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ROOT SYSTEM ARCHITECTURE AND STEM TRAITS OF BREAD WHEAT SEEDLINGS UNDER CONTRASTING WATER REGIMES

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ABSTRACT

Differential response of bread wheat seedlings to contrasting water regimes can be used to determine traits related to drought tolerance. The F_1 offspring from eight crosses and 11 selected parental genotypes were simultaneously grown in hydroponic cultivation-polyethylene glycol (PEG) induced drought stress vs. control conditions. Criteria for selection of maternal and paternal genotypes were desirable traits in terms of increased tolerance to drought: longer stem length, longer primary root, large number of seminal roots, large root dry mass and stem dry mass, beginning of root branching at the greatest possible distance from beginning of the root, contrasting angle of seminal roots. The traits were measured on 14 day old seedlings: primary root length (PRL), distance to the first branch on the primary root (DFBR), number of seminal roots (NSR), total seminal root length (TSRL), angle of seminal roots (ASR), stem length (SL), root dry mass (RDM), stem dry mass (SDM), and the ratio of root dry mass to stem dry mass (RDM/SDM). Drought stress led to a decrease in the mean values of all root and stem traits by 11% on average, except for RDM/SDM which was increased. The most sensitive trait to drought stress was DFBR (25% reduction), causing root systems to branch at a shallower depth. In conditions of induced drought stress, the strongest statistically significant correlation was found among RDM and RDM/SDM ($r = 0.794$), SL and ASR ($r = 0.708$), RDM and TSRL ($r = 0.673$). The stress tolerance index had the strongest positive correlation with the SDM, PRL, TSRL, SL, and the stress susceptibility index with the NSR. The highest heterosis mean value was observed for PRL (24.6%) and for SL (15.6%) under drought stress. The different directions of average heterosis in induced drought stress vs. control conditions were observed for DFBR, RDM, and SDM, indicating differential traits to account for when planning breeding bread wheat for drought tolerance. Selected traits as criteria for selection and favorable combinations can be incorporated into pre-breeding and breeding schemes, directly or indirectly, aiming to achieve drought tolerance.

Keywords: bread wheat; drought stress; heterosis; hydroponics; root system architecture.

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INTRODUCTION

The main limiting environmental factor that negatively affects wheat (*Triticum aestivum* L.) production is drought, due to reduced precipitation and rising temperatures, threatening global food security (Farooq *et al*., 2014, 2017; Wan *et al*., 2022). Research data published in the period from 1980 to 2015 show that from 21% to 39% of yield loss in wheat and corn (*Zea mays* L.) in the world is the result of the adverse effects of drought (Hussain *et al*., 2019; Johnson *et al*., 2022), as the consequence of climate change manifested through global warming.

Root system architecture (RSA) traits are increasingly becoming a key target for researchers and breeders to improve drought tolerance in wheat (Ober *et al*., 2021); through phenotyping of desirable traits and incorporating them into new cultivars using pre-breeding. Root length at the seedling stage is a key agronomic trait for increasing yield in arid environmental conditions (Ahmed *et al*., 2019; Hussain *et al*., 2019). It was shown that the characteristics of the RSA, such as the number and angle of growth of seminal roots, are genetically determined and related to drought resistance (Maqbool *et al*., 2022). The narrow seminal root growth angle and their greater number represent reference traits for selection in the early stages of wheat growth in breeding programs, as seminal root growth angle is closely linked to the RSA of mature plants (Pais *et al*., 2022). The primary root length is also a genetically highly determined trait (Chen *et al*., 2020; Zhu *et al*., 2019). Greater root mass and density improve yield components by increasing the rate of water uptake when subsoil water is limited (Fang *et al*., 2017). Total root length is also a trait associated with drought tolerance because it affects the distribution of roots in the soil and the amount of water absorbed (Wasaya *et al*., 2018). The most reliable and indirect screening methods for deep roots are the above-ground traits that are easily measured and represent indicators of favorable water relation (Li *et al*., 2019).

Seedling survival, or vigor, is a simple germplasm testing method that enables the assessment of drought tolerance of genotypes under laboratory conditions. Early growth (vigor), in regard to the size of leaves and stems produced in the early stages of plant development, is an important crop trait because it is associated with greater water utilization, higher biomass and grain yields (Zhao *et al*., 2019). Faster early growth will promote faster leaf area development, reduces water evaporation directly below the plant leaf canopy, thus conserving moisture for later stages of development, increases plant competition with weeds, improves nutrient uptake (Farooq *et al*., 2015; Hussain *et al*., 2016; Li *et al*., 2019), and most vegetative growth occurs under low evapotranspiration.

In the study of Xie *et al*. (2017) significant links between the seedling RSA, and field yield and yield-related traits of bread wheat were identified, suggesting that the RSA of 13-day-old seedlings grown in the growth pouches could be predictive of field performance of these agronomic traits.

Taking into consideration the difficulties of accessing mature root systems in soil and the finding that the number of seminal roots, primary root length, root growth angle and other root architecture traits at the seedling stage are closely linked to the architecture of mature plant root systems (Pais *et al*., 2022) the hypothesis was set. The main hypothesis is that differential responses of wheat genotypes in P and F_1 generations at the seedling stage to drought stress vs. control conditions, will point on the most important RSA and stem traits with the highest heterosis values and contributing to drought tolerance. Selected traits serve as criteria for selection and favorable combinations can be incorporated in pre-breeding and breeding schemes, directly or indirectly, aiming to achieve drought tolerance.

The aims of this research were: i) assessment of variability and correlation of RSA and stem traits of the parents, chosen from a previously screened collection of 101 wheat genotypes for drought tolerance, and their F_1 progeny, in the seedling stage under contrasting water regimes; ii) analysis of association of the stress indices and root system architecture and stem traits under induced drought stress; iii) determination of combinations of parental genotypes with the best heterotic progeny, with better early growth of roots and stems and tolerance to drought.

