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GENETIC DIVERGENCE IN WHEAT GENOTYPES BASED ON SEED BIOCHEMICAL PROFILES APPRAISED THROUGH AGGLOMERATIVE HIERARCHICAL CLUSTERING AND ASSOCIATION ANALYSIS AMONG TRAITS

ANAM KHALID[1,2*] AND AMJAD HAMEED[1,2]

[1]*Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan*
[2]*Pakistan Institute of Engineering and Applied Sciences, Islamabad, Pakistan*
[*]*Corresponding author's email: anamkhalidrai@gmail.com*

Abstract

For biochemical traits improvement, presence of variability has primary significance in plant breeding. Data of various seed biochemical constituents in 77 wheat genotypes were analysed for correlation and agglomerative hierarchical clustering to choose varieties and characters for future breeding program. Correlation analysis revealed significant positive relationship of catalase (CAT) with reducing sugars (RS), total soluble sugars (TSS), and total soluble proteins (TSP) while negative association with total phenolic content (TPC), protease and ascorbic acid (AsA). Peroxidase (POD) activity displayed highly significant positive correlation with superoxide dismutase (SOD), TPC, protease and AsA but showed negative correlation with malondialdehyde (MDA) content. Cluster analysis clustered all genotypes into four different groups. The D^2 statistics confirmed highest distance between cluster- II and cluster-III whereas maximum similarity was found in cluster-III as well as cluster-IV. Hence, it is suggested that crosses between genotypes of cluster-II and cluster-III with those of cluster-III and cluster-IV may be chosen for wheat breeding program and for superior genotypes selection in subsequent population.

Key words: Cluster analysis, Correlation, Enzymatic antioxidants, Non-enzymatic antioxidants.

Introduction

Triticum aestivum L. (bread wheat), is the most significant cereal crop in the world and because of its multidimensional usage and nutritional importance, it is primary food for greater than one third of the population in the world including Pakistan (Asif *et al.*, 2005; Rehman *et al.*, 2013). Pakistan is the eighth major wheat producer (Uddin *et al.*, 2015), with a total production of 25.195 million tonnes from an area of 8,740 thousand hectares under cultivation (Economic survey of Pakistan 2018-2019). It is famous as the 'King of cereals' owing to its production, acreage as well as the discernable place in international food grain trade (Afridi & Khalil, 2007; Rehman *et al.*, 2013). Due to rapid rise in human population, world demand for wheat is estimated to be much elevated in near future but the wheat production resources are expected to be considerably lower. The challenge of food security is perilous in the developing countries as compared to developed world while wheat is the central crop for ensuring food constancy particularly in Pakistan. Pakistan population increasing at a rate of 2.4 % per year is expected to be 212.82 million in 2030 which demand an increase in its production correspondingly (Economic survey of Pakistan 2017-2018).

Wheat holds antioxidation property against the significant bio-molecules like DNA, membrane lipids and proteins. It deters the super oxide anion (O^{2-}) and oxidation of low density lipoprotein (LDL) cholesterol in humans (Yu *et al.*, 2005; Sedej *et al.*, 2011). Environmental conditions like average daily radiation of sun, hour's number and growing area as well as genotype of wheat are the key features which influence the percentage of scavenging of antioxidants found in the grains of whole wheat. A wide range of bioactive compounds are responsible for rendering antioxidant ability which include phytic acids, phenolic acids, tocopherols, carotenoids, tocotrienols, flavonoids and phytosterols. Antioxidant capacity differs in respect of wheat genotypes and biologically active compounds for instance phenolic acids, carotenoids and anthocyanins and tocopherols (Rao *et al.*, 2013).

Enhancement of germplasm and genetic variability is crucial for consistent and viable production of food crops. For productive estimation and use of germplasm, extent of existing genetic divergence measurement is of ultimate importance (Zubair *et al.*, 2007). Primary objective of any breeding program is the production of high yielding and disease resistant genotypes with wider adaptation and these diverse goals can merely be attained in the presence of sufficient genetic diversity and variability. When the patterns of genetic variation in a community are recognized, the efficiency of genetic gain through random selection can be excelled. Varieties with wider genetic base can withstand disease stress more effectively and are more adaptive to exacting agro-climatic conditions (Uddin *et al.*, 2015).

