Journal of Pure and Applied Agriculture (2023) 8(4): 20-29
ISSN (Print) 2617-8672, ISSN (Online) 2617-8680
http://jpaa.aiou.edu.pk/

Early selection and assessment of drought tolerance in bread wheat germplasm

Hafiz Ghulam Muhu-Din Ahmed[1]*, Muhammad Moaaz Mushtaq[1], Aziz Ullah[2], Zain Ul Abdeen[1], Muhammad Ajmal[1], Muhammad Hamza Rafiq[1] and Ali Usama[1]

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

*Corresponding Author: Hafiz Ghulam Muhu-Din Ahmed (ghulam.muhudin@iub.edu.pk)

Abstract

Drought can significantly impact on wheat and can lead to reduced yields, lower quality grains, increased susceptibility to pests and disease in many parts of the world. An experiment was conducted to investigate the response of 100 wheat genotypes in the seedling stage under normal and drought conditions using a Completely Randomized Design. The analysis of variances (ANOVA) showed a highly significant difference among the genotypes in all studied traits. The results of correlation displayed a positive result with all traits expect root length in normal and drought conditions. Among the genotypes namely G-217 (Chakwal-97), G-5 (Pakitan-13), G-307 (Kohsar-95), G-17 (NIFA-LALMA) and G-38 (Shahkar-13) showed highest mean values, while the genotypes namely G-301 (BARS-09), G-104 (AS-02), G-10 (AARI-2011), G-202 (Sehar-06) and G-37 (Miraj-2008) exhibited the lowest means values. The Pearson correlation results indicated that all seedling variables, except for root length under normal and drought stress, displayed high positive and significant coefficients of association. However, root length exhibited negative and non-significant association with other studied traits and its selection seems not to be promising criteria for this germplasm for drought stress. Overall results suggested that selection for chlorophyll content, shoot length, root fresh and dry weight, shoot fresh and dry weight, turgid weight and seedling dry weight at seedling stage would improve genetic gain for drought tolerance.

Keywords: Drought, Genotypes, Grain, Variability, Wheat

To cite this article: Ahmed, H. G. M. D., Mushtaq, M. M., Ullah, A., Abdeen, Z. U., Ajmal, M., Rafiq, M. H., & Usama, A. (2023). Early selection and assessment of drought tolerance in bread wheat germplasm. *Journal of Pure and Applied Agriculture, 8*(4), 20-29.

Introduction

Common wheat (*Triticum aestivum* L.), commonly known as bread wheat, is an annual grass species that is primarily autogenous and a member of the Triticale tribe of the Poaceae family (Shehzad et al., 2022; Shafqat et al., 2023; Shaheen et al., 2023; Shehzad et al., 2023). It has 21 chromosomal pairs grouped into three sub genomes, A, B, and D, making it hexaploid species (genome BBAADD, 2n = 6x = 42) (Khan et al., 2023). The polyploidy levels of wheat include bread wheat (hexaploid) (2n = 6x = 42), tetraploids (2n = 4x = 28), and diploids (2n = 2x = 14). Its domestication began in the Fertile Crescent, and extended throughout the world, being cultivated in an unparalleled range of locations, ranging from 45° S in Argentina to the furthest north of 67° N in Norway, Finland, and Russia. As part of the "Neolithic Revolution," which saw a transition from searching for livelihood to established agriculture, wheat was first planted around 10,000 years ago. The top two countries for wheat production were China and India, with yearly production reaching a record high of 770 MT in 2020 and 2021. The amount of wheat produced worldwide in 2022 has decreased by 2.7 million tonnes. Winter wheat plantings are still going on in the European Union due to generally favorable weather conditions that favor crop emergence (Ahmed et al., 2022b).

Wheat production in Pakistan not only fulfills the nation's food requirements but also plays a pivotal role in contributing to the country's GDP. The wheat crop constitutes 1.8 percent of Pakistan's GDP and makes up a noteworthy 9.2 percent of its overall agricultural output. This contribution significantly influences both the GDP and the broader agricultural sector, highlighting the crucial role that wheat farming plays in the economic landscape of the country (Khan et al. 2023). Pakistan is one of the agricultural nations with a sizable quantity of land that is cultivated. It is often believed that the main purpose of wheat is to provide energy in the form of carbohydrates, and this is true. However, it also contains significant amounts of other vital nutrients, including fibre, proteins, and minor components like lipids, vitamins, minerals, and phytochemicals, all of which may promote a healthy demise (Shewry & Hey, 2015; Khan et al., 2016; Anser et al., 2018; Ullah et al., 2023). Wheat is a crucial crop that provides 19.0% of the world's daily calories and 20.8% of its protein needs (Farrukh Saleem et al. 2022). 12.6% water, 1.7% minerals, 11.3% protein, 13.2% fibre, 1.8% fats, and 59.4% carbohydrates make up its composition (Koehler & Wieser 2012; Shafqat et al., 2019; Mehmood et al., 2020; Shehzad et al., 2022). Estimates of World population in

2050 is estimated to 9.5 billion and demand for wheat to be reached about 3 billion tons (Elferink & Schierhorn, 2016). But wheat production decreases due to diseases mostly caused by rust. Brown rust and yellow rust decreased the production of wheat significantly (Victoria et al., 2023). Environmental stress exerts a substantial impact on the yield which is a complex characteristic. The production of essential crops faces heightened challenges due to environmental factors becoming primary concerns in the wake of a changing climate. Global climate changes, an increasing scarcity of water, and deteriorating environmental conditions all had a negative impact on wheat production. This has threatened the rising population's ability to eat nutritiously (Khan et al., 2023). Drought is a primary abiotic stress that reduces global wheat quality and yield. The mechanisms of drought in wheat are complex and involve physiological, biochemical, and molecular processes.

