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Full Length Research Papers

Assessment of Drought Tolerance in Spring Wheat (Triticum Aestivum L.) At Different Growth Stages under the Rainfed Conditions of Rawalakot Azad Kashmir Pakistan

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Twenty wheat genotypes were sown in field conditions to assess the variability for drought tolerance at various growth stages under the rainfed conditions of Rawalakot Azad Kashmir Pakistan. A multivariate approach including correlation, factor, cluster and principle component analysis was used to quantify the amount of variability among the genotypes. Correlation analysis showed that grain yield was positively correlated with plant height. Days to 50% heading showed positive correlation with drought traits like residual transpiration and relative water content. Principle component analysis showed that seven factors contributed 82.66% of the total variability with the Eigen value greater than 1. Cluster analysis grouped 20 genotypes into six clusters. Cluster 1,4 and 5 consist of 3 genotypes, cluster 2 contained 7 while cluster 3 and 6 had 2 genotypes. Cluster 1 genotype showed highest value of grain yield while cluster 4 genotype showed drought tolerant trait like high relative water content. Less residual transpiration was shown in cluster 2 genotype. Cluster 4 and cluster 2 genotypes will be used for further breeding programs.

Keywords: Wheat, Drought, Multivariate Analysis, Morpho-physiological traits, Correlation

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is the main staple food of the world population, feeding more than one billion people of the world. Wheat is rich in protein and calories than any other cereal crop (Abd-El-Haleem et al., 2009). In Pakistan wheat contributes 14.4% to agriculture and 3.1% to GDP. Wheat production increased to 24.231 thousand tonnes in 2012-13 as compared to 23.471 thousand tonnes in 2011-12 showing an increase of 3.2% (MINFA, 2013).

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The main environmental restraint for the wheat crop throughout the world is drought, salinity and heat stresses (Sial et al., 2005). Drought is main ecological restraint to decrease the production of various crops. Drought stress brings about effect in growth rate, stem elongation, leaf expansion and stomatal movements and cause changes in a number of physiological and biochemical processes which leads to plant growth and productivity (Balouchi, 2010).

Drought tolerance phenomenon is difficult in which morphological and physiological characters are involved. The physiological measures are involved for the detection of tolerant and susceptible wheat cultivars to drought. Decreased stem length and diameter and decreased number of tillers due to drought stress resulted to a very low dry matter value. Yield production in different environments should be used to check the performance of wheat genotypes under drought stress (Voltas et al., 2005).

Genotypes resistant to drought were selected can increase production (Rajaram, 2001). Several morphological parameters such as plant height, number of tillers plant^{-1}, days taken to maturity, number of grains spike^{-1}, number of spikelets spike^{-1}, 1000-grain weight and grain yield has been identified in some way contributing to moisture stress tolerance of the wheat plants (Ahmed et al., 2007).

Field experiment is helpful to evaluate the degree of drought tolerance. The preference should be given to the relationship between the crop yield obtained under drought stress (Moradi et al., 2015). At vegetative stage drought stress has no direct effect on yield but on reproductive stage it cause serious damage to yield 30-40% (Jatoi et al., 2011;Ayranci et al., 2014).

Simple plant characteristics such as kernel weight, emergence percentage, seedling weight and seedling height have been identified as good indicators of seedling vigor. However, under field conditions selection for seedling vigor can mask the genetic potential of seedlings but controlled conditions make such practice easy (Khan et al., 2002; Awan et al., 2007).

For a successful breeding program, the presence of genetic diversity and variability play a vital role. Genetic diversity is essential to meet the diversified goals of plant breeding such as breeding for increasing yield, wider adaptation, desirable quality, pest and disease resistance. Genetic divergence analysis estimates the extent of diversity existed among selected genotypes (Mondal, 2003). Assessment of the genetic variability can also be invaluable for analysis for genetic variability in cultivars and introgression of desirable traits from diverse germ plasm into the available base (Sajjad et al., 2011).