MATERIALS AND METHODS

Plant material, choice of superior parents, biparental crosses and F_1 hybrids: A total of 11 genotypes were chosen for parents in this investigation and eight crosses were performed (Table 1). Criteria for selection of maternal and paternal components in crosses were desirable traits in terms of increased tolerance to drought: longer stem length, longer primary root, large number of seminal roots, large root dry mass and stem dry mass, beginning of root branching at the greatest possible distance from the beginning of the root. Contrasting genotypes that had the widest or narrowest angle between the outermost seminal roots were selected.

Growing wheat seedlings of parental and F_1 generations in hydroponic cultivation: drought stress vs. control conditions: The F_1 offspring from eight crosses and a total of 11 selected parental genotypes were simultaneously grown in hydroponic cultivation conditions (induced drought stress vs. control conditions) in the laboratory of the Plant GeneBank of the Directorate for National Reference Laboratories of the Ministry of Agriculture, Forestry and Water Management of the Republic Serbia, in the year 2020. Seedlings of wheat genotypes were grown in a chamber for growing plants under controlled conditions-phytotron (KBW 720, Binder GmbH), with adjustable temperature, humidity, and lighting. Seeds from parental and F_1 generation for all crosses were germinated on filter paper soaked in distilled water for four days at 20˚C. Fifteen uniformly germinated seeds *per* genotype were placed on the perforated lid of the plastic box, rhizobox (38 × 29 × 11 cm) divided in the middle by a plastic partition, for the purpose of providing control conditions and conditions for applying stress treatment. The experiment was set as a completely randomized design with three replicates of each growth condition. Prior to placing seedlings on the perforated lid, the primary roots were marked with a marker. The seedlings were placed in such a way that the entire root system was immersed in an aqueous solution. The container with the aqueous solution was lined on all sides with dark crêpe paper and connected to an oxygen pump. In one half of the box, there was an aqueous nutrient culture that represented control. The seedlings were grown in the absence of stress. The hydroponic culture had a modified Knop's solution with the following composition and concentration: 14.4 g/L $Ca(NO_3)_2 \times 4H_2O$; 2.5 g/L KNO_3; 9.5 g/L $(NH_4)_2SO_4$; 1.2 g/L KCl; 2.5 g/L KH_2PO_4; 4.7 g/L $MgSO_4 \times 6H_2O$; 5.07 g/L $MgSO_4 \times 7H_2O$. The pH value of the solution was between 5.6 and 5.8. In the second half of the box (on the right), the genotypes were first grown only in nutrient culture for three days after setting up the experiment, and then after the expiration of three days (that is, on the seventh day from the start of germination), polyethylene glycol 6000 (PEG-6000, ACROS Organics™) was added in an amount that causes moderate desiccation stress (Peršić *et al.*, 2022) which resulted in an osmotic potential of -0.4 MPa. PEG is chemically inert and nontoxic for plant cells and changes the water potential of solutions by inducing potential osmotic pressure. Osmotic stress represents a surrogate for drought stress in the plant (Li *et al.*, 2019; Johnson *et al.*, 2022). The mode of operation of the phytotron was set: for the first 11 hours, there was light in the chamber at a temperature of 20°C; for the next hour, the temperature dropped from 20°C to 16°C in the dark; for the next 11 hours, the chamber was in the dark at a temperature of 16°C; and then for the next hour, the temperature increased from 16°C to 20°C in the light. The relative air humidity was 75%. The cycle was completed after 24 hours and repeated. The mode of operation lasted 7 more days after PEG treatment, after which the plants were removed from the growth chamber. After 10 days of cultivation in the phytotron, 10 representative seedlings *per* genotype were selected, washed under running water, scanned, and the photographs were processed in the program Image J (Rasband, 2020). The following traits were measured: primary root length (PRL), distance to the first branch on the primary root (DFBR), number of seminal roots (NSR), total seminal root length (TSRL), angle of seminal roots (ASR), and stem length (SL). After drying samples for 24 h at a temperature of 80°C, root dry mass (RDM) and stem dry mass (SDM) were measured, and the ratio RDM/SDM was calculated.

Table 1. Crosses of wheat parental genotypes with their country of origin and selection criteria.

Cross	Female parent/Origin	Selection criteria	Male parent/Origin	Selection criteria
1.	Euklid (FRA)	PRL; RDM/SDM	CHI 4 (CHN)	DFBR; ASR
2.	Dika (SRB)	TSRL; DFBR	Ingenio (FRA)	RDM; TSRL; RDM/SDM
3.	Pobeda (SRB)	DFBR	Donska semi-dwarf (RUS)	SDM
4.	Phoenix (USA)	SL	NS 40S (SRB)	PRL; ASR
5.	Pobeda (SRB)	DFBR	Brigant (GBR)	SDM; ASR
6.	Dika (SRB)	TSRL	Donska semi-dwarf (RUS)	SDM
7.	Zemunska rosa (SRB)	RDM; RDM/SDM	Ingenio (FRA)	RDM; TSRL; RDM/SDM
8.	WWBMC2 (USA)	TSRL; NSR; ASR	Ingenio (FRA)	RDM; TSRL; RDM/SDM

[a]PRL-primary root length, DFBR-distance to the first branch on the primary root, NSR-number of seminal roots, TSRL-total seminal root length, ASR-angle of seminal roots, SL-stem length, RDM-root dry mass, SDM-stem dry mass, RDM/SDM-the ratio root dry mass to stem dry mass. FRA-France, SRB-Serbia, USA-United States of America, CHN-China, RUS-Russian Federation, GBR-United Kingdom of Great Britain and Northern Ireland.