In numerous countries around the world, drought and water shortage constitute edaphic stresses that exerts highly adverse effect on plant growth and agricultural productivity (Comas et al., 2013). Drought stress diminishes the amount of available water for the plant, which can lead to reduced water uptake by the roots. This decline in water absorption may be attributed to alterations in root morphology, including reductions in root length, density, and surface area (Ali et al., 2023). Moreover, drought stress can also lead to stomata closure, which reduces transpiration and conserves water. Nevertheless, an excessive closure of stomata can lead to diminished photosynthesis and hindered growth (Chaves et al., 2002). In response to the accumulation of solutes like sugars, amino acids, and proline during circumstances of drought stress, plant cells may suffer an osmotic imbalance. This osmotic stress can lead to cell dehydration, reduced cell expansion, and ultimately, reduced plant growth (Liu et al., 2015). This study aims to contribute to the early selection and evaluation of drought tolerance in bread wheat germplasm by identifying key traits and genotypes. These can serve as valuable indicators for breeding programs focused on enhancing wheat resilience to drought stress.

Material and Methods

The experiment was carried out at the experimental block of the Department of Plant Breeding and Genetics (PBG), The Islamia University of Bahawalpur (IUB), Pakistan, which is located at 29.24°N and 71.41°E. In this study, 100 different genotypes (Table 1) were grown in triplicate fashion using a complete randomized design (CRD) under normal conditions (100% field capacity) and drought conditions (50% field capacity) at the seedling stage. Experimental wheat accessions were planted in 10×10 cm polyethylene bags filled with sand. Each bag containing three seeds was placed and after the germination process was completed, to achieve one wheat seedling, the thinning operation was carried out. Three bags were utilized for each genotype for each replication. After applying water upon sowing, one group of genotypes received regular irrigation (100% of the field's capacity), whereas another group of genotypes with similar characteristics was kept under water-deficient stress (at 50% of the field's capacity). The pressure chamber device was used to determine the soil's field capacity (Gugino et al., 2009). Data of the different attributes like shoot length, root length, fresh shoot weight, fresh root weight, dry shoot weight, dry root weight, chlorophyll content, and turgid weight were recorded after 21 days of the germination from both of the normal and drought conditions. Data that were recorded was analyzed for analysis of variances (ANOVA) (Steel & Torrie 1986) using statistix 8.1 software. For highly significant effects, $\alpha = 0.01$ was the significance level, while for significant effects, $\alpha = 0.05$. RADAR-graph was developed using Excel-Stat (Ahmed et al. 2019). Pearson's correlation coefficients (r) were used in order to determine the relationship between the studied seedlings attributes under normal and drought conditions.

Table 1 A list of 100 studied wheat genotypes (code and genotypes name mentioned)

Sr. No.	Code	Variety Name	Sr. No	Code	Variety Name	Sr. No	Code	Variety Name
1	G-1	SHALKOT-14	35	G-35	S-24	69	G-119	ASS-15-STRN
2	G-2	AZRC-1	36	G-36	BARSAT-10	70	G-120	SILVER-BLUE
3	G-3	LALMA-13	37	G-37	ATTA-HABIB-10	71	G-201	TD-01
4	G-4	PIRSABAK-2013	38	G-38	Ufaq	72	G-202	FSD-08
5	G-5	AAS-2011	39	G-39	NARC 2009	73	G-203	NISHAN-E-BAKHAR
6	G-6	GALAXY-2013	40	G-40	BARS 2009	74	G-204	SEHER-6
7	G-7	BENAZIR	41	G-41	FAISALABAD 2008	75	G-205	SHAHFAQ
8	G-8	HAMAL-FAQIR	42	G-42	LASANI 2008	76	G-206	UGALA
9	G-9	NIFA-LALMA	43	G-43	CHAKWAL 50	77	G-207	BALHAR-STAR
10	G-10	NIA SAARANG	44	G-44	MAIRAJ 2008	78	G-208	MH-21
11	G-11	SHAHKAR-2013	45	G-45	GOMAL 08	79	G-209	AKBAR
12	G-12	MILLAT-2011	46	G-46	BATHUR	80	G-210	GALAXY-13
13	G-13	DHARABI-2011	47	G-47	HASHIM	81	G-211	JOHER-16

14	G-14	GOLD16	48	G-48	GOMAL 7	82	G-212	BOURLAG
15	G-15	IHSAN16	49	G-49	PIRSABAK 08	83	G-213	ANNAJ-17
16	G-16	JAUHAR16	50	G-50	PIRSABAK BARANI 05	84	G-214	DILKASH
17	G-17	SEHER 2006	51	G-101	IMDAD 2005	85	G-215	GHAZI-19
18	G-18	NN GANDAM I	52	G-102	KHIRMAN	86	G-216	SUBHANI-19
19	G-19	SINDHU16	53	G-103	SASSUI	87	G-217	FAKHR-e-BAKHAR
20	G-20	DHARABI-2011	54	G-104	SKD-1	88	G-218	SUBHANI-21
21	G-21	PUNJAB-2011	55	G-105	TIJABAN-2010	89	G-219	NAWAB
22	G-22	NIA-SUNDER	56	G-106	SHAFAQ 2006	90	G-220	AS-02
23	G-23	NARC-2011	57	G-107	FAREED 2006	91	G-301	SUPER
24	G-24	AARI-2011	58	G-108	RASKOH 2005	92	G-302	MARKAR
25	G-25	NIFA-BARSAT-10	59	G-109	AARI	93	G-303	ZINKOL
26	G-26	JANBAZ-10	60	G-110	15-STRN-NAWAB	94	G-304	BORLAUG
27	G-27	SIRAN-2007	61	G-111	178	95	G-305	V-16164
28	G-28	ATA HABIB 2010	62	G-112	204171	96	G-306	UJALA-16
29	G-29	KT 2009	63	G-113	204164	97	G-307	NARC-11
30	G-30	NIA AMBER	64	G-114	SADIQ-15-STRN	98	G-308	MH-97
31	G-31	NIA-SUNEHRI	65	G-115	214313	99	G-309	PASBAN
32	G-32	AMIN-2008	66	G-116	10-SATY	100	G-310	NARC-2009
33	G-33	SUREN 2010	67	G-117	HTYT-9			
34	G-34	KT-2010	68	G-118	GOLD			

