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Root traits contributing to drought tolerance of synthetic hexaploid wheat in a greenhouse study

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Abstract Drought stress imposes major limits on wheat (*Triticum aestivum* L.) yield and is predicted to increase in frequency due to climate change. The aim of this study was to explore the potential of synthetic hexaploid wheat (SHW) to improve productivity of winter wheat under drought stress. Six SHW lines and four winter wheat cultivars from the U.S. Great Plains were evaluated in 1 m × 10 cm plastic tubes under drought-stressed and well-watered conditions in a greenhouse study. Root morphology, biomass, stomatal attributes, plant water relations, and the response of these traits to drought stress were measured. Traits significantly ($P < 0.05$) correlated with a drought tolerance index included root biomass in the bottom third of the tubes, length of the longest root, stomatal conductance, and production of small diameter roots. Plasticity for root biomass allocation to greater depths showed a strong association with maintenance of plant water status. Synthetic line SYN-201 ranked highest for deep root biomass and length of the longest root under stress, and demonstrated plasticity by shifting root biomass production from the upper third to the bottom third of the tubes when stressed. Digital analysis of root morphology indicated that SYN-201, SYN-290, and cultivar Byrd produced large amounts of small diameter roots at depth. SYN-396 showed high stomatal density and reduced stomatal aperture while maintaining leaf growth when stressed despite a lack of deep roots. Trait variation in the SHW lines may contribute beneficial drought tolerance to Great Plains-adapted cultivars through introgression of novel allelic diversity.

Keywords Drought tolerance · Root morphology · Root tubes · Trait plasticity · *Triticum aestivum*

Introduction

Wheat (*Triticum* spp.) accounts for about 20 % of the human food supply and is produced on about 215 million ha worldwide (WHEAT 2014). It is often grown under limited soil moisture conditions, and the

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frequency of drought in many regions is predicted to increase with climate change (Dai 2013). Thus, improving the tolerance of wheat to drought stress is essential for future global food security.

The term drought represents a large diversity of conditions where soil moisture is limiting and reduces plant growth, reproduction, and yield (Blum 2011). Traits that help plants tolerate the effects of drought stress are determined in large part by the population of target production environments (Chenu et al. 2011). Ideally the expression of drought tolerance traits will be plastic, allowing crop plants to adapt to seasonal variation of precipitation in moisture-limiting environments without sacrificing yield under favorable conditions. Drought tolerance can be conferred by reducing leaf water loss and/or increasing root water uptake, among other processes.

A primary focus in drought tolerance research has been root morphology and biomass, which may be beneficial to improving yield stability. However, Reynolds et al. (2007) demonstrated that an overall increase in root biomass does not indicate an increase in water uptake. The authors reported that synthetic hexaploid-derived wheat lines produced less overall root biomass under drought, yet yielded more root mass at depth, enabling more water extraction from deeper in the soil profile. Maximum root depth was shown to contribute to improved water extraction and had a higher heritability than other root traits (Ekanayake et al. 1985; Lopes and Reynolds 2010; Sayar et al. 2007). Although the contribution of increased root dry weight at depths below 90 cm has been associated with cooler canopy temperatures and yield improvements under drought stress (Lopes and Reynolds 2011), shallow root systems may also be beneficial for capturing rainfall that does not infiltrate to deeper soil layers (Ehdaie et al. 2012). Research in Australia showed that for each mm of moisture absorbed throughout the grain filling period, a 55 kg ha^{-1} increase in grain yield occurred in rainfed environments (Manschadi et al. 2006). Therefore, identifying a rooting structure that will lead to maximum extraction of soil moisture at key growth stages in the target environment will be beneficial to improvements in productivity under water stress.

Given the logistical challenges of phenotyping root systems in the field, a number of alternative approaches have been developed (Gregory et al. 2009). One of the most employed methods has been growing plants in plastic cylinders or root tubes, which were used to evaluate variation of root traits in germplasm collections and near-isogenic lines of wheat (Ehdaie et al. 2012; Narayanan et al. 2014; Narayanan and Prasad 2014; Ytting et al. 2014).

Leaf water loss through transpiration is driven by atmospheric demand and can be minimized by reduction in stomatal conductance. Variation in stomatal conductance can be due to constitutive variation in stomatal size and/or density or to plastic responses in stomatal aperture. Franks et al. (2009) reported that an increase in stomatal density will increase maximum potential conductance (g_WMax), whereas an increase in stomatal size will reduce g_WMax (Hetherington and Woodward 2003). Therefore, greater stomatal control relates to smaller stomates, as smaller guard cells more rapidly adjust their turgor pressure to minimize water loss and maintain adequate levels of CO_2 influx (Franks et al. 2009; Hetherington and Woodward 2003).

The genetic bottleneck due to the origin and subsequent domestication of bread wheat resulted in a limited pool of genetic and phenotypic diversity available for breeding for drought tolerance and other important traits (Cavanagh et al. 2013; Gill et al. 1991). A possible remedy to reverse this reduction in variation is the use of synthetic hexaploid wheat (SHW) (*X Aegilotriticum* spp.) lines produced by resynthesizing the cross between accessions of cultivated tetraploid wheat (*T. turgidum*) and accessions of *Aegilops tauschii* (Dreisigacker et al. 2008; McFadden and Sears 1944; Mujeeb-Kazi and Hettel 1995; Reynolds et al. 2007; Van Ginkel and Ogbonnaya 2007). Subsequent crossing of SHW to domesticated hexaploid wheat opens the door to introduction of genotypic diversity and variation in traits needed to improve adaptation in elite breeding lines (Dreisigacker et al. 2008; Reynolds et al. 2007; Trethowan and Mujeeb-Kazi 2008; Van Ginkel and Ogbonnaya 2007). To date, the potential to increase the drought tolerance of winter wheat in the Great Plains region of North America through beneficial traits present in SHW has not been fully explored.

