



Root architecture traits and genotypic responses of wheat at seedling stage to water-deficit stress

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Abstract

This study was aimed to identify genotypic differences in shoot growth rate and root architecture traits in response to water-deficit stress at early growth stages of wheat seedling, and to quantify relationships between genotypic variation in shoot physiological traits and root system architecture with a drought tolerance at several development stages. Eight bread wheat cultivars, namely Bam, Mahooti, Roshan, Tabbasi, Atrak, Falat, Shiraz and Qods, were grown in polyvinyl chloride tubes filled with soil in the greenhouse under well-watered and water-deficit stress conditions. Water stress elicited genotypic variation in root traits and shoot growth across cultivars. Drought stress decreased root architecture traits, with greater effects in drought-sensitive cultivars compared with those that were drought-tolerant. Branch root length was less influenced compared with seminal root length. We showed that cultivars Roshan and Bam were most tolerant to drought due to their shorter distance between the first branch root and the root tip, higher branch root length, longer seminal roots and higher stomatal conductance compared to the other cultivars. Positive relationship between root growth and shoot physiological responses was quantified upon drought stress, highlighting the role of leveraging more efficient root systems as a strategy to enhance resource uptake under water-deficit conditions. Thus, root growth responses can be used as a drought tolerance selection criterion at the seedling stage of employed genotypes.

Keywords Drought tolerance · Root branch · Root length · Stomatal conductance · Grain yield · Food security

Introduction

Plant growth and development are mostly affected by different types of abiotic stresses such as temperature extremes, drought and salinity (Rahimi et al. 2021; Salehi et al. 2023). Crop productivity in semiarid areas is threatened primarily by water availability, which cumulatively over the growing season should be assessed as a quantum and frequency distribution (Harrison et al. 2012a; 2012b). Key extreme weather events impacting on global food security are varied depending on time of year and location, ranging from heat waves, to waterlogging to frost (Langworthy et al. 2018; Liu et al. 2020a; 2020b; Harrison 2021), but the most predominant is drought (Phelan et al. 2015; Bell et al. 2015; Yan et al. 2022). Drought stress induces osmotic and nutritional constraint that have resultant effects on growth, phenology and yield (Ibrahim et al. 2018, 2019). Reduced water uptake causes osmotic stress, and consequently, plants experience cellular dehydration (Xiong and Zhu 2002). Drought stress imposes various biochemical and physiological limitations

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and negatively affects chlorophyll content, stomatal conductance, the photosynthesis rate, cell division, and wall and protein synthesis (Li et al. 2006; Jin et al. 2017; Ghorbanpour et al. 2020; Luz et al. 2023). Plants display a broad range of molecular and cellular responses under osmotic stress (Bohnert et al. 1999; Hasegawa et al. 2000; Hatami et al. 2017) including stomatal regulation, thus influencing leaf photosynthetic capacity (Rahnama et al. 2010a).

Root traits play a vital role in drought tolerance and yield improvement in cereals under water-limited conditions (Blum 2009; Comas et al. 2013). The development of crops with suitable root traits enable them to increase their sustainability and have higher yields in soils with contrasting water regimes (Liu et al. 2020a; 2020b; Langworthy et al. 2018; Sun et al. 2023). As such, the role of root growth traits for maintaining crop production is of rising interest to plant breeders (Gewin 2010). However, the ability of plant roots to exploit deep water depends on root architectural traits, such as the root length and root dry weight at depth (Carvalho et al. 2014; Zhao et al. 2018), as well as their development over time (Harrison et al. 2012a; 2012b; Ho et al. 2014).

Extensive root systems with higher root mass at depth facilitate greater water extraction capacity that allows sustained transpiration; together these factors can lead to higher stomatal conductance and photosynthesis rate (Lopes and Reynolds 2010; Falster et al. 2021). Deeper and extensive roots are important when plants are grown in soils containing inadequate water or nutrients supply, particularly at the seedling stage (Bengough et al. 2004). Identification

of causes of genotypic variation in root traits through plant breeding could significantly enhance seedling establishment in dry soils, leading to improved crop production (Richards 2008). However, contemporary techniques for phenotyping root architectural traits in the field are challenging, suggesting that root traits studied in the laboratory may not translate to comparable outcomes in mature plants in the field (Wasson et al. 2012).

Plant adaptations in response to drought stress categorize into drought recovery, drought avoidance, drought escape and drought tolerance (Fig. 1). A suitable breeding program to improve root systems and increase water uptake in water-limited crops has been suggested as the most important strategy to cope with drought and enhance yields in dry land regions (Wasson et al. 2012). Therefore, identifying genes that result in beneficial root characteristics would be of massive value to crop breeding efforts around the world. Previous work has shown that deeper root length may lead to more water extraction from various soil during the grain filling period, thus increasing dry matter partitioned to grain (Manschadi et al. 2006).

Hitherto screening processes for root traits has been constrained by available phenotyping technologies for diagnosing root systems in the field, mandating the use of time-consuming and destructive techniques (Rahnama et al. 2019). Such inhibitions have delayed the advance in knowledge of mechanisms underlying changes in root architecture.