Greater the genetically different parents, more will be the probabilities of attaining greater heterotic manifestation in F1s and broad range of variation in dividing population (Shekhawat *et al.*, 2001). For developing new population, the study of genetic difference can help in the genotypes selection to be employed in breeding programs because it evaluates the extent of diversity present among selected genotypes (Khan *et al.*, 2019). Various genetic divergence studies have been administered on diverse species of crops depending upon qualitative and quantitative traits so as to select genetically distant parentages for hybridization (Singh & Salgotra, 2014).

The use of multivariate statistical algorithms is a crucial approach for classifying germplasm besides analysing genetic relationships amongst breeding material. The most frequently practiced algorithms for this objective are principal component analysis, canonical variable analysis and clustering methods. Clustering is a multivariate analysis which employs grouping of traits otherwise genotypes information for classifying a population into main clusters on the basis of similarities (Jaynes *et al.*, 2003; Ghaed Rahimi & Heidari, 2014).The cluster analysis is a suitable method to find out family relations i.e. to figure out the wideness of genetic affinity or genotypes distance from one another (Mishra *et al.*, 2015). The D^2 statistic usage (Mahalanobis, 1936) is one of the most significant biometrical methods in order to estimate genetic difference existent in a population. Clustering and correlation have also been previously reported in tomato as well as wheat for genetic diversity assessment (Khodadadi *et al.*, 2011; Ajmal *et al.*, 2013; Iqbal *et al.*, 2014). Selection of parents based on the extent of genetic divergence has been successfully utilised in different crop species (Gashaw *et al.*, 2007).

Evaluating the whole wheat grains for health advantages and potential bioactive constituents can help in developing functional diet based on grain. The current study was directed to classify the accessible germplasm into separate clusters/groups based on genetic variation amongst their seed biochemical attributes using agglomerative hierarchical clustering.

Material and Methods

Seventy seven wheat genotypes as reported earlier (Khalid & Hameed, 2017) belonging to diverse geographical origin were collected for testing antioxidant activity. Seeds of wheat genotypes (0.2 g) were extracted in 2 ml (50 mM) potassium phosphate buffer (pH 7.4). Samples were centrifuged at 14,462 ×g for 10 min at 4°C. The supernatant was parted and employed for the evaluation of diverse enzymatic activities like catalase (CAT) (Chance & Maehly, 1955), superoxide dismutase (SOD) (Giannopolitis & Ries, 1977), peroxidase (POD) (Beers & Sizer, 1952), ascorbate peroxidase (APX) (Dixit *et al.*, 2001) and other biochemical attributes i.e. malondialdehyde content (MDA) (Heath and Packer, 1968 and Dhindsa *et al*., 1981), alpha amylase activity (Varavinit *et al.*, 2002), esterase (Van Asperen, 1962), protease (Drapeau, 1974), total oxidant status (TOS) (Erel, 2005), total phenolic content (TPC) (Ainsworth & Gillespie, 2007), Ascorbic acid (AsA) (Mindlin and Bulter, 1937), non-reducing sugars (NRS), reducing sugars (Lever, 1972; Miller, 1972), total soluble sugars (Dubois *et al.*, 1951), Albumins, Globulins, salt soluble protein (SSP) and total soluble protein (TSP) (Bradford, 1976). Data for different seed quality and nutritive parameters were recorded.

Finally, data was subjected to analysis of variance and genetic divergence was computed through cluster analysis via agglomerative hierarchical clustering through computer software Microsoft Excel along with XLSTAT Version 2012.1.02, Copyright Addinsoft 1995-2012 (http://www.xlstat.com).

Results

Order of correlations/associations amid traits: Analysis of variance showed substantial genotypic mean square values for entire characters presenting importance of genetic divergence (Table 1) to be utilized for wheat enhancement. Simple values of correlation coefficient revealed remarkable associations to plan breeding approach (Table 2). Significantly positive correlation was revealed by CAT with RS, TSS and TSP. However, it had significantly negative association with TPC, protease and AsA. POD activity showed highly significant and positive relationship with SOD, TPC, protease and AsA however, displayed extremely significant negative association with MDA. APX, alpha amylase and NRS had positive correlation with TSS. Nevertheless, APX showed significantly negative association with MDA while NRS revealed significant negative correlation with RS. SOD had significantly positive correlation with SSP. Esterase and albumin revealed insignificant correlation with entire traits in the current study.

