The Journal of Animal & Plant Sciences, 25(2): 2015, Page: 431-441
ISSN: 1018-7081

MORPHO-PHYSIOLOGICAL DIVERSITY IN ADVANCED LINES OF BREAD WHEAT UNDER DROUGHT CONDITIONS AT POST-ANTHESIS STAGE

M. A. Ali[1,2*], M. Zulkiffal[3], J. Anwar[3], M. Hussain[3], J. Farooq[4] and S. H. Khan[2]

[1]Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan, [2]Centre of Agricultural Biochemistry & Biotechnology, University of Agriculture, Faisalabad, Pakistan, [3]Wheat Research Institute, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan [4]Cotton Research Institute, AARI, Faisalabad, Pakistan,
[*]Corresponding Author: Amjad.Ali2001@gmail.com, Amjad.Ali@uaf.edu.pk

ABSTRACT

Fifty two advanced lines of bread wheat including 6 approved varieties were used to study morpho-physiological diversity under drought conditions at post-anthesis stage based on 18 plant characters. A multivariate approach including correlation, principal component and cluster analysis was used to quantify the variability among the wheat lines. Correlation analysis showed that grain yield was positively correlated with yield attribute like head weight, spike length, spikelets per spike, grains per spike, days to heading and days to maturity. Grain yield, on the other hand, did not show any positive association with physiological traits. Relative water content was positively correlated with head weight and negatively associated with excised leaf weight loss. Head weight was negatively correlated with residual transpiration. Principal component (PC) analysis resulted in six principal components (PCs) having Eigen value >1 which contributed 70.908% of the total variability amongst the wheat genotypes assessed for various morph-physiological traits. The PC I contributed maximum towards the variability (24.108%) and this variability was mainly conditioned by yield attributes like spikelets per spike, grains per spike, days to heading, head weight, flag leaf area, spike length and grain yield. PC analysis also supported the results of correlation analysis that yield components were not correlated with the physiological characters. Cluster analysis grouped 52 genotypes into 4 clusters. Cluster 1 comprised of 17 genotypes, cluster 2 of 16 while cluster 3 had 6 and cluster 4 contained 13 genotypes. Cluster 1 comprised of lines having high values for grain yield attribute, while cluster 2 lines showed drought tolerance characters like high relative water content and less residual transpiration. Altogether, this research showed that enormous amount of morpo-physiological variation was present in this group of genotypes which could be helpful for selection of favourable cultivars for drought tolerance in bread wheat.

Keywords: Bread wheat, drought tolerance, morpho-physiological traits, multivariate analysis.

INTRODUCTION

In cereal crops like wheat, water stress sometimes coupled with heat stress at critical growth stages like seedling establishment, tillering and reproductive stages may result in considerable effects ranging from yield reduction to lethality for crops (Farooq *et al*., 2011). Moreover, in contrast to other stages of plant growth, the moisture deficit at the reproductive stage results in the major decline in the crop productivity (Ali *et al.*, 2009b; 2011). Therefore, to ensure the food demands of ever increasing population there is a dire need to develop crop cultivars which can yield better under scarce water availability (Shao *et al.*, 2006). The mechanism of drought tolerance is very complex. So quantification of drought tolerance should be based on the grain yield under both stress and non-stress environments that may result in the selection of genotypes which can yield better under stress condition because the response of selection under non-stress condition is maximum and heritability of the yield under these conditions is high (Shirinzadeh *et al.* 2010; Geravandi *et al.*, 2011).

Grain yield in wheat, which is an ultimate parameter for measurement of drought tolerance, is the outcome of combined effect of different parameters (Anwar *et al.*, 2009). Significant progress has been made to develop models that can estimate wheat grain yield and distinguish the ideal plant (Leilah and Al-Khateeb, 2005). The proper knowledge of genetic correlation between grain yield and its related traits under water stress would improve the efficiency of breeding programs by identifying suitable indices for selecting wheat varieties (Evans and Fischer, 1999). Ali *et al.* (2010) reported that flag leaf area and presence of awns are significant indicators of high grain yield in wheat. However, according to Moghaddam *et al.* (1998) negative correlation was found between plant height and grain yield due to the lower number of grains/spike.

Various statistical procedures like correlation, regression, path analysis, factor analysis, principal components and cluster analysis have been adopted in modeling crops yield. Association analysis is an important statistical tool to initiate and weigh up breeding programs for high yield, as well as to study direct and indirect contribution of the yield attributes (Anwar *et al.*

2009). Many researchers exploited these techniques for sorghum (Ali *et al.*, 2009b, 2011) and wheat (Mohamed, 1999, Anwar *et al.* 2009). The principal component (PC) analysis is a multivariate statistical tool for examining and simplifying complex data sets. This analysis transforms the larger number of correlated variables into smaller ones. The principal components have been explained by Everitt and Dunn (1992). Similarly cluster analysis can be utilized to select variables that can be categorized into main and subgroups based on homogeneity and dissimilarity. For crop modeling and parental selection in breeding programs, cluster analysis can serve the purpose (El-Deeb and Mohamed, 1999; Jaynes *et al.*, 2003, Ali *et al.* 2011). Based on the importance of these statistical techniques and their implication to uncover morphological and physiological variation in wheat, a research was planned under water stress conditions. The aim of present study was to clarify the relationship between wheat grain yield, its components and some drought related physiological parameters using these statistical procedures.

MATERIALS AND METHODS

Plant cultivation: The present research was carried out at Wheat Research Institute (WRI), Faisalabad, Punjab, Pakistan (31º24´ N and 73º02´ E) during 2008-2009 under irrigated and drought (irrigated only at the time of sowing and afterwards entirely depending on rainfall) conditions.