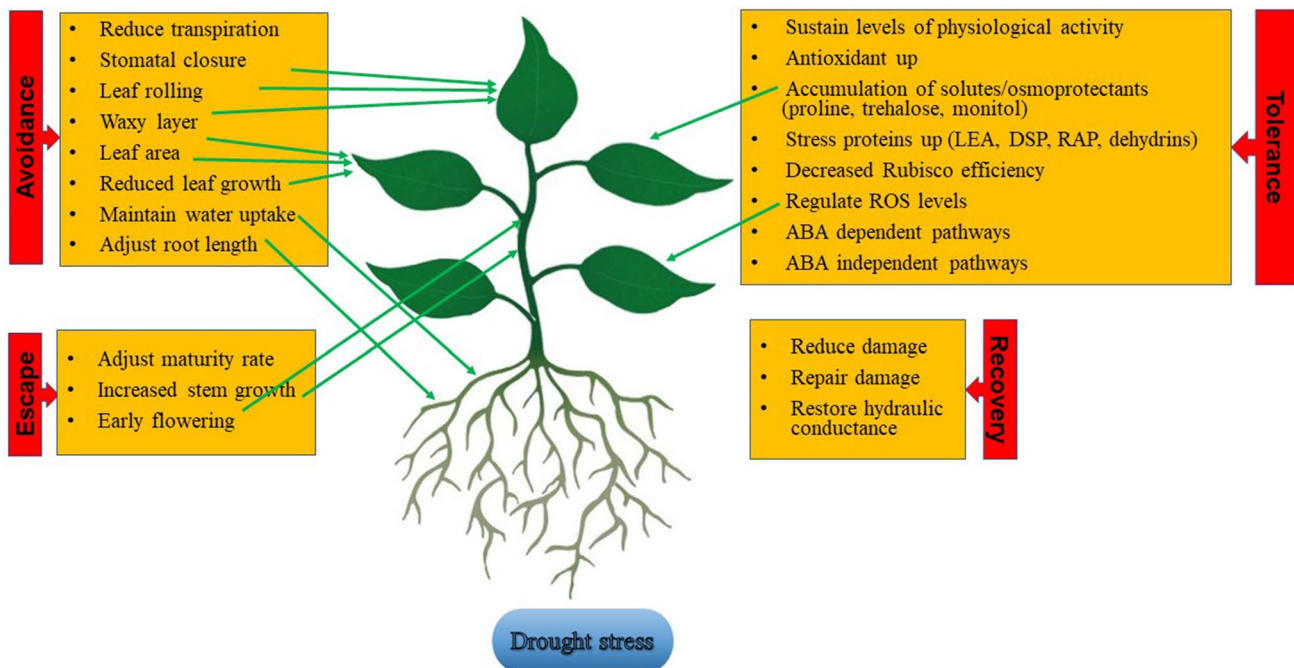


Fig. 1 Schematic representation of plant responses to drought stress (modified from Khan et al. 2016)

The current study aimed to (1) identify genetic differences in shoot physiological traits, seminal and branch roots in response to drought stress at seedling stage; (2) quantify a relationship between genotypic variation in shoot physiological traits and root architecture traits; and (3) identify the corollary of the second aim for drought tolerance during subsequent crop growth stages. Successful realization of these aims would be expected to facilitate and fast-track the screening of large numbers of plants for drought stress tolerance.

Materials and methods

Plant materials

Eight contemporary bread wheat (*T. aestivum* L.) cultivars with known differences in drought tolerance, namely Bam, Roshan, Mahooti and Tabasi (drought-tolerant cultivars) and Atrak, Falat, Qods and Shiraz (drought-sensitive cultivars), were selected; and seeds were provided by the National Plant Gene Bank of Iran.

Growth conditions

Soil was thoroughly mixed with adequate amounts of nutrients including N, P, K and S. Soil samples were passed through a 2 mm standard test sieve to remove stones and gravels. Electrical conductivity (EC) was measured by using a WTW Inolab conductivity meter Level 1 (the Netherlands). Conductivity of the soil samples was $\sim 1.5 \text{ dS}\cdot\text{m}^{-1}$. PVCTM tubes (12.5 cm diameter, 60 cm depth) with small basal drainage holes were carefully filled with plastic bags also with holes containing a mixture of clay loam and sandy soils (40:60 by volume). The deliberate use of plastic bags enabled us to remove the whole root system from the PVC tubes (Rahnama et al. 2019).

Uniformly weighed and sized seeds were selected, surface-sterilized with 2% hypochlorite, washed with sterile ultrapure water-imbibed for 18–24 h, transferred to sterile filter paper (Whatman, Maidstone, UK) in square Petri dishes with distilled water and germinated for two days at room temperature ($20 \pm 2 \text{ }^\circ\text{C}$) in the dark. After two days, four uniform seedlings with 10 mm long radicles were selected, sown in the center of the PVC tubes at 2 cm soil depth (Rahnama et al. 2019). PVC tubes were then placed in a greenhouse with adequate light intensity and exposed to consistent day/night temperatures of $24/14 \pm 2 \text{ }^\circ\text{C}$. Tubes were irrigated every other day with tap water. After seedling establishment, seedlings were thinned to two healthy seedlings per tube (four days after emergence). Plants were grown for 21 days under ambient light with supplementary

light to get an average daily total radiation of 900–1200 $\mu\text{mol m}^{-2} \text{ s}^{-1}$.

