Pak. J. Agri. Sci., Vol. 60(4), 517-526; 2023
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI:10.21162/PAKJAS/23.71
http://www.pakjas.com.pk

Identification and characterization of morphophysiological and yield-oriented traits for drought improvement in wheat

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Drought is a significant abiotic stress that negatively impacts plant growth and can lead to substantial crop yield losses. Our study aimed to establish selection criteria for 200 spring-wheat (*Triticum aestivum* L.) genotypes exposed to three different field-capacity (FC) levels (100%FC, 50%FC, 25%FC) in a growth chamber (seedling traits) and in the field under withhold-irrigation conditions (yield traits). Variance, correlation, and principal component analysis (PCA) was performed to identify genotypes with high tolerance to drought stress. The stress induction significantly affects morphophysiological traits i.e., root length, root weight (fresh, dry), fresh shoot weight, relative water contents and root shoot ratio in spring wheat genotypes at seedling stage. The traits flag leaf area, spike weight, grain weight/spike, biological and grain yield were interlinked and strongly influenced by drought in the field. The mean grain yield performance decreased from 21.85 g to 19.46 g under variable water regimes; however, the differences in harvest index and spikelets/spike were not significant. Among the tested genotypes, 9970 and 36-Eswyt-42 showed better root architecture development, leaf water management system, biological yield (66.07 g, 56.23 g) and grain yield (27.47 g, 28.78 g), respectively. The traits we identified may serve as an indirect selection index to enhance current semi-arid wheat germplasm and develop high-yielding drought-tolerant lines in advanced generations.
Keywords: Mini core collection, Seedling and yield traits, Selection criteria, Variability analysis.

INTRODUCTION

Wheat (*Triticum aestivum* L.), the beloved staple crop of 2.5 billion people across 89 countries worldwide (CGIAR, 2021), is a nutritional powerhouse that boasts high levels of carbohydrates, protein, and amino acids. With its low management cost and exceptional caloric value, wheat remains a vital source of sustenance for millions around the globe (Fernandes *et al*., 2018). The 21st century is marked by global scarcity of water resources, environmental pollution, and increased salinization of soil and water. By the year 2050, around 65% of the world's population is expected to face absolute water scarcity (Alexandratos and Bruinsma, 2012), indicating that water scarcity is one of the most critical limiting factors for food supply (Lei *et al*., 2016). Although wheat is widely grown worldwide, drought, heat, and other abiotic stresses significantly contribute to low wheat productivity from one season to the next (Hendawy *et al.*, 2017). Wheat has been grown in the widest area across the world with about 766 million tons of total wheat production (FAO, 2019). Water scarcity affects 20–25% of the world's wheat-growing areas (Schmidt *et al*., 2020), reducing wheat yield by 50–90%, depending on the growth stage of the plant (Reynolds *et al*., 2012). Additionally, other abiotic factors, such as low temperature (7%), high temperature (20%), salinity (10%), and other environmental extremes (9%), account for wheat production losses (Kajla *et al*., 2015). Drought has a significant impact on the agronomic attributes of wheat, such as plant growth, development, pre-anthesis, terminal phase, and yield (Ali *et al*., 2021). Water deficiency during early anthesis negatively affects seed setting (Weldearegay *et al*., 2012). Terminal water stress causes leaf senescence, grain development, and grain-filling rate reduction, ultimately resulting in lower total grain weight (Hendawy *et al*., 2019). In arid and semiarid agricultural regions, water scarcity is the primary constraint to wheat yield, which worsens with increasing stress intensity and duration (Rana *et al*., 2013). Due to seasonal climatic changes, heat and other abiotic stresses, it is crucial to develop water-stress resilient germplasm (Mansour *et al*., 2020).
Breeding wheat for high productivity and resistance to biotic and abiotic stresses, including drought, requires careful

Ishaaq, I., R. Maqbool, A. Shakeel and F.S. Awan. 2023. Identification and characterization of morphophysiological and yield-oriented traits for drought improvement in wheat. Pakistan Journal of Agricultural Sciences 60: 517-526.
[Received 10 Mar 2023; Accepted 10 Aug 2023; Published 11 Dec 2023]

consideration of yield and yield-related traits, such as leaf area, productive tillers, and grains per spike (Noorka and Silva, 2014). These traits can be used as indirect criteria to access diverse germplasm in drought-prone areas. Several studies have reported that morpho-physiological traits play a significant role in improving germplasm under drought, either directly or indirectly, in crops such as millet (Seghatoleslami *et al*., 2008), wheat (Shahryari *et al*., 2011), barley (Vaezi *et al*., 2010), and common beans (Ramirez and Kelly, 1998). There is a lack of in-depth information on the phenotypic characterization of germplasm based on seedling and yield-related traits. Principal component, correlation, and biplot analysis are useful approaches for estimating the impact of predominant characteristics, variance components, and heritability (Mather and Jinks, 2013).

There is a pressing need to develop new cultivars that perform well in changing climatic conditions and have a high production potential. Therefore, the current study aimed to uncover the direct and indirect roles of morpho-physiological and yield attributes in 200 diversified spring wheat genotypes under variable field capacities and natural field conditions. The results of this study can be used in future wheat breeding programs to develop selection criteria and a mini-core collection, and to disseminate information for varietal introduction in semiarid and rainfed areas of Pakistan.