Fifty two wheat genotypes belonging to local trials (A, B and Micro trials) were grown in 5 x 1.62 meter^2 plot in three repeats. The genotype accession number and pedigree information is given in Supplementary Table 1 for various lines. Production technology of wheat has been given in Anwar *et al.* (2009). Soil texture of the site was loam. At the depth of 0-15 cm soil electrical conductivity (EC) was 2.25 ms/cm, pH 8.1, organic matter 0.91, available phosphorus and potassium was 8.9 ppm and 340 ppm respectively. The water holding capacity at this depth was 37%. However, at the depth of 15-30 cm, EC and organic matter were same as at 0-15 cm depth while pH, water holding capacity, available phosphorus and potassium were 8.0, 38%, 6.3 ppm and 300 ppm respectively. The previous crop history was Wheat- Fallow-Barley-Fallow. The field was irrigated prior to sowing for both normal and drought conditions and after attaining the proper soil moisture, sowing was completed. Date of sowing was 12-15 November for both the years. For normal conditions, first irrigation was applied to the crop after 35 days and subsequent irrigations were applied at the start of flowering, anthesis and filling stages. There was a distance of 10 feet between the two blocks to avoid seepage of water between the blocks. On the other hand, no irrigation was applied for maintaining the water stress conditions and the wheat genotypes were solely dependent on the rainfall throughout the plant growth. The weeds were controlled chemically. For this purpose Bactril SUPER® was applied at post emergence after 1st irrigation at optimum moisture (‘watter’) conditions. At maturity 5 guarded plants were randomly selected from each plot and data were collected for different physiological and morphological plant traits.

Weather conditions: The meteorological data during both the growing seasons of 2008 and 2009 at Ayub Agricultural Research Institute, showed Faisalabad nearly similar patterns of rainfall (mm), temperature (ºC) and relative humidity (%) (Fig 1). The outline of Fig 1 clearly demonstrated that at different stages of plant growth (more pronounced at anthesis and post-anthesis stages), significant drought stress was induced naturally due to lower rainfall accompanied by high temperature.

Measurement of physiological characters: During both the years, at post-anthesis stage, flag leaves of 5 randomly selected plants from the plot were used for measurement of various physiological parameters in both normal and drought conditions. Flag leaf area (FLA) of 5 randomly selected plants from each replication was measured during early morning hours (h) when leaves were fully turgid. FLA (cm^2) was measured according to the procedure adopted by Ali *et al.* (2011). The flag leaves were oven dried at 80 ºC for 48 h and specific flag leaf area (SFLA) was calculated as a ratio of FLA to the oven dry weight (DW) of the leaves in grams. The specific flag leaf weight (SFLW) was calculated as SFLW = DWF/FLA. The estimation of excised leaf weight loss (ELWL) was done according Clarke and Townley-Smith (1986) and followed by Ali *et al.* (2009b).

The “residual transpiration” (RT) was measured according to Clarke *et al.* (1991) and recently Ali *et al.* (2011). For this purpose, flag leaves were excised and immediately brought to the laboratory and were placed in darkness for stomatal closure for 30 minutes at ambient room temperature. After 30 minutes the leaves were weighed (W1 in g) and were placed open at room temperature in the laboratory for 180 min, the leaves were again weighed (W2 in g) along with measurement of leaf area (LA.180). The RT on leaf area basis (g $H_2O/min/cm^2/10^5$) was calculated as given below;

$$RT = (W1 – W2)/ (LA.180)$$

Relative water contents (RWC) were determined for detached leaves. The percentage relative RWC were calculated from flag leaf blades using the method devised by Mata and Lamattina (2001). Similarly, the relative dry weight of the leaves (RDW) was calculated according to the formula as described by Ali *et al.* (2009b);

$$RDW = DW/ (TW-DW)$$

Morphological and yield traits: At maturity, data was collected from 5 guarded plants for plant height (cm), days to heading, days to maturity, peduncle length (cm), spike length (cm), awn length (cm), head weight (g), spikelets per spike, grains per spike, and seed index (1000 grain weight in gram). The date of harvesting of both years was 2-5 May. Grain yield was determined on kg/ha basis. For the assessment of grain yield, whole plot (5 x 1.62 m^2) was harvested and the grain weight was measured and afterwards was converted in to kg per hectare.

Statistical analysis: The average data of both the years were subjected to basic statistics, correlation analysis, cluster analysis and principal component (PC) analysis using statistical software packages of SPSS version 12 and STATISTICA version 5.0 (Sneath and Sokal, 1973). Cluster analysis was performed using K-means clustering while tree diagram based on elucidation distances was developed by Ward's method. The D^2 statistics was calculated according to Rao (1952). First two principal components were plotted against each other to find out the patterns of variability among genotypes and association between different clusters using SPSS version 12.

RESULTS AND DISCUSSION

Maximum temperature over 30 ºC and less rainfall during the month of March and early April exerted enough heat stress coupled with drought stress during the terminal stages of the crop (Fig. 1). These stresses resulted in enormous effects on various morpho-physiological characters in four varieties (Chakwal50, Auqab2000, Seher06, Fsd.08) grown under both normal and drought stressed conditions. On an average, the varieties grown under normal conditions showed higher values for all morpho-physiological traits as compared to the averages of the varieties grown under stress conditions.

Correlation studies: The basic statistics of various morpho-physiological traits demonstrated considerable range and variability among 52 wheat genotypes (Supplementary Table 2). The information regarding correlation among various traits is an important aspect for the initiation of any breeding program as it provides an opportunity for the selection of desirable genotypes having desirable traits (Ali *et al*., 2009c). Simple correlation coefficients revealed significant relationships among 18 traits (Table 1). Flag leaf area exhibited positive and significant association with specific flag leaf area, head weight, spikelets per spike and grains per spike while it showed negative significant association with specific flag leaf weight. Ali *et al.* (2010) reported that the removal of flag leaf have negative effects on the grain yield and its attributes in wheat, therefore displayed the important correlation of flag leaf with grain yield. Similarly, Aruna and Audilakshm (2008) emphasized on the importance of yield related traits in the development of high yielding cultivars in sorghum. The specific flag leaf area showed significant positive correlation with spike length and grains per spike and negative association with specific flag leaf weight while specific flag leaf weight, relative dry weight, excise leaf weight loss, residual transpiration and awn length did not show positive and significant correlation with any of the traits. For relative water content and plant height only positive association was found with head weight. The days to heading were positively and significantly correlated with days to maturity, spike length, awn length, head weight, spikelets per spike, grains per spike, and grain yield. Days to maturity exhibited significant positive associations with spike length, spikelets per spike and grain yield. For peduncle length positive correlation was found with spike length and awn length while spike length showed highly significant association with awn length, spikelets per spike, grain per spike and grain yield. Awn length was positively correlated with days to heading and days to maturity and peduncle length which showed that might be the lines maturing early have lengthy awns. The awns are reported to enhance grain yield in the cultivars having lengthy awns (Ali *et al.*, 2010) but in this study awn length did not show any positive association with grain yield and its attributes. Head weight showed positive and highly significant correlation with yield and yield components like spikelets per spike and grains per spike and these two traits showed positive association with grain yield. The positive correlation among the yield contributing traits suggested that these characters are important for direct selection of high yielding genotypes (Anwar *et al.*, 2009).

