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Research Article

Characterization of Wheat Genotypes for PEG-induced Physiological Drought Stress at Seedling Stage

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Abstract

Abiotic stresses like drought frequently reduce the productivity of wheat, an important global cereal crop farmed for food, feed and raw materials. Therefore, attempt should be made to search for drought tolerance in wheat cultivars. The purpose of the study was to look into how morphological features of thirty wheat genotypes were affected by PEG-induced physiological drought stress at germination and seedling stages. Seven morphological traits viz. germination percentage, root number, shoot length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight was measured at 14 days of stress condition and significant variation was observed in most of the traits due to genotypes, treatments, and genotype × treatment interaction. Drought stress causes a significant decrease in almost all morphological traits during the germination and early seedling stages, and among the genotypes SA-2 and SA-3 were performed best. A positive significant correlation was found among the morphological traits, except for fresh and dry weight of shoot. Principle Component Analysis (PCA) showed that only first two PCs were significant and had eigenvalues > 1. The first two PCs cover the 72.78% of total variation. Multi Trait Stability Index (MTSI) showed that SA-3, SA-2, BL-1020 and PV-79 performed well in drought stress condition and these genotypes can be further used for breeding program to develop drought tolerant high yielding wheat variety.

Introduction

Wheat (*Triticum aestivum* L.) is one of the world's most important cereal crops, providing a staple food supply for billions of people while also contributing significantly to global food security. In 2021-2022, wheat output is expected to reach 1.85 million tons with a total area of 3, 14, 865 hectares. The wheat research center of the Bangladesh Agricultural Research Institute (BARI) has released a total of 24 improved wheat varieties with a yield potential of 3 tons per hectare. In contrast, several affluent nations achieve yields of 8 tons per hectare (FAO, 1999). Wheat can grow in a number of soil types and environmental conditions. In Bangladesh, wheat is cultivated as a rabi crop, which is sown in November and harvested in March. It requires a daily water supply of 250-350 mm and evapotranspiration necessitates 1.5-4.0 mm of water (Hossain and Teixeira da Silva, 2013a). The main wheat-growing regions in Bangladesh include Rajshahi, Dinajpur, Thakurgaon, Pabna, and Faridpur, which are also recognized as the country's draught-prone area. Of the 5.46 million hectares of drought-prone areas of Bangladesh the northwest's Barind tract is the most drought prone (BBS, 2018), and, it is anticipated that as more land becomes afflicted by drought in the twenty-first century, the problem would get worse (Dai and Zhao, 2017).

Global wheat production is significantly impacted by water shortages brought on by climate change, with 45% of wheat-growing territories in developing nations experiencing drought (Macharia and Ngina, 2017). Drought impairs the plant's capacity to absorb water, disrupting regular physiological functions and changing the wheat plant's developmental cycle (Seleiman *et al.*, 2021). Numerous plant traits, including flowering duration, cell water retention, and water uptake efficiency, are impacted by drought stress (Pennisi, 2008). Drought modifies the wheat crop's developmental cycle, resulting in smaller leaves and

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stems, less root proliferation, less cell division and expansion, and less efficient use of water (Farooq *et al*., 2009; Li *et al*., 2009). A moderate drought stress might reduce wheat yield by up to 61% (Selvaraju and Baas, 2007). Reduced yields are the outcome of the wheat-growing regions becoming more susceptible to climate change-related events such droughts and monsoon loss (Hossain and Teixeira da Silva, 2013b). To overcome limiting conditions and preserve wheat output, it is essential to comprehend how drought affects plant morphological and physiological responses (Yamaguchi-Shinozaki and Shinozaki, 2006). As a result, efforts must be made to overcome a variety of limiting conditions, such as abiotic pressure, which causes wheat production regions to shrink.

The development and production of crops are significantly impacted by drought stress during the germination stage. This crucial stage of the plant life cycle impacts the overall stability and yield of the plant (Gholamin *et al*., 2010). To study osmotic stress, seeds can be subjected to drought solutions like polyethylene glycol. According to studies (Aziz *et al*., 2008; Ghanifathi et al., 2011), seed germination in the presence of polyethylene glycol can be utilized as a stand-in for seed germination in soil with a comparable osmotic potential. To increase the crop's resilience and productivity in demanding conditions, it is crucial to comprehend wheat genotypes' tolerance to osmotic stress during these stages. The results of this study will be useful in identifying wheat genotypes that are stress-tolerant and in enhancing the crop's productivity. These insights can then be utilized to create strategies that will increase the crop's yield and stability in abiotic stress situations.

Materials and Methods

The experiment was conducted in a well-equipped plant culture laboratory situated at the Department of Genetics and Plant Breeding, Bangladesh Agricultural University (BAU), Mymensingh-2202. A total of 30 different wheat genotypes collected from germplasm stock of Genetics and Plant Breeding Department, Bangladesh Agricultural University, Mymensingh, as they were not characterized for drought tolerance yet. The collected genotypes were cultivated using a Completely Randomized (CRD) design. Seeds of 30 wheat genotypes were surface sterilized immersing the seeds in 70% Ethanol solution (according to Davoudpour *et al*., 2020) for 2 minutes and washed well with sterilized water. Germination of seeds occurred on a petri plate. Tissue paper was used to cover the Petri dishes. The tissue papers become moist by sprinkling water over it. These moist tissue papers were utilized for germination of seedlings. Twelve seeds were put on each petri plate. Periodically a water splash was put on the seeds to speed up the germination. The Petri plates were watered everyday with required amount (3 ml) of each solution. The treatment was duplicated thrice.