Therefore, the objectives of this research were to (1) compare selected SHW lines to locally adapted cultivars for greenhouse-evaluated traits that may improve drought tolerance in the U.S. Great Plains; (2) determine the physiological and morphological traits that are most closely associated with drought tolerance as evaluated in a greenhouse root tube study; and (3) assess whether trait plasticity or constitutive expression of SHW drought tolerance traits is more important for improving productivity under ideal and drought-stressed conditions.

Materials and methods

Germplasm

A collection of 412 primary SHW lines was grown under dryland conditions at Akron, CO (40.1548°N, 103.142°W, elevation 1383 m)in 2007 as a single replication of two-row plots, 75 cm long. These SHW lines were developed by the International Maize and Wheat Improvement Center in Mexico (Mujeeb-Kazi and Hettel 1995) and kindly provided to us by Dr. Art Klatt, Oklahoma State University. The 45 most promising lines were evaluated the following year at Akron in a three-replication yield trial with the same plot dimensions. Selection of a subset of six promising lines for further investigation was based on a qualitative assessment of head type, tiller density, lodging resistance, seed yield, and proportion of well-filled seeds. Entries (genotypes) in the following study included the subset of six spring-habit SHW lines and four locally adapted winter-habit cultivars (Table 1).

Experimental design

Six replications of a split-plot design were grown with well-watered (fully saturated on a daily basis) and drought-stressed (irrigation withheld 1 week after emergence) conditions as the main plot treatments and genotype as the subplot factor. Three seeds of each genotype were planted in polyvinyl chloride (PVC) tubes, 10.2 cm inside diameter and 99 cm tall in a greenhouse at Colorado State University (Fort Collins, CO) on 13 October 2011. For easy removal of root masses, each tube was lined with a 4 mil polytube liner (Uline, Pleasant Prairie, WI) with drainage holes. Tubes were filled with 5.3 kg of dry GreensGrade media, a calcined, nonswelling illite and silica clay (Profile Products LLC, Buffalo Grove, IL) and packed to a height of 98 cm, resulting in a bulk density of approximately 0.74 g/cm^3 after irrigation. Greenhouse conditions were a 16 h/8 h of light/darkness photoperiod at a temperature range from 18.3 to 25.5 °C. Seven days after planting, seedlings were thinned to a single plant per tube. Drip irrigation with Peters Professional 15-16-17 fertilizer (The Scotts Company, Marysville, OH) was applied at a concentration of 0.20 g/L with a 1:100 injector ratio. The water stress treatment was initiated 14 days after planting by terminating irrigation for a span of 18 days, corresponding to approximate Zadoks growth stages 12 (two leaves unfolded) to 31 (first node detectable) (Zadoks et al. 1974).

Physiological and morphological measurements

Tubes in the drought stress treatment were weighed every 3 days for a total of six gravimetric water content measurements. Just prior to final collection of biomass samples, approximately 2-cm leaf segments were collected from the fifth leaf for measuring relative water content (RWC), following the procedure of Barrs and Weatherley (1962). Leaf elongation rates of the fifth leaf were estimated by dividing the final length of the leaf by the number of days from its emergence above the fourth leaf collar until appearance of the fifth collar, similar to Praba et al. (2009).

Measurement of stomatal conductance (g_s) on the fourth fully developed leaf from the base of the plant began 11 days after treatment imposition. Measurements of g_s continued every other day, sampling complete replicates at midday in a randomized order each day, for a total of three repeated measurements. Both abaxial and adaxial surfaces of the leaf were measured with a Decagon SC-1 steady-state leaf porometer (Decagon, Inc., Pullman, WA). However, abaxial conductance for the drought-stressed treatment was too low to detect; thus, only adaxial conductance is reported. Leaf peels for measurement of stomatal density and aperture were collected through nail polish imprints adjacent to the site of porometer measurement on the fourth leaf. Stomatal counts were made under a light microscope at 16× magnification. Stomates were counted in five viewing areas of 2.18 mm^2 across the with of the leaf and were averaged for both adaxial and abaxial leaf surfaces. Stomatal length as an indication of aperture was estimated by measuring and averaging the total length of 10 closed stomates on both the adaxial and abaxial leaf surfaces.

Above-ground biomass samples were collected at the termination of the experiment (Zadoks growth stage 31) and dried at 70 °C for at least 24 h prior to weighing.

Table 1 Pedigrees of entries used in this study

Entry name[a]	Pedigree	GRIN ID	WGRC[b] *Ae. tauschii* ID
SYN-172	Snipe/Yavaros 79//Dackiye/Teal/3/Ae. tauschii (904)[c]	NSGC 9711	TA2477
SYN-201	68112/Ward//Ae. tauschii (369)	PI 648646	[d]
SYN-290	Garza/Boyeros//Ae. tauschii (241)	PI 648733	TA2382
SYN-320	Laru/Ae. tauschii (333)	PI 648758	TA2482
SYN-380	Decoy 1/Ae. tauschii (256)	PI 648810	TA2400
SYN-396	Decoy 1/Ae. tauschii (322)	PI 648823	TA2471
Byrd	TAM112/CO970547-7	PI 664257	
Goodstreak	SD3055/KS88H164//NE89646	PI 632434	
Hatcher	Yuma/PI372129//Tam-200/3/4*8Yuma/r/KS91H184/Vista	PI 638512	
Ripper	CO940606/TAM107R-2	PI 644222	

Synthetic hexaploid pedigrees are from the USDA-ARS GRIN database (http://www.ars-grin.gov/). Cultivar pedigrees are from Baenziger et al. (2004) and Haley et al. (2005, 2007, 2012)

[a] Synthetic hexaploid designations are from Mujeeb-Kazi and Hettel (1995)

[b] Wheat Genetics Resource Center, Kansas Wheat Innovation Center, Manhattan, KS 66502

