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Evaluation of wheat genotypes for drought tolerance

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ABSTRACT:
Drought stress is one of the main abiotic constraints for wheat. Water deficit stress especially at early developmental stage affects forthcoming physiological and morphological attributes of wheat and considerably lessens overall performance of wheat. Breeding for drought stress tolerance can be possible with the help of conventional breeding tools. There is a dire need to notify water deficit stress tolerant germplasm which perform better under drought conditions. Fifty wheat genotypes were screened for drought tolerance when evaluated for three physiological and three morphological attributes. Out of all the studied attributes and genotypes, ETAD232, ETAD19 and ETAD211, shoot length, seedling length, root shoot ratio and relative water contents contributed more towards diversity. These genotypes and attributes could be exploited for drought tolerance wheat breeding programs.

Key Words: Drought stress, Genetic diversity, Principal component analysis

INTRODUCTION: Wheat is regarded as vital cereal and it is one of the most important cereal crops of world. It is mainly grown in rain-fed conditions in which drought and heat stress occurs frequently which ultimately results in yield reduction (Rana *et al.*, 2013). Plants may also experience water deficit problem in certain period of time even in habitats with relatively high rainfall (Balouchi, 2014). Water deficit is a common environmental phenomenon encountered by wheat all over the world (Nouri *et al.*, 2011).

Drought stress is one of the most important yield limiting factors for crops such as wheat. Long period of water stress leads to lessen in the performance of wheat in arid and semi arid regions (Nezhadahmadi *et al.*, 2013).

Moderate to stern water deficit condition significantly affects different morpho-physiological attributes in wheat such as chlorophyll contents, relative water contents or water potential, dry matter yield etc (Ehdaie *et al.*, 1991). Since genotypic variations for the attributes have been reported for different crops including wheat, these attributes have been utilized to recognize drought tolerant germplasm in different crops. Drought tolerance is a quantitative attribute, with a complex phenotype, often confounded

by plant phenology, and there is no undeviating technique for measuring it. This makes it easier said than done to recognize drought tolerant genotypes (Takeda and Matsuoka, 2008). Gan *et al.*, 2003 reported that drought tolerant wheat genotypes had higher relative water content, chlorophyll a/b contents and dry matter weight than water susceptible wheat genotypes but the selection criterion must be identified that are allied with better yield under drought stress, having a sky-scraping heritability and can be measured simply and precisely in a large group of individuals. Several researchers concluded that those genotypes are desirable and sustainable which performs best under both normal and stress conditions (Nezhadahmadi *et al.*, 2013).

One of the main goals of plant breeders is to make genotypes suitable to changing needs and environment by ensuring a healthy yield. For this they have to exploit different genotypes. Mostly plant breeders are utilizing principal component analysis as a pattern finding process because it is more useful (Sajjad *et al.*, 2011).

Germplasm diversity evaluation based on morphological attributes requires a high degree of accuracy of field trials through design and analysis. In this study, the effects of water stress on wheat at seedling stage were carried out to scrutinize the genetic variation by treatments. The relative water contents, chlorophyll a/b ratio, cell membrane stability, fresh and dry seedling weight, and root and shoot length and root shoot ratio were analyzed and compared with those of untreated seedlings.

MATERIALS AND METHODS: Fifty wheat genotypes were utilized to evaluate genetic diversity of wheat for drought tolerance. The experiment was conducted at Department of Plant Breeding and Genetics, PMAS Arid Agriculture University, Rawalpindi (Table 1). Wheat seeds were sown in disposable plastic pots with three replications in growth chamber at 18/24°C day/night temperature with 60% relative humidity. Fifteen seeds were sown and thinned after one week to get final population of 10 uniform seedlings per pot. Moisture level was maintained by adding water daily in the morning. Two weeks after sowing, one set of seedlings was subjected to drought shock by limiting irrigation for one week, while the other set was provided with an adequate amount of water. One week after stress treatment, the data were collected for following parameters from stressed as well as unstressed seedlings.

Table 1: Wheat genotypes used in current study

Sr. #	Genotypes	Sr. #	Genotypes
1	AAS11	**26**	ETAD51
2	CHAKWAL50	**27**	ETAD55
3	MILLAT11	**28**	ETAD170
4	PUNJAB11	**29**	ETAD211
5	SEHER06	**30**	ETAD213
6	SHAFAQ06	**31**	ETAD215
7	FSD08	**32**	ETAD218
8	CB2	**33**	ETAD219
9	CB5	**34**	ETAD225
10	CB24	**35**	ETAD226
11	CB28	**36**	ETAD230
12	CB32	**37**	ETAD232
13	CB39	**38**	ETAD233
14	CB40	**39**	ETAD236
15	CB51	**40**	ETAD239
16	CB321	**41**	LLR2
17	ETAD1	**42**	LLR18
18	ETAD4	**43**	LLR31
19	ETAD7	**44**	LLR42
20	ETAD8	**45**	WC2
21	ETAD19	**46**	WC5
22	ETAD30	**47**	WC11
23	ETAD48	**48**	WC16
24	ETAD49	**49**	WC18
25	ETAD50	**50**	WC19

Relative water contents: Youngest emerging wheat seedling leaves of uniform size were

detached to measure relative water contents (RWC). Leaves were detached, fresh weight was recorded spontaneously. Those leaves were then dipped in 15 ml of distilled water for 24 hours in test tubes at room temperature to allow rehydration and turgid weight was calculated. Following rehydration, the leaves were wrapped in aluminum foil and dried in hot air oven for 48 hours at 75 °C to measure dry weight. Same procedure was applied to both stresses as well as normal seedlings. RWC % was calculated by using below mentioned formula following (Rahimi *et al.*, 2010).