Experimental set up and treatments

Based on pre-experimental results with two wheat cultivars (Roshan and Qods), a water potential of -7 to -8 MPa was selected to obtain a significant difference in shoot and root growth response at vegetative stage (Supplementary Tables S1, S2). The current experiment was thus carried out using eight bread wheat cultivars and two treatments (0 to -0.3 MPa as a control and -7 to -8 MPa as the water-deficit treatment). There were six tubes per treatment per genotype comprising a total of 96 tubes for the experiment. We also prepared 16 tubes without plants as null controls to estimate the soil water potential and the amount of soil surface evaporation.

Soil moisture was continuously monitored using TDR soil moisture meters (Delta-T Devices Ltd, Cambridge, UK); TDR probes were installed horizontally and vertically into the soil around the roots. Controls were watered every other day to keep the soil moisture close to field capacity. Before sampling, tubes were allowed to drain until soil water content reached desired percentage of field capacity, corresponding to a pre-defined volumetric soil moisture. Readings were taken daily with the TDR soil moisture meter to maintain the desired volumetric moisture content, and the amount of water lost was added to each tube to ensure that target soil moisture was maintained (Porcel et al. 2004).

The water stress treatment was initiated from the point at which the fourth leaf was fully expanded. The sampling time corresponded to the day when water remained at a water potential of -7 to -8 MPa . Exactly 28 days after emergence, stomatal conductance was measured using a porometer (Delta-T Devices, Burwell, Cambridge, CB25 0EJ, UK) on the abaxial leaf 4 surface. Six replicate plants for each genotype and treatment were harvested, one from each tube. Leaf area measurement was taken using a leaf area meter (Delta-T Devices, UK). Leaf chlorophyll content was recorded using a nondestructive, SPAD-502 m (Minolta, Osaka, Japan). Average SPAD chlorophyll records were calculated from four measurements from different parts of leaf blade.

Leaf relative water content (RWC)

RWC was calculated using 1 cm^2 segment of leaf tissue; each leaf segment was weighed immediately to record a fresh weight (FW). The relative water content was calculated as:

$$\text{RWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}}$$

where TW is turgid weight when leaf segments were floated in distilled water for 4 h and DW is dry weight when leaf segments were dried at 80 °C for 2 days.

Plants were harvested 28 days after emergence. Plastic bags containing roots and soil were carefully removed from the PVC tubes, placed horizontally, cut vertically and washed slowly with running water from a tap followed by washing with deionized water. Roots were removed from the soil without losing or destroying any material and then were preserved in 50% ethanol for further measurements (Rahnama et al. 2019).

Root system architecture

The seminal roots length and the distance between the first branch root and the root tip was measured with a ruler. Root surface area, root diameter and total root length were quantified using a Delta-T Scan (Delta-T Devices, Burwell, UK). Total branch root length was calculated by subtracting the seminal root length from the total root length. Shoots were cut below the crown, and shoot and root dry weight was calculated after the shoot and root samples were oven-dried at 72 °C for 2 days.

Statistical analysis

Statistical analysis was conducted using SAS V9.2. Mean values were compared using a Duncan's tests at the $p < 0.05$ probability level unless stated otherwise.

Results

Genotypic variation in root traits in response to water-deficit stress

Effects of water deficit and cultivars were significant for all root traits examined. The water deficit \times cultivar interaction

was significant in all root traits except for the distance between the first branch root and the tip (Table 1).

Root architecture traits

For the controls, Bam, Tabbasi, Qods and Mahooti had the highest total seminal axile root length; axile root length was lower for Falat and Roshan. Total seminal axile root length was significantly reduced for all cultivars in response to -7 to -8 MPa at the vegetative stage, with larger reductions in sensitive cultivars (Atrak, Shiraz, Qods and Falat with 41, 38, 37 and 32%, respectively) than tolerant cultivars (Roshan, Bam, Tabbasi and Mahooti with 22, 25, 27 and 28%, respectively) (Table 2). Total seminal axile root length was decreased by 22–41% for all cultivars, with cultivar Roshan maintaining the highest total seminal axile root length relative to the control (78%). In contrast, Falat had the lowest total seminal axile root length relative to the control (57%; Table 2).

Total root length was decreased in response to water-deficit conditions (Table 2). Total root length of all tolerant cultivars declined by 8.2% for all tolerant cultivars, compared with a 17.9% decline in total root length for all sensitive cultivars. Total root length was longer in Roshan than in Atrak, mainly due to the larger seminal root length and branch root length under water-deficit conditions (Table 2). Propensity for growth of deeper roots would seem to be a target trait for improving drought tolerance, notwithstanding the fact that genotypic variation for drought tolerance may be impacted by a number of traits, suggesting a need for studies that examine multiple metrics at the same time (viz. Harrison et al. 2021b).

