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Exploration of genetic diversity and drought tolerance potential of bread wheat genotypes at seedling stage using the multivariate analyses tools

Mueen Alam Khan[1]*, Ayesha Bashir[1]* and Muhammad Umar Ayyub[1]

[1]Department of Plant Breeding & Genetics, Faculty of Agriculture & Environment, The Islamia University of Bahawalpur, Pakistan

***Corresponding author(s): Mueen Alam Khan (mueen_1981@yahoo.com);
Ayesha Bashir (ayeshabasheer.1030@gmail.com)

Abstract

Drought stress is one of the major limiting factors in plant growth. The endeavor of the present research was to identify bread wheat drought-tolerant genotypes by screening on the basis of seedling characteristics. The experiment was conducted during the cropping season (2021-2022) in the wire house of the Department of Plant Breeding & Genetics, Faculty of Agriculture & Environment, The Islamia University of Bahawalpur, Pakistan. Fifty-five genotypes were screened at the seedling stage under normal and drought stress conditions. Three treatments (T1, T2 and T3) were used with different polyethylene glycol (PEG-6000) solution concentrations i.e., (T1 was the controlled condition, T2 had 15% PEG solution, and T3 had 25% PEG solution). The results depicted the presence of significant differences among the genotypes related to drought tolerance and genetic diversity in response to the stress conditions using various morpho-physiological traits. Shoot fresh weight and chlorophyll content index were positively correlated under all treatments. Thus, the selection of these traits at the seedling stage would improve genetic gain for drought tolerance. Based on the results of principal component analysis (PCA), the promising genotypes identified were G19 (T1), G47 (T2) and G35 (T3). The identified traits could well serve as potential drought tolerance indicators and identified genotypes can be further utilized in future wheat breeding programs to develop high-yielding and drought-resilient genotypes.

Keywords: Biplot, Correlation, Genetic diversity, PCA Wheat

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Introduction

Wheat (*Triticum aestivum* L.) is a vital cereal crop grown worldwide (Khan et al., 2016; Anser et al., 2018; Shafqat et al., 2019). It is being utilized as a staple food in many regions of the world including Pakistan (Nowsherwan et al., 2018; Mehmood et al., 2020; Shehzad et al., 2022; Shafqat et al., 2023). It fulfills almost 55-60% of the total carbohydrates requirement (Baniwal et al., 2021). It is a decent source of diet enriched in proteins, vitamins and minerals, furthermore its bread-making ability increases its value to be used as a staple food for many countries (Budhwar et al., 2020; Shaheen et al., 2023; Shehzad et al., 2023). With this, thiamine, niacin, calcium, vitamin B6, riboflavin, and other small portion of vitamin A are the constituents of its grain (Baniwal et al., 2021).

According to the estimate, abiotic stresses account for more than 50% of the overall stresses. The most commonly occurring abiotic stresses are the high/low temperature, salinity and drought stresses (Kajla et al., 2015). It is estimated that the demand for wheat production will be increased up to 50% by 2050. The growth and production of wheat have been critically influenced by the abiotic stresses which reduce the yield (Ur Rehman et al., 2021). Increasing the land is not the solution as it has a lot of complications and not all land can be prepared to be used as agricultural land, but improving and incorporating desirable traits in plants can increase the production to meet the burgeoning population demand (Hickey et al., 2019). Among all the abiotic stresses causing damage to the plant growth and development, drought and heat stresses are the most severe ones. A major and rapid reduction in crop yield (more than 50%) occurs due to low water availability to the plant at critical growth stages (Amjad et al., 2021). Drought affects more than fifty percent wheat production area of world. It is the most common reason of yield losses in wheat crop (Janjua et al., 2014). Water is essential for plant growth and development because it makes up between 80 and 95 percent of a plant's total biomass. Sometimes the soil has enough water, but the plant becomes unable to uptake due to the high ionic concentration or excessive fertilizer called pseudo or physiological drought (Bakhshayeshan-Agdam & Salehi-Lisar, 2020).

Drought affects different growth stages of plants differently and the severity of the stress depends upon its intensity, duration, cultivar, and developmental stages. Stress occurring at each stage disturbs the development and causes reduction in the yield. The early stages of development are more prone to be highly effected by the drought stress as less water availability decreases seed water potential and decline the resistance (Nowsherwan et al., 2018). Roots are mostly affected by moisture deficiency. Root length is an important seedling characteristic for increasing yield under water stress condition. Roots stimulate signal by producing abscisic acid (ABA) hormone at the root tip and triggers closing of stomata (Sadok & Schoppach, 2019). Drought stress can be induced by several methods, treating with ABA, using Polyethylene glycol (PEG), water soluble polymer, or by restricting the water availability (Robin et al., 2015). Inducing drought stress by using PEG is more efficient as it does not enter the plant cells and induces water stress in the plants without having any toxic effects on the plant. It mimics the drought stress and thus lowers the water potential of plants by osmotic pressure (Ahmad et al., 2020). This triggers the formation of ABA and proline contents in plants which helps the plant to tolerate drought stress. Several researches have reported the use of PEG solution in varying concentration, to identify drought tolerant wheat lines (Sharma et al., 2022).