MATERIALS AND METHODS

The study was conducted at the research area of the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan, located at an altitude of 184 meters, longitude of 73.8˚ East, and latitude of 31.43˚ North during cropping season 2016-2017 and 2017-2018. The experiment used two different approaches: a lab-based experiment to evaluate the seedling growth assay and a field-based experiment to evaluate yield and related traits. The experiment included 200 spring wheat genotypes (Supplementary Table 1), including checks for drought resistance and susceptibility, elite lines, advanced lines, and historical Pakistani varieties with good yield potential.

Seedling experiment: This experiment was carried out in a growth chamber under the following conditions: light (16 hours of light and 8 hours of darkness), temperature (25^{o}C). Humidity (80%) was maintained by the dehumidifier. The seeds of 200 genotypes were sown in sand-filled cups (11.5 cm × 9 cm) to assess morpho-physiological traits at the six-week-old seedling stage. The plastic cups were filled with 480g/cup of well-sieved sand (E.C 1.7dSm-1, pH 7.8). The water-holding capacity of sand was estimated using the method described by Veihmeyer and Hendrickson for three field capacity levels, FC1:100%, FC2:50% and FC3:25% (Veihmeyer and Hendrickson, 1949). The volume of 20 mL of water was applied to 480 g soil and checked for any moisture retain. After that, another 20 mL of water was applied, and the process was repeated until the water level at 85.5 mL revealed 100% FC (control). After that, 42.5 mL of water were added to create 50% FC. Similarly, 21.25 mL of water was applied to maintain 25% FC (Ahmad *et al*., 2022). The water stress was imposed right after 15 days of sowing (at the 3-leaf stage of the wheat seedling). Plants in the control condition were watered regularly to keep soil-water contents near the estimated field capacity. The irrigation frequency maintained was 2 irrigations/week (Ahmed *et al*., 2019).

Table 1. The irrigation levels, and its application stage for both experiments.

	Normal Plot	Drought Plot
Number of Irrigations applied	4	1
Roani Irrigation applied	Yes	Yes
Stage of Roani irrigation	before sowing (411 m^3)	before sowing (411 m^3)
	Stage of Irrigation treatments	
1st irrigation application stage	At tillering stage (308 m^3)	At tillering stage (308 m^3)
2nd irrigation application stage	At booting stage (308 m^3)	--
3rd irrigation application stage	At milking stage (308 m^3)	--
4th irrigation application stage	At dough stage (308 m^3)	--

Determination of Morpho-physiological Traits: A comprehensive array of seedling traits were meticulously measured and recorded, including root length (RL; cm), shoot length (SL; cm), fresh root weight (FRW; g), fresh shoot weight (FSW; g), dry root weight (DRW; g), dry shoot weight (DSW; g), root-shoot ratio (RSR), coleoptile length (CL; cm), chlorophyll content (CC), excised leaf water loss (ELWL) and relative water content (RWC). These data points provide a rich and detailed understanding of the wheat seedling's morpho-physiological characteristics, allowing for a more nuanced analysis of its drought tolerance potential (Noorka and Silva, 2014).

Rigorous and precise measurement techniques were employed to collect data on various seedling traits. Root length (RL) was meticulously measured from the root-shoot joints to the root tip, while shoot length (SL) was measured from that joint to the upper leaf tip of the same plant using a graduated ruler. Fresh shoot weight (FSW) and fresh root weight (FRW) were determined using an electrical balance (Ogawa. Seiki-Co. Ltd, 1991), while dry shoot weight (DSW) and dry root weight (DRW) were obtained by oven-drying samples at 65˚C for 48 hours. The root shoot ratio (RSR) was calculated by dividing root length over shoot length. The coleoptile length was measured in triplicate using a ruler. The emerged coleoptile was carefully straightened to measure the point of emergence at the seed to the tip of the coleoptile, ensuring accuracy and consistency across multiple measurements. Additionally, proportional relative water content (RWC) was calculated by measuring the fresh weight (FW), soaking samples in distilled water for 4 hours to calculate turgid weight (TW), and then drying them in an oven at 60◦C for 24 hours (Barrs, 1968). These meticulous measurement techniques provide a robust dataset that can be used to identify key morpho-physiological traits associated with drought tolerance in wheat:

$$RWC\ (\%) = \frac{FW-DW}{TW-DW} \times 100 \qquad (1)$$

To obtain accurate measurements of excised leaf water loss (ELWL), samples were weighed immediately to obtain their fresh weight (FW), then subjected to a controlled incubation environment at 28◦C and 50% relative humidity for 6 hours. Following this, the samples were dried in an oven at 70˚C for 24 hours to determine ELWL (Clarke, 1987).

$$ELWL = [(Fresh\ weight - Weight\ after\ 6h) - Dry\ weight] \times 100 \qquad (2)$$

***Determination of chlorophyll concentration (SPAD units)*:** The chlorophyll contents were measured using a SPAD meter. It measures the relative chlorophyll content based on the light absorbance of the leaf tissue. Measurements were often taken in the morning to minimize the potential influence of changing light conditions on the readings. SPAD meters operate on a specific wavelength, typically around 650 nm, which is in the red region of the visible spectrum. This wavelength is sensitive to chlorophyll absorbance and provides a reliable estimate of chlorophyll content. The samples preparation method for SPAD is different from spectrophometer method of chlorophyll measurement. Here, we focused on healthier fully expanded leaves of similar position in plant and do measurement using SPAD machine in the field. To obtain accurate measurements of chlorophyll content, three readings were meticulously taken from three distinct leaf parts excluding midrib (tip, middle, and base) and averaged. The resulting data were then represented as chlorophyll SPAD units (Ling *et al*., 2011).

