Journal of Experimental Botany, Vol. 70, No. 18 pp. 4963–4974, 2019
doi:10.1093/jxb/erz232 Advance Access Publication May 14, 2019

RESEARCH PAPER

Deeper roots associated with cooler canopies, higher normalized difference vegetation index, and greater yield in three wheat populations grown on stored soil water

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Received 1 February 2019; Editorial decision 7 May 2019; Accepted 8 May 2019

Editor: Ian Dodd, Lancaster University, UK

Abstract

Simple and repeatable methods are needed to select for deep roots under field conditions. A large-scale field experiment was conducted to assess the association between canopy temperature (CT) measured by airborne thermography and rooting depth determined by the core-break method. Three wheat populations, C306×Westonia (CW), Hartog×Drysdale (HD), and Sundor×Songlen (SS), were grown on stored soil water in NSW Australia in 2017 (n=196–252). Cool and warm CT extremes (‘tails’) were cored after harvest (13–32% of each population). Rooting depth was significantly correlated with CT at late flowering (r= –0.25, –0.52, and –0.23 for CW, HD, and SS, respectively, P<0.05 hereafter), with normalized difference vegetation index (NDVI) at early grain filling (r=0.30–0.39), and with canopy height (r=0.23–0.48). The cool tails showed significantly deeper roots than the respective warm tails by 8.1 cm and 6.2 cm in CW and HD, and correspondingly, greater yields by an average 19% and 7%, respectively. This study highlighted that CT measured rapidly by airborne thermography or NDVI at early grain filling could be used to guide selection of lines with deeper roots to increase wheat yields. The remote measurement methods in this study were repeatable and high throughput, making them well suited to use in breeding programmes.

Keywords: Canopy temperature, core-break, deep root, drought adaptation, NDVI, thermal imaging, wheat.

Introduction

Soil water shortage is a major constraint limiting crop productivity on large tracts of farmland around the globe. Variability in crop productivity is predicted to be exacerbated in some parts of the world by both climate change (Olesen *et al.*, 2011; Ray *et al.*, 2015) and the increased demand for water resources for non-arable crops and non-agricultural uses. Meanwhile, globally, rates of genetic gain in wheat potential yield have slowed to <1% per year during the last two decades (Fischer and Edmeades, 2010; Richards *et al.*, 2014). Hence, developing wheat varieties with better drought adaptation together with improved management tools to mitigate impacts of water deficits are essential to increase wheat yields (Richards *et al.*, 2014). Numerous traits have been proposed and evaluated to increase the productivity of wheat grown in drought-prone environments. These have predominantly been easily measurable above-ground traits (reviewed in, for example, Araus *et al.*, 2002; Richards *et al.*, 2010; Reynolds and Langridge, 2016; Fischer and Rebetzke, 2018). Root traits are still underexploited primarily due to challenges in methodology, in particular for mature field-grown plants (Wasson *et al.*, 2014).

Deeper rooting has been demonstrated to be an important trait to capture water stored at depth. This is especially important during later growth stages when water is used for grain filling as it directly impacts on grain yield (Lopes and Reynolds, 2010; Wasson *et al.*, 2012, 2014; Hendriks *et al.*, 2016). It has been reported that under moderate drought, access to additional water from the subsoil during grain filling can contribute to an increase in wheat yield as high as 59 kg ha^{-1} mm^{-1} (Kirkegaard *et al.*, 2007), although lower values may be more typical [e.g. 12 kg ha^{-1} mm^{-1} calculated from data presented in Morgan and Condon (1986)]. Such yield gains indicate that deeper rooting may be an important trait to pursue in regions with a terminal drought and unused subsoil moisture. Challenges in phenotyping root traits in field environments are numerous: laborious and time-consuming soil coring to extract roots; difficulty in sampling the root system; high plasticity of the root system; soil spatial variability and variability associated with experimental conditions; and the root measurement itself (e.g. core-break versus root washing have differing abilities to discriminate genotypic differences, as shown in Hodgkinson *et al.*, 2017). All these challenges make phenotyping root traits for selection purposes in pre-breeding and breeding programmes a daunting task at a large scale where there may be hundreds and even thousands of genotypes being evaluated. Therefore, simple, fast, and repeatable methods are required to measure relevant root traits directly or indirectly under realistic field environments.

Despite some progress in measuring soil moisture that implies genetic differences in root activity (Whalley *et al.*, 2017), the most reliable and rapid indirect screening methods for deep roots are likely to be above-ground traits that are easily measured and are indicators of which lines have the most favourable water relations and therefore are likely to have root systems accessing greater soil water. Two obvious candidates are canopy temperature (CT) and the retention of green leaf area using the normalized difference vegetation index (NDVI) as a key measurement (Lopes and Reynolds, 2012; Wasson *et al.*, 2012). Previously, CT has been used as a surrogate for stomatal conductance that controls the exchange of water for CO_2 in the plant canopy (e.g. Fischer *et al.*, 1998; Rebetzke *et al.*, 2013). CT is a direct function of the evaporation or transpiration from the leaf surface and thereby is strongly related to stomatal conductance (Rebetzke *et al.*, 2013). Limited access to soil water under dry conditions, increasing ambient temperature, and vapour pressure deficit cause stomata to close and subsequently increase the CT. Accordingly, lines with deep roots accessing soil water may maintain more open stomata and will have cooler canopies. Cool CTs in wheat have been reported to be associated with increased root dry weight at deep soil layers in a few small-scale studies (e.g. with ~10 genotypes) using hand-held instruments to measure CT (e.g. Lopes and Reynolds, 2010; Pinto and Reynolds 2015; Clarke *et al.*, 2017) and with increased root depth in rice (Hirayama *et al.*, 2006). Pinto and Reynolds (2015) reported that under droughted conditions, genotypes with cool canopies extracted 35% more water from the 30–90 cm soil layer than genotypes with warm canopies as a result of more roots at depth. However, as discussed in Rebetzke *et al.* (2013) and Deery *et al.* (2016), hand-held thermometers become problematic to measure CT in large experiments because of the sensitivity of CT to weather fluctuations over the period required to measure large trials. In Deery *et al.* (2016), a new airborne thermal imaging platform was shown to be more suitable for CT measurements in large-scale field studies, demonstrating measurements to be cost-effective, rapid, and with high heritability.

The NDVI is a commonly used trait for high-throughput screening. It is related to leaf chlorophyll content, due to the differential absorption of light in the visible wavelengths by chlorophyll, and also to the green leaf area and thus the photosynthetic capacity of the plant canopy. NDVI has been correlated with vegetative biomass, ground cover, N status, and senescence rate (Babar *et al.*, 2006; Lopes and Reynolds, 2012; Tattaris *et al.*, 2016). For instance, positive relationships between NDVI and crop yield or biomass have often been reported under either irrigated or drought conditions (e.g. Gutierrez-Rodriguez *et al.*, 2004; Babar *et al.*, 2006; Christopher *et al.*, 2016). Under terminal drought, high NDVI late in the growing season (indicating high retention of green leaf area) is likely to be a result of access to extra water; however, few studies have investigated the relationship between NDVI and root traits in the field.

