

## GENETIC DISSECTION OF YIELD ENHANCING TRAITS IN PAKISTANI SPRING WHEAT GENOTYPES

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Genetic gains of crops could be improved by pyramiding physiological, agronomic and stress tolerant traits in single genotypes. Comprehensive foreground and background phenotypic information is a prerequisite for any molecular and strategic breeding program. A population of 465 local spring wheat (*Triticum aestivum* L.) genotypes was characterized using various agro-morphological and yield traits for two cropping seasons. Average values ranged from 92%-112%, 80%-240%, 48%-57% and 34%-42% for plant height, harvest index, grain per spike and 1000 grain weight, respectively. In both years, there was a strong correlation of yield per plant with plant height, harvest index, 1000 grain weight and grain per spike. Factor1 and factor2 of principal component analysis (PCA) explained the maximum variability ranges from 21%-19% for the first year and 19%-16% for the second year. Plant height and yield per plant showed a significantly positive correlation with genotypes during both cropping seasons. Cluster analysis divided genotypes into three distinct groups indicating the existence of considerable variation. The extent of variation and correlation among genotypes is required for effective utilization and conservation of the genetic resource. Based on PCA, genotypes with highest score and desirable characters can be selected for further breeding programs.

**Keywords:** PCA, Cluster analysis, Wheat, Multivariate, Genetic diversity, Yield, Correlation.

### INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is a staple dietary source for 2.5 billion peoples of 89 countries. It single handedly provides 20% of the total protein and energy requirement for human diet (Shewry and Hey, 2015). Current annual spring wheat genetic gain is around 0.5-1% (Crespo *et al.*, 2017); however, in view of promptly growing human needs, annual wheat production must increase with a similar pace at a rate of 2% annum (Gill *et al.*, 2004). The rapid increase of almost 2.4% per annum in the population of Pakistan raises the demand for agricultural products. In Pakistan, wheat, maize, rice cotton and sugarcane are some of the significant cash crops of farmer community which adds 23.60% value to the agriculture sector with 4.4% to national GDP. In Pakistan, the total area hosted for wheat cultivation was 8.7 million hectares and accounted for an annual production of 25 million tonnes for the year 2017-2018. Wheat alone adds 8.9% value to agriculture and 1.6% to Pakistan's GDP (Pakistan Economic Survey 2018-19). In Pakistan 30 to 40% calories intake comes from wheat and it covers 40% of the crop area of Pakistan (Hussain *et al.*, 2013; Qadir *et al.*, 2019).

Morphological as well as genetic diversity analysis acts as a prerequisite for efficient management and utilization of potent genetic resources. Accurate phenotypic characterizations of genotypes is very important for plant breeders from parent selection to final utilization of genotypes in breeding

programs (Haussmann *et al.*, 2004; Haider *et al.*, 2015; Mehmood *et al.*, 2016). Primary requirement to increase wheat production in wheat improvement program is to select the cultivar with high yield in changing climate and different environments (Enghiad *et al.*, 2017). Major yield contributing agronomic traits are thousand grain weight, spikes per plant, grains per spike, grain weight, days to heading and days to maturity (Gulnaz *et al.*, 2019). Multivariate analysis uses statistic approach to estimate the underline genetic variation in given population. Principle component analysis (PCA) distribute the data and genotype in two-dimensional graph to explain the correlation, dissociation and variability amongst the genotypes (Chakravorty *et al.*, 2013). Agronomical trait information not only complements the process of strategic trait-based breeding but also helps to increase the chances of positive allele pyramiding in comparison to uncharacterized parents (Reynolds and Langridge, 2016). Recognizing the economic importance of morphological relationship, among bread wheat genotypes for enhanced yield potential, the current study was conducted to find out the genetic diversity confined by Pakistani spring wheat germplasm by dissociation of yield traits and selection of germplasm based on improved morphological traits for further breeding programs.