Drought tolerance is a complex mechanism as it is a multigenic trait that involves genetic, physiological and biochemical responses (Khadka et al., 2020). Plant breeding is aided in these circumstances by the identification of morpho-physiological and biochemical markers associated with the improved performance of crops under drought conditions (Lopez et al., 2003). The best way to improve yield under drought stress is to produce tolerant cultivars but it requires enormous resources and time. However, another aspect is to evaluate already existing wheat genotypes for drought tolerance. Selection of genotypes based on better performance could ultimately improve yield of crop. Elucidating plant responses and adaptative mechanisms to moisture deficit stress is imperative for developing drought-resilient genotypes. The intricacy of drought tolerance arises from several modulating factors, including crop-specific traits, the intensity and duration of water limitation, as well as the developmental stage at which the stress ensue (Ahmed et al., 2019).

The association between specific seedling morphological and physiological traits and drought tolerance in wheat remains unclear. There is a need to identify morpho-physiological traits that are associated with drought tolerance, and to develop selection criteria for drought-tolerant wheat genotypes. The aim of this study was therefore to assess the performance of different wheat genotypes under normal and drought conditions based on some morpho-physiological traits at seedling stage using the multivariate analysis tools. In order to characterize the association of the seedling indices and to provide appropriate selection criteria for both normal and drought conditions, the experiment was designed to screen different wheat accessions for drought tolerance using seedling attributes. From this study, we identified some promising performance genotypes that can be used in future breeding programs.

Material and Methods

Experimental conditions and data recording

The experiment was carried out in the wire house facility of the Department of Plant Breeding and Genetics, Faculty of Agriculture and Environment at The Islamia University of Bahawalpur, Pakistan. The wire house provided a controlled environment to conduct the screening of wheat genotypes under normal and simulated drought stress conditions. The use of the wire house allowed control over experimental variables and reduced environmental noise that could influence results in field conditions. Fifty-five genotypes were screened at the seedling stage under normal and drought stress conditions during wheat growing season 2021-2022. Pedigree of the studied genotypes is provided in Table 1.

Table 1 Pedigree and origin of genotypes used in the experiment

Code	Pedigree	Origin
G1	PFAU/MILAN//FISCAL/3/VORB/4/MUTUS CMSS07Y01253T-009TOPM-099Y-099M-099Y-16M-0RGY	Pakistan
G2	MUCUY/BORL14//MUCUY	Pakistan
G3	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1/4	Pakistan
G4	CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ6/6/WH576/7/WH542/8/WAXWING/9/ATTTILA*2/PBW65//PIHA/3/ATTILA/2*PASTOR/10/UP2338*2/KKTS*2/YANAC CMSS10B00824T-099TOPY-099M-099NJ-099NJ-19WJY-0B	Pakistan
G5	KHP/D31708//CM74A370/3/CIAN079/4/RL6043/*4NAC PBD 795-23A-1A-0A.	Pakistan
G6	T.DICOCCONC19309/AE.SQUAROSSA (409)//MUTUS/3/2*MUTS CMSS08Y01129T-099M-099Y-3M-0Y-5M-0Y	Pakistan
G7	OASIS/5*BORL95/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI/6/KACHU/BECARD//WBLL1*2/BRAMBLING/7/KACHU/BECARD//WBLL1*2/BRAMBLING	Pakistan
G8	MUU/KBIRD//2*KACHU/KIRITATI	Pakistan
G9	MUTUS*2/MUU//2*MUCUY CMSS13Y01148T-099TOPM-099Y-099M-0SY-21M-0WGY	Pakistan