Correlation studies is an important tool for the starting of breeding programme as it provides a tool for the selection of desirable genotypes having desirable traits (Ali et al., 2009). Correlation is also helpful to identify the effective characters in order to indirect selection of superior genotypes (Salehi et al., 2013).

The study was carried out with following objectives:

- To quantify the amount of variability existing in the genetic material for drought tolerance.
- To quantify associations between physiological traits and yield responses to drought.

2. MATERIAL AND METHODS

Study Area

The research work was conducted in the experimental field of the Department of Plant Breeding and Molecular Genetics, Faculty of Agriculture, Rawalakot, University of Poonch. The material was provided by the Department of Plant Breeding and Molecular Genetics. Twenty genotypes were evaluated for drought tolerance under field conditions mentioned in the Table number 1.

Twenty genotypes were sown in well prepared seed bed according to Randomized complete block design (RCBD) in three replications. Row length was two meter and row to row distance was 30cm. Seed rate was 125 kg ha^{-1}.Single row of each entry was sown. The following parameters were taken at various stages of crop.

- **Flag Leaf Area (cm^2)**

Flag leaf area was measured as (Muller, 1991) by using the following formula:

Flag leaf area= L x W x 0.74

- **Flag leaf weight (g)**

Flag leaf was taken from the field and weight was measured by using the electronic balance in grams.

- **Specific flag leaf area**

Specific flag leaf area was calculated as

SFLA= FLA/DW

- **Specific flag leaf weight**

Specific flag leaf weight was calculated as

SFLW= DW/FLA

- **Residual transpiration**

The “residual transpiration” was measured according to (Clarke et al., 1991). Leaves were taken from the field and kept in darkness for stomata closure for half an hour. W_1 was taken in grams after half an hour and again W_2 was recorded in grams after 180 min. Then the leaf area (LA in cm^2) was determined. Residual transpiration was recorded as follows:

$RT = (W_1 – W_2)/ (LA.180 \text{ min})$

Table 1. Genotypes used for Experiment

Sr. No	Genotypes	Sr. No	Genotypes
1.	03FJ26	11.	SH-2003
2.	Blue silver	12.	Pirsabak-2004
3.	Tandojam-83	13.	Raskoo-05
4.	Chakwal-86	14.	Sehar-2006
5.	Pasban-90	15.	Fareed-2006
6.	Inqlab-90	16.	Chakwal-50
7.	Wafaq-2001	17.	PBW-343
8.	Panjnad-1	18.	NARC-2009
9.	AS-2002	19.	BARS-2009
10.	GA-2002	20.	Zarlashta

- **Osmotic adjustment**

Osmotic adjustment was determined by following formula:

OA = TW-FW

- **Relative water contents**

First the fresh weight **(FW)** and leaf area **(LA)** of flag leaf was measured. The full turgid weight **(TW)** was recorded. Leaves were placed in beaker containing distilled water for 24 hours at 4^0C. Then relative water contents (RWC) of flag leaf was calculated by the following equation:

RWC (%) = $(FW – DW_2)/ (TW– DW_2) \times 100$

- **Leaf venation**

The leaf strips taken from the flag leaf of preselected plants was dipped into Carnoy's solution in order to remove the chlorophyll from the leaf tissues. After one week the strips were removed from the solution, washed in acetone and stored in formaline solution for further examination. The leaf strips were used for recording leaf venation. The leaves were examined under low power (10X) objective of the microscope in order to count the number of parallel veins. Five observations were taken from each foliar strip and average was calculated.

- **Stomatal frequency**

The leaf strips taken for studying leaf venation were used for counting the stomata. Low power microscopic field (10X) was used as a unit of area for stomatal frequency.

- **Days to 50% heading**

Days to 50% heading was counted for each genotype in days from the date of sowing till 50% of plants started heading.