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

Chlorophyll contents: Chlorophyll of stresses as well as normal seedlings was extracted in 80% acetone and chlorophyll concentration was obtained by measuring its absorbance at λ 645 nm and λ 663 nm in as spectrophotometer and calculations were made using following functions formulated by (Arnon, 1949)

$$Chla = 12.7A_{663} - 2.69A_{645}$$

$$Chlb = 22.9A_{645} - 4.68A_{663}$$

Cell membrane stability: Cell membrane stability (CMS) of the roots of stressed as well as control wheat at seedling stage was determined by relative electrolyte leakage method following (Bajji *et al.,* 2002). Roots of water stressed and control seedling were washed with tap water to remove attached soil particles. The roots were then excised to get equal weight (0.1g each) and rinsed with double distilled water to get rid of electrolytes from root surface. Root samples were dipped in 20 ml distilled water in test tubes overnight at room temperature. The test tubes were gradually shaken and conductivity of the solution was measured after 24 h using an electrolyte meter (YSI Model 32). Samples were then killed in an autoclave at 110 °C for 10 min and allowed cool down to room temperature to measure final conductivity (complete electrolyte leakage). CMS was calculated by using following equation.

$$CMS\% = \{(1 - T1/T2)/(1 - C1/C2)\} \times 100$$

Whereas, T refer to treatment; C refers to control; 1 refer to initial conductance reading; 2 refer to final conductance reading.

Seedling fresh and dry weight: Fresh seedlings taken from water stressed as well as control pots and weighed in grams immediately using electrical weighing balance. The seedlings were then oven dried at 70 °C for 48 hours to calculated dry seedling weight in grams (Bashan and de-Bashan, 2005).

Root and shoot length: Root and shoot length for both conditions (water stress and control) were measured in centimeter by using scale (Bashan and de-Bashan, 2005).

Root/shoot ratio: Root/shoot ration was calculated by dividing root length to shoot length.

Statistical Analysis: Descriptive statistics and principal component analysis (PCA) were carried out with the help of SPSS V 16 and XLSTAT respectively as outlined by Umer *et al.,* 2014.

RESULTS AND DISCUSSION: Fifty wheat genotypes screened for aforementioned parameters and their performance was

compared for drought tolerance. The ratio of RWC under drought versus control conditions was calculated and used to assess the genotype response under stress as compared to control. The results indicated eight genotypes showing increment in their RWC under drought as compared to control seedlings. The most promising genotypes were ETAD232 with 1.631 drought/control ratio followed by WC5 with 1.204. Other genotypes showing increased RWC are ETAD215, LLR2, WC16, ETAD49, ETAD211 and ETAD8 with 1.178, 1.091, 1.043, 1.012, 1.009 and 1.005 respectively. Whereas, AAS11, CHAKWAL50, ETAD170 and PUNJAB11 showed maximum reduction in their drought to control ratio with 0.402, 0.476, 0.476 and 0.520 respectively (Figure 1). The ratio of chlorophyll a/b ratio under drought versus control conditions was calculated and used to assess the genotype response under stress as compared to control. The results indicated that ETAD232 showed maximum chlorophyll a/b ratio with 3.787 followed by ETAD211, ETAD248, and ETAD239 with 3.757, 3.325 and 2.774 respectively. Minimum chlorophyll a/b ratio was shown by genotypes CB32, ETAD30, ETAD215 and ETAD230 with 0.110, 0.242, 0.283 and 0.341 respectively (Figure 2). The percentage of cell membrane stability drought versus control conditions was calculated and used to evaluate the genotype response under drought stress. Genotype ETAD232 showed maximum cell membrane stability with 85.972% followed by genotype WC19 with 82.532%. Other promising genotypes are CB51, LLR2 and ETAD7 which showed values more than 80% having, 82.123%, 81.237% and 81.236% respectively. Whereas notorious genotypes in this regard were ETAD239, ETAD226, ETAD213 and ETAD170 with 27.949%, 37.089%, 41.262% and 41.586% respectively (Figure 3). Fifty wheat genotypes were also screened for fresh seedling weight and their performance was compared for drought tolerance. The ratio of fresh seedling weight under drought versus control conditions was calculated and used to assess the genotype response under stress as compared to control. Most promising genotype which showed maximum ratio was ETAD232 with 0.987 followed by WC19 with 0.565. Whereas MILLAT11, ETAD8, ETAD7 and PUNJAB11 showed maximum reduction fresh seedling ratio having 0.0212, 0.0312, 0.0346 and 0.0352 respectively (Figure 4).

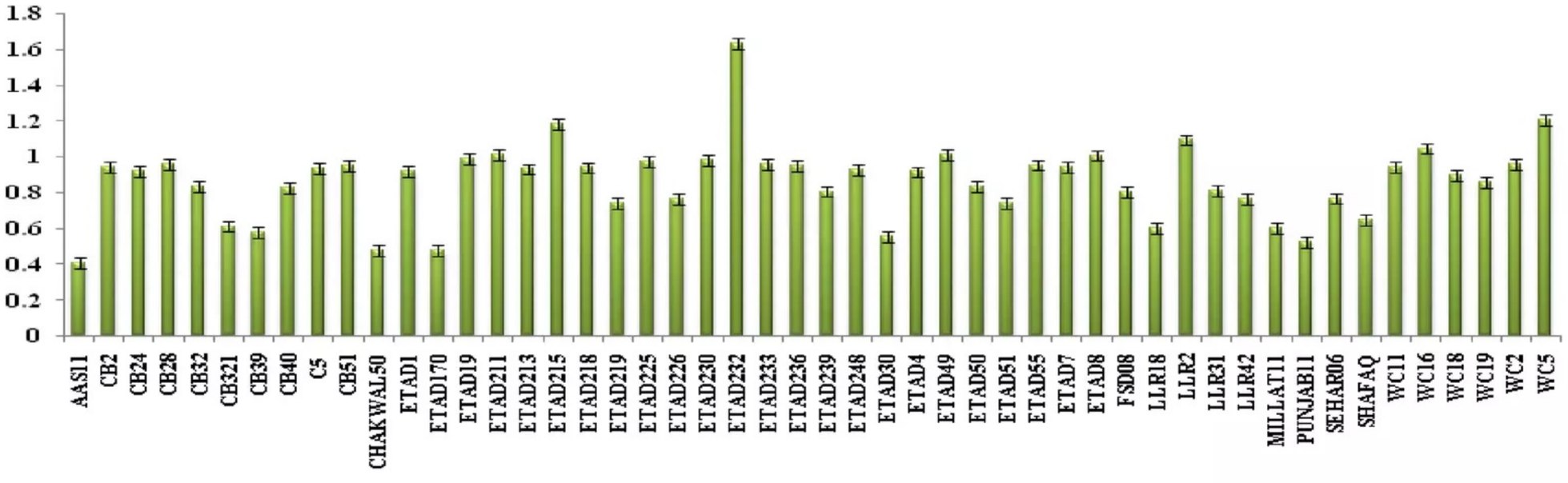

Figure 1: Percent relative water contents variation under drought stress condition as compared to control seedlings