Protease and ascorbic acid revealed significant positive correlation with TOS. Significantly positive correlation was shown by TPC with POD and AsA however, it had significant negative association with CAT, TSS and TSP. Protease and AsA showed significant positive correlation with POD while it showed significantly negative correlation with CAT. The RS had significantly positive association with CAT and significantly negative correlation with NRS whereas TSS showed significantly positive correlation with CAT, APX, alpha amylase and NRS. It had significant negative association with TPC and AsA. Globulin showed highly positive correlation with SSP however; SSP had significant positive correlation with SOD and globulins. Total soluble proteins showed significantly positive correlation with CAT and negative correlation with TPC and AsA.

Cluster analysis: Genotypes grouping on the basis of studied traits are shown in (Fig. 1). Cluster analysis assembled 77 wheat genotypes into 4 groups as presented in (Table 3). Cluster-I encompassed 46 genotypes followed by 21, 3 and 7 genotypes correspondingly in cluster-II, III in addition to cluster-IV. Cluster-I showed least diversity for all the traits. Cluster-II comprised of genotypes with higher esterase, AsA, NRS. The genotypes in cluster-III encompasses large albumin, globulin, POD, SOD, TPC, TOS, while the genotypes in cluster-IV possessed larger CAT and APX, MDA, alpha amylase, RS, NRS, TSS, SSP and TSP. In cluster analysis, diverse biochemical traits average values regarding wheat genotypes have been presented in (Table 4).

Pairwise Mahalanobis distances (D^2 statistics) are shown in (Table 5). Cluster-IV genotypes illustrated maximum divergence against cluster-III genotypes. Though, least variation was detected between cluster II and III due to minimum value of genetic diversity.

Table 1. Analysis of variance for different biochemical parameters in wheat genotypes.

	CAT	POD	APX	MDA	alpha amylase	SOD	ESTR.	TOS	TPC	PROT.	AsA	NRS	RS	TSS	ALB.	GLOB.	SSP	TSP
R^2	0.825	0.904	0.886	0.941	0.980	0.942	0.927	0.960	0.964	0.985	0.989	0.924	0.919	0.918	0.853	0.939	0.950	0.973
F	9.555	19.167	15.679	32.258	101.802	33.096	25.770	48.254	53.508	129.629	183.781	24.566	22.897	22.574	11.792	30.913	38.621	71.818
Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Significant at 0.0001% level of probability

CAT= catalase; POD= peroxidase; APX= ascorbate peroxidase; SOD= superoxide dismutase; ESTR.= esterase; TOS= total phenolic content; PROT.= protease; AsA= ascorbic acid; NRS= non reducing sugras; RS= reducing sugars; TSS= total soluble sugars; ALB.= albumin; GLOB.= globulin; SSP= salt soluble protein; TSP= total soluble proteins

Table 2. Correlation matrix among different biochemical parameters in wheat genotypes.

Variables	CAT	POD	APX	MDA	AA	SOD	ESTR.	TOS	TPC	PROT.	AsA	NRS	RS	TSS	ALB.	GLOB.	SSP	TSP
CAT	**1**																	
POD	-0.208	**1**																
APX	0.170	0.002	**1**															
MDA	0.028	**-0.236**	**-0.425**	**1**														
AA	0.018	-0.216	-0.196	0.218	**1**													
SOD	-0.138	**0.278**	0.049	-0.076	-0.026	**1**												
ESTR.	0.064	0.162	0.007	-0.020	-0.184	0.070	**1**											
TOS	-0.198	0.186	0.131	-0.200	-0.176	0.099	0.051	**1**										
TPC	**-0.535**	**0.279**	-0.076	-0.153	-0.144	0.151	-0.018	0.098	**1**									
PROT.	**-0.378**	**0.333**	0.000	-0.105	-0.174	0.078	0.022	**0.260**	0.201	**1**								
AsA	**-0.725**	**0.287**	-0.093	**-0.250**	-0.066	0.221	0.051	**0.317**	**0.616**	**0.256**	**1**							
NRS	0.104	-0.108	0.121	0.099	0.203	-0.106	0.072	0.056	-0.221	-0.109	-0.220	**1**						
RS	**0.238**	-0.088	0.148	-0.086	-0.003	-0.027	-0.088	0.003	-0.153	-0.144	-0.171	**-0.479**	**1**					
TTS	**0.275**	-0.180	**0.235**	0.054	**0.228**	-0.138	0.023	0.066	**-0.351**	-0.218	**-0.362**	**0.814**	0.121	**1**				
ALB.	-0.097	0.118	0.142	0.097	0.030	-0.115	-0.069	-0.065	0.214	-0.014	-0.118	-0.091	0.028	-0.084	**1**			
GLOB.	0.113	0.122	-0.023	0.033	-0.011	0.181	-0.006	-0.159	-0.201	-0.005	-0.155	-0.054	0.216	0.082	-0.002	**1**		
SSP	0.187	0.128	0.143	-0.086	-0.128	**0.228**	0.211	-0.059	0.063	0.103	-0.137	-0.053	-0.080	-0.113	0.008	**0.283**	**1**	
TSP	**0.568**	-0.056	0.029	-0.003	0.117	-0.146	-0.047	-0.202	**-0.356**	-0.153	**-0.668**	-0.009	0.195	0.119	0.001	0.175	0.193	**1**