Water stress reduced total branch roots for all cultivars, although with differences between tolerant and sensitive cultivars. There was a 2% decline in total branch roots for tolerant cultivars compared with a 10% decline in total branch roots for sensitive cultivars (Table 2). No significant differences were found between control and drought stress effects on branch root length, while tolerant

Table 1 Analysis of variance for total seminal axile root length (TSARL), total root length (TRL), branch root length (BRL), distance from the tip (DT), root diameter (RD), root surface area (RSA), root

dry weight (RDW), stomatal conductance (gs), relative chlorophyll content (SPAD number), relative water content (RWC) and leaf area (LA) of wheat cultivars under water-deficit stress

SOV	Mean square											
	Df	TSARL	TRL	BRL	DT	RD	RSA	RDW	gs	SPAD	RWC	LA
Water deficit (A)	1	892**	992**	40,665**	0.67 ns	0.93*	321*	0.0006**	26,295**	25.04**	35.08*	0.006**
Cultivar (B)	7	1013**	28,846**	262,069**	10.93**	11.61**	496**	0.007**	72,825**	56.76**	127.5**	0.086**
A \times B	7	483*	471**	13,086*	0.40 ns	1.61**	166*	0.0002**	16,520**	1.75 ns	4.83 ns	0.006**
Error	32	116	45.92	761	0.39	0.28	25.2	0.00003	2216	2.30	14.53	0.0009
CV (%)		8.23	6.16	6.3	16	14.3	11.3	8.8	22.9	3.66	4.69	6.96

* and ** Significant at the 0.05 and 0.01 probability levels, respectively; ns, nonsignificant

Table 2 Effects of water deficit on total seminal axile root length (TSARL), total root length (TRL), branch root length (BRL), distance from the tip (DT), root diameter (RD), root surface area (RSA) and root dry weight (RDW) of eight bread wheat cultivars

Cultivars	Water potential (MPa)	TSARL (cm)	% Change	TRL (cm)	% Change	BRL (cm)	% Change	DT (cm)	% Change	RD (mm)	% Change	RSA (cm ²)	% Change	RDW (gr)	% Change	
<i>Drought-tolerant</i>																
Roshan	-0.3	130 bc	-	446 e	-	316 de	-	2.5 b-d	-	0.36 a	-	50 c	-	0.083 b	-	
	-8	101 d	-22	412 ef	-7.5	312 d-f	-1.5	1.66 d-f	-33.6	0.21 h	-41.6	38 de	-24	0.068 cd	-18	
Bam	-0.3	144 a	-	632 ab	-	488 ab	-	3.83 a	-	0.35 a	-	66 a	-	0.104 a	-	
	-8	108 d	-25	577 c	-8.7	469 b	-3.9	1.93 c-f	-49.6	0.24 b	-31.4	43 cd	-34	0.081 b	-22	
Tabbasi	-0.3	144 a	-	433 de	-	289 e-g	-	2.33 b-f	-	0.27 g	-	48 c	-	0.079 bc	-	
	-8	104 d	-28	387 ef	-10.7	283 f-h	-2.4	1.66 d-f	-28.7	0.18 i	-33.3	37 de	-23	0.062 d	-21	
Mahooti	-0.3	142 ab	-	652 a	-	511 a	-	3.83 a	-	0.31 c	-	69 a	-	0.080 bc	-	
	-8	102 de	-28	612 b	-6.2	510 a	-0.2	2.43 b-d	-36.5	0.23 h	-25.8	48 c	-30	0.079 bc	-1	
<i>Drought-sensitive</i>																
Qods	-0.3	141 a	-	414 fg	-	273 gh	-	3.16 ab	-	0.27 e	-	63 ab	-	0.069 cd	-	
	-8	89 e	-37	345 g	-16.6	256 h	-6.3	1.36 f	-57	0.20 k	-25.9	28 ef	-55	0.049 e	-28	
Atrak	-0.3	135 ab	-	436 ef	-	301 d-g	-	3.2 ab	-	0.25 c	-	46 cd	-	0.075 c	-	
	-8	79 f	-41	344 fg	-21.1	265 gh	-12	1.5 ef	-57.1	0.19 i	-24.0	24 ef	-45	0.043 ef	-42	
Falat	-0.3	121 d	-	433 ef	-	312 d-f	-	2.83 bc	-	0.23 d	-	47 cd	-	0.071 c	-	
	-8	83 ef	-32	356 fg	-17.9	273 gh	-12.4	1.53 d-f	-45.9	0.17 i	-26.1	27 ef	-47	0.052 e	-26	
Shiraz	-0.3	137 ab	-	493 d	-	356 c	-	3.2 ab	-	0.27 a	-	59 b	-	0.086 b	-	
	-8	86 e	-38	414 fg	-16.1	328 cd	-7.9	1.66 d-f	-48.1	0.21 i	-22.2	36 de	-39	0.059 d	-31	

Means labeled with the same letter are not significantly different according to the Duncan multiple range tests at the significance level of $P \leq 0.05$

cultivars exhibited greater ability to elongate branch roots at -8 MPa (Table 2).