### MATERIALS AND METHODS

**Seed Material:** A total of four hundred and sixty-five (named L1 to L465) genotypes of bread wheat (*Triticum aestivum* L.) were collected from Wheat Research Institute, Ayub Agricultural Research Institute (AARI), Faisalabad. Healthy seeds of all genotypes were sown in November at Centre of Agricultural Biochemistry and Biotechnology (CABB) experimental area, University of Agriculture, Faisalabad (31° 26' N, 73° 6' E) for two consecutive years 2015-16 and 2016-17. Each genotype was planted in a one meter long row by keeping row to row distance of thirty six cm and sowing was done by planting two seeds per hole and maintaining eight cm as plant to plant distance. The crop was raised by following all standard agronomic practices. The experiment was conducted following the augmented design (Federer, 2002) where twelve checks were repeated in each block with the whole material divided into eight blocks.

Three healthy plants from each genotype were selected and tagged for phenotypic data. Data of ten agronomical traits were recorded for each genotype (Laghari *et al.*, 2010; Ain *et al.*, 2015) which were, days to heading, days to maturity, plant height (cm), peduncle length (cm), number of tillers per plant, spike length (cm), grain per spike, 1000 grain weight (g), yield per plant (g) and harvest index (HI). The harvest index

was calculated according to Donald (1965) by using the formula:

$$\text{Harvest Index} = (\text{Grain yield/Biological yield}) \times 100$$

Where, the biological yield was considered as weight of harvested plant in grams for each genotype.

**Statistical Analysis:** Statistical parameters including minimum, mean and maximum value of data, standard deviation, variance and coefficient of variation were calculated for agronomic traits using XLSTAT (Addinsoft, 2019). Analysis of variance (ANOVA) with significance  $P < 0.001$  was done using augmented RCBD package in R software. Genetic diversity of morphological data accessed using principal component analysis (PCA) (Khodadadi *et al.*, 2011). Ward's clustering method was used to construct the dendrogram based on morphological data for both years.

## RESULTS

Descriptive statistics was performed on 465 Pakistani spring wheat genotypes to estimate the behavior of ten agronomical important traits during cropping season 2015-16 and 2016-17 (Table 1). Genotype L421 showed highest plant height; while, genotype L224 showed minimum plant height. Minimum yield per plant was observed in L278 while L435 had higher

**Table 1. Statistical summary of ten agronomical traits of 465 of wheat genotypes.**

	Year 2015-2016					Year 2016-2017				
	Range	Mean	Var. (n)	SD (n)	CV	Range	Mean	Var. (n)	SD (n)	CV
<b>DTH</b>	78-103	84.267	17.017	4.125	0.049	81-105	94.806	14.303	3.778	0.040
<b>DTM</b>	123-141	128.230	5.174	2.275	0.018	127-148	140.505	3.824	1.953	0.014
<b>PH</b>	35-127	85.393	92.416	9.613	0.113	49-134	101.241	112.959	10.617	0.105
<b>PL</b>	8-44	23.819	30.637	5.535	0.232	9-49	22.256	43.109	6.559	0.295
<b>T/P</b>	2-30	11.772	22.800	4.775	0.406	6-32	16.658	17.527	4.182	0.251
<b>G/S</b>	34-73	54.312	48.318	6.951	0.128	43-92	58.211	57.649	7.585	0.130
<b>SL</b>	7.3-14.2	10.542	1.408	1.187	0.113	6-20	11.656	4.215	2.051	0.176
<b>TGW</b>	19.3-66.9	35.154	34.509	5.874	0.167	24.7-63.5	43.159	42.506	6.513	0.151
<b>Y/P</b>	1.7-31.7	10.044	19.035	4.363	0.434	4.7-46.8	23.193	51.839	7.192	0.310
<b>HI</b>	6-87.1	26.871	78.999	8.888	0.331	6.7-79.8	34.698	240.746	15.499	0.447

Var.: Variance, SD: Standard deviation, CV: Variation coefficient, DTH: Days to heading, DTM: Days to maturity, PH: Plant height, PL: Peduncle length, T/P: Tillers per plant, G/S: Grain per spike, SL: Spike length, TGW: 1000 grain weight, Y/P: Yield per plant, HI: Harvest index