- **Plant height (cm)**

Plant height of ten randomly selected plants from each plot was recorded in centimeters from the base of the plant to the tip of tallest tiller without awns.

- **Number of tillers plant^{-1}**

Number of tillers plant^{-1} was counted for ten randomly selected plants from each plot. Then average was calculated.

- **Spike length (cm)**

Spike length of mother shoot of ten randomly selected plants from twenty rows was calculated in centimeters from base of the spike to the tip excluding awns at the time of maturity.

- **Number of spikelets spike^{-1}**

Number of spikelets spike^{-1} of ten randomly selected tillers from each plot was counted manually.

- **1000-Grain weight (g)**

After threshing 1000-grains were counted and their weight was recorded in grams on electronic balance.

- **Harvest Index (%age)**

Harvest index was calculated by applying the following formula:

$$\text{Harvest Index (\%age)} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

- **Grain yield plant^{-1}(g)**

Ten plants from each row were threshed, grains were separated and weighed by using an electronic balance and average grain yield plant^{-1} was calculated in grams.

- **Statistical Analysis**

The data was analyzed with the help of Software "SPSS" using correlation, Cluster, factor and Principal component analysis.

3. RESULTS AND DISCUSSION

- **Principle Component Analysis (PCA)**

Principle component analysis for some morpho-physiological traits in various wheat genotypes was shown in table 2. Seven components with Eigen values greater than 1 were selected (Figure 1). Factor loadings for morpho-physiological traits in various wheat genotypes were displayed in table 3. Factor 1 showed maximum Eigen value of 3.22. The sum of the Eigen values is usually equal to the number of variables. Factor 1 explained

Table 2. Principle component analysis for morpho-physiological traits in wheat genotypes

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
Eigen value	3.22	2.73	2.20	2.09	1.64	1.16	1.01
Total Variance %	18.97	16.07	12.95	12.27	9.64	6.80	5.96
Cumulative Eigen value	3.22	5.96	8.16	10.24	11.88	13.04	14.05
Cumulative %	18.97	35.03	47.99	60.99	69.90	76.65	82.65