[c] Parentheses indicate *Ae. tauschii* accession number assigned by CIMMYT

[d] *Ae. tauschii* accession 369 does not correspond to any WGRC germplasm

Water use efficiency was calculated by dividing the above-ground biomass (mg) by the total water loss (g) in the drought-stressed treatment. Following collection of the above-ground biomass, the root systems were removed from the polytube liners, washed free of growth medium, and measured for the longest seminal root length. The roots were divided into the upper (0–33 cm depth), middle (33–66 cm depth), and bottom (66–98 cm depth) sections of the tube. Individual root sections were floated on approximately 1 cm of water in a 30 × 40.5 cm plexiglass tray and scanned with a MicroTek Scanmaker 9800XL (Microtek, Santa Fe Springs, CA). Digital images were analyzed with WinRhizo Regular software (Regent Instruments Inc., Quebec, Canada). Root morphology measurements recorded by WinRhizo included total root length, average root diameter, and root length of the following diameter classes: 0.00–0.25, 0.25–0.50, 0.50–0.75, 0.75–1.00, and >1.00 mm. A complete list of traits measured or calculated and their descriptions can be found in Online Resource 1.

Statistical analysis

Unless otherwise stated, all statistical procedures were conducted in SAS software v. 9.2 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) for each trait was conducted with the MIXED procedure, where effects for genotype (G), treatment (T), and the G x T interaction were analyzed as fixed effects, and replicate was considered a random effect. Least squares means of genotypes were estimated with the MIXED procedure and compared at the 0.05 probability level. Plasticity of each trait and genotype between the well-watered and drought-stressed treatments was quantified by subtracting the drought-stressed mean value from the well-watered mean value and dividing by the grand mean for that trait.

In order to quantify the response of water status of each entry under drought stress, least squares mean values of RWC were used to calculate a modified drought stress index (DI) similar to the formula of Ehdaie et al. (2012):

$$DI = 1 - \left(\frac{RWC_D}{RWC_W}\right) \times 100$$

where RWC_D is the RWC in the stress treatment and RWC_W is the RWC of the well-watered treatment.

Traits associated with changes in plant water status were identified based on phenotypic correlations between each trait and DI in JMP Pro 9.0.2 (SAS Institute Inc., Cary, NC).

Results

Analysis of variance

For nearly all the traits, the predominant sources of variation were genotype and moisture treatment (Tables 2, 3). Significant genotypic differences ($P < 0.05$) were found for all physiological and morphological traits except root length for the 0.25–0.50 and 0.75–1.00 mm root diameter classes of the bottom tube section (Tables 2, 3). The effect of moisture treatment was significant ($P < 0.05$) except for 0.75–1.00 mm roots at the middle depth, and the 0–0.25 and 0.75–1.00 mm diameter classes in the deepest section. The G × T effect was significant for fewer than half the traits and in general at lower levels of significance than for the main effects. Traits that showed significant ($P < 0.05$) interaction were above-ground biomass, RWC, leaf elongation rate, adaxial stomatal aperture, top tube section root biomass, root length for all diameter classes in the top tube section, and root length for the 0–0.25, 0.50–0.75, and >1.00 mm root diameter classes in the middle section (Tables 2, 3).

Comparison of treatment means

Plants in the drought-stressed treatment showed a major reduction in growth, as seen by the decreased mean above-ground biomass from 1.22 g in the well-watered treatment to 0.67 g under drought stress, a decrease of 45 % (Table 4). Similarly, the elongation rate of the fifth leaf decreased by 28 % in the drought treatment (Table 4). Total root biomass, however, declined by only 8 % (Fig. 1). This resulted in a decrease in the ratio of above- to below-ground biomass from a mean of 4.9 (range of 4.3–6.3) in the well-watered treatment to a mean of 3.0 (range of 2.4–3.9) under drought stress. Root length of all diameter classes in the top section declined from wet to dry conditions (Fig. 2a). In the middle section, length of all diameter classes increased in the drier conditions, except the 0.75–1.00 mm class, which was unchanged (Fig. 2b). In the bottom section, three of the five diameter classes were observed to increase in the drought treatment (Fig. 2c). Thus the overall effect of withholding water was a reduction of shoot biomass and a reallocation of root biomass from the upper to lower sections of the profile. Distribution of root biomass shifted considerably from 79 % top-17 % middle-3 % bottom (well-watered) to 51–38–11 % (drought-stressed).

Comparison of genotype means

For most traits, variation occurred within both cultivars and synthetic lines, such that significant differences between groups were not found. However, analysis of the upper root section for length of various diameter classes showed a significant ($P < 0.05$) difference between cultivars and synthetics. In the well-watered treatment cultivars produced greater root lengths for all diameter classes, and in the drought-stressed treatment cultivars produced significantly ($P < 0.05$) longer fine roots (0.00–0.25 and 0.25–0.50 diameter classes) than the synthetic lines.

Under well-watered conditions, differences among entries were apparent for top, middle, and total root

Table 2 Analysis of variance for 13 physiological and morphological traits under well-watered and drought-stressed treatments

Source	ABM	TRBM	MRBM	BRBM	TotRBM	LR	RWC	LER	Adaxial g_s	TSA	BSA	TSD	BSD
Genotype (G)	***	***	***	**	***	***	**	**	*	***	***	***	***
Treatment (T)	***	***	***	***	**	*	***	***	***	***	**	**	*
G × T	**	**	NS	NS	NS	NS	*	**	NS	*	NS	NS	NS

ABM above-ground biomass, *TRBM* top tube section root biomass, *MRBM* middle tube section root biomass, *TotRBM* total root biomass, *BRBM* bottom tube section root biomass, *LR* length of longest seminal root, *RWC* relative water content, *LER* leaf elongation rate, g_s stomatal conductance, *TSA* adaxial (top) stomatal aperture, *BSA* abaxial (bottom) stomatal aperture, *TSD* adaxial (top) stomatal density, *BSD* abaxial (bottom) stomatal density

*, **, ***, *NS* significant at the 0.05, 0.01, and 0.001 probability levels, and not significant, respectively