**Table 2. Analysis of variance (ANOVA) of ten agronomical traits in 465 genotypes of Pakistan spring wheat.**

	Df	DTH	DTM	PH	PL	SL	T/P	G/S	TGW	Y/P	HI
<b>2015-16 Block</b>	7	47.19	17.46	240.9	344.50	19.09	113.01	156.6	57.32	71.63	2990.70
<b>Genotype</b>	476	22.93	6.25	98.2	37.90	2.33	22.82	60.8	42.22	21.34	277.20
<b>Check</b>	11	232.15	9.5	421.8	409.30	9.45	50.09	617.1	268.96	121.57	2866.10
<b>Genotype*Check</b>	465	17.98	6.1	90.5	29.10	2.15	22.17	47.6	36.85	18.97	215.00
<b>Residuals</b>	77	0.27	0.85	2.2	2.70	0.15	0.79	0.9	0.71	0.36	0.02
<b>2016-17 Block</b>	7	58.17	23.22	458.7	168.61	39.45	198.90	770.2	157.20	149.75	2988.80
<b>Genotype</b>	476	18.45	4.69	148.9	44.85	5.28	23.60	55.6	51.10	51.39	277.10
<b>Check</b>	11	171.17	8.31	678.3	274.54	55.07	127.80	301.6	367.20	69.03	2866.10
<b>Genotype*Check</b>	465	14.83	4.61	112.7	44.14	4.10	16.40	49.8	43.60	50.97	215.80
<b>Residuals</b>	77	0.05	0.03	1.2	0.80	0.01	0.40	1.1	0.06	0.20	0.02

\*Significance at  $P < 0.001$

**Table 3. Proximity matrix of various agronomical traits of 465 wheat genotypes of Pakistani spring wheat for two consecutive years.**

	DTM	PH	PL	T/P	G/S	SL	TGW	Y/P	HI
<b>DTH</b>	0.511**	-0.009	-0.012	-0.026	0.197**	0.037	-0.274**	-0.189**	-0.047
	0.341**	0.051	-0.047	0.087	0.077	-0.030	-0.202**	-0.093*	0.057
<b>DTM</b>	1.000	0.141**	0.010	0.101*	0.149**	0.115*	-0.135**	0.118*	-0.014
		0.059	0.002	0.030	-0.006	-0.060	0.020	0.072	-0.002
<b>PH</b>		1.000	0.444**	0.296**	0.141**	0.329**	0.024	0.297**	-0.120*
			0.393**	0.161**	-0.021	-0.112*	0.217**	0.199**	-0.005
<b>PL</b>			1.000	0.168**	0.005	0.207**	-0.036	0.046	-0.076
				-0.036	-0.188**	-0.163**	0.138**	0.161**	0.019
<b>T/P</b>				1.000	0.027	0.046	0.091	0.320**	-0.063
					0.171**	0.036	0.051	0.021	0.100*
<b>G/S</b>					1.000	0.252**	-0.092	0.051	-0.018
						0.517**	-0.234**	0.061	0.096*
<b>SL</b>						1.000	0.044	0.148**	-0.138**
							0.071	-0.071	-0.030
<b>TGW</b>							1.000	0.295**	0.320**
								0.340**	-0.030
<b>Y/P</b>								1.000	0.394**
									0.393**
<b>HI</b>									1.000

\*\* Significant at 0.01; \* Significant at 0.05. Top value of each box from the year 2015-16 and bottom value from the year 2016-17.

yield for the first year. Likewise, the genotype L184 had the maximum 1000 grain weight (66.9g) among all 465 genotypes. Variance revealing the deviation of traits from their normal trend, such as plant height, harvest index, grain per spike and 1000 grain weight showed the variation as 92%, 80%, 48% and 34% respectively.