G10	BORL14*2/FITIS	Pakistan
G11	ELVIRA/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI/6/VEE/PJN//KAUZ/3 /PASTO R/7/KIRITATI/4/2*SERI.1B*2/3/KAUZ*2/BOW//KAUZ/8/ELVIRA/5/CNDO/R1 43//ENT E/MEXI75/3/AE.SQ/4/2*OCI/6/VEE/PJN//KAUZ/3/PASTOR/9/BORL14 CMSS13B00475S-099M-0SY-5M-0WGY	Pakistan
G12	MUU/KBIRD//2*KACHU/KIRITATI	Pakistan
G13	SERI-1-B-2/3KAUZ/08FJS26	Segregating line or population from CIMMYT
G14	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1/4	Pakistan
G15	Sehar-06/inqlab-91//KINGBIRD#1/3/PARULA	Pakistan
G16	Becard/Quaiu#1	Jointly developed by CIMMYT and Pakistan
G17	MUTUS*2//TAM200/TURACO*2/3/WBLL1*2/BRAMBLING*2//BAVIS	Pakistan
G18	BORL14*2/6/BABAX/LR42//BABAX*2/3/ KUKUNA/4/CROSBILL #1/5/BECARD CMSS12B00635T-099TOPY-099M-0SY-16M-0WGY	Pakistan
G19	Pastor/6/Pvn/ear-422Mna‟‟‟s/Bow/erm//Bue/…..	Pakistan
G20	MUCUY	Pakistan
G21	KUTZ//KFA/2*KACHU	Pakistan
G22	SUP152/BAJ #1//KFA/2*KACHU CMSS13B00067S-099M-0SY-2M-0WGY	Pakistan
G23	BECARD #1/51KIRITATI/4/ 2*SERI.1B*2/3/KAUZ*2/BOW//KAUZ*2/6/KFA/2*KACHU CMSS12B00911T-099TOPY-099M-099NJ-099NJ-31Y-0WGY	Heat Yield Trial advanced line
G24	AU/UP301//GLL/Sx/3/PEW „S‟/4/MAI „S‟/MAY A „S‟//PEW‟S‟ CM.67245-C-2M-OY	Advanced material from CIMMYT
G25	WBLL*2/4/4YACO/PBW.65/3/KAUZ*2/TRAP//KAUZ/5/….. CMSS05B00061S-099Y-099M-099Y-099ZTM-5WGY-0B	Pakistan
G26	KAUZ/PASTOR//V.3009	Cross made in the country, one CIMMYT parent
G27	KUTZ//KFA/2*KACHU	Pakistan
G28	WBLL1*2/SHAMA//BAJ#1/3/BORL14	Pakistan
G29	WBLL1*2/SHAMA//KACHU/3/SUP152*2/TECUE #1/4/WBLL1/KUKUNA//TACUPETO F2001/3/BAJ #1	Pakistan
G30	MTI “S” CM47634-1-2M-3Y-1M-2Y-1Y-1M-0Y	Advanced material from CIMMYT
G31	SHI4414/CROW/4/NIF/3/SOTY//NAD/CHR/5/FRAME/6/SUNCO/2*PASTOR/7/ WBLL1*2/BRAMBLING/4/BABAX/LR42//BABAX*2/3/SHAMA/8/KACHU/BECARD//WBLL1*2/BRAMBLING	Pakistan
G32	ALTAR 84/AE.SQ (221)//3*BORL95/3/URES/ JUN//KAUZ/4/WBLL1/5/MUTUS/6/ SUP152/BAJ #1 CMSS12B00092S-099M-099NJ-099NJ-31Y-0WGY	Heat Yield Trial advanced line
G33	CMH84.3379/CMH78.578// MILAN CMSS93Y006285-7Y-010Y-010M-010Y-10M0Y-3KBY-0KBY	Cross made in the country, two CIMMYT parents
G34	KUTZ*2/5/UP2338*2/VIVITSI/3/FRET2/TUKURU//FRET2/4/MISRI	Heat Yield Trial advanced line
G35	ELVIRA/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI/6/VEE/PJN//KAUZ/3 /PASTO	Heat Yield Trial advanced line

G36	W98/3/PBW343*2/KUKUNA*2//FRTL/PIFED/4/QUAIU #1	Pakistan
G37	T.DICOCCONC19309/AE.SQUAROSSA (409)//MUTUS/3/2*MUTS CMSS08Y01129T-099M-099Y-3M-0Y-5M-0Y	Heat Yield Trial advanced line
G38	SNTL/3/KACHU//WBLL1*2/BRAMBLING CMSS12B00561S-099M-099NJ-099NJ-3Y-0WGY	Heat Yield Trial advanced line
G39	ZINCOL//BECARD/QUAIU #1/7/INQALAB91*2/TUKURU//WHEAR/6/BAV92//IRENA/KAUZ/3/HUITES/4/T.SPEL TAPI348764/5/BAV92//IRENA/KAUZ/3/HUITES	Pakistan
G40	KUTZ*2/5/UP2338*2/VIVITSI/3/FRET2/TUKURU//FRET2/4/MISRI CMSS12Y00813T-099TOPM-099Y-099M-0SY-1-M-0WGY	Heat Yield Trial advanced line
G41	MUTUS*2/MUU//2*MUCUY CMSS13Y01148T-099TOPM-099Y-099M-0SY-21M-0WGY	Pakistan
G42	MUCUY/BORL 14//MUCUY CMSS12B00870T-099TOP-099M-099NJ-099NJ-16Y-0WGY	Heat Yield Trial advanced line
G43	MAYIL/5/PFAU/WEAVER*2/4/BOW/NKT//CBRD/3/CBRD/6/KINDE*2/SOLALA/3/UP 2338*2/KKTS*2//YANAC/4/UP2338*2/SHAMA//2*BAJ #1	Heat Yield Trial advanced line
G44	QUAIU # /5/KIRITATI/4/2*BAV92//IRENA/KAUZ/3/…	Pakistan
G45	HUIRIVIS #1*2/MURGA/3/TACUPETO F2001/BRAMBLING*2//KACHU CMSS11Y00343S-099Y-099M-099NJ-099NJ-11WGY-0B	Pakistan
G46	1G094.7.1.12/2*QUAIU#/3/VIUAJUAREZ F22009/SPLALA//..	Jointly developed by CIMMYT and Pakistan
G47	29SAWSN 11-12/56 x PBI09C048-BC-0C-6N-99N// 29SAWSN 11 12/56	Pakistan
G48	PBW343*2/KUKUNA*2//FRTL/PIFED	Pakistan
G49	Pastor/6/pvn/ear-422/Mna”s/Bow/erm//Buc/..	Heat Yield Trial advanced line
G50	1154388/NA/3/YT54/N10B/LR64 II 18427-0PAK	Segregating line or population from CIMMYT
G51	OASIS/5*BORL95/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI/6/KACHU/BECARD//WBLL1*2/BRAMBLING/7/KACHU/BECARD//WBLL1*2/BRAMBLING	Pakistan
G52	BAV92/SERI+Aas-11//F1-E-12	Pakistan
G53	QUAIU/5/UP2338*2/SHAMA/3/MILAN/KAUZ/CHIL/…	Developed through selection from exotic germplasm
G54	MUCUY/BORL 14//MUCUY	High Temperature Wheat Yield Trial line
G55	NADI/COPIO//NADI CMSS11B00910T-099TOPY-099M-099NJ-099NJ-37WGY-0B	Elite Spring Bread Wheat Yield Trial line