The main objective of the present study was to determine if fast and accurate measurements of CT could be used to guide selection for deep rooting traits in large-scale field screening as well as the relationships with grain yield of these two traits. We explored this question in three diverse wheat populations developed for either genotypic variation in rooting depth, transpiration efficiency, or osmotic adjustment. In addition, rooting depth was also related to other above-ground traits, such as NDVI and canopy height. Populations were grown in a semi-arid environment in south-eastern Australia on a site with a full profile of soil water at sowing. The results should be of interest to scientists exploring the physiological and molecular basis of root system traits and to breeders as a selection tool to develop future wheat varieties adapted to environments where deep soil moisture may be available during grain filling.

Materials and methods

Experimental site

The field trials were conducted at Yanco Agricultural Research Institute in SE Australia, an experimental station of the NSW Department of Primary Industries (34.62°S, 146.43°E, elevation 164 m) with access to irrigation. The soil is classified as chromosol with a clay–loam texture (Isbell, 2002). The average annual rainfall is 418 mm (1981–2010) distributed relatively uniformly across months, and the average annual maximum and minimum temperatures are 24.0 °C and 11.4 °C (1999–2010), respectively (Bureau of Meteorology, 2018). Daily meteorological data during May–November 2017 are presented in Fig. 1. Of note is the relatively below-average rainfall recorded for this period in 2017.

Wheat populations and genotypes

Three populations including the respective parent lines were sown. Two populations (CW and SS) were derived from unrelated parents and thus are relatively variable, whereas the third population (HD) was developed from closely related parents. The CW population is a doubled-haploid population developed from crossing C306, a tall wheat grown widely

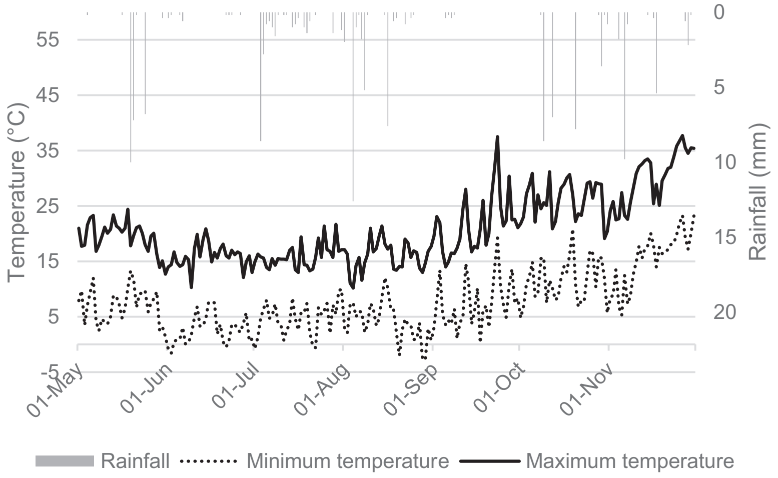

Fig. 1. Daily minimum and maximum air temperature and rainfall at Yanco Agricultural Institute, NSW, Australia, 1 May–30 November 2017.

Table 1. *The total number of genotypes, replicates, and plots in the rainfed experiments, and in the canopy temperature (CT) tails selected for coring in the three populations*

	CW		**HD**		**SS**	
Rainfed experiment						
Genotypes	196		198		252	
Replicates	1–6		3		3	
Columns	18		18		18	
Rows	24		33		42	
Plots	432		594		756	
CT tails selected for coring						
CT tail	Cool	Warm	Cool	Warm	Cool	Warm
Genotypes	32	32	19	19	15	15
Replicates	1–3	1–3	3	3	3	3
Plots	40	40	57	57	45	45

The three populations were CW, C306×Westonia; HD, Hartog×Drysdale; and SS, Sundor×Songlen. The CW population was sown in a partial replicate design, and the HD and SS in randomized complete block design with three blocks each. All plots were arranged in the same number of columns but different rows in the same paddock. The two parent lines were not included in the CT tails of both HD and SS but cored.

in India with Westonia, a semi-dwarf wheat previously grown widely in Western Australia. Earlier studies showed C306 to have deep roots and Westonia to have relatively shallow roots in SE Australia (Wasson *et al.*, 2012; Rich *et al.*, 2016). The SS population is also a doubled-haploid population developed from two unrelated wheats, Sundor and Songlen, which were previously both grown widely in the northern Australian wheat belt. Songlen has been reported to have high osmotic adjustment whereas Sundor lacks the osmotic adjustment trait, and lines selected from an earlier developed population from this cross were shown to differ in their ability to extract water from depth (Morgan and Condon, 1986). The HD population was derived from Hartog and its backcross derivative Drysdale. The two parents are genetically related, having a co-ancestry of 87.5%. Hartog was a successful wheat introduced from CIMMYT and grown in northern NSW, whereas Drysdale was a backcross derivative of Hartog selected for low carbon isotope discrimination and therefore high transpiration efficiency arising because of generally reduced stomatal conductance when water was not limiting (Rebetzke *et al.*, 2002). The three populations were all developed to study their performance under water-limited conditions; they are of interest because the primary trait of interest for crossing each set of parents is different. The populations were at different stages of development, and this resulted in different amounts of available seeds. Seed was a limiting factor for the CW population but not for the HD and SS populations. This resulted in some differences in trial design between populations and also altered the strategy for selecting which plots to core to determine rooting depth (see below).

Experiments

The three populations were sown in the same field as separate but adjacent experiments. There were 196, 198, and 252 genotypes in the CW, HD, and SS populations, respectively (Table 1). Each experiment was arranged in a row–column design and sown East–West. Due to seed limitations, the CW population was sown as a partially replicated design with an average 2.2 replicates; the HD and SS experiments were each arranged in three fully replicated blocks. Plot size was 1.8×1.8 m for the CW and 1.8×6.0 m for the HD and SS populations. Plots contained 10 rows with row spacing of 18 cm, and the sowing density was 160 seed m^{-2}. Plots were sown on 16 May 2017 in a field which previously had been planted to lucerne and then killed the previous spring. The field was fully irrigated on 5 May 2017 to help fill the soil profile and to ensure uniform establishment. No further irrigation was supplied during the season, thereby allowing the wheat to grow on stored water and in-season rainfall (hereafter 'rainfed' experiments). Plots were given adequate fertilizer, and pests were managed with recommended chemicals. All CW plots were harvested for grain yield on 23 November, and HD and SS on 28 November, excluding the outside rows in each plot. For other purposes, the HD and SS lines were also sown in an adjacent field in two fully replicated blocks. Irrigation water was supplied during the season to minimize drought stress (hereafter 'irrigated' experiments). Airborne CTs were measured on all rainfed and irrigated plots, and root measurement was conducted in selected plots of the rainfed trials (for details, see below).