Result and Discussion

Shoot length

The measurement of shoot length is vital, as it serves as a reliable indicator of a plant's overall growth and development under drought stress. Longer shoots often signify better adaptation and resilience to water scarcity. Data recorded for analysis of variance (ANOVA) is represented in Table 2. The result of ANOVA showed that shoot length had a highly significant effects between genotypes and genotypes ×environments interaction. The genotypes G217 (19.80 cm), G5 (19.10 cm), G307 (18.90 cm), G17 (18.30 cm) and G38 (17.90 cm) showed the highest mean values of shoot length as mentioned in Fig. 1 in the non-stressed environment (Fig. 1). The genotypes G301 (9.20 cm), G104 (9.10 cm), G37 (8.90 cm), G202 (8.70 cm) and G10 (8.32 cm) showed the lowest mean values of shoot length indicated that they showed poor performance in the same environment. The genotypes G17 (16.76 cm), G307 (16.17 cm), G5 (15.54 cm), G217 (15.23 cm) and G38 (15.23 cm) had the highest mean values of shoot length against drought stress environment which indicated that these genotypes were drought tolerant due to best performance. The genotypes G301 (6.99 cm), G104 (6.98 cm), G10 (6.87 cm), G202 (5.99 cm) and G37 (5.98 cm) had the lowest mean value for shoot length (Figure 1). In a study by (Ahmed et al., 2022a, Ahmed et al., 2022b), wheat seedlings were subjected to drought and salinity stress for varying durations. They found that shoot length was significantly reduced in all drought treatments compared to well-watered plants. Furthermore, the longer the duration of drought stress, the greater the reduction in shoot length as compared to well-watered plants.

In normal environment shoot length showed the positive and highly significant association with the shoot fresh weight (0.72^{**}), root fresh weight (0.73^{**}), seedling weight (0.47^{**}), turgid weight (0.64^{**}), shoot dry weight (0.62^{**}) and root dry weight (0.57^{**}) while root length (0.12ns) showed non-significant association with shoot length. While in drought stress environment root length (0.28^{**}), shoot fresh weight (0.75^{**}), root fresh weight (0.75^{**}), seedling weight (0.55^{**}), turgid weight (0.68^{**}), shoot dry weight (0.66^{**}) and root dry weight (0.63^{**}) showed the highly significant and positive relationship with shoot length (Table 3). Khan et al. (2011) discovered a positive and highly significant association between fresh shoot weight and dry shoot weight under both normal and drought conditions (Khan et al., 2011). A prolonged seedling and a more extensive root system might lead to improved adaptability to dry land conditions through selection (Ahmed et al., 2022b).

Root length

Root length is a critical trait, reflecting the plant's ability to explore and extract water from the soil. Longer roots contribute to improved water absorption and are essential for maintaining plant health in drought-prone conditions. Table 2 displays the results of an analysis of variance showing that there are statistically highly significant differences between genotypes and treatments for this trait. The genotypes with the highest mean values of root length in a normal environment were G307 (18.56 cm), G17 (18.10 cm), G5 (17.97 cm), G38 (17.90 cm) and G217 (17.78 cm). The lowest mean values of root length in the normal environment were G10 (10.21 cm), G301 (10.20cm), G104 (10.00 cm), G37 (9.98 cm) and G202 (9.30 cm), indicating that these genotypes were not good in performance. The genotypes which were good in performance in drought environment were G307 (19.87 cm), G217 (19.27 cm), G38 (16.25 cm), G17 (15.43 cm) and G5 (15.33 cm) due to the highest mean values of root length (Fig. 1). The genotypes G10 (11.75 cm), G301 (11.12 cm), G202 (10.98 cm), G104 (10.71 cm) and G37 (10.32 cm) had the lowest mean values of this trait in drought environment which indicated that these genotypes were not good in performance (Fig. 1). Previously wheat scientists (Farooq et al., 2009) examined that drought stress significantly reduced root length in wheat seedlings. The study suggested that reducing root length is one of the adaptive mechanisms of wheat plants to cope with drought stress. They found that wheat seedlings under mild drought stress had longer roots compared to those under non-stress conditions. In normal environment root length showed the positive and highly significant association with shoot fresh weight (0.74^{**}), root fresh weight (0.76^{**}), seedling weight (0.93^{**}), turgid weight (0.83^{**}), shoot dry weight (0.85^{**}) and root dry weight (0.87^{**}). In drought stress environment, shoot fresh weight (0.84^{**}), root fresh weight (0.84^{**}), seedling weight (0.91^{**}), turgid weight (0.89^{**}), shoot dry weight (0.90^{**}) and root dry weight (0.92^{**}) showed the highly significant and positive relationship with shoot length (Table 3). The findings of wheat scientists (Khan et al., 2023) in terms of correlation coefficients showed that root length had a highly positive and significant relationship with shoot fresh and dry weight under drought conditions. They concluded that there was a positive correlation between root length and shoot length, shoot fresh weight, and shoot dry weight as some of our findings. Scientists stated that (Ahmed et al., 2023, Khan et al., 2023, Victoria et al., 2023) that a barrier to cell division and elongation that prevents tuberization may be the cause of the decrease in root length during dry stress. The root system's lignification and tuberization allow the circumstances to return favorably.

Shoot fresh weight

The measurement of shoot fresh weight is crucial in assessing the plant's immediate response to drought stress. A higher fresh shoot weight indicates better water retention and overall stress tolerance. Results of analysis of variance showing that there are highly significant differences between genotypes and treatments (Table 2) also showed the significant interaction between the studied genotypes and the environments. In a normal environment the genotypes G5 (0.22 g), G307 (0.22 g), G38 (0.20 g), G17 (0.19 g) and G217 (0.19 g) exhibited the highest mean values of shoot fresh weight which indicated that these genotypes were good in performance. The genotypes G104 (0.10 g), G301 (0.09 g), G37 (0.09 g), G10 (0.08 g) and G202 (0.08 g) indicated the lowest mean values of this trait indicated that they were poor performance in a normal environment (Fig. 2). The genotypes G217 (0.18 g), G307 (0.18 g), G17 (0.17 g), G5 (0.16 g) and G38 (0.17 g) had the highest mean values of shoot fresh weight in drought stress environment, indicated that they were drought tolerance. The genotypes G10 (0.07 g), G202 (0.07 g), G37 (0.06 g), G104 (0.06 g) and G301 (0.05 g) exhibited the lowest mean values of shoot fresh weight demonstrating their poor performance in a drought stress environment (Fig. 2). In the previous study, the scientists (Almaghrabi, 2012) examined how several wheat genotypes responded to drought stress at the seedling stage. The findings demonstrated that all tested genotypes significantly decreased shoot fresh weight under drought stress as results obtained in the present study. Shoot fresh weight showed highly positive and significant association with root fresh weight (0.88^{**}), seedling weight (0.91^{**}), turgid weight (0.93^{**}), dry shoot weight (0.90^{**}) and dry root weight (0.95^{**}). In drought stress environment root fresh weight (0.85^{**}), seedling weight (0.87^{**}), turgid weight (0.91^{**}), dry shoot weight (0.89^{**}) and dry root weight (0.91^{**}) showed the highly significant and positive relationship with shoot length (Table 3). In an experiment conducted by wheat breeders (Ahmed et al., 2022b), in both normal and drought conditions, they identified a favorable and highly significant correlation between seedling fresh weight and seedling dry weight.