Interestingly, most of the drought related traits did not show significant negative or positive correlation with any of the yield related traits. This is in correspondence with the reports of Ali *et al.* (2011) and Karamanos and Papatheohari (1999), who demonstrated yield and drought tolerance are not correlated with each other in cereal crops. On the basis of current findings, selection under drought stress could be made on the basis of more flag leaf area, larger spikes, higher number of grains per spike, awn length and grain yield per plant. But Ali *et al.* (2011), Karamanos and Papatheohari (1999) suggested that for evolving cultivars under stress, the selection may not be based on yield.

Principal component analysis: The conservation and exploitation of genetic resources could be made by partitioning the total variance into its components. It also provides opportunity for utilization of appropriate germplasm in crop improvement for particular plant traits (Sneath and Sokal, 1973; Pecetti *et al.*, 1996). The principal component (PC) analysis divides the total variance into different factors.

In this study, out of total 18, six principal components (PCs) were extracted having Eigen value >1. These 6 PCs contributed 70.908% of the total variability amongst the wheat genotypes assessed for various morph-physiological traits (Table 2). However, the remaining 12 components contributed only 29.92% towards the total morph-physiological diversity for this set of wheat genotypes. The PC I contributed maximum towards the variability (24.108%) followed by PC II (12.641%), PC III (10.861%), PC IV (8.857%), PC V (7.801%) and PC VI (6.641%) respectively. High contribution of first few PCs in total variability based on various plant traits has already been reported in the literature (Ali *et al.*, 2011). In our experiment, 1st PC was mainly due to variations in yield components like spikelets per spike, grains per spike, days to heading, head weight, flag leaf area, spike length and grain yield per plant. These results are in agreement with the results of correlation analysis which showed positive association among yield related traits. But contrasting results were obtained in the studies of Brock and Galen (2005) which reported that the variations in the 1st PC were mainly due to transpiration rate and relative water content of leaves in *Taraxacum ceratophorum* and *Taraxacum officinale* under drought stress. However, Ali *et al.* (2011) reported the importance of negative loadings of relative water content in PC1 in sorghum. The 2nd PC was related to the diversity among wheat genotypes due to relative water content, relative dry weight, excise leaf weight loss and residual transpiration. The PC 3 was explained by variation among genotypes due to specific flag leaf area, specific flag leaf weight and days to maturity. Similarly PC 4 was explicated by variation in seed index, peduncle length and awn length with their considerable positive factor loadings. The PC 5 was elucidated by diversity among the genotypes for relative dry weight and plant height with some considerable negative loadings. Similarly, plant height had the considerable positive weight and awn length exhibited negative loadings on PC 6. The PC analysis ultimately confirmed the amount of variation for the traits among the material in hand. This variability could be utilized in designing a breeding program aimed at improving water stress tolerance in wheat as it is generally assumed that maximum variation yields maximum heterotic effects.

Cluster analysis: The cluster analysis also corroborates sufficient morpho-physiological diversity for this group of genotypes at terminal growth stages. Fifty two wheat genotypes were grouped into 4 clusters based on various morph-physiological traits (Table 3). Cluster analysis showed that cluster 1 comprised of 17 genotypes, cluster 2 of 16 while cluster 3 had 6 and cluster 4 contained 13 genotypes (Table 4). The genotypes in cluster 1 demonstrated larger flag leaf area, specific flag leaf area, plant height, days to heading, spike length, awn length, head weight, spikelets per spike and grains per spike. This suggested that the advanced lines corresponding to this group could be used for improving grain yield attributes. According to Leileh and Al-Kahteeb (2005), higher yield of wheat plants under drought conditions could be obtained by selecting advanced lines with high spikes/m^2, 100-grain weight, weight of grains/ spike and biological yield. Similarly, the 2nd cluster comprised of genotypes with more relative water content and seed index while less days to maturity and residual transpiration (Table 3). Both cluster 1 and 2 contained contrasting groups of genotypes for various characters showing negative correlation between yield traits and drought tolerance. A negative correlation was reported for grain yield with morpho-physiological characters contributing towards drought tolerance in bread wheat (*Trificum aestivum* L.), durum wheat (*T. turgidum* L.), triticale (*X. Tritosecale* Wittmack), barley (*Hordeum vulgare* L) and faba bean (*Vicia faba* L.) (Karamanos and Papatheohari 1999) and sorghum (Ali *et al.* 2009b, 2011). This suggested that member of these clusters could be utilized in breeding programs due to availability of potential regarding yield in cluster 1 and some degree of drought tolerance in cluster 2. The contrasting characters for drought tolerance and high yield in both these clusters suggest that the member of these clusters could be subjected to hybridization and selection of high yielding drought tolerant cultivars from the segregating populations. Both these cluster could also be utilized for identification of quantitative trait loci (QTLs) for the contrasting characters.

The members of 3rd cluster were characterized by lowest relative dry weight and days to heading while more specific flag leaf weight. The cluster 4 was characterized by maximum days to maturity peduncle length and grain yield. The traits like spike length, spikelets per spike and grains per spike showed 2nd best values among all clusters. The pairwise Mahalanobis distances (D^2 statistics) among four clusters of 52 wheat genotypes (Table 3) revealed that genotypes of cluster 3 showed maximum diversity against the members of cluster 2 followed by cluster 4 for most of the studied characters. On the contrary, minimum differences were found between the members of cluster 2 and cluster 4 due to the least value of D^2 statistics. The pair wise Mahalanobis distances (D^2 statistics) among four clusters revealed that genotypes of cluster 3 showed maximum diversity against the members of cluster 2 followed by cluster 4 for most of the studied characters which could be exploited to obtain transgressive segregates with wide range of adaptation to water scarcity.