Experimental design and treatments

The experimental wheat genotypes were planted in pots filled with sand under a complete randomized factorial design. Three seeds were sown in each pot. For each genotype, three replications were used with three treatments. The 1st treatment (T1) was considered as control condition and was regularly watered. The 2nd set of treatments (T2) was of 15% PEG solution while the 3rd set of treatment (T3) was of 25% PEG solution. The PEG solutions were applied after 7 days of sowing while the controlled condition was given water normally.

Data recording

The data on the traits like shoot and root lengths were recorded using a meter scale, shoot and root fresh weights were measured through electric balance, chlorophyll content index through SPAD meter, and stomatal conductance through using a portable leaf porometer.

Measuring chlorophyll content

The flag leaves from each wheat genotype were selected and SPAD meter readings were taken from 3 spots on each leaf, avoiding the midribs. The sensor was clamped flat on the leaves and the 'measure' button pressed to get each reading. The readings were averaged per leaf and this process was repeated on 5 replicate plants of each genotype under each treatment. The SPAD meter directly reports a chlorophyll content index value (Parry et al., 2014). Higher readings indicate more leaf chlorophyll. The average SPAD values for each genotype and treatment were compared to see differences in chlorophyll content in response to the treatments.

Measuring stomatal conductance

A portable leaf porometer was used to measure the stomatal conductance of the wheat leaves. The sensor head was clamped onto the leaf surface, ensuring full contact. Measurements were taken on fully expanded leaves, avoiding veins and edges. The 'measure' button was pressed which activates the sensors to take a reading. The porometer directly reports stomatal conductance in mmol/m^{2}s. Higher values indicate more open stomata. Readings were taken from multiple spots on each leaf and averaged. This process was repeated on replicate plants of each genotype under each treatment. The average stomatal conductance values were compared across genotypes and treatments to evaluate differences in response to the treatments.

Procedure and measurement of root and shoot related traits

The dry weights of the seedling roots and shoots were determined after oven drying the samples at 70°C for 24 hours. For the determination of relative water content (RWC), fresh leaves were randomly sampled from each genotype in each replication and weighed immediately to record the fresh weight (FW). These leaf samples were then immersed in distilled water for 4 hours to induce full turgidity, after which the turgid weight (TW) was measured. Subsequently, the leaf samples were oven dried at 70°C for 24 hours and the dry weight (DW) was recorded. The immersion of the leaves in distilled water allowed the leaves to reach full hydration, enabling a standardized measurement of the TW across samples. The oven drying method allowed accurate determination of the DW by removing all moisture content from the leaves. The fresh leaf was weighed to calculate the relative water content (RWC). The leaves were packed in paper bags and dried in oven for three to four days at 60 °C and then weighed again to obtain the dry weight and RWC was measured using the following equation (Turner, 1986):

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

Statistical analysis

Data taken from the plants for specific traits were subjected to analysis of variance (ANOVA) (Steel et al., 1997) to evaluate the variations present in the genotypes in response to treatments by using the software Statistix 8.1. Mean comparison test using least significant difference (LSD) was performed for the genotypes and treatments found significant. Pearson's correlation analysis was conducted using R statistical software version 4.1.0 (http://CRAN.R-project.org) in RStudio 1.3.1039 (https://rstudio.com) to evaluate the relationships between the morphological and physiological traits assessed in this study (Mohi-Ud-Din et al., 2021). Principal component analysis (PCA) enabled multivariate data reduction and visualization of relationships between genotypes, treatments, and traits. PCA biplots were generated using XLSTAT statistical software on Excel. The use of the R software and XLSTAT add-in enabled robust statistical analysis and graphical representation of the complex dataset obtained from this experiment.

Results and Discussion

Estimation of variability and association analysis

We executed the multivariate tools on the data obtained from the morphological and physiological traits of the plants by applying different concentration of PEG-6000 solution at the seedling stage and the interpreted results showed the effects of drought stress. The mean square values of ANOVA (Table 2) for all genotypes showed highly significant differences for all the traits. Despite a significant decline in all the studied parameters under drought stress, the root length, root fresh and dry weights and root to shoot ratios increased which is a clear manifestation of plant’s ability to sustain drought stress (Table 3). Ahmad et al., (2013) also observed a decreasing trend in seedling traits in response to increasing concentrations of PEG. Genotypes with maximum root length and minimum shoot length are considered to be desirable and thus could well be suited to arid and dry environments (Ahmed et al., 2019).