Field experiment: The plots in the field were simply separated by bunds to restrict the water flow and create normal and drought plots. Polythene sheets were used to cover the drought plots in case of any forecasted precipitation to avoid rainfall contributions. The irrigation control for both plots is mentioned in Table 1. The r × r and p × p distances were maintained at 9 inches and 6 inches, respectively.

Determination of Yield and Cognate Traits: At maturity, data were measured for yield-oriented traits, viz., plant height (PH; cm), spike length (SL; cm), peduncle length (PL; cm), flag leaf area (FLA; cm^2), spikelets/spike (SPS), tillers/plant (NTP), grain weight/spike (GWS; g), spike weight/plant (SW; g), 1000-grain weight (TGW; g), grain yield/plant (GY; g), biological yield (BY; g) and harvest index (HI; %). A comprehensive morphometric analysis of the wheat plant was conducted, encompassing various parameters such as the PH of the main mother shoot tiller, the length of the spike (SL) excluding awns. The upper first node's to the base of the spike represents PL (Farooq *et al*., 2018). Furthermore, the measurement of the flag leaf area (FLA) was performed at peak growth, when the plant was still vibrant green (Muller, 1991) i.e.

$$Flag\ leaf\ area = [Maximum\ Length \times Maximum\ Width] \times 0.74 \qquad (3)$$

A thorough assessment of yield-related traits was conducted on various genotypes of interest at maturity. The investigation included the counting of productive tillers and spikelets per spike, excluding basal sterile spikelets (Farooq *et al*., 2018). In addition, the weight of each mother spike was precisely measured using an electronic balance from Ogawa. Seiki-Co. Ltd in combination with the computation of the spike's grain weight (GW) and the number of grains per spike (NGS). The thousand grain weight (TGW) was determined by counting and weighing a representative sample of grains. To estimate the grain yield (GY), the reproductive produce of selected plants was manually harvested and threshed separately. Moreover, for biological yield (BY) determination, the weight of the whole plant was meticulously measured (Farooq *et al*., 2019). Harvesting operation was done in the 3^{rd} week of April (2016-2017) and the 1^{st} week of May (2017-2018) for both field experiments, and the harvest index was measured.

$$Harvest\ index\ (\%) = \left[\frac{Grain\ Yield\ (g)}{Biological\ Yield\ (g)}\right] \times 100 \qquad (4)$$

***Statistical Analysis*:** For lab-based seedling experiment the average data of 5 plants was used. To determine the field capacity (FC), yearly and varietal effects on the measured parameters, factorial (2-factor) analysis design (replicated) was implemented using Statistix 8.1 (User's Manual. (2003) Analytical Software, Tallahassee) (Steel *et al*., 1997). The field capacity (FC), genotype (G) and FC × G interaction effects with all pairwise possible combinations were estimated. For field-based evaluation of yield-related trait, split plot design was implemented using irrigation in main plot factor and 200-wheat genotypes as sub-plots factor. However, the samples were arranged randomly in triplicate in the field conditions to avoid the epigenetic environmental effects, as well as vertical and horizontal soil fertility. The least significant difference (LSD) test was performed to compare means at 5% probability level, while mean square values are expressed in tables (Supplementary Table 2, 3). The standard deviation and scatter plots were used to identify the outliers (Supplementary Fig. 1, 2).

The significant traits were further investigated to find out association between traits and genotypes following correlation and principal component and biplot analysis (PCA). The data were then fitted into linear y = ax + b models to analyze the correlations. The significance levels for correlation analyses were α = 0.01 (for extremely significant effects) and α =0.05 (for significant effects). Principal component analysis was computed using GenStat (10th statistical package). Significant principal components (PCs) were defined as those having an eigenvalue greater than one in the PCA. Drought-tolerant genotypes and favorable seedling features were chosen based on the results of correlation and principal component analysis to identify the selection criteria for drought stress tolerance. Graphs were drawn using GenStat and Microsoft Excel 2016 (Clewer and Scarisbrick, 2013).

RESULTS

Variability analysis and mean performance**:** The results disclosed highly significant differences for eleven seedling traits studied (Table 2). The main effects were highly significant. While the interactive effects were significant for Y × FC and FC × G. The mean performance of all seedling traits decreased under extreme FC stress (50% FC, 25% FC) except for ELWL, RSR, FRW and DRW. The lines classified as drought-tolerant (9866, 9797, and 36-Eswyt-42) showed the maximum mean value of RL (21 cm, 24.25 cm, 39.6 cm). On the other hand, lines (9796, 9764 and 9751) were classified as drought-susceptible due to their low performance (6 cm, 9.1 cm, 8.1 cm) in terms of RL. The mean performance of traits, i.e., SL (11.26 cm), CL (1.25 cm), FSW (0.013 g) and DSW (0.006) decreased under 50% FC stress. However, under 25% FC the observations were (6.5 cm), (0.7 cm), (0.003 g) and (0.001 g) respectively. The data collected for RWC and CC among 200 genotypes varied significantly. A high percentage of RWC was observed in 9803 (97%), however, 9751 was extremely susceptible under 25% FC with 0.69% RWC. The genotypes 10119, 9860, and 9740 showed better performance for CC.