Water stress reduced the distance between the first branching point and the root tip; across cultivars, this distance declined by 45% on average (Table 2). Our results demonstrate that water-deficit stress inhibited seminal root elongation, inducing lateral root initiation twice as close to the root tip (Table 2).

Root diameter was significantly reduced in response to water deficit (declining by 22–42%), with Roshan maintaining the highest root diameter relative to the control (58%) and Shiraz showing the greatest reduction (78%) (Table 2). Root surface area was similarly reduced in response to water stress, but more so in drought-sensitive cultivars (47%) than tolerant cultivars (28%; Table 2). Cultivar Roshan maintained the highest surface area relative to the control (76%) while cultivar Qods had the lowest root surface area relative to the control (45%).

Root dry weight was reduced in response to water-deficit stress for all cultivars except for Mahooti, again with lower effects on tolerant (16%) compared with sensitive cultivars (32%). Mahooti generally exhibited greater stress tolerance than other cultivars in terms of biomass production.

Genetic variation in shoot physiological traits in response to water stress

The effects of water deficit and cultivars were significant for shoot physiological traits. The water deficit \times cultivar interaction was significant for all traits except for relative water content and relative chlorophyll content (SPAD number; Table 1).

Stomatal conductance

Significant genotypic differences in stomatal conductance were observed among cultivars and across treatments (Table 3). For the controls, Atrak and Roshan had the highest stomatal conductance while Shiraz and Tabbasi had the lowest. Water-deficit exposure reduced stomatal conductance by 16–44% with Roshan maintaining the highest stomatal conductance over the control (84%) and Qods the lowest (56%). Reductions in stomatal conductance were lower in drought-tolerant cultivars (23%) than for drought-sensitive cultivars (41%; Table 3).

SPAD number

Genotypic differences in SPAD value were observed among cultivars. Qods and Atrak exhibited lower SPAD values than other cultivars (Fig. 2A). For the controls, Tabbasi had the

Table 3 Effects of water-deficit stress on stomatal conductance (gs), relative chlorophyll content (SPAD number), relative water content (RWC) and leaf area (LA) of eight bread wheat cultivars

Cultivars	Water potential (MPa)	gs (mmol m ⁻² s ⁻¹)	% Change	SPAD number	% Change	RWC (%)	% Change	LA (cm ²)	% Change
<i>Drought-tolerant</i>									
Roshan	-0.3	323 a	–	43.1 ab	–	82.9 a	–	30.5 b	–
	-8	270 b	-16.4	42.4 ab	-1.6	81.3 a	-1.9	21.8 c–e	-28.5
Bam	-0.3	286 b	–	44.0 ab	–	83.3 a	–	37.1 a	–
	-8	233 c	-18.5	40.7 b–d	-7.5	80.5 a	-3.3	21.3 de	-42.5
Tabbasi	-0.3	95 f	–	45.7 a	–	83.9 a	–	25.1 c	–
	-8	69 g	-27.4	42.1 a–c	-7.9	82.7 a	-1.4	19.1 e	-24
Mahooti	-0.3	233 c	–	43.9 ab	–	84.8 a	–	34.5 a	–
	-8	166 d	-28.7	41.9 a–c	-4.5	81.8 a	-3.5	21.8 c–e	-36.8
<i>Drought-sensitive</i>									
Qods	-0.3	170 d	–	39.8 b–d	–	78.2 a	–	25.0 c	–
	-8	110 f	-35.3	37.0 d	-7.1	75.0 a	-4.1	13.6 f	-45.6
Atrak	-0.3	233 c	–	39.9 b–d	–	83.4 a	–	24.5 cd	–
	-8	143 e	-38.6	37.6 d	-5.7	79.5 a	-4.7	14.0 f	-44.9
Shiraz	-0.3	280 b	–	40.4 b–d	–	83.2 a	–	21.7 c–e	–
	-8	156 de	-44.3	39.8 b–d	-1.5	78.3 a	-5.9	13.8 f	-36.4
Falat	-0.3	320 a	–	42.7 a–b	–	83.0 a	–	19 e	–
	-8	179 d	-44	40.6 b–d	-4.9	76.8 a	-7.5	11.9 f	-37.4

Means labeled with the same letter are not significantly different according to the Duncan multiple range tests at the significance level of $P \leq 0.05$