Polyethylene glycol 6000 (PEG-6000, Merck-Schuchardt, Hohenbrunn, Germany) (previously used by Dodig *et al*., 2015, Nupur *et al*., 2020) was administered at the germination and seedling stage of plant in order to introduce osmotic stress. PEG-6000 was applied at 10% concentration as treatment and 0% PEG- 6000 was applied in remaining containers as control from germination up to 14 days. Two weeks after treatments plants were collected and data were taken on different parameters as Robin *et al.,* 2021. Germination percentage, germination speed, germination index, and relative germination rate were determined by the following formula (Li *et al*., 2008) just after emergence of seedlings. The data were analyzed using MS-Excel and R-package.

Results

Analysis of variance for studied traits

According to the P values, all of the traits of these 30 genotypes are significant and most of them are highly significant (Table 1) under control and treatment.

Table 1. Analysis of variance (mean squares) in thirty genotypes of wheat-

SOV	df	PG	RN	SL	FWS	FWR	DWS	DWR
Genotype	29	3604.05***	4.3303***	45.71***	0.0041***	0.0010***	0.0006 ***	0.000028***
Treatment	1	31.25**	15.0222***	1600.86***	0.3400***	0.0191***	0.0034***	0.000448***
Genotype x Treatment	29	419.18***	1.0797*	11.33*	0.0045***	0.0003***	0.00002*	0.000019***
Error	120	3.47	0.6611	6.91	0.0001	0.000060	0.000013	0.000002

Legend: SOV= Source of variation, df= Degree of freedom, PG= Germination percentage, RN= Root number, SL= Shoot length, FWS= Fresh weight of shoot, FWR= Fresh weight of root, DWS= Dry weight of shoot, DWR= Dry weight of root. ***= P value ≤ 0.001 (Highly significant), **= P value ≤ 0.01 (Significant), *= P value ≤ 0.05 (Less significant)]

Effect of PEG induced drought stress on different traits

Percent germination was highest (100%) under both in control and treatment (Figure 1). Among the genotypes, Bijoy had 100% germination in both control and treatment. Lowest percent germination was observed in BAU 456 in both control and treatment. Highest germination percentage was observed in genotype SA-8. On the contrary, the highest reduction in percent germination was observer in BAU 1004, BAU 1006, BAU 457, BAU 966 and SADH-24. As BAU 456 showed 0% germination in both control and treatment (Figure 1), therefore the lowest value of this genotype was not considered for other traits.

In maximum cases, root number was less in treatment among these interactions (Figure 2). But, in case of BAU 898, Bijoy and DSN-76 root number was increased. Among the genotypes, KAV-2 had the highest root number in control and BAU 966 had the lowest root number in treatment (Figure 2). Highest increase in root number was observed in BAU 898 and DSN-76. On the contrary, highest decrease in root number was observed in KAV-2.

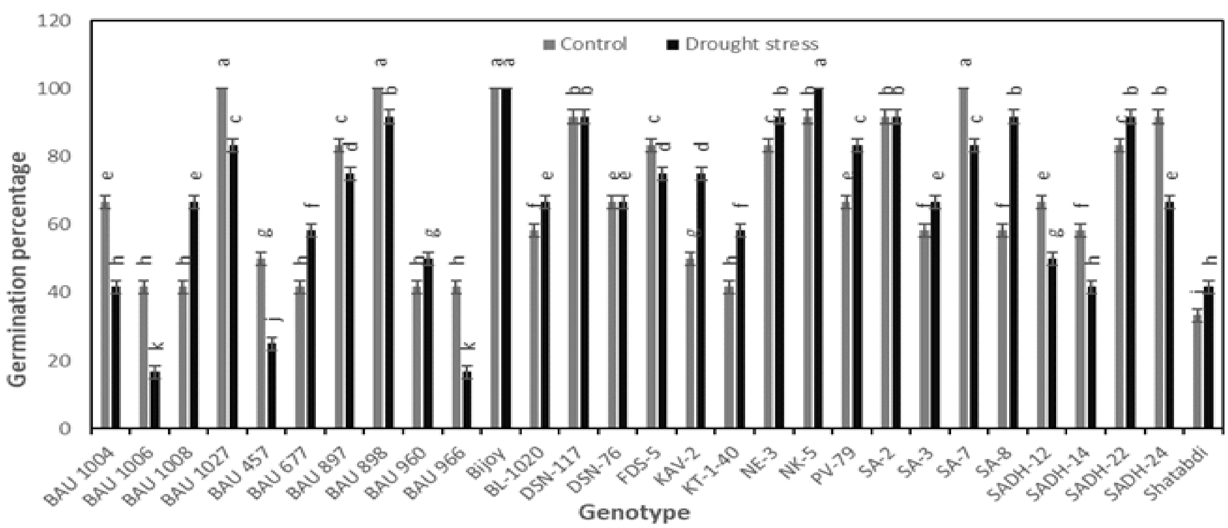

Figure 1. Mean performance of percent germination (%) of thirty wheat genotypes under control and 10% PEG induced drought stress condition. Plotted data represent the average of three replicates of each treatment (n=5) of each genotype. Vertical bar indicates standard error and different letter states the significant difference at 5% level of probability following Tukey's test.

