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Research Paper

Genetic variability and correlation studies for seedling traits of wheat (*Triticum aestivum* L.) genotypes under normal and water stress conditions

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Abstract. Drought is the major abiotic limiting factors for wheat crop production. This study aimed to evaluate the wheat germplasm for drought tolerance and the association of seedling parameters. An experiment was conducted in wire house, Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan. Seventy five genotypes were sown under normal (control) and water stress conditions. Data were collected for shoot length, root length, root-shoot ratio, fresh weight, dry weight and leaf water contents of seedlings. On the basis of these parameters cluster analysis was done for grouping of genotypes. Under normal conditions 12 clusters were formed while in water stress conditions genotypes were grouped into 11 clusters. Formation of large number of clusters indicates huge variation among the genotypes. Results revealed that all the studied parameters differed significantly ($P < 0.05$) among all genotypes. Under water stress conditions, performance of shoot length, root length, root-shoot ratio, fresh weight, dry weight and leaf water contents were suppressed by 21, 6, 33, 39, 8 and 16% respectively as compared to normal conditions. Most of the parameters studied were significantly correlated among themselves. Consequently, adequate attention should be given to the negatively correlated seedling parameters during selection.

Keywords: Correlation, seedling, stress, wheat germplasm.

INTRODUCTION

Wheat is the largest consumable cereal in the world. It is grown on both irrigated and arid lands. In Pakistan, it is grown on an area of 9.062 million hectares with an annual production of about 25 million tons. It contributes about 13% to the value added in agriculture and 2.8% to gross domestic product (GDP) of the country. Due to rapid increase in population, gap between supply and demand is increasing. In contrast, the land for cultivation is decreasing due to rapid urbanization and colonization on the agricultural lands (Economic Survey, 2009-2010). So there is dire need to increase per acre yield of wheat by using available resources.

The wheat yield attained by the Pakistani farmers is far below the potential yield as reported by the breeders. There are many reasons behind this low yield; however, the major reason for is the unavailability of irrigation water especially at critical growth stages. Seedling parameters are the useful criteria for screening of wheat germplasm at early stages of growth (Khan et al., 2010). Adverse effect of water stress on early stages of wheat growth is reported by many scientists (Blum, 1979; Dhanda and Sethi, 1998; Dhanda et al., 2004; Rauf et al., 2007). So we can significantly increase the national wheat yield by developing drought tolerant varieties.

In Pakistan, drought is a major threat to wheat productivity. Drought tolerance of wheat varieties can be estimated from the response of varieties under varying environments (Talouizite and Champigny, 1988; Ahmad et al., 2003). Several physiological and morphological criteria have been described by the breeders to select genotypes that can perform well under drought conditions. Various researchers used seedling traits of wheat like root length, shoot length, fresh and dry shoot weight, root to shoot ratio, leaf water contents for screening germplasm under water stress conditions (Sojka et al.,1981; Khan et al., 2002; Khan et al., 2010; Rauf et al., 2007; Dhanda et al., 2004).

The main objectives of the current research were to: (i) determine the performance of wheat genotypes under water stress conditions to select genotypes that can be used for breeding under water stress conditions; and also to (ii) find the association between different seedling traits that are related to germination and seedling establishment parameters.

MATERIALS AND METHODS

To explore the objectives outlined above a wire house experiment was carried out in the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan. Seventy five wheat genotypes having diverse genetic base were collected from the gene pool of the National Agriculture Research Council (NARC) Islamabad, Ayub Agriculture Research Institute (AARI) Faisalabad and University of Agriculture Faisalabad to study different seedling parameters under control and water stress conditions. This experiment was conducted using a factorial experiment based on a completely randomized design (CRD) with two replications. Seeds of the genotypes were sown in 9” × 4” polythene bags in two soil environments, that is, control and water stress. Polythene bags were filled with sand and at first watering nutrients (N, P and K) were applied. To create water stress conditions in the pot, the irrigation was withheld for every four weeks (75% water stress). In control water was applied weekly while on the stressed environment it was applied after four weeks. Eight weeks after the sowing data for following parameters were collected:

Shoot and root length (SL and RL): Shoot and root length of three seedlings was measured in centimeters (cm) from the base of shoot to the top from each replication and then average was calculated.

Root-shoot ratio (R-S-R): Root shoot ratio was measured by dividing root length to shoot length.