The range of data for agronomical traits for the year 2016-17 was: days to heading (81 to 105), days to maturity (127 to 148), plant height (49 to 134), peduncle length (9 to 49), tillers per plant (6 to 32), grain per spike (43 to 92), spike length (6 to 20), 1000 grain weight (24.7 to 63.5), yield per plant (4.7 to 46.8) and harvest index (6.7 to 80). The genotype L423 had the maximum 1000 grain weight; while, L290 showed maximum yield per plant for the second year. Analysis of variance (ANOVA) of ten morphological traits revealed significant response of all genotypes for both years (Table 2).

**Analysis of correlation coefficient:** Correlation among ten agronomical traits was studied by Pearson correlation coefficient for pairwise alignment. Proximity/ correlation coefficient matrix of ten agronomic traits for two consecutive years is presented in Table 3. Plant height was positively correlated with all the other traits and negatively with the harvest index. Plant height was highly correlated with peduncle length (0.44) followed by spike length (0.329) and yield per plant (0.297). Minimum positive correlation was found between peduncle length and grain per spike (0.005). Yield per plant had a positive correlation with harvest index (0.394), tillers per plant (0.32), plant height (0.297), 1000 grain weight (0.295), grain per spike (0.061) and spike length

(0.148) but a negative correlation with days to heading (-0.274).

A positive correlation (0.34) between days to heading and days to maturity, plant height and peduncle length (0.39), grain per spike and spike length (0.517), yield per plant and 1000 grain weight (0.34) and yield per plant and harvest index (0.393) was observed for the year 2016-17 (Table 3). Grain per spike had positively correlated with spike length for the year 2016-17 followed by tillers per plant (0.171) and harvest index (0.096). Plant yield had a positive correlation with harvest index (0.393) same as for the year 2015-16 followed by 1000 grain weight (0.340), plant height (0.199) and peduncle length (0.161). Minimum correlation was observed between peduncle length and days to maturity (0.002). A strong negative correlation was observed between days to heading and 1000-grain weight for both consecutive years (Table 3).

#### **Principal Component Analysis (PCA) of Wheat Agronomical Traits Estimation of Cumulative Variability Based on Eigenvalue:**

In this study, the two year's data was divided into ten principal components (PCs) or factors (Fs) based on eigenvalue and variability. First three PCs contribute more in variability having Eigenvalue of >1 represented in bold (Table 4) for both years 2015-16 and 2016-17. Eigenvalue one was used as the cutoff value for selecting PCs for further analysis. These three factors contribute a total of 54% variability for the year 2015-16 and 49% variability for the year 2016-17. The first two factors F1 (20.8%) and F2 (18.9%) contribute higher variability for the year 2015-16 indicating their importance in constructing the biplot. In the

**Table 4. Percentage variability contribution, eigenvalue, variability and cumulative variability by agronomical traits of 465 Pakistani spring wheat genotypes.**

	Year 2015-16				Year 2016-17			
	F1	F2	F3	F1+F2	F1	F2	F3	F1+F2
DTH	3.466	21.265	17.393	24.731	2.363	4.006	41.691	6.369
DTM	10.036	6.486	24.064	16.522	0.083	3.177	35.272	3.260
PH	27.635	2.846	3.688	30.481	10.167	15.704	0.007	25.871
PL	13.792	0.815	12.348	14.607	18.109	0.904	0.181	19.013
T/P	11.802	2.499	0.375	14.301	0.460	14.377	0.686	14.837
G/S	7.548	1.988	4.692	9.536	18.270	20.264	3.991	38.534
SL	16.337	0.000	1.105	16.337	11.405	14.649	12.906	26.054
TGW	1.417	23.580	2.508	24.997	20.251	0.102	4.230	20.353
Y/P	6.684	25.526	9.025	32.210	17.524	12.395	1.032	29.919
HI	1.282	14.996	24.803	16.278	1.370	14.422	0.004	15.792
<b>Eigenvalue</b>	2.082	1.892	1.415		1.946	1.574	1.393	
<b>Variability %</b>	20.820	18.923	14.154		19.463	15.739	13.931	
<b>Cumulative %</b>	20.820	39.742	53.897	39.742	19.463	35.202	49.134	35.202