CAT= catalase; POD= peroxidase; APX= ascorbate peroxidase; SOD= superoxide dismutase; ESTR.= esterase; TOS= total phenolic content; PROT.= protease; AsA= ascorbic acid; NRS= non reducing sugras; RS= reducing sugars; TSS= total soluble sugars; ALB.= albumin; GLOB.= globulin; SSP= salt soluble protein; TSP= total soluble proteins

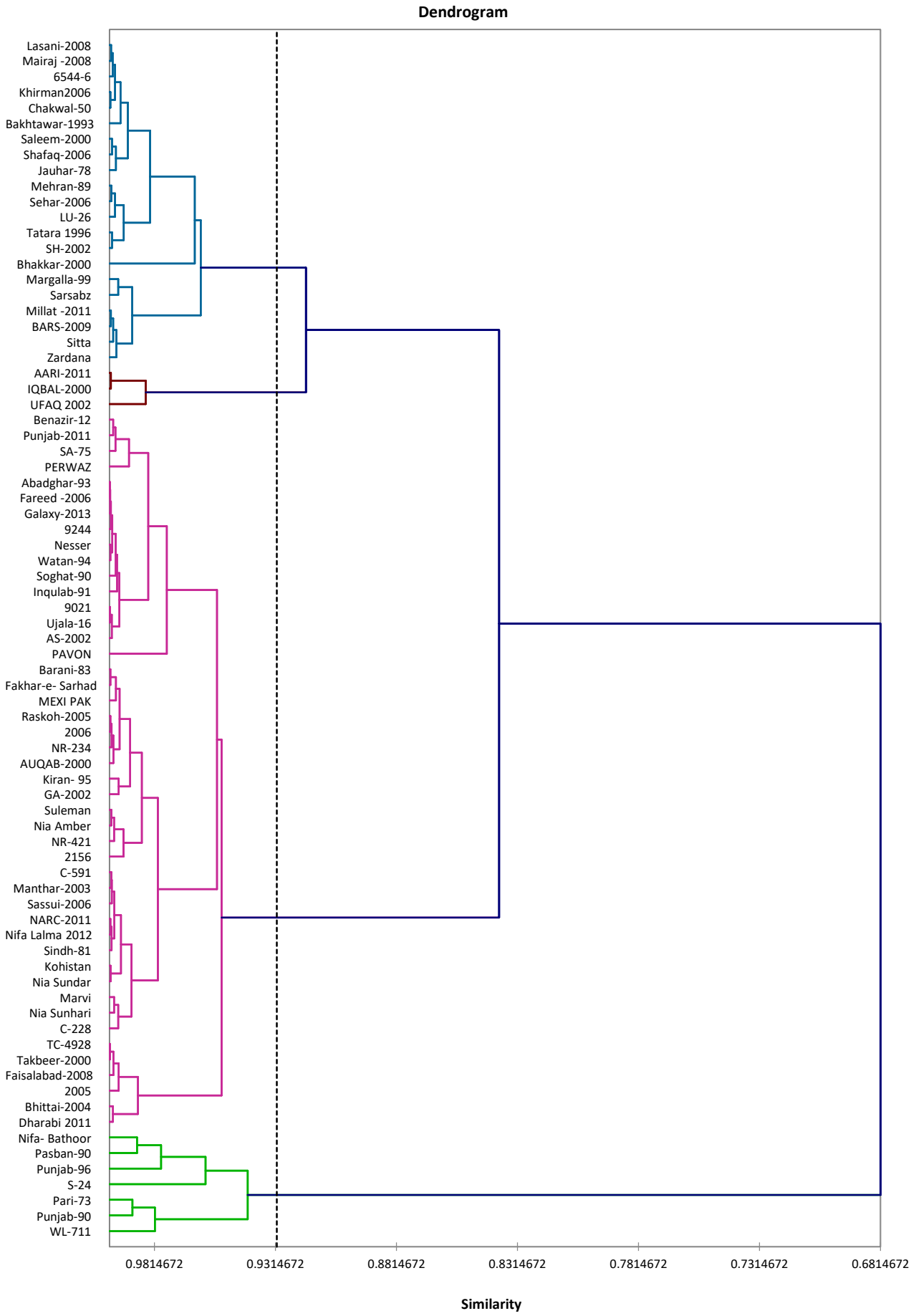

Fig. 1. Tree diagram based on eighteen traits for different wheat genotypes.

Table 3. Distribution of wheat genotypes in different clusters.

Cluster	Genotypes
I	PAVON, PERWAZ, MEXI PAK, AS-2002, Manthar-2003, Fareed -2006 ,Inqulab-91, Dharabi 2011, Faisalabad-2008, Punjab-2011, Galaxy-2013, Ujala-16, Fakhar-e-Sarhad, Takbeer-2000, Nifa Lalma 2012, Sindh-81, Soghat-90, Kiran-95, Bhittai-2004, Barani-83, Sassui-2006, Nia Amber, Nia Sunhari, Nia Sundar, Benazir-12, NARC-2011, Watan-94, Marvi, Abadghar-93, C-228, C-591, 2006, 2005, 2156, Kohistan, 9021, TC-4928, 9244, Suleman NR-234, NR-421, Nesser, Raskoh-2005, SA-75
II	LU-26, Bhakkar-2000, SH-2002, Shafaq-2006, Mairaj-2008, Lasani-2008, Sehar-2006, Chakwal-50, BARS-2009, Millat-2011, Bakhtawar-1993, Tatara 1996, Jauhar-78, Sarsabz, Khirman-2006, 6544-6, Margalla-99, Sitta, Zardana, Mehran-89, Saleem-2000
III	IQBAL-2000, UFAQ 2002, AARI-2011
IV	Punjab-90, Pasban-90, Nifa- Bathoor, Punjab-96, S-24, Pari-73, WL-711

Table 4. Mean values of different biochemical traits of wheat genotypes in cluster analysis.

Cluster	CAT	POD	APX	MDA	Alpha Amylase	SOD	Estr.	TOS	TPC	Prot.	AsA	NRS	RS	TSS	Alb.	Glob.	SSP	TSP
1	121.01	6956.80	498.26	520.62	214.35	166.73	574.76	297.89	16180.07	7273.26	669.08	8.80	5.54	14.34	272.68	165.27	90.46	291.49
2	129.21	15763.06	476.19	480.57	230.65	182.78	615.88	310.31	13040.48	7692.86	678.09	10.33	5.03	15.35	259.38	170.22	94.02	308.46
3	144.44	39597.40	631.11	452.82	139.29	191.01	595.26	330.50	16438.89	8533.33	672.17	6.45	6.82	13.27	297.78	191.78	92.82	316.15
4	413.33	3161.91	668.57	529.99	240.72	146.69	547.32	258.27	2216.67	5904.29	479.43	12.28	7.33	19.61	279.08	180.73	99.16	459.75