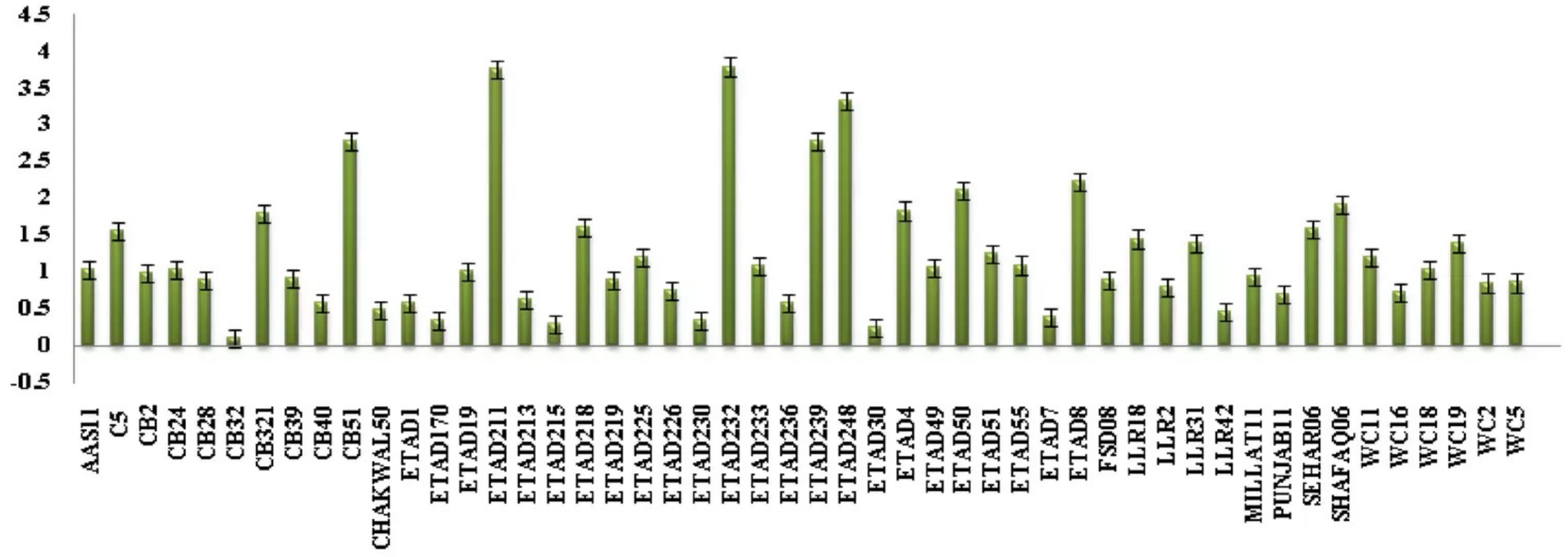

Figure 2: Percent chlorophyll a/b ratio variation under drought stress condition as compared to control seedlings