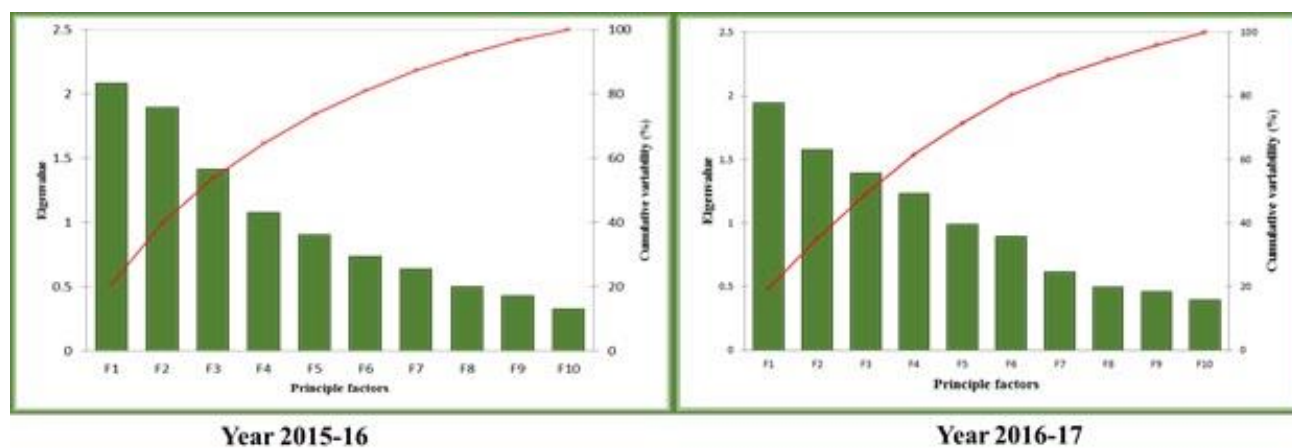
F: Principal factors, DTH: Days to heading, DTM: Days to maturity, PH: Plant height, PL: Peduncle length, T/P: Tillers per plant, G/S: Grain per spike, SL: Spike length, TGW: 1000 grain weight, Y/P: Yield per plant, HI: Harvest index.

year 2016-17 factor F1 contributed higher variability 19.4% followed by F2 (15.7%) and F3 (13.9%). The first two factors F1 and F2 for the year 2016-17 were used for biplot generation and further analysis.

Plant height (27%), spike length (16%) and peduncle length (13%) contributed majorly in the variability of principal factor F1 for the year 2015-16 (Table 4). However, minimum variability (1%) was contributed by 1000 grain weight and harvest index; whereas, for F2 the highest variability was contributed by yield per plant, 1000 grain weight and days to heading with the value 25%, 23% and 21% respectively. Maximum contributing variables for F1 of the year 2016-17 were 1000 grain weight (20%), grain per spike (18%), peduncle length (18%) and yield per plant (17%). Days to heading had the minimum contribution in total variability (Table 4). Grain per spike, plant height, spike length, harvest

index and tillers per plant contributed considerably in the total variability of F2 with the value of 20%, 15.7%, 14.6%, 14.4% and 14.3% respectively for the year 2016-17. Observed 1000-grain weight and peduncle length had the minimum contribution in total variability with the values 0.9% and 0.1%.

Scree plot based on eigenvalue and cumulative variability revealed the ten principal factors and their contributions (Fig. 1). The first three principal factors (F1, F2, F3) touching the curve line had the highest contribution in distributing the genotypes based on agronomic traits. The remaining six factors with eigenvalue <1 had a small contribution in variability that accounts for 35% of cumulative variability for the year 2015-16 and 38% cumulative variability for the year 2016-17 not worthwhile for further analysis.