Statistical analyses: The environment represented the contrasting growth conditions of wheat seedlings in phytotron-controlled conditions vs. induced drought stress in the balanced experimental design with two check genotypes (NS 40S and Zemunska Rosa) tolerant to drought. Pearson's correlation analysis was used to examine the degree of linear dependence of the examined root and stem traits of wheat seedlings under contrasting water regimes of growth. The stress susceptibility index (SSI) and stress tolerance index (STI) were calculated for the wheat genotype seedlings using total seedling biomass as a yield parameter under control conditions and under induced drought stress (Sánchez-Reinoso *et al.*, 2020). The principal component analysis was used to present an association between stress indices and root system architecture and stem traits under induced drought stress. Mid-parent heterosis, or hybrid vigor, in the F_1 progeny obtained from the crossing of the selected parental genotypes for the examined traits was calculated. All statistical analyses were performed in the program MINITAB V. 16 (Minitab Inc., State College, PA, USA, 2021).

RESULTS AND DISCUSSION

Mean values of RSA and stem traits: drought stress vs. control conditions: A statistically highly significant difference between the mean values of traits measured under induced drought stress and under control conditions was shown for DFBR, SL, and SDM (Table 2). All the mean values of the studied RSA and stem traits in wheat seedlings of parental and F_1 generation were lower under induced drought stress compared to control, except RDM/SDM.

The most sensitive trait to induced drought stress was DFBR, in terms of the highest decrease of this trait (24.5%), in comparison to other observed traits. This indicates that PEG treatment causes root systems to branch at a shallower depth. The examined root traits, except DFBR, were more resistant to the effect of induced drought stress (on average, the reduction was 9% compared to the control). The examined stem traits showed greater sensitivity to induced drought stress (on average, the reduction was 13% compared to the control). Ahmad *et al*. (2013) investigated root and stem traits of 40 wheat genotypes in the seedling stage under three different PEG treatments and showed that root length was less affected by all three treatments compared to stem length, which decreased significantly in all three treatments. These authors emphasized that root length should be taken as a trait that contributes to drought tolerance and incorporated into high-yielding wheat cultivars to obtain high yields under water-deficit conditions. Ahmed *et al*. (2019) also suggested that root length has proven to be an important trait for achieving drought tolerance. A higher ratio of RDM/SDM under conditions of induced drought stress was due to a smaller adverse effect on root growth compared to stem growth, as was also confirmed by Chen *et al*. (2021).

Correlations of RSA and stem traits of wheat seedlings: drought stress vs. control conditions: Pearson's coefficients of correlations of RSA and stem traits of 19 wheat genotypes grown in control conditions and under PEG-induced drought stress is shown in Table 3. Information on the association of traits under optimal growth conditions and under drought stress conditions can further help to develop strategies for indirect selection for drought tolerance.

Table 2. Mean values of RSA and stem traits-drought stress vs. control conditions.

Trait	Control	Drought stress	Drought stress/Control
PRL (cm)	12.7	11.8	0.93
DFBR (cm)	1.51^{**}	1.14	0.75
NSR	3.00	2.8	0.93
TSRL (cm)	23.6	22.2	0.94
ASR (°)	94.7	92.7	0.98
SL (cm)	24.3^{**}	20.9	0.86
RDM (mg)	6.40	6.00	0.93
SDM (mg)	23.1^{**}	20.4	0.88
RDM/SDM	0.28	0.30	1.07

^{a**} $P < 0.01$, PRL-primary root length, DFBR-distance to the first branch on the primary root, NSR-number of seminal roots, TSRL-total seminal root length, ASR-angle of seminal roots, SL-stem length, RDM-root dry mass, SDM-stem dry mass, RDM/SDM-the ratio of root dry mass to stem dry mass.

Table 3. Pearson's coefficients of correlations of RSA and stem traits of wheat parental genotypes and F_1 offspring's from different crosses grown in control conditions and under drought stress.

Trait	Growth conditions	PRL	DFBR	NSR	TSRL	ASR	SL	RDM	SDM
DFBR	Control	-0.012							
	Stress	0.428							
NSR	Control	-0.485^{*}	0.316						
	Stress	-0.487^{*}	-0.220						
TSRL	Control	0.197	0.295	0.286					
	Stress	0.519^{*}	0.456^{*}	0.107					
ASR	Control	0.138	-0.724^{**}	-0.295	-0.261				
	Stress	0.243	-0.580^{**}	-0.261	0.034				
SL	Control	-0.198	-0.602^{**}	-0.106	-0.475^{*}	0.624^{**}			
	Stress	0.404	-0.407	-0.382	0.197	0.708^{**}			
RDM	Control	0.236	0.579^{**}	0.320	0.465^{*}	-0.391	-0.567		
	Stress	0.573^{**}	0.484^{*}	0.103	0.673^{**}	-0.062	-0.109		
SDM	Control	-0.060	0.288	0.263	-0.033	-0.067	0.104	0.156	
	Stress	0.327	-0.031	-0.028	0.442	0.419	0.480^{*}	0.126	
RDM/SDM	Control	0.222	0.308	0.118	0.420	-0.294	-0.546	0.763	-0.516
	Stress	0.297	0.447	0.110	0.319	-0.323	-0.395	0.794^{**}	-0.498^{*}

$^{a*}P < 0.05$, ** $P < 0.01$, PRL-primary root length, DFBR-distance to the first branch on the primary root, NSR-number of seminal roots, TSRL-total seminal root length, ASR-angle of seminal roots, SL-stem length, RDM-root dry mass, SDM-stem dry mass, RDM/SDM-the ratio of root dry mass to stem dry mass.