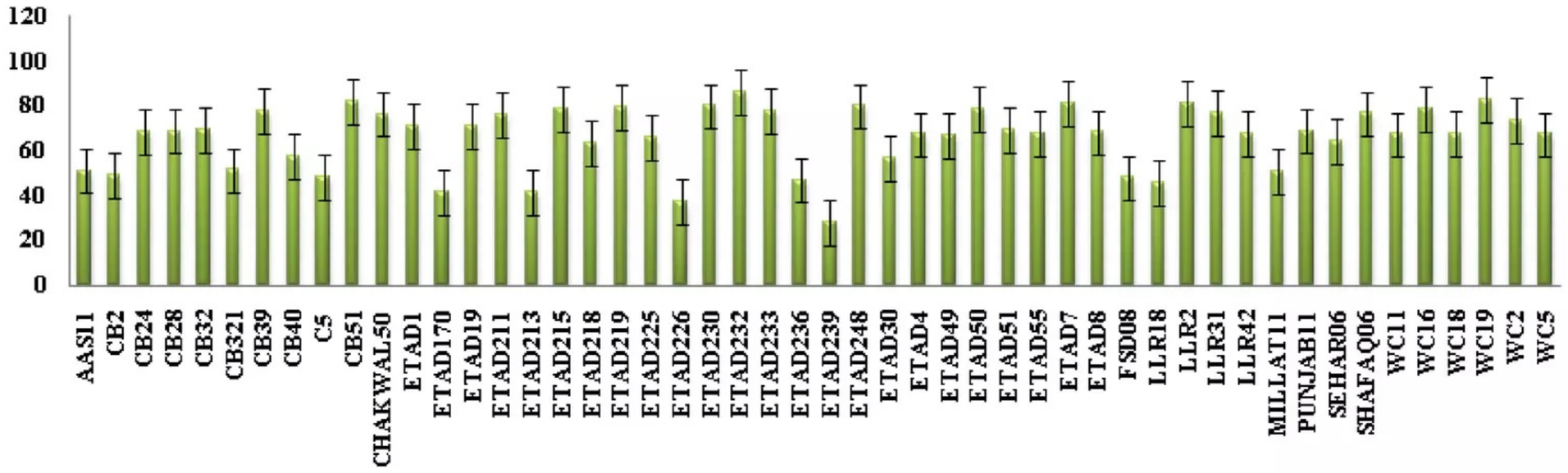

Figure 3: Percent cell membrane stability of wheat genotypes

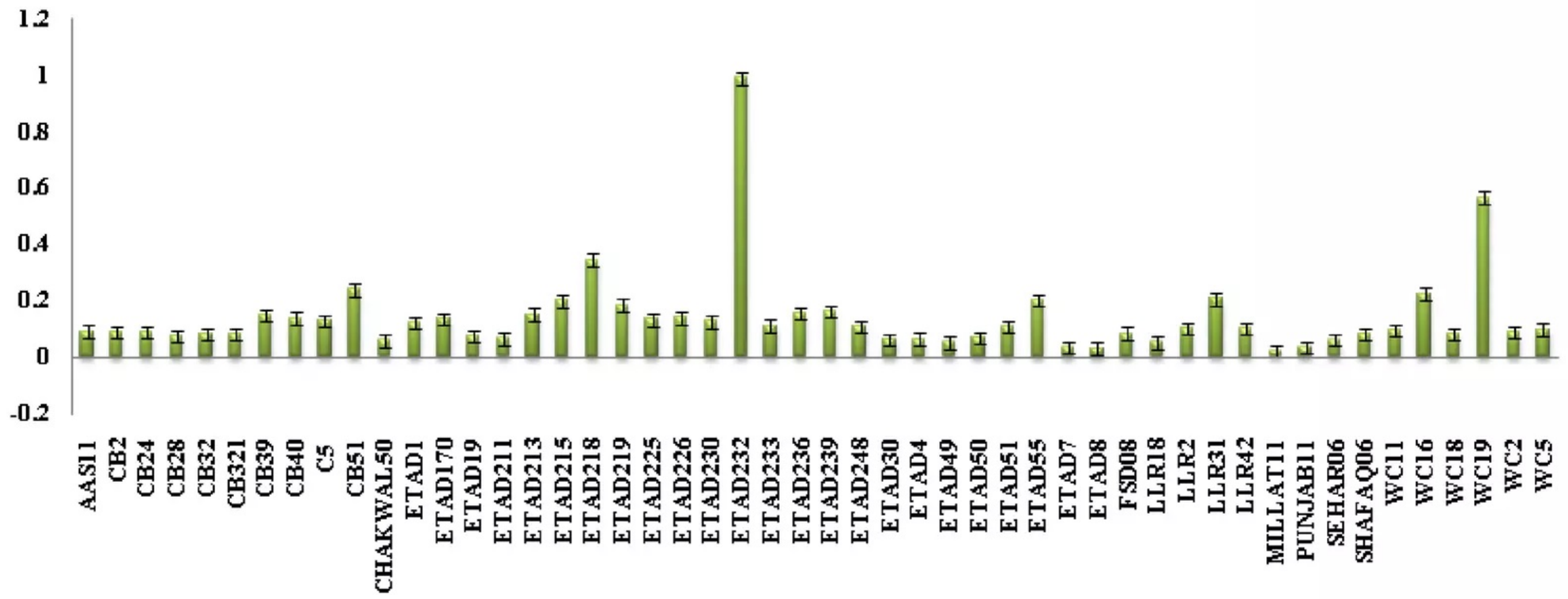

Figure 4: Percent fresh seedling weight variation under drought stress condition as compared to control seedlings

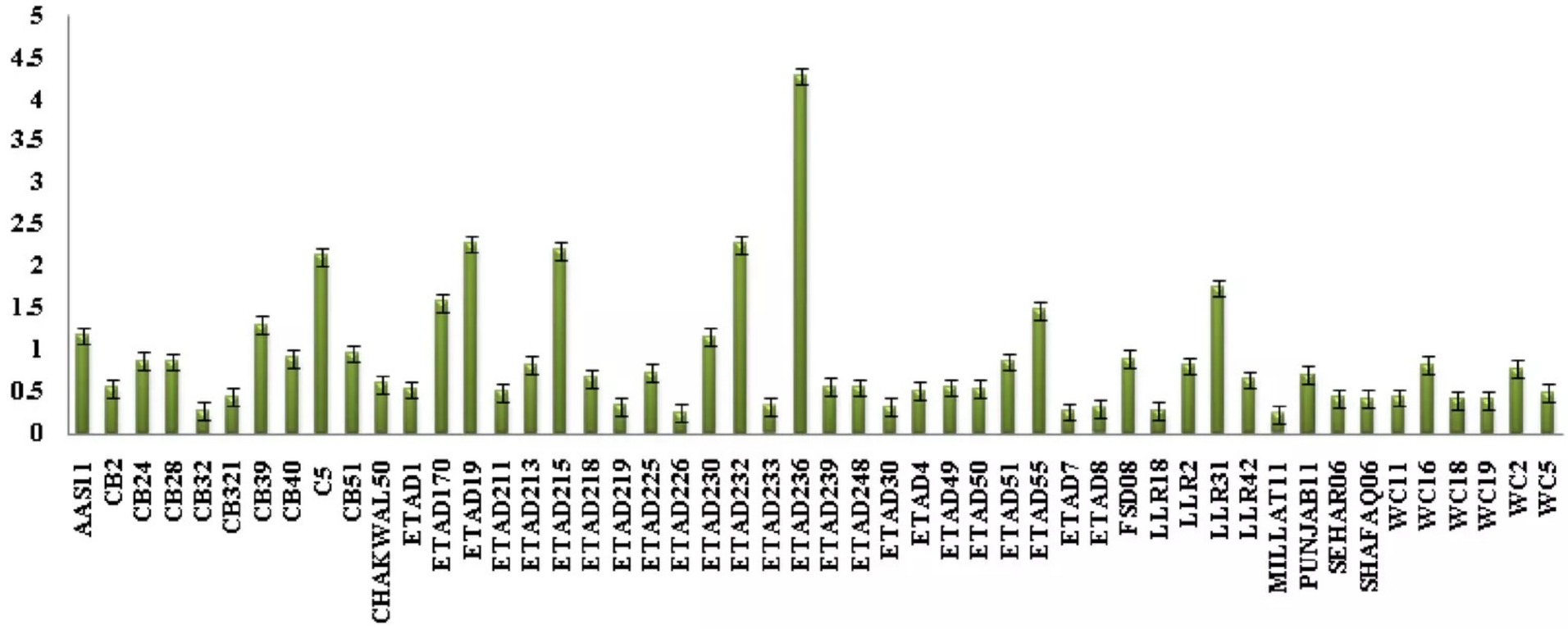

Figure 5: Percent dry seedling weight variation under drought stress condition as compared to control seedlings

The ratio of dry seedling weight under drought versus control conditions was calculated and used to assess the genotype response under stress as compared to control. The results illustrated that ETAD236 showed maximum ratio with 4.273 followed by ETAD19, ETAD232 and ETAD215 with 2.271, 2.266 and 2.189 respectively. Whereas minimum dry seedlings weight ratio were shown by MILLAT11, ETAD226, ETAD7 with 0.236, 0.247 and 0.265 respectively (Figure 5). The ratio of root length under drought versus control conditions was calculated and used to assess the genotype response under stress as compared to control. The results demonstrated that AAS11 showed maximum ratio for root length with 1.077 value followed by LLR2, ETAD232, ETAD55 with 1.075, 1.073 and 1.06. Whereas the genotypes which showed lowest ratio for root length was ETAD51 followed ETAD236 with 0.989 and 1.0007 respectively. Other low ratio genotypes were ETAD230, ETAD239, ETAD211 and LLR18 having 1.001, 1.002, 1.010 and 1.013 respectively (Figure 6).