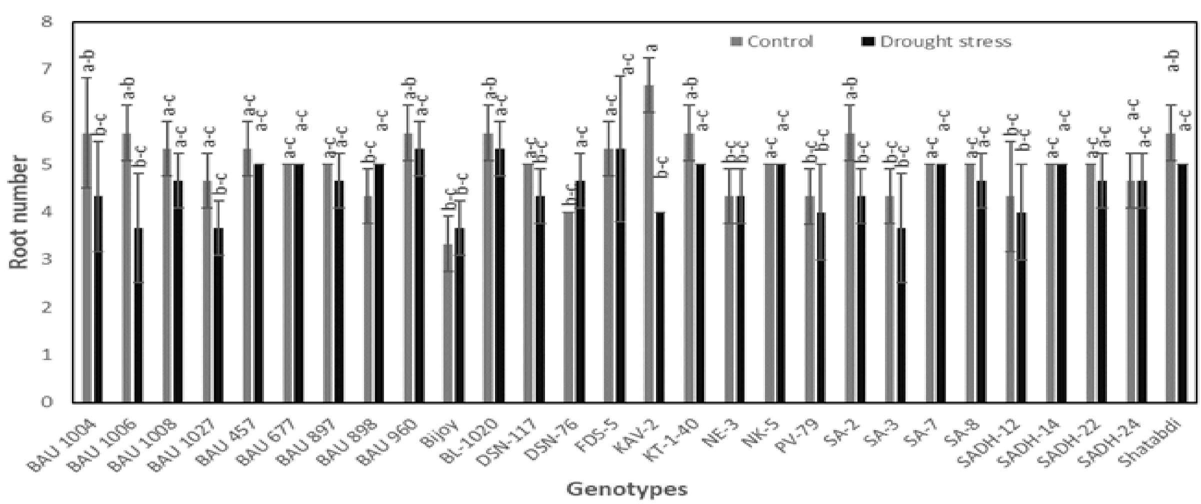

Figure 2. Mean performance of root number of thirty wheat genotypes under control and 10% PEG induced drought stress condition. Seeds were grown on control and stress condition for 14 days and root numbers were counted. Plotted data represent the average of three replicates of each treatment (n=5) of each genotype. Vertical bar indicates standard error and different letter states the significant difference at 5% level of probability following Tukey's test.

Shoot length was always less in treatment in every interaction (Figure 3) and highest reduction was observed in genotype Bijoy. Genotype Bijoy had the highest shoot length in control and BAU 1008 had the highest shoot length in treatment. Contrarily, SA-7 had the lowest shoot length in both control and treatment. BAU 897 had the highest shoot fresh weight in treatment though in other all interactions the rest genotypes had less shoot fresh weight in treatment (Figure 4). The maximum difference between control and treatment in shoot fresh weight was also observed in BAU 897. Genotype NK-5 had the highest shoot fresh weight in control. Genotype BAU 1006 and SA-7 had the lowest shoot fresh weight, respectively in treatment and control. Genotype SA-7 had the highest fresh weight of root in control and in maximum interactions fresh weight of root was less in treatment except BAU 960 and FDS-5 (Figure 5). SADH-22 had the highest fresh weight in treatment. BAU 966 and BAU 1006 had the lowest fresh weight of root respectively in control and treatment. The highest reduction of fresh weight of root in treatment was observed in BAU 1006. Dry weight of shoot was always less in treatment in every interaction (Figure 6) and highest reduction was observed in genotype BAU 677. The highest dry weight of shoot was observed in BAU 966 in control and in NK-5 in treatment. On the contrary, BAU 1027 and PV-79 had the lowest dry weight of shoot in control and Bijoy had the lowest dry weight of shoot in treatment. In maximum interactions dry weight was higher in control and the highest dry weight of root among the interactions was observed in BAU 898 (Figure 7). The highest reduction in dry weight of root was also observed in BAU 898. Contrarily, the highest induction in dry weight of root was observed in NK-5. The lowest dry weight of root was observed in BAU 1027 and PV-79 in control and the lowest dry weight of root was observed in DSN-76 in treatment.