The results of the field experiment were somehow divergent. The genotype, irrigation, and year main effects were significant for the majority of traits (Table 3). The interactive effects (Y × I and Y × G) were highly significant. While the I × G interactive effect was non-significant for the majority of traits except for TGW and HI. Kohsar-95 showed maximum mean values of PH (112.15 cm, 104.72 cm) and PL (47.85 cm, 42.27 cm) under normal irrigation and drought, respectively. The performance of line 10120 for traits GWS (3.68 g) and NGS (90.20) was efficient under drought. The

Table 2. Performances of 200 wheat genotypes in growth chamber for seedling traits†

	d.f	CC	CL	SL	RL	RSR	FSW	FRW	DSW	DRW	RWC	ELWL
Y	1	1805.4**	11.97**	983.2**	1383.6**	0.085**	0.058**	0.044**	0.0036**	0.039**	3291.7**	262.0*
FC	2	29508.9**	4.96**	10332.2**	11382.1**	0.578**	0.677**	0.033**	0.0163**	0.0018**	50031.1**	15405.0**
G	199	74.6**	0.34**	10.9**	442.3**	0.1009**	0.0027**	0.0009**	7.288E-05**	0.0001**	890.9**	222.1**
Y×FC	2	198.6**	1.04**	163.0**	166.5**	0.0058^NS	0.0051**	0.0010**	3.147E-04**	0.0001**	527.3**	380.3**
Y×G	199	0.5^NS	0.01^NS	0.3^NS	0.5^NS	0.0029^NS	0.0000^NS	0.0000^NS	2.38E-06^NS	0.0000*	56.2^NS	46.7^NS
FC×G	398	48.7**	0.17**	6.3**	39.1**	0.103**	0.0019**	0.0009**	3.235E-05**	0.0000**	726.7**	206.4**
Error	398	3.2	0.01	0.4	1.0	0.0062	0.0001	0.0001	3.245E-06	0.0000	88.8	46.9

*, ** Significant at the 5% and 1% probability levels, respectively. N.S = Non-significant. †Y; Two years under observation in 2016 and 2017, FC; field capacity levels, G; 200 spring wheat genotypes, ELWL; Excised leaf water loss, RWC; Relative water content, DRW; Dry root weight (g), DSW; Dry shoot weight (g), FRW; Fresh root weight (g), FSW; Fresh shoot weight (g), RSR; Root-shoot ratio, RL; Root length (cm), SL; Shoot length (cm), CL; Coleoptile length (cm), CC; Chlorophyll content.

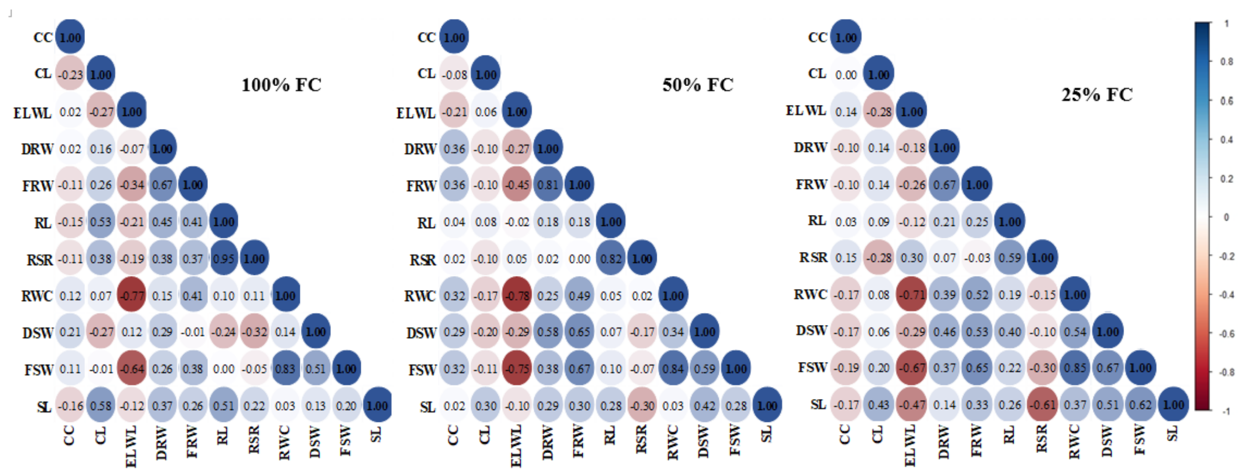

Figure 1. Correlation among various morpho-physiological traits of 200 wheat genotypes for seedling traits at three field capacity levels (100% FC, 50 % FC, 25% FC).

Legends: Chlorophyll content (CC); Coleoptile length (CL); Excised leaf water loss (ELWL); Dry root weight (DRW); Fresh root weight (FRW); Root length (RL); Root-Shoot ratio (RSR); Relative water content (RWC); Dry shoot weight (DSW); Fresh shoot weight (FSW); Shoot length (SL)

