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RESEARCH ARTICLE

Exploring the variability of root system architecture under drought stress in heat-tolerant spring-wheat lines

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Abstract

Background and aims Global wheat production is under threat due to climate change, specifically heat and drought and their combination. This study aims to address the root trait responses of heat-tolerant wheat genotypes to drought.

Methods The variability in root traits of CIMMYT wheat lines, which were previously developed for heat stress tolerance (HTWL), was evaluated alongside 10 Pakistani-approved varieties under three cultivation conditions and soil moisture levels.

Results Our findings revealed that the plasticity of the wheat root system is highly pronounced, with rhizosphere conditions exerting a more substantial influence (5–49%) than the genotypic response (1–14%). Furthermore, in the hydroponic and pot system, we noted higher maximum-root length (1.5–1.8 fold) and root-to-shoot ratio (3.4–10.6 fold) as compared to field condition, while the root biomass was substantially higher in the field trial (3-57 fold). Nonetheless, persistent drought conditions exerted contrasting impact with reduction in most of the traits except specific root length and harvest index which were increased under drought.

Conclusions The variation in root traits against drought indicates the potential for the development of improved genotypes that can withstand multiple stresses. Furthermore, it is crucial to consider rhizosphere conditions when selecting genotypes, as the plasticity of wheat roots may lead to misinterpretations if rhizosphere conditions are disregarded. Root dry weight and root-to-shoot ratio are more stable traits as compared to maximum root length and specific root length. It is recommended to evaluate a

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broader range of rhizosphere conditions to select tolerant genotypes.

Keywords Root traits · Field trial · Pots · Hydroponics · Moisture-stress

Introduction

Wheat is the utmost essential and largest cereal crop from a food security point of view and it is cultivated under almost all ecological zones of Pakistan and worldwide. Climate change threatens wheat production with an estimated 16% reduction in the next three decades, especially in the semi-arid tropics of Asia where it is consumed daily as a staple food (Pequeno et al. 2021). This susceptibility to harsh weather conditions has long been observed in wheat. Drought is the primary factor but not only the player in climate change, both heat and drought are coexisting factors adversely influencing yield and productivity, contingent upon the plant's growth stage and the severity of the stress (Daryanto et al. 2016; Bakhshandeh et al. 2019; Ciais et al. 2005). Major wheat growing area of the world is affected by both stresses where heat and drought events occur simultaneously causing yield losses of up to 69% and 86% (Prasad et al. 2011; Tricker et al. 2018). Drought and higher temperatures than 30°C at later developing stages, especially at spike differentiation and grain filling stage in wheat reduce the final yield (Barnabás et al. 2008; Ferris et al. 1998). Higher temperature also reduces the downward partitioning that affects the root development by reducing the root number, thickness and length (Batts et al. 1998). Combined stress of both factors: heat and drought have even more detrimental effect that increases the electrolyte leakage reduce the relative water content (Grigorova et al. 2011), seminal roots during germination (Fábián et al. 2008), and wheat grain yield to 50% (Mondal et al. 2015). In a review on physiological and genetic bases for combined drought and heat stress, various phenotypic traits and their qualitative trait loci were discussed. Most of the common traits affected by the combined stress were the phonological, shoot architectural and yield attributes, while root traits were only reported related to solo-drought stress. Traits playing role in tolerance are often in contrast between drought and heat stress such as cool canopy vs stomatal closure and transpiration efficiency vs water use efficiency (Tricker et al. 2018), therefore, the genetic and phenotypic bases are likely to be different for combined stress than either of individual stress.

Despite the significant adverse impact of heat and drought on wheat growth, metabolism and yield, breeding has mainly been focused on individual stress while the combined stress has rarely been addressed (Bakhshandeh et al. 2019; Mondal et al. 2015; Zahra et al. 2021). Heat avoidance such as canopy cooling is a trait obtained by increasing stomatal conductance and water uptake from the ground (Amani et al. 1996). Excessive and deep root growth may help to uptake the required amount of water for canopy cooling (Lopes and Reynolds 2010). The genotypes with cooler canopy traits exhibited a deeper root system, under water stress, enabling them to extract 35% more water from the soil profile at depths of 30–90 cm. Conversely, in response to heat stress, these genotypes concentrated more roots near the surface, where water was more readily available from surface irrigation. Given the superior agronomic performance of these genotypes, it can be inferred that their QTL are linked to an optimal distribution of roots, aligning with the availability of water during specific stress (Pinto and Reynolds 2015). Multiple stress conditions such as drought, salt and heat have additive effects on crops. Previously, barley and rice accessions have been evaluated against drought and salt stress (Kaur et al. 2020; Manju et al. 2023; Sonia et al. 2023).

The use of novel genetic resources and breeding for heat and drought resistance are the most viable strategies to cope with the changing climatic conditions (Mwadzingeni et al. 2016; Pequeno et al. 2021). However, due to the limited availability of resistance sources, progress in breeding drought-tolerant cultivars is not satisfactory yet. As a consequence, many crop improvement programs have prioritized the development of wheat genotypes with the primary objectives of enhancing adaptation to water stress conditions and achieving higher yields. In the breeding history of wheat, another aspect that have mostly been ignored by the plant breeders are the contribution of the underground part of a plant and its association with other aboveground traits against abiotic stresses such as root system architecture and length under drought-affected regimes. Roots are vital for water procurement, uptake of supplements and can be focused on improving plant profitability under a wide scope of developing environments. Various studies have acknowledged relationships between root characteristics and crop productivity which includes soil properties, rendition under drought and grain yield (Liu et al. 2022; Paez-Garcia et al. 2015). Several root traits have been identified for future plant breeding research against drought. Improving root traits is the most vital aspect for continuing crop yield under water stress environments (Comas et al. 2013; Liu et al. 2021). Root traits contributing to plant stand under drought conditions include root length to fetch water from deeper moisture zones, diameter of fine roots for water retention and active root growth for water availability (Comas et al. 2013; Xiong et al. 2006).

Investigating plant roots presents a unique challenge that demands specialized tools, making it a labor-intensive and time-consuming endeavor compared to studying other plant tissues. Additionally, the complexity of root structures and their interactions with the surrounding soil further complicates the process, necessitating careful handling and precise methodologies. Different cultivation techniques facilitate specific root measurements which are not possible in the field. Moreover, techniques such as hydroponics and pot systems as compared to field conditions also affect the root growth and development probably due to variations in penetration resistance (Colombi et al. 2019). In recent years root traits have received much attention therefore, several techniques have been used for root studies at an early vegetative stage such as hydroponic, ebb flow system, agar plates, grown in plastic tubes and soil-filled rhizoboxes (Becker et al. 2016; Chen et al. 2020; Colombi et al. 2019; Fang et al. 2017; Liu et al. 2021; Shah et al. 2015a, 2015b; Singh Grewal 2010; Zhu et al. 2011). Root trait studies at the full vegetative growth stage are only possible either in bigger pots or in the field-grown plants. Extraction of wheat roots grown in the field is quite burdensome, tiresome, and seems laborious (Severini et al. 2020), in contrast, isolation and handling of wheat roots without damage is quite easier under hydroponic culture but may not be ecologically comparable to the field (Girdthai et al. 2010; Zabłudowska et al. 2009).