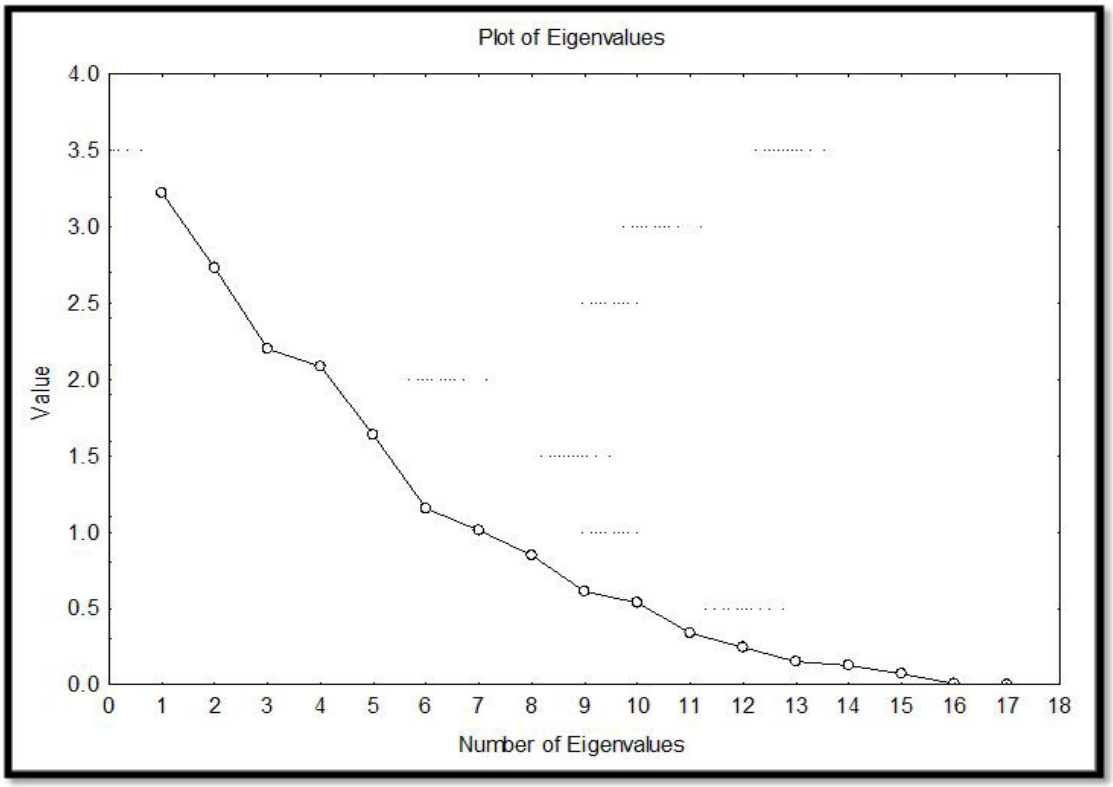

Figure 1: Plot of Eigen values of 17 traits in Wheat.

Table 3. Factor loadings for morpho-physiological traits in various wheat genotypes

Parameters	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
DTH	-0.26	-0.59	-0.10	0.32	0.16	0.35	0.42
NT	-0.24	0.77	-0.03	0.21	-0.45	-0.01	-0.05
PH	0.49	0.05	-0.12	0.57	-0.11	0.37	0.18
SL	0.57	0.05	0.23	0.55	0.03	-0.20	-0.36
NS	0.42	-0.06	0.35	0.58	-0.27	-0.35	-0.09
RT	-0.41	-0.30	0.37	0.08	-0.29	-0.49	0.32
RWC	-0.49	-0.62	-0.10	0.05	-0.01	0.31	-0.10
OA	0.48	0.06	0.44	-0.51	0.12	-0.27	0.01
FLA	0.30	-0.64	-0.35	0.26	0.14	0.18	0.11
FLW	0.67	-0.52	-0.38	-0.26	-0.18	-0.09	-0.02
SFLA	-0.50	0.25	0.18	0.42	-0.01	0.18	0.31
SFLW	0.56	0.06	-0.41	-0.37	-0.42	-0.08	0.23
LV	-0.35	0.32	-0.44	-0.04	0.49	-0.29	-0.18
SF	-0.31	0.10	-0.77	0.19	0.15	-0.39	-0.15
1000 grain.wt	0.30	0.51	0.06	-0.21	0.38	0.02	0.55
GY	0.45	0.44	-0.50	0.37	0.12	-0.05	0.20
HI	0.35	-0.09	0.40	0.17	0.76	0.03	-0.04

Where,
DTH= Days to 50% heading SLW= Specific leaf weight RWC= Relative water contents
NT= Number of tillers per plant LV= Leaf venation OA= Osmotic adjustment
PH= Plant height SF= Stomatal frequency FLA= Flag leaf area
SL= Spike length FLW= Flag leaf weight NS= Number of spikelets per spike GY=Grain yield SLA= Specific leaf area 1000-grain.wt= 100-Grain weight
RT= Residual transpiration HI= Harvest index

18.97% variation was strongly associated with flag leaf weight and spike length. This factor was named as grain yield. Factor 2 accounted 16.07% variability was indicated as an affective factor for spike yield because it is consisted of no of tillers per plant. Factor 3 contributed 12.95% variability and this factor is related to osmotic adjustment. So, it is an effective factor for drought tolerance. Factor 4 showed 12.27% variation was named as spike yield because it consisted of no of spikelets per spike. Factor 5 indicated 9.64% variation and maximum positive load was shown by harvest index so this factor called as yield potential factor. Factor 6 explained 6.80% variation was name as grain yield because it consisted of plant height. Factor 7 explained 5.96% of total variation and maximum positive load was shown by 1000-grain weight so this factor was named as grain yield. These factors contributed 82.66% variability. Results were matched with the results of (Sheykhi et al., 2014) in terms of total variation.