Above-ground measurements

The CT was measured 5–6 times at 1–2 h interval from 9.00 h to 15.00 h on eight dates commencing from late tillering and concluding near the end of grain filling. The dates were: 24 August; 11, 20, 28 September; 3, 10, 24 October; and 9 November. Detailed information on the thermal imaging and thermal data extraction is reported in Deery *et al.* (2016). Briefly, thermal images were obtained at cloud-free times with a thermal infrared camera (FLIRR SC645, FLIR Systems, Wilsonville, OR, USA) mounted to view vertically through the floor of a cargo pod fitted onto a Robinson R44 Raven helicopter. Selected technical specifications of the camera were: ±2 °C or ±2% of reading; <0.05 °C pixel sensitivity; 640×480 pixels; 0.7 kg without lens; 13.1 mm lens. The flying was typically at 60–90 m above ground level and at a flight velocity of 45–65 km h^{-1}. The resolution was ~100–204 temperature pixels per m^2 of plot canopy. From the thermographic images, the CT data were extracted for each plot using a custom software, ChopIt, which was developed with Python 2.7 (Python Software Foundation, https://www.python.org/). The ChopIt software is designed for high quality control in a way that the user can manually exclude sections of the plot during the semi-automated plot segmentation for data extraction, such as edge margins or areas with prior biomass cutting.

Crop height was estimated by a LiDAR (Light Detection and Ranging) sensor (LMS400-2000, SICK AG, Waldkirch, Germany) mounted vertically on the Phenomobile Lite (http://www.plantphenomics.org.au/services/phenomobile/; Deery *et al.*, 2014; Jimenez-Berni *et al.*, 2018) at a height of 2.1 m above ground. NDVI was measured using an active NDVI sensor (GreenSeeker, Trimble, USA) also attached to the Phenomobile Lite but mounted at a height of 1.5 m. The LiDAR and NDVI measurements were carried out on eight dates, namely 15, 30 August; 20 September; 5, 11, 18, 25 October; and 1 November 2017, representing both pre- and post-anthesis developmental periods. There was no measurement for HD and SS on 15 August and 18 October. The rate of senescence (RS) was defined as the rate of NDVI decay during post-anthesis, and estimated as the slope of a linear regression of NDVI against the date of measurement (11 October–1 November), and the value was negative (modified from Lopes and Reynolds, 2012). The height on the last measurement date was used later in the analysis.

Development scores using the Zadoks scale were recorded for the CW lines on 19 September and 5 October 2017 and for the irrigated SS plots on 27 September (Zadoks *et al.*, 1974). The development stage was very close in the BC$_3$ HD population as revealed in previous multi-year and multi-site trials across Australia (G. Rebetzke, personal communication), and thus was not recorded in this experiment. Descriptive statistics including the minimum, maximum, and mean of these traits in the selected CT tails (see below) from each population can be found in Table 2.

CT tails selected for coring

Based largely on the CT measurements, a subset of genotypes representing extremes in each population were selected and grouped into 'tails' (after Rebetzke *et al.*, 2017) to measure their rooting depth. Considering the high correlation among the multiple CT measurements during each day and across dates (Supplementary Table S1 at *JXB* online), the mean CT of three measurements during 12.00–14.00 h was used in the selection procedure.

Two different strategies were employed to select the CT tails for coring in the three populations due to the different experimental designs and availability of CT in the irrigated environment. For the CW population, the cool and warm tails were selected based on the rankings of CT at anthesis (3 October) and the average post-anthesis NDVI (11 October–1 November) in individual plots. The cool tails were selected from the one-third plots with the lowest CT at anthesis and the one-third plots with the highest post-anthesis NDVI, and warm tails with the opposite

Table 2. *The minimum (Min), maximum (Max), and mean values for selected traits in the canopy temperature (CT) tails selected for coring from the three populations*

Trait	CT tail	CW		HD		SS		Date
		Cool	Warm	Cool	Warm	Cool	Warm	
Depth	Min	80.0	72.5	87.5	84.2	100.0	98.3	After harvest
(cm)	Max	125.0	117.5	118.3	111.4	119.2	115.8	
	Mean	99.1	93.0	103.3	96.0	107.2	105.5	
Z-score	Min	45	45	NA		55	55	CW: 19 September
	Max	51	55			66	66	SS: 27 September
	Mean	47	51			62	59	
CT	Min	28.6	31.1	28.9	29.7	28.4	29.7	CW: 3 October
(°C)	Max	30.3	34.0	30.2	31.2	29.6	30.5	HD: 10 October
	Mean	29.9	31.9	29.4	30.4	29.1	30.1	SS: 10 October
NDVI	Min	0.4487	0.3320	0.3896	0.3597	0.3772	0.3516	11 October
	Max	0.6033	0.4455	0.4876	0.4526	0.4959	0.4684	
	Mean	0.5280	0.3882	0.4455	0.3910	0.4121	0.3950	
RS	Min	–0.0211	–0.0140	–0.0146	–0.0122	–0.0146	–0.0117	11 October–1 November
(NDVI units d^{-1})	Max	–0.0099	–0.0070	–0.0097	–0.0086	–0.0090	–0.0074	
	Mean	–0.0159	–0.0108	–0.0124	–0.0104	–0.0106	–0.0095	
Height	Min	67.0	58.0	62.3	54.3	62.3	56.7	1 November
(cm)	Max	91.0	81.0	68.3	65.3	74.7	68.3	
	Mean	79.5	69.7	65.4	60.0	68.7	60.5	
Yield	Min	2.8	2.2	2.9	2.7	2.8	2.7	CW: 23 November
(t ha^{-1})	Max	4.9	4.3	4.0	3.8	3.9	3.8	HD: 28 November
	Mean	3.7	3.1	3.5	3.3	3.3	3.4	SS: 28 November

Traits included: the rooting depth (Depth) determined by the core-break method after harvest, development score (*Z*-score), CT at about late flowering/early grain filling (mean values of three measurements during 12.00–14.00 h), NDVI at around the onset of grain filling (NDVI), rate of senescence (RS) using the slope of NDVI decay during post-anthesis, plant height (Height), and grain yield at maturity. The Min and Max reported were from individual plots in CW, and genotype means in HD and SS. Rooting depth was the average of the four cores per plot. Date indicates when the measurement was conducted.

combination. Additionally, to control the potential confounding factors of plant height and development on CT and grain yield, plots with extreme heights and development scores (scored on 19 September) were excluded (i.e. 10% of the plots with the largest and the smallest values). Eventually, 40 plots for each of the cool and the warm tails were selected, respectively, covering 62 genotypes in the CW population (Table 1).