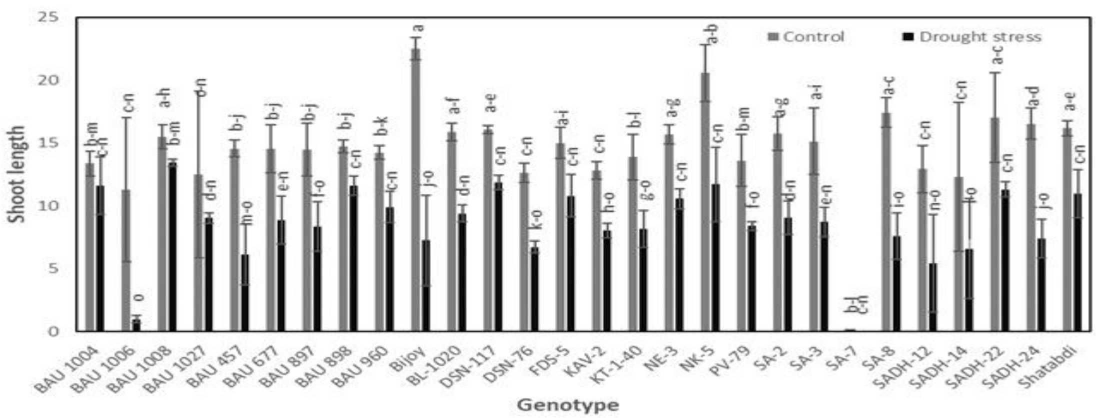

Figure 3. Mean performance of shoot length (cm) of thirty wheat genotypes under control and 10% PEG induced drought stress condition. Plotted data represent the average of three replicates of each treatment (n=5) of each genotype. Vertical bar indicates standard error and different letter states the significant difference at 5% level of probability following Tukey's test.

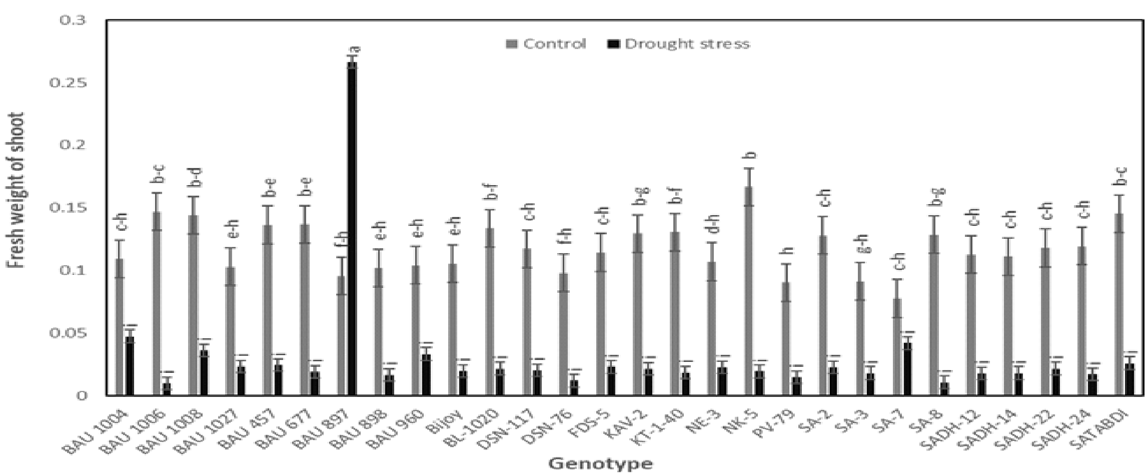

Figure 4. Mean performance of thirty wheat genotypes for fresh shoot weight (gm) under control and 10% PEG induced drought stress condition. Plotted data represent the average of three replicates of each treatment (n=5) of each genotype.

Vertical bar indicates standard error and different letter states the significant difference at 5% level of probability following Tukey's test.

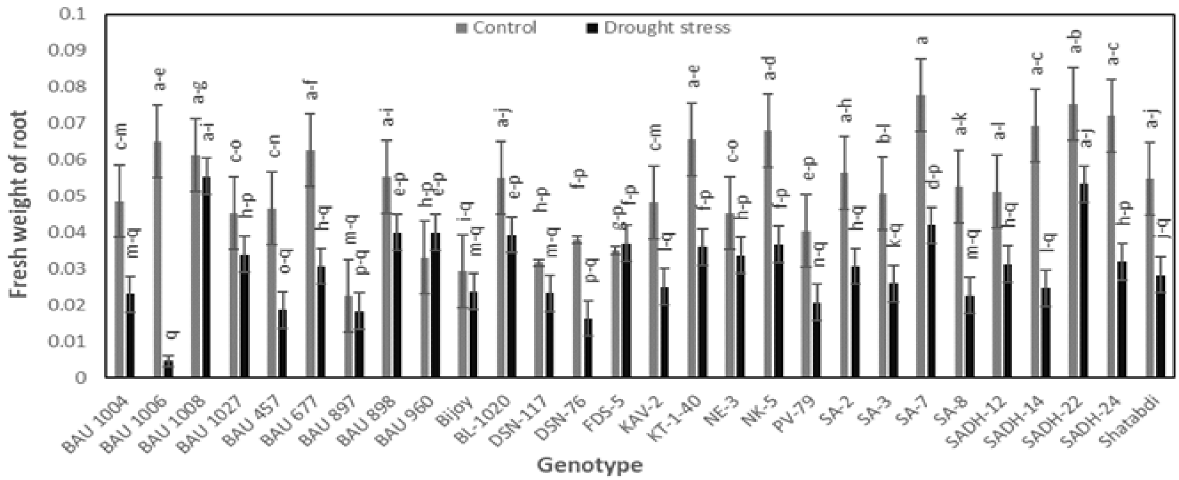

Figure 5. Mean performance of thirty wheat genotypes for fresh root weight (gm) under control and 10% PEG induced drought stress condition. Plotted data represent the average of three replicates of each treatment (n=5) of each genotype. Vertical bar indicates standard error and different letter states the significant difference at 5% level of probability following Tukey's test. Figure 6. Mean performance of thirty wheat genotypes for shoot dry weight (gm) under control and 10% PEG induced drought stress condition. Plotted data represent the average of three replicates of each treatment (n=5) of each genotype. Vertical bar indicates standard error and different letter states the significant difference at 5% level of probability following Tukey's test.