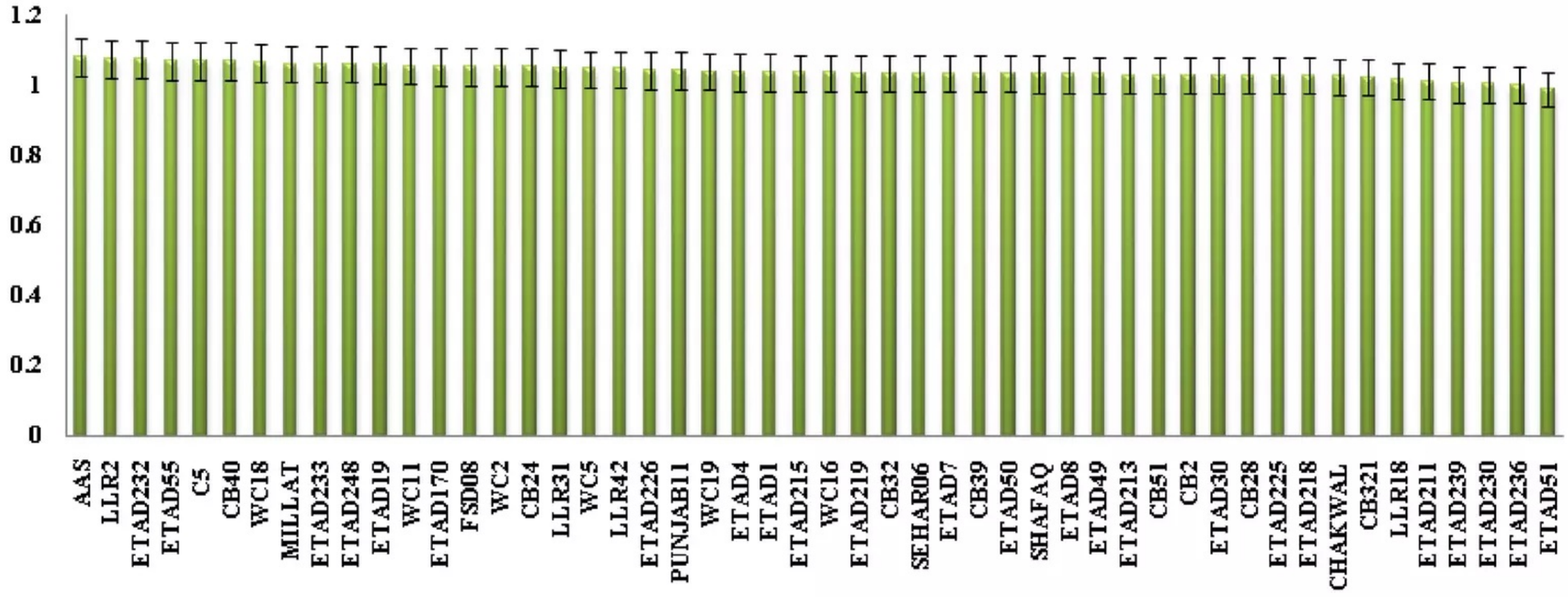

Figure 6: Percent root length variation under drought stress condition as compared to control seedlings

The ratio of shoot length under drought versus control conditions was calculated and used to assess the genotype response under stress as compared to control. Results illustrated that ETAD19 showed maximum ratio for shoot length with 1.1 value followed by CB28 with 1.030. Other promising genotypes were WC18, ETAD248, CB321 and CB51 with 1.022, 1.005, 1 and 1 respectively. ETAD213, ETAD211, ETAD1 and CB2 showed minimum shoot length ratio with 0.877, 0.887, 0.9 and 0.9 respectively (Figure 7). The ratio of root/shoot length under drought versus control conditions was calculated and used to assess the genotype response under stress as compared to control. Results illustrated that, ETAD232 showed maximum root/shoot length ratio with 1.179 followed by ETAD213 with 1.171. Other prominent genotypes were LLR2, ETAD170, ETAD1 and ETAD233 with 1.169, 1.164, 1.151 and 1.442 respectively (Figure 8).

