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Genotype by trait analysis and estimates of heritability of wheat (*Triticum aestivum* L.) under drought and control conditions

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ABSTRACT

In order to observe the behaviour of 100 wheat genotypes under drought stress, an experiment was conducted following randomized complete block design (RCBD) with three replications. The outcomes of analysis of variance (ANOVA) depicted highly significant variation among wheat genotypes for all studied attributes in both control and drought stress conditions. Heritability assessed the relative significance of gene action in genetic variation and plays vital role in selection process for yield improvement in collaboration with genetic advance. Principle component analysis (PCA) was also performed to explore the divergent genotypes in wheat germplasm. Positive and significant correlation observed between peduncle length, spike length, spikelet per spike and grain yield under both control and drought stress conditions. PCA and heritability along with genetic advance suggested that these traits and diverse genotype should be given importance in selection programs to get better grain yield in wheat under rainfed conditions.

Keywords: Broad sense heritability; Drought; Genetic advance; Principle component analysis (PCA).

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important cereal crop of the world, and it regarded as the significant food commodity in Pakistan (Ajmal et al., 2013). At present, food security is one of the main concerns throughout the world. As the global climatic changes and an ever-increasing population challenged plant breeders to develop crop genotypes having tolerance against biotic and abiotic stress factors (Rana et al., 2013). As compared to biotic stresses, the pace of improvement in

crop yield under abiotic stresses i.e. drought, has been found low due to the complex nature of molecular behaviours controlling abiotic stress response (Budak et al., 2013). A gradual loss in crop yield, especially in wheat, due to drought is predicted to increase in forthcoming times. Critical understanding of mechanism underlying drought effects is important for breeding of drought tolerant wheat varieties. The complex response against drought condition is commenced by an immense

transcriptional reprogramming upon the intuition of water scarcity and is progressed by many physiological and anatomical changes *i.e.* synthesis of compatible antioxidants and stomatal closure (Ergen and Budak, 2009).

Plant's response to water stress depends on many factors *i.e.* duration and severity of stress, plant developmental stage and genetics of cultivars (Beltrano and Marta, 2008). Drought stress during early vegetative stages has no direct effect on yield but at reproductive stage it drastically causes serious damages as a consequence up to 20-40% reduction in grain yield (Jatoi et al., 2011; Ayranci et al., 2014). Severe water deficient condition affects many features of plant growth and morphological attributes such as days to heading, plant height, spike length, number of spikelets per spike, grains number per spike, 1000 grain weight, peduncle length and ultimately crop yield (Kilic and Yagbasanlar, 2010).

Exploitation of genetic parameters enables us to develop heritable improvements in economically vital traits through selection. In wheat breeding programme, genetic variation among parental lines is regarded as key component to get unique gene combinations necessary for the development of superior cultivars. Many techniques are available to evaluate genetic parameters and their transmissibility. Heritability is a good index for transmission of traits from parents to offspring. It has been reported that only heritability estimates doesn't provide useful information about selection of cultivars unless aided by genetic advance (Eid 2009). Arshad and Chowdhry (2003) reported the presence of high heritability and genetic advance about various yield related traits in wheat.

When coping with mammoth amount of genotypes, multivariate analytical and biometrical techniques are mostly utilized by plant breeders to exploit genetic variability irrespective of data set. Among various biometrical techniques, a non-hierarchical analytical approach named principal component analysis (PCA) is usually utilized to assess the pattern of diversity in available germplasm (Khan et al., 2015; Umer et al., 2014). This approach (PCA) is effective in determining which trait had positive contribution towards due important trait, subsequently; these traits could be employed in the crop breeding program (Bilal et al., 2015). In addition to PCA, genotype by trait (GT) biplot is also an efficient biometrical technique for resolving data (Khan et al., 2015). It graphically depicts the utility of genotypes for production and helps to identify traits that are suitable for indirect selection of trait (Khodarahmpour et al., 2011).

As the improvement in wheat yield under drought is still a complicated task to achieve, therefore the main purpose of this study was to screen wheat genotypes with better grain yield and to identify reliable selection criteria for drought tolerant wheat genotypes.