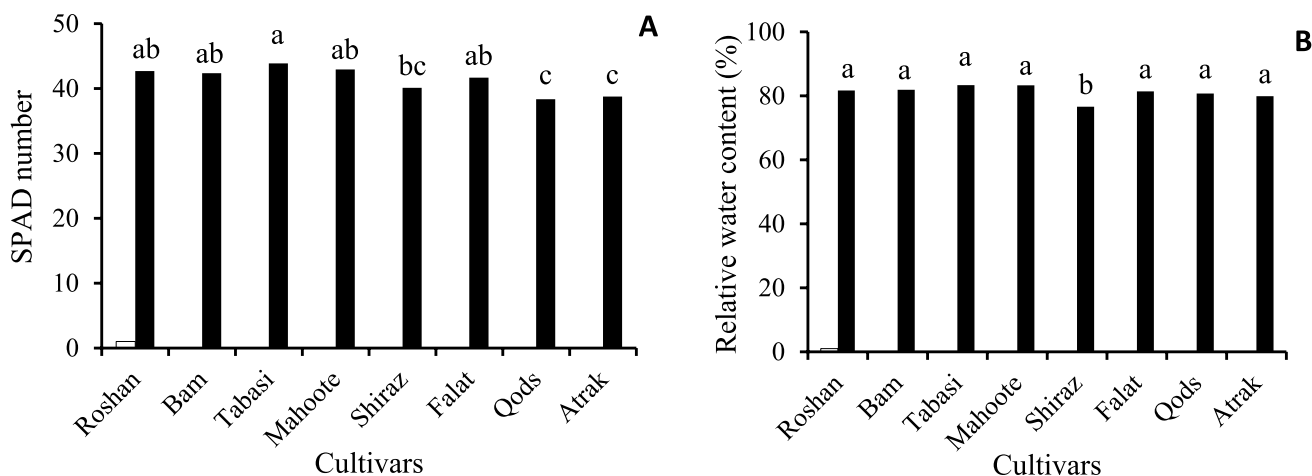


Fig. 2 Mean comparison (main effects) of SPAD number (**A**) and relative water content (**B**) among wheat cultivars

highest SPAD number while Atrak and Qods had the lowest (Table 3). Relative chlorophyll content decreased by 5% relative to controls for all cultivars in response to water stress (Fig. 3A). Slight differences in the chlorophyll content of control and drought-treated plants were measured here.

Relative water content

There were no significant differences in relative water content among cultivars except for Shiraz (Fig. 2B). Relative water content slightly decreased by 4% relative to controls in response to water stress (Fig. 3B). For the controls, relative water content was similar for all cultivars except for Qods, which had lower water content. Relative water content was reduced by 6% for sensitive cultivars, with Falat maintaining 92.5% of the level achieved by the control and Qods, 95.9% (Table 3).

Leaf area

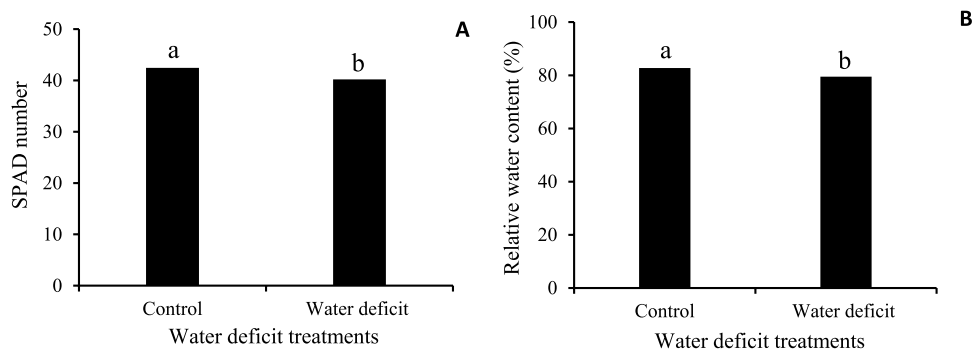
For the controls, Bam and Mahoote had the greatest leaf area, while Qods and Atrak had the lowest. Leaf area of the cultivars was reduced to 37% of control levels for

all cultivars under water deficit (Table 3). No statistical differences were found between the leaf areas of sensitive cultivars under water stress conditions (Table 3).

Discussion

Here we have documented remarkable genotypic variation in total seminal axile root length and total root length of cultivars subjected to water stress, similar to previous results for barley (Bengough et al. 2004), maize (Li et al. 2015) and rice (Kano-Nakata et al. 2011). The differences we observed in total seminal root length between cultivars may be attributed to genetic variability. Our results also indicate that root plasticity is a key trait under drought conditions to benefit plant growth. Data gathered in the present study has thus added valuable genetic resources including a range of agronomic and morphological traits to the scientific literature; these data could be used in future to improve crop stress tolerance. The effects of water stress on total seminal axile root length observed here are consistent with previous reports that show decreased total seminal axile root length

Fig. 3 Mean comparison (main effects) of SPAD number (**A**) and relative water content (**B**) between water-deficit treatments



of bread wheat (Shahzad et al. 2012; Ehdai et al. 2012) and rice (Kano-Nakata et al. 2011).

We also showed that water deficit reduced total root length (except for cultivar Roshan). Drought-tolerant cultivars exhibited larger root systems than drought-sensitive cultivars. This phenomenon increased water uptake as shown by positive correlations between relative water content and total root length ($r=0.47^{**}$), which in turn contributed to greater stomatal conductance and photosynthesis under water deficit (Table 4). Similar effects of defoliation on the alleviation of water stress have been observed for rainfed winter wheat in Australia (Harrison et al. 2010; Harrison et al. 2011a; 2011b). In this experiment, it is possible that exposure longer to water deficit than previous studies provided sufficient time to fully realize the genotypic sensitivity of total root length to water deficit. Future studies could consider pursuing such treatments over the entire crop life cycle to examine whether cumulative effects of genetic variability in root architecture and water uptake translate into dry matter production and yield. Such studies could also be explored in the field and using simulation frameworks to extrapolate results across management, genotypes and environment types (Liu et al. 2020a, b; Ibrahim et al. 2019; Farina et al. 2020).