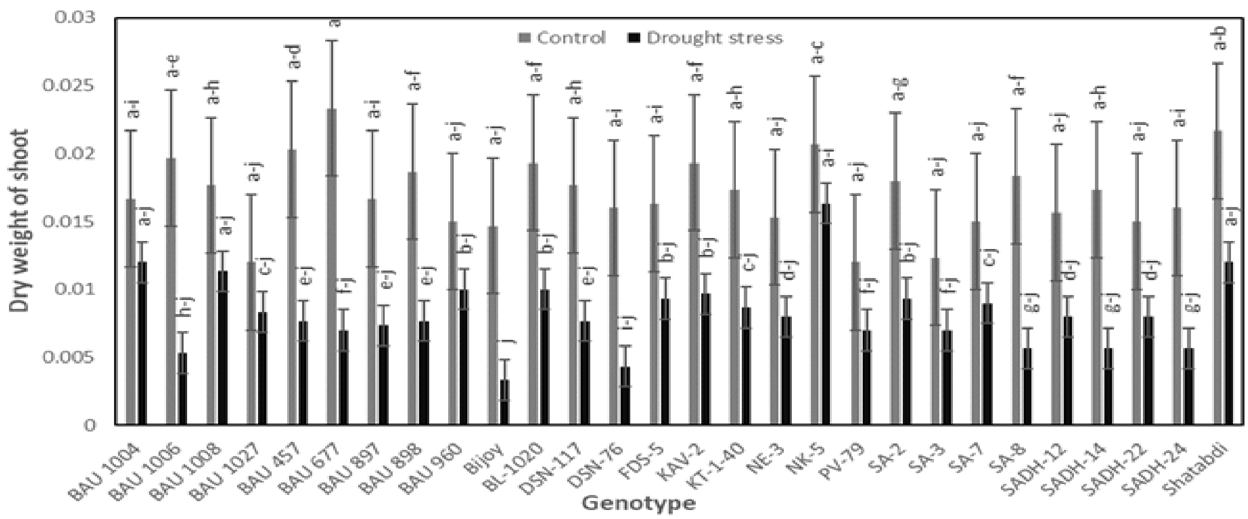

Figure 6. Mean performance of thirty wheat genotypes for shoot dry weight (gm) under control and 10% PEG induced drought stress condition. Plotted data represent the average of three replicates of each treatment (n=5) of each genotype. Vertical bar indicates standard error and different letter states the significant difference at 5% level of probability following Tukey's test.

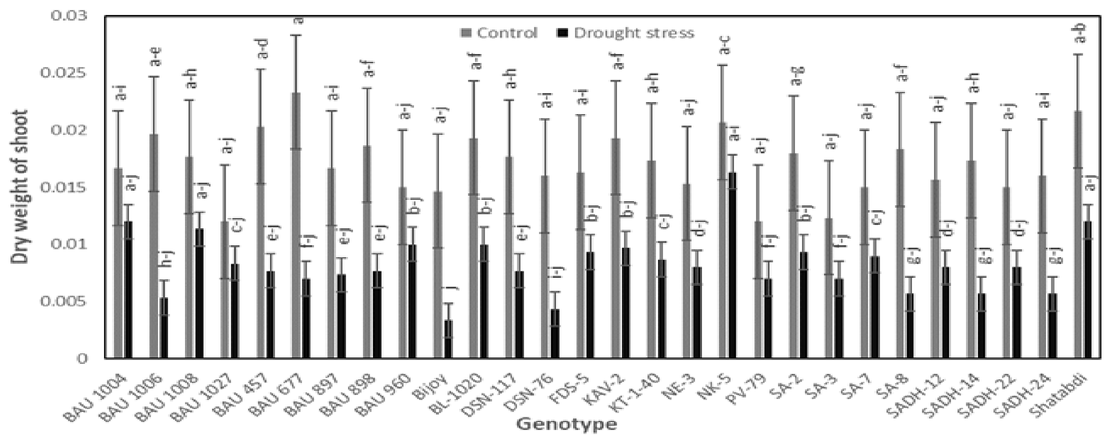

Figure 7. Mean performance of thirty wheat genotypes for root dry weight (gm) under control and 10% PEG induced drought stress condition. Plotted data represent the average of three replicates of each treatment (n=5) of each genotype. Vertical bar indicates standard error and different letter states the significant difference at 5% level of probability following Tukey's test.

Association between different studied traits under stress condition and Principle component analysis

Correlation coefficients were generated to determine the link between the studied traits. In this study, all the traits showed positive and significant correlation among them, except fresh shoot weight and dry shoot weight which showed non-significant association with germination percentage (Table 2). One moderate and less significant correlation was observed for dry weight of root with germination percentage.

We also performed Principle Component Analysis (PCA) for these 30 wheat genotypes considering both control and stress condition. The first two principle component having the Eigen value more than one covered about 73% to total variability (Table 3). DWS and PG had the most contribution respectively in PC1 and PC2 (Figure 8). On the other hand, PG and RN had the lowest contribution to PC1 and PC2 (Figure 8). According to the PCA biplot, DWR, FWS and DWS were at the same axis (Figure 9). Again, RN, FWR, SL, PG were at the same axis but PG was more far from other traits as it had more contribution to PC2 (Figure 9).