In control conditions, the strongest statistically significant positive correlation was shown between SL and ASR. Genotypes with a longer stem had higher values of ASR, i.e., root systems that grew wider, while those with a shorter stem had narrower root systems. It has previously been shown that root systems with a narrower growth angle of seminal roots are longer, while those with a greater width distribution have shorter roots (Zhu *et al*., 2019). SL was negatively correlated with all other measured root traits: DFBR, NSR, TSRL, and RDM, similar as in a study of Wiener *et al*. (2017) who reported that above-ground and below-ground traits in wheat are not highly linked and can evolve independently. A positive statistically significant relationship between RSA traits and aboveground biomass, between NSR and TSRL (r = 0.710), and between PRL and TSRL (r = 0.540), was established in the investigation of Xie *et al*. (2017), but in our study these correlations proved to be non-significant. Zhu *et al*. (2019) reported statistically significant correlation between TSRL and PRL (r = 0.870), and between ASR and NSR (r = 0.732), while in our study statistically significant correlation between these traits was absent.

A statistically highly significant positive correlation was observed between RDM and DFBR, as well as between RDM and TSRL. In the study by Li *et al*. (2019) on mature wheat plants, RDM was not significantly correlated with root depth. This indicates the complexity of the root architecture, i.e., the diversity of the distribution of RDM in different soil layers.

A statistically highly significant negative correlation was determined between ASR and DFBR, SL and DFBR, PRL and NSR, and between SL and TSRL. Zhu *et al*. (2019) found statistically significant ($P < 0.01$) negative correlation between ASR and TSRL (r = -0.680), between ASR and PRL (r = -0.873), and between TSRL and NSR (r = -0.819). Genotypes that had a narrower ASR had a deeper root system, and vice versa, genotypes with a shallower root system had a wider ASR. Also, genotypes with better-developed above-ground growth had a shallower root system and a smaller TSRL. This is contrary to the view of Zhao *et al*. (2019) that genotypes with faster early root growth result in deeper roots, faster root penetration, and greater total root length, whereas seedlings with slower early root growth result in shallower roots, slower penetration, and smaller total root length.

In drought stress conditions, the strongest positive statistically significant correlation was between RDM and RDM/SDM. This is consistent with previously reported results showing that plants under drought stress tend to produce more root biomass and increase the root-to-stem ratio (Boudiar *et al*., 2020). Within the functional balance theory, when a certain plant resource is limited, an increased allocation of biomass to the plant part that is responsible for the supply of the limiting resource is observed. The fact that the correlation between RDM and RDM/SDM was not statistically significant under control conditions contributes to this theory. A statistically significant positive correlation was also shown for SL and ASR, RDM and TSRL, RDM and PRL, PRL and TSRL, RDM and DFBR, SL and SDM, and TSRL and DFBR. Similar correlations of investigated root traits in the presence of drought stress, induced by PEG, were reported by Fernandes *et al.* (2020). They found a higher statistically significant ($P < 0.01$) correlation between SDM and SL (r = 0.785) and RDM and PRL (r = 0.700), than in our study. In conditions of induced drought stress, most of the root traits were positively correlated with each other, and to a greater degree compared to their correlations under control conditions. It was shown that the induced drought stress affected all the examined root traits and that the selection of any of these traits would improve the performance of other root characteristics under drought stress conditions. Ahmed *et al.* (2019) reported that root dry weight, maximum root length, total root length, root-to-stem ratio, and number of seminal roots were considered appropriate root traits for improving drought tolerance.

As in control conditions, the strongest statistically significant negative correlation was shown between ASR and DFBR, although the degree of correlation was somewhat lower. This indicates that roots with a narrow ASR will branch at a greater depth than roots with a wide ASR. In early growth stages, genotypes with a narrow ASR tend to grow deeper under water deficit conditions compared to those with a wider ASR. SDM and RDM/SDM were statistically significantly negatively correlated, similarly to the study of Chen *et al.* (2021). This confirms the fact that the degree of decrease in stem growth due to drought stress was greater than that of roots, which affected a greater reduction of above-ground biomass compared to below-ground biomass and led to an increase in the root-to-stem ratio. SDM and RDM/SDM correlations were not statistically significant in the absence of drought stress, thus confirming that drought stress significantly affects the redistribution of assimilates between roots and stems. NSR and PRL were also statistically significantly negatively correlated. Genotypes with a longer primary root had a lower NSR, while genotypes with a shorter primary root had a higher NSR. Although longer root systems with a narrow ASR are thought to be vital for drought resistance in wheat, Khadka *et al.* (2020) reported that root systems with a great NSR in the root crown, located close to the soil surface, are more drought-tolerant, in the early stages of growth.

Principal component analysis of RSA and stem traits and stress-tolerance indices: All examined traits except NSR were positively associated with the STI index. The strongest positive association with this index was shown by the traits: SDM, PRL, TSRL, SL (Figure 1).

Zhu *et al*. (2019) found significant (P < 0.01) positive correlation between grain yield (GY) and PSRL, between GY and TSRL, significant (P < 0.01) negative correlation between GY and NSR, and between GY and ASR. A positive association between the STI index and PRL indicated its importance for assessing drought stress intensity. Xie *et al*. (2017) found that yield components (number of grains per unit area, number of grains per ear, and plant biomass) were lower in wheat genotypes with shorter PRL, while lines with longer PRL had the highest yield and biomass. Jain *et al.* (2014) found a significant positive correlation between the STI index and RDM, and indicated that root biomass and its components have a positive correlation with grain yield under drought conditions. They showed that, as yield is a complex trait, selection based only on yield in drought conditions will not lead to an increase in yield. However, selection based on RDM, combined with above-ground traits, such as SDM, under severe drought stress, will improve the efficiency of selection for drought tolerance. In contrast to the STI index, the SSI index had negative associations with all tested traits, except NSR. Taking into account both stress indices and their associations with RSA and stem traits, preference could be given to the STI index. Mohammadijoo *et al.* (2015) showed that the STI index had more advantages for the selection of superior genotypes in variable environmental conditions, when dry and favorable years alternate.