Table 1. Simple coefficients of correlation among morph-physiological traits under drought stress in spring wheat

	FLA	SFLA	SFLW	RWC	RDW	ELWL	RT	PH	DTH	DTM	PL	SL	AL	HW	SPS	GPS	SI
SFLA	0.43**	1.00															
SFLW	-0.42**	-0.95**	1.00														
RWC	0.24	0.02	-0.03	1.00													
RDW	-0.07	-0.09	0.03	0.15	1.00												
ELWL	-0.28*	0.16	-0.09	-0.45**	-0.16	1.00											
RT	-0.26	-0.14	0.17	-0.14	-0.22	0.26	1.00										
PH	0.03	0.04	0.00	0.01	0.11	-0.08	-0.24	1.00									
DTH	0.26	0.06	-0.11	0.21	0.03	-0.20	-0.14	0.25	1.00								
DTM	0.12	0.07	-0.10	0.00	0.23	-0.06	-0.07	0.25	0.74**	1.00							
PL	0.11	-0.02	0.02	0.19	0.07	-0.04	0.10	0.11	0.10	0.16	1.00						
SL	0.25	0.32*	-0.25	-0.16	-0.33*	0.13	0.20	0.22	0.38**	0.37**	0.28*	1.00					
AL	0.21	0.15	-0.18	-0.06	-0.13	-0.04	0.15	-0.08	0.32*	0.25	0.27*	0.37**	1.00				
HW	0.50**	0.18	-0.23	0.32	0.04	-0.35**	-0.42**	0.28*	0.28*	0.10	0.17	0.14	-	1.00			
SPS	0.34*	0.15	-0.19	0.12	-0.18	-0.26	-0.11	0.04	0.56**	0.28*	0.07	0.43**	0.21	0.45**	1.00		
GPS	0.35**	0.27*	-0.30*	0.00	-0.27*	0.01	-0.19	0.13	0.39**	0.19	0.17	0.38**	0.03	0.51**	0.68**	1.00	
SI	-0.05	-0.07	0.12	0.15	0.11	0.09	0.15	0.07	-0.22	-0.11	0.14	-0.02	0.04	0.08	-0.34*	-0.38**	1.00
GY	0.15	0.25	-0.33*	-0.07	0.00	-0.17	0.01	0.12	0.29*	0.29*	0.16	0.35*	0.12	0.37**	0.32*	0.43**	-

Where as FLA-Flag leaf area; SFLW-Specific flag leaf weigh; SFLA-Specific flag leaf area; ELWL-Excise leaf weight loss; RDW-Relative dry weight; RWC-Relative water content; RT-Residual transpiration; PH-Plant height; DTH-Days to heading; DTM-Days to maturity; PL-Penduncle length; SL-Spike length; AL-Awn length; HW-Head weight; SPS-Spikelets per spike; GPS-Grain per spike; SI- Seed index (1000 grain weight) and GY-Grain yield per hectare, * = Significant at p≥5%, ** = Significant at p≥1%

The members of different clusters are given in the Table VI. The first two principal components contributing almost 36.749% towards the total variance were plotted to observe the relationships between different clusters with PC 1 on X-axis and PC 2 on Y-axis (Fig 2 and Supplementary Fig. 1). Not a single cluster showed obvious separation. The scatter plot showed association between different clusters. It could be explained by the fact that a large number of genotypes with varying degree of adaptation against water stress were used, based on different morpho-physiological traits or some sort of similarity among the genotypes. However, cluster analysis brings together those genotypes showing considerable tolerance against drought stress at one place and on the basis of yield related traits at other place. Amurrio *et al.* (1995), Rabbani *et al.* (1998) and Ali *et al.* (2011) reported lack of relationship between various clusters based on morpho-physiological traits and origins of genotype in peas (*Pisum sativum*), mustard (*Brassica juncea*) and sorghum (*Sorghum bicolor*) respectively. The tree diagram showed more or less similar results comprising of two main groups A and B each of which is further subdivided into two clusters (Fig 3). The genotypes of the 2nd cluster showed considerable tolerance against drought stress. The occurrence of this wide variation between the clusters is of great genetic value in providing materials aimed at wheat selection for adaptation to water stress conditions.

Table 2. Principle component analysis of various morpho-physiological traits in spring wheat under water stress

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Eigen value	4.339	2.275	1.955	1.594	1.404	1.195
% of total variance	24.108	12.641	10.861	8.857	7.801	6.641
Cumulative variance %	24.108	36.749	47.609	56.466	64.267	70.908
Factor loading by various traits						
Flag leaf area	0.617	-0.132	-0.346	0.184	0.245	-0.113
Specific flag leaf area	0.512	0.373	-0.641	0.279	-0.200	-0.109
Specific flag leaf weigh	-0.554	-0.318	0.627	-0.240	0.216	0.176
Relative water content	0.177	-0.602	-0.095	0.279	0.353	-0.169
Relative dry weight	-0.112	-0.515	0.029	0.284	-0.514	-0.208
Excise leaf weight loss	-0.249	0.670	-0.045	0.044	-0.281	0.253
Residual transpiration	-0.274	0.537	0.359	0.171	0.303	-0.058
Plant height	0.268	-0.233	0.175	0.120	-0.442	0.566
Days to heading	0.682	-0.143	0.475	-0.042	-0.182	-0.249
Days to maturity	0.505	-0.041	0.528	0.143	-0.473	-0.231
Peduncle length	0.250	-0.031	0.319	0.479	0.252	0.255
Spike legth	0.591	0.491	0.299	0.177	0.067	0.203
Awn length	0.332	0.309	0.301	0.392	0.210	-0.372
Head weight	0.631	-0.452	-0.198	0.025	0.159	0.364
Spikelets per spike	0.737	-0.018	0.167	-0.351	0.251	-0.063
Grains per spike	0.735	0.106	-0.043	-0.395	0.123	0.269
Seed index	-0.281	-0.103	0.005	0.705	0.131	0.317
Grain yield (kg/ha)	0.580	0.110	0.066	-0.082	-0.115	0.101

PC= Principal component

Table 3. Cluster analysis of different morpho-physiological traits in spring wheat under water stress along with D^2 statistics among four clusters