Table 2. Correlation coefficient (Pearson) among the studied traits under PEG-induced drought stress condition

	PG	RN	SL	FWS	FWR	DWS
RN	0.200**					
SL	0.406***	0.460***				
FWS	0.080NS	0.357***	0.581***			
FWR	0.288***	0.438***	0.585***	0.573***		
DWS	0.068NS	0.432***	0.591***	0.733***	0.706***	
DWR	0.149*	0.432***	0.452***	0.495***	0.690***	0.807***

Legend: PG= Germination percentage, RN= Root number, SL= Shoot length, FWS= Fresh weight of shoot, FWR= Fresh weight of root, DWS= Dry weight of shoot, DWR= Dry weight of root

Table 3. Eigenvalues, percentage of variability, and percentage of cumulative variability of seven principle component as analyzed for six different traits of thirty wheat genotypes

PCs	Eigenvalues	% of Variance	Cumulative % of variance
PC1	4.068	58.117	58.117
PC2	1.027	14.667	72.784
PC3	0.676	9.657	82.440
PC4	0.434	6.197	88.637
PC5	0.368	5.254	93.891
PC6	0.305	4.352	98.243
PC7	0.123	1.757	100.000

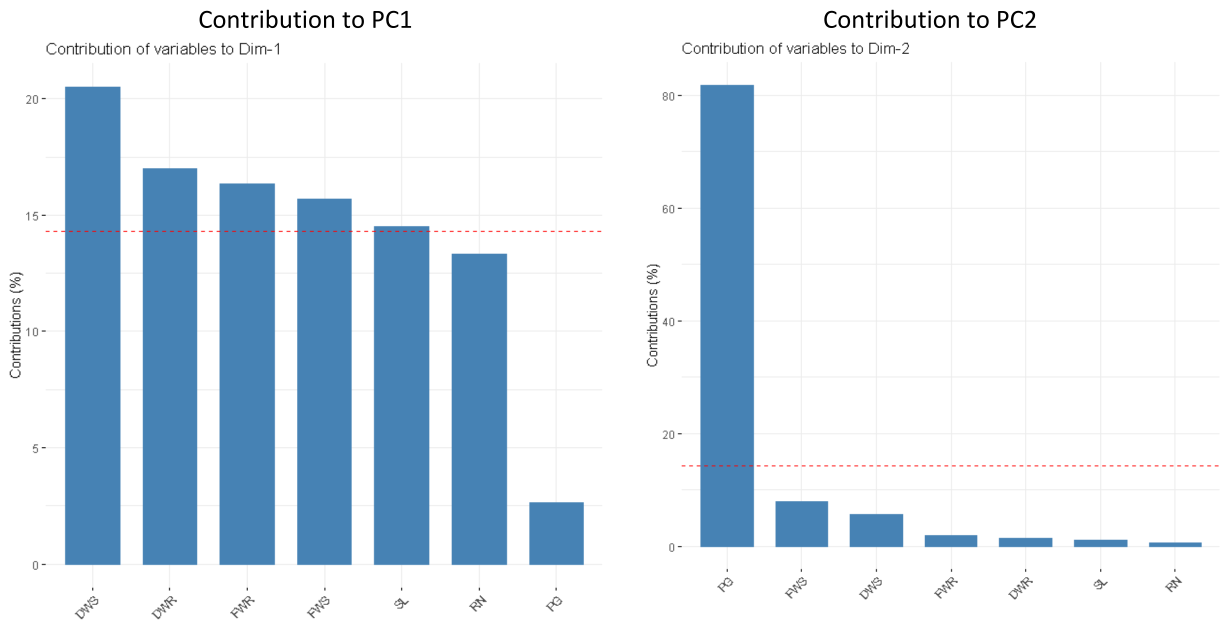

Figure 8. Graphical representation of different traits to the contribution of PC1 and PC2

