RESPONSE OF BREAD WHEAT GENOTYPES TO CELL MEMBRANE INJURY, PROLINE AND CANOPY TEMPERATURE

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Abstract

Genetic diversity for physiological traits is imperative in reducing genetic vulnerability and improve the plant species against stressed environments. The present study was designed to assess genetic divergence for some physiological and yield related traits among thirty wheat cultivars. Data were collected for cell membrane injury, proline content, flag leaf area, canopy temperature, spike length, spikelets per spike[-1], plant height, grains per spike[-1], 1000-grains weight, and yield plant[-1]. Principal component analysis depicted that five components were responsible for 74.54% variability. Traits that contributed greatest diversity in grain yield followed by flag leaf area and cell membrane injury. Correlation study revealed that direct selection for spikelets spike[-1], 1000-grain weight, grains per spike[-1] and spike length can contribute towards grain yield. The dendrogram constructed through simple sequence repeats markers categorized 30 wheat cultivars into three clusters based on their similarity. Simple sequence repeats markers were found suitable for characterization and discrimination of wheat cultivars for genetic diversity which would be useful in further breeding programs.

Key words: Genetic diversity, PCA, Cluster analysis, Physio-morphic, Correlation, Wheat.

Introduction

Wheat is a major cereal of the world after corn and constitutes 20-30% of annual production among cereals. It provides proteins and 20% calories to world (Braun *et al.*, 2010). Food security is very important issue and thus need enhanced wheat production is required to feed increased population. Genetic variability and diversity play a vital role in breeding program for wider adaptability, desirable quality, tolerance to abiotic and biotic stresses and increased yield. Development and cultivation of climate resilient and drought tolerant cultivars is necessary for sustainable wheat production (Ahmed *et al.*, 2019).

Physiological trait like cell membrane injury, proline content and canopy temperature are important indicators of drought stress tolerance. High chlorophyll content, cell membrane stability, more proline content and lower canopy temperature are major contributors of wheat stress tolerance. Genetic diversity among cultivars can be determined through various analyses. Principal component analysis (PCA) transforms large number of variables into a few variables and retain maximum variation. It also explains variation among crop cultivars on the basis of different variables. This approach helps to identify the trait that would be targeted for improvement through breeding program. Various researchers have used principal component analysis to evaluate genetically diverse wheat genotypes (Mishra *et al.*, 2015; Fahim, 2014; Beheshtizadeh *et al.*, 2013; Rymuza *et al.*, 2012; Hailegiorgis *et al.*, 2011). Yield is greatly affected by different genetic and environmental factors. So, direct selection of grain yield would be misleading. Correlation among traits is useful to improve yield through indirect selection of different yield related traits. Researchers observed positive correlation of yield with flag leaf area (Ghallab *et al.*, 2017), spike length (Degewione *et al.*, 2013), spikelets per spike[-1] (Desheva, 2016), grains spike[-1] (El-Mohsen *et al.*, 2011) and 1000-grains weight (Kalimullah *et al.*, 2012).

Genetic diversity studies are essential in selection strategies for genetic differentiation and development of new cultivars in wheat. Genetic variation among different wheat genotypes have been achieved through RAPD (Bhutta *et al.*, 2006) and ISSR (Maqsood *et al.*, 2017; Abou-Deif *et al.*, 2013). SSR markers are exceedingly polymorphic, co-dominant and dispersed in whole genome of wheat. Genetic diversity has been studied through SSRs in wheat (Drikvand *et al.*, 2015; Sardouie Nasab *et al.*, 2013; Zhang *et al.*, 2002). Cluster analysis is used to differentiate genotypes on their similarity. Construction of dendrogram using micro-satellite markers saves the time and labor for screening large number of genotypes. It maximizes the variation between groups and minimizes variation within the group which helps to identify the genetically diverse genotypes. Hence, the objectives of present research work was to study genetic variability in wheat cultivars and to improve the yield under rain-fed conditions and to estimate the association among different physio-morphic attributes.