Root fresh weight

Root fresh weight is a tangible measure of a plant's capacity to produce new root biomass, demonstrating its ability to adapt and thrive in challenging drought conditions. The analysis of variance results presented in Table 2 which showed highly significant differences among genotypes and environments for root fresh weight. Under normal environment the genotypes G307 (0.09 g), G17 (0.09 g), G217 (0.08 g), G38 (0.07 g) and G5 (0.07 g) had the highest mean values of root fresh weight indicated that these genotypes were good in performance. The genotypes G104 (0.03 g), G301 (0.03 g), G10 (0.03 g), G37 (0.03 g) and G202 (0.03 g) had the lowest mean values of this trait indicated that they were poor performance in a normal environment (Fig. 2). The genotypes G38 (0.13 g), G17 (0.11 g), G217 (0.11 g), G5 (0.09 g) and G307 (0.09 g), act as superior genotypes, due to showing the largest mean values against drought stress environment for this attribute. The genotypes G37 (0.06 g), G104 (0.06 g), G10 (0.06 g), G301 (0.05 g) and G202 (0.05 g) showed the lowest mean values of root fresh weight demonstrated their poor performance in drought stress environment (Fig. 2). Mahpara et al., (2022) was revealed that wheat seedlings at various growth stages had their root fresh weight reduced significantly by drought stress. Root fresh weight showed the positive and highly significant association with seedling weight (0.94^{**}), turgid weight (0.87^{**}), shoot dry weight (0.91^{**}) and root dry weight (0.97^{**}). On the other hand, seedling weight (0.91**), turgid weight (0.83**), shoot dry weight (0.87**) and root dry weight (0.92**) showed the highly significant and positive relationship with root fresh weight in drought environment (Table 3). Previously, wheat scientists found that (Rauf et al., 2007) significant positive correlation between root fresh weight and shoot fresh weight and shoot dry weight in wheat seedlings grown under drought stress as present results accordance with their results.

Table 2 Mean sum of squares of analysis of variance (ANOVA) for studied genotypes

Source	DF	SL	RL	CC	RFW	RDW	SFW	SDW	TW
REP	2	4.41	35.83	0.009	0.003	0.0001	0.00466	0.00148	0.00996
VAR	99	9.05**	13.52**	26.52**	0.0004**	0.00005**	0.002**	0.00016*	0.003**
ENV	1	1231.06**	59.97**	0.47**	0.24**	0.03**	0.24**	0.023**	0.17**
VAR*ENV	99	10.85**	12.73**	0.50**	0.0004**	0.00005**	0.003**	0.00028**	0.0043**
Error	398	5.33	7.285	0.033	0.0002	0.00003	0.00114	0.00012	0.00197
Total	599								

Significant **Highly Significant **=p (0.00-0.01) *=p (0.05-0.05), DF= Degree of freedom, SS= Sum of square, MS= Mean of square, F= Calculated value, SL= Shoot length, RL= Root length, SFW= Shoot Fresh weight, RFW= Root Fresh weight, TW= turgid weight, SDW= Shoot Dry weight, RDW= Root Dry weight, CC=Chlorophyll contents

Table 3 Pearson correlation under normal and drought conditions

		CC	SL	RL	SF	RF	TW	SD
SL	N	0.73**						
	D	0.67**						
RL	N	-0.07ns	0.12ns					
	D	-0.14ns	0.28*					
SFW	N	0.71**	0.72**	0.74**				
	D	0.57*	0.75**	0.84**				
RFW	N	0.01ns	0.73**	0.76**	0.88**			
	D	0.11ns	0.75**	0.84**	0.85**			
TW	N	0.72**	0.64**	0.83**	0.93**	0.87**		
	D	0.63**	0.68**	0.89**	0.91**	0.83**		
SDW	N	0.71**	0.62**	0.85**	0.90**	0.91**	0.93**	
	D	0.67**	0.66**	0.90**	0.89**	0.87**	0.90**	
RDW	N	0.01ns	0.57**	0.87**	0.95**	0.97**	0.88**	0.92**
	D	0.18ns	0.63**	0.92**	0.91**	0.92**	0.88**	0.90**

Significant **Highly Significant **=p (0.00-0.01) *=p (0.05-0.05), ns= non-significant SL= Shoot length, RL= Root length, SFW= Shoot Fresh weight, RFW= Root Fresh weight, TW= turgid weight, SDW= Shoot Dry weight, RDW= Root Dry weight, CC=Chlorophyll contents

Shoot dry weight

Shoot dry weight is an important metric for evaluating a plant's ability to allocate resources effectively and sustain growth even when water is limited. This trait reflects the plant's resilience over the long term. The analysis of variance presented in Table 2 showed the significant differences among genotypes and treatments for shoot dry weight. In the normal environment, the genotypes which were good in performance G217 (0.08 g), G307 (0.07 g), G5 (0.07 g), G17 (0.06 g) and G38 (0.05 g) had the highest mean values of shoot dry weight as shown in Fig. 3. The genotypes G37 (0.02 g), G202 (0.02 g), G104 (0.02 g), G10 (0.01 g), G301 (0.01 g) showed the lowest mean values of shoot dry weight (Fig. 3) indicated their poor performance in a normal environment. The genotypes which were good in performance were G307 (0.06 g), G17 (0.06 g), G5 (0.05 g), G38 (0.05 g) and G217 (0.05 g) due to their highest mean values. The genotypes G301 (0.02 g), G104 (0.02 g), G10 (0.02 g), G37 (0.01 g) and G202 (0.01 g) showed the lowest mean values of shoot dry weight which indicated that these genotypes performed best in the drought stress conditions (Fig. 3). In previous research studies conducted by Ahmed et al. (2022b), Ahmed et al., 2023, drought stress significantly reduced the shoot dry weight in wheat seedlings. They reported that drought stress reduced shoot dry weight by 35-40% compared to well-watered conditions. Shoot dry weight showed a positive association with root dry weight. Also, shoot dry weight (0.92**)

showed the strong correlation with root dry weight in normal environments (Table 3). Previously wheat scientists (Farooq et al., 2009) found that both root length and root dry weight had a significant association with shoot dry weight, and plant height in wheat seedlings under drought conditions.