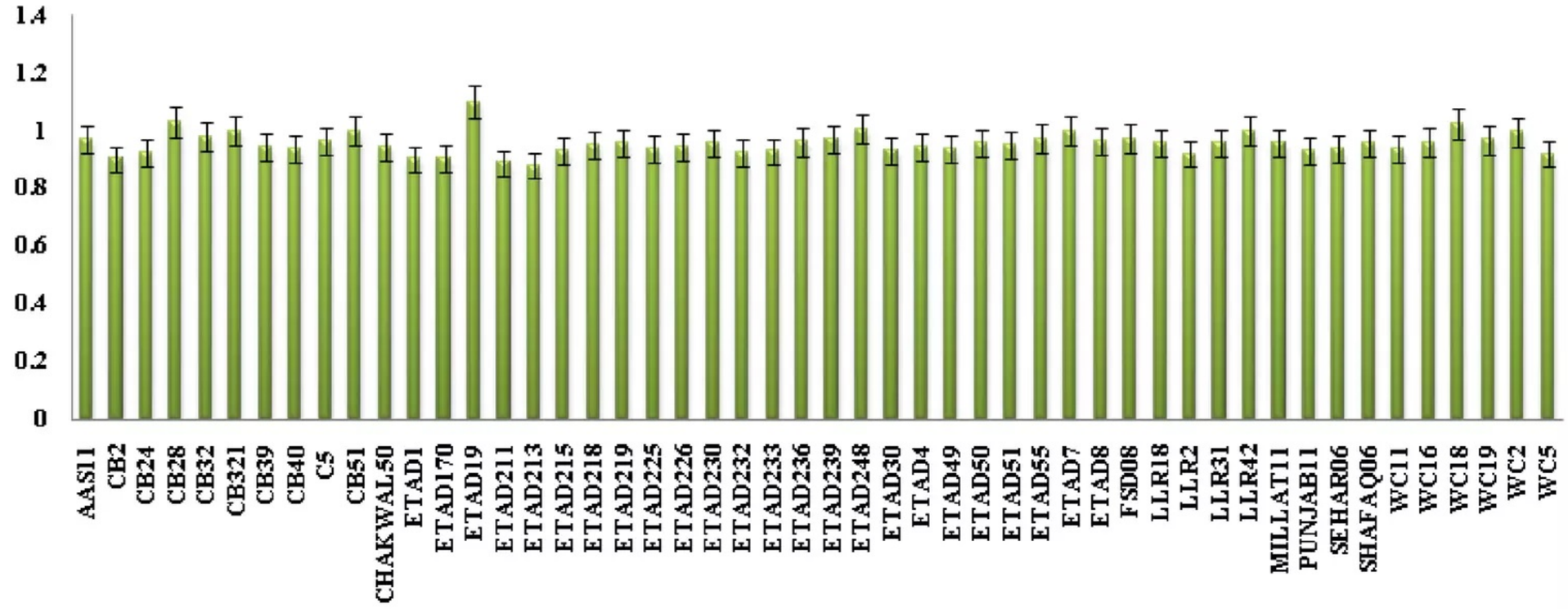

Figure 7: Percent shoot length variation under drought stress condition as compared to control seedlings

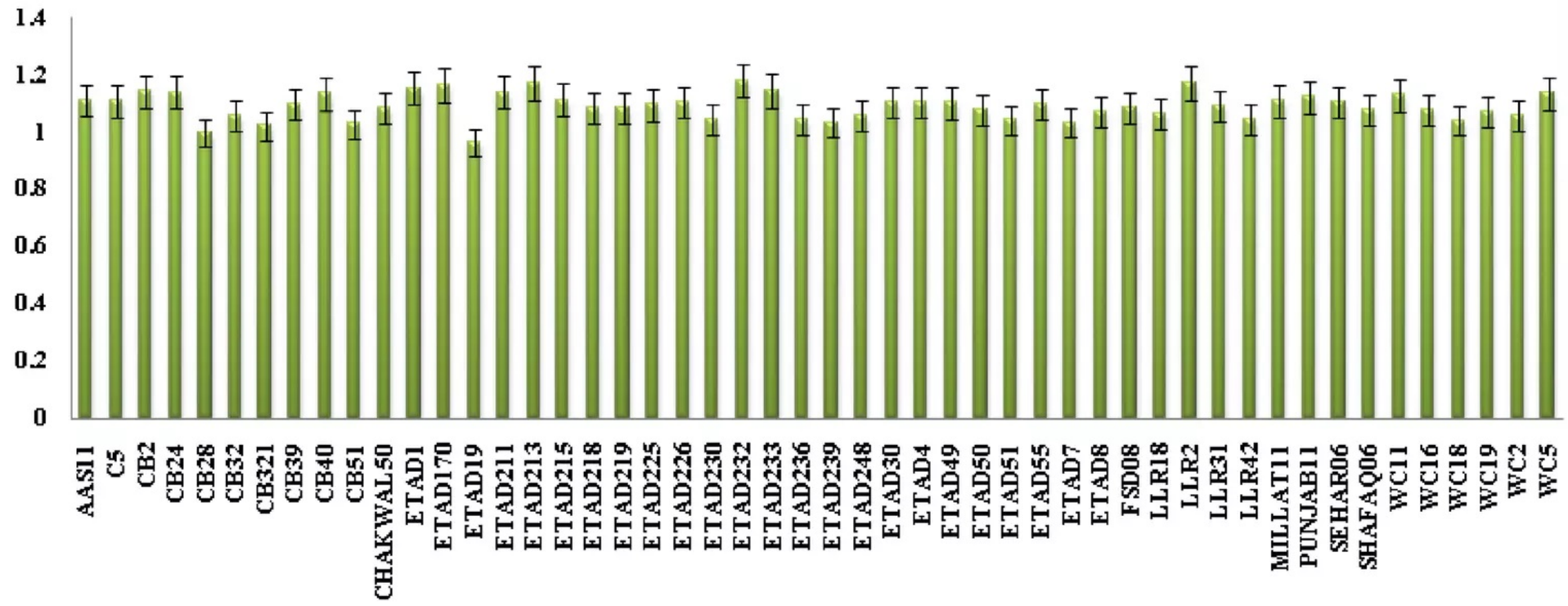

Figure 8: Percent variation in root/shoot length ratio under drought stress condition as compared to control seedlings

Table 2: Eigen values and proportion of variability

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Eigen value	2.799	1.645	1.536	1.175	0.821	0.492	0.421	0.103	0.009
Variability (%)	31.096	18.275	17.066	13.061	9.127	5.461	4.675	1.143	0.095
Cumulative %	**31.096**	**49.371**	**66.437**	**79.498**	**88.625**	**94.086**	**98.762**	**99.905**	**100**

Principal Component Analysis: Out of nine principal components (PCs), first 4 components exhibited eigen values greater than 1 which can be regarded as significant while rest (Table 2). First 2 PCs exhibited 49.371% variation in these genotypes. First PC accounted for 31.096% variation followed by 2nd PC with 18.275% variation (Table 2). First PC was highly related to studied attributes such as fresh seedling weight; shoot length, root length/shoot length ratio and seedling length (Table 2). This implies that PC1 is a weighted mean of these four attributes. The attributes of significant vitality in 2nd PC was relative water contents and cell membrane stability (Table 3).

Table 3: Squared cosines of the variables

	F1	F2	F3	F4	F5
RWC	0.01	0.73	0.03	0.02	0.00
FSDLW	0.53	0.01	0.00	0.12	0.00
DSDLW	0.09	0.08	0.01	0.66	0.00
RL	0.07	0.00	0.83	0.04	0.02
SL	0.87	0.01	0.05	0.03	0.00
RT/SHT	0.57	0.00	0.38	0.01	0.00
SDL	0.64	0.08	0.18	0.03	0.01
chlA/chlB	0.00	0.37	0.05	0.08	0.47
CMS	0.03	0.37	0.00	0.19	0.32

RWC= relative water contents, FSDLW= fresh seedling weight, DSLW= dry seedling weight, RL= root length, SL= Shoot length, RT/SHT= root shoot ratio, SDL= seedling length, chlA/chlB= chlorophyll A and B. CMS= cell membrane stability.