Here, we showed that water stress caused a moderate reduction in total branch roots, which demonstrates that water stress impacted root system morphology of wheat upon exposure to water stress. The results may be a compensation strategy for the reduction to total seminal root length under salinity stress (Rahnama et al. 2011) noting that suppression of lateral root meristems can reduce root branching (Malamy et al. 2005). Given that enhanced lateral root production plays a main role for adaptation to drought stress (Suralta et al. 2010), we suggest that further work

should aim to identify genetic causes of variability in such traits (Table 4).

It was shown that water stress reduced the distance from the root tip. Reduced primary root elongation results from a decrease in cell numbers in the root elongation zone leading to a decrease in the distance between the first branch root and the seminal root tip (Rahnama et al. 2011). Previous work has shown that the distal branch roots were shortened by twofold for bread wheat (Rahnama et al. 2019) and sixfold for durum cultivars (Rahnama et al. 2011) in response to salinity. It should be noted here that root system architecture is primarily determined by root length, seminal root number and root branching (Rahnama et al. 2019). It has been previously suggested that osmotic conditions may cause a loss of apical dominance in the axile root tips, while carbohydrates and water continued to be delivered to the branch root primordia via the phloem to enable growth (Boyer et al. 2010).

The results of the present study revealed that water deficit reduced the seminal axile root elongation more than total root length, while there was a slight reduction in branch root length. Branch root length increased slightly in all eight cultivars under water stress, probably to counteract reduced seminal root length. Conversely, water deficit modified the proportion of the root system component through promotion of the lateral roots and inhibition of the seminal roots to maintain a large root system for access to water. Such plasticity may underpin a comparative advantage for crops grown under abiotic stress (Rahnama et al. 2011, 2019). Zolla et al. (2010) found that root systems in *Arabidopsis* proliferated branch roots while axile root length slowed when grown on agar with basal nutrient medium with 50 mM NaCl. It was suggested that the axile tips are more sensitive than the branch roots and that differentiation of

Table 4 Correlation coefficient between shoot and root traits of eight wheat cultivars under water deficit

Traits	SC	SPAD	RWC	LA	TSRL	TRL	RB	DT	RSA	RD	RDW
SC	1										
SPAD n	0.12 n.s	1									
RWC	0.40**	0.65**	1								
LA	0.48**	0.66**	0.62**	1							
TSRL	0.42**	0.58**	0.63**	0.77**	1						
TRL	0.41**	0.49**	0.47**	0.66**	0.49**	1					
RB	0.35**	0.33*	0.36*	0.53**	0.27 n.s	0.57**	1				
DT	0.47**	0.36 n.s	0.59**	0.69**	0.62**	0.57**	0.43**	1			
RSA	0.85**	0.52**	0.38**	0.68**	0.63**	0.26 n.s	0.12 n.s	-0.56**	1		
RD	-0.71**	-0.03 n.s	-0.62**	-0.67**	-0.83**	-0.59**	-0.43**	0.78**	-0.41**	1	
RDW	0.33*	0.081 n.s	-0.37*	-0.20 n.s	0.38**	0.23 n.s	0.35 n.s	0.27 n.s	0.36 n.s	-0.38**	1

n.s Nonsignificant; SC stomatal conductance; SPAD n. SPAD number; RWC relative water content; LA leaf area; TSRL total seminal root length; TRL total root length; RB root branch; DT distance from the tip; RSA root surface area; RD root diameter; RDW root dry weight

‡* and ** Significant at the 0.05 and 0.01 probability levels, respectively

stellar pericycle cells was maintained to stimulate formation of new branch root primordia. These results suggest that root system architecture is a complex trait that affects water capture and is mediated by environmental stresses.

Water deficit caused a significant reduction in root diameter for all cultivars we examined. Production of thinner roots has been proposed as an advantage in facilitating water and nutrient uptake under conditions of limiting resources (Bonifas and Lindquist 2009; Wasson et al. 2012; Corneo et al. 2016). Thinner roots with small diameter and greater specific root length enable plants to increase hydraulic conductance by increasing root surface area in contact with soil water, thus generating greater area for water and resource uptake (Bonifas and Lindquist 2009; Comas et al. 2013). Here, we found a negative correlation between stomatal conductance with root diameter ($r = -0.71$) and relative water content ($r = -0.62$), suggesting that reduced root diameter enabled greater relative water content and enhancement of stomatal aperture. Production of thinner root systems and consequently a larger root surface area in response to water-limited conditions has been previously reported for bread wheat (Becker et al. 2016), and barley and durum wheat (Carvalho et al. 2014).