Root dry weight

Root dry weight is a reliable indicator of a plant's resilience, as it reflects the ability to maintain essential structures even in the absence of water. This trait highlights the long-term adaptability of the plant to drought stress. The result of the analysis of variance (ANOVA) showed that root dry weight had a highly significant interaction between genotypes and environments as presented in Table 2. The highest mean values of root dry weight were found for the genotypes G17 (0.07 g), G307 (0.07 g), G217 (0.06 g), G5 (0.05 g) and G38 (0.05 g). This indicated that these genotypes performed well in a normal environment. The lowest mean values of root dry weight showed the poor performance of the genotypes G202 (0.01g), G37 (0.01g), G301 (0.01 g), G104 (0.01 g) and G10 (0.01 g) under normal environment (Fig. 3). The genotypes which were good in performance G17 (0.05 g), G307 (0.05 g), G38 (0.05 g), G217 (0.04 g) and G5 (0.04 g) had the highest mean values of root dry weight (Fig. 3). The genotypes G202 (0.01 g), G301 (0.01 g), G37 (0.01 g), G104 (0.01 g) and G10 (0.01 g) showed the lowest mean values of root dry weight among the genotypes indicated their poor performance in a drought stress environment. Drought stress can reduce the root dry weight of wheat seedlings (Liu et al. 2015). The reduction in root dry weight may be attributed to the fact that drought stress limits water availability, which in turn affects plant growth and development, including root growth (Wang et al., 2017).

Chlorophyll content

Chlorophyll content is a key indicator of a plant's photosynthetic activity. Maintaining adequate chlorophyll levels, even under drought stress, is essential for sustaining efficient photosynthesis and overall plant productivity. The analysis of variance presented in Table 2 showed higly significant differences among genotypes and treatments. The highest mean values of chlorophyll content were found for the genotypes G38 (2.38 cm), G17 (1.94 cm), G217 (1.52 cm), G307 (1.47cm) and G5 (1.33cm) and considered to be a good genotype. The lowest mean values of chlorophyll content showed the poor performance of the genotypes G202 (0.28 cm), G301 (0.23 cm), G104 (0.21 cm), G10 (0.20 cm) and G37 (0.18 cm). The genotypes G307 (0.77 cm), G5 (0.68 cm), G217 (0.66 cm), G17 (0.61 cm) and G38 (0.53 cm) had the highest mean values of chlorophyll content. This indicates their high performance under drought-stressed conditions (Fig. 4). The genotypes G10 (0.12 cm), G202 (0.11 cm), G104 (0.09 cm), G301 (0.07 cm) and G37 (0.05 cm) had the lowest mean values of chlorophyll content, demonstrating their poor performance under drought stress situations (Fig. 4). Previously wheat scientists (Sattar et al. 2019) found that drought stress significantly reduced chlorophyll contents in wheat seedlings. Similarly, (Prasad et al., 2011) found that drought stress decreased chlorophyll content in wheat leaves and that this effect was more pronounced at higher levels of stress.

In the normal environment chlorophyll content showed a positive and highly significant association with all the traits except root length. Shoot length, root length, turgid weight, shoot fresh weight and turgid weight and shoot dry weight these all traits are significant association with chlorophyll content. Root fresh weight and root dry weight exhibited non-significant correlation with chlorophyll content as shown in Table 3. On the other hand, in drought stress condition chlorophyll content showed positive associations with all the studied traits except root length. Turgid weight (0.72^{**}), Shoot length (0.73**), and shoot dry weight (0.71**) showed highly significant and positive association with chlorophyll content in drought stress environment while the root length (-0.14 ns) showed the negative and non-significant correlation with chlorophyll content. Remaining parameters like root dry weight (0.18 ns) and root fresh weight (0.11ns) showed the positive but non-significant association with chlorophyll content shown in Table 3. Many scientists (Kaur & Asthir, 2015) also stated the same results that wheat seedlings were subjected to drought stress for varying durations. He also found that shoot length was significantly reduced in all drought treatments compared to well-watered plants. When the degree of water stress increased, the amount of chlorophyll in all wheat genotypes decreased substantially because the thylakoid membranes break down when cells get dehydrated (Liu et al., 2016).

Turgid weight

Turgid weight is a key parameter in understanding the water status of plant cells. Maintaining turgor pressure, especially under drought stress, is essential for preventing wilting and ensuring the overall structural integrity of the plant. The result of the analysis of variance showed that the turgid weight had a highly significant differences between genotypes and the environment (Table 2). The highest mean values of turgid weight were found for the genotypes G17 (0.29 g), G307 (0.29 g), G38 (0.28 g), G217 (0.27 g) and G5 (0.27 g) mentioned in Fig. 4. This indicated that these genotypes performed well in a normal environment. The lowest mean values of turgid weight showed by the genotypes G104 (0.12g), G37 (0.11g), G202 (0.10 g), G10 (0.09 g) and G301 (0.08 g) indicated their poor performance in a normal environment. The genotypes G217 (0.21 g), G17 (0.18 g), G307 (0.17 g), G5 (0.17 g) and G38 (0.17 g) had the highest mean values of turgid weight as shown in Fig. 4 which were good genotypes for drought stressed conditions. The lowest mean values of turgid weight showed by the genotypes G104 (0.09 g), G202 (0.09 g), G10 (0.09 g), G37 (0.08 g) and G301 (0.08 g), illustrating their poor performance in a drought stress environment. Previously wheat scientists (Anjum et al., 2017) stated that water shortage significantly decreased the turgid weight of seedlings, indicating that water deficit can negatively impact plant growth at an early developmental stage as found in the present study. Turgid weight was positively correlated with root length and shoot length in wheat seedlings under drought stress (Awad et al., 2018). This suggests that wheat seedlings with higher turgid weight may be more resilient to drought because they had longer roots and shoots, which can help them access more water and nutrients.