CAT= catalase; POD= peroxidase; APX= ascorbate peroxidase; SOD= superoxide dismutase; ESTR.= esterase; TOS= total phenolic content; PROT.= protease; AsA= ascorbic acid; NRS= non reducing sugars; RS= reducing sugars; TSS= total soluble sugars; ALB.= albumin; GLOB.= globulin; SSP= salt soluble protein; TSP= total soluble proteins

Table 5. D2 statistics among different clusters.

	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I	0			
Cluster II	9358.863	0		
Cluster III	32666.431	24090.824	0	
Cluster IV	14540.792	16713.304	39203.136	0

Discussion

Correlation studies provide information on the nature and extent of relationship between any two sets of metric/quantitative characters. From this, it might be likely to generate genetic advancement in one trait by selecting the other pair (Baloch *et al.*, 2014). In present study, significantly positive correlation was revealed by CAT with RS, TSS and TSP. POD activity showed greatly significant and positive association with SOD, TPC, protease and AsA. SOD had significantly positive correlation with SSP. Protease and ascorbic acid revealed significant positive correlation with TOS. Significantly positive correlation was shown by TPC with POD and AsA. Protease and AsA showed significant positive correlation with POD. The RS had significantly positive association with CAT. TSS showed significantly positive correlation with CAT, APX, alpha amylase and NRS. Globulin showed highly positive correlation with SSP however; SSP had significant positive correlation with SOD and globulins. Total soluble proteins showed significantly positive correlation with CAT. Presence of significant correlations for POD, TPC, protease and AsA reflects that increase in either of these traits will results in an increase of other characters. Hence, these qualitative and nutritive traits can be employed for the selection of superior wheat genotypes in breeding program which renders an excellent opportunity to assemble desirable characters.