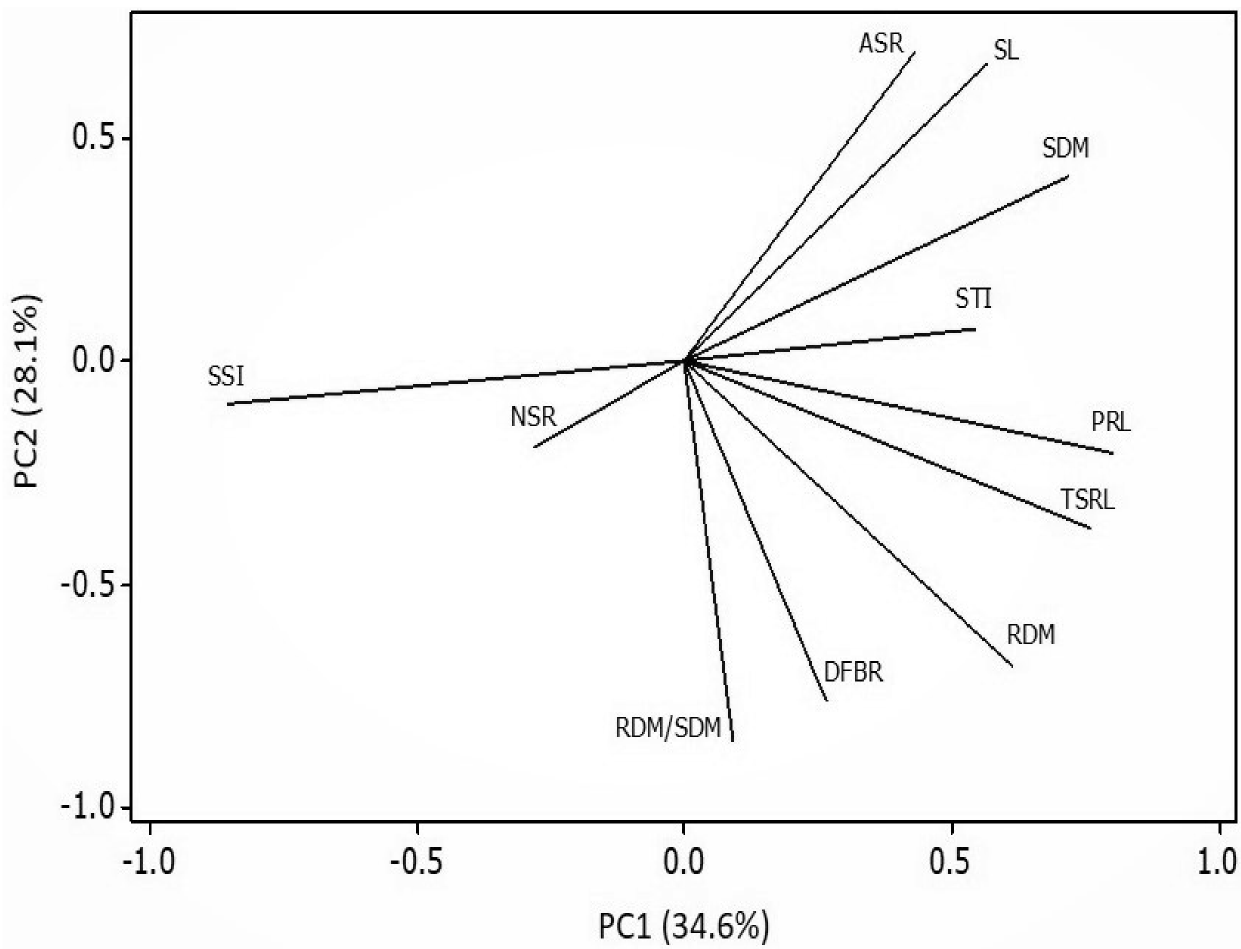

Figure 1. Principal component analysis of associations between stress indices and RSA and stem traits under PEG-induced drought stress. Legend: SSI-stress susceptibility index, STI-stress tolerance index, PRL-primary root length, DFBR-distance to the first branch on the primary root, NSR-number of seminal roots, TSRL-total seminal root length, ASR-angle of seminal roots, SL-stem length, RDM-root dry mass, SDM-stem dry mass, RDM/SDM-the ratio of root dry mass to stem dry mass.

Table 4. Heterosis (%) evaluated for wheat seedlings of F_1 offspring from eight crosses for RSA and stem traits: drought stress vs. control.

Cross	Trait														
	PRL (cm)		DFBR (cm)		NSR		TSRL (cm)		ASR (˚)*	SL (cm)		RDM (mg)		SDM (mg)	
	Contr.	PEG	Contr.	PEG	Contr.	PEG	Contr.	PEG	Contr.	Contr.	PEG	Contr.	PEG	Contr.	PEG
1.	10.85	10.04	-27.20	1.64	-8.47	0.00	-8.51	-1.07	13.16	3.13	5.85	-4.29	6.35	3.11	12.95
2.	2.98	10.48	-32.20	2.59	-16.13	-24.10	0.40	6.81	16.75	2.88	11.11	-14.30	4.13	-2.47	13.15
3.	16.81	46.89	6.25	61.45	-5.88	-25.00	20.29	30.99	2.04	1.70	10.26	-8.53	9.91	-7.16	10.71
4.	-0.72	7.57	-43.45	-14.2	-16.36	-19.30	-11.50	-4.94	28.05	8.07	22.08	-0.84	7.14	-11.40	-0.98
5.	13.51	52.44	-30.10	19.50	0.00	-19.30	8.16	9.04	11.67	-4.01	11.00	-2.48	27.50	-21.20	-8.59
6.	12.93	16.59	-38.30	-8.99	-13.43	-22.60	1.70	4.35	4.50	6.36	13.87	-0.80	11.50	-9.54	-1.65
7.	3.85	26.92	-46.48	-0.44	-11.76	-1.64	-2.41	9.54	25.15	4.86	22.99	-15.20	6.56	-2.25	9.46
8.	17.03	25.47	-37.20	3.42	4.61	0.00	9.92	17.02	20.75	9.61	27.76	-8.72	1.47	-3.07	14.21
Mean	9.65	24.55	-31.10	8.12	-8.43	-13.99	2.26	8.97	15.26	4.07	15.62	-6.90	9.32	-6.75	6.16

[a]Contr.-Control; PEG-induced drought stress by applying PEG treatment; *Angle of seminal roots was measured only in control conditions; PRL-primary root length, DFBR-distance to the first branch on the primary root, NSR-number of seminal roots, TSRL-total seminal root length, ASR-angle of seminal roots, SL-stem length, RDM-root dry mass, SDM-stem dry mass, RDM/SDM-the ratio root dry mass to stem dry mass.