	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Flag leaf area	17.185	14.864	13.304	14.619
Specific flag leaf area	4.649	4.425	3.633	4.424
Specific flag leaf weigh	0.225	0.242	0.307	0.239
Relative water content	77.230	78.326	73.549	73.876
Relative dry weight	0.429	0.476	0.411	0.454
Excise leaf weight loss	28.487	28.717	33.013	28.415
Residual transpiration	0.062	0.060	0.070	0.071
Plant height	72.059	69.271	69.444	70.385
Days to heading	100.039	95.375	94.222	97.410
Days to maturity	136.118	133.521	134.222	136.179

Peduncle length	27.901	26.977	26.444	27.726
Spike legth	9.949	8.502	8.850	9.618
Awn length	5.469	5.063	4.733	5.087
Head weight	7.059	5.937	4.833	6.179
Spikelets per spike	14.961	13.042	13.000	14.026
Grains per spike	46.333	35.000	37.889	43.205
Seed index	41.839	44.332	42.349	40.574
Grain yield (kg/ha)	1590.882	1304.375	988.500	1849.615
	D^2 statistics among different clusters			
	Cluster 1	**Cluster 2**	**Cluster 3**	**Cluster 4**
Cluster 1	0.000			
Cluster 2	286.831	0.000		
Cluster 3	602.531	315.970	0.000	
Cluster 4	258.812	545.347	861.159	0.000

Table 4. Germplasm accessions related to various clusters based on various morpho-physiological traits

Clusters	No. of accessions	Accessions
Cluster 1	17	V-8002, V-8003, V-8006, V-8008, V-8011, V-8012, V-8013, V-8014, V-8016, V-8158, V-8064, Bwp line, 6C002, 6C015, 6C017, V-7155, Shafaq06
Cluster 2	16	V-8001, V-7006, V-7007, V-7015, V-8155, V-8156, V-8159, V-8160, V-8161, V-8162, V-8163, V-8065, V-6007, Auqab2000, Lasani08, Fsd.08
Cluster 3	6	V-7002, V-7013, V-8157, NR358, NR360, Chakwal50
Cluster 4	13	V-8004, V-8005, V-8007, V-8009, V-8010, V-8015, 25FJ3035, 05FJ051, 05FJ3074, 05BT014, 05BT015, V-7151, Seher06

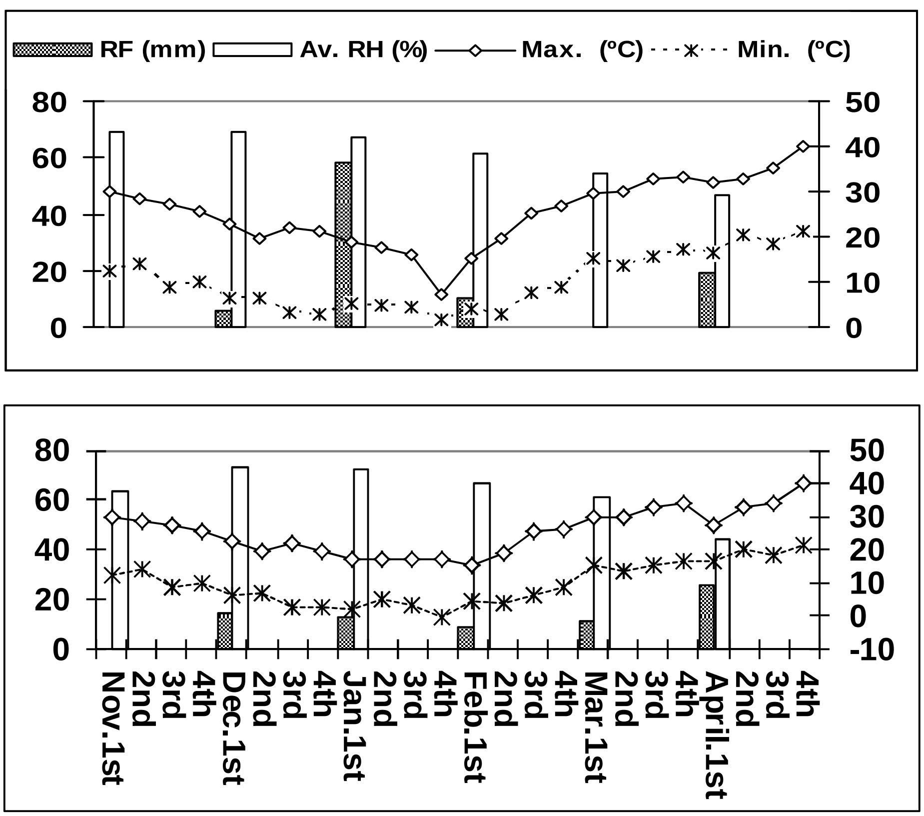


Fig. 1. Meteorological data regarding rainfall (mm), relative humidity (%),and temperature (ºC) during both wheat growing seasons: upper) 2008 and lower) 2009