Materials and Methods

Thirty wheat cultivars were grown at the University Research Farm Chakwal Road of PMAS-Arid Agriculture, Rawalpindi - Pakistan (33.11 ºN, 73.01 ºE). Randomized complete block design (RCBD) was followed with three replications keeping row length 5 meters and row to row distance 30 cm. Before planting, field was ploughed, fertilized with mixture of urea and farm yard manure in the soil. Sowing was done in first week of November during 2015-16 and 2016-17. Data were recorded at different stages of crop following the standard protocols i.e., canopy temperature (Neukam *et al.*, 2016), flag leaf area (Francis *et al.*, 1969), cell membrane injury (Deshmukh *et al.*, 1991) and proline content (Bates *et al.*, 1973) before maturity. Data regarding Plant height, spike length, spikelets per spike[-1], grain per spike[-1], 1000-grain weight and yield plant[-1] were

recorded at maturity. DNA was extracted following Stein *et al.*, (2001). Six SSR markers viz., *barc*281, *wmc*168, *wmc*83, *wmc*405, *wmc*790 and *wms*233 were used to study the genetic diversity at genomic level. PCR was done following Roder *et al.*, (1998).

Data were collected for physio-morphic attributes and analyzed following Steel *et al.*, (1997). Correlation coefficients were estimated following Singh & Chaudhary (1985). Principal component analysis (PCA) for extraction of component was performed following Harman (1976) using statistical software Statistica 7.0. Cluster analyses were done using UPGMA derived from genetic similarities and construction of dendrogram with NTSYS-PC.

Results and Discussion

All 30 wheat cultivars differed significantly (*p*<0.01) for physio-morphic traits as revealed by analysis of variance suggesting existence of genetic variation (Table 1).

Principal component analysis: PCA revealed maximum contribution of total variability and eigen values ascertain that how many factors to be retained. In present study the first five factors having eigen values greater than unity (2.865 followed by 1.777, 1.263, 1.190 and 1.106) explained maximum variation (Table 2). Ahmad *et al.,* (2014) performed principal component analysis and identified first three components with Eigen value 6.66 of original variable. In present study, eleven principal components were extracted from original data in which first five PC's contained 74.54% variability. Khodadadi *et*

al., (2011) identified 97% of total genetic variability in the first five components whereas Fahim (2014) identified 64% of total variation in first four components which contained mostly yield related traits. Rymuza *et al.,* (2012) suggested traits contributed towards genetic diversity that contained high negative and positive load.

In our study, the PC1 retained 26.04% variability and it contained mostly yield and yield related traits. The PC2 contained 16.15% of total variation which had high negative load of grains spike^{-1} and spike length while high positive load of flag leaf area and plant height (Fig. 1). Third principal component (PC3) accounted for 11.48% variability and major contributing trait contributed toward diversity was cell membrane injury whereas PC4 retained 10.82% variability and major contributing trait was proline content. The fifth component (PC5) had 10.05% of total variation carries information about canopy temperature and spikelets per spike.

These traits can be utilized to identify high yielding diversified genotypes which may be useful in development of wheat cultivars for rain-fed areas. Chahal & Gosal (2002) reported that traits with high value in principal component have greater effected in clustering than traits with lower value. Bhanupriya *et al.,* (2014) concluded that plant height, and yield related traits whereas Bibi *et al.*, (2017) of the view that spike related traits contributed more to diversity. El-Hendawy *et al.*, (2015) found that spike length, grain number, grain weight, canopy temperature and grain yield contributed more toward diversity and these attributes could be used as selection criteria for developing high yielding genotypes of wheat.

Table 1. Analysis of variance for physio-morphic traits in wheat.