Table 3 Analysis of variance for 15 root traits under well-watered and drought-stressed treatments

Source	Top section root length per diameter class					Mid-section root length per diameter class					Bottom section root length per diameter class				
	0.0–0.25	0.25–0.50	0.50–0.75	0.75–1.00	>1.00	0.0–0.25	0.25–0.50	0.50–0.75	0.75–1.00	>1.00	0.0–0.25	0.25–0.50	0.50–0.75	0.75–1.00	>1.00
Genotype (G)	***	***	**	**	***	***	***	***	**	***	*	NS	**	NS	**
Treatment (T)	***	***	***	***	***	**	***	***	NS	***	NS	**	**	NS	**
G × T	**	**	*	**	**	**	NS	***	NS	*	NS	NS	NS	NS	NS

*, **, ***, *NS* significant at the 0.05, 0.01, and 0.001 probability levels, and not significant, respectively

biomass, but there were no significant differences among entries in bottom section root biomass (Fig. 1). Synthetic line SYN-290 had significantly ($P < 0.05$) greater total root biomass than all other genotypes. SYN-290 and SYN-320 produced the greatest amounts of above-ground biomass (Table 4). Leaf elongation rate and RWC were similar for all entries (Table 4).

Under drought stress conditions, entries differed for above-ground biomass, total root biomass, and root biomass at all depths, with high values found among both cultivars and SHW lines (Table 4, Fig. 1). Byrd, Ripper, SYN-201, and SYN-290 had greater total root biomass than other entries. Line SYN-201 ranked highest for length of the longest root, as well as root biomass in the bottom tube section under drought stress. Synthetic line SYN-172 had the highest adaxial g_s of all entries, followed by Byrd, Hatcher, and Ripper (Table 4). Goodstreak, Hatcher, and SYN-396 had the lowest RWC at the end of the drought treatment, while Byrd, Ripper, and SYN-172 had high values for RWC.

Lines SYN-201 and SYN-290 and cultivar Byrd were consistently large producers of fine roots (diameter classes 0.00–0.50 mm) in the middle and bottom tube sections of both treatments (Fig. 2b, c). When well-watered, synthetics SYN-201 and SYN-396 were the only two entries to rank above average for total root biomass and above average for percentage of roots produced in the upper section (Fig. 1). However, SYN-201 was able to reallocate production of root biomass to greater depths under drought, ranking third in total root production with the largest percentage of roots produced in the bottom section (Table 4). Lines SYN-396 and SYN-172 showed statistically ($P < 0.05$) smaller stomatal aperture than most other entries in both treatments (Table 4).

When plasticity was calculated, some traits were found to be more plastic than others. For example, plasticity of bottom root biomass had a much greater magnitude (mean of −0.93) than that of total root biomass (mean of 0.09), and as expected, mean plasticity of stomatal conductance (0.80) was much greater than that of stomatal density or aperture (0.04 and −0.08, respectively). There were no obvious differences in plasticity between SHW lines and cultivars, as a similar range of plasticity values was observed for most traits within both groups (data not shown).

Table 4 Means for physiological and morphological traits under well-watered and drought-stressed conditions

Genotype	ABM (g)	LR (mm)	WUE (mg/g)	RWC (%)	LER (mm/day)	Adaxial g_s (μM/m^{2}s)	TSA (μm)	BSA (μm)	TSD (no./mm^2)	BSD (no./mm^2)
Well-watered										
SYN-172	1.02 *c*[a]	860*ab*	NA[b]	95.6*a*	52.0*ab*	404*abc*	49.8*e*	47.1*ef*	10.7*ab*	7.9*ab*
SYN-201	1.24*b*	941*ab*		961*a*	51.8*ab*	374*bc*	50.6*e*	48.6*ef*	10.3*abc*	6.9*c*
SYN-290	1.57*a*	1009*a*		95.9*a*	55.2*ab*	330*c*	58.1*a*	55.9*ab*	8.9*c*	7.1*bc*
SYN-320	1.44*a*	835*b*		96.2*a*	56.2*ab*	361*bc*	56.3*abc*	53.1*bc*	9.6*bc*	7.7*abc*
SYN-380	1.25*b*	865*ab*		97.0*a*	52.7*ab*	521*a*	54.0*cd*	57.3*a*	10.6*ab*	7.5*abc*
SYN-396	1.14*bc*	578*c*		95.5*a*	57.6*a*	370*bc*	45.0*f*	45.5*f*	11.2*a*	8.2*a*
Byrd	1.20*b*	983*ab*		96.0*a*	55.9*ab*	350*bc*	58.0*a*	55.4*abc*	9.9*abc*	7.1*bc*
GS	1.14*bc*	899*ab*		95.3*a*	57.7*a*	312*c*	55.1*bcd*	49.7*de*	10.7*ab*	7.1*bc*
Hatcher	1.16*bc*	838*b*		96.3*a*	53.9*ab*	385*abc*	53.4*d*	52.4*cd*	11.1*ab*	7.9*ab*
Ripper	1.02*c*	891*ab*		96.0*a*	50.3*b*	449*ab*	57.2*ab*	56.0*ab*	10.9*ab*	8.2*a*
Mean	1.22	870		96.0	54.3	386	53.8	52.1	10.4	7.6
Drought-stressed										
SYN-172	0.62*abcd*	941*abcd*	1.37*abc*	93.7*a*	38.8*ab*	211*a*	46.6*d*	46.9*e*	11.5*ab*	8.7*ab*
SYN-201	0.75*ab*	1064*a*	1.29*abcd*	92.6*ab*	38.0*ab*	143*cd*	51.8*bc*	49.4*cde*	9.7*c*	6.9*e*
SYN-290	0.73*abc*	942*abcd*	1.40*ab*	93.0*ab*	44.5*a*	158*abcd*	53.0*abc*	52.6*abc*	9.3*c*	7.3*de*
SYN-320	0.77*a*	821*cd*	1.24*cd*	906*bc*	41.6*ab*	150*bcd*	53.7*ab*	51.6*abc*	10.0*c*	8.1*bcd*
SYN-380	0.69*abcd*	1016*ab*	1.19*d*	93.5*a*	40.4*ab*	150*bcd*	53.9*ab*	53.6*a*	10.1*bc*	7.6*cde*
SYN-396	0.59*cd*	794*d*	1.36*abc*	89.0*c*	44.3*ab*	144*cd*	46.4*d*	46.3*e*	12.3*a*	9.1*a*
Byrd	0.76*a*	981*abc*	1.25*cd*	94.5*a*	37.5*b*	196*ab*	54.8*a*	50.1*bcd*	12.5*a*	8.4*abc*
GS	0.60*bcd*	863*bcd*	1.41*a*	89.8*c*	34.3*b*	125*d*	50.5*c*	47.2*de*	12.3*a*	8.9*ab*
Hatcher	0.56*d*	937*abcd*	1.35*abc*	89.9*c*	32.0*c*	185*abc*	51.6*bc*	50.7*abc*	12.4*a*	8.6*ab*
Ripper	0.66*abcd*	1012*ab*	1.41*a*	93.9*a*	39.8*ab*	183*abc*	55.7*a*	53.1*ab*	12.1*a*	8.2*bcd*
Mean	0.67	937	1.33	92.1	39.1	165	51.8	50.2	11.2	8.2