Fresh shoot weight (FSW): Fresh seedlings were taken from the polythene bags and weight of three seedlings from each replication was measured in grams (g) with the help of a digital balance and then average was calculated.

Dry shoot weight (DSW): Firstly, seedlings were sun-dried and then in oven for 72 h to attain constant weight. Weight of three seedlings was measured in g with the help of electronic balance and then average was calculated.

Leaf water contents (LWC): leaf water contents were measured by using the following formula,

$$LWC = 100\ (FSW - DSW)/FSW. \qquad (1)$$

Statistical analysis

Data obtained from the current investigation was subjected to analysis of variance and simple correlation coefficients were calculated between the seedling traits and their significance was tested by using t-test (Steel et al., 1997). To find the variability among the genotypes cluster analysis was carried out by using Ward's method and squared Euclidian distance and identification the cutting point was done using discriminate analysis (Kumar et al., 2009). This analysis was done by using the statistical software SPSS version 17.0 (SPSS, Chicago, USA) program and graphs were prepared by using Sigmaplot 12.0.

RESULTS AND DISCUSSION

The results revealed a huge substantial variation ($P < 0.01$) in seedling parameters among the genotypes. The effect of environment (water conditions) was also highly significant on all traits. Similarly genotype and environment interaction is also significant which indicates that seedling parameters are severely affected due to stress (Table 1). However, most of the genotypes grouped differently under both conditions which showed that they performed differently under normal and water stress conditions (Table 2 and 3). Under normal conditions, average shoot length varies from 24 to 37 cm while under water stress conditions it ranged from 19 to 33 cm (Figures 1 to 7). The respective values in case of root length ranged from 20 to 28 cm in normal conditions (Figure 2) and 21 to 23 cm (Figure 8) under water stress conditions. Root to shoot ratio also affected by water stress conditions and it ranges from 0.6 to 0.95 in normal conditions (Figure 3) and in water stress conditions (Figure 9) it ranges from 0.48 to 0.54. Averages value of fresh weight for the clusters ranges 0.3 to 0.9 g (Figure 4)

Table 1. Mean squares of 30 wheat genotypes under controlled and water stress environments for seedling characters.

Character	Genotype (74 d.f)	Environment (1 d.f)	G x E (74 d.f)	Error (74 d.f)	CV%
Shoot length	30.699**	3550.355**	18.494**	1.218	4.09
Root length	5.583**	132.495**	5.162**	0.642	3.66
Root-shoot ratio	0.045**	2.145**	0.023**	0.002	5.63
Fresh weight	0.044**	3.493**	0.031**	0.003	11.03
Dry weight	0.002**	0.007**	0.001**	0.0003	13.26
Leaf water content	68.411**	10254.235**	74.578**	40.753	9.13

d.f = Degree of freedom. ** = Significant at 1%. G × E = Genotype × environment interaction

Table 2. Grouping of genotypes grown under controlled conditions by cluster method.

Cluster	Genotypes
1	6117, 6016, 7200, 76314, 6111, V-05BT006, AUP-4008, B-07, V-05066, CT-03457, 9247, Manthar 2003, GA 2002
2	Sehar 2006, DN-62, PR-90
3	6068, 4770, 9316, 9436
4	7178, B-08, V05067, V-05068, KOHISTAN 97, 9258, 9407, 9272, 9372, 9451, 94444
5	9242, Pb-96, Uqab 2000, 9446, 9438, 05BT06, V-NR, 70312, Shafaq 2006, 33010
6	05BT06, LU26, 9268, 9437
7	Inqlab 91, 9250, 4943, LU26
8	SD-408513, , 9268, V-05082, NR-356, M H 97, 9267, 5039, Barani 83, Lasani-2008, Chakwal-50, V-04022
9	ZAS-70, 22-03, NIA-817, 66284, 6103, 9381
10	9437, 9435-2, Chakwal 86, Pak 81, 7012, 9452
11	7189, 9244
12	MH 99, 9277

Table 3. Grouping of genotypes under water stress condition by cluster method.