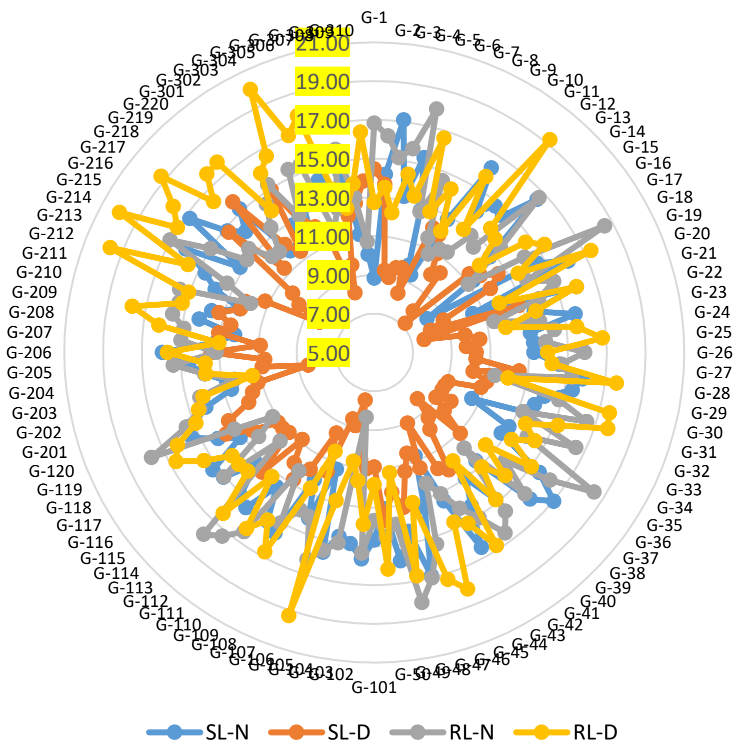

Fig. 1 RADAR graph for shoot length (SL) and root length (RL) under normal and drought conditions

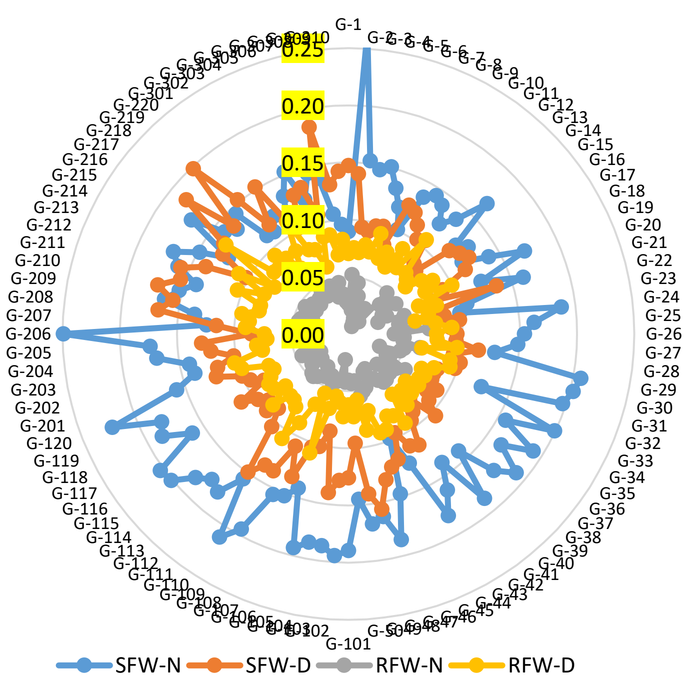

Fig. 2 RADAR Graph for shoot fresh weight (SFW) and root fresh weight (RFW) under normal and drought conditions

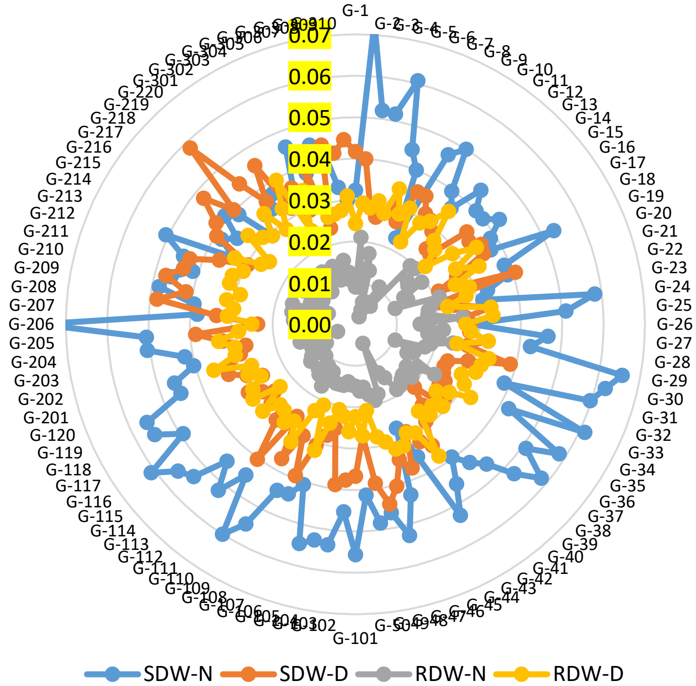

Fig. 3 RADAR Graph for shoot dry weight (SDW) and Root dry weight (RDW) under normal and drought conditions