	CT	RI	Pro	PH	FLA	SL	SPP	GPS	TGW	GYP
Replications	0.15	137.94	0.001	153.76	2.52	0.24	0.31	18.49	2.73	40.08
	0.71	44.16	0.133	12.87	620.01	0.71	46.04	71.1	9.11	0.27
Cultivars	13.3**	104.7**	0.03**	54.9**	133.5**	1.6**	5.9**	48.3**	25.4**	8.7**
	1.1**	461.1**	0.03**	77.1**	73.7*	2.9**	6.3**	46.3**	21.5**	6.3**
Error	3.95	37.73	0.009	14.21	14.61	0.46	1.88	16.78	1.83	1.64
	0.32	8.38	0.001	7.62	30.81	0.001	1.07	0.11	0.001	2.21

ANOVA for CT (canopy temperature), RI (Relative cell injury), Pro (proline content), PH (plant height), FLA (flag leaf area), SL (spike length), SPP (spikelets per spike), GPS (grains per spike), TGW (1000-grain weight) and GYP (grain yield per plant) at 5% level of probability

Table 2. Factor load for physio-morphic traits in wheat.

Traits	F1	F2	F3	F4	F5	F6	F7
CT	-0.200	0.131	-0.220	-0.026	0.741	0.375	0.218
CMS	-0.073	-0.125	0.678	0.214	0.037	0.552	-0.222
PRO	0.185	0.076	-0.280	0.591	-0.394	0.418	0.018
PH	0.315	0.380	0.077	0.322	0.024	-0.265	0.445
FLA	0.143	0.568	-0.042	-0.129	-0.052	0.328	0.136
SL	0.279	-0.379	-0.524	0.033	0.107	0.232	-0.116
SPS	0.430	0.014	-0.042	0.179	0.375	-0.130	-0.471
GPS	0.302	-0.414	0.249	0.012	0.168	0.071	0.615
TGW	0.430	0.237	0.251	0.009	0.230	-0.136	-0.257
GYP	0.447	-0.283	0.062	-0.273	-0.161	0.049	0.070
Eigen value	2.865	1.777	1.263	1.190	1.106	0.822	0.634
Variability (%)	26.04	16.155	11.484	10.819	10.050	7.471	5.765
Cumulative %	26.04	42.196	53.680	64.499	74.549	82.020	87.786

CT (canopy temperature), RI (Relative cell injury), Pro (proline content), PH (plant height), FLA (flag leaf area), SL (spike length), SPP (spikelets per spike), GPS (grains per spike), TGW (1000-grain weight) and GYP (grain yield per plant)
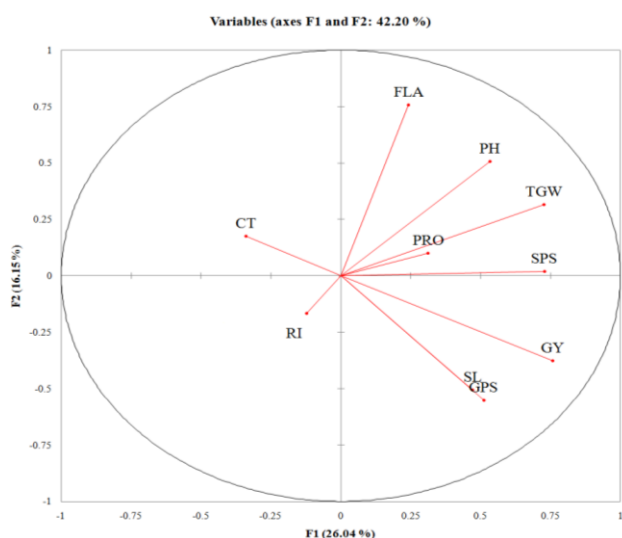
Fig. 1. Factor coordinate for PC1 and PC2 for physio-morphic traits.