Cluster analysis could be considered as an effective tool to classify germplasm which provides consistent foundation in the selection of base material to design breeding tactics in future (Susic *et al.*, 1999; Jin *et al.*, 2006). Cluster analysis grouped 77 wheat genotypes into 4 clusters. According to Pairwise Mahalanobis distances (D^2 statistics), cluster-IV genotypes demonstrated maximum divergence against cluster-III genotypes. Though, the authors are the view that during the selection of base material, one should beware of breeding techniques besides genetic constraints to acquire likely genetic least diversity for all the traits. Outcomes of current study showed that multivariate analysis aids in placing the genotypes in various clusters.

Conclusion

Hence, it is suggested that cluster III and IV genotypes are complementary for maximal biochemical parameters and can be chosen for hybridization to establish potential hybrids in subsequent generation.

References

Afridi, N. and I.H. Khalil. 2007. Genetic improvement in yield related traits of wheat under irrigated and rainfed environments. *Sarhad J. Agri.*, 23: 965-972.

Ainsworth, E.A. and K.M. Gillespie. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nat. Protocols*, 2: 875-877.

Ajmal, S.U., N.M. Minhas, A. Hamdani, A. Shakir, M. Zubair and Z. Ahmad. 2013. Multivariate analysis of genetic divergence in wheat (*Triticum aestivum*) germplasm. *Pak. J. Bot.*, 45: 1643-1648.

Asif, M., Mehboob-ur-Rahman and Y. Zafar. 2005. DNA fingerprinting studies of some wheat (*Triticum aestivum* L.) genotypes using random amplified polymorphic DNA (RAPD) analysis. *Pak. J. Bot.*, 37: 271-277.

Baloch, A.W., M. Baloch, I.A. Baloch, S.N. Mari, D.K. Mandan and S.A. Abro. 2014. Association and path analysis in advance Pakistani bread wheat genotypes. *Pure & App. Biol.*, 3: 115-120.

Beers, R.F. and I.W. Sizer. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.*, 195: 133-140.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72: 248-254.

Chance, B. and A. Maehly. 1955. Assay of catalases and peroxidases. *Methods in Enzym.*, 2: 764-775.

Dhindsa, R.S., P. Plumb-Dhindsa and T.A. Thorpe. 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.*, 32: 93-101.

Dixit, V., V. Pandey and R. Shyam. 2001. Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L. cv. Azad). *J. Exp. Bot.*, 52: 1101-1109.

Drapeau, G. 1974. Protease from Staphylococcus aureus. *Academic Press, NY.*

Dubois, M., K. Gilles, J. Hamilton, P. Rebers and F. Smith. 1951. A colorimetric method for the determination of sugars. *Anal. Chem.*, 28: 350-356.

Erel, O. 2005. A new automated colorimetric method for measuring total oxidant status. *Clin. Biochem.*, 38: 1103-1111.

Gashaw, A., H. Mohammed and H. Singh. 2007. Genetic divergence in selected durum wheat genotypes of Ethiopian plasm. *Afr. Crop Sci. J.*, 15.

Ghaed Rahimi, L. and B. Heidari. 2014. Genetic Advance Prediction and Multivariate Analysis for Antioxidants and Agronomic Traits in Wheat. *Ann. Res. & Rev. Biol.*, 4: 2427-2449.

Giannopolitis, C.N. and S.K. Ries. 1977. Superoxide dismutases I. Occurrence in higher plants. *Plant Physiol.*, 59: 309-314.

Heath, R.L. and L. Packer. 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.*, 125: 189-198.

Iqbal, Q., M.Y. Saleem, A. Hameed and M. Asghar. 2014. Assessment of genetic divergence in tomato through agglomerative hierarchical clustering and principal component analysis. *Pak. J. Bot.*, 46: 1865-1870.