MATERIALS AND METHODS

Present investigation was conducted at Regional Agriculture Research Institute, Bahawalpur. In this study, 100 wheat (*Triticum aestivum* L.) genotypes collected from different research centres were sown under drought and control conditions following randomized complete block design (RCBD) with three replications. Recommended irrigations were given to control treatment and soil moisture was maintained to field capacity (100%) until harvest. All suggested agricultural practices were followed as and when required. Drought stress was given at booting stage by preventing irrigation for fifteen days by maintain field capacity of 35%. At booting stage wheat crop was also covered with polythene sheet to protect it from rain. Data was collected from tagged plants of three replications for following attributes days to heading, plant height (cm), peduncle length (cm), spike length (cm), number of spikelets per spike, grain number per spike, 1000 grain weight (g) and grain yield.

The analysis of variance (ANOVA) for each attribute was executed using Statistix 8.1. Genetic coefficient of variance (GCV), phenotypic coefficient of variance (PCV) and environmental coefficient of variance (ECV) were calculated using the formulae specified by Cochran and Cox (1957). Broad sense heritability (h^2) and genetic advance (GA %) was carried out as reported by Hansen *et al.* (1956) and Falconer, (1989). The mean data of both control and drought stress were subjected to principle component analysis (PCA) using PAST software to observe genetic variation and to notify promising genotype (Bilal et al., 2015). The formulas for CV%, GCV, PCV, h^2_{BS} and GA% are below mentioned.

$$CV\% = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

$$GCV =$$

$$\frac{(\text{Genotypic variance}/\text{mean value of trait}) \times 100}{CV\%}$$

$$PCV = \frac{(\text{Phenotypic variance}/\text{mean value of trait}) \times 100}{CV\%}$$

$$h^2_{BS} = \frac{(\text{Genotypic variance}/\text{Phenotypic variance}) \times 100}{PCV\%}$$

$$GA\% = k \times (\text{Phenotypic variance})^{0.5} \times h^2_{BS}$$

Where 'k' is selection intensity at 5% level (value = 2.06)

RESULTS

Analysis of variance

The outcomes of analysis of variance depicted highly significant genetic variation ($P < 0.01$) for all studied traits in both drought and control conditions thus exhibited the presence of considerable genetic diversity for all traits in the studied materials (Table 1).

Table 1. Analysis of variance based on mean square values, broad sense heritability and genetic advance percent for studied traits under control and drought stress condition

S.O.V	d.f	Days to heading		Plant height		Peduncle length		Spike length	
		Control	Drought	Control	Drought	Control	Drought	Control	Drought
Replication	2	4.17	6.41	0.3	4.81	3.93	1.01	1.67	2.53
Genotypes	99	45*	28*	176**	225**	44.6**	49.1**	2.55**	3.53**
Error	198	4.5*	2.56*	3.83*	3.15*	3.23**	1.85**	0.76*	1.24**
ECV		2.12	1.81	2.06	2.12	5.13	4.39	7.91	11.81
GCV		3.67	3.29	7.98	10.3	10.6	12.7	7.03	9.255
PCV		4.24	3.76	8.24	10.5	11.8	13.4	10.6	15.01
G.A (5%)		6.54	5.94	15.9	20.7	19.8	24.7	9.61	11.76
h ² (b.s)		74.9	76.8	93.7	95.9	81.1	89.3	44.1	38.04
CV		2.12	1.74	2.56	2.08	5.89	4.14	12.17	7.77

ECV = environmental variance, GCV = genotypic variance, PCV = phenotypic variance, G.A = genetic advance, h² (b.s) = broad sense heritability, *** = significant at 5% and 1% respectively

Table 1. Continue

S.O.V	d.f	No. of spikelet/spike		Grains/spike		TGW		Yield	
		Control	Drought	Control	Drought	Control	Drought	Control	Drought
Replication	2	1.6	6.1	0.97	2.41	44	7.4	4613	5155
Genotypes	99	7.36**	7.6**	136**	140.3**	109**	60**	155694**	71635**
Error	198	2.73*	1.9*	6.74*	3.78*	11**	2.3*	2484**	1702.7*
ECV		8.9	8.2	4.37	4.028	7.9	4.7	3.99606	4.709
GCV		6.69	8.3	11.1	13.98	14	14	18.1197	17.423
PCV		11.1	12	11.9	14.55	16	14	18.5551	18.049
G.A (5%)		8.27	12	21.2	27.67	24	26	36.4507	34.649
h ² (b.s)		36.1	50	86.5	92.33	74	89	95.3619	93.193
CV		9.27	8.93	4.67	4.74	9.51	8.56	7.77	5.89