Limited information exists regarding the variability of root traits considering the multiple stresses such as heat and drought stress. This study represents the responses of root traits in CIMMYT-HTWL lines (Singh et al. 2019) to different soil moisture levels and compare various cultivation conditions. Establishing connections between root traits and above-ground traits under drought stress will provide valuable insights for the breeders aiming to enhance heat and drought tolerance in wheat by combining different desirable traits.

Materials and methods

Germplasm

The genotypes included: 49 CIMMYT wheat lines (C2-C50) of CIMMYT-HTWT (Singh et al., 2019) and 10 approved Pakistani varieties used as check including V1 (Shalkot-14), V2 (Tijaban-10), V3 (Zardana), V4 (Faisalabad-208), V5 (Ujala Faisalabad), V6 (Zarlashta), V7 (Sarsabz), V8 (Zarghoon), V9 (LS-101), V10 (Rasko). The seeds were received from Ayub Agricultural Research Institute Faisalabad, Nuclear Institute of Agriculture Tandojam, Agricultural Research Institute, Quetta and Arid-zone Research Center, Quetta. The seeds of the respective lines were stored in the seed bank of the Department of Plant Breeding and Genetics, Lasbela University of Agriculture Water & Marine Sciences.

Experiments undertaken

The study was comprised of two experiments: *Experiment-1* was performed to compare the three culture conditions and *Experiment-2* against moisture stress conditions to assess genetic variability- and impact of drought. The experiments were performed at the experimental site of the Department of Plant Breeding and Genetics, Lasbela University of Agriculture Water Marine Sciences, Uthal, Balochistan.

Experiment-1

The concerned experiment was conducted under three culture conditions: Hydroponic system, Pot system and Field condition as Treatments. The plants were grown for four consecutive weeks of germination while the plants for the Pot system and Field conditions were grown till anthesis before harvesting for root and shoot measurements. Normalized data was used to compare the three culture conditions.

For the hydroponic system, plants were grown under controlled growth conditions at room temperature (20–25°C) with light and dark periods of 16 and 8 h. Seeds were sterilized with 20% diluted solution of chlorine for 20 min followed by three times rinsing with sterile water. Glass wool was used in this experiment to sustain moisture surrounding the seeds. Seeds were kept in water-soaked moist glass-wool plugs (IKEA Sweden) and wrapped with plastic foil for five days until seedlings emerged. Then, the seeds trays were unwrapped, and each tray (containing 50 plants) were placed on their respective containers. The distilled water zone of hydroponic tanks was continuously aerated with an aquarium air pump. Blomstra as plant growth medium (500mL water with 1mL media) was supplemented to each hydroponic container only once after one week of germination. The chemical composition of "Blomstra" constitutes of Nitrogen (N) 2.5g (Nitrate–N:1.5g; Amonium-N:1.0g), Phosphorus (P) 0.5g, Potassium (K) 2.1g and balanced micronutrients per 100ml.

For the pot system, wheat seeds of each genotype were sown in polythene bags, containing five kg sandy loam soil used as pots, which were wrapped with two bottom perforated polythene bags to maintain moisture, to promote aeration and drainage of excessive water. Similarly the field trials were conducted on the same soil type. Water was supplied to both pot and field trial on every alternative week. Both pots and field trials were supplemented with urea, di-ammonium phosphate (DAP), and potassium sulphate (SOP) at the rate of 150, 100, and 140 kg as a source of N, P_2O_5 and K_2O ha^{-1} respectively. The experiment was carried out in a controlled manner with a Completely Randomized Design (CRD) with three biological replicates.

Experiment-2

To evaluate the wheat genotypes against water availability, a field experiment was performed at the Department of Plant Breeding and Genetics, Lasbela University of Agriculture, Water & Marine Sciences, Balochistan (GPS coordinates:25.842981, 66.626487). The concerned experiment was carried out in the field with two factorial (RCBD) Randomized Complete Block Design with three biological replicates during the Rabi season, 2018–19. Seeds were sown at the rate of 125 kg seed ha^{-1} with four rows through hand drill with a 10cm distance kept between rows as well as plants. The distance between the sub-plots was kept as 30cm. Individual plots were fertilized with urea, DAP, and SOP at the rate of 150, 100, and 140 kg as a source N, P_2O_5 and K_2O ha^{-1} respectively.

Drought treatments

Two applications of moisture stress were designed as treatments along with a well-watered as control. The field selected for this experiment has a gradient in moisture conservation towards the river bank. Three sites were selected with varying water-holding capacity along the gradient. The control (C) treatment was designed with 12 ± 2% water retention capacity of field with full irrigation, Moisture-stress-1 (D1) with 12 ± 2% water retention with drought stress at spike initiation to anthesis stage, and Moisture-stress-2 (D2) with 6 ± 1% water retention capacity of field and drought stress at spike initiation to anthesis stage.

Root-sampling and image analysis

Root samples were taken from *Experiment-2* after irrigation at the anthesis stage. Roots were dug out with soil with the help of a spade at 30cm depth and washed by placing the soil block in water. The soil around the roots was carefully removed and roots were washed and surface dried with a paper towel. Root images were analysed through software Wang and Zhang (2009) and the measurements were taken on root-diameter, root surface area density, and root length density (Himmelbauer et al. 2004; Kalhoro et al. 2018).