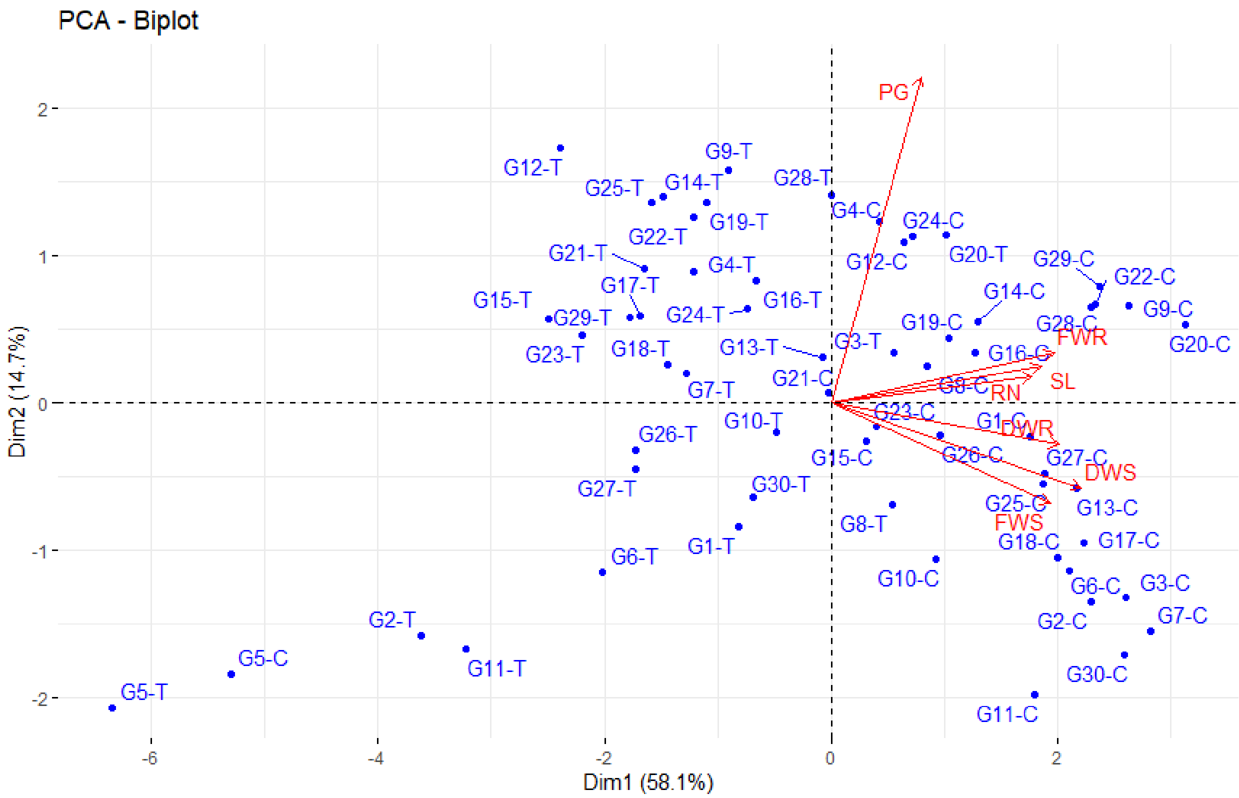

Figure 9. Principal Component Analysis (PCA) biplot of thirty wheat genotypes and the measured traits under control and drought stress condition. The length of arrow indicates the contribution of the traits and varietal response to traits were indicated by arrows direction. Abbreviations: PG= Germination percentage, RN= Root number, SL= Shoot length, FWS= Fresh weight of shoot, FWR= Fresh weight of root, DWS= Dry weight of shoot, DWR= Dry weight of root. G1=BL-1020, G2= NK-5, G3=KT-1-40, 4=PVV-70, G5=KAV-2, G6=DSA-117, G7=SA-2, G8= SA-3, G9=SA-7, G10= SA-8, G11= NE-3, G12=BAU457, G13= BAU677, G14= BAU 897, G15= BAU898, G16= BAU960, G17= BAU456, G18= B AU966, G19= BAU1004, G20= BAU1008, G21= FDS-5, G22=Bijoy, G23= Shatabdi, G24= BAU1006, G25= BAU1027, G26= DSN-76, G27= SADH-12, G28= SADH-14, G29= SADH-22 and G30= SADH-24

Multi Trait Stability Index (MTSI) Analysis

MTSI analysis was performed among the 30 wheat genotypes. Here are 4 genotypes (SA-3, SA-2, BL-1020, PV-79) are located out of the red circle and identified with red dots that mean selected (Figure 10). On the other hand, the rest genotypes were remained inside the circle and marked as non-selected with black dots (Figure 10).

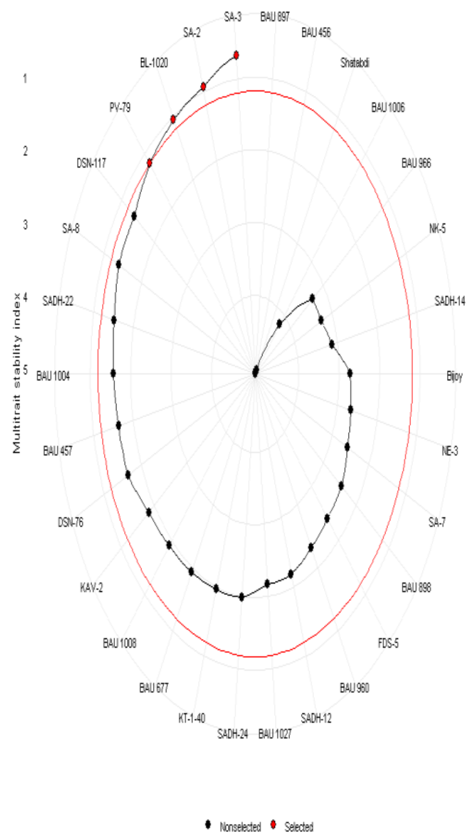

Figure 10. Ranking of stable and best performing genotypes based on MTSI (Multi Trait Stability Index) considering all the studied traits across control and drought stress condition. The red small circle showed the selected stable genotypes, and central red circle represents the cut point