- **Cluster analysis**

- **Hierarchical Cluster**

Different morpho-physiological traits in wheat genotypes were displayed in Figure 2. Cluster 1 consisted of Days to heading, residual transpiration and relative water content. Cluster 2 contained No of tillers per plant, specific leaf area, leaf venation and stomatal frequency. Cluster 3 included traits like Plant height, grain yield, spike length and no of spikelets. Cluster 4 consisted of Osmotic adjustment. Harvest index and 1000-grain weight. Cluster 5 comprised of Flag leaf area, flag leaf weight and specific flag leaf weight. Our data indicated the tendency of each grouped variables in one cluster to express their close relationships. Results were matched with the results of (Zarei et al., 2013) in terms of spikelets and spike length relationship.

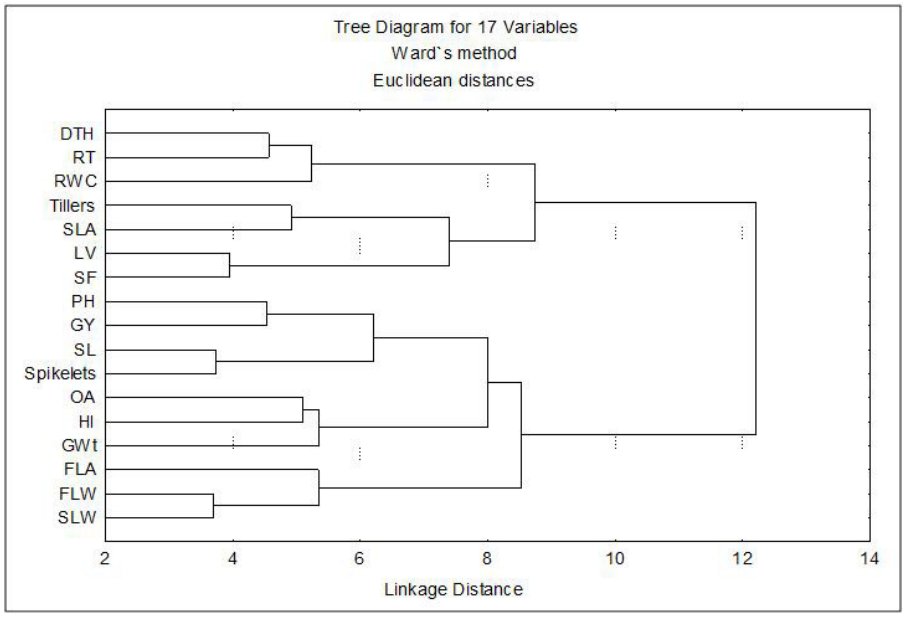

Figure 2: Dendrogram based on average linkage distance for morpho-physiological traits.

Table 4. Euclidean distances between clusters

	Cluster.1	Cluster.2	Cluster.3	Cluster.4	Cluster.5	Cluster.6
Cluster. 1	0					
Cluster.2	0.99	0				
Cluster. 3	1.38	1.09	0			
Cluster. 4	1.21	0.91	1.09	0		
Cluster. 5	1.34	0.92	1.27	1.02	0	
Cluster. 6	1.21	1.03	1.38	1.10	1.36	0

- **Euclidean distances between clusters**

Euclidean distances between clusters were shown in Table 4. In this table cluster 1, cluster 5 showed maximum Euclidean distances and minimum distance was showed by cluster 2, followed by cluster 4 and cluster 5. In cluster 2, clusters 3 indicate maximum Euclidean distances. In cluster 3, highest Euclidean distance showed by cluster 2, while cluster 3 followed by 4 indicate lowest Euclidean distance. Cluster 4 displayed maximum Euclidean distance by cluster 3 and minimum distance showed by cluster 2.Maximum Euclidean distance in cluster 5 was shown by cluster 3 followed by cluster 3, 4 while minimum distance was displayed by cluster 2. In cluster 6 maximum Euclidean indicate by cluster 3 followed by cluster 5, 1 and 3 and minimum Euclidean distance was displayed by cluster 4