Statistical analysis

For all the experiments, analysis of variance (ANOVA) and coefficient of variance (CV) were followed for an appropriate and systematic breakdown of findings of the research that was carried out for examining morphological parameters using Statistix

8.1 (USA). Fisher's analysis of variance method at 1% and 5% probability levels were used to test the significant differences among treatments, genotypes and between their interactions (Steel et al. 1997) along with Pearson correlation coefficient analysis to assess the association among various parameters. Multivariate analysis was performed through BioVinci software and Ward Method using the R programme and JNP software. Ranking of the genotypes for root traits is shown as bar graphs of top and least the then genotypes using MS-excel. Selected genotypes with top and least ten genotypes under well-watered conditions were selected for detailed analysis of roots and yield traits in response to moisture stress and the plots were generated using Origin software. Genetic variance (δ^2g)was calculated by following the method of (Kown and Torrie, 1964) given below as:

$$\delta^2 g = \frac{\mathrm{MSg} - \mathrm{MSe}}{\mathrm{r}}$$

whereas, the heritability (h^2_{BS}) was assessed according to Hanson et al. (1956) which is given as under:

$$\mathrm{h}^2\mathrm{BS} = \frac{\delta 2\mathrm{g}}{\delta 2\mathrm{p}} \times 100$$

Results

The results obtained from various phenotypic traits were used for multivariate analysis. The results on root parameters from three different culture conditions indicated a clear shift in root dry weight to specific root length as contrasting parameters. Under the hydroponic system, root length was enhanced while higher root weight was obtained under field conditions. Similarly, hierarchical clustering and XY plot of the data showed three major clusters based on cultivation systems (Fig. 1A and B). Significant differences were observed for root attributes when grown under different culture conditions. The Root-to-shoot-ratio was also higher in the hydroponic system followed by pot system as compared to the field condition. Under the hydroponic culture, root length was higher and was associated with root fresh weight. By concluding experiment one, it is revealed that hydroponically grown seedlings with large root systems can be a very local response to the resistance free rhizosphere environment.

It was expected that genotypes with deeper rooting length have the potential to contribute towards yield traits under drought. We have observed that, the same genotypes produced a lower root length when grown under field conditions or the moisture stress treatment and were clustered separately according to the culture conditions (Fig. 1A and B). In contrast, under hydroponic and pot system, roots were overgrown. Comparing the genotypes under different culture conditions, we have found that the hydroponic and pot system cannot be the representative of field conditions (Fig. S. 1A and B). Therefore, for the drought experiment, field conditions were used and the samples for root trait measurements were taken at the anthesis stage.

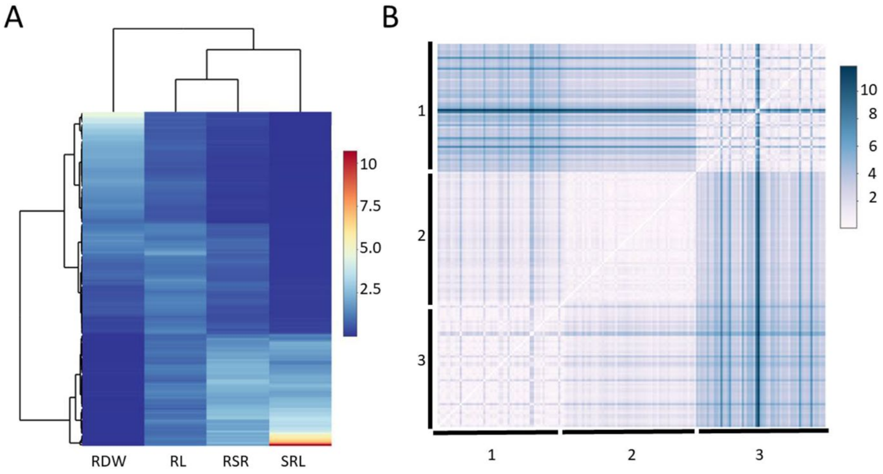


Fig. 1 Root traits of wheat genotypes under different culture conditions. Hierarchical cluster analysis (**A**) and XY plot (**B**). Root traits:Root dry weight (RDW), maximum root length (RL), root-to-shoot ratio (RSR) and specific root length (SRL) are presented on X axis while culture conditions and genotypes are presented on Y axis

Analysis of variance (ANOVA) and Correlation

In the hydroponic experiment, maximum root length was significantly ($P < 0.01$) correlated with root-to-shoot ratio (r = 0.31**), however root dry weight showed a negative correlation with root length (r = -0.73**) and root-to-shoot ratio (r = -0.41**). Under field conditions, significant differences were observed among all studied plant traits at ($P < 0.01$) probability level (Table 1).

Furthermore, in a field experiment, results on correlation coefficient (Table 2) indicated a significant association for maximum root-length with root-shoot ratio, root dry weight, plant height, number of spikes plant^{-1}, biological yield, and grain yield ($P < 0.01$). Moreover, root dry weight showed a significant relationship with root-to-shoot ratio, plant height, spike length, grain yield, and biological yield ($P < 0.01$) while the association with flag leaf area, thousand-grain weight, and number of grains spike^{-1} was significant at $P < 0.05$. However, root to shoot ratio indicated a negative relationship with biological yield, grain yield, thousand-grain weight, plant height, and flag leaf area whereas showed a positive correlation only with harvest index. Similarly, the two-way hierarchical clusters of individual treatments for wheat lines and the traits shown in Fig. S. 2, have reflected the results. Genetic diversity permitted the estimation of genetic variation and heritability for the studied root traits as presented in Table 3. Yield components such as number of grains plant^{-1} or thousand grain weight are the well-known heritable traits. In our study, broad-sense heritability of root traits was observed in the same range as other above ground traits.

Higher heritability of root traits across the three field conditions indicates that the root traits are under strong genetic control. Under the moisture stress-1 (D1) condition, a decrease in variance and heritability was observed in all of the traits. Genetic variance of maximum root-length and root-to-shoot ratio was decreased by 22 and 9% and heritability by 23 and 6% respectively.

In addition, genetic correlation (Table 4) indicated a significant association for root-to-shoot ratio with root dry weight and harvest index whereas a negative relationship was noted with number of grain plant^{-1} and biological yield ($P < 0.01$). However, root length was associated with root dry weight, root-to-shoot ratio, thousand grain weight and grain and biological yield ($P < 0.01$) under D1 and D2, it was negatively correlated with number of grains per sike (D1). Root-to-shoot ratio was negatively associated with grain

Table 2 Correlation coefficients for root traits with above ground parameters under field condition

Traits	RTL	RDW	RSR
RDW	0.25**		
RSR	0.16**	0.76**	
FLA	0.14*	0.13*	-0.12*
SPL	NS	0.14**	NS
PHT	0.15**	0.15**	-0.11*
BLY	0.18**	0.23**	-0.35**
GRY	0.18**	0.22**	-0.17**
HI	NS	NS	0.20**
TGW	NS	0.12*	-0.10*
NSP	0.16**	NS	NS
NGR	NS	0.10*	NS

* = Significant ($p < 0.05$), ** = Highly Significant ($p < 0.01$), ^{NS} = non-Significant ($p > 0.05$), n = 177

Abbreviations: *RTL* maximum root length, *GRY* grain yield, *BLY* biological yield, *RDW* root dry weight, *RSR* root-to-shoot ratio, *HI* harvest index, *FLA* flag leaf area, *SPL* spike length, *PHT* plant height, *TGW* thousand grain weight, *NSP* number of spikes per plant, *NGR* number of grains per plant

Table 1 ANOVA for mean square values of morphological traits under field condition