Jaynes, D., T. Kaspar, T. Colvin and D. James. 2003. Cluster analysis of spatiotemporal corn yield patterns in an Iowa field. *Agron. J.*, 95: 574-586.

Jin, F.,44 J. Xue, Y. Jia and Z. Liu. 2006. The cluster analysis on tomato germplasms. *Acta Agri. Boreali-Sin.*, 21: 49-54.

Khalid, A. and A. Hameed. 2017. Seed biochemical analysis based profiling of diverse wheat genetic resource from Pakistan. *Front. Plant Sci.*, 8: 1-15.

Khan, A., M. Ahmad, M.K.N. Shah and M. Ahmad. 2019. Performance of wheat genotypes for Morpho-Physiological traits using multivariate analysis under terminal heat stress. *Pak. J. Bot*., 52: 1981-1988.

Khodadadi, M., M.H. Fotokian and M. Miransari. 2011. Genetic diversity of wheat (*Triticum aestivum* L.) genotypes based on cluster and principal component analyses for breeding strategies. *Aust. J. Crop Sci.*, 5: 17-24.

Lever, M. 1972. A new reaction for colorimetric determination of carbohydrates. *Anal. Biochem.*, 47: 273-279.

Mahalanobis, P.C. 1936. On the generalized distance in statistics. *Proceed. Nat. Inst. Sci., (Calcutta)*, 2: 49-55.

Miller, G. 1972. Analytical Chemistry. New York: McGraw Hill Book.

Mindlin, R.L. and A.M. Butler. 1937. *Journal of Biological Chemistry*, 122: 673.

Mishra, C., V. Tiwari, S. Kumar, V. Gupta, A. Kumar and I. Sharma. 2015. Genetic diversity and genotype by trait analysis for agro-morphological and physiological traits of wheat (*Triticum aestivum* L.). *SABRAO J. Breed. & Gen.*, 47: 40-48.

Rao, A., S.D. Ahmad, S.M. Sabir, S. Awan, A.H. Shah, M.F. Khan, S.A. Khan, S. Shafique, S. Arif and S.R. Abbas. 2013. Antioxidant activity and lipid peroxidation of selected wheat cultivars under salt stress. *J. Med. Plants Res.*, 7: 155-164.

Rehman, R., K.N. Shah, M.S. Masood, M. Arshad and A. Ghafoor. 2013. Genetic divergence among Pakistani bread wheat varieties and advanced line for randomly amplified polymorphic DNA (RAPD) markers. *Pak. J. Bot.*, 45: 327-332.

Sedej, I., M. Sakač, A. Mandić, A. Mišan, V. Tumbas and M. Hadnađev. 2011. Assessment of antioxidant activity and rheological properties of wheat and buckwheat milling fractions. *J. Cereal Sci.*, 54: 347-353.

Shekhawat, U., V. Prakash and D. Singhania. 2001. Genetic divergence in barley (*Hordeum vulgare* L). *Ind. J. Agri. Res.*, 35: 121-123.

Singh, P. and S. Salgotra. 2014. Genetic divergence study in improved bread wheat varieties (*Triticum aestivum*). *Afr. J. Agri. Res.*, 9: 507-512.

Susic, Z., J. Zdravkovic, N. Pavlovic and S. Prodanovic. 1999. Selecting features for estimating genetic divergence of tomato genotypes (*Lycopersicum esculentum* Mill.). *Genetika*, 31: 235-244.

Uddin, F., F. Mohammad and S. Ahmed. 2015. Genetic divergence in wheat recombinant inbred lines for yield and yield components. *Acad. J. Agri. Res.*, 3: 303-307.

Van Asperen, K. 1962. A study of housefly esterases by means of a sensitive colorimetric method. *J. Ins. Physiol.*, 8: 401-416.

Varavinit, S., N. Chaokasem and S. Shobsngob. 2002. Immobilization of a thermostable alpha-amylase. *Sci. Asia*, 28: 247-251.

Yu, L.L., K. Zhou and J.W. Parry. 2005. Inhibitory effects of wheat bran extracts on human LDL oxidation and free radicals. *LWT-Food Sci. & Technol.*, 38: 463-470.

Zubair, M., S.U. Ajmal, M. Anwar and A. Haqqani. 2007. Multivariate analysis for quantitative traits in mungbean [*Vigna radiata* (L.) Wilczek]. *Pak. J. Bot.*, 39: 103-113.

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