Cluster	Genotypes
1	6117, V-05066, 9316, , 9242, 6500, 9446, Inqalab 91, 4943
2	6016, 7200, Sehar 2006, 6111, V-05068, Kohistan 97, 9272, Uqab 2000
3	76314, 7178, 4770, 9372, 9451, 9444, pb-96, 9438
4	V-05BT006, AUP-4008, B-08, V-05067, 9436, 9258, 9247, 9407, Manthar 2003, GA 2002
5	DN-62, PR-90, B-07, CT-03457, Chanab 2000, 9250
6	05BT06, LU26, 9268, 9437
7	V-NR, 33010, 22-03, shafaq 2006
8	70312, 66285, SD-308513, V-04022, NR-356, NIA-817
9	66284, 6103, Pak 81, MH 97, 9267, 5039, 9244, MH 99, 9277, 9381
10	7189, 9435-2, 7012, 9452, Barani 83, Lasani 2008
11	Chkwal 86, Chaakwal 50

which is a huge variation while in water stress conditions it ranges 0.20 to 0.55 g (Figure 10). Means values of dry weight ranges 0.10 to 0.17 (Figure 5) in normal conditions while in water stress condition it varied from 0.10 to 0.17g (Figure 11). Leaf water contents varied 62 to 84% in normal conditions (Figure 6) while in water stress conditions it ranges from 0.50 to 0.74% (Figure 12). Table 4 shows the magnitude of decrease in the seedling performance under stress environment. Shoot length, root length, root shoot ratio, fresh weight and dry weight decreased up to 22.6, 5.88, 33.27, 38.8 and 7.61% respectively due to stress environment. The broad sense heritability also decreased greatly in traits root length, root to shoot ratio, fresh weight, dry weight and leaf moisture content from 84.02 to 62.3, 90.13 to 84.83, 89.39 to 81.3, 74.68 to 71.79 and 47.02 to 22.04% respectively showing the influence of drought stress on genome of different genotypes. However in case of shoot length, broad sense heritability (h^2) increased from 83.68 to 94.26%. This loss in performance might be ascribed to

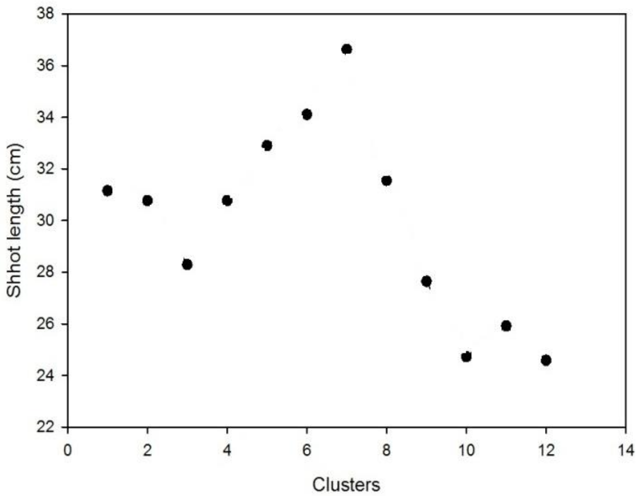

Figure 1. Shoot length under normal conditions.

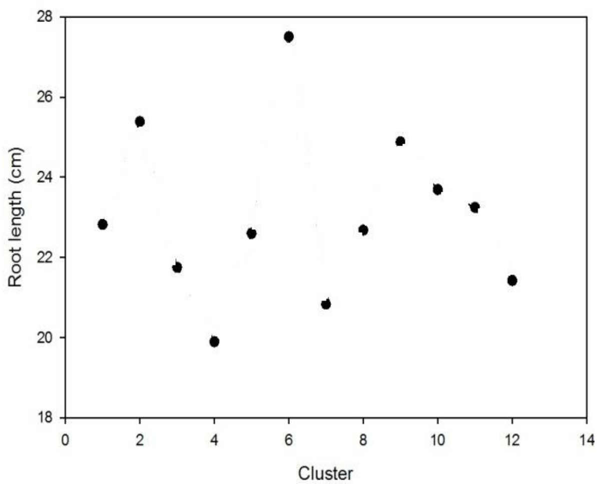

Figure 2. Root length under normal conditions.