ECV = environmental variance, GCV = genotypic variance, PCV = phenotypic variance, G.A = genetic advance, h² (b.s) = broad sense heritability, *** = significant at 5% and 1% respectively

Coefficient of variation, broad sense heritability and genetic advance of traits

The estimates of genetic parameters i.e. coefficient of variability (CV%), genotypic mean square (GMS), environmental coefficient of variation (ECV), genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), broad sense heritability (h²_{B.S}) and genetic advance (GA%) are mentioned in Table 1. Under control conditions, all traits showed maximum values for PCV followed by GCV and ECV except spike length and number of spikelets per spike. Similar results were obtained under drought stress conditions except for spike length. Under control conditions, 1000 grain weight, peduncle length and grain yield exhibited higher values for GCV and PCV while days to heading showed lower values for GCV and PCV. In case of drought stress conditions, grain yield, 1000 grain weight, grain per spike and peduncle length showed higher values for GCV and PCV whereas days to heading showed lower values for GCV and PCV. Plant height, peduncle length, grains per spike and grain yield showed higher values for broad sense heritability and genetic advance under stress and

non-stress conditions. Whereas spike length and number of spikes per plant showed lower values of broad sense heritability and genetic advance under both conditions (Table 1).

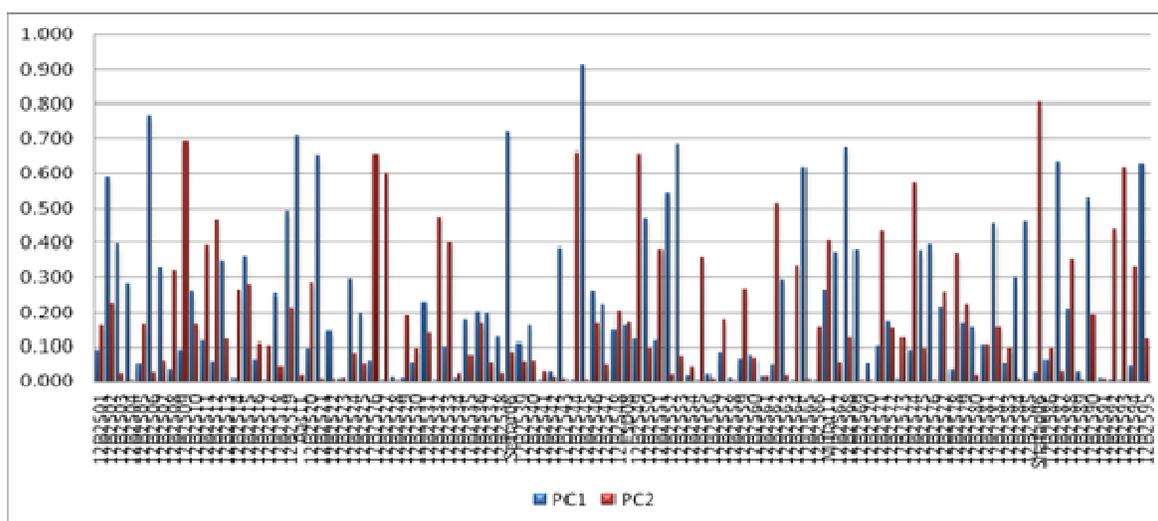
Principal component analysis (PCA)

Out of eight principal components (PCs), three PCs showed eigen value more than 1. These 3 PCs, contributed 54.66% towards total variability. Whereas, remaining 5 PCs contributed 45.34% as a whole. Under control conditions, 1st and 2nd PC exhibited 41.02% variability (Table 2) so these PCs were selected for further explanation. First principal component had a high match with thousand grain weight (0.4703), spike length (0.4107) and plant height (0.4014). Second PC was more related to days to heading (0.6672) and number of grains per spike (0.4044). Genotypes 12B2502 (0.588), 12B2506 (0.766), AAS11 (0.707), 12B2521 (0.652), SEHAR06 (0.717), 12B2545 (0.915), 12B2553 (0.683), 12B2665 (0.617), 12B2568 (0.671), 12B2587 (0.635) and 12B25 (0.627) were more related to PC1 under control

Table 2. Eigen values, proportion of variability and traits that contributed to principal components under control and drought condition