ABM above-ground biomass, *LR* length of longest seminal root, *WUE* water use efficiency, *RWC* relative water content, *LER* leaf elongation rate, g_s stomatal conductance, *TSA* adaxial (top) stomatal aperture, *BSA* abaxial (bottom) stomatal aperture, *TSD* adaxial (top) stomatal density, *BSD* abaxial (bottom) stomatal density

[a] Values followed by the same letters within a treatment are not statistically different at $P = 0.05$

[b] Not applicable because the well-watered treatment was not measured for water loss

Trait associations with drought stress index

To understand the traits contributing to variation in drought tolerance (as expressed by maintenance of RWC), we examined correlations of DI with the traits in well-watered and drought-stressed conditions and the plasticity of each trait (Tables 4, 5). In the drought-stressed treatment, traits significantly correlated ($r = -0.64$ to -0.86, $P < 0.05$, n = 10) with DI included bottom root biomass, longest root length, g_s, root length of the 0.00–0.25 mm root diameter class in the middle tube section, and root length of the 0.00–0.25, 0.25–0.50, and 0.50–0.75 mm diameter classes in the bottom tube section (Table 5, Online Resource 2). In both treatments root length of the 0.00–0.25 and 0.25–0.50 mm root diameter classes in the bottom tube section as well as length of the longest root were negatively correlated with DI. Traits that were significantly ($P < 0.05$) correlated with DI also showed high correlations among themselves (Table 5). However, these correlations were inconsistent across treatments; for example, no significant correlation with g_s was found in the WW treatment. Positive correlations were seen under drought conditions between plant water use (as estimated by water loss) and both root biomass and fine root growth in the bottom tube section. Correlations among trait plasticity and DI were significant only for bottom root

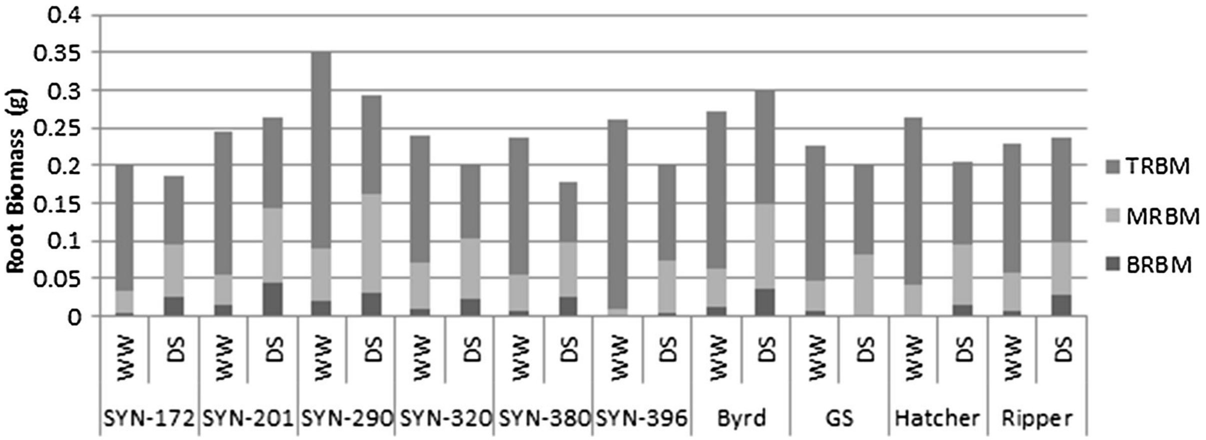

Fig. 1 Distribution of root biomass in the *top, middle* and *bottom* root tube sections for each genotype in each treatment. *TRBM* top root biomass, *MRBM* middle root biomass, *BRBM* bottom root biomass, *WW* well-watered treatment, *DS* drought-stressed treatment, *GS* Goodstreak

biomass and total root biomass ($r = 0.72$ and 0.70, respectively, $P < 0.05$, $n = 10$).

Discussion

Both the SHW lines and the cultivars in our study showed variation for several drought tolerance-related traits. We also found significant G × T interaction for some above- and below-ground traits, indicating that genotypes responded differently to the imposition of moisture stress. This study addressed our objectives by identifying beneficial traits in SHW and demonstrating that both constitutive traits, such as longest root length, and plastic traits, such as fine roots at depth, in SHW lines may improve plant water status under drought stress.