The stereotypical impact of drought stress on plant performance is suboptimal dry matter production (Langworthy et al. 2018; 2020). Here, we showed that seminal and total root length decreased in Mahooti, but root biomass was not affected by water deficit due to increased branch rooting, a compensatory result that maintained root biomass. This result was also partially due to the ability of cultivar Mahooti to develop a larger root system, allowing greater water uptake and photosynthesis under water stress. Previous reports have shown that root biomass in dry soils may be greater than well-watered conditions (Munns and Cramer 1996), perhaps a consequence of stimulation of lateral root initiation in dry soil (Jupp and Newman 1987). As well, the metabolic costs of a thinner root system (lower root diameter with lower root biomass) are lower than the cost of a system of roots with larger diameters (Elazab et al. 2016).

Stomatal conductance was reduced under water deficit; higher reductions were observed in drought-sensitive cultivars compared with drought-tolerant cultivars. Several studies have shown a similar reduction in stomatal conductance as a result of salinity (Rahnama et al. 2010b) and water stress (Xu et al. 2008; Pour-Aboughadareh et al. 2017). In this study, tolerant cultivars maintained the highest total and seminal root length in response to water deficit. Hence, roots of drought-tolerant cultivars can extract more water from the soil to maintain higher stomatal conductance during water stress compared to the other cultivars. It is generally recognized that stomatal response to water potential of the soil is determined by

root signals, with roots being involved in the perception and transduction of the drought stress signals (Dubos and Plomion 2003). While it has been postulated that ABA plays a central role in such phenomena (Davies et al. 2005), stomatal conductance is also responsible for other plant functions, including canopy-level evaporative cooling through control of transpiration (Langworthy et al. 2020). Here, we found a positive correlation between stomatal conductance and seminal root length ($r = 0.42$) and relative water content ($r = 0.40$), suggesting that stomatal conductance is tightly linked with drought tolerance at the pot scale. Further work remains in elucidating whether such effects hold true under field conditions.

Genotypic variability in relative chlorophyll content (SPAD readings) were found between cultivars under water deficient, similar to previous reports (Pour-Aboughadareh et al. 2017). Drought also reduces leaf area, so reductions in chlorophyll concentrations possibly linked with smaller cell size and higher chloroplast concentration per unit area (James et al. 2002). Water-deficit stress is thought to reduce chlorophyll content through lipid peroxidase and electrolytic leakage from chloroplasts and thylakoid membranes (Ristic et al. 2007; Djanaguiraman et al. 2010).

Leaf relative water content as an estimate of plant water status is used as an important index for water stress tolerance (Anjum et al. 2011). In all cultivars studied here, leaf relative water content decreased under water stress, although this result may have been offset by changes in stomatal conductance, given known effects of drought on stomatal apertures (Rahnama et al. 2010a; Torres-Ruiz et al. 2013; Nemeskeri et al. 2015; Clauw et al. 2015). Stomatal closure reduces cell enlargement, leaf area production and growth rate and—depending on the extent and duration of stomatal closure over the crop life cycle—can severely reduce yield and biomass (Nemeskeri et al. 2015). Indeed, Ball et al. (2000) reported that limitation of assimilate production and storage during the seed filling period can penalize yields.

In the present study, shoot physiological traits were an indicator of root growth traits. Stomatal conductance, SPAD number and relative water content were found to be correlated with root length, root specific area, root diameter and root branching under water-deficit conditions, suggesting that root growth traits might be driving factors for improving plant growth and productivity in drought conditions. Cultivars Bam and Roshan experienced the lowest inhibition in seminal and total root length and the lowest inhibition in stomatal conductance and relative water content in response to water deficit. It was previously reported that longer root length is useful when screening for yield potential in plants under salinity stress conditions (Rahnama et al. 2011, 2019). These results suggest that the relatively higher root length and degree of branching under

drought conditions confers drought tolerance to current cultivars in terms of shoot physiological traits.

Conclusion

We showed that exposure to water deficit during the seedling stage reduced root growth and negatively impacted on shoot physiological traits in wheat cultivars. We have shown the interaction between water stress and root growth, including effects of root system architecture on perceived water stress through inhibition of seminal root length, promotion of branch root elongation and shortening distal branch roots. Such root plasticity in response to variation in soil moisture stress may engender increased soil water acquisition and reduce negative effects of water deficit. We identified cultivars Bam and Roshan as the most tolerant to water deficit due to their shorter seminal axile root tips, longer seminal roots and higher branch roots, higher stomatal conductance and relative water content compared with other cultivars. A positive relationship was found between genotypic variation in shoot physiological traits and root growth traits. We believe these relationships represent a drought tolerance strategy in which root growth results in a greater collective root surface area to harvest water in drying soils. We showed that root traits are useful indicators for proxies that may improve crop productivity, highlighting the role of root systems to improve water and nutrient uptake under water deficit. We suggest that genetic variability in root traits during early phenology could be used by plant breeders to drought tolerance.

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Author contributions AR planned and designed the research, collected and analyzed the data and wrote the manuscript. BH performed the experiment and conducted fieldwork, AFF wrote the manuscript, and MTH and MG revised the manuscript critically.

Declarations

Conflict of interest The authors declare no conflict of interest.

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