SOV	DF	RTL	RDW	RSR	GRY	TGW	NSP	NGR	PHT	BLY
GEN	58	53.27**	0.35**	0.014**	4.86 **	523.55**	1.35**	2700.1**	103.93 **	79.08 **
TRT	2	6681.04**	67.08**	16.9**	294.35 **	2598.48**	46.53**	78682.9**	5093.69 **	2562.85 **
GEN X TRT	116	51.81**	0.37**	0.02**	1.43 **	255.44**	1.16**	3213.2**	38.43 ^{NS}	17.97 **
Error	395	12.08	0.04	0.02	0.7	158.62	0.56	123.6	60.71	8.4
C.V		16.64%	11.43%	15.86%	16.27%	29.89%	9.87%	8.31%	11.26%	15.17%

** = Highly Significant ($p < 0.01$)

Table 3 Genotypic variance and heritability for wheat traits under field condition

Traits	Control		Moisture-stress-1		Moisture stress-2	
	Genotypic Variance	Heritability	Genotypic Variance	Heritability	Genotypic Variance	Heritability
RTL	0.7	0.88	0.48	0.65	0.86	0.94
RDW	0.93	0.96	0.9	0.94	0.92	0.98
RSR	0.85	0.92	0.76	0.86	0.88	0.95
GRY	0.66	0.79	0.46	0.63	0.43	0.69
TGW	0.58	0.85	0.53	0.65	0.48	0.55
NSP	0.46	0.57	0.45	0.56	0.52	0.67
NGR	0.6	0.64	0.57	0.64	0.55	0.63
PHT	0.73	0.84	0.03	0.06	0.36	0.51
BLY	0.68	0.8	0.45	0.62	0.58	0.8

Table 4 Correlation coefficients for root trait parameters with above-ground traits within each applied treatment

	Traits	ANT	BLY	FLA	GRY	HI	PHM	PHT	TGW	NSP	NGR	RDW	RSR
Control	RDW	NS	NS	-0.23*	0.18*	NS	NS	NS	0.25*	NS	NS		
	RSR	NS	-0.40**	-0.19*	NS	0.38**	NS	NS	NS	NS	-0.21*	0.79**	
	RTL	NS	NS	NS	NS	NS	NS	0.42**	NS	NS	NS	NS	0.23*
Drought 1	RDW	NS	NS	NS	NS	NS	NS	-0.20*	NS	NS	NS		
	RSR	NS	-0.44**	NS	NS	0.34**	NS	NS	NS	NS	NS	0.84**	
	RTL	NS	NS	NS	0.22*	NS	NS	NS	0.20*	NS	-0.21*	0.20*	0.19*
Drought 2	RDW	NS	0.16*	NS	NS	NS	NS	NS	NS	NS	NS		
	RSR	NS	-0.28**	NS	-0.18*	NS	NS	NS	NS	NS	NS	0.86**	
	RTL	0.16*	0.18*	NS	NS	NS	0.15**	NS	NS	NS	NS	0.30**	0.20**

* = Significant ($p < 0.05$), ** = Highly Significant ($p < 0.01$), ^{NS} = non-Significant ($p > 0.05$), n = 177

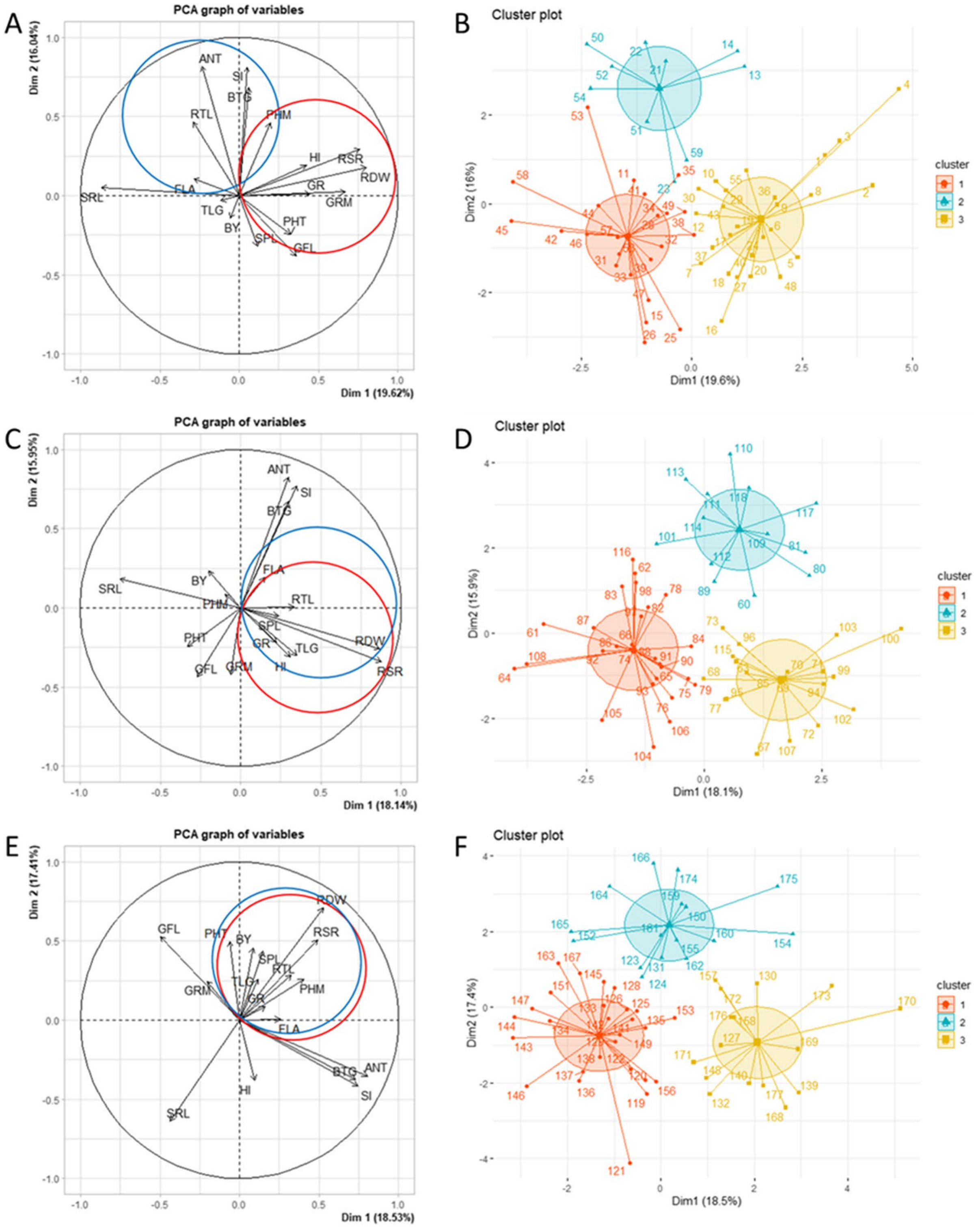
Abbreviations: *RTL* maximum root length, *GRY* grain yield, *BLY* biological yield, *RDW* root dry weight, *RSR* root-to-shoot ratio, *HI* harvest index, *FLA* flag leaf area, *SPL* spike length, *PHT* plant height, *TGW* thousand grain weight, *NSP* number of spikes per plant, *NGR* number of grains per plant

yield under moisture stress-2 conditions. Genotypic correlation with below and above ground phenotypic traits, represented as hierarchical clusters is shown in Fig. S. 2-4.