Table 3. Performance of 200 wheat genotypes in field conditions for yield-related traits.†

	d.f	PH	PL	SL	FLA	SPS	SW	GWS	NGS	NTP	GY	BY	TGW	HI
Block	2	1109.2^{NS}	73.2^{NS}	35.8^{NS}	1421.9^{NS}	9.9^{NS}	0.9^{NS}	0.1^{NS}	67.6^{NS}	70.8^{NS}	201.3^{NS}	1216.1^{NS}	10.4^{NS}	13.9^{NS}
Y	1	424.9^{**}	3068.2^{**}	29.0^{**}	30615.4^{**}	192.1^{**}	35.7^{**}	31.7^{**}	34.1^{NS}	120.8^{**}	1389.5^{**}	3109.3^{**}	7802.8^{**}	773.1^{**}
I	1	13407.8^{**}	2275.8^{**}	9.2^{**}	0.01^{NS}	125.7^{**}	9.3^{**}	10.2^{**}	5119.8^{**}	3.1^{NS}	397.3^{**}	1388.2^{**}	97.2^{**}	136.9^{**}
G	199	114.7^{**}	33.7^{**}	3.8^{**}	56.5^{**}	4.9^{**}	0.5^{**}	0.3^{**}	119.3^{**}	4.6^{**}	20.0^{**}	90.1^{*}	41.4^{**}	19.4^{**}
Y×I	1	3175.6^{**}	23.1^{**}	1.2^{*}	865.1^{**}	257.0^{**}	13.7^{**}	6.5^{**}	4425.2^{**}	579.3^{**}	2890.7^{**}	17376.4^{**}	13.0^{NS}	51.1^{**}
Y×G	199	17.2^{**}	3.0^{**}	0.4^{**}	21.4^{**}	1.4^{NS}	0.1^{**}	0.0^{**}	36.7^{*}	2.3^{NS}	11.6^{NS}	62.5^{NS}	9.9^{**}	3.2^{*}
I×G	199	12.1^{NS}	1.8^{NS}	0.2^{NS}	7.9^{NS}	1.1^{NS}	0.1^{NS}	0.1^{NS}	24.9^{NS}	2.6^{NS}	14.1^{NS}	67.8^{NS}	7.4^{*}	6.3^{*}
Error	199	12.3	1.6	0.2	8.1	1.2	0.1	0.1	28.9	2.1	12.9	71.6	5.6	2.3

*, ** Significant at the 5% and 1% probability levels, respectively. N.S = Non-significant. †Y; Two years under observation in 2016 and 2017, I; Irrigation factor (normal irrigation and drought condition), G; 200 spring wheat genotypes, PH; Plant height (cm), PL; Peduncle length (cm), SL; Spike length (cm), FLA; Area of Flag Leaf (cm^2), SPS; Spikelets / spike, SW; Spike weight (g), GWS; Weight of grain / spike (g), NGS; Number of Grains / Spike, NTP; Tillers number / plant, GY; Grain yield / plant (g), BY; Biological yield (g), TGW; Thousand grain weight (g) and HI; Harvest Index.

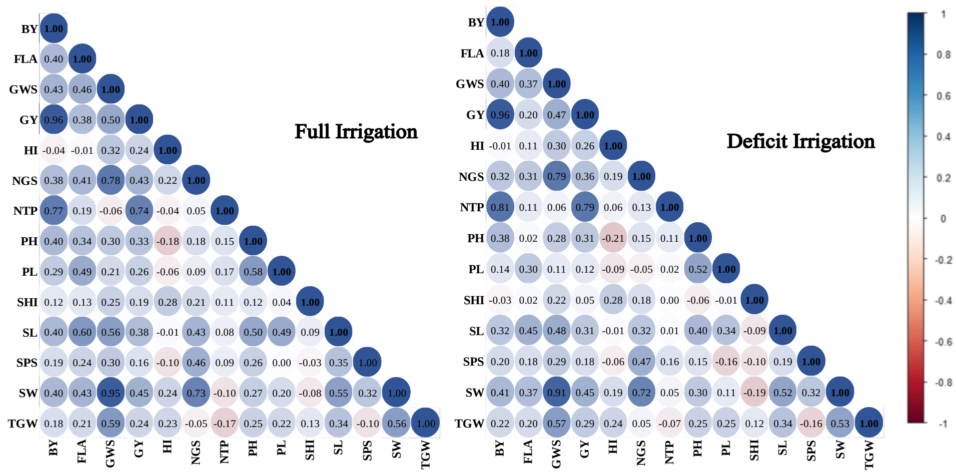

Figure 2. Correlation among various yield-related traits of 200 wheat genotypes under different field conditions (Normal irrigation, Drought).

Legends: Biological yield (BY); Area of Flag Leaf (FLA); Weight of grain / spike (GWS); Grain yield (GY); Harvest Index (HI); Grains/ Spike (NGS); Tillers / plant (NTP); Plant height (PH); Peduncle length (PL); Spike harvest index (SHI); Spike length (SL); Spikelets / spike (SPS); Spike weight (SW) and Thousand grain weight (TGW).

varieties Maxi-Pak-65 and Chakwal-86 were effective under normal irrigation for spike-related yield traits. The genotype 9862 showed the highest GY (34.44 g) and BY (75.08 g) under normal conditions.

Correlation studies: The simple correlation coefficient of RL showed a strong positive association with RSR under 100% FC (r=0.95) and 50% FC (r=0.82) (Fig. 1). A significant but negative correlation of RL was found with DSW (r= -0.24), ELWL (r= -0.21) and CC (r= -0.15). The correlation of RWC with FSW was strong and positive, which increased with extreme FC (50% and 25%). The association was positive and strong between BY and GY (r= 0.96), GWS and SW (r= 0.95). However, positive, and moderate correlations were observed between NTP and BY (r= 0.81), NTP and GY (r=0.79), SW and NGS (r= 0.73), SW and SL (r=0.55), TGW and SW (r= 0.56), TGW and GWS (r= 0.59), PH and PL (r= 0.58). A significant negative and weak association was found between SPS and TGW (r= -0.18) (Fig. 2).