**Figure 1. Scree plots indicating ten principal factors based on eigenvalue and cumulative variability of agronomical traits.**

**Variability Analysis Based on Principal Factors, Variability and Correlation:** Total variability depicted by the first two principal factors F1 (20.8%) and F2 (18.9%) for the year 2015-16 (Table 4) was used to construct the biplot based on the correlation of variable/ traits with principal factors. Variable were levied as vectors whose length revealing the combined variability in both principal factors and its effect on yield (Yan and Tinker, 2005). Positive and negative factor loading explains the trends of correlation among the variables and factors. Biplot was divided into four groups based on factor loading values. The vector that were close to the origin including tillers per plant, peduncle length, grain per spike and spike length had less variability as compared to yield per

plant, plant height, 1000 grain weight and days to heading for the year 2015-16.

For the year 2016-17, the factors F1 and F2 with variability as 19.4% and 15.7% respectively (Table 4) were used to construct the variable biplot. Vectors of plant height, yield per plant, grain per spike and spike length had more contribution in variability based on their length and combined variability. Days to maturity, days to heading, tillers per plant, and harvest index had less combined variability for both principal factors. The variability and correlation of traits depicted through principal component analysis can be exploited for further selection of genotypes.

**Biplot Analysis of Variables and Genotypes:** Principal factors (F1 and F2) scattered the 465 wheat genotypes in two-

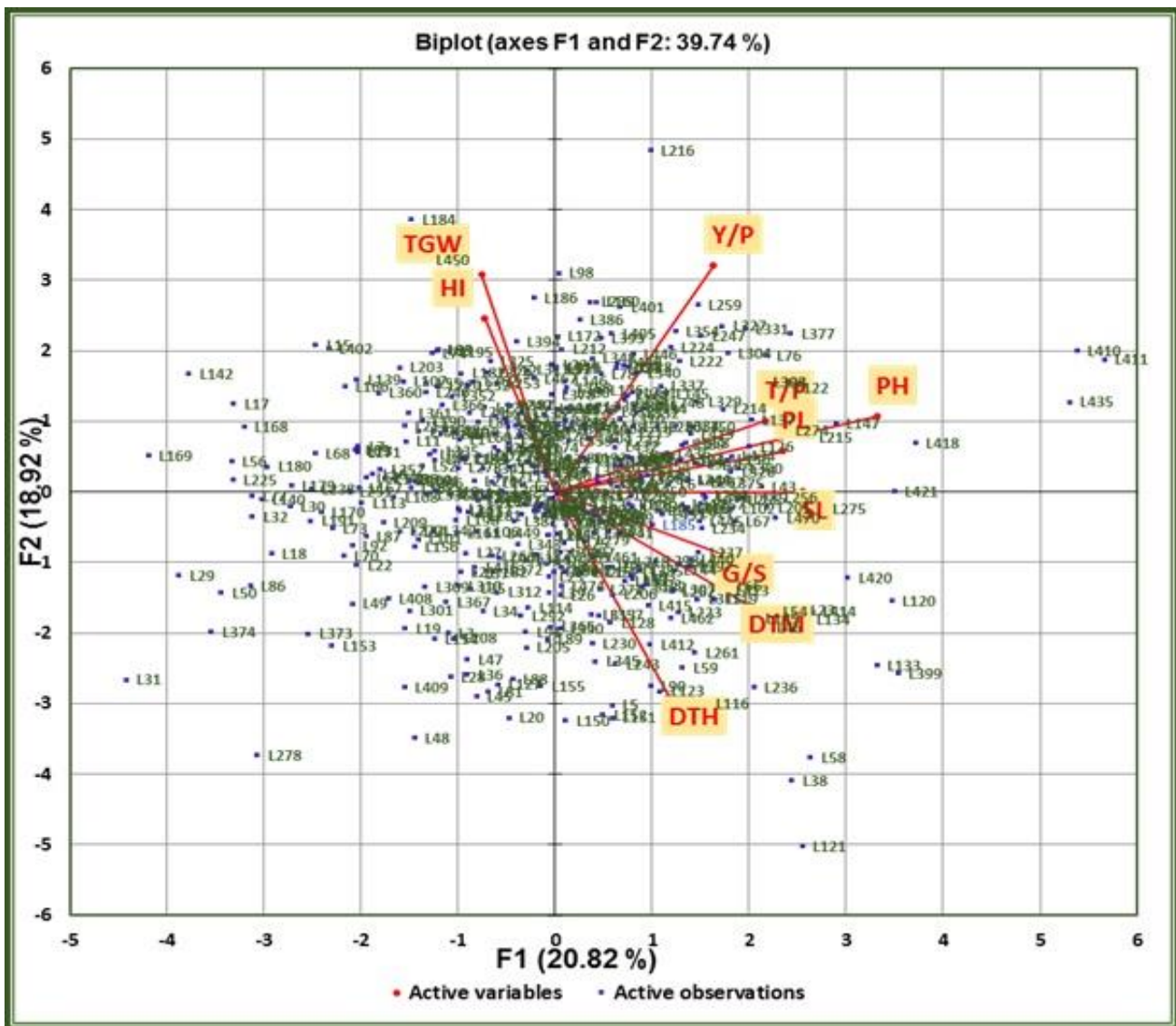


Figure 2. Biplot representing the correlation of ten agronomical traits with 465 wheat genotypes for the year 2015-16.