Heterosis for RSA and stem traits of F_1 offspring from different crosses: drought stress vs. control conditions: Based on the results from the first phase of testing seedlings of the 101 wheat genotype, the best genotypes were selected according to several criteria for crossing (Table 1). The idea was to combine or accumulate as many positive alleles for root and stem traits as possible, in one genotype, by targeted crossing, in order to select lines with better early growth in the early generations. The estimated values of heterosis in the eight F_1 progeny resulting from the crossing of selected parental genotypes for RSA and stem traits of wheat seedlings in control conditions and under induced drought stress are shown in Table 4.

Control Conditions: Looking at all investigated RSA and stem traits, four out of eight traits showed a positive average value of heterosis (the average of all eight crosses): PRL, TSRL, ASR, and SL. A negative average value of heterosis was determined for DFBR, NSR, RDM, and SDM. Most traits had an average value of heterosis less than 10%, except ASR (15.3%) and DFBR (-31.1%), which also represented the highest average positive value of heterosis and the highest average negative value of heterosis, respectively. On average, the F_1 progeny obtained from crossing the selected parental genotypes had a shorter DFBR, i.e., shallower branching depth of the root system, and a higher ASR, i.e., spread root systems. The highest values of both positive and negative heterosis in control conditions were determined by traits that describe the architecture of the root system. The root system with a wider and shallower root system is more suitable for the conditions of intermittent rainfall, when it occurs during the spring period of the year, which can absorb surface water that quickly drains into the deeper layers of the soil (Pais *et al*., 2022). Negative heterosis for DFBR cannot be considered desirable, given that greater branching depth and a greater number of seminal roots contribute to a better overall uptake of water from the soil in optimal conditions, and especially under drought stress conditions (Zhu *et al*., 2019). From all crosses in control conditions, two combinations were singled out that had the highest heterosis values, both positive and negative, for several traits. It can be said that those two crossing combinations stood out as antagonistic in terms of RSA. The first, Phoenix × NS 40S, showed the highest positive heterosis value for ASR, but also the only negative heterosis value for PRL. It had the highest negative heterosis values for DFBR and NSR. The progeny from this combination had a shorter PRL, a smaller depth of root system branching, a smaller number of NSR, and a wider ASR. Thus, this crossbreeding combination can be considered superior in terms of RSA and adaptability for surface water extraction in conditions of intermittent rainfall.

However, for conditions where the absorption of deeply stored water is required, the offspring from this cross will not be heterotic, i.e., they will be more sensitive to stress. In contrast to this, the combination Pobeda × Donska had the lowest heterosis value for SL, the highest heterosis values for PRL and TSRL, and was the only one with a positive heterosis value for DFBR. It had the lowest heterosis value for ASR. The offspring from this combination had a shorter stem, but a longer primary root, narrower and longer seminal roots, and in greater numbers, a more favorable architecture of the root system for the extraction of deeply stored water, when adverse conditions due to drought occur. It has been shown that selection for deeper root systems can significantly improve water and nitrogen uptake (Ahmed *et al*. 2019). Higher NSR and narrow ASR were considered reference traits for selection at early growth stages in wheat breeding programs (Wasaya *et al*., 2018).

Drought stress: For most of the RSA and stem traits, the heterosis values were higher in the conditions of induced drought stress than in the conditions without applied PEG treatment. For all traits, with the exception of NSR, a positive average value of heterosis was determined. The highest average values of heterosis were shown for PRL (24.6%) and SL (15.6%), while the lowest average values of heterosis were shown for SDM (6.2%). The resulting F_1 progeny had, on average, a longer primary root and a longer stem compared to their parents under drought stress conditions. As root length in the seedling stage is a key feature for increasing adaptability in dry environmental conditions, especially in regions with terminal drought and unused subsoil moisture (Bapela *et al*., 2022; Hussain *et al*., 2019; Li *et al*., 2019), the obtained F_1 progeny was heterotic in terms of early vigor (strength and speed of early growth) under conditions of drought stress. For PRL, positive heterosis was established in all crossing combinations. For DFBR, five out of eight combinations had positive heterosis values, and three combinations had negative values. Positive values of heterosis were below average, except in the case of the combination Pobeda × Donska, where the estimated value of heterosis for DFBR was very high (61.4%). It was also the highest value of heterosis assessed in control conditions and under induced drought stress. The progeny from this combination can be considered superior in terms of the depth at which the root system begins to branch, which is a desirable trait in drought conditions. The only trait for which the estimated average value of heterosis was negative under drought stress conditions is NSR. The average estimated value of heterosis for TSRL was positive but low, less than 10%. A high value of heterosis for TSRL was estimated in the combination Pobeda × Donska (31%), and this combination can be considered superior for this trait in the conditions of induced drought stress. After PRL, the second trait with the highest average value of heterosis in conditions of drought stress was SL (15.6%). Heterosis values for SL in conditions of drought stress were higher in all crossing combinations compared to conditions without applied treatment, and we can conclude that heterotic offspring with better above-ground growth in conditions of drought stress are expected. The best crossbreeding combination for RDM was Pobeda × Brigant, whose heterosis value was 27.5%, while for SDM it was WWBMC2 × Ingenio (14.2%). If we compare the studied traits with heterosis for yield, where research in the 1980s and 1990s showed that the best wheat hybrids had a heterosis of 13.5% for yield (Gimenez *et al*., 2021), then we can establish that the heterosis values for the studied traits in our investigation were relatively high, especially in drought stress conditions. The best crosses to single out were Pobeda × Donska and WWBMC2 × Ingenio. They had significant heterotic effects both in conditions without and with PEG treatment. The combination of Pobeda × Donska showed a significant level of heterosis for most of the investigated root traits, especially under conditions of drought stress, and WWBMC2 × Ingenio for PRL and SL. By crossing the F_1 offspring of these two combinations, the accumulation of positive alleles in one genotype could be carried out further. Such complex crosses could produce desirable transgressive segregants for the development of drought-tolerant genotypes.