Statistical Parameters	Control Condition			Drought stress		
	PC1	PC2	PC3	PC1	PC2	PC3
Eigen value	1.932	1.349	1.0915	2.0246	1.259	1.0433
Variability (%)	24.156	16.864	13.64	25.308	15.742	13.041
Cumulative %	24.156	41.021	54.665	25.308	41.051	54.093
Traits						
Days to heading	0.0833	0.6672	0.00426	0.001	0.588451	0.0940
Plant height (cm)	0.4014	0.0139	0.0189	0.3165	0.0197	0.2417
Peduncle length (cm)	0.2101	0.0543	0.2275	0.2793	0.2063	0.0970
Spike length (cm)	0.4107	0.0470	0.00135	0.363	0.00480	0.0115
No. spikelets/ spike	0.1732	0.0986	0.1499	0.3309	0.0663	0.0696
No. grains/ spike	0.0857	0.4044	0.1122	0.2743	0.1170	3.54
1000 grain weight(g)	0.4703	0.0522	0.1022	0.4316	0.2483	0.0055
Yield (kg/ha)	0.0974	0.01114	0.4750	0.1270	0.00826	0.5237

Values in bold correspond for each variable to the factor for which the squared cosine is largest

**Figure 1.** Squared Cosines of genotypes under control condition

conditions (Figure 1). Whereas 2nd PC had high matches with 12B2509 (0.690), 12B2526 (0.654), 12B2537 (0.598), 12B2549 (0.656), SHAFQA06 (0.803) and 12B2593 (0.617) (Figure 1). Under drought stress conditions, 3 PCs, from a total of 8 PCs, showed eigen value more than 1. These 3 PCs showed a cumulative variation of 54.093% (Table 2). While remaining 5 PCs showed 45.907% variability. First two PCs accounted for major portion (41.051%) of variability, hence due importance was given to those PCs for further explanation. Traits of significant values in 1st PC under water deficit conditions were plant height (0.3165), peduncle length (0.2793), spike length (0.363), number of spikelets per spike (0.3309) grain number per spike (0.2743) and 1000 grain weight (0.4316). Whilst days to heading (0.58845) and 1000 grain weight (0.2483)

showed maximum values for 2nd PC (Table 2). Genotypes having more vitality to 1st PC under drought condition were 12B2506 (0.829), AAS11 (0.747), SEHAR06 (0.900), FSD (0.905), SHAFQA06 (0.699), 12B2587 (0.588) and 12B2595 (0.540). While second PC, was more related to 12B2520 (0.621), 12B2532 (0.528), 12B2562 (0.657) and 12B2594 (0.618) (Figure 2).

Relationship among studied traits

The line originating from point of origin and ending at trait clogged position is known as trait vector. The degree of angle determines whether traits have either positive or negative association. Acute angle between traits is

