	<0.001	<0.001
	High	Indian irrigated	Z-scores	3.7	3.4	3.9	6.2
	High	Indian rainfed	*P*-values	0.003	<0.001	<0.001	<0.001
	High	Indian rainfed	Z-scores	3.0	3.4	3.7	5.4
	Low	Australian North	*P*-values	<0.001	<0.001	<0.001	<0.001
	Low	Australian North	Z-scores	–3.7	–4.1	–3.8	–7.2
	Low	Australian South and West	*P*-values	<0.001	<0.001	<0.001	<0.001
	Low	Australian South and West	Z-scores	–4.0	–3.9	–3.7	–6.0
	Low	Indian irrigated	*P*-values	<0.001	<0.001	<0.001	<0.001
	Low	Indian irrigated	Z-scores	–4.1	–4.1	–3.9	–6.2
	Low	Indian rainfed	*P*-values	<0.001	<0.001	<0.001	<0.001
	Low	Indian rainfed	Z-scores	–5.0	–4.2	–4.5	–7.6
South-east Australian Hillplots 2013	High	Australian North	*P*-values	0.01	0.041	<0.001	<0.001
	High	Australian North	Z-scores	2.6	2.0	3.3	3.6
	High	Australian South and West	*P*-values	<0.001	0.012	<0.001	0.027
	High	Australian South and West	Z-scores	23.1	2.5	23.7	2.2
	High	Indian irrigated	*P*-values	0.008	0.001	0.002	<0.001
	High	Indian irrigated	Z-scores	2.7	3.2	3.0	3.7
	High	Indian rainfed	*P*-values	0.009	0.004	<0.001	<0.001
	High	Indian rainfed	Z-scores	2.6	2.9	3.4	3.5
	Low	Australian North	*P*-values	0.008	0.005	0.006	0.001
	Low	Australian North	Z-scores	–2.6	–2.8	–2.7	–3.2
	Low	Australian South and West	*P*-values	<0.001	ns	<0.001	0.026
	Low	Australian South and West	Z-scores	20.4	-0.8	2.0	–2.2
	Low	Indian irrigated	*P*-values	0.001	0.012	<0.001	0.002
	Low	Indian irrigated	Z-scores	–3.2	–2.5	–4.5	–3.1
	Low	Indian rainfed	*P*-values	<0.001	0.001	<0.001	<0.001
	Low	Indian rainfed	Z-scores	–3.3	–3.2	–4.2	–4.7
South-east Australian mini-plots 2013	High	Australian North	*P*-values	<0.001	<0.001	<0.001	ns
	High	Australian North	Z-scores	4.2	4.9	4.3	1.5
	High	Australian South and West	*P*-values	<0.001	0.003	<0.001	ns
	High	Australian South and West	Z-scores	3.8	3.0	3.4	1.9
	High	Indian irrigated	*P*-values	<0.001	<0.001	<0.001	ns
	High	Indian irrigated	Z-scores	3.5	4.3	4.1	1.5
	High	Indian rainfed	*P*-values	<0.001	<0.001	<0.001	0.038
	High	Indian rainfed	Z-scores	3.7	4.1	3.9	2.1
	Low	Australian North	*P*-values	0.004	ns	0.019	0.006
	Low	Australian North	Z-scores	–2.9	–2.0	–2.4	–2.7
	Low	Australian South and West	*P*-values	ns	ns	0.044	ns
	Low	Australian South and West	Z-scores	–1.3	–2.0	–2.0	–1.0
	Low	Indian irrigated	*P*-values	0.004	ns	<0.001	0.031
	Low	Indian irrigated	Z-scores	–2.9	–1.9	–3.3	–2.2
	Low	Indian rainfed	P-values	<0.001	<0.001	<0.001	0.015
	Low	Indian rainfed	Z-scores	-4.4	–3.8	–4.6	–2.4

Table 7. Genotype groups in post-hoc testing (Table 6)

Highs and lows for various root traits and trials were defined by the top and bottom 10th percentile of the ranked means