Because resources for root coring and scoring were limited, selection of tails was based on a different strategy for the HD and SS populations. It was reasoned that canopies could be cool after anthesis for two reasons, either because water was conserved before anthesis, and so leaving more water available to transpire after anthesis, or because roots were better at extracting deep water after anthesis. The interest here was in identifying differences in the latter scenario, so lines that were warm under irrigation before anthesis were excluded from selection into tails because they were likely to have been conserving soil water before anthesis. The cool tails were selected from those genotypes with a negative CT deviation from the grand mean of the irrigated population both before and after anthesis and with a negative CT deviation from the grand mean of the rainfed population after anthesis (i.e. genotypes that maintained a cool CT not only under irrigation but also under drought). The warm tails were selected from those genotypes with a negative CT deviation from the grand mean of the irrigated population but with a positive deviation from the grand mean of the rainfed population, which indicates that these genotypes maintained a cool CT with sufficient water but not under water limitation. Genotypes with extreme heights were also excluded in the SS during the selection, whereas there was very little height variation in the HD population. There were 40 and 32 genotypes including the corresponding parents selected for coring in the HD and SS populations, respectively (Table 1).

Core-break method for rooting depth

Four soil cores were taken from well inside each selected plot, two along the crop rows and two between the rows. Soil cores of ~180 cm length were extracted by a tractor-mounted, hydraulic corer. The internal diameter of the coring tube was 4.2 cm. Design of the coring equipment was detailed in Wasson *et al.* (2014). The intact soil cores were transferred onto a 2 m long tray with 10 cm increment marks. The cores were scored from the bottom up because the study was most interested in deep roots and not roots near the soil surface, and also to minimize the time and labour required for scoring. Cores were broken by hand in 10 cm intervals and visually checked for the presence of roots. The number of live wheat roots from the current season was recorded on both broken core faces. The counts on both faces were then summed for each depth and used in later analysis. The break count was continued upwards along the cores until non-zero counts were recorded at no less than three depths. Wheat roots of the current season were determined as those being supple and white coloured, according to Watt *et al.* (2008). The core-break count on a total of 1184 cores was completed with a team of 5–7 people in two campaigns in late January–early February 2018 (~5–6 d in total).

Quality control of core-break count data

A conservative quality control was applied to the core-break count data using the following two criteria. First, if zero counts were recorded consecutively at ≥2 depths (20 cm core) above where roots were identified, the count of roots at depth was changed to zero. Secondly, counts with a question mark were changed to zero. The question marks had been noted at the time of counting when a root count operator had doubted if a root was from the current season. Maximum rooting depth in each core was determined as the maximum depth where presence of root was recorded following quality control corrections.

Soil moisture

At sowing, four soil cores down to 170 cm were taken transversely over the CW field to determine the soil water content. The soil cores were sectioned every 10 cm, stored in sealed plastic bags, and dried subsequently at 70 °C for at least 96 h (Fig. 2). To determine the soil moisture content after the crop, the 90–100 cm soil section was saved from one core per plot of the HD population after the core-break count in sealed plastic bags and later dried at 105 °C for at least 48 h.

Statistical analysis

Statistical analyses were performed using the R software v3.4.1 (R Core Team, 2017). Linear mixed models were applied separately to the rooting depth data and to above-ground traits to test the difference between the cool and warm tails of each population. The linear mixed model was fitted using the lme function in the nlme package (Pinheiro *et al.*, 2017). For the rooting depth, the CT tails (cool versus warm), replicate (or block) of each genotype, and the root count operator were taken as the fixed effects, and row and column number of individual plots as linear covariates. The structure of four cores nested in each plot was included as a random effect. For the above-ground traits, after checking the Akaike information criterion (AIC) of the models with/without the column/row covariates, a mixed model was decided with only the CT tails and the block (in HD and SS) as the fixed effect terms and the plot ID as the random effect term. To test the significance of the fixed effects, the Anova function in the car package was used (Fox and Weisberg, 2011).

Phenotypic correlations were estimated between rooting depth and above-ground traits across the selected CT tails within each population, whereas for all pairs of above-ground traits the data from all plots in the rainfed trial of each population were used. Correlation matrices were obtained using the rcorr function in the Hmisc package (Harrell, 2017). Additionally, principal component analysis (PCA) was performed for the rooting depth and above-ground traits in the selected CT tails within each population using the prcomp function. Biplots representing the two largest principal components were then generated to summarize the relationships across the different traits measured on the CT tails using the factoextra package (Kassambara and Mundt, 2017) in R.

Results

Weather and deep stored soil water for wheat growth

At the experimental site, the total rainfall was 738 mm in 2016 but only 292 mm in 2017 compared with 418 mm for the long-term average (1981–2010). The very wet 2016 secured storage of deep soil water for the 2017 season. In particular, there was 184 mm of rainfall in September–October 2016 alone at the time of the removal of lucerne, and 164 mm in the 6 months of November–April prior to sowing of the 2017 trial. In contrast to this, there was only 132 mm during the 191 growing days of the wheat crop. There was 24 mm rain within 1 week after sowing in May (17–24 May), but only 1 mm during 26 August–7 October (Fig. 1) which encompassed the stem elongation, booting, heading, and flowering stages in the

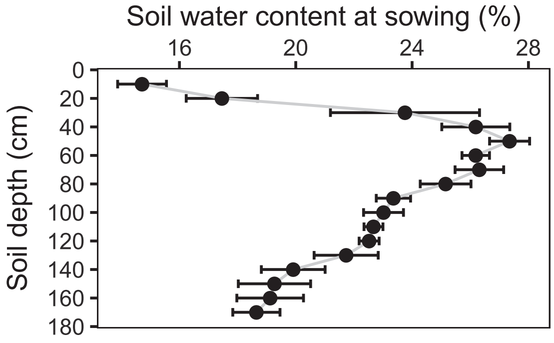

Fig. 2. Soil water content in the profile of the CW field at sowing on 17 May 2017 (*n*=4).

majority of the genotypes. Such conditions would encourage deep root growth and provided favourable conditions to differentiate genotypes with deeper roots.

The soil was fully saturated at sowing due to a wet 2016 season, and ~100 mm pre-irrigation. At sowing, the soil water content was 22–27% at 30–130 cm depth (Fig. 2). After harvest, the soil cores from the HD trial showed that the residual moisture content at the 90–100 cm soil layer was 12–16%, which was not significantly different among the cored genotypes or between the cool and warm tails (data not shown).

To provide some context for the impact of the dry conditions on grain yield in the rainfed experiments, the average yields in the adjacent irrigated HD and SS populations were 7871 kg ha^{-1} and 6991 kg ha^{-1} compared with 3356 kg ha^{-1} and 3090 kg ha^{-1} for the rainfed experiments, respectively.