Multivariate analysis of phenotypic traits

The genotypes showed a range from higher to lower levels in terms of root length and root biomass. In this experiment, the top 10 long-root producing lines and the top 10 lines with more biomass were identified for further analysis. Similarly, 10 bottom-most lines were also selected to compare root traits with above-ground traits. The traits correlating with root traits were used for multivariate analysis. Figure 2 (A, C and E) shows the correlation of different traits from well-watered control, D1 and D2. Under the control conditions, maximum root length is clustered with flag-leaf area and days to anthesis while root dry weight and root-to-shoot ratio with grain yield and number of grains. Under drought conditions traits affected by droughts have been clustered together. Most of the wheat traits showed correlation except specific root length and grain fill duration.

Principle component analysis helped the distribution of wheat lines to different classes. For root traits against different water treatments, genotypes were categorized into three clusters (Fig. 2. B, D and F). Comparing the top and least genotypes (Table. S. 1), those with lower dry weight and root-to-shoot ratio


A
PCA graph of variables
Dim 2 (16.04%)
Dim 1 (19.62%)
ANT
SI
BTG
RTL
PHM
HI
RSR
RDW
GR
GRM
SRL
FLA
TLG
BY
PHT
SPL
GFL
B
Cluster plot
Dim2 (16%)
Dim1 (19.6%)
cluster
1
2
3
C
PCA graph of variables
Dim 2 (15.95%)
Dim 1 (18.14%)
ANT
SI
BTG
FLA
SRL
BY
PHM
RTL
SPL
GR
TLG
HI
RDW
RSR
PHT
GFL
GRM
D
Cluster plot
Dim2 (15.9%)
Dim1 (18.1%)
cluster
1
2
3
E
PCA graph of variables
Dim 2 (17.41%)
Dim 1 (18.53%)
RDW
GFL
PHT
BY
RSR
SPL
RTL
PHM
GRM
TLG
GR
FLA
BTG
ANT
SI
HI
SRL
F
Cluster plot
Dim2 (17.4%)
Dim1 (18.5%)
cluster
1
2
3

◂**Fig. 2** Multivariate analyses of wheat genotypes under different water regimes. PCA plot of wheat plant traits (**A**, **C** and **E**), among the genotypes (**B**, **D** and **F**). Data used for multivariate analysis was from maximum root length (RTL), root dry weight (RDW), root-to-shoot ratio (RSR), days to tillering (TLG), days to booting (BTG), days to spike initiation (SI), days to anthesis (ANT), days to physiological maturity (PHM), grain Filling Duration (GFL), flag leaf area (FLA), spike length (SPL), plant height (PHT), biological yield (BLY), grain yield (GRY), Harvest Index (HRI)

are grouped with genotypes, higher in specific root length.

Within maximum-root length, genotypes with lower length are grouped in red or yellow clusters while the genotypes with higher length are grouped in blue and red cluster. Similarly, most of the longer root genotypes are grouped in the blue cluster while lower lye in the red and yellow cluster. Scanning of root images of selected genotypes ranked in the top and least 10 for root-shoot ratio and specific root length, indicated distribution of phenotypic traits into two major clusters with further division into sub-clusters (Fig. S. 2). Most of the traits including root length are grouped with maximum-total root length (cm), total root surface area (cm^2) and root volume ($V > 3.5$–4.5 and < 0.5 cm^3) while root weight and root-shoot ratio were grouped with root surface area of 1.5–3.0 cm^3.

Results for the averaged mean performance and common genotype frequency (CGF) among the traits of 20 genotypes ranked in the top and least ten (Fig. 3 A-C) revealed a range from least to top values of individual traits and genotypic frequency. The CGF between root dry weight and root-to-shoot ratio remained highest across the treatments and ranks except for least ranked under well-watered condition. These are the two traits which only had higher GCF with maximum root length under well-watered condition while the frequency decreases under drought stress. Harvest index showed higher GCF with grain yield while lower with biological yield. Specific root length had least GCF with most of the traits except plant height and root length.

Genotypic comparisons

Among the 20 genotypes (Figs. 4 and 5), the top significantly higher than 50% of the genotypes were V1, C23 and C47 in RTL (14.7- 18.5 cm), C5, V6, C37 and C49 in RDW (1.3–2.1 g), C4 in SRL (43.2), C49 and C37 in RSR (0.1 and 0.2), C9, C40 and C10 in GY (6–6.3 g plant^{-1}), C6, C40 and V6 in BLY (20–26.5 g plant^{-1}) and C9 in HRI (44.6%).

Effect of drought

The averaged results presented in Fig. 3A-C revealed that the moisture stress condition-1 reduced the average performance of individual trait in top and least genotypes in similar fashion with maximum reduction in biological yield and root dry weight, followed by grain yield, maximum root length, plant height and root-to-shoot ratio respectively. The two traits: specific root length and harvest index were increased. The moisture stress-2 reduced the performance of mainly above ground traits in top, and both above and below ground traits in least ranked genotypes (almost two-fold) than moisture stress-1, while below ground traits showed the opposite trend with lower reduction than moisture stress-1 in top ranked genotypes. Harvest index in top ranked lines while specific root length in both top and least were increased under moisture stress-2. The genotypic comparisons for below ground traits, within ranking order is presented in Fig. S. 6–9.

Results for the mean performance of 20 genotypes including top and least ten in ranking for maximum root length (RTL) under well-watered conditions, were further analyzed for other root and above ground traits under three field conditions of water availability (Figs. 4 and 5). Either of the two drought treatments significantly reduced the averaged RTL; (14.5–12.6 cm), RDW (1.5–0.87 g plant^{-1}), GY (6.5–3.5 g plant^{-1}), BLY (23.8–13.4 g plant^{-1}) and PHT (74.7–61.2 cm) while the SRL was increased (13–21 cm g^{-1}) under drought treatment.