Trial	Group	Maximum depth (cm)	Maximum depth (90th percentile, cm)	Root penetration rate (cm$^{-°C\ day}$)	Total root length (m)
South-east Australian hill-plots 2012	High	Gregory	C306	HI 8638	DBW 17
		WH 1021	HW 2004	DBW 17	Syn29589
		COW (W) –1	C306	HW 2004	HW 2004
		33404	Beaufort	PBW 550	Orion
		HI 8638	30276	C306	DL 153–2
		NIL3–14	HS 420	MACS 6145	Wyalkatchem
		Stampede	30374	Stampede	Hunter
		VL 829	UP 2338	WH 1021	PBW 550
		C306	33404	30276	VL 804
	Low	K 9107	Kennedy	Lincoln	HW 2045
		Mace	Seri	Kennedy	CV98
		Impala	HW 2044	K 9107	RAJ 3765
		Lincoln	RAJ 3765	Impala	Lincoln
		MACS 1967	K 9107	DBW 16	HI 8627
		Seri	Lincoln	38–19	38–19
		38–19	NIAW 34	RAJ 3765	K 9107
		HI 8627	Speedee	WH 147	HUW 234
		RAJ 3765	38–19	Seri	B. Yellow
		WH 147	WH 147	HI 8627	HD 4672
South-east Australian hill-plots 2013	High	MACS 6145	HUW 234	MACS 6145	NI 5439
		HI 1500	Kennedy	HI 1500	Syn 29589
		HS 420	Syn 29589	HS 420	DBW 17
	Low	HD 2888	Dhawardry	NIL3–14	NIAW 34
		HW 2045	NIL3–14	Westonia	HD 4672
		Westonia	A 9–30–1	Beaufort	HS 277
		HS 277	HS 277	HS 277	DBW 16
South-east Australian mini-plots 2013	High	HI 1500	HI 1500	HI 1500	HS 277
		COW (W) –1	COW (W) –1	COW (W) –1	C306
	Low	HW 2045	A 9–30–1	A 9–30–1	DBW 17
		Kennedy	Kennedy	Kennedy	Syn 29589
		A 9–30–1	HW 2045	Janz	A 9–30–1

with 32 mm in Pune; Table 2). Although the length of the growth season at the two sites is similar (100–110 days; Table 1), the conditions at the Pune site were far warmer (1671 vs 812 Celsius degree days). The transpiration burden on the Pune may drive the increase in total root length amongst the rainfed genotypes, as the plants struggle to obtain sufficient water, whereas the root systems of the irrigated genotypes are comparatively fixed. However, that explanation is seemingly contradicted by the total root length data from Indore, where it was the irrigated genotypes that had the longer root length. These data suggest that if easier coring methods could be employed, further root studies in India would be of interest to tease apart the development and role of wheat roots across the geographic regions encompassed by the wheat growing regions. Given the prevalence of irrigation in India is would be of interest to study the roots of these lines under different management regimes as well.

Indian rainfed wheats were significantly deeper rooting than Indian wheats grown under irrigation in the 2013 mini-plot trial, which is the most agronomically relevant trial in terms of management. However, that was the sole Australian trial and trait where the two categories of Indian wheat differed, although (as discussed below) there were also few differences between the Indian and Australian material. The categories in these contrasts, as they are based on geography (Australian) and management (Indian), may simply encompass variation in a too wide a range of traits to generate detectable contrasts in the particular traits of interest here; the significance of the narrower category based on the C306 variety background (discussed below) contrasted with other categories would seem to suggest that there are genetic drivers that can be identified.

Do the root systems of Indian rainfed wheats differ from Australian wheats in the Australian field environment?

We hypothesised that the rainfed Indian wheat genotypes would show the deepest rooting traits. We could only differentiate

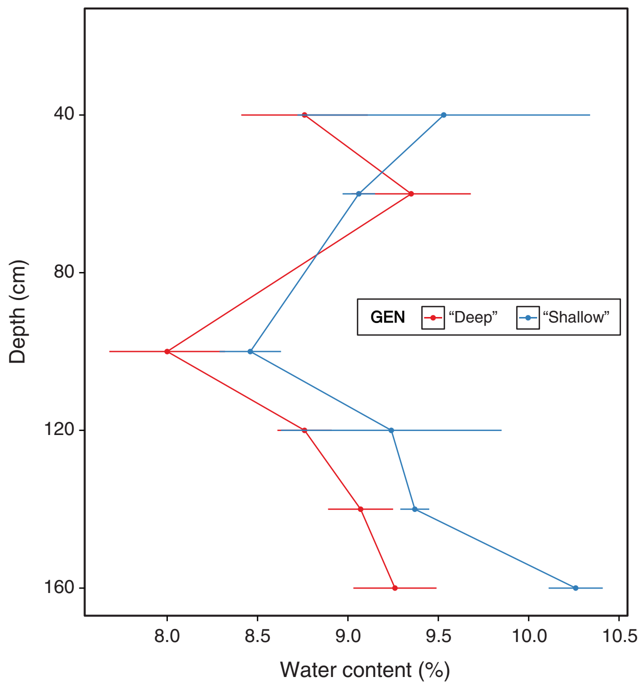

Fig. 5. Gravimetric water content of soil beneath selected lines post-harvest in the 2013 mini-plots. The water content (*x*-axis) is plotted against the depth of the sample (reverse *y*-axis). Six lines were cored for water content ($n = 5$) and then were classed as either deep rooting (red line; C306, COW(W)-1, HW2004, NIL3–14) or shallow rooting (blue line; Westonia and Kennedy). Error bars are the s.e. of the mean across replicates.