Conclusion: All the mean values of the studied RSA and stem traits in wheat seedlings of parental and F1 generation from different crosses were lower under PEG treatment compared to control, except root dry mass/stem dry mass. The most sensitive trait to induced drought stress was distance to the first branch on the primary root, in terms of its highest decrease. This indicated that induced drought stress caused root systems to branch at a shallower depth. A strong positive association with the stress tolerance index showed stem dry mass, primary root length, total seminal root length, and stem length. The differential responses of wheat genotypes in P and F_1 generations from different crosses at the seedling stage to induced drought stress vs. control conditions, pointed to the most important RSA and stem traits, with the highest heterosis values, contributing to drought tolerance. The highest mean values of heterosis under drought stress were shown for primary root length and stem length. The crosses Pobeda × Donska and WWBMC2 × Ingenio had significant heterotic effects in both conditions, which distinguished them as potential starting material in the further selection process for drought tolerance. Selected traits as criteria for selection and favorable combinations can be incorporated into pre-breeding and breeding schemes, directly or indirectly, aiming to achieve drought tolerance.

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Authors' Contributions: MB conceived experiment, measured traits, conducted statistical analysis. DD choose experiment design, provide resources, supervised experiment. VK provide resources and measured traits. GB conceptualized and wrote manuscript. TŽ supervised experiment and analyses.

REFERENCES

Ahmad, M., G. Shabbir, N.M. Minhas and M.K.N. Shah (2013). Identification of drought tolerant wheat genotypes based on seedling traits. Sarhad J. Agric. 29: 21-27.

Ahmed, H.G.M.D., M. Sajjad, M. Li, M.A. Azmat, M. Rizwan, R.H. Maqsood and S.H. Khan (2019). Selection criteria for drought-tolerant bread wheat genotypes at seedling stage. Sustainability 2019(11): 2584. Doi: 10.3390/su11092584.

Bapela, T., H. Shimelis, T.J. Tsilo and I. Mathew (2022). Genetic improvement of wheat for drought tolerance: progress, challenges and opportunities. Plants (Basel) 2022(11): 1331. Doi: 10.3390/plants11101331.

Boudiar, R., A.M. Casas, T. Gioia, F. Fiorani, K.A. Nagel and E. Igartua (2020). Effects of low water awailability on root placement and shoot development in landraces and modern barley cultivars. Agron. 2020(10): 134. Doi: 10.3390/agronomy10010134.

Chen, Y., J. Palta, P.V.V. Prasad and K.H.M. Siddique (2020). Phenotypic variability in bread wheat root systems at the early vegetative stage. BMC Plant Biol. 20: 185. Doi: 10.1186/s12870-020-02390-8.

Chen, X., Y. Zhu, Y. Ding, R. Pan, W. Shen, X. Yu and F. Xiong (2021). The relationship between characteristics of root morphology and grain filling in wheat under drought stress. PeerJ 9: 12015. Doi: 10.7717/peerj.12015.

Farooq, M., M. Hussain and K.H.M. Siddique (2014). Drought stress in wheat during flowering and grain-filling periods. Cri. Rev. Plant Sci. 33(4): 331-349. Doi: 10.1080/07352689.2014.875291.

Farooq, S., M. Shahid, M.B. Khan, M. Hussain and M. Farooq (2015). Improving the productivity of bread wheat by good management practices under terminal drought. J. Agron. Crop Sci. 201(3): 173-188. Doi: 10.1111/jac.12093.

Farooq, S., M. Hussain, K. Jabran, W. Hassan, M.S. Rizwan and T.A. Yasir (2017). Osmopriming with CaCl2 improves wheat (*Triticum aestivum* L.) production under water-limited environments. Environ. Sci. Poll. Res. 24(15): 13638-13649. Doi: 10.1007/s11356-017-8957-x.

Fang, Y., Y. Du, J. Wang, A. Wu, S. Qiao, B. Xu, S. Zhang, K.H.M. Siddique and Y. Chen (2017). Moderate drought stress affected root growth and grain yield in old, modern and newly released cultivars of winter wheat. Front. Plant Sci. 8: 672. Doi: 10.3389/fpls.2017.00672.

Fernandes, R.C., V.F. de Oliveira, L.D. Schroeder, A.P. de Jesus, C. Busanello, M. Ferreira Santos, V.E. Viana, A.C. de Oliveira and C. Pegoraro (2020). Morphologically accessing wheat genotypes in response to drought on early stages of development. Commun. Plant Sci. 10(2020): 105-113. Doi: 10.26814/cps2020014.

Gimenez, K., P. Blanc, O. Argillier, J.B. Pierre, J. Le Gouis and E. Paux (2021). Dissecting bread wheat heterosis through the integration of agronomic and physiological traits. Biology (Basel) 10(9): 907. Doi: 10.3390/biology10090907.