Genotype x Drought interactions

The genotype x drought interactions revealed significant reduction under drought in RTL (34 and 36%) of V1 and C5 respectively, RDW (70–92%) of V6, C2, C3, C4, C5, and GY (66%) of C3 genotypes. Furthermore, mean performances of the top and least 10

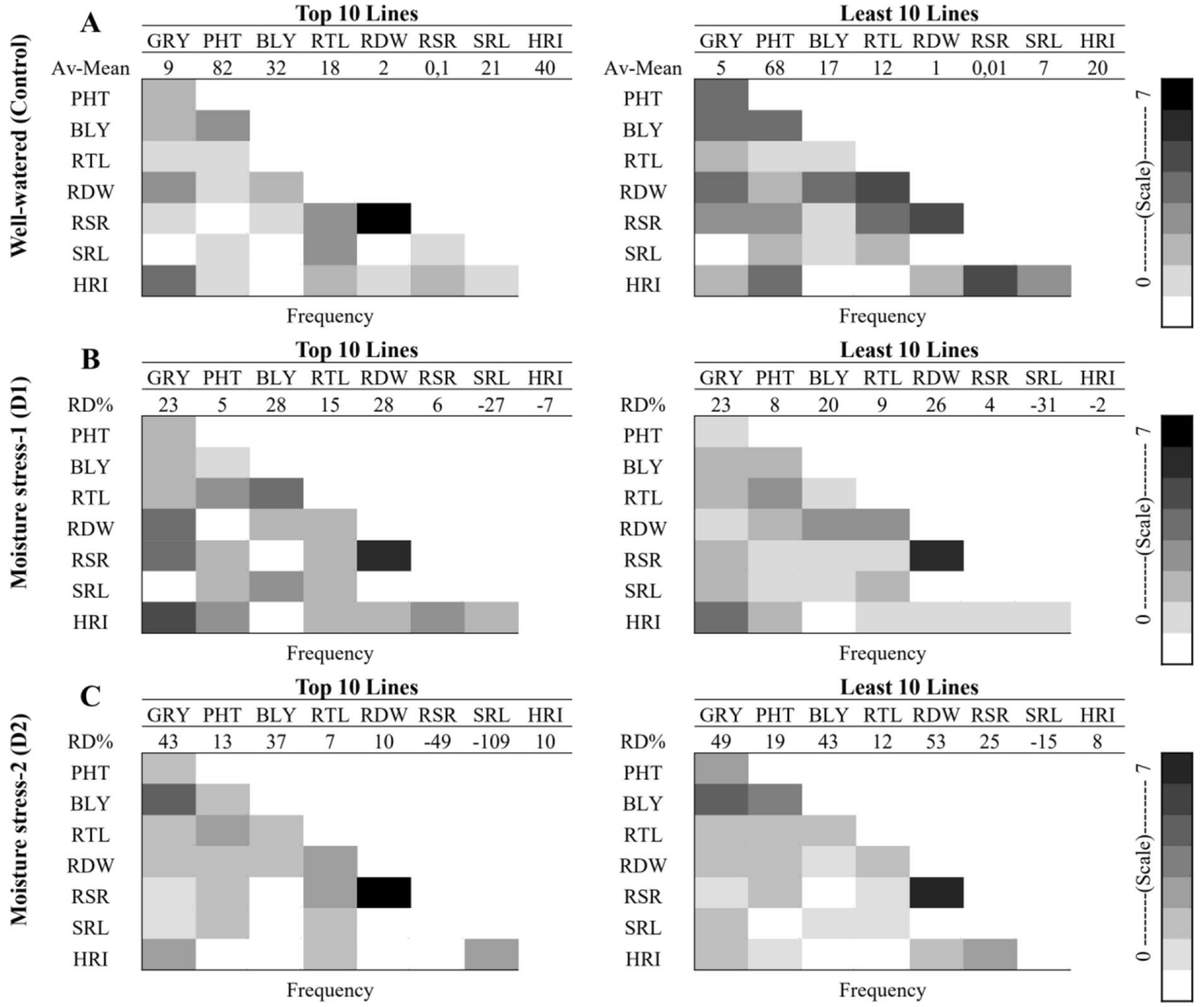


Fig. 3 Frequency matrix shown as heat map of common genotypes among the traits ranked in top and least ten under well-watered (**A**), moisture stress-1 (**B**) and moisture- stress-2 (**C**). Averaged mean values of individual traits under well-watered conditions and percent reduction under moisture stress conditions are presented under each trait. Abbreviations: RTL maximum-root length, GRY grain yield, BLY biological yield, RDW root dry weight, RSR root-to-shoot ratio, HRI harvest index, PHT plant height and %RD percent decrease

genotypes for other root traits under three soil moisture conditions are presented in Fig, S. 6–9.

Discussion

Reponses of root traits to culture conditions

Root is an important supportive organ for nutrient and water accusation and its phenotyping is as important as shoot phenotyping, due to the dependency of plant's development on it (Zhu et al. 2011). A better understanding of root systems is critical to crop improvement in water-limited environments (Fang et al. 2017). The present study revealed significant differences in above and below-ground traits among the genotypes, cultivation conditions and soil moisture stress levels. Transitory changes in root traits such as root length to root biomass from hydroponic system to field condition revealed rhizosphere effects. The root phenotypes change with the change in rhizosphere conditions (Shah et al. 2015a). In our study, large variations in root length and its biomass under different culture conditions such as hydroponic and field conditions could be due to the variation in rhizosphere penetration