Table 2. Analysis of variance for various morpho-physiological traits of bread wheat

Traits	Mean sum of square			
	Genotypes	Treatment	Genotypes × Treatment	Error
	DF = 54	DF = 2	DF = 108	DF = 330
Stomatal conductance	0.30**	569**	0.32**	0.17

Chlorophyll content index	0.06**	18.3**	0.04**	0.01
Relative water content (%)	0.02**	23.8**	0.03**	0.00
Root length (cm)	7.56**	4047**	4.05**	2.57
Shoot length (cm)	4.09**	2721**	3.17**	2.14
Root fresh weight (g)	0.03**	10.5**	0.02**	0.00
Shoot fresh weight (g)	0.06**	18.2**	0.04**	0.01
Root dry weight (%)	0.00*	1.13**	0.00**	0.00
Shoot dry weight (%)	0.00**	1.29*	0.00**	0.00
Root to shoot ratio by length	0.69**	279**	0.62**	0.37

DF: Degree of freedom; $p<0.05$=significant (*), $p<0.01$=highly significant (**)

Table 3 Minimum, maximum and mean values of morpho-physiological traits under three different treatments

Trait	Treatment	Minimum	Maximum	Mean
Stomatal conductance	T1	4.53	5.90	5.22
	T2	2.83	4.50	3.67
	T3	0.80	2.66	1.73
Dry root weight %	T1	0.01	0.93	0.47
	T2	1.18	7.00	4.09
	T3	7.53	28.0	17.8
Dry shoot weight %	T1	7.92	30.0	18.96
	T2	1.38	7.73	4.56
	T3	0.01	1.08	0.55
Chlorophyll content index	T1	3.53	5.53	4.53
	T2	2.32	4.32	3.32
	T3	2.20	3.20	2.70
Relative water content %	T1	54.7	59.9	57.3
	T2	50.5	53.7	52.1
	T3	45.7	48.6	47.1
Fresh root weight (g)	T1	0.06	0.14	0.10
	T2	0.16	0.31	0.24
	T3	0.35	0.86	0.61
Shoot fresh weight (g)	T1	0.53	1.29	0.91
	T2	0.32	0.52	0.42
	T3	0.13	0.31	0.22
Root Length (cm)	T1	2.07	9.64	5.85
	T2	9.66	12.5	11.1
	T3	13.7	20.1	16.9
Shoot Length (cm)	T1	11.6	16.9	14.3
	T2	7.77	10.5	9.15
	T3	3.54	7.74	5.64
Root to Shoot	T1	0.19	0.92	0.56
	T2	0.94	1.86	1.40
	T3	1.98	5.42	3.70

T1 (control); T2 (15% PEG solution); T3 (25% PEG solution)

Simple linear correlation analysis was performed to find out the association of different quantitative attributes under three treatments (Fig.1, 2 & 3). Correlation estimates among various variables, notably yield and yield components, and the assessment of cause-and-effect correlations between them assist breeders in selecting the most optimal yield components, resulting in increased yield. The present study found that shoot fresh weight was positively correlated with chlorophyll content index, and relative water content was highly significantly associated with dry shoot weight under normal conditions (T1) (Fig. 1). These results concur with previous studies by Ahmed et al., (2019), Belay et al., (2021), and Mohi-Ud-Din et al., (2021) who reported similar correlations among these traits. Relative water content is considered an important selection criterion for drought tolerance at the seedling stage, as concluded by Almeselmani et al. (2011). Under moderate drought stress (T2), dry shoot weight exhibited a significant positive association with dry root weight. Chlorophyll content index was also positively correlated with shoot fresh weight under both T2 (Fig. 2) and severe drought stress (T3) (Fig. 3). Fresh weights have been characterized as major indicators for selecting drought-tolerant seedlings by Ahmed et al., (2022). The positive associations between chlorophyll content index, relative water content, and fresh weights suggest that these traits may be useful indicators for identifying wheat genotypes capable of maintaining growth and physiological function under increasing moisture limitation stress in future studies.

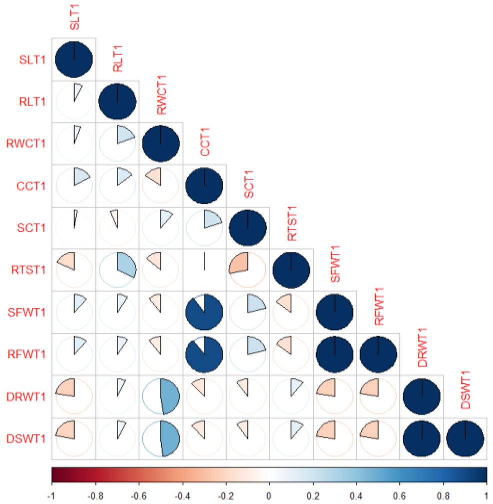

Fig. 1 Pearson's correlation coefficients between all studied traits under 1st using pie graphs for correlation to compare the sizes of categories to the entire dataset. These consist of a circle (i.e., the pie) with slices representing subgroups. The size of each slice is proportional to the relative size of each category out of the whole. Each circle represents the individual variables with different color. Correlation values ranged from -1 to 1 which also indicated by different colors for respective associated traits. (Traits Abbreviations: SL: shoot length, RL: root length, RWC: relative water contents, CC: chlorophyll content index, SC: stomatal conductance, RTST: root to shoot ratio, SFW: shoot fresh weight, RFW: root fresh weight, DRW: dry root weight, DSW: dry shoot weight).