Hussain, M., M. Waqas-ul-Haq, S. Farooq, K. Jabran and M. Farooq (2016). The impact of seed priming and row spacing on the productivity of different cultivars of irrigated wheat under early season drought. Exp. Agric. 52(3): 477-490. Doi:10.1017/S0014479716000053.

Hussain, M., A. Latif, W. Hassan, S. Farooq, S. Hussain, S. Ahmad and A. Nawaz (2019). Maize hybrids with well-developed root system perform better under deficit supplemental irrigation. Soil Environ. 38(2): 203-213. Doi: 10.25252/SE/19/61578.

Jain, N., G.P. Singh, R. Yadav, R. Pandey, P. Ramya, M.B. Shine, V.C. Pandey, N. Rai, J. Jha and K.V. Prabhu (2014). Root trait characteristics and genotypic response in wheat under different water regimes. Cereal Res. Commun. 42: 426-438. Doi: 10.1556/CRC.42.2014.3.6.

Johnson, S.N., Z.H. Chen, R.C. Rowe and D.T. Tissue (2022). Field application of silicon alleviates drought stress and improves water use efficiency in wheat. Front. Plant Sci. 13: 1030620. Doi: 10.3389/fpls.2022.1030620.

Khadka, K., M.N. Raizada and A. Navabi (2020). Recent progress in germplasm evaluation and gene mapping to enable breeding of drought-tolerant wheat. Front. Plant Sci. 2020(11): 1149. Doi: 10.3389/fpls.2020.01149.

Li, X., C.H. Ingvordsen, M. Weiss, G.J. Rebetzke, A.G. Condon, R.A. James and R.A. Richards (2019). Deeper roots associated with cooler canopies, higher normalized difference vegetation index, and greater yield in three wheat populations grown on stored soil water. J. Exp. Bot. 70(18): 4963-4974. Doi: 10.1093/jxb/erz232.

Maqbool, S., S. Ahmad, Z. Kainat, M.I. Khan, A. Maqbool, M.A. Hassan, A. Rasheed and Z. He (2022). Root system architecture of historical spring wheat cultivars is associated with alleles and transcripts of major functional genes. BMC Plant Biol. 22: 590. Doi: 10.1186/s12870-022-03937-7.

Minitab Statistical Software, (2021). Release 16 for Windows. Minitab Inc.; State College (USA). Available at: https://www.minitab.com.

Mohammadijoo, S., A. Mirasi, R. Saeidi-Aboeshaghi and M. Amiri (2015). Evaluation of bread wheat (*Triticum aestivum* L.) genotypes based on resistance indices under field conditions. Int. J. Biosci. 6: 331-337. Doi: 10.12692/ijb/6.2.331-337.

Ober, E.S., S. Alahmad, J. Cockram, C. Forestan, L.T. Hickey, J. Kant, M. Maccaferri, E. Marr, M. Milner, F. Pinto, C. Rambla, M. Reynolds, S. Salvi, G. Sciara, R.J. Snowdon, P. Thomelin, R. Tuberosa, C. Uauy, K.P. Voss-Fels, E. Wallington and M. Watt (2021). Wheat root systems as a breeding target for climate resilience. Theor. Appl. Genet. 134(6): 1645-1662. Doi: 10.1007/s00122-021-03819-w.

Pais, I., R. Moreira, J.N. Semedo, F.H. Reboredo, F.C. Lidon, J. Coutinho, B. Maçãs and P. Scotti-Campos (2022). Phenotypic diversity of seminal root traits in bread wheat germplasm from different origins. Plants (Basel) 11(21): 2842. Doi: 10.3390/plants11212842.

Peršić, V., A. Ament, J. Antunović Dunić, G. Drezner and V. Cesar (2022). PEG-induced physiological drought for screening winter wheat genotypes sensitivity – integrated biochemical and chlorophyll a fluorescence analysis. Front. Plant Sci. 13: 987702. Doi: 10.3389/fpls.2022.987702.

Rasband, W. S. (2020). ImageJ. National Institutes of Health; Maryland (USA). Retrieved on January 01, 2021 from: https://imagej.nih.gov/ij/.

Sánchez-Reinoso, A.D., G.A. Ligarreto-Moreno and H. Restrepo-Díaz (2020). Evaluation of drought indices to identify tolerant genotypes in common bean bush (*Phaseolus vulgaris* L.). J. Integr. Agric. 19(1): 99-107. Doi: 10.1016/S2095-3119(19)62620-1.

Wan, C., P. Dang, L. Gao, J. Wang, J. Tao, X. Qin, B. Feng and J. Gao. (2022). How does the environment affect wheat yield and protein content response to drought? A meta-analysis. Front. Plant Sci. 13: 896985. Doi: 10.3389/fpls.2022.896985.

Wasaya, A., X. Zhang, Q. Fang and Z. Yan (2018). Root phenotyping for drought tolerance: A review.

Agron. 8(11): 241. Doi: 10.3390/agronomy8110241.

Weiner, J., Y.L. Du, C. Zhang, X.L. Qin and F.M. Li (2017). Evolutionary agroecology: Individual fitness and population yield in wheat (*Triticum aestivum*). Ecol. 98(9): 2261-2266. Doi: 10.1002/ecy.1934.

Xie Q., K.M.C. Fernando, S. Mayes and D.L. Sparkes (2017). Identifying seedling root architectural traits associated with yield and yield components in wheat. Ann. Bot. 119(7): 1115-1129. Doi: 10.1093/aob/mcx001.

Zhao Z., G.J. Rebetzke, B. Zheng, S.C. Chapman and E. Wang (2019). Modelling impact of early vigour on wheat yield in dryland regions. J. Exp. Bot. 70(9): 2535-2548. Doi: 10.1093/jxb/erz069.

Zhu Y.H., J. Weiner, M.X. Yu and F.M. Li (2019). Evolutionary agroecology: Trends in root architecture during wheat breeding. Evol. Appl. 12(4): 733-743. Doi: 10.1111/eva.12749.