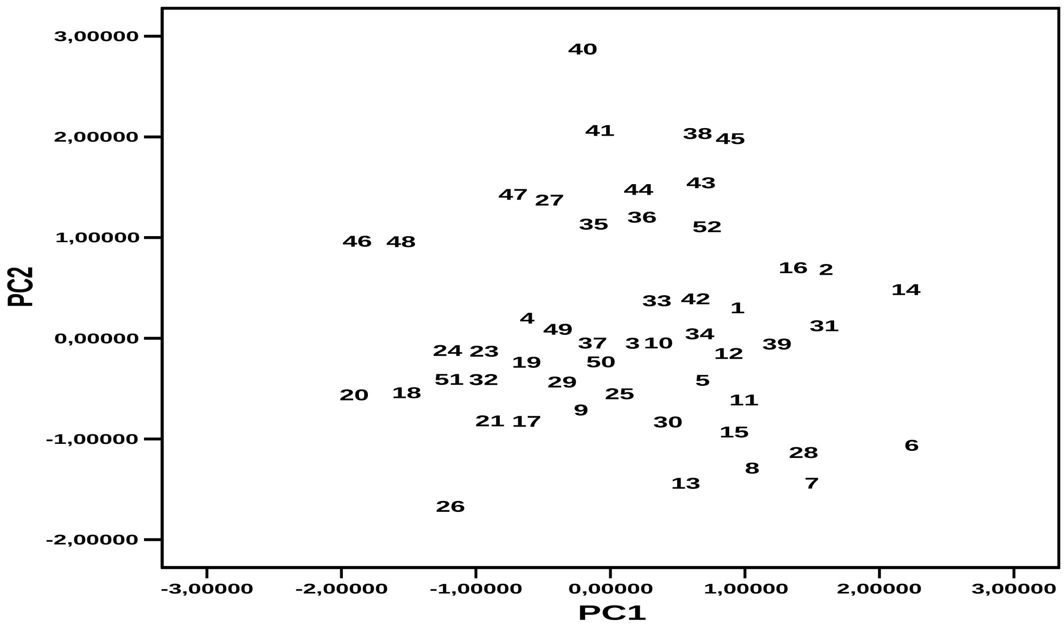


Fig.2. Placement of wheat genotypes in scatter plot plotted using first two Principal components which contributed maximum to the total variability.

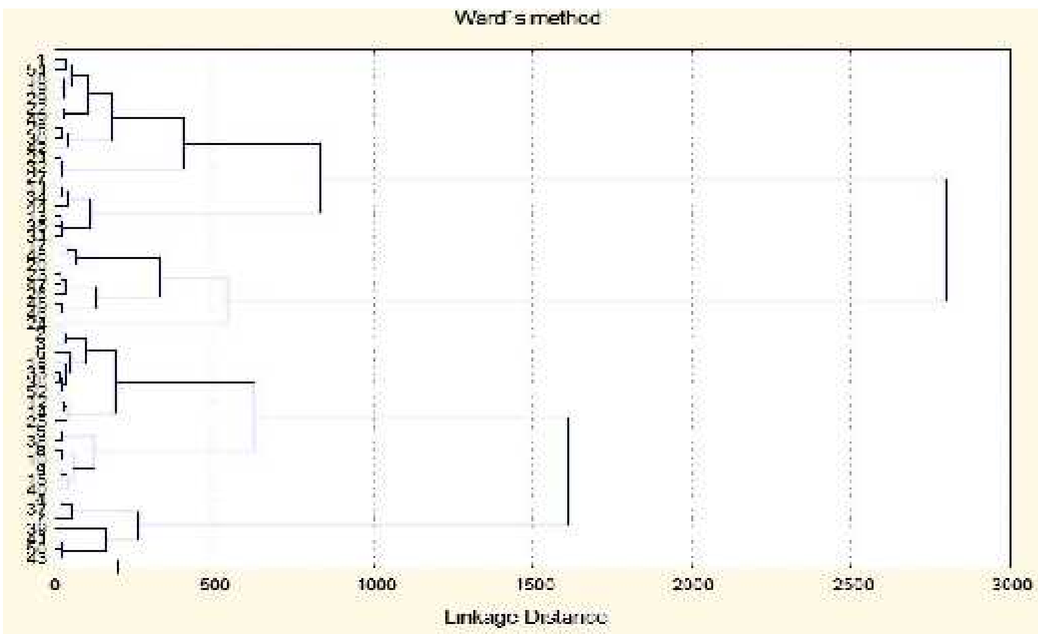


Fig. 3: Tree diagram for wheat genotypes for 18 morpho-physiological characters recorded under drought conditions. The numbering of varieties is shown in the supplementary Table 1.

Conclusion and Future Prospects: The results concluded that water stress significantly affect different morphological and physiological characters. Correlation analysis represented significant correlations between grain yield and its components. The PC analysis demonstrated significant amount of variation conditioned by various traits in different PCs. The results suggested the utilization of the member of first two clusters as parents to build up populations for selection of transgressive segregants in subsequent generations for drought tolerance. The results proposed that utilization of genetic diversity for various morphological as well as physiological characters contributing towards drought tolerance would be important for variety development with considerable drought tolerance a terminal growth stages.

REFERENCES

Ali, M. A., K. Jabran, S.I. Awan, A. Abbas, Ehsanullah, M. Zulkiffal, T. Acet, J. Farooq and A. Rehman (2011). Morpho-physiological diversity and its implications for improving drought tolerance in grain sorghum at different growth stages. Aust. J. Crop Sci., 5(3): 311-320.

Ali, M. A., A. Abbas, S. Niaz, M. Zulkiffal and S. Ali (2009a). Morpho-physiological criteria for drought tolerance in sorghum (*Sorghum bicolor*) at seedling and post-anthesis stages. Int. J. Agric. Biol., 11 (6): 674–680.

Ali, M. A., S. Niaz, A. Abbas, W. Sabir and K. Jabran (2009b). Genetic diversity and assessment of drought tolerant sorghum landraces based on morph-physiological traits at different growth stages. Plant Omics J., 2 (5): 214-227.

Ali, M.A., N.N. Nawab, A. Abbas, M. Zulkiffal and M. Sajjad (2009c). Evaluation of selection criteria in *Cicer arietinum* L. using correlation coefficients and path analysis. Aust. J. Crop Sci., 3(2): 65-70.

Ali, M.A., M. Hussain, M. I. Khan, Z. Ali, M. Zulkiffal, J. Anwar, W. Sabir and M. Zeeshan (2010). Source-sink relationship between photosynthetic organs and grain yield attributes during grain filling stage in spring wheat (*Triticum aestivum*). Int. J. Agric. Biol., 12(3): 509–515.

Amurrio, J.M., A.M. de Ron and A.C. Zeven (1995). Numerical taxonomy of Iberian pea landraces based on quantitative and qualitative characters. Euphytica. 82: 195–205

Anwar, J., M.A. Ali, M. Hussain, W. Sabir, M.A. Khan, M. Zulkiffal and M. Abdullah (2009). Assessment of yield criteria in bread wheat through correlation and path analysis. J. Anim. Plant Sci., 19(4): 129-132.

Aruna, C. and S. Audilakshm (2008). A strategy to identify potential germplasm for improving yield attributes using diversity analysis in sorghum. Plant Genet. Resour., 6: 187-194.

Brock, M.T. and C. Galen (2005). Drought tolerance in the alpine dandelion, *Taraxacum ceratophorum* (Asteraceae), its exotic congener *T. officinale*, and interspecific hybrids under natural and experimental conditions. Amer. J. Bot. 92: 1311–1321.