Six of the eight traits correlated with DI were root traits such as bottom section root biomass and small diameter root length (Table 5, Online Resource 2). The phenotypic correlations were all negative, indicating that an increase in those traits is related to a beneficial decrease in DI. This study confirms previous research on SHW that showed the importance of greater root biomass at depth (Lopes and Reynolds 2011; Reynolds et al. 2007) and adds to that information by demonstrating the value of fine root production for maintaining plant water status. This study also demonstrated that trait variability, both constitutive and plastic, is present in diverse SHW lines that may prove useful for improvement of key traits. Allocation of assimilates to deeper root growth, especially fine roots, enhances water capture as seen by the correlations between DI, total water loss, and bottom tube section root traits (Table 5).

The plasticity of only two traits (total root biomass and bottom tube section biomass) was found to be significantly correlated with DI ($P < 0.05$). Positive correlations of these plasticity values with DI indicate that increasing the amount of relative root biomass under drought stress is associated with maintenance of leaf water status. However, an overall increase in root biomass may only be beneficial if the change occurs at deeper soil profiles. Plastic allocation of assimilates to deeper roots can improve water capture, with no reduction in productivity (Blum 2009; Ehdaie et al. 2012; Lopes and Reynolds 2011). Synthetic line SYN-172 and cultivar Byrd demonstrate this by showing relatively small reductions in above-ground biomass along with large increases in bottom section root biomass and the smallest two DI values. Synthetic line SYN-201 produced the largest amount of bottom section root biomass under drought stress and ranked second in bottom root biomass production under well-watered (WW) conditions. However, this synthetic line also produced as much above-ground biomass as the cultivars when well-watered and was among the largest producers of above-ground biomass in the drought stress treatment.

Lengths of smaller diameter roots (0.00–0.50 mm) in the middle and bottom tube sections were correlated with DI (Table 5). Negative correlations for these

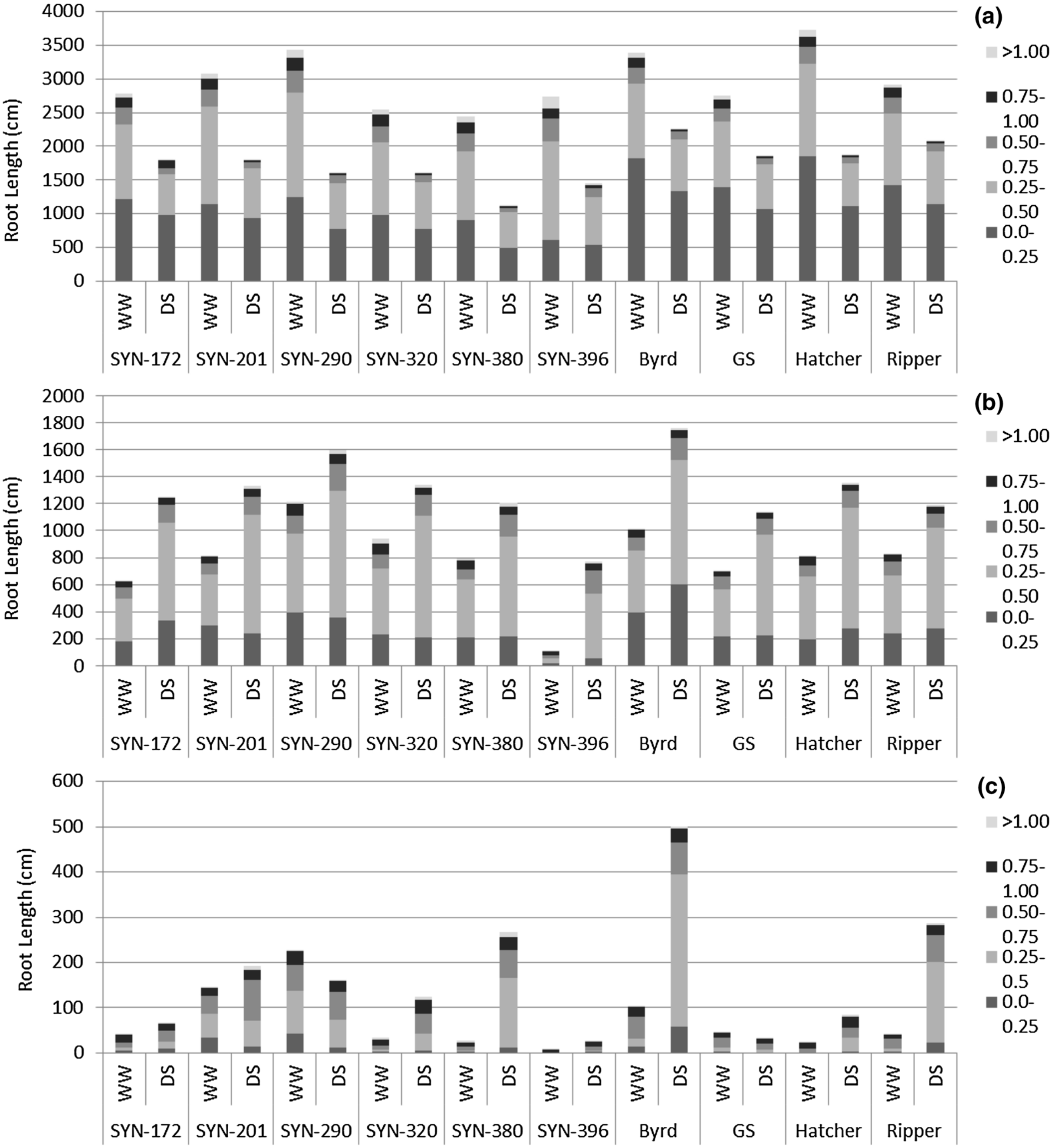

Fig. 2 Root length per diameter class (0.00–0.25, 0.25–0.50, 0.50–0.75, 0.75–1.00, >1.00 mm) in the top (**a**), middle (**b**), and bottom (**c**) tube sections for each treatment. *WW* well-watered treatment, *DS* drought-stressed treatment, *GS* Goodstreak

traits indicate improved drought tolerance from production of a greater length of fine roots. The importance of increased production of finer roots at depth can be seen in Table 5 as the length of fine roots is positively correlated with an increase in g_s and water use in the drier treatment, whereas overall bottom section biomass was not significantly correlated with g_s in either treatment. Synthetic line SYN-290 and cultivar Byrd consistently produced the largest amount of fine roots in both treatments (Fig. 2).