Variation in canopy temperature

Canopy temperature was highly correlated across eight measurement dates (P<0.05), with stronger correlations between measurements closer in time (e.g. r=0.7–0.9 for CT measured on any two adjacent dates; Supplementary Table S1), confirming high repeatability of the airborne CT measurements. The hourly CT data were also highly correlated on any date (data not shown), and particularly among those between 12.00 h and 14.00 h (r=0.7–0.9 on most dates). Moreover, the range in CT values in each population was greatest during the period 12.00–14.00 h compared with other times of the day (data not shown), which suggests that together with the stronger correlations here, the CT at this time of the day can display a larger variability and therefore greater suitability for selection of genotypes differing in CT. Therefore, the plot selection for coring and further analysis was based on the average CT within this early afternoon period. Because most of the genotypes in CW were at late flowering or post-flowering on 5 October, and the CT on 3 and 10 October was highly correlated (r=0.9, P<0.001 for both CW and SS; Supplementary Table S1), the CT on 3 October was used for plot selection to core in CW, and the CT on 10 October was used for HD and SS. The CT range for the cool versus warm tails was greatest in CW and smallest in SS (Table 2). The CT difference between the two CT tails were on average 2 °C for CW and 1 °C for the other two populations (Table 3), although it must be noted that data compared here were from individual plots of CW and the mean CT from three plots of each genotype in the HD and SS populations.

NDVI and rate of canopy senescence

NDVI showed high repeatability, and stronger correlations were observed between measurements closer in time (e.g. r=0.6–0.9 for NDVI measured on any two adjacent dates; Supplementary Table S2). The NDVI values generally decreased from the first measurement in August in all three populations (Fig. 3), and were consistently higher in the cool than the warm tails (P<0.001 for CW and HD, but P>0.05 for SS; Table 3; Fig. 3). Additionally, NDVI (11 October) was negatively correlated with CT (10 October), which was consistent across all three populations with correlation coefficients between –0.60 and –0.62 (P<0.001; Table 4, based on data from each rainfed population; r= –0.69, P<0.001 when analysing the whole set of genotypes in the rainfed experiments). This suggests that canopies retaining more green area were associated with cooler temperatures.

The RS was negatively correlated with NDVI measured on all dates (except on 1 November), and the correlation was highly significant. For instance, the correlation coefficients between RS and NDVI on 11 October were –0.9 in both CW and HD (P<0.001; Table 4). This indicates that the greener the canopy at about early grain filling, the faster the NDVI decays after flowering (i.e. the greater the absolute value of the RS).

Plant height

The range of plant heights was 52–94 cm across all individual plots of CW, and 53–72 cm and 51–75 cm across all genotype means in the HD and SS populations (data not shown). A similar negative correlation was seen between plant height and CT (10 October) across the three populations (r= –0.5 to –0.7, P<0.001, Table 4; r= –0.7, P<0.001 for the whole set of rainfed genotypes), indicating that taller plots are associated

Table 3. *The fixed effect of the canopy temperature (CT) tails on selected traits and the correlation (Corr) between these traits and the rooting depth (Depth) based on the data from the cored CT tails*

Trait	Fixed effect	CW	HD	SS
Depth	Warm–Cool	–8.1 cm***	–6.2 cm**	–0.5 cm^{NS}
	Replicate	*	*	^{NS}
	Column	***	^{NS}	^{NS}
	Row	^{NS}	*	*
	Root count operator	^{NS}	***	***
Z-score	Warm–Cool	4.5***	NA	–2.4*
	Replicate	–		–
	Corr	0^{NS}		–0.13^{NS}
CT	Warm–Cool	2.0 °C***	1.0 °C***	1.0 °C***
	Replicate	–	***	***
	Corr	–0.25*	–0.52***	–0.23*
NDVI	Warm–Cool	–0.1398***	–0.0516***	–0.0148^{NS}
	Replicate	–	*	*
	Corr	0.30**	0.39***	0.35**
RS	Warm–Cool	0.0051***	0.0021***	0.0011***
	Replicate	–	^{NS}	^{NS}
	Corr	*–0.33***	*–0.39****	*0.03*^{NS}
Height	Warm–Cool	–9.0 cm***	–5.4 cm***	–8.1 cm***
	Replicate	–	*	^{NS}
	Corr	0.23*	0.48***	0.13^{NS}
Yield	Warm–Cool	–628 kg ha^{-1***}	–258 kg ha^{-1***}	30 kg ha^{-1NS}
	Replicate	–	***	^{NS}
	Corr	0.12^{NS}	0.56***	0.21*

Selected traits are as per Table 2. 'Warm–Cool' indicates the estimated difference between the CT tails in each trait using a linear mixed model. Replicate is the replicate or block of each genotype. Column and row represent the coordinate of each plot. Root count operator was n=4–5 for each population. *** significant at P<0.001, ** significant at P<0.01, * significant at P<0.05. NS, not significant.

Table 4. *Correlation matrices for selected above-ground traits in the three populations based on the data from all genotypes in each rainfed population*

	Yield	CT	RS	NDVI
		CW (*n*=432)		
CT	–0.40***			
RS	–0.42***	0.52***		
NDVI	0.44***	–0.61***	–0.91***	
Height	0.24***	–0.51***	–0.21***	0.24***
		HD (*n*=594)		
CT	–0.70***			
RS	–0.51***	0.48***		
NDVI	0.68***	–0.62***	–0.94***	
Height	0.67***	–0.67***	–0.49***	0.59***
		SS (*n*=756)		
CT	–0.21***			
RS	–0.34***	0.36***		
NDVI	0.34***	–0.60***	–0.57***	
Height	0.25***	–0.59***	–0.33***	0.28***

Selected above-ground traits are as per Table 2 excluding the *Z*-score. Values are correlation coefficients. *** significant at $P<0.001$.

with cooler canopies. Even with a reduced range in plant height in the selected CT tails, the cool tails were ~5–9 cm taller than the warm tails across populations (Table 3). Moreover, taller lines were also associated with a greater NDVI (r=0.2–0.6, $P<0.001$; Table 4).

Grain yield

The cooler tails significantly outyielded the warmer tails by 628 (+19%) kg ha^{-1} and 258 (+7%) kg ha^{-1} in CW and HD populations, respectively ($P<0.001$; Table 3). In contrast, no significant difference in yield of cool and warm tails was observed in SS ($P>0.05$). Greater yield was significantly correlated with deeper rooting in HD and SS populations but not in CW (Table 3). Additionally, grain yield showed consistent correlations with above-ground traits measured on all genotypes across the three populations (Table 4). For example, yield was negatively correlated with variation in CT and RS, but positively correlated with NDVI and plant height ($P<0.001$). Genotypes that maintained a cooler and greener canopy at late flowering/early grain filling had higher grain yields. Plant height under these growing conditions was also an indicator of increased yield.