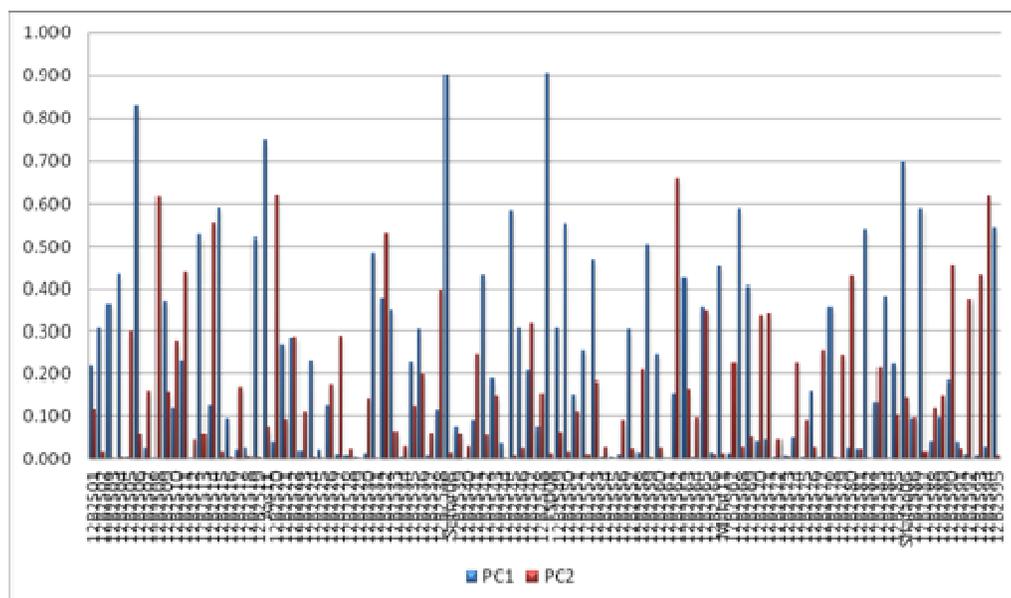


Figure 2. Squared Cosines of genotypes under drought stress condition

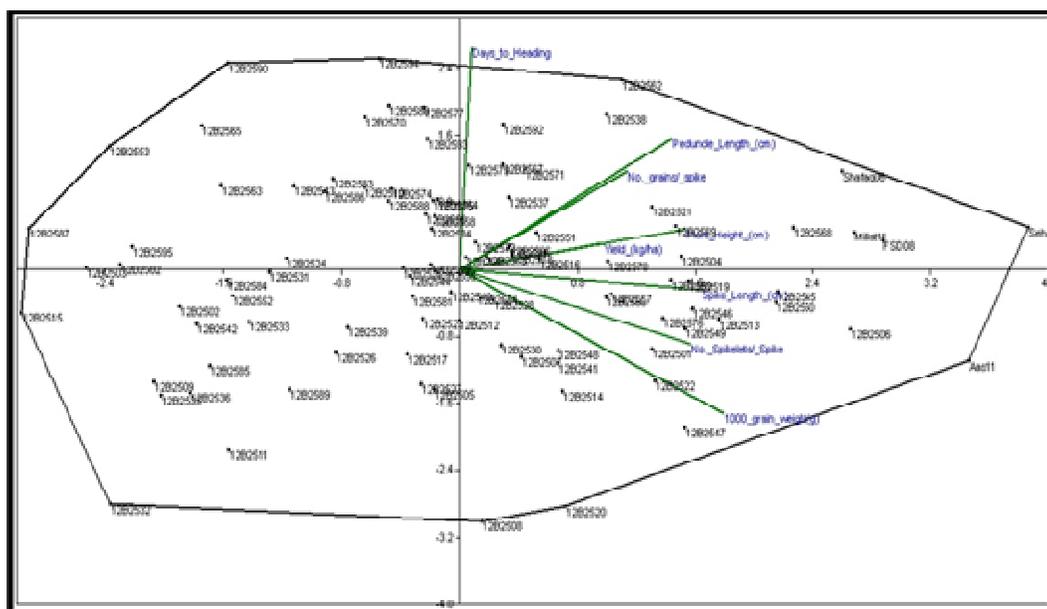


Figure 3. Biplot based on mean data of hundred wheat genotypes under control condition

responsible for positive association while obtuse angle is responsible for negative association. While right angle between traits shows that these traits act autonomously (Khan et al., 2015). Under control conditions positive and significant (0.199) association was observed between plant height and peduncle length. Spike length had significant and positive (0.229) association with plant height. While spike length, had significant and negative

association (-0.245) with days to heading having obtuse angle between them (Figure 3). Thousand grains weight was significantly and positively correlated (0.283) with plant height. Thousand grains weight showed significant and negative correlation (-0.283) with days to heading. Thousand grains weight also showed significant and positive association with peduncle length and spikelets per spike with 0.284 and 0.225 correlation matrix values

Table 3. Correlation matrix (Pearson (n)) under control conditions

Variables	Days to Heading	Plant Height	Peduncle Length	Spike Length	No. Spikelets/ Spike	No. grains/ spike	1000 grain weight	Yield (kg/ha)
Days to Heading	1							
Plant Height (cm)	-0.028	1						
Peduncle Length (cm)	0.027	0.199	1					
Spike Length (cm)	-0.245	0.229	0.198	1				
No. Spikelets/ Spike	0.049	0.167	0.053	0.083	1			
No. grains/ spike	0.235	0.098	0.110	0.129	0.158	1		
1000 grain weight(g)	-0.283	0.283	0.111	0.284	0.225	0.122	1	
Yield (kg/ha)	-0.006	0.168	0.166	0.077	0.060	-0.010	0.052	1