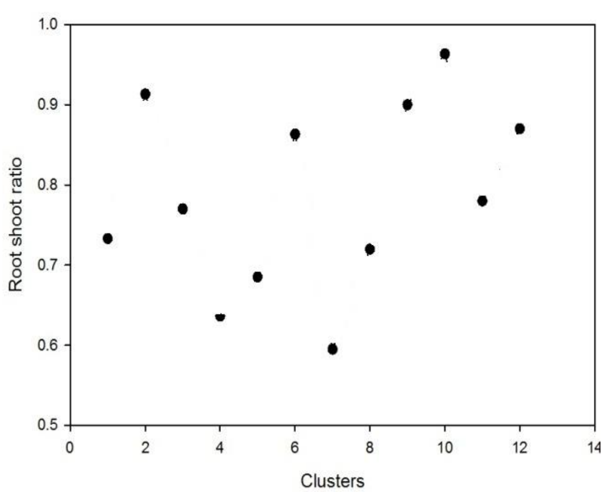

Figure 3. Root shoot ratio under normal conditions.

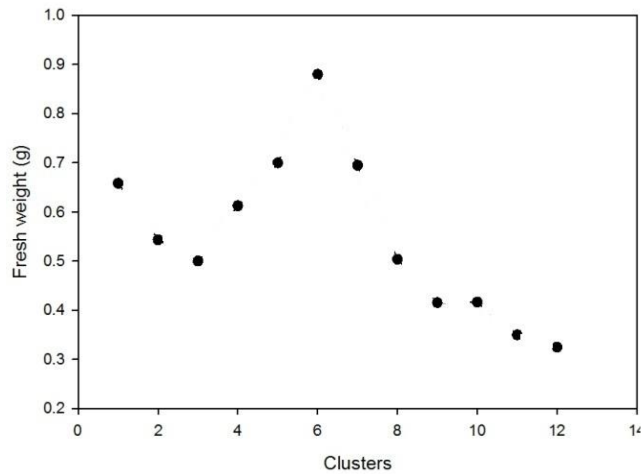

Figure 4. Fresh weight under conditions.

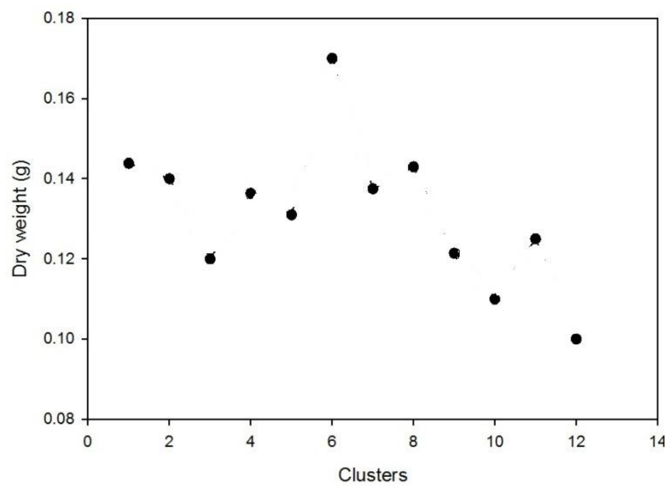

Figure 5. Dry weight under normal conditions.

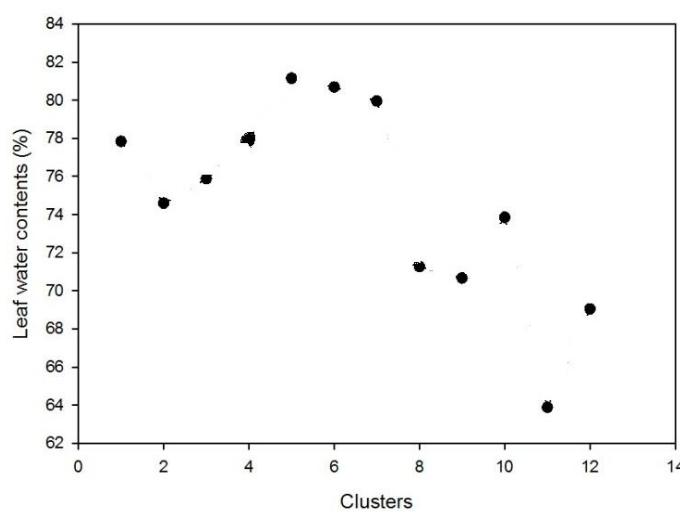

Figure 6. Leaf water contents under normal conditions.