Deeper roots in the cool than the warm extremes

Core-break count was undertaken on 320, 480, and 384 cores in the CW, HD, and SS populations, respectively, and the range in rooting depth among cores was large, namely 60–160, 50–160, and 70–170 cm across the three populations. Roots were significantly deeper ($P<0.05$) in the cool than in the warm tail in the CW (by 8.1 cm) and in the HD (by 6.2 cm) populations, but were not statistically different in the SS population (Table 3). Additionally, the linear mixed models identified statistically significant effects of the row–column spatial factors and individual root count operators on the rooting depth, emphasizing the importance of taking into account the environmental and human variation when analysing field root data.

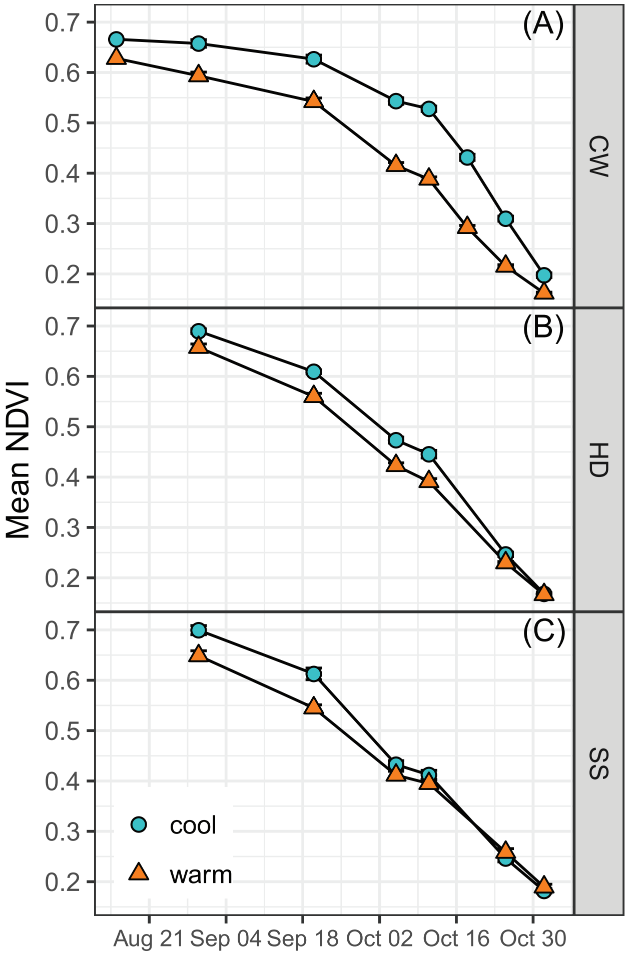

Fig. 3. The declining trend of NDVI in the cool and warm tails in the CW (A), HD (B), and SS populations (C) during August–November. The points represent the means of all plots or genotype means in each tail, and bars the SE (*n*=40 for CW, 19 for HD, and 15 for SS). (This figure is available in colour at *JXB* online.)

Associations between rooting depth and above-ground traits

The biplots derived from PCA summarized relationships between different variables measured for the selected CT tails of each population grown on stored soil water (Fig. 4). The first two principal components explained 71–85% of the total phenotypic variance in the three populations. Some consistent patterns were seen for rooting depth and above-ground traits across the three populations: for example, negative associations between rooting depth and both CT and RS (a >90° angle between the vectors representing the corresponding traits), and positive associations between rooting depth and both NDVI and plant height (a <90° angle). The rooting depth was also positively associated with grain yield, more closely in HD and SS than in CW.

The PCA confirmed the correlation between rooting depth and above-ground traits shown in Table 3 that deeper roots were significantly correlated with lower CT (r= –0.2 to –0.5,

$P<0.05$; Table 3; Fig. 5) and with greater NDVI ($r=0.3–0.4$, $P<0.01$) in each population. Rooting depth was also correlated positively with plant height in CW and HD ($r=0.2$ and 0.5, respectively, $P<0.05$), but was not significant in SS (Table 3). This probably reflects the beneficial value of deeper rooting on crop height when water supply is limiting. Moreover, greater grain yield was associated with deeper rooting in HD and SS ($r=0.6$ and 0.2, respectively, $P<0.05$, Table 3). Rooting depth was not correlated with variation in flowering time in CW and SS (as indicated by the Z-score, Table 3). Besides the correlation between plant traits, a weak but significant negative association was recorded between the rooting depth in HD and the residual soil moisture content at depth after harvest (i.e. moisture in the 90–100 cm soil layer, $r=-0.2$, $P<0.05$, data not shown). The regression slope indicated that each 1.7 cm of additional rooting depth was associated with a 1% drop in soil moisture content at this depth. This indicated a depletion in soil water as roots progressed deeper in the soil profile.

Discussion

CT as a proxy for rooting depth

In water-limited environments, deep roots are essential to access soil moisture at depth, so as to maximize crop yield. A few studies have demonstrated that greater yields are associated with deeper roots in wheat (e.g. Lopes and Reynolds, 2010; Lilley and Kirkegaard, 2011; Ober *et al.*, 2014). To measure rooting depth and other root traits accurately and efficiently is difficult, particularly when assessing a large number of genotypes, under field conditions. Cooler CT measured in field plots has been suggested as a surrogate for deep rooting as a genotype that is able to maintain transpiration via access to additional water deep in the soil profile will be cooler (e.g. Lopes and Reynolds, 2010; Wasson *et al.*, 2014; Pinto and Reynolds, 2015). However, these cited studies used either a single bi-parental wheat population with root measurements in only 8–10 selected wheat lines, or a diverse range of cultivars that were genetically unrelated, and the CT was measured with a hand-held thermometer. It is now possible to scale up the frequency of CT measurements using airborne thermography such that thousands of lines can be screened with a high degree of precision and repeatability (see Deery *et al.*, 2016). In this study, we demonstrated the consistent negative correlation between CT and rooting depth and the value of cool CT as a surrogate for the deep rooting trait in large-scale trials under realistic field conditions by using the novel airborne thermography as described in Deery *et al.* (2016). The small R^2 values of the correlation between CT and rooting depth (Fig. 5) were probably due to the large variation associated with the rooting depth measurement.