- **Members of six clusters and Mean values**

Members of six different clusters were displayed in Table-5 and mean values were shown in table-6. Twenty genotypes were grouped into six clusters. Cluster 1,4 and 5 consist of 3 genotypes, cluster 2 contained 7 while cluster 3 and 6 had 2 genotypes. The member of cluster 1 showed maximum specific flag leaf weight and grain yield. Similarly the cluster 2 comprised of genotypes with larger plant height, harvest index and less flag leaf weight. Cluster 3 consisted of genotypes with more 1000-grain weight and less flag leaf area. Less flag leaf area results in less transpiration under stress. Cluster 4 included the genotype showed drought tolerant traits like high relative water content. It can be further used for drought tolerance. Cluster 5 indicated the genotypes with less osmotic adjustment, leaf venation and more stomatal frequency.

Table 5. Members of clusters based on different genotypes

Cluster	Total Members	Name of Genotypes
1	3	03FJ26, Chakwal-86, Wafaq-01
2	7	Blue silver, Tandojam-83, AS-2002, GA-2002, Pirsabak-2004, Sehar-2006, PBW-343
3	2	Fareed-2006, BARS-2009
4	3	Pasban-90, SH-2003, Zarlashta
5	3	Inqlab-91, Panjnad-1, NARC-2009
6	2	Raskoo-05, Chakwal-50

Table 6.Simple Correlation coefficients of morph-physiological traits in wheat.

	DTH	NT	PH	SL	NS	RT	RWC	OA	FLA	FLW	SLA	SLW	LV	SF	1000-G. wt	GY
DTH	1															
NT	-.358	1														
PH	-.016	.060	1													
SL	-.065	.071	.414*	1												
NS	.053	.023	.333	.634**	1											
RT	.452*	-.022	-.189	-.142	.202	1										
RWC	.389*	-.240	-.078	-.247	-.319	.254	1									
0A	-.227	-.140	-.272	.193	.069	-.035	-.346	1								
FLA	.303	-.567**	.232	.128	.149	-.162	.265	-.109	1							
FLW	.092	-.495*	.120	.170	.096	-.213	-.012	.286	.586**	1						
SFLA	.100	.364	-.048	-.131	.022	.154	.037	-.253	.020	.629**	1					
SFLW	-.242	-.015	.248	-.061	-.008	-.127	-.295	.260	.085	.640**	-.389*	1				
LV	-.078	.058	-.193	-.179	-.319	-.047	-.002	-.174	-.148	-.330	.060	-.068	1			
SF	.293	.170	-.174	-.099	-.181	-.139	.018	-.440*	.126	.021	.032	-.035	.591**	1		
1000-G.wt	-.169	.085	.080	-.031	-.136	-.227	-.484*	.295	-.182	-.101	-.020	.131	.090	-.129	1	
GY	-.072	.317	.460*	.303	.128	-.388*	-.358	-.080	.129	.161	-.021	.333	.190	.326	.335	1
HI	.072	-.462*	.186	.363	.152	-.154	-.104	.314	.081	-.071	-.106	-.303	.054	-.302	.234	.104

* Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.

DTH= Days to 50% heading SFLW= Specific flag leaf weight RWC= Relative water content
NT= Number of tillers LV= Leaf venation OA= Osmotic adjustment
PH= Plant height SF= Stomatal frequency FLA= Flag leaf area
SL= Spike length FLW= Flag leaf weight NS= Number of spikelets per spike
GY= Grain yield SFLA= Specific flag leaf area 1000-G. wt= 100-Grain weight
RT= Residual transpiration HI= Harvest index

Cluster 6 consisted of genotypes with more spike length and no of spikelets per spike. This suggested that member of these clusters could be utilized in breeding programs due to availability of potential like yield in cluster 1 and some degree of drought tolerance in cluster 4 and 3. Results were matched with the results of (Ali *et al*., 2015) in terms of traits in cluster 1. (Khavarinejad & Babajanor, 2011) grouped the genotypes into 6 clusters.