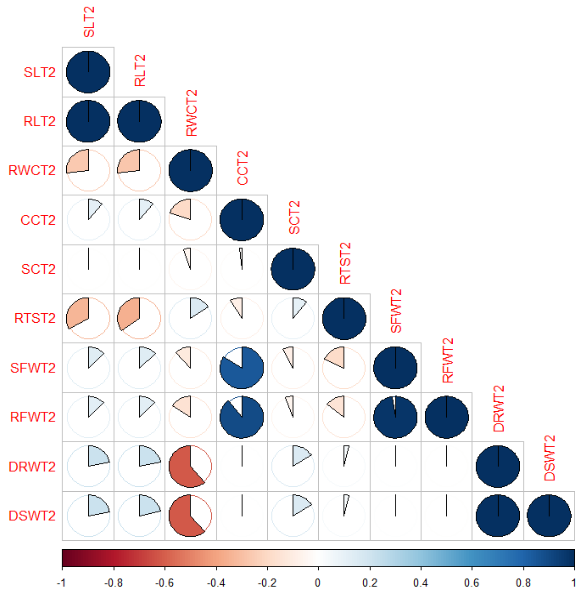

Fig. 2. Pearson's correlation coefficients between all studied traits under 2nd treatments respectively using pie graphs for correlation to compare the sizes of categories to entire dataset (See traits abbreviations in Fig. 1).

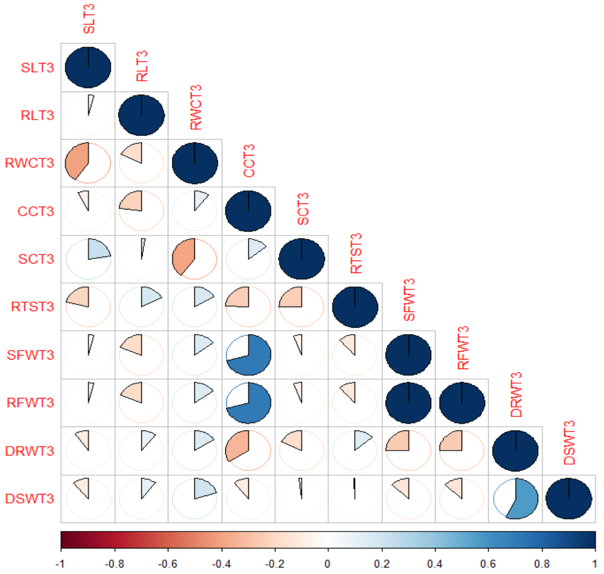

Fig. 3. The figures show Pearson's correlation coefficients between all studied traits under 3rd treatment (See traits abbreviations in Fig. 1).

Principal Component Analysis (PCA)

Principal component analysis (PCA) was performed to transform the data into principal components or factors. This transformation represents different proportions of data variation. The factors (Fs) correspond to the number of variables studied. The present study transformed the data into 10 Fs which followed the pattern that the 1st factor (F1) showed the most variability followed by F2 and so on until the last fact (Fn) corresponding to the least present variability in the data set. Here in the analysis, the factors from 1 to 4 had the effective data transformation with more variability (Eigen value greater than 1) (Table 4). These first 4 PCs showed 66.4%, 82.9% and 65.9% variation under 1st, 2nd and 3rd treatments respectively.

Table 4 Eigenvalues, variability % and cumulative % of traits under three different treatments

		F1	F2	F3	F4
Eigenvalue	T1	2.19	1.76	1.55	1.14
	T2	2.95	2.78	1.50	1.07
	T3	2.15	1.81	1.52	1.12
Variability (%)	T1	21.9	17.6	15.5	11.4
	T2	29.5	27.7	15.0	10.7
	T3	21.5	18.0	15.2	11.2
Cumulative (%)	T1	21.9	39.6	55.1	66.5
	T2	29.5	57.3	72.2	82.9
	T3	21.5	39.5	54.7	66.0

T1 (control); T2 (15% PEG solution); T3 (25% PEG solution)

To explain the percentage variability for each component, biplots were constructed. Usually, maximum variation can be attributed to the PC1, being most beneficial due to its maximum diversity to select the genotypes. In PCA biplots (Fig.4, 5 & 6), the variables in the graphs were shown as lines called vectors. The genotypes closer to the origin showed least amount of variation in terms of genetic diversity and drought tolerance potential and those far away from the origin referred to as best performing with reference to their respective traits. This use of multivariate PCA enabled effective data reduction and extraction of the key sources of variation from the intricate dataset. The PCA biplots clearly distinguished diverse and potentially drought-tolerant genotypes for further targeted study, demonstrating the value of this technique for dissecting complex trait inter-relationships. Using PCA for genotype evaluation provides breeders with visual tools to simplify complex data into informative principal components that capture the key response patterns.