Table 5 Phenotypic correlations among traits that were significantly correlated with the drought stress index (n = 10)

	DI	BRBM	LR	g_s	Mdia1	Bdia1	Bdia2	Bdia3	Water loss[a]
DI		−0.73*	−0.68*	−0.73*	−0.70*	−0.86**	−0.70 *	−0.67 *	−0.64*
BRBM	−0.45		0.78**	0.55	0.63	0.87**	0.79**	0.84**	0.86**
LR	−0.64*	0.78**		0.57	0.57	0.70*	0.73*	0.80**	0.49
g_s	−0.44	−0.03	0.00		0.84**	0.72*	0.64*	0.51	0.41
Mdia1	−0.56	0.68*	0.96***	0.03		0.69*	0.67*	0.65*	0.71*
Bdia1	−0.69*	0.90**	0.82**	0.08	0.70*		0.93**	0.86**	0.74*
Bdia2	−0.64*	0.87**	0.93**	−0.03	0.85**	0.95***		0.95***	0.66*
Bdia3	−0.61	0.78**	0.97***	−0.15	0.91**	0.83**	0.94***		0.73*

Correlations above and below the diagonal are associated with the drought-stressed and well-watered treatments, respectively

DI Drought stress index, *BRBM* bottom root biomass, *LR* length of longest root, g_ss stomatal conductance, *Mdia1* middle tube section 0–0.25 mm diameter, *Bdia1* bottom tube section 0–0.25 mm diameter, *Bdia2*, bottom tube section 0.25–0.50 diameter, *Bdia3* bottom tube section 0.0.50–0.75

[a] Measured only under drought stress

*, **, ***, significant at the 0.05, 0.01, and 0.001 probability levels, respectively

Synthetic lines such as SYN-201 and SYN-290, which show greater root length in the 0.00–0.25 and 0.25–0.50 mm diameter classes in the middle and bottom tube sections, may contribute novel variation for beneficial root traits useful in breeding for drought tolerance.

Analysis of the upper root section (Fig. 2a) indicated that the adapted cultivars had significantly greater ($P < 0.05$) root lengths than the synthetic lines for all diameter classes in the well-watered treatment and for fine roots (0.00–0.25 and 0.25–0.50 diameter classes) in the drought-stressed treatment. This may indicate selection for improved moisture and nutrient extraction from the upper soil layers, as fine roots near the soil surface are known to be a primary location of moisture and nutrient uptake (Hopkins and Hüner 2008). Selection for increased yield potential under more favorable conditions may have caused an indirect selection for increased shallow root biomass (Palta et al. 2011). However, as reported by Ehdaie et al. (2012), constitutive dispersion of fine, lateral roots throughout the soil profile, may contribute to improved moisture uptake due to greater soil surface area contact.

Stomatal aperture is proportional to g_WMax (Franks et al. 2009; Hetherington and Woodward 2003). It would be expected that genotypes with larger apertures would lose more moisture and thus have a lower water status (lower RWC) (Hetherington and Woodward 2003). However, we observed large apertures in some entries with the highest RWC under drought stress, including Byrd, Ripper, and SYN-380. It was unclear if there was a causal relationship in this study between stomatal aperture or density and an increase in g_s as no statistically significant($P < 0.05$) correlations among these traits were found (data not shown). Under drought stress, water uptake traits may have overshadowed the effects of minor traits, such as stomatal attributes (Blum 2011). Thus, the large effect of longer fine diameter roots at depth, which was correlated with g_s (Table 5), may have reduced the relative effects of stomatal size and density. It can be argued that line SYN-396 had an ideal combination of the densest stomates (12.3 adaxial and 9.1 abaxial stomata per mm^2) and the smallest aperture (46.4 µm adaxial and 46.3 µm abaxial) under drought stress (Table 4). This would create a large leaf porosity with greater stomatal density and the potential for a quicker response time under drought stress due to a smaller aperture (Franks et al. 2009). SYN-396 had among the lowest g_s and second smallest bottom tube section root biomass under drought (Table 3). However, it was still able to maintain a high leaf elongation rate, indicating that it may have minimized conductance and water loss while maintaining a sufficient amount of CO_2 uptake to continue photosynthetic activity and leaf growth. However, the lack of any correlation between DI and both stomatal density and aperture supports the need for further study of the beneficial stomatal attributes of SHW lines.

Conclusion

Synthetic hexaploid wheat was shown previously to improve yield as well as resistance to biotic and abiotic stresses. In this study, root morphological traits of both SHW lines and cultivars that increased water extraction from deeper depths in drying soil media contributed to avoidance of plant water stress and thus maintenance of productivity. These traits included increased deep root biomass, longer seminal roots, and increased production of small diameter roots at depth. The SHW lines in our study showed variation both in constitutive and plastic root traits that may be beneficial for improving drought stress tolerance in elite cultivars. The genetic diversity contributed by SHW for these traits may be novel in many breeding programs and may increase productivity and stability in variable drought-stressed regions of the U.S. Great Plains.