Discussion

Highly significant differences were observed among the thirty wheat genotypes for all the studied traits *viz.* root number, shoot length, fresh weight of shoot, fresh weight of root, dry weight of shoot, dry weight of root except germination percentage for treatment that was also observed before by Biligili *et al*. (2019), Alaei *et al.* (2010) & Emami *et al*. (2010). Droughtstress lowered the rate of germination (Rana *et al*. 2017), but in some cases germination percentage was increased after the treatment in some genotypes (BAU 1008, BAU 677, BAU 960, BL-1020, KT-1-40, NE-3, NK-5, PV-79, SA-3, SA-8, SADH-22, Shatabdi) (Figure 1). In case of Bijoy, DSN-117, DSN-76, SA-2, there was similar performance in treatment same as control which may due to the production of stress responsive proteins that can improve the seed's chances of survival. On the contrary, as the highest reduction in percent germination was observed in BAU 1004, BAU 1006, BAU 457, BAU 966 and SADH-24 (Supplementary table 2 & Figure 1). In maximum cases, root number was less in treatment (Figure 2) which was also previously tested by researchers and it has found that PEG stress greatly inhibited new root development activity under drought stress (Robin *et al*., 2021). But contrarily, in case of BAU 898, Bijoy and DSN-76 root number was increased (Figure 2 and Supplementary Table 3). So, they may have the tolerance to drought stress.

Shoot length is decreased due to PEG stress which was also found previously and a sort of tuberization caused by an obstruction to cell division and elongation could be the cause of the reduction in shoot length (Khakwani *et al*., 2011.). A minor decrease in shoot development during drought stress is a sign of drought tolerance (Sassi *et al*.,2012, Ming *et al*.,2012, Mouchesh *et al*., 2012 and Saghafikhadem, 2012) which was observed in SA-7. Several other researchers (Ahmad *et al*., 2013a, Kamran *et al*., 2009a) have also seen a decline in shoot and root dry weight, indicating that water stress has a notable impact on these parameters. The decrease in the fresh and dry weight of the shoots and roots was ascribed to the growth of fewer and smaller leaves together with an elevated *PEG (6000)* concentration in the growing medium (Chachar *et al*., 2016). Other researchers (Ahmad et al., 2013b, and Kamran *et al*., 2009b) who discovered that water stress had a substantial impact on root and shoot dry matter production also observed a declining trend in root and shoot dry weight. Contrarily, increase in shoot/root fresh or dry weight may be a sign of stress tolerance.

Correlation analysis elucidates the connection between two variables, which is valuable in the field of plant sciences as it establishes associations that may be used to investigate the relationship between many features (Ahmed *et al*., 2019). Understanding the correlation among these traits was very important to improve the efficiency of breeding for drought tolerance in wheat (Sallam *et al*., 2019). This experiment investigated the correlations among germination percentage, root number, shoot length, fresh weight of roots and shoots, and dry weight of roots and shoots under drought stress condition. Most of the correlations were positive and highly significant (Table 2) which indicates that these traits tend to move in the same direction together and have tendencies to increase or decrease together. Germination percentage showed positive and significant relationship with fresh and dry weight of root that was similarly found by previous research (Rauf *et al*., 2007).

Principle component analysis is a statistical technique that converts a set of correlated variables into a smaller set of uncorrelated variables (Kamel *et al*., 2009). The principle components with eigenvalues greater than 1 are deemed significant, but the other components are not (Pour-Aboughadareh *et al*., 2021) and in this experiment, the eigenvalues of first two PCs were greater than 1 and had the most percent of variance. Variables that may be divided into major groups and subgroups according to homogeneity and dissimilarity can be chosen using a PCA biplot analysis (Sisodia and Rai ,2017). The lesser the angle between the traits, the stronger the correlation (Teleghani *et al*., 2023). So, shoot dry weight and root dry weight had the highest co-relation value. Germination percentage had the higher angle with other traits (Figure 9), so it had non-significant co-relationship with fresh and dry weight of shoot (Table 2).

Plant breeders would find MTSI to be highly helpful in selecting superior genotypes for numerous attributes based on data from multiple environments (Sharifi *et al*., 2011 and Koundinya *et al*., 2011). The experimental genotypes are arranged in descending order based on their MTSI values, with the genotype having the greatest MTSI value positioned at the center and the genotype with the lowest MTSI value placed in the outermost circle and the MTSI scores were used to select the genotypes shown in red dots (Teleghani *et al*., 2023). That means genotype having the lower value of MTSI, there is higher chance to be selected of that genotype. According to the Figure 10, SA-3 was in the first rank followed by SA-2, BL-1020 and PV-79 as the most ideal stable genotypes. Average value of all attributes in selected genotypes has increased which was aimed at the intended goals. Contrarily, BAU-456, BAU-897 had the highest MTSI values as they were positioned in the center of the circle. For every attribute, the genotypes that were chosen produced a favorable selection differential.

Conclusion

This study has shed light on the complex mechanisms underlying drought tolerance in thirty wheat genotypes. From the study, it can be concluded that, there is a significant reduction in germination percentage, root number, shoot length, fresh and dry weight of root and shoot due to PEG treatment (drought stress). Among these genotypes, SA-3 followed by SA-2, BL-1020 and PV-79 had the ability to survive potentially in drought stress which was found from different analysis like PCA, MTSI etc. Furthermore, the genotypes BAU-456, BAU-897, BAU 966 are the sensitive genotypes under drought stress. So, it is highly advisable to include evaluated genotypes into future breeding programs to generate cultivars that are tolerant to drought.

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