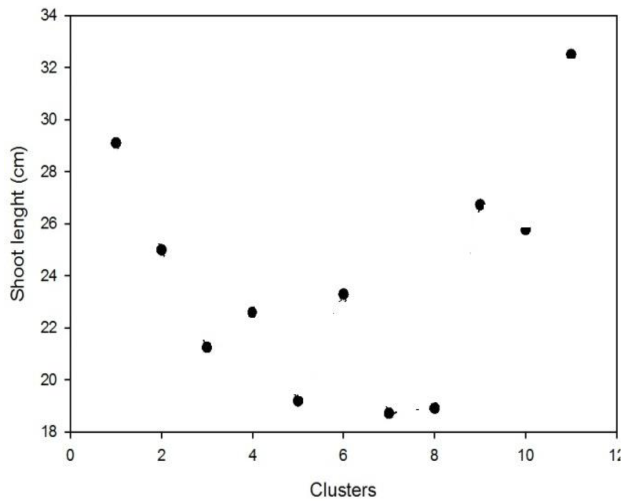

Figure 7. Shoot length under water stress conditions.

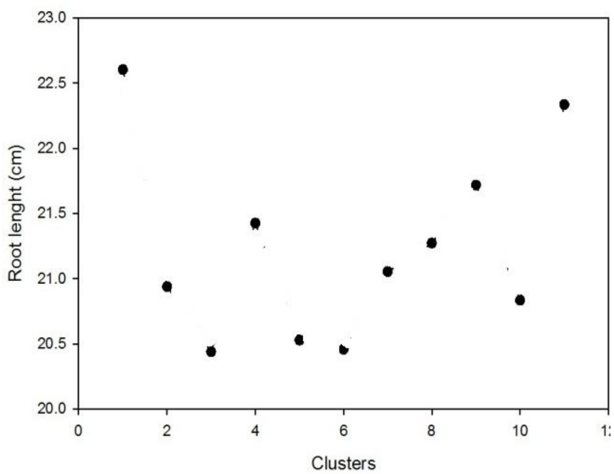

Figure 8. Root length under water stress conditions.

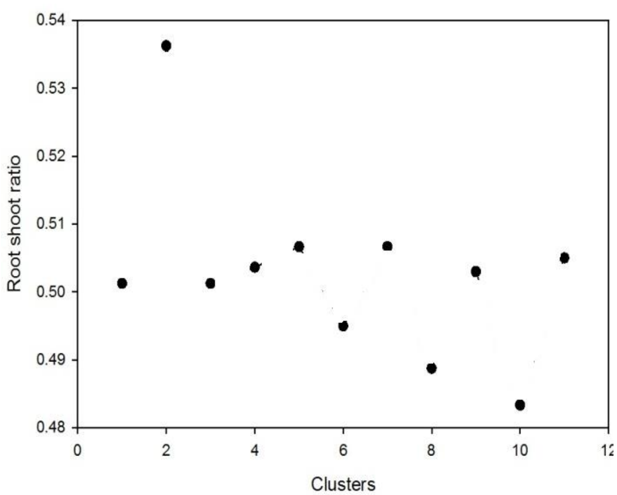

Figure 9. Root shoot ration under stress conditions.

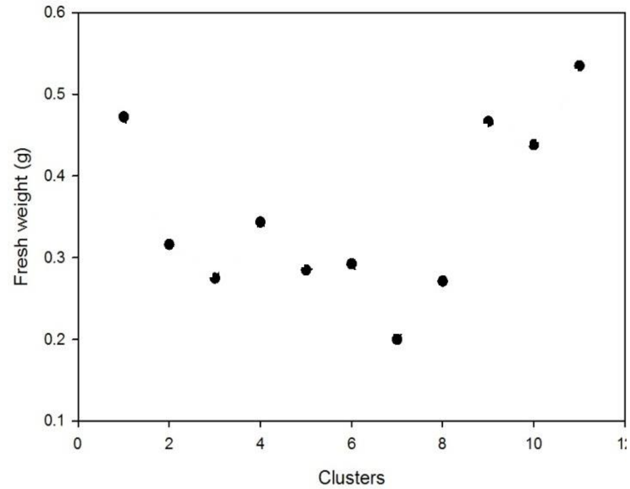

Figure 10. Fresh weight under water stress conditions.