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References

Baenziger PS, Beecher B, Graybosch RA, Baltensperger DD, Nelson L, Krall JM, Mcvey DV, Watkins JE, Hatchett JH, Chen M (2004) Registration of Goodstreak wheat. Crop Sci 44:1473–1474

Barrs HD, Weatherley PE (1962) A re-examination of relative turgidity technique for estimating water deficits in leaves. Austr JBiol Sci 15:413–428

Blum A (2009) Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. Field Crops Res 112:119–123

Blum A (2011) Plant breeding for water-limited environments. Springer Science + Business Media, LLC, New York

Cavanagh CR, Chao S, Wang S, Huang BE, Stephen S, Kiani S, Forrest K, Saintenac C, Brown-Guedira GL, Akhunova A, See D, Bai G, Pumphrey M, Tomar L, Wong D, Kong S, Reynolds M, da Silva ML, Bockelman H, Talbert L, Anderson JA, Dreisigacker S, Baenziger S, Carter A, Korzun V, Morrell PL, Dubcovsky J, Morell MK, Sorrells ME, Hayden MJ, Akhunov E (2013) Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proc Natl Acad Sci USA 110:8057–8062

Chenu K, Cooper M, Hammer GL, Mathews KL, Dreccer MF, Chapman SC (2011) Environment characterization as an aid to wheat improvement: interpreting genotype-environment interactions by modelling water-deficit patterns in North-Eastern Australia. J Exp Bot 62:1743–1755

Dai A (2013) Increasing drought under global warming inobservations and models. Nature Climate Change 3:52–58

Dreisigacker S, Kishii M, Lage J, Warburton M (2008) Use of synthetic hexaploid wheat to increase diversity for CIMMYT bread wheat improvement. Austr JAgric Res 59:413–420

Ehdaie B, Layne AP, Waines JG (2012) Root system plasticity to drought influences grain yield in bread wheat. Euphytica 186:219–232

Ekanayake IJ, O'Toole JC, Garrity DP, Masajo TM (1985) Inheritance of root characters and their relations to drought resistance in rice. Crop Sci 25:927–933

Franks PJ, Drake PL, Beerling DJ (2009) Plasticity in maximum stomatal conductance constrained by negative correlation between stomatal size and density: an analysis using Eucalyptus globulus. Plant Cell Environ 32:1737–1748

Gill KS, Lubbers EL, Gill BS, Raupp WJ, Cox TS (1991) A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). Genome 34:362–374

Gregory PJ, Bengough AG, Grinev D, Schmidt S, Thomas WTB, Wojciechowski T, Young IM (2009) Root phenomics of crops: opportunities and challenges. Funct Plant Biol 36:922–929

Haley SD, Quick JS, Johnson JJ, Peairs FB, Stromberger JA, Clayshulte SR, Clifford BL, Rudolf JB, Seabourn BW, Chung OK, Jin Y, Kolmer JA (2005) Registration of 'Hatcher' wheat. Crop Sci 45:2654–2655

Haley SD, Johnson JJ, Peairs FB, Quick JS, Stromberger JA, Clayshulte SR, Butler JD, Rudolph JB, Seabourn BW, Bai G, Jin Y, Kolmer J (2007) Registration of 'Ripper' Wheat. J Plant Reg 1:1–6

Haley SD, Johnson JJ, Peairs FB, Stromberger JA, Hudson EE, Seifert SA, Kottke RA, Valdez VA, Rudolph JB, Bai G, Chen X, Bowden RL, Jin Y, Kolmer JA, Chen M-S, Seabourn BW (2012) Registration of 'Byrd' Wheat. JPlant Reg 6:302–305

Hetherington AM, Woodward FI (2003) The role of stomata in sensing and driving environmental change. Nature 424:901

Hopkins WG, Hüner NPA (2008) Introduction to plant physiology. John Wiley & Sons, Hoboken

Lopes MS, Reynolds MP (2010) Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. Funct Plant Biol 37:147–156

Lopes MS, Reynolds MP (2011) Drought adaptive traits and wide adaptation in elite lines derived from resynthesized hexaploid wheat. Crop Sci 51:1617–1626

Manschadi AM, Christopher J, Devoil P, Hammer GL (2006) The role of root architectural traits in adaptation of wheat to water-limited environments. Funct Plant Biol 33:823–837

McFadden ES, Sears ER (1944) The artificial synthesis of *Triticum spelta*. Rec Genet Soc Amer 13:26–27

Mujeeb-Kazi A, Hettel GP (eds) (1995) Utilizing wild grass biodiversity in wheat improvement: 15 years of wide cross research at CIMMYT. CIMMYT Research Report No. 2, Mexico

Narayanan S, PrasadPV Vara (2014) Characterization of a spring wheat association mapping panel for root traits. Agron J106:1593–1604

Narayanan S, Mohan A, Gill KS, Vara Prasad PV (2014) Variability of root traits in spring wheat germplasm. PLoS ONE 9:e100317

Palta JA, Chen X, Milroy SP, Rebetzke GJ, Dreccer MF, Watt M (2011) Large root systems: are they useful in adapting wheat to dry environments? Funct Plant Biol 38:347–354

Praba ML, Cairns JE, Babu RC, Lafitte HR (2009) Identification of physiological traits underlying cultivar differences in drought tolerance in rice and wheat. J Agron Crop Sci 195:30–46

Reynolds M, Dreccer F, Trethowan R (2007) Drought-adaptive traits derived from wheat wild relatives and landraces. J Exp Bot 58:177–186

Sayar R, Khemira H, Kharrat M (2007) Inheritance of deeper root length and grain yield in half-diallel durum wheat (*Triticum durum*) crosses. Ann Appl Biol 151:213–220

Trethowan RM, Mujeeb-Kazi A (2008) Novel germplasm resources for improving environmental stress tolerance of hexaploid wheat. Crop Sci 48:1255–1265

van Ginkel M, Ogbonnaya F (2007) Novel genetic diversity from synthetic wheats in breeding cultivars for changing production conditions. Field Crops Res 104:86–94

WHEAT (2014) Wheat: vital grain of civilization and food security 2013 Annual Report, CGIAR Research Program on Wheat, Mexico

Ytting NK, Andersen SB, Thorup-Kristensen K (2014) Using tube rhizotrons to measure variation in depth penetration rate among modern North-European winter wheat (*Triticum aestivum* L.) cultivars. Euphytica 199:233–245

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421