The projection of pattern of the attributes on PC1 depicted that the vital drought tolerance contributing attributes are relative water contents, chlorophyll a/b, and fresh and dry seedling weight (Figure 9) as they congested far away from the point of origin hence of more breeding value. The projection of genotypes exhibited population structure (Figure 10 and 11).

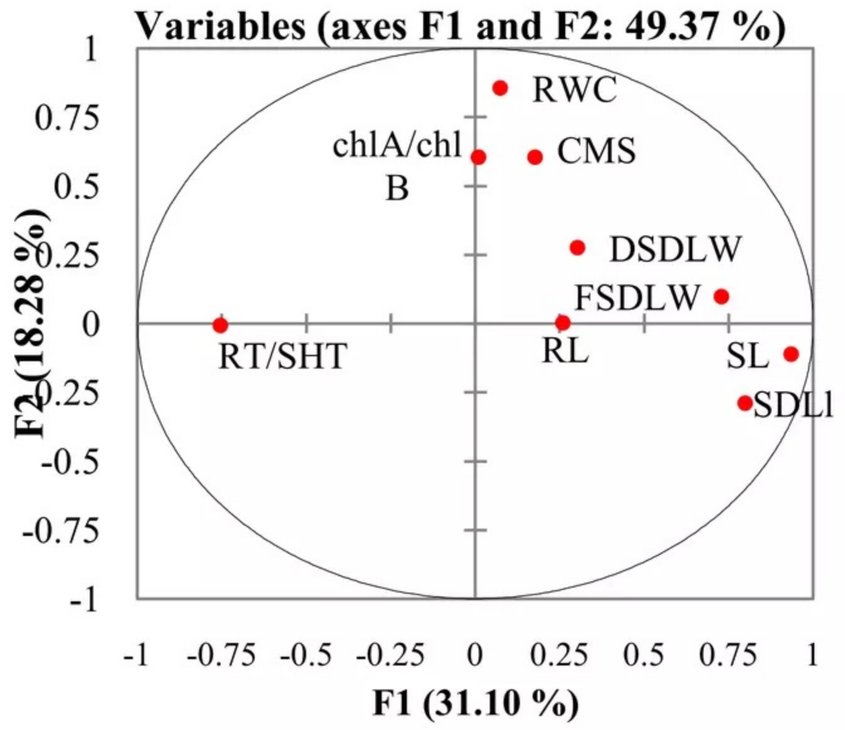

Figure 9: Projection pattern of attributes
RWC= relative water contents, FSDLW= fresh seedling weight, DSLW= dry seedling weight, RL= root length, SL= Shoot length, RT/SHT= root shoot ratio, SDL= seedling length, chlA/chlB= chlorophyll A and B. CMS= cell membrane stability

According to the observation on axes PC1 and PC2, the genotype ETAD232 is in contrast to the ETAD170, AAS11, and ETAD226. ETAD19 is opposite to ETAD211, ETAD248, CB51, ETAD215, ETAD55 and ETAD8 are in contrast with WC18, CB28, CB321, LLR42, CB40 and ETAD239 (Figure 11).