Estimation of Principal Component Analysis and Biplot studies: Based on a significant eigenvalue, PCs were identified. Only such PCs with eigenvalues greater than one were deemed important (significant) under both experimental scenarios. The PCs showed total variation of 79.45% in FC1 (first four), 79.41% in FC2 (first four), and 67.15% in FC3 (first three) (Table 4). The first PC was highly related to parameters; RL (0.41), FRW (0.39), and RSR (0.35). Based on the highest squared cosine value, highest variable axes; F1 and F2 were 55.75% in 100% FC, 54% in 50% FC, and 56.77% in 25%FC stress. The projection of seedling traits FSW, DSW, FRW, and DRW on F1 and F2 indicated a strong positive correlation between them (Fig. 3). The clustering indicated ELWL was in opposing quadrants of the RWC under water-scarce conditions (50% FC and 25% FC stress). The same was true for CL and CC. A biplot and scatter plot indicated the genotypic potential based on the traits observed. Under controlled conditions (100% FC), the genotypes exhibited a diverse range of performances, with greater variability observed among (9493, 9610, 9638, 9664, 9708, 9731, 9751, 9680, 10121, 10131, 10137, Pirsabak-2004, Sind-81, FD-83, AARI-2011, Darwar-97, and 9940). Under 50% FC, genotypes such as 9508, Pak-81, GA-2002, 9787, Hashim-2008, FD-83, 36-Eswyt-42, 9724, 9803, Saleem-2000, 10137 exhibited better performance. Furthermore, advanced lines (Saleem-2000, 9437, Darwar-97, Abadgar-93, 36-Eswyt-41, 9633, Gomal-2008, 9866, 9727, and 10111) were found to be efficient under 25% FC. The genotypes 9797 and 36-Eswyt-42 showed effective performance for higher FRW and DRW under 25% FC. Notably, there were varying responses between advanced lines (9860, LU26S), (9806, 9930) and (FD-83, 9882) suggesting differences in their adaptation to varying water regimes, and hence, were grouped in the opposite quadrant (X: + ve, Y: - ve).

Table 4. Eigenvalues, variability (proportional and cumulative) for wheat seedling traits.

Variable	PC_1	PC_2	PC_3	PC_4
100% Field capacity (Seedling experiment)				
Eigenvalue	3.61	2.51	1.48	1.12
Proportion of σ^2	32.90	22.84	13.51	10.19
Cumulative σ^2	32.90	55.74	69.26	79.45
50% Field Capacity				
Eigenvalue	4.09	1.85	1.58	1.22
Proportion of σ^2	37.19	16.82	14.34	11.06
Cumulative σ^2	37.19	54.00	68.35	79.41
25% Field Capacity				
Eigenvalue	4.31	1.94	1.14	--
Proportion of σ^2	39.16	17.60	10.39	--
Cumulative σ^2	39.16	56.77	67.15	--

For yield and related attributes, significant PCs accounting for total variability were 80.76% (first four) under normal irrigation and 86.18% (first five) under drought (Table 5). The first PC was highly related to GWS (0.39 for normal irrigation and 0.42 for drought). The highest variability observed was 56.23% under normal irrigation and 52.10% under drought. Overall, the projection of traits on PC1 and PC2 revealed a difference of 2.82% in F1 variable axes (Normal irrigation: 38.48%; Drought: 35.66%) and 1.32% in the F2 (Normal irrigation: 17.75%; Drought: 16.43%) (Fig. 4). The synchronous biplot analysis for genotypic performance under different field conditions indicated that the germplasm (Pirsabak-2004, Bakhtawar-94, 9860, 9793, 10130, Shafaq-2006, 9868, 10114, 9759, Watan-2001, Fareed-2006, Millat-2011, Bahawalpur-2000, Aas-2002, Bars-2009, 9705, 9872 and 9882 exhibited non-significant differences and shared same region. However, the varieties far from origin that showed less similarity with other varieties were 9637, Pasban-90, 9970, 10123, Aas-2011, Bahatwar-94, Rohtas-90, and 9930 performed better under normal irrigation. The data analysis for the drought experiment revealed that the accessions (9495, 9875, 9871, Nifa-Barat 2010, Mehran-89, 9883, 9707, Sariab-92, 9749, 9496, 9778 and Inq-91) were very close to each other on PC1 and PC2. While accessions 9866, 9885, 9970, 10060, 10116, 10120, 10122, 97766, LU26S, Pirsabak-2008, SKD-1, 36-Eswyt-42, Aas-2011, Bakar-2002, and Chakwal-50 were more diverse.