- **Simple Correlation Coefficient of Morpho-physiological Traits in 20 Wheat Genotypes**

Simple correlation coefficients of morpho-physiological traits are shown in Table 6. Days to 50% heading showed positive and significant correlation with residual transpiration and relative water content. Number of tiller per plant showed negative and highly significant correlation with flag leaf area while number of tillers per plant showed negative and significant correlation with flag leaf weight and harvest index. Plant height showed positive and significant correlation with grain yield and spike length. Spike length indicated positive and highly significant correlation with number of spikelets per spike. Residual transpiration showed negative and significant correlation with grain yield. Relative water content indicated negative and significant correlation with 1000-grain weight. Osmotic adjustment showed negative and significant correlation with stomatal frequency. Flag leaf area indicated positive and highly significant correlation with flag leaf weight. Flag leaf weight showed negative and highly significant correlation with Specific flag leaf area while positive and highly significant correlation with specific flag leaf weight. Specific leaf area showed negative and significant correlation with specific flag leaf weight. Leaf venation

Table 6 Mean Values of Morpho-Physiological Traits of Wheat

Parameters	Maximum value	Minimum value
DTH	Inqilab-91	SH-2003
NT	Panjnad-01(6)	Chakwal-86(5)
PH	GA-2002(78.73)	Pasban-90(62.12)
SL	Raskoo-05(10.63)	Pasban-90(8.47)
NS/S	Raskoo-05(17)	BARS-2009(14)
RT	Panjnad-01(0.07)	GA-2002(0.01)
RWC	Zarlashta(93.31)	BARS-2009(75.05)
OA	Chakwal-50(0.82)	NARC-2009(0.23)
FLA	Sehar-2006(28.97)	BARS-2009(17.94)
FLW	Chakwal-86(0.87)	PBW-343(0.47)
SFLA	Panjnad-01(52.98)	Chakwal-86(29.50)
SFLW	Chakwal-86 (0.03)	Blue silver(0.02)
LV	NARC-2009(23.37)	Panjnad-01(23.27)
SF	Inqilab-91(21.13)	Chakwal-50(13.67)
1000-G. WT	Fareed-2006(51.81)	Pasban-90(33.66)
GY	Chakwal-86(55)	Zarlashta(21.60)
HI	GA-2002(44)	Panjnad-01(24.10)

Whereas,

DTH= Days to 50% heading SFLW= Specific flag leaf weight RWC= Relative water content
NT= Number of tillers LV= Leaf venation OA= Osmotic adjustment
PH= Plant height SF= Stomatal frequency FLA= Flag leaf area
SL= Spike length FLW= Flag leaf weight NS= Number of spikelets per spike
GY= Grain yield SFLA= Specific flag leaf area 1000-G. wt.= 100-Grain weight
RT= Residual transpiration HI= Harvest index

showed positive and highly significant correlation with stomatal frequency. Results were matched with the results of (Sinha et al., 2006) for the trait like 1000-grain weight. Similar results were reported by (Ali et al., 2007) for the traits like Residual transpiration, Flag leaf area and flag leaf weight. The positive correlation among the traits indicated that these characters are important for direct selection of high yielding genotypes (Anwar et al., 2009).

4.CONCLUSION

Correlation analysis represented significant correlation with grain yield and its components. The PC analysis shows significant amount of variability by various traits. Fareed-2006 and Chakwal-86 shown highest value of grain yield and 1000-grain weight.GA-2002 and Zarlashta displayed high relative water content and less residual transpiration. So these varieties can be used for further breeding programs.

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