Clarke, J. M., R. A. Richards and A. G. Condon (1991). Effect of drought on residual transpiration and its relationship with water use of wheat. Can. J. Plant Sci., 71: 695-702.

Clarke, J.M. and T.F. Townley-Smith (1986). Heritability and relationship to yield of excised leaf water retention in durum wheat. Crop Sci., 26:289-292.

El-Deeb A.A. and N.A. Mohamed (1999). Factor and cluster analysis for some quantitative characters in sesame (*Sesamum indicum* L.). The Annual Conference ISSR, Cairo University. 34: Part (II).

Evans, L.T. and R.A. Fischer (1999). Yield potential: its definition, measurement and significance. Crop Sci., 34: 1544-1551.

Everitt, B.S. and G. Dunn (1992). Applied Multivariate Data Analysis, Oxford University Press, New York, NY.

Farooq, J., I. Khaliq, M.A. Ali, M. Kashif, A. Rehman, M. Naveed, Q. Ali, W. Nazir and A. Farooq (2011). Inheritance pattern of yield attributes in spring wheat at grain filling stage under different temperature regimes. Aust. J. Crop Sci., 5(13):1745-1753.

Geravandi, M., E. Farshadfar and D. Kahrizi (2011). Evaluation of some physiological traits as indicators of drought tolerance in bread wheat genotypes. Russ. J. Plant Physiol., 58(1): 69-75.

Mata, C.G. and L. Lamattina (2001). Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress. Plant Physiol., 126:1196-1204.

Jaynes, D.B., T.C. Kaspar, T.S. Colvin and D.E. James (2003). Cluster analysis of spatio temporal corn yield pattern in an Iowa field. Agron. J., 95(3): 574-586.

Karamanos, A.J. and A.Y. Papatheohari (1999). Assessment of drought resistance of crop genotypes by means of the water potential index. Crop Sci., 39: 1792-1797.

Leilah, A.A. and S.A. Al-Khateeb (2005). Statistical analysis of wheat yield under drought conditions. J. Arid Environ., 61: 483-496.

Moghaddam, M., B. Ehdaie, J.G. Waines (1998). Genetic variation for and inter relationships among agronomic traits in landraces of bread wheat from southwestern Iran. J. Genet. Breed., 52(1): 73–81.

Mohamed, N.A. (1999). Some statistical procedures for evaluation of the relative contribution for yield components in wheat. Zagazig J. Agri. Res., 26(2): 281–290.

Pecetti, L., P. Annicchiario and A.B. Damania (1996). Geographic variation in tetraploid wheat (*Triticum turgidum* spp. *Turgidum* convar. Durum) landraces from two provinces in Ethiopia. Genet. Resour. Crop. Evol., 43: 395-407

Rabbani, M.A., A. Iwabuchi, Y. Murakami, T. Suzuki, K. Takayanagi (1998). Phenotypic variation and the relationships among mustard (*Brassica juncea* L.) germplasm from Pakistan. Euphytica, 101: 357-366.

Rao, C.R. (1952). Advanced Statistical Methods in Biometrical Research. John Wiley and Sons. New York USA.

Shao, H.B., X.Y. Chen, L.Y. Chu, X.N. Zhao, G. Wu, Y.B. Yuan, C.X. Zhao and Z.M. Hu (2006). Investigation on the relationship of proline with wheat anti-drought under soil water deficits. Colloids Surf. B: Biointerf., 53: 113-119.

Shirinzadeh, A., R. Zarghami, A.V. Azghandi, M.R. Shiri and M. Mirabdulbaghi (2010). Evaluation of drought tolerance in mid and late mature corn hybrids using stress tolerance indices. Asian J. Plant Sci., 9(2):67–73.

Sneath, P.H.A. and R.R. Sokal (1973). Numerical Taxonomy: The Principles and Practice of Numerical Classification. W.F. Free-Man and Co. San Francisco. pp: 573.

Morpho-physiological diversity in advanced lines of bread wheat under drought conditions at post-anthesis stage
Supplementary material
Supplementary Table I: The germplasm accessions and their parentage