The PCA biplot facilitates visual interpretation of trait correlations based on the angles between their vector lines, an established analytical approach in previous studies (Abdi & Williams 2010; Gholizadeh & Dehghani, 2016; Shirvani et al., 2021). Acute angles under 90° represent positive correlations, while obtuse angles over 90° denote negative correlations between trait vectors. In the biplot for the control treatment (Fig. 4), the traits of chlorophyll content index and shoot fresh weight exhibited long vector lines pointing in similar directions. This indicates strong positive correlation and high variability, as validated by the correlation analysis. In contrast, the fresh root weight vector was short and plotted near the origin, indicating minimal diversity for this trait across genotypes, aligning with previous observations of constrained root variability under normal conditions (Watt et al., 2005; Yang et al., 2020)**.** The genotypes themselves can also be interpreted based on their spatial distribution. Genotype 19 (G19) displayed the longest vector distant from the origin, signifying maximum variability and a diverse response. Meanwhile, G14 and G28 clustered near the origin, indicative of minimal genotype variation under control conditions (Mwadzingeni et al., 2016; Zhou et al., 2018; Mansour et al., 2020). Thus, the PCA biplot effectively encapsulated the key patterns of trait relationships and genotype diversity from the complex dataset into a simplified visual output, as demonstrated in previous studies. This aids rapid interpretation of the differences in genotypic response to the moisture regimes. Similar to the control treatment, the vectors for chlorophyll content index and shoot fresh weight also exhibited longer lengths compared to other trait vectors in the PCA biplots for the moderate (T2) and severe (T3) drought stress treatments Fig 5 and 6, respectively). The continued maximal variability and strong positive correlation between chlorophyll content and shoot biomass under moisture limitation aligns with prior studies. Previous researchers have reported that these traits are tightly coupled, as chlorophyll aids photosynthetic carbon assimilation to support growth processes (Akram 2011; Karimpour 2019; Chowdhury et al., 2021).

Maintenance of chlorophyll content and shoot weight is therefore considered indicative of continued physiological function and growth despite low water availability (Farooq et al. 2008; Sattar et al. 2020). The visual patterns in the PCA biplots concur, consistently showing these traits as key and positively correlated determinants of plant productivity and health even as moisture decreases. The PCA vector patterns thereby validate chlorophyll content and shoot fresh weight as useful selection criteria to identify wheat genotypes capable of sustaining physiological activity under both moderate and severe drought stress in breeding programs. These traits display continued diversity and positive correlation across all three moisture regimes examined.

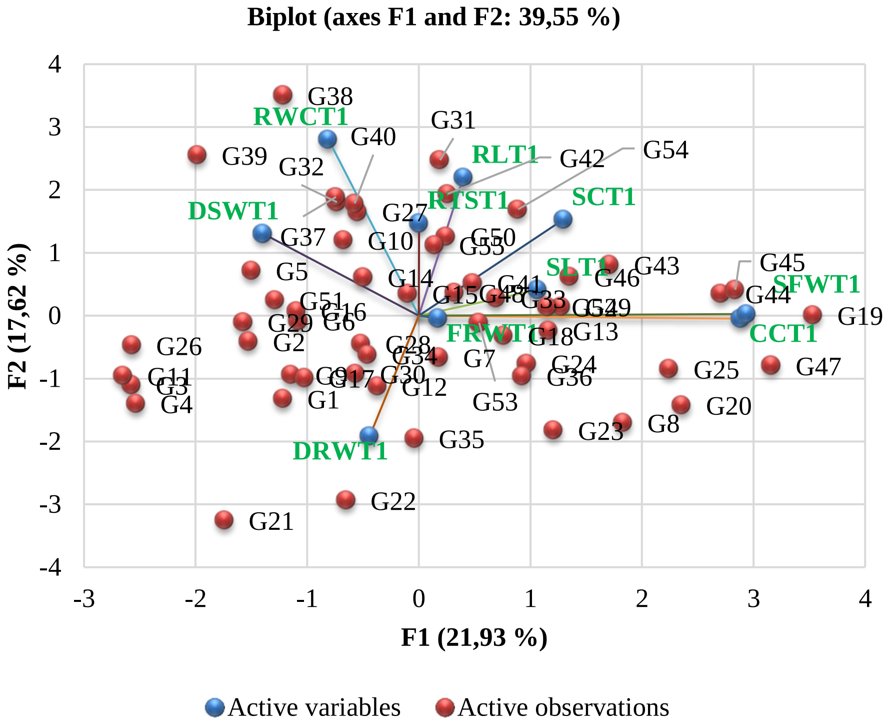

Fig. 4. PCA biplots for 55 genotypes of wheat under stress condition (1st) treatment (Please see Fig. 1 for trait abbreviations).