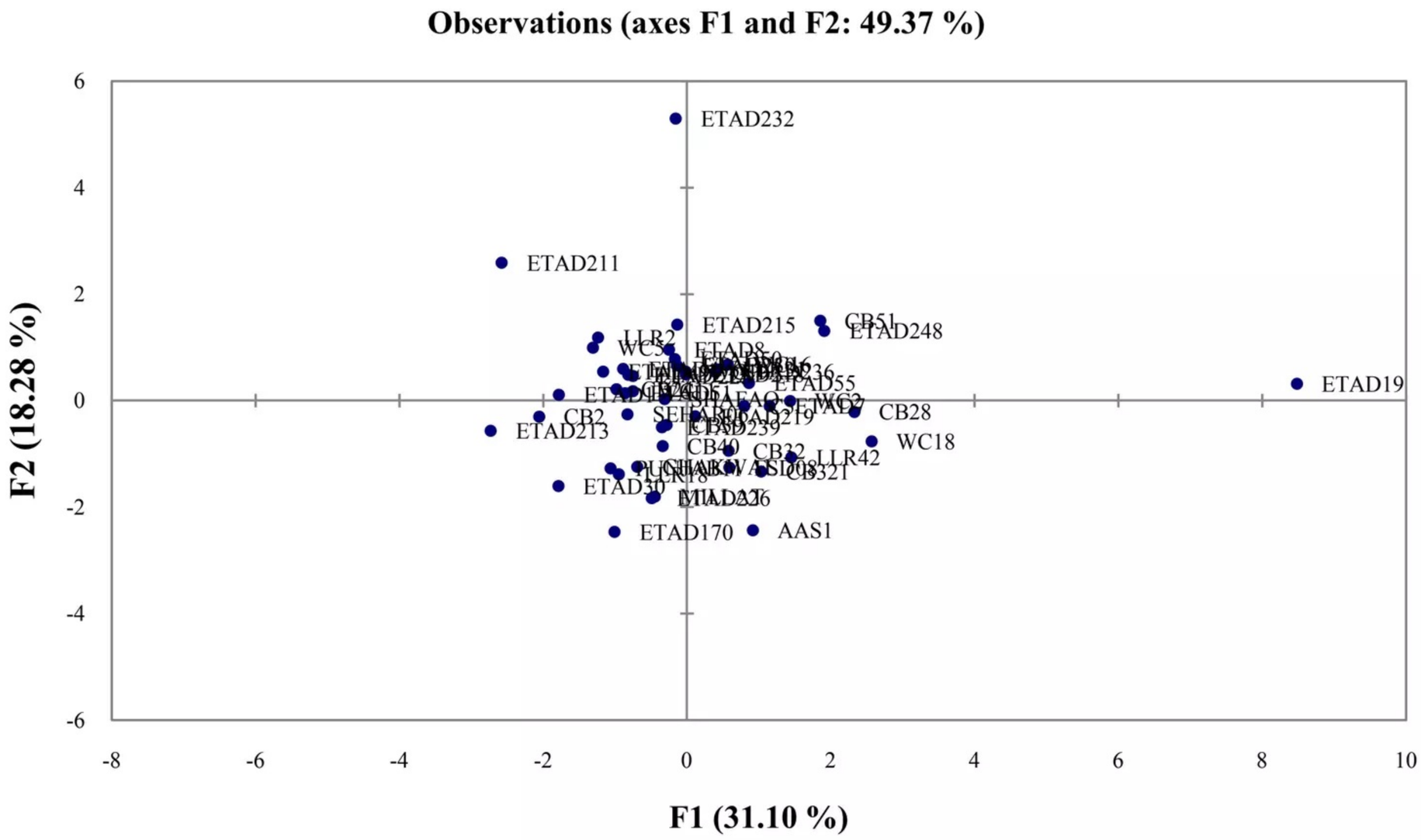

Figure 10: Two dimensional ordinations of 50 wheat genotypes on PC1 and PC2

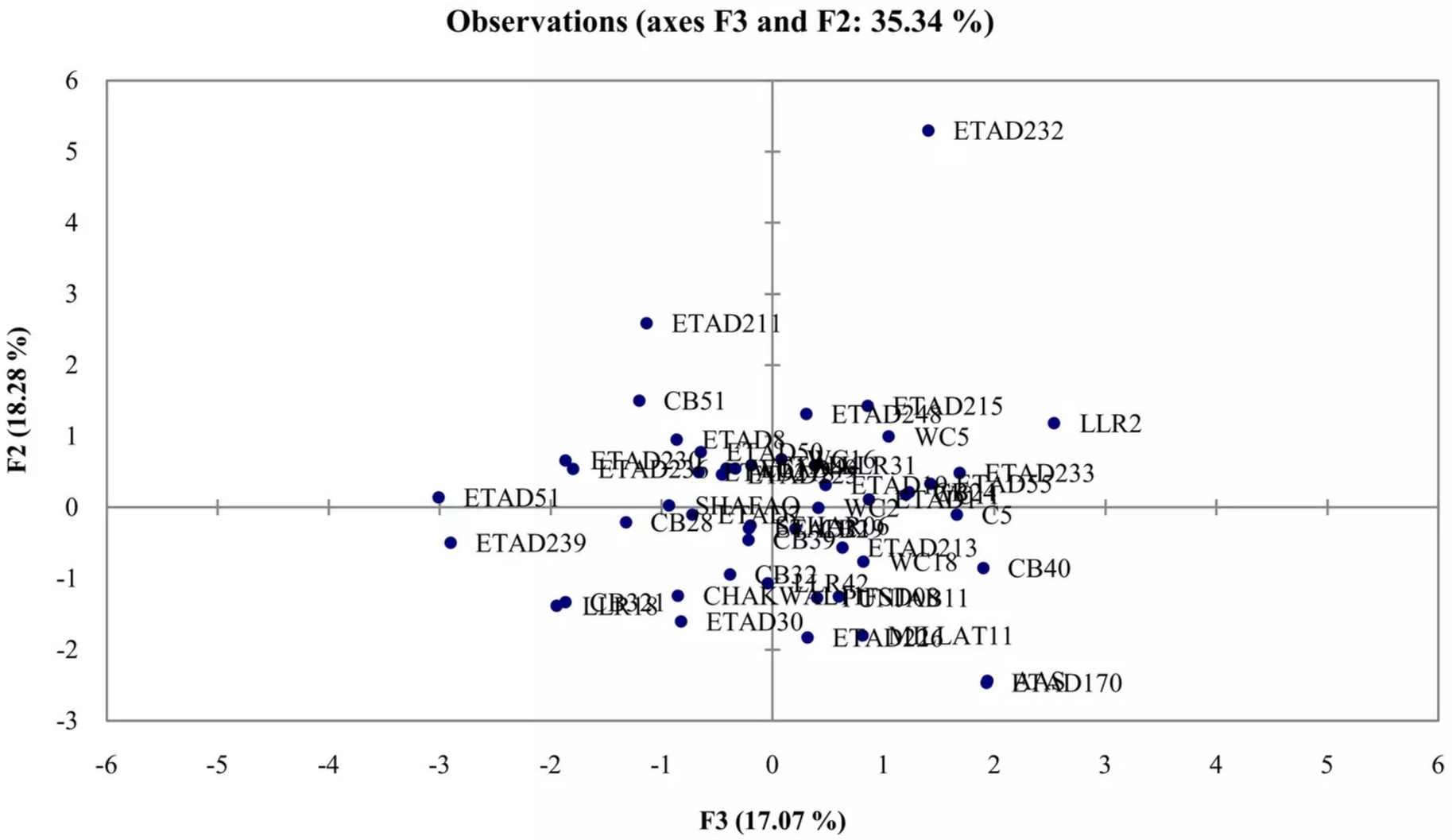

Figure 11: Two dimensional ordinations of 50 wheat genotypes on PC2 and PC3

Whereas on the basis of PC2 and PC3 , the contrasting genotypes are, ETAD232 and ETAD170. LLR2 is opposite to CB51. ETAD215, WC5, ETAD233 and ETAD248 are in contrast to ETAD211, ETAD8, ETAD51, SHAFAQ06, and ETAD30. MILLAT11, CB40, ETAD213, LLR42 and ETAD211 are opposite to the ETAAD239, LLR18 CHAKWAL50 and CB32 (Figure 11). From figure 10 and 11 it is clear that ETAD232 congested away from point of origin on the graph hence more diversified among rest of all. On the other hand wheat genotypes clogging very near to the point of origin are less diversified hence of less breeding value. From figures 10 and 11 it is clear that wheat genetic background is becoming narrowing due to extensive breeding for selected number of genotypes. (Sajjad *et al.,* 2011) also reported similar results in his findings.

CONCLUSION: After evaluating wheat germplasm on given attributes i.e. shoot length, seedling length, relative water contents and root shoot ratio were noted as vital traits for drought tolerance breeding programs. The range of variation for the given traits was higher in the germplasm and could be utilized for transgressive segregation but the germplasm utilized in present investigation were structured. Hybridization among diversified genotypes could make the breeding material free of population structure. This hybridization could be carried out for the development of promising genotypes and could be utilized to create variability for forthcoming wheat improvement programs.

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