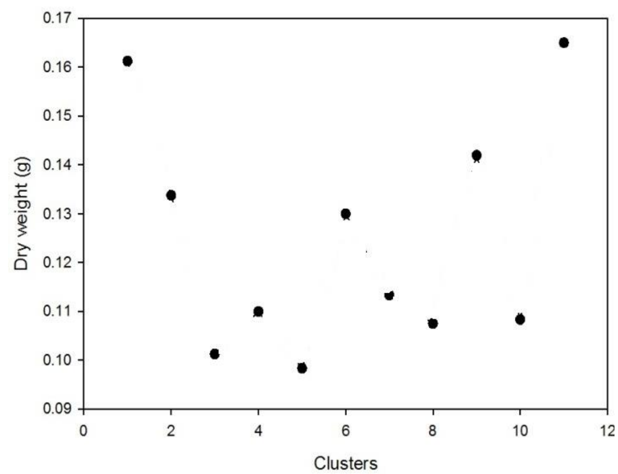

Figure 11. Dry weight under stress conditions.

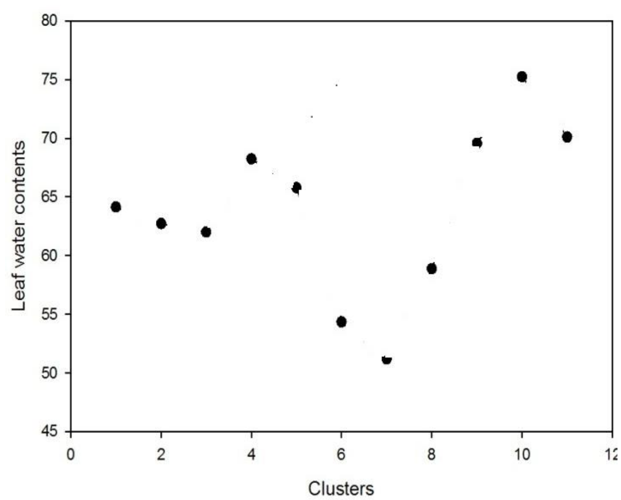

Figure 12. Leaf water contents under water stress conditions.

Table 4. Range and decrease in mean values and heritability of 75 wheat genotypes in control (E_1) and water stress (E_2) environments for various characters.

Character	Environment	Range	Mean ± SE	% Decrease in E_2	% h^2
SL (cm)	E_1	37.17 - 21.17	30.4 ± 0.38	22.6	83.68
	E_2	33.50 - 17.00	23.56 ± 0.43		94.26
RL (cm)	E_1	27.67 - 17.33	22.57 ± 0.23	5.88	84.02
	E_2	23.5 - 18.5	21.25 ± 0.142		62.3
R-S-R	E_1	1.1 - 0.45	0.75 ± 0.014	33.27	90.13
	E_2	0.84 - 0.41	0.503 ± 0.006		84.83
FW (g)	E_1	0.98 - 0.22	0.57 ± 0.019	38.8	89.39
	E_2	0.58 - 0.04	0.35 ± 0.012		81.3
DW (g)	E_1	0.22 - 0.07	0.13 ± 0.0035	7.61	74.68
	E_2	0.2 - 0.07	0.12 ± 0.0035		71.79
LWC (%)	E_1	85.71 - 55.56	75.75 ± 7.49	15.45	47.02
	E_2	80 - 33.34	64.05 ± 8.82		22.04

drought or water shortage which slows down the physiological process and ultimately the growth decreases. Characters that show high heritability should be selected as selection criteria for improvement through breeding.

Presence of genetic variation among the genotypes for all the traits studied indicates that there is room for improvement for these traits. Significance of genotype and environment interaction showed that water stress severely affects the seedling growth, but significant results of analysis of variance indicate the dissimilar response of genotypes for different traits under stress. There are some genotypes that showed equal performance under both conditions for some traits. Genotypes performing best under normal conditions do not necessarily perform best under stress. So there is dire need to evaluate germplasm under stress before using it in breeding program for stress. Maximum effect of water stress was found on fresh weight. The reason of this may be that most of the water is used by the leaf so it is severely affected. Dry weight was least affected by water stress. Dry weight does not contain water but reduction in dry weight is due to the decrease in growth caused by water stress. Maximum broad sense heritability was found in fresh weight and shoot length under controlled and stress conditions respectively that validates their importance as selection criteria for improvement. Effect of water stress on the performance for different traits of wheat under stress environment to different extents is also described by different researchers (Blum et al., 1980; Katerji et al., 1994; Chandrasekar et al., 2000; Khan et al., 2006; Khan et al., 2010).