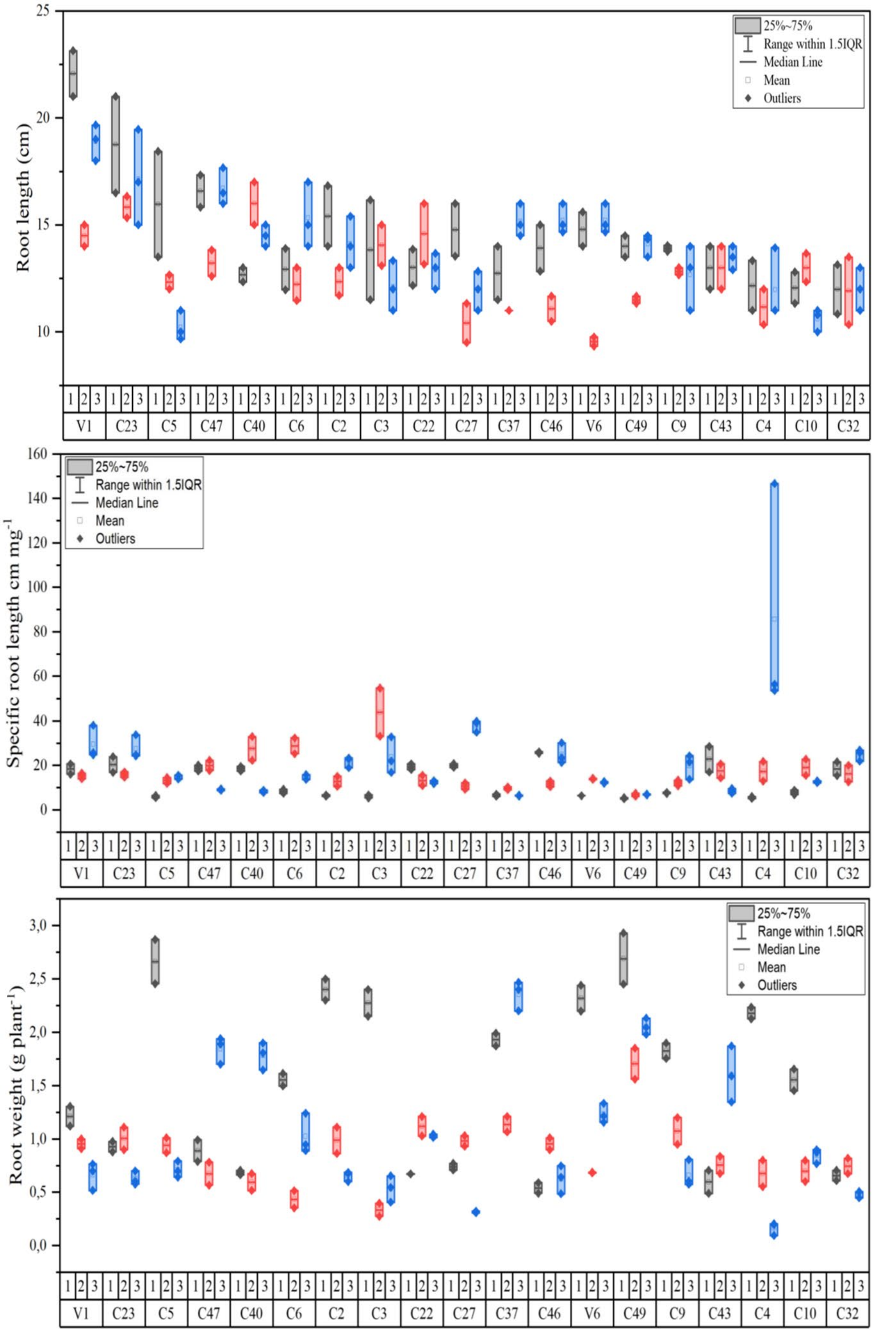


Fig. 4 Mean performance of wheat genotypes for maximum root length, specific root length and root dry weight in response to well-watered control (1-black), moisture stress 1 (2-red) and moisture stress 2 (3-blue) conditions

resistance such as soil compaction (Colombi et al. 2019) and water availability (Liu et al. 2022; Xiong et al. 2006). No relationship has been observed between data from the laboratory root screens and root depth in the field (Bai et al. 2019; Mian et al. 1994) who grew wheat genotypes under field conditions based on the result found hydroponically. In our study longer roots under a hydroponic system as

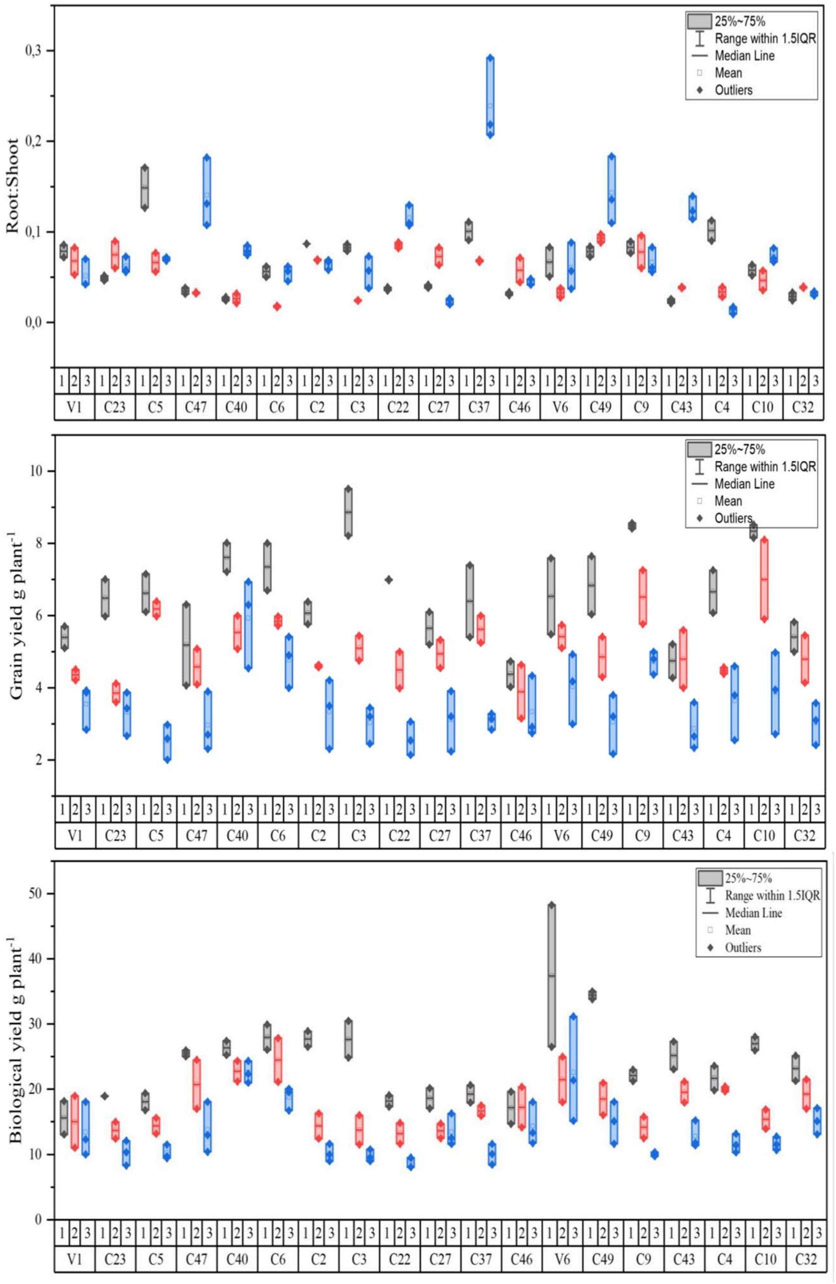


Fig. 5 Mean performance of wheat genotypes for root-to-shoot (root:shoot), grain yield and biological yield of wheat genotypes in response to well-watered control (1-black), moisture stress 1 (2-red) and moisture stress 2 (3-blue) conditions

compared to pot or field could be due to soil structural arrangement, uneven distribution of porosity, soil hydraulic conductivity, air-filled porosity, and gaseous diffusion rate affecting root development under field conditions (Liu et al. 2022; Taylor and Brar 1991).