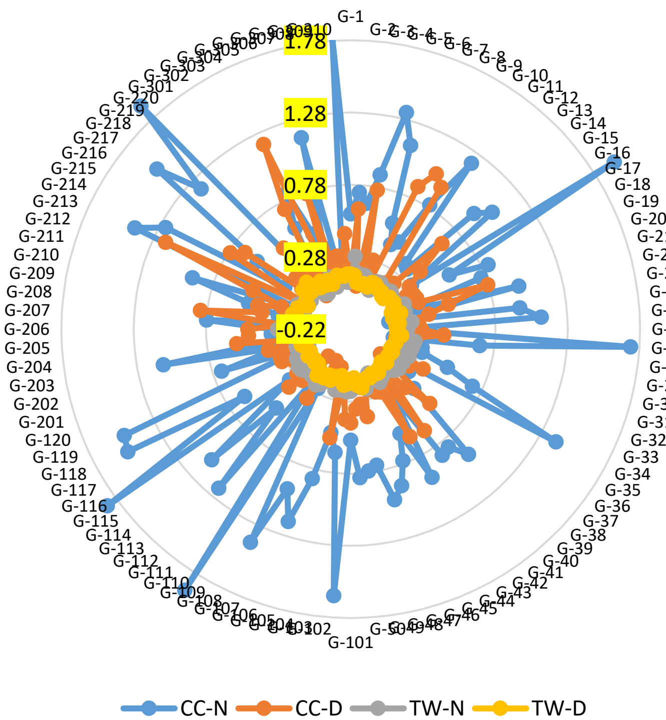

Fig. 4 RADAR Graph for chlorophyll contents (CC) and turgid weight (TW) under normal and drought conditions

Conclusion

In this study, 100 wheat genotypes were evaluated for drought stress using shoot length, root length, chlorophyll content, seedling weight, root fresh weight, root dry weight, shoot fresh weight, shoot dry weight and turgid weight as drought indices. Based on the performance of 100 genotypes, the genotypes with the highest mean values among them were considered as drought tolerant like genotype G-217 (Chakwal-97), G-5 (Pakitan-13), G-307 (Kohsar-95), G-17 (NIFA-LALMA), and G-38 (Shahkar-13), while the genotypes with the lowest mean values were classified as drought sensitive, like G-301 (BARS-09), G-104 (AS-02), G-10 (AARI-2011), G-202

(Sehar-06), and G-37 (Miraj-2008). Overall results suggested that positively associated traits at seedling stage would improve genetic gain for drought tolerance. In order to meet the demand for wheat and provide sustained food security, these best performer genotypes would also be helpful in the development of the highest-yielding, drought-tolerant wheat cultivars.

References

Ahmed, H. G. M.-D., Fatima, N., Faisal, A., Ullah, A., Ali, M., Ameen, M.,……. & Imran, M. (2023). Characterization of bread wheat genotypes using spike related traits for sustainable yield potential. *Journal of Applied Research in Plant Sciences, 4*(1), 469-476.

Ahmed, H. G. M.-D., LI, M.-j., Khan, S. H., & Kashif, M. (2019). Early selection of bread wheat genotypes using morphological and photosynthetic attributes conferring drought tolerance. *Journal of Integrative Agriculture, 18*(11), 2483-2491.

Ahmed, H. G. M.-D., Zeng, Y., Raza, H., Muhammad, D., Iqbal, M., Uzair, M., . . . & El Sabagh, A. (2022). Characterization of wheat (*Triticum aestivum* L.) accessions using morpho-physiological traits under varying levels of salinity stress at seedling stage. *Frontiers in Plant Science, 13*, 953670.

Ahmed, H. G. M.-D., Zeng, Y., Shah, A. N., Yar, M. M., Ullah, A., & Ali, M. (2022). Conferring of drought tolerance in wheat (*Triticum aestivum* L.) genotypes using seedling indices. *Frontiers in Plant Science, 13*, 961049.

Ali, Z., Naeem, M., & Ahmed, H. G. M.-D. (2023). Determination of salinity tolerance in pigmented rice genotypes at seedling stage. *Journal of Crop Health, 76*(1), 297-308.

Almaghrabi, O. A. (2012). Impact of drought stress on germination and seedling growth parameters of some wheat cultivars. *Life Science Journal, 9*(1), 590-598.

Anjum, S. A., Ashraf, U., Zohaib, A., Tanveer, M., Naeem, M., Ali, I., . . . & Nazir, U. (2017). Growth and developmental responses of crop plants under drought stress: a review. *Zemdirbyste-Agriculture, 104*(3).

Anser, M. R., Ahmad, I., Shah, S. H., Abuzar, M. K., Raza, M. S., & Malik, M. A. (2018). Weed control measures for controlling the density of Canada thistle (*Cirsium arvense* (L.) Scop.) in wheat (*Triticum aestivum* L.). *Pakistan Journal of Botany, 50*(1), 355-363.

Awad, W., Byrne, P. F., Reid, S. D., Comas, L. H., & Haley, S. D. (2018). Great plains winter wheat varies for root length and diameter under drought stress. *Agronomy Journal, 110*(1), 226-235.

Chaves, M. M., Pereira, J. S., Maroco, J., Rodrigues, M. L., Ricardo, C. P. P., Osório, M. L., . . . & Pinheiro, C. (2002). How plants cope with water stress in the field? Photosynthesis and growth. *Annals of botany, 89*(7), 907.

Comas, L. H., Becker, S. R., Cruz, V. M. V., Byrne, P. F., & Dierig, D. A. (2013). Root traits contributing to plant productivity under drought. *Frontiers in Plant Science, 4*, 442.

Elferink, M., & Schierhorn, F. (2016). Global demand for food is rising. Can we meet it. *Harvard Business Review, 7*(4), 2016.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. (2009). Plant drought stress: Effects, mechanisms and management. *Sustainable Agriculture*, 153-188.

Farrukh Saleem, M., Bilal Hafeez, M., Ramzan, Y., & Nadeem, M. (2022). Impact of soil applied humic acid, zinc and boron supplementation on the growth, yield and zinc translocation in wheat. *Asian Journal of Agriculture and Biology* (Online).

Gugino, B. K., Abawi, G. S., Idowu, O. J., Schindelbeck, R. R., Smith, L. L., Thies, J. E., . . . & Van Es, H. M. (2009). *Cornell soil health assessment training manual*: Cornell University College of Agriculture and Life Sciences.

Kaur, G., & Asthir, B. (2015). Proline: a key player in plant abiotic stress tolerance. *Biologia plantarum, 59*, 609-619.

Khan, M. A., Waseem Akram, M., Iqbal, M., Ghulam Muhu-Din Ahmed, H., Rehman, A., Arslan Iqbal, H. S. M., & Alam, B. (2023). Multivariate and association analyses of quantitative attributes reveal drought tolerance potential of wheat (Triticum aestivum L.) genotypes. *Communications in Soil Science and Plant Analysis, 54*(2), 178-195.