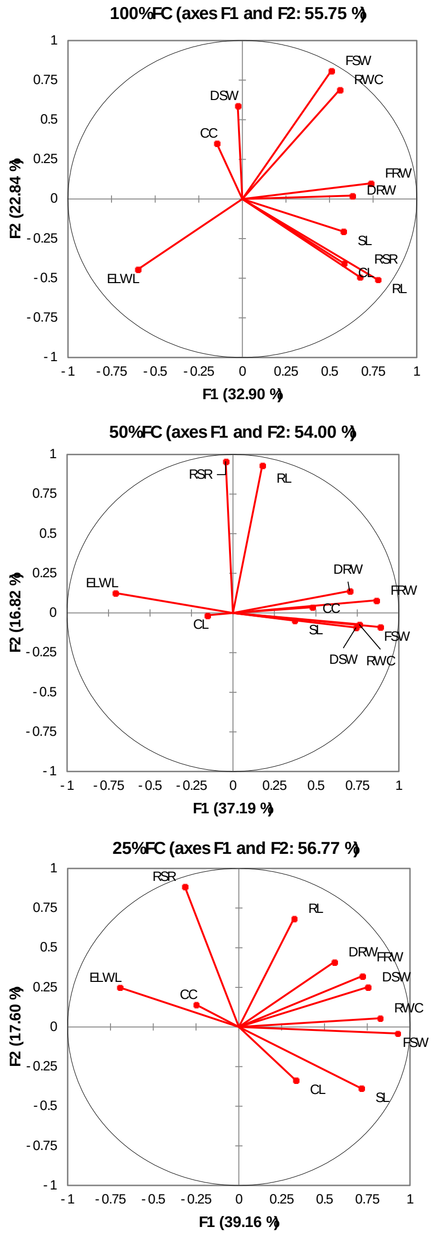

Figure 3. PCA analysis and projection of the variables on the factor-plant for seedling traits at 100% FC, 50% FC & 25% FC stress.

***Legends*: ELWL; Excised leaf water loss, RWC; Relative water content, DRW; Dry root weight (g), DSW; Dry shoot weight (g), FRW; Fresh root weight (g), FSW; Fresh shoot weight (g), RSR;

Root-Shoot ratio,RL; Root length (cm), SL; Shoot length (cm), CL; Coleoptile length (cm), CC; Chlorophyll content.

Table 5. Eigenvalues, variability (proportional and cumulative) for wheat yield traits.

Variable	PC_1	PC_2	PC_3	PC_4	PC_5
Normal Irrigation (Field Experiment)					
Eigenvalue	5.00	2.31	1.68	1.51	--
Proportion of σ^2	38.48	17.75	12.96	11.58	--
Cumulative σ^2	38.48	56.23	69.19	80.76	--
Drought					
Eigenvalue	4.64	2.14	1.82	1.56	1.05
Proportion of σ^2	35.66	16.43	13.99	12.02	8.07
Cumulative σ^2	35.66	52.10	66.09	78.12	86.19

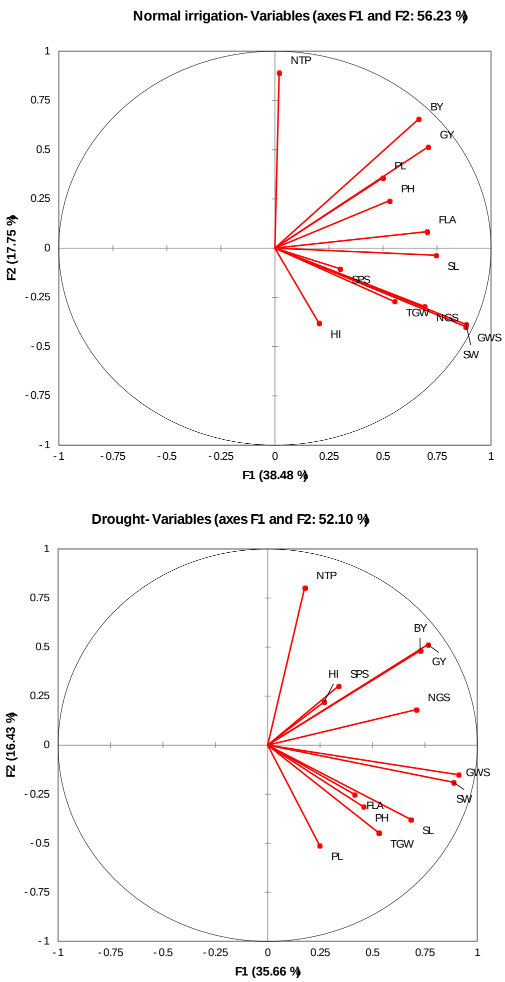

Figure 4. PCA and Biplot analysis of the variables on the factor-plant under normal irrigation and drought conditions.

Legends: BY; Biological yield (g), FLA; Area of Flag Leaf (cm^2), GWS; Weight of grain / spike (g), GY; Grain yield / plant (g), HI; Harvest Index, NGS; Grains Number / Spike, NTP; Tillers number / plant, PH; Plant height (cm), PL; Peduncle length (cm), SHI; Spike harvest index, SL; Spike length (cm), SPS; Spikelets / spike, SW; Spike weight (g) and TGW; Thousand grain weight (g).

DISCUSSION

A wide range of adaptation and mitigation strategies are required to utilize available arable and drought-affected areas effectively (Fernandes *et al.*, 2018). Under all major crops, the impact of root architecture and its mechanism on yield and yield-related parameters, particularly in deficit irrigation, is result oriented (Dorlodot *et al.*, 2007). Root length appeared to be an important trait for drought stress tolerance (Leishman and Westoby, 1994). Water scarcity decreases root length, root-shoot ratio and weight (fresh and dry) (Faisal *et al.*, 2017). Root morphology and biomass are very important traits while selecting drought-tolerant genotypes. A promising strategy to enhance drought tolerance is to selectively breed for maximal root length (Dhanda *et al.*, 2004). The phenotypic response of coleoptile length, shoot length, and shoot weight (fresh and dry) is due to the interaction of genotype and environment (Khokhar *et al.*, 1985). Stress induction reduces the shoot length to a great extent. Other wheat experts have also described wheat genotypes with stimulated root development in water-deficient environments (Ahmad *et al.*, 2013). For drought tolerance, minimum shoot length, excised leaf water loss and maximum root length are favourable traits (Ahmed *et al.*, 2019). In rainfed locations, screening based on seedling length, along with a vast root system, leads to good adaptability.