Values in bold are different from 0 with a significance level $\alpha = 0.05$

Table 4. Correlation matrix (Pearson (n)) under drought stress condition

Variables	Days to Heading	Plant Height	Peduncle Length	Spike Length	No. Spikelets/ Spike	No. grains/ spike	1000 grain weight	Yield (kg/ha)
Days to Heading	1							
Plant Height	0.120	1						
Peduncle Length	0.108	0.250	1					
Spike Length	-0.026	0.167	0.209	1				
No. Spikelets/ Spike	0.023	0.236	0.030	0.167	1			
No. grains/ spike	0.099	0.087	0.202	0.194	0.126	1		
1000 grain weight	-0.206	0.255	0.235	0.301	0.354	0.098	1	
Yield (kg/ha)	0.008	0.001	0.228	0.111	0.153	0.042	0.108	1

Values in bold are different from 0 with a significance level $\alpha = 0.05$

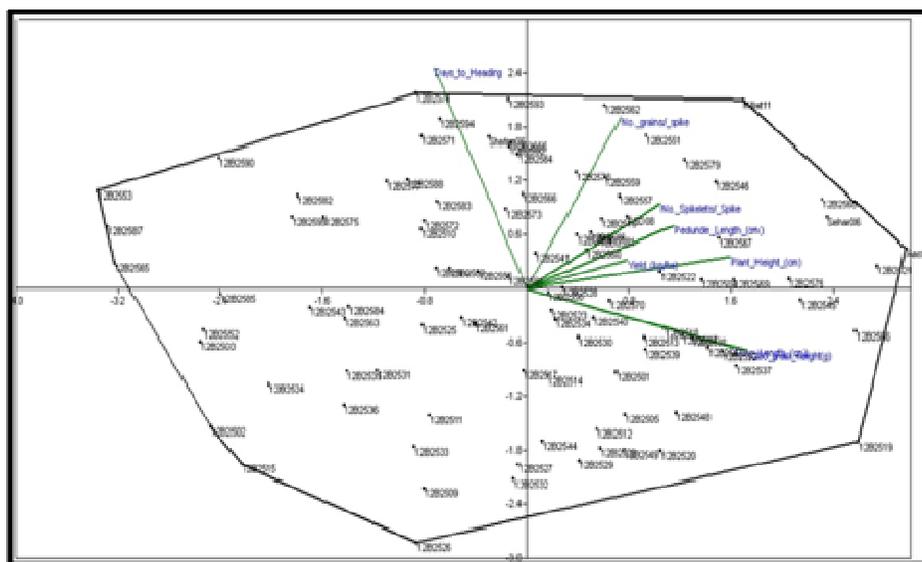


Figure 4. Biplot based on mean data of hundred wheat genotypes under drought stress

respectively (Table 3). In case of drought conditions, plant height and peduncle length showed significant and positive association between them having correlation value of 0.250 (Table 4). These traits also has acute

angle between them (Figure 4). Thousand grains weight was significantly and positively correlated with plant height, peduncle length, spike length and spikelets per spike with correlation matrix values of 0.235, 0.301, and

0.354 respectively. Days to heading showed negative but non-significant and significant correlation with spike length (-0.026) and thousand grain weight (-0.206) respectively.

Genotype by trait analysis

On the basis of data collected on given traits for control and as well as drought conditions, evaluation and identification of diverged genotypes was carried out with the help of GT biplot analysis (Figure 3 and 4). Under control conditions, 12B2537, 12B2569, 12B2576, 12B2567, 12B2521, AAS11, SEHAR06 and 12B2568 were found in close proximity of 1000 grain weight, plant height and spike length, hence these genotypes have high match with these traits (Figure 3). Whereas under drought conditions, 12B2568, AAS11, SEHAR06, MILLAT11, 12B2521, 12B2545, 12B2550 and 12B2547 were clogged near the vicinity of plant height, number of spikelets per spike, spike length and 1000 grain weight. Therefore these genotypes are more related to these traits (Figure 4). Moreover, all the aforementioned genotypes and traits lie far away from point of origin on the graph indicating greater breeding value as compared to other genotypes and traits which clogged near the point of origin.