Khan, M., Shabbir, G., Akram, Z., Shah, M., Ansar, M., & Cheema, N. (2011). Character Association Studies of Seedling Traits in Different Wheat Genotypes under Moisture Stress Conditions. *SABRAO Journal of Breeding and Genetics, 45*(3), 458-467.

Khan, Q., Mumtaz, A. S., Khurshid, H., Jan, S. A., Ahmad, N., Khan, S. A., Saleem, N., Shah, S. H., Ibrahim, M. I., Ilyas, M., & Arif, M. (2016). Exploring durable genetic resistance against leaf rust through phenotypic characterization and Lr34 linked STS marker in wheat germplasm. *Bioscience Journal, 32*(4), 986-998.

Koehler, P., & Wieser, H. (2012). Chemistry of cereal grains *Handbook on sourdough biotechnology* (pp. 11-45): Springer.

Liu, B., An, G., & Gao, X. (2016). Morpho-physiological responses of Alhagi sparsifolia Shap.(leguminosae) seedlings to progressive drought stress. *Pak. J. Bot, 48*(2), 429-438.

Liu, H., Searle, I. R., Mather, D. E., Able, A. J., & Able, J. A. (2015). Morphological, physiological and yield responses of durum wheat to pre-anthesis water-deficit stress are genotype-dependent. *Crop and Pasture Science, 66*(10), 1024-1038.

Mahpara, S., Zainab, A., Ullah, R., Kausar, S., Bilal, M., Latif, M. I., . . . & Elshikh, M. S. (2022). The impact of PEG-induced drought stress on seed germination and seedling growth of different bread wheat (Triticum aestivum L.) genotypes. *PloS One, 17*(2), e0262937.

Mehmood, K., Arshad, M., Ali, G. M., Shah, S. H., Zia, M. A., Qureshi, A. A., & Qureshi, R. (2020). Drought stress tolerance in transgenic wheat conferred by expression of a dehydration-responsive element-binding 1a gene. *Applied Ecology and Environmental Research, 18*(2), 1999-2024.

Prasad, P., Pisipati, S., Momčilović, I., & Ristic, Z. (2011). Independent and combined effects of high temperature and drought stress during grain filling on plant yield and chloroplast EF-Tu expression in spring wheat. *Journal of Agronomy and Crop Science, 197*(6), 430-441.

Rauf, M., Munir, M., ul Hassan, M., Ahmad, M., & Afzal, M. (2007). Performance of wheat genotypes under osmotic stress at germination and early seedling growth stage. *African journal of biotechnology, 6*(8).

Sattar, A., Cheema, M. A., Sher, A., Ijaz, M., Ul-Allah, S., Nawaz, A., . . . & Ali, Q. (2019). Physiological and biochemical attributes of bread wheat (Triticum aestivum L.) seedlings are influenced by foliar application of silicon and selenium under water deficit. *Acta Physiologiae Plantarum, 41*, 1-11.

Shafqat, N., Ahmed, H., Khan, Z., Shehzad, A., Shah, S. H., Islam, M., & Masood, R. (2023). Characterization of wheat-thinopyrum bessarabicum genetic stock for stripe rust and karnal bunt resistance. *Brazilian Journal of Biology, 83*, e246440. https://doi.org/10.1590/1519-6984.246440

Shafqat, N., Ahmed, H., Shehzad, A., Chaudhry, S. K., Shah, S. H., Islam, M., Khan, W., Masood, R., & Khan, U. (2019). Screening of wheat-Thinopyrum bessarabicum addition and translocation lines for drought tolerance. *Applied Ecology and Environmental Research, 17*(5), 10445-10461.

Shaheen, A., Shahzad, A., Shah, S. H., Khattak, S. H., Idrees, S., & Khan, S. U. (2023). Molecular and morphological evaluation of salt tolerance genes in advanced wheat lines. *Pakistan Journal of Botany, 55*(3), 813-823.

Shehzad, R. A., Sarwar, G., Shah, S. H., Tahir, M. A., Sabah, N. U., Muhammad, S., Aftab, M., Manzoor, M. Z., & Shehzad, I. (2022). Efficacy of P enriched organic manures to improve soil health and nutrient acquisition of wheat. *Pakistan Journal of Agricultural Research, 35*(2), 266-273.

Shehzad, R. A., Sarwar, G., Shah, S. H., Tahir, M. A., Sabah, N. U., Muhammad, S., Aftab, M., Manzoor, M. Z., & Shehzad, I. (2022). Efficacy of P enriched organic manures to improve soil health and nutrient acquisition of wheat. *Pakistan Journal of Agricultural Research, 35*(2), 266-273.

Shehzad, R. A., Sarwar, G., Shah, S. H., Tahir, M. A., Sabah, N.-u.-S., Muhammad, S., Aftab, M., Manzoor, M. Z., Shehzad, I., & Saleem, U. (2023). Growth and yield response of wheat to organic manures (farm yard manure, phospho-compost (PROM), and press mud) alone and in combination with mineral fertilizer. *Pakistan Journal of Agricultural Research, 36*(1), 1-8.

Shewry, P. R., & Hey, S. J. (2015). The contribution of wheat to human diet and health. *Food and energy security, 4*(3), 178-202.

Steel, R. G. D, & Torrie, J. H. (1986). *Principles and procedures of statistics: a biometrical approach*: McGraw-Hill New York, NY, USA.

Ullah, G., Khan, E. A., Shah, S. H., Kiran, M., & Haq, F. (2023). Unlocking the secrets of wheat production: Evaluating phosphorus levels and seeding rates in alluvial soil conditions. *Trends in Horticulture, 6*(1), 34-45.

Victoria, O., Idorenyin, U., Asana, M., Shuoshuo, L., & Yang, S. (2023). Seed treatment with 24-epibrassinolide improves wheat germination under salinity stress. *Asian Journal of Agriculture and Biology*.

Wang, M., Schoettner, M., Xu, S., Paetz, C., Wilde, J., Baldwin, I. T., & Groten, K. (2017). Catechol, a major component of smoke, influences primary root growth and root hair elongation through reactive oxygen species-mediated redox signaling. *New Phytologist, 213*(4), 1755-1770.