Photosynthesis is the primary process in plant cells that regulates the low concentration of water in the culture medium. Photosynthetic mechanisms will be more efficient if chlorophyll contents are higher (Said and Hefny, 2021). The water scarcity reduced the chlorophyll contents in wheat genotypes (Farshadfar and Amiri, 2015), which directly changed the color of leaves from a greenish to a yellowish appearance (Hassanzadeh *et al.*, 2009).

The genetic variability and association present in the germplasm significantly influence the expression and efficacy of selection (Fischer and Rebetzke, 2018). The average mean reduction in biological and grain yield under drought was observed in wheat (Raza *et al.*, 2019), cotton (Mubarik *et al.*, 2021) and maize (Ahmad *et al.*, 2020). The drought induction stage significantly impacts the yield pattern. Drought put the anthesis stage at risk because it might decrease pollen grain viability, which reduces the grain quantity per spike (Youldash *et al.*, 2020). The tolerant genotypes insensitive response to drought condition at later stages might be due to their ability to speed up the transition time between stages (booting and milking), which were more vulnerable to drought.

Evidence of the association among traits in variable field capacity circumstances could enhance the chances of varietal selection with desirable traits (Khan *et al*., 2011). The high RWC is an important indicator for drought tolerance and escape mechanisms adapted by plants (Arjenaki *et al*., 2012). High osmotic regulation or low elasticity of cell-wall tissue might be a reason for the changed RWC and FSW observations (Ahmed *et al.*, 2019). The extreme stress significantly switches the physiological (CC, ELWL) and morphological (CL and RSR) response, reduction in stomatal conductance, acceleration of leaf senescence, and dehydration of plant tissues among genotypes (Farooq *et al*., 2014). It might be due to the nitrogen nutrient transition capability in genotypes. Insufficient nitrogen availability disrupts the vital process of chlorophyll synthesis, causing a decline in chlorophyll content in leaves (Shamuyarira *et al.*, 2019). This impairs the integrity of chloroplasts, hastening the onset of leaf senescence, and ultimately promoting premature plant maturation (Aboughadareh *et al*., 2020).

The negatively correlated parameters can affect the performance of other traits during the selection process. The weak association between TGW, and GY, medium for SPS, and high for NGS (Shamuyarira *et al*., 2019). Leaf properties directly influence other physiological traits; the correlation of FLA with other studied yield traits was weak to moderate under both water regimes.

Selection of different parents for hybridization and other plant breeding strategies was supported by PCA (Khodadadi *et al*., 2011). The genotype projection on PC1 and PC2 helped in the selection of the several groups of parents in wheat seedling attributes (Sisodia and Rai, 2017). The deviation in RWC, ELWL, CL and CC may be attributed to varietal differences in soil water uptake and ability to control its loss (Ahmed *et al.*, 2019). The imposition of severe water stress reduces grain weight, and plant yield (Keyvan, 2010). Exposure of wheat to moisture stress during stem elongation, booting and pollen stage resulted in reduced spikelets/spike, dry matter accumulation, and overall plant distress in comparison to normal irrigation (Dong *et al*., 2017). The genotypes identified in the experiment will lay the basis for future wheat breeding and molecular characterization of the screened germplasm.

Conclusions**:** The selection for maximal root length, root-shoot ratio, weight (fresh, dry) and relative water contents could impart key features for stress avoidance and tolerance. The minimal excised leaf water loss is an important indicator for tolerance to drought. Drought onset at booting stage crucially influences important yield traits i.e., spike weight, grain weight and yield. Ten genotypes were chosen as drought-tolerant based on multivariate analysis and selection criteria (36-Eswyt-42, 9970, 9797, 9803, 9866, 10120, LU26S, Saleem-2000, SKD-1, Chakwal-50) and five as drought-susceptible (9751, 9764, 9796, Mehran-89, Sariab-92) for mini-core collection. This phenotypic characterization provides a basis for the identification and screening of QTLs/SNPs/QTNs associated with drought tolerance and yield-oriented traits in existing wheat germplasm for further molecular studies.

Conflict of Interest: The authors declare that no conflicts of interest exist for this research.

Author's Contributions Statement: The project's conception and experimental design were the brainchild of RM and II, while the execution of the experiment was carried out by II. RM and II took charge of statistical analysis and manuscript writing. AS, and FSA wrote the discussion section and proofread the manuscript. All authors read and approved the final manuscript.

Supplementary Material: Supplementary Table 1. Pedigree record of 200 spring wheat genotypes. Supplementary Table 2: Descriptive statistics (Min, Max, Mean, S.D) for Lab-based seedling experiment. Supplementary Table 3: Descriptive statistics (Min, Max, Mean, S.D) for field-based yield traits. Supplementary Fig. 1: Scatterplot of 200 wheat genotypes for seedling traits at 100% FC, 50 % FC & 25% FC. Supplementary Fig. 2: Scatterplot of 200 wheat genotypes for yield traits under field conditions (Normal irrigation & Drought).

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