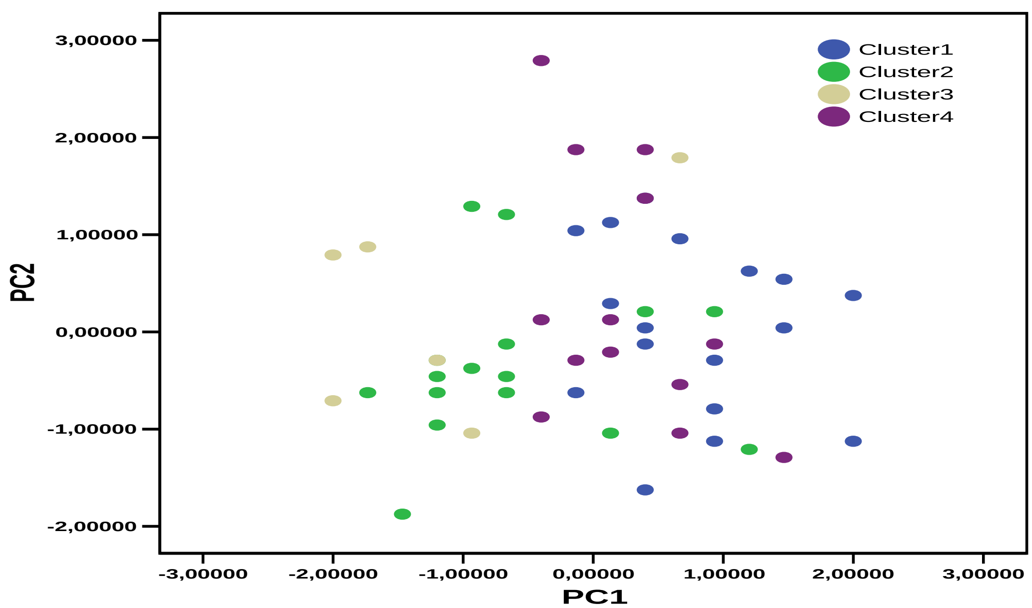
Sr. No.	***Accession name***	**Parantage**
1	08001	MRNG/BUC'S'//BLOS/PSN'S'/3/HD.2179//*2INQ.91
2	08002	SUNBIRD/SHAFAQ.06
3	08003	LU.26/BOW'S'/3/CNO/HD832//HD832/HD832/BB/4/IQBAL
4	08004	LU.26/BOW'S'/3/CNO/HD832//HD832/HD832/BB/4/IQBAL
5	08005	LU26/BOW'S'/3/CNO/HD832//HD832/BB/4/INQ.91
6	08006	LU26/HD2179//TTR'S'/JUN'S'/3/HP1744
7	08007	CHEN/AE.SQ(205)//3*KAUZ'S'/3/SHAFAQ.06
8	08008	PFAU/WEAVER/3/SH88/90A204/MH.97
9	08009	PRINIA/WEAVER//STAR/3/WEAVER/4/SUPER SARI #1
10	08010	URES/BOW'S'/3/BOW'S'//CMH.75A.142/ CMH74A.14214/..
11	08011	RABE/2*M088/8/CH.70/6/K.NOOR.83
12	08012	SUNCO/2*PASTOR
13	08013	KRICHAUFF/2*PASTOR
14	08014	BABAX/3/PRL/SARA//TSIVEE#5/4/CROC_1/…
15	08015	BUC/CHR'S'//PRL'S'/3/AMSEL/*2BAU
16	08016	BUC/CHR'S'//PRL'S'/3/AMSEL/*2BAU
17	07002	LU26/HD2179/5/BABAX/3/MANGO/VEE#10// PRL/4/ BABAX
18	07006	VEE/MRL//84133/3/OASIS/S.KAUZ//4*BCN
19	07007	KAUZ/RAYON/8/CH.70/6/KN83/CH70//ALD /5/CH.70/4/INIA/CNO/3//LR/SON64/7/INQ.91
20	07013	BABAX/4/BOW/CROW//BUC/PVN/3/VEE#10/5/BABAX/6/LU26/HD2179
21	07015	FRET2/TUKURU//FRET2
22	08155	VEE/PJN//2*TUI/3/PIFED
23	08156	MILAN/SHA7/3/CROC_1/AE.SQ (224)//…
24	08157	BJY/COC//PRL/BOW/3/SARA/THB//VEE/4/PIFED
25	08158	KRICHAUFF/2*PASTOR
26	08159	BABAX/3/PRL/SARA//TSI/VEE#5/4/WBLLI
27	08160	CROC_1/AE.SQ(205)//KAUZ/3/PIFED
28	08161	JNRB.5/PIFED
29	08162	ALTER84/AE.SQ (TAUS)//OPATA/3/…
30	08163	VEE/PJN//2*TUI/3/PIFED
31	08064	T.DICOCCON P194625/AE.SQ (372)//TUI/…
32	08065	T.DICOCCON P194625/AE.SQ (372)//TUI/…
33	Bwp line	---
34	6C002	HXL/2*BAU//PASTOR
35	6C015	HXL/2*BAU//PASTOR
36	6C017	HXL/2*BAU//PASTOR
37	05FJ3035	PASTOR//HXL7573/2*BAU
38	05FJ051	---
39	05FJ3074	---
40	05BT014	---
41	05BT015	---
42	06007	KOH97/MILAN
43	07151	KAUZ//ALTAR84/AOS/3/PASTOR/4/TILHI
44	07155	RL6043/4*NAC//2*PASTOR
45	NR358	PFAU/WEAVER*2//KIRITATI
46	NR360	PFAU/SERI.1B//AMAD/3/WAXWING

47	Auqab2000	CROW 'S'/NAC//BOW 'S'
48	Chakwal50	ATTILA/3/HUI/CARC//CHEN/CHTO/4/ATTILA
49	Lasani08	LUAN/KOH97
50	Seher06	CHIL/2*STAR/4/BOW/CROW//BUC/PVN/3
51	Fsd.08	PBW65/2*PASTOR
52	Shafaq06	V87094/2* INQ-91

Supplementary Table 2: Basic statistics for various morpho-physiological characteristics in spring wheat under water stress

	Range	*Minimum*	*Maximum*	*Mean± S.E*	*Std. Deviation*
FLA (cm^2)	13.813	9.503	23.315	15.382±0.406	2.931
SFLA	3.958	2.494	6.452	4.406±0.120	0.864
SFLW	0.245	0.159	0.404	0.243±0.007	0.052
RWC (%)	26.317	60.444	86.761	76.304±0.829	5.977
RDW	0.377	0.264	0.641	0.448±0.009	0.066
ELWL (%)	22.171	19.983	42.154	29.062±0.840	6.054
RT	0.150	0.017	0.168	0.064±0.003	0.023
PH (cm)	21.667	61.667	83.333	70.481±0.714	5.149
DTH	23.333	88.000	111.333	97.276±0.710	5.121
DTM	15.333	127.000	142.333	135.115±0.505	3.641
PL	12.133	21.633	33.767	27.405±0.407	2.932
SL	5.700	6.367	12.067	9.294±0.201	1.446
AL	4.033	3.133	7.167	5.163±0.139	\0.999
HW	5.333	3.667	9.000	6.237±0.197	1.418
SPS	6.667	11.000	17.667	13.910±0.258	1.858
GPS	41.000	24.000	65.000	41.090±1.230	8.870
SI	24.410	31.970	56.380	42.349±0.672	4.846
GY (kg/ha)	1311.667	740.000	2051.667	1497.904±40.555	292.443

Where as FLA-Flag leaf area; SFLW-Specific flag leaf weigh; SFLA-Specific flag leaf area; ELWL-Excise leaf weight loss; RDW-Relative dry weight; RWC-Relative water content; RT-Residual transpiration; PH-Plant height; DTH-Days to heading; DTM-Days to maturity; PL-Penduncle length; SL-Spike length; AL-Awn length; HW-Head weight; SPS-Spikelets per spike; GPS-Grain per spike; SI- Seed index (1000 grain weight) and GY-Grain yield per hectare, * = Significant at p≥5%, ** = Significant at p≥1%



Supplementary Fig. 1: Scatter plot of wheat genotypes for first two Principal components contributing maximum to the total variability. None of the cluster displayed clear separation that revealed some degree of association between different clusters.