Correlation

Cell membrane injury showed positive correlation with proline content and negative correlation with plant height during both years of study (Table 3). It is concluded that cell membrane would be more stable, decrease the injury which could increase the accumulation of proline content. Naeem *et al*., (2016) also found positive association of cell membrane stability and proline content. Canopy temperature had positive association with plant height and flag leaf. It showed negative correlation with spike length, spikelets spike[-1] and grain yield. Plant height showed negative relation with grains spike[-1]. It is suggested that increase in canopy temperature could enhance flag leaf area but reduce spikelets spike[-1] and spike length and grain yield. Azimi *et al*., (2017) found negative relationship among grains spike[-1] and plant height. Similarly, Habibpour *et al*., (2012) reported negative relationship of plant height with grains spike[-1].

Flag leaf area manifested positive association with 1000-grain weight. Flag leaf is responsible for the production of photosynthetic products which are translocated to grains during grain filling and enhance the size of grains. Kalimullah *et al.,* (2012) also found positive relationship of flag leaf area with 1000-grain weight and suggested that increased in flag leaf area could increase grain weight. Spike length was positively

associated with spikelets spike[-1], grains spike[-1] while spikelets per spike indicated positive relationship with grains spike[-1]. It suggested that increased spike length can increases the number of grains in spikes and spikelets spike[-1]. Similarly, enhancement in spikelets spike[-1] can increase grains per spike. Nowsherwan *et al.,* (2017) and Dabi *et al.,* (2016) also found positive relationship between yield contributing traits.

Grain yield plant[-1] is an important trait which indicates negative relationship with proline content and flag leaf area. Furthermore, yield plant[-1] was positively associated with all the yield contributing traits during both years (Table 3). It is suggested that increase in yield related traits could have enhance yield. Various researchers studied positive correlation among yield plant[-1] and yield contributing traits (El-Mohsen *et al.*, 2011; Degewione *et al.*, 2013; Albokari *et al*., 2016; Hama *et al*., 2016; Mecha*, et al.*, 2017; Khan, 2018).

Cluster analysis: Cluster analysis was performed to evaluate genetic diversity among different at DNA level in wheat cultivars. The UPGMA cluster analysis developed the dendrogram which divided the 30 wheat cultivars into three distinct groups (Fig. 2). First cluster A included cultivar Anmol-91 which was distinct from other cultivars of wheat. Second cluster B was further divided in 2 sub-clusters 1B and 2B. The 1B contained cultivars i.e., Millet-11, NARC-09, Chakwal-50, Pirsabak-08, Shahkar-13 and Punjab-11 which were distinct from 2B. The Sub-cluster 2B included the cultivars Fakhre-Sarhad, Pisabak-05 and Bhakhar-02 with maximum similarity. Third cluster C consisted of two sub-clusters 1C and 2C. Sub cluster 1C consisted of cultivar Sariab which was distinct from second sub-cluster. The second sub-cluster 2C contained the cultivars viz., Chakwal-86, Barani-83, LU-26, AARI-11 and Pirsabak-91 with maximum similarity. Abou-Deif *et al.,* (2013) assessed 20 wheat cultivars using 8 inter simple sequence repeat markers to identify the similarity among these cultivars according to their origin and genetic background. Various researchers used SSR markers to evaluate the genetic diversity in wheat and found useful for the study of morphological and physiological traits (Bertin *et al.,*2001; Islam *et al.*, 2012; Sardouie-Nasab *et al.*, 2013; Drikvand *et al.*, 2015). This information helps to evaluate diversity in elite germplasm which would be used in future wheat breeding programs.

Table 3. Correlation coefficient of physio-morphic traits during 2015-16 (Lower diagonal) and 2016-17 (upper diagonal).