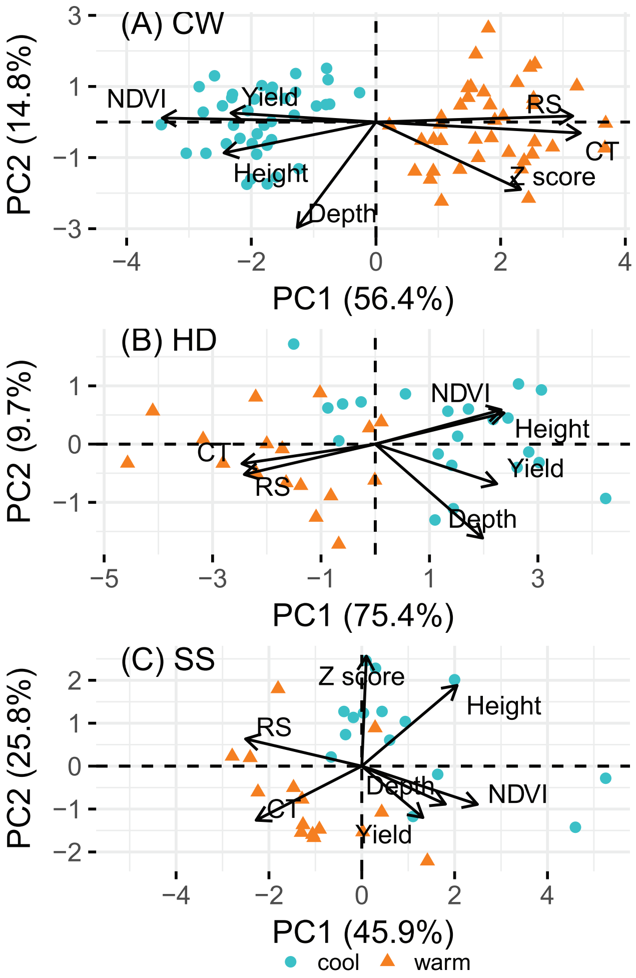

Fig. 4. Biplots showing the principal component analysis of rooting depth and selected above-ground traits in the CW (A), HD (B), and SS populations (C). Selected traits are as per Table 2. Data from individual plots were used for CW, and means of replicated plots for HD and SS. This figure is available in colour at *JXB* online.)

The core-break method

Rooting depth was determined by visually identifying the wheat roots on the surfaces after breaking successive 10 cm segments of soil cores. The significant effect of the root count operator on rooting depth (Table 3) emphasizes how difficult it is to identify roots in the soil cores. Optimally, roots from every 10 cm soil core segment would be washed out and scanned to determine rooting depth and root length density (RLD) at given depths. However, the time and resources needed for such an exercise are not feasible for screening multiple large wheat populations. Moreover, high RLD does not always indicate high water uptake, and a small number of deep roots can contribute to a significant portion of water uptake by the plants (e.g. Gregory *et al.*, 1978; Ober *et al.*, 2014), particularly under conditions where soil water in the upper soil layers has been depleted. Evidence for this is a weak but significant correlation between rooting depth and soil moisture at the 90–100 cm layer observed in HD at the time of root coring ($r=-0.2$, $P=0.03$; data not shown), indicative of soil water depletion as the roots grow into deeper soil layers. Based on the needs when processing a large number of lines under field conditions, the core-break count method used for determining the rooting depth is the optimal compromise for precision and time for the purpose of the present study.

Relationship of CT to rooting depth and yield

Statistically significant differences in rooting depth were identified between the cool and warm CT tails (Table 3) in two

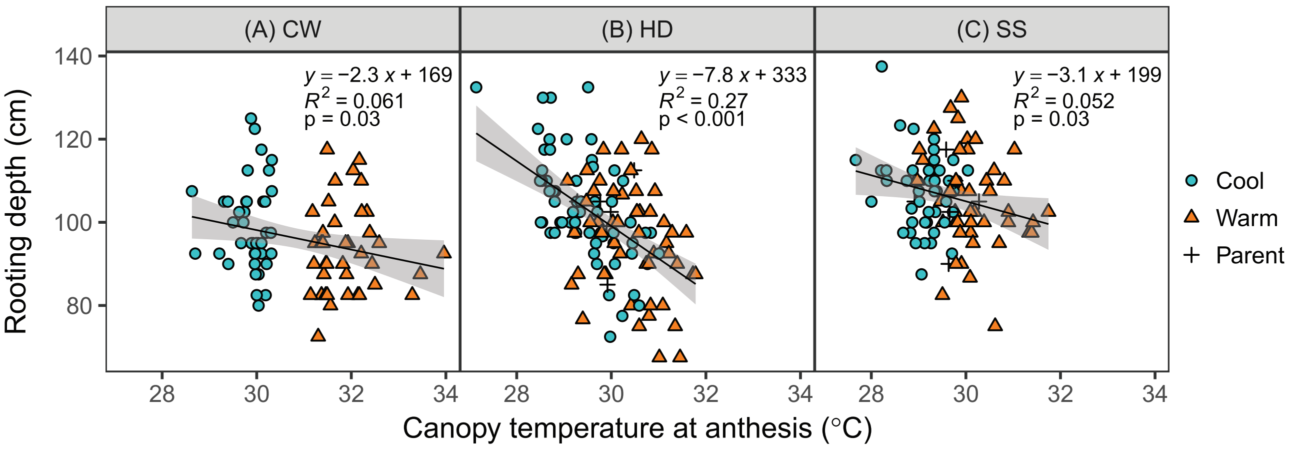

Fig. 5. Relationship between the rooting depth and CT at anthesis in the three populations. The grey band indicates the 95% confidence interval. This figure is available in colour at *JXB* online.)

(CW and HD) of the three examined populations, which accords with previous studies. For example, significantly greater root biomass at the 60–120 cm soil layer was observed in the cool CT group identified from a small number of wheat genotypes drawn from a single population grown in Mexico (Lopes and Reynolds, 2010). Similar results have been reported by Clarke *et al.* (2017) using a doubled-haploid wheat population in a UK field environment. Moreover, we found that greater rooting depth in the cool tail coincided with an increase in grain yield of 628 kg ha^{-1} and 258 kg ha^{-1} in the CW and HD populations, respectively (Table 3). A similar increase of 620 kg ha^{-1} in wheat yield has been reported due to an extra water extraction of 11 mm from the subsoil under moderate post-anthesis drought in Australia (Kirkegaard *et al.*, 2007). The correlation between rooting depth and grain yield in CW was not significant despite there being significant differences in both variables between the cool and warm tails, which may be attributed to large sampling variation considering that data from individual plots were used rather than means of replicated plots as in the other two populations. These findings further demonstrate that deeper roots that can access soil water at depth can contribute to yield increases in a terminal drought.

Although the rooting depth did not differ between the CT tails in the SS population, there was a weak but significant positive association between rooting depth and grain yield (r=0.2, P<0.05; Table 3). This may have been associated with sampling variation. It could also be due to other unknown factors influencing the relationship between CT and rooting depth.

NDVI and rooting depth

The consistent positive association between rooting depth and NDVI in the tails of all three populations (r=0.3–0.4; Table 3) was encouraging, despite the fact that NDVI was not used as a criterion in the selection of tails for the HD and SS populations. The simplest interpretation of this is that the deeper roots extracted additional water to reduce the negative impact of drought on the retention of leaf greenness. Cooler CT in genotypes with deeper roots indicates that the extra available water resulted in more favourable water relations and a higher stomatal conductance to sustain photosynthesis activity for longer. Similar findings were reported in Hendriks *et al.* (2016), and are supported by the consistent observation in all populations of a high negative correlation between CT and NDVI (Table 3).