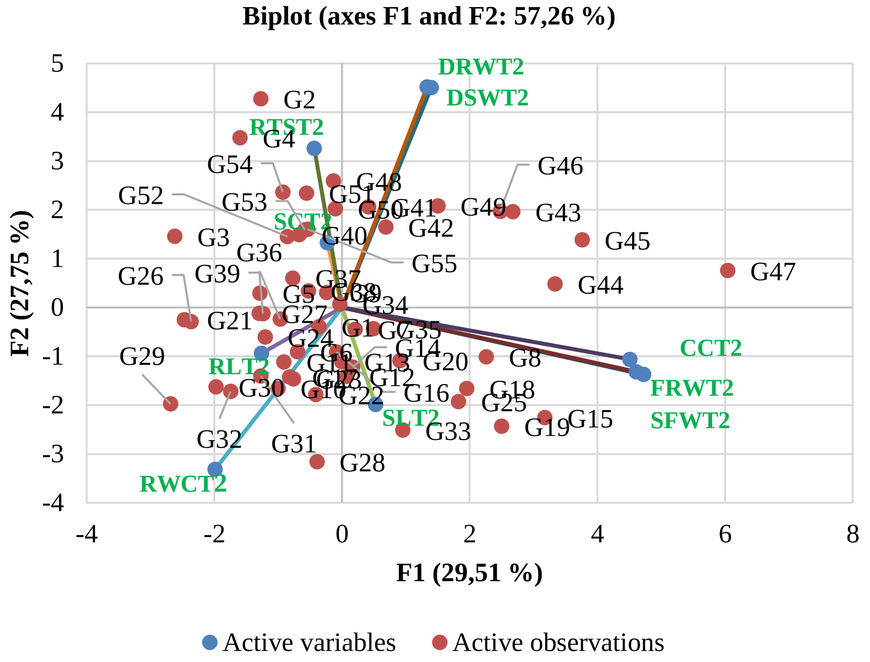

Fig. 5. PCA biplots for 55 genotypes of wheat under stress condition (2nd) treatment (Please see Fig. 1 for trait abbreviations).

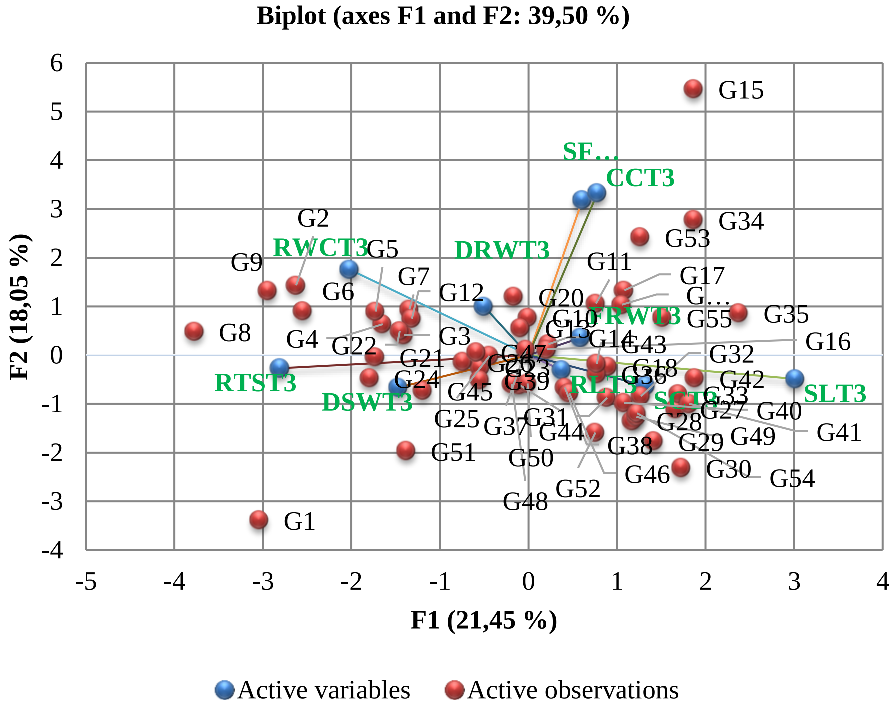

Fig. 6. PCA biplots for 55 genotypes of wheat under stress condition (3rd) treatment (Please see Fig. 1 for trait abbreviations)

These two traits were also positively associated as discussed in the previous section. The majority of the genotypes fall in the 3rd quadrant (negative quadrant) and 2nd quadrant (positive quadrant) under T2 and T3 respectively. The genotypes with maximum vector length and fewer angles were G47 and G35 under T2 and T3 respectively (Fig. 4, 5 & 6). The results of the PCA in the present study align with the findings of Khan et al., (2023), who also employed multivariate analytical techniques for genotype evaluation. In their work, Khan et al., (2023) found that out of 12 initial PCs, the first 4 PCs explained 83.33% and

79.12% of total variation under normal and drought conditions respectively.

Similarly, this experiment revealed that the first 4 PCs extracted from the 10 original traits accounted for 66.4-82.9% of variance across the three moisture regimes examined. Both studies demonstrate that the majority of the variation in complex multi-trait datasets can be effectively captured in just the first few PCs. This confirms the power of multivariate PCA to simplify intricate datasets down to the key drivers of variability. The congruence in findings also validates the utility of evaluating just the first few major PCs, rather than attempting to interpret all 10+ original traits independently. Focusing on the leading PCs as demonstrated by Khan et al., (2023) and this study provides an efficient and informative reflection of the genotypes' overall performance.

Conclusion

The study utilized multivariate techniques to examine the effects of drought stress on morphological and physiological traits of bread wheat at the seedling stage. The results showed a significant decline in all parameters under drought stress, except for root length, root fresh and dry weights, and root to shoot ratios which were increased. Furthermore, among all genotypes the promising and diverse genotypes were G19, G47 and G35 under different PEG concentrations, highlighting the importance of genotypic variation in plant responses to drought stress. The results of the association analysis can assist breeders in selecting optimal yield components leading to increased yield. The study's findings also have important implications for developing strategies to enhance crop yield under different environmental conditions, which is critical for ensuring sustainable food security in the context of climate change.

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