Traits	RI	CT	PRO	PH	FLA	SL	SPS	GPS	TGW	GYP
RI	1.00	0.01	0.08	-0.07	-0.11	-0.08	0.05	0.31	-0.01	0.11
CT	-0.13*	1.00	-0.06	0.16	0.19	-0.02	-0.03	-0.05	0.02	-0.39*
PRO	0.44**	0.08	1.00	0.14	-0.01	0.51**	0.14	-0.04	-0.06	-0.07
PH	-0.33**	0.26*	-0.26*	1.00	0.27	-0.06	0.26	0.18	0.35	-0.03
FLA	0.12	0.05	0.19	-0.24	1.00	-0.21	0.06	-0.35*	0.21	-0.19
SL	-0.29	-0.17	-0.02	0.05	-0.13	1.00	0.35	0.16	0.11	0.31
SPS	-0.36*	-0.09	-0.42*	0.17	-0.20	0.01	1.00	0.31	0.49**	0.31
GPS	-0.22	-0.25	0.14	-0.01	-0.36*	0.32	0.54**	1.00	0.26	0.41*
TGW	0.03	-0.03	0.31	-0.27	0.24	-0.30	-0.47**	-0.31	1.00	0.27
GY	-0.38*	0.17	-0.18	0.01	-0.48**	0.21	0.17	0.40	0.15	1.00

CT (canopy temperature), RI (Relative cell injury), Pro (proline content), PH (plant height), FLA (flag leaf area), SL (spike length), SPP (spikelets per spike), GPS (grains per spike), TGW (1000-grain weight) and GYP (grain yield per plant)
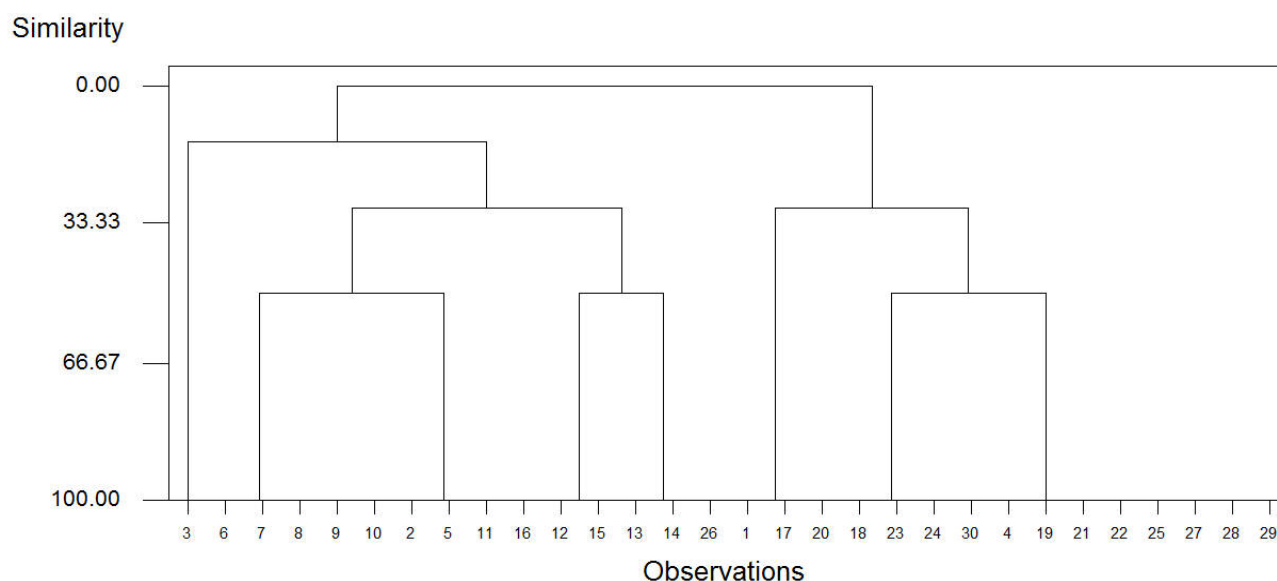
Fig. 2. Dendrogram showing genetic similarity among different wheat cultivars.

Conclusion

Genetic diversity helps to select efficient cultivars with wider adaptability and variation. Principal component analysis and cluster analysis confirmed that studied wheat cultivars were genetically diverse for various physiological and morphological traits. Correlation among traits revealed positive relationship between yield and yield related traits. Dendrogram generated by SSRs markers data was found useful to classify 30 wheat cultivars in 3 clusters on the basis of their genetic similarity.

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