However, it is not possible to rule out that this association between CT and NDVI may also be due to interference from the background soil. For instance, when the NDVI began to decrease in August due to the drying conditions (Fig. 3), a higher fraction of background soil and yellow or dead leaves could bias toward lower NDVI because of the higher reflectance in NIRs of the soil. Similarly, a higher fraction of background soil could bias toward a higher CT as the dry soil is normally warmer than the plant canopy (Deery *et al.*, 2016). Such a confounding effect will diminish when the NDVI is higher, such as in the cool tails. However, Deery *et al.* (2016) found high correlations between CTs extracted by three different methods from the temperature pixels within a plot in order to investigate the influence of background soil. All three methods also showed high heritability. Although the accuracy of CT and NDVI measurements was affected, the consistent relationship between these two traits and rooting depth under current experimental settings still confirm that the current instrumentation is sufficiently accurate.

It is noteworthy that NDVI was, in general, more closely correlated with both grain yield and rooting depth than CT across all three populations (Tables 3, 4). The NDVI was measured at early grain filling on 11 October. This value was also negatively and closely related to RS (Table 4). Hence, plots with a greener canopy (greater NDVI) at early grain filling lost greenness faster (accelerated senescence) than the genotypes with lower NDVI. Similar trends were also noticed in other studies. For example, Christopher *et al.* (2016) reported that some high-yielding wheat genotypes commenced senescence later but completed senescence (and maturity) at a similar time to low-yielding genotypes, suggesting an increased senescence rate. Lopes and Reynolds (2012) reported a significant albeit weak association between RS and estimated NDVI at maturity under heat stress, but not under combined heat and drought. Physiological factors affecting these changes and the underlying mechanisms warrant further investigation.

Duration versus speed of root growth

A deeper root system is suggested to be attributed to a longer duration of root growth (i.e. an extended period in crop development from sowing to anthesis) (Thorup-Kristensen *et al.*, 2009), or to a greater root penetration rate (Kirkegaard and Lilley, 2007). We found no association between rooting depth and anthesis date (as indicated by *Z*-score) in the CW and SS populations under the current experimental settings (Table 3). Similarly, this association in the HD population is also unlikely, considering the significant variation in rooting depth but little variation in flowering time within this population. The lack of association between rooting depth and flowering time implies that a deep root system found here more probably resulted from greater root penetration rates rather than a longer time to flowering within these populations which were sown at the same time. Variation in root penetration rate in the field has previously been reported at a location near to these experiments (Richards *et al.*, 2007). Such potential genetic variation in the root penetration rate can provide opportunities to breed for deeper rooted wheat. However, other factors might be in play, such as subsoil constraints that may hinder some late-flowering genotypes from expressing a deep rooting trait (Kirkegaard and Lilley, 2007), leading to no association between flowering time and rooting depth.

Furthermore, a negative association between development stage (*Z*-score) and grain yield was apparent in CW and SS (Fig. 4), suggesting that early flowering genotypes had lower grain yields. This is contradictory to the generally held notion that early flowering genotypes may escape the terminal drought and show higher grain yields. A possible reason for the negative relationship is that there was insufficient accumulation of biomass in the earliest lines and this penalized yield in this environment.

Role of plant height

Plant height was negatively correlated with CT and positively correlated with NDVI across the three tested populations (Table 4). It has been argued that plant height might obscure the expression of drought-adaptive traits, and that the variation in plant height needs to be constrained (Lopes and Reynolds, 2010). This was achieved in the HD population where there is restricted variation in plant height under favourable conditions and extremes in height were eliminated from the other two populations for coring. It is notable that the strongest association between CT and plant height occurred in the most invariant population for plant height (HD). Differences in canopy height also result in confounding of leaf-to-air temperature differences strongly driven by the boundary layer effects of genotypes with varying heights as discussed in detail by Giunta *et al.* (2008) and Rebetzke *et al.* (2013). It is therefore possible that this may also contribute to the cooler CT in the taller lines in this study, although this is not expected to be very important here as height differences were <10 cm for the majority of the HD lines (data not shown).

A significant positive correlation was observed between plant height and rooting depth in two populations, CW and HD (Table 3). This contrasts with a study of only eight contrasting genotypes, which reported that shorter genotypes tended to have greater rooting depth where soil water was not such a major limitation (Severini, 2015). A likely explanation is that under more extreme water limitation, lines do not reach their potential heights, and a deeper root system that can extract more water from deep in the soil profile enables the plant to grow taller. This is illustrated by the plant height of the rainfed HD lines which were generally <70 cm and more variable (Table 2), whilst under favourable conditions it was less variable and closer to 90 cm according to our previous experience with this population.

Conclusion

We used three diverse populations to explore the use of proxies to select for deeper growing roots under terminal drought. The parents of these populations were previously identified to be varying in either (i) rooting depth in the field, (ii) transpiration efficiency, or (iii) osmotic adjustment.

The CT measured by airborne thermography at about 12.00–14.00 h of the day was found most suitable for selection of the cool and warm plot or genotype extremes due to the identified large variability of CT within each population. We demonstrated small but significant associations between greater rooting depth, cooler CT, and higher NDVI at the late-flowering to early grain-filling stage across the three populations in a natural terminal drought and available deep stored soil water environment. Further, the rooting depths in the cool tails were ~8 cm and 6 cm deeper than the warm tails in the CW and HD populations, respectively. Correspondingly, the cool tails significantly outyielded the warm tails by 19% and 7%, respectively, but the yield versus rooting depth relationship was only strong (r=0.52) in one population. When considering all genotypes from each population, NDVI and CT were consistently correlated with grain yield.

The findings of the present study highlight the value of using CT and NDVI, both of which can be measured quickly and accurately, in breeding programmes as proxies for deep rooting. The study also highlights the importance of deep roots to access water stored at depth to increase the yield of wheat during a terminal drought.

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. Correlation matrices for canopy temperature (CT) on eight dates.

Table S2. Correlation matrices for NDVI on eight dates.

Acknowledgements

The authors would like to acknowledge the many needed hands providing excellent technical assistance: Gilbert Permalloo, Tom McLucas, Byron Corcoran, Savannah McGuirk, David Smith, Jamie Scarrow, Carol Blake, and David Lewis. This work was supported by the Australian Centre for International Agricultural Research (ACIAR) via the 'Indo-Australian project on root and establishment traits for greater water use efficiency in wheat – Phase 2' project (project no. CIM/2006/071). We thank Tony Fischer and John Passioura for valuable comments on the manuscript.

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