between rainfed and irrigated Indian wheats in one trial (as discussed above). Likewise, the wheats from the two Australian geographic regions performed similarly in all trials. This result is not surprising as they were chosen as the highest yielders across very broad sowing regions. Although there was little difference between the classes, when the Indian and Australian wheats were compared, differences did emerge. For depth traits (maximum depth and 90th percentile maximum depth) the rainfed Indian wheats outperformed all the Australian lines in the 2013 mini-plots and some Australian classes in the 2013 hill-plots, although no differentiation was possible in 2012. Both Indian classes performed very well for their root penetration rate, outperforming all Australian lines in the 2013 hill-plot and the Northern lines in the mini-plots. Root penetration rate takes the flowering time into consideration (maximum depth over thermal time at anthesis) as it is believed by many authors that downward extension of the root system ceases just after anthesis (Troughton 1962; Gregory *et al.* 1978; Kirkegaard and Lilley 2007; Thorup-Kristensen *et al.* 2009) although there are contrary observations (e.g. Manschadi *et al.* 2006). Indian lines generally have shorter growing seasons than Australian lines, and this was reflected in the flowering times in 2013 where the Indian lines flowered significantly earlier than the other lines. This was not evident in 2012, possibly because it was a particularly cold and dry season. Our root penetration results show that the Indian lines, although they may not have reached depths greater than the Australian lines, were possibly growing faster and this root vigour could be a highly beneficial trait.

Total root length takes into account the proliferation of roots in the middle and upper soil layers. This trait was included in this study to indicate entire root system vigour. The Indian lines outperformed the northern Australian lines in the 2012 trial but showed no differentiation in the 2013 trials. It is likely that this result was driven by environmental conditions as the very dry conditions in 2012 likely inhibited production of roots in the shallower soil layers, and encouraged the growth of deeper roots as demonstrated in studies by Blum and Arkin (1984 in sorghum roots), and Asseng *et al.* (1998 in wheat roots).

Genotypes and genetic material associated with valuable root traits

The seasonal, site, trial and trait variation made determining overall best performers difficult, however our analysis, combined with ranking genotype performance across traits and trials and overall plant performances in the field allowed us to shortlist several genotypes which stood out as strong performers for root traits. Although Indian high yielding lines did not consistently show high performance across all root traits and trials, the dominance of rainfed Indian lines in our top picks is indicative that the selection pressures in Indian rainfed breeding systems may lead to lines with vigorous deep root systems.

In earlier Australian root trials (Wasson *et al.* 2014) the Indian genotype C306 was flagged as a high performer, and in both the northern and south-eastern Australian trials in 2012 it performed very well (deepest rooted in northern trials and second deepest in the south-eastern, with the deepest rooted being HW 2004, a line backcrossed to C306 seven times). Although it was not a strong a performer in our 2013 trials, C306 still performed well considering these trials were of a funnelled selection of high performers (plus several low performing checks). Released in 1965 for the Indian central zone, C306 is a tall genotype (1.12 ± 0.2 m in the 2013 mini-plots), that was developed to yield well on retreating water and high temperatures (Nagarajan 2006). Our analysis of those lines with a C306 background showed these lines performing better than almost all other groups for all traits in 2012. Although these lines did not perform as well in the 2013 trials, they were still best performers for root penetration, which reflects that these lines penetrate relatively deeply, whilst having a short maturity time (in all our trials C306 was one of our earliest flowering genotypes), indicating strong root vigour. The high performance of these related lines is strong evidence of a genetic driver for the traits contributing to root penetration rate. The genotype HI1500 was a consistent high performer over the four Australian trials and across all traits. This genotype (known as Amrita) was released in 2003 for low fertility soils in rainfed regions. Like C306 it is a tall (1.24 m ± 0.1 m in the 2013 mini-plots), early maturing genotype. Other lines of interest were the rainfed Indian lines COW(W)-1 and NI 5439 and the CM18/Magnif-derived NIL, NIL3–14.

All the high performing lines show variation in their success for the different root traits from trial to trial. However, they are all likely candidates to examine further for both breeding purposes and as key genotypes in elucidating the genetic and physiological cues driving fast root penetration and deep rooting systems in wheat.

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