Reponses of root traits to drought stress

Root length and density are the primary traits, facilitating nutrients and water uptake from the soil (Liu et al. 2021; Manske et al. 2000) in contrast to traits that help the plants to avoid water deficit, such as the decrease the leaf area, transpiration efficiency, early stomata closure, leaf waxiness and trichome density, which are likely to have a greater impact on yield (Bowne et al. 2012; Sinclair and Muchow 2001) but not mimicing the canopy cooling against heat (Tricker et al. 2018), so may not be dominating in heat tolerant genotypes. Root are the traits may have a dual effect against combine stress of drought and heat. A vigorous root system in the early growth stage, with significant root mass and length can access more water and nutrients to facilitate crop establishment and growth such as faster leaf area development and shoot-biomass increment (Comas et al. 2013; Fang and Xiong 2015; Ober et al. 2021).

The correlation coefficient among different parameters indicated that root traits were positively correlated with yield-contributing traits. The positive correlation of grain yield with root dry weight under well-watered condition and root length under moisture-stress condition indicates plants with longer roots under drought have performed better under moisture-stress conditions. Our results are in line with those of (Arai-Sanoh et al. 2014; Bacher et al. 2023; Bai et al. 2019) who found that genotypes with deep rooting produced more grain yield.

The contrasting genotypes for root traits identified in this study can be exploited to improve drought and heat tolerance and/or resource capture in wheat breeding programs. The cluster analysis revealed the distribution of root traits where most of the traits including root length are grouped with the total root length (cm), total root surface area (cm^2) and root volume ($V > 3.5$–4.5 and < 0.5 cm^3). However, in 2nd cluster, root weight and root-to-shoot ratio were grouped with root surface area of 1.5–3 cm^3 (S. Fig. S. 2). Studies about the root traits in relation to plant height reported that differences in root dry weight between a tall wheat landrace with greater root dry weight and a short wheat cultivar with less root dry weight were largely due to genetic control on chromosome or ploidy level (Monyo and Whittington 1970; Xiong et al. 2006). The total root length and rooting depth affect the distribution of roots in the soil profile and influence the access and uptake of water (Yu et al. 2007). Among the longer root genotypes V1 (Shalkot-14), and V8 (Zarghoon) were developed for dry areas. Some of the genotypes already selected against drought might have an association of root traits with other drought-resistant traits. Moreover, different genotypes may have different levels of tolerance and adaptability to drought varying their ranking order in performance under control and drought conditions in our study.

Under drought-stressed conditions, cultivars with higher root growth are particularly important to avoid drought-stress (Dhanda et al. 2004). A great reduction was observed for root biomass when grown under field and within field conditions against drought treatments in contrast to perineal grasses with different adaptive strategies under drought (Hanslin et al. 2019).

Specific root length is an indicative of the density of fine roots (Chen et al. 2020; Ostonen et al. 2007). It did not only vary between the genotypes but also under different water availability which contrasts with previous reports given that the observations has been made at early vegetative stages or under controlled conditions (Chen et al. 2020; Løes and Gahoonia 2004). Early deep routing may mimic the drought resistance but not in every case due to a complex and polygenic control of drought tolerance in wheat, moreover, the traits linking to crop yield under drought need further validation (Chen et al. 2020; Comas et al. 2013; Fang and Xiong 2015; Løes and Gahoonia 2004). The averaged SRL was increased (27–109%) under drought and for the top and least ten lines selected for SRL, root dry weight and root-to-shoot ratio was also increased under drought. An increase in variation of several root traits between the top and bottom lines under drought also reflects the variability of genotypes in tolerance to drought. A barley germplasm study against moisture stress shows similar results where a decrease of 51–58% was observed in total-root length, root surface area and root volume (Manju et al. 2023).

Furthermore, we investigated the genotype frequencies among the morphological traits of 20 genotypes, ranked in top and least 10, to show the distribution of genetic variation in a specific group. Results revealed that root dry weight and root-to-shoot ratio remained highest across the treatments and ranks except for least ranked under well-watered condition. Specifically, the highest ranked genotypes exhibited an average root dry weight of 2g under well-watered conditions, which slightly decreased to 28% under drought conditions. The root-to-shoot ratio also followed this trend, with values of 0.1 and 6%, respectively. In contrast, the least ranked genotypes under well-watered conditions showed significantly lower root dry weight (1g) and root-to-shoot ratio (0.01). These results suggest that genotypes with higher root biomass and favorable root-to-shoot ratios are better adapted to fluctuating water availability, maintaining growth and resource allocation more efficiently in contrast to maximum root length which had lower frequency with most of the traits except root dry weight and root-to-shoot ratio only under control conditions. Least GCF was observed for specific root length with yield traits and root dry weight due to its opposite response to drought. It also indicates its genetic instability.

Root/rhizosphere management is an effective approach to increase crop productivity for sustainable crop production (Shen et al. 2012) which supported our work in terms of root plasticity under varying rhizosphere conditions. Wheat root plasticity may also mislead while selecting genotypes if the rhizosphere conditions are ignored where rhizosphere conditions may have a greater impact (Severini et al. 2020). Most of the root traits consistently showed negative correlations with yield parameters. This could be due to competition for assimilation between these traits for growth and development.

Conclusions

In this study we have investigated the wheat genotypes that were selected for high temperatures and the varieties developed for different ecological regions against drought and root responses.

We have highlighted the importance of diverse rhizosphere conditions while selecting genotypes for stable root traits. Although maximum root length and root dry weight are heritable traits, most of the traits are very plastic where the rhizosphere conditions may have a greater impact than the genotypic response. The deeper root may help with a short-term of moisture deficiency or terminal drought while persistent drought, adversely affects most of the plant traits except specific root length and harvest index which were increased under drought.

We have also highlighted the importance of field studies for root traits, systems like hydroponics cannot be representative of field conditions, and therefore, such systems should be used in combination with field data in wheat breeding against root traits.

The variation in root traits of HTWL against drought indicates their potential for the development of improved genotypes that can withstand multiple stresses. Root traits showed a significant but lower correlation with above-ground parameters, due to variations under field conditions.

It is important to consider rhizosphere conditions for genotype selection, due to the plasticity of wheat roots to the rhizosphere. Therefore, for the selection of root traits under persistent drought conditions, it is recommended to evaluate a broader range of rhizosphere conditions in addition to controlled studies.

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Authors' Contribution AW, A and S conducted the trials and collected data A, AI and SRUS interpreted the results and wrote a manuscript, SRUS and WF designed the experiment and received funding. SAK analysed the root images and MR provided a critical evaluation.

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