DISCUSSION

Existence of considerable degree of variations in all give genotypes for all traits showed abundant scope for characterization of wheat genotypes for drought tolerance. The coefficient of variability (CV %) is an effectual statistical tool for assessment of studied attributes. In current investigation the attributes having lower values of CV% might be more repeatable as compared to those traits having higher values of CV% and these attributes with lower values of coefficient of variability could be trustworthy markers for successful breeding programmes (Bilgin et al., 2011). GCV, PCV and ECV values also have significant impact on breeding programs. In this study most of the traits have lower values of ECV as compared to GCV and PCV values indicating that these traits are less affected by environmental factors and selection might be effectual for wheat breeding (Bilgin et al., 2011). Heritability (broad sense) values of traits are of vital significance because it determines response to selection (Yao et al., 2014). In present experiment, high as well as low heritability of traits has been observed. High heritability (above 80%) value suggested that selection could be done in earlier generation. Moderate heritability values (60-79%) of traits suggested that selection should be delayed to few more progenies for these traits. While low heritability values

(less than 59%) depicted that selection for these trait will result in useless outcomes. High heritability (broad sense) coupled with higher values for genetic advance indicate that there could be predominance of additive gene action and might not much prejudiced by environmental fluctuations, therefore might be selected for wheat drought tolerance improvement programs through these attributes. Bilgin et al. (2011) also reported high heritability coupled with high genetic advance for plant height, grain per spike and grain yield. Iftikhar et al., (2012) published contrasting results as compared to outcomes of our investigation for spike length and number of spikelets per spike. The contrasting results might be environmental effects which constituted major portion of whole phenotypic variation for these traits. Ahmed et al., (2007) also reported higher estimates of heritability and genetic advance for grains/spike.

Knowledge of range of genetic biodiversity within given germplasm is of vital significance for effectual utilization, conservation and triumph of any breeding programme. For this purpose, principal component analysis was used to identify genotypes having diversity for drought tolerance. This analytical technique is a powerful tool to select parental lines for triumphant breeding programme and to identify best genotypes. Geographical distribution of genotypes is not the only factor upon which we conclude that there exists genetic diversity. Genetic variability might be consequence of various other factors i.e. environmental fluctuations, genetic drift or exchange of breeding material. Hence selection of parental lines for forthcoming wheat programs should be on the basis of genetics rather than geographical variability. Squared cosine values of traits and genotypes also provide obvious estimates about them. Genotypes or traits having higher values in 1st PC and lower values in 2nd are regarded as promising genotype or trait irrespective of conditions. On the other hand, if genotype has lower square cosine value in first PC and have higher squared cosine value in second are regarded as notorious genotype (Khodarahmpour et al., 2011). For instance, in drought conditions one genotype has lower values for squared cosine in first PC and higher values in second PC, then that genotype is not suitable for cultivation in rainfed conditions. While if a genotype has higher value of squared cosine in 1st PC and lower value in 2nd PC than that genotype will perform better under specified condition. GT biplot depicts that genotypes which clogged farthest from the point of origin on graph are regarded as more diversified and vice versa (Maqbool et al., 2010). Genotype by trait analysis compares germplasm on the basis of various attributes and correlation among and between the attributes. Under both conditions i.e. stress and non-stress, plant height showed positive and significant association with thousand grain weight. These outcomes were in agreement with the findings of Akram et al. (2007); Zafarnaderi et al. (2013). Alike results were

obtained for grain yield with spike length and number of spikelets per spike, these results were in accordance with the work of Tazeen et al. (2009). Therefore it could be said that improvement in any of these traits might result in grain yield boost up. Under control and as well as water deficit conditions, thousand grain weight exhibited negative association with days to heading (Zafarnaderi et al., 2013). Plant height also showed positive and considerable association with spikelets per spike under both conditions. Keeping in view the behaviour of traits under both conditions it might be concluded that gene expression changed considerably but no significant difference was observed among association of studied traits in both stress and non-stress conditions.

CONCLUSION

Studying genetic diversity is not an optional, but essential. It will assist to select and breed for drought stress. In this investigation, drought stress caused considerable effects on all studied attributes. Traits having lower values of ECV than GCV and PCV are more effectual in selection. High heritability coupled with high genetic advance also gives unambiguous estimates about performance of traits. In current exploration, higher heritability was recorded for plant height, peduncle length, grains/spike and thousand grain weight under drought stress conditions. Moreover these traits also should positive and significant correlation with yield under osmotic stress conditions. SHAF106, AAS11, 12B2568, 12B2545, 12B2550 and 12B2521 clogged in close proximity of aforementioned traits indicating that these genotypes are more related to these traits under drought stress conditions suggesting that above mentioned genotypes could be utilized for drought tolerance wheat breeding programs.

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