The correlation is the most common and useful statistics. A correlation is a single number that describes the degree of relationship between two variables. It is useful because it can indicate a predictive relationship that can be exploited in practice. Correlation study may help for selection of suitable genotypes. The correlation study provides the information about the association among different traits. The simple correlation coefficient among different traits was found to be highly significant in the control environment except between dry weight and leaf water contents (-0.017), the root length and fresh weight (-0.151) and root length and dry weight (-0.049). Shoot length is negatively correlated with root length and root shoot ratio while positively correlated with other traits. Root length was negatively correlated with all traits except root shoot ratio. Fresh weight has positive correlation only with shoot length in controlled environment (Table 5). Characters that are positively correlated with each other can be selected together for the improvement. The parameters that are negatively correlated can affect the performance of other during selection process. But correlation of seedling traits with yield should be considered important in selecting parameters for selection.

Under water stress environment, value of correlation coefficient between shoot length and root length changed from r = - 0.225 to r = 0.327. Correlation of root length with root-shoot ratio and leaf water contents change from highly significant value (r = 0.686) and (r = - 0.223) to non-significant value (r = 0.093) and (r = 0.085) respectively. Similarly correlation between root length and fresh weight (r = - 0.151), between root length and dry weight (r = -0.049), between leaf water contents and

Table 5. The simple correlation coefficients among seedling parameters in control conditions.

	SL	RL	R-S-R	FW	DW
RL	-0.225**				
R-S-R	-0.847**	0.686**			
FW	0.560 **	-0.151 NS	-0.493**		
DW	0.348 **	-0.049 NS	-0.293**	0.758**	
LWC	0.437 **	-0.223**	-0.438**	0.604**	-0.017NS

** = Significant at 1%. * = significant at 5%. NS = Non-significant.

Table 6. Simple correlation coefficients among seedling parameters under water stress.

	SL	RL	R-S-R	FW	DW
RL	0.327**				
R-S-R	-0.895 **	0.093 NS			
FW	0.704 **	0.358**	-0.590**		
DW	0.505**	0.308 **	-0.411 **	0.641**	
LWC	0.299**	0.085 NS	-0.277 **	0.509 **	-0.293**

** = Significant at 1%. * = significant at 5%. NS = Non-significant.

dry weight (r = - 0.017) change from non-significant to highly significant with coefficient value (r = 0.358), (r = 0.308) and (r = -0.293) respectively. The negative correlation of root length with fresh weight (r = - 0.151), dry weight (r = - 0.049) and leaf water contents (r = - 0.223) change to positive correlation, that is, (r = 0.358), (r = 0.308) and (r = 0.085) respectively (Table. 6). These results are in accordance with Tavakol and Pakniyat (2007) and Khan et al*.* (2010) who worked on wheat. Different value of correlation coefficient in different environments may be due to different response of genes to different environments.

Under controlled conditions, highest correlation was found in shoot length and root shoot ratio which means that with the increase of shoot length root-shoot ratio decreases. Similarly fresh weight and dry weight are positively correlated showing that increase in fresh weight also increases the dry weight. So selection for fresh weight also improves the dry weight. Correlation of root length with shoot length, fresh weight, dry weight and leaf water contents changed from negative to positive in stress conditions. This follows the functional equilibrium in plants which shows that under drought plant invest more on root and in response root goes deeper to absorb more water and nutrients that are used in performance of shoot. Some stress genes might be activated in stress conditions to cause these changes. These results are in agree with the findings of Khan et al*.* (2002), Awan et al*.* (2007), Rauf et al. (2007), Tavakol and Pakniyat (2007) and Khan et al*.* (2010) who worked on wheat.

CONCLUSION

The analysis of variance and large number of clusters indicates the presence of very high variability among genotypes. The higher values of broad sense heritability indicate the selected traits are highly heritable and change in heritability values indicates interaction between genotypes and environment. The higher values of correlation coefficient show that the traits are highly correlated. The highly significant negative correlation of root shoot ratio indicates that selection for this trait may affect the performance of others. The change of correlation trend from positive to negative or negative to positive due to stress condition reflects the importance and strong effect of stress on genotypes. Further evaluation should be done for other yield parameters with correlation effect in field conditions.

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