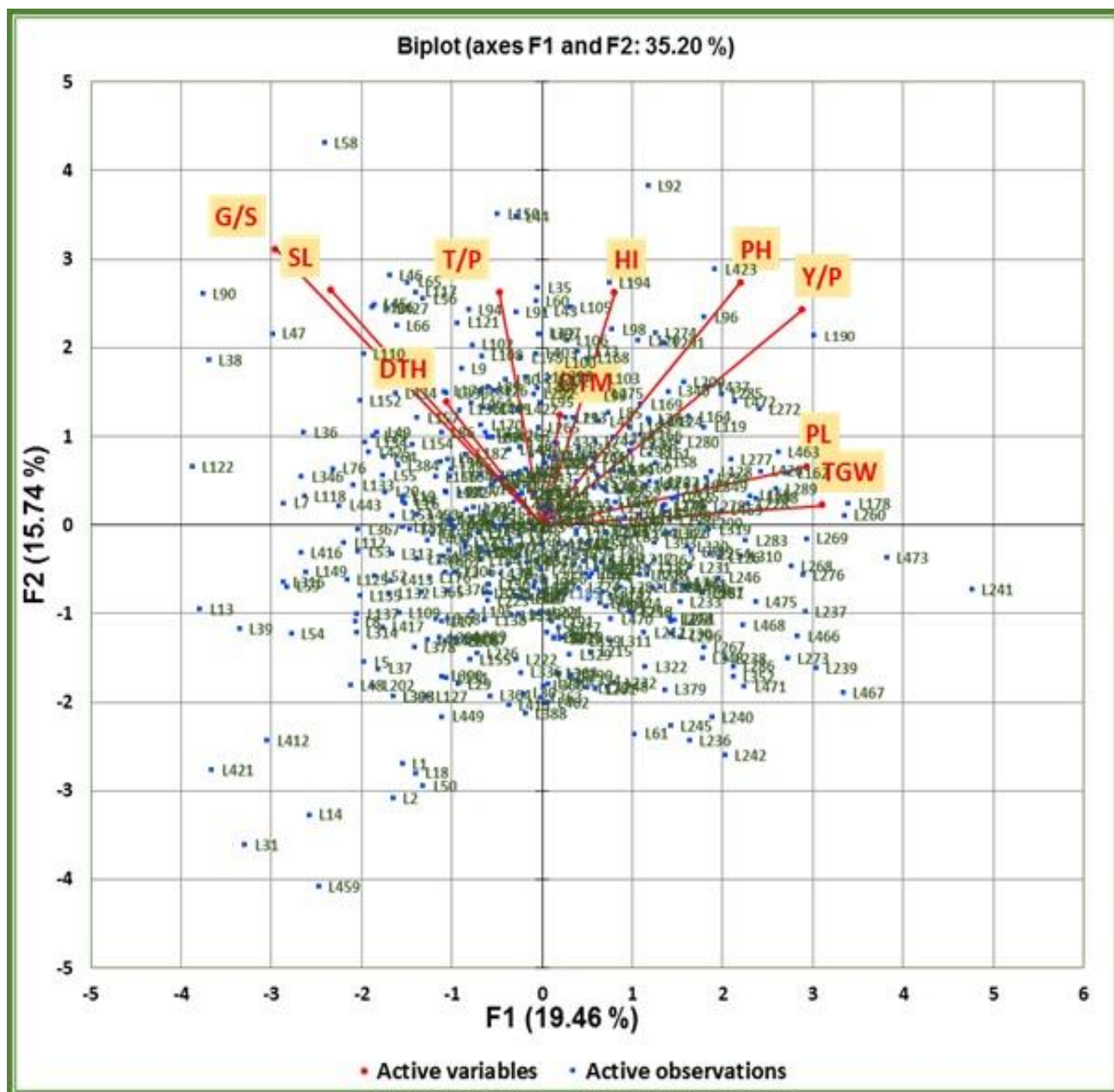


Figure 3. Biplot representing the correlation of ten agronomical traits with 465 wheat genotypes for the year 2016-17.

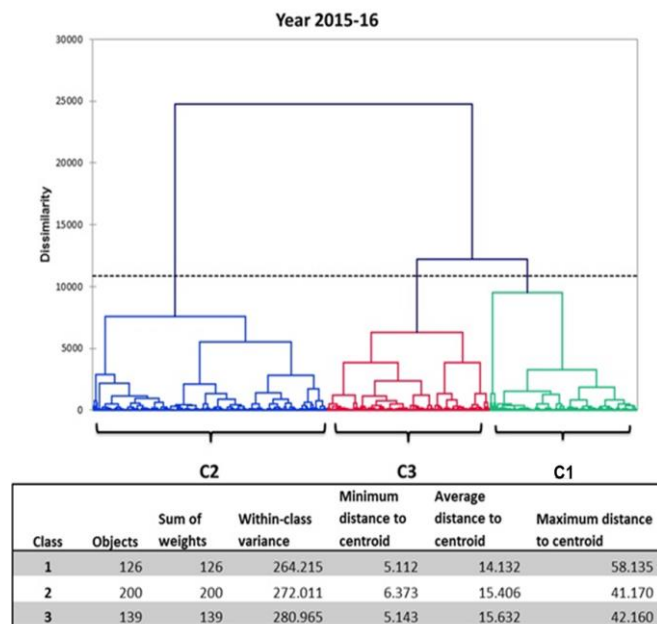
dimensional scale for graphical representation of diversity among the genotypes. Biplot depicts the association of genotypes with each other as well as with the vectors of traits. Biplot graph for the year 2015-16 and 2016-17 scattered the genotypes in four different groups based on x-y plane using the variability of the first two principal factors. The genotypes that were in the first group (positive axis) performed well in that year and are more likely linked with plant height, yield per plant and peduncle length for both year. The genotypes present away from origin and towards negative axis were

negatively linked with that traits. In the biplot for the year 2015-16, 123 genotypes out of the total 465 were present in group 1, 114 in group 2, 121 in group 3 and 107 in group 4 (Fig. 2) likewise for year 2016-17 total 111 genotypes were present in group 1, 118 in group 2, 116 in group 3 and 120 in group 4 (Fig. 3). In group 1 of both years (2015-16 and 2016-17), 32 wheat genotypes were common including L43, L85, L98, L105, L124, L146, L160, L173, L194, L200, L224, L258, L259, L274, L280, L305, L331, L332, L337, L339, L340, L354, L375, L382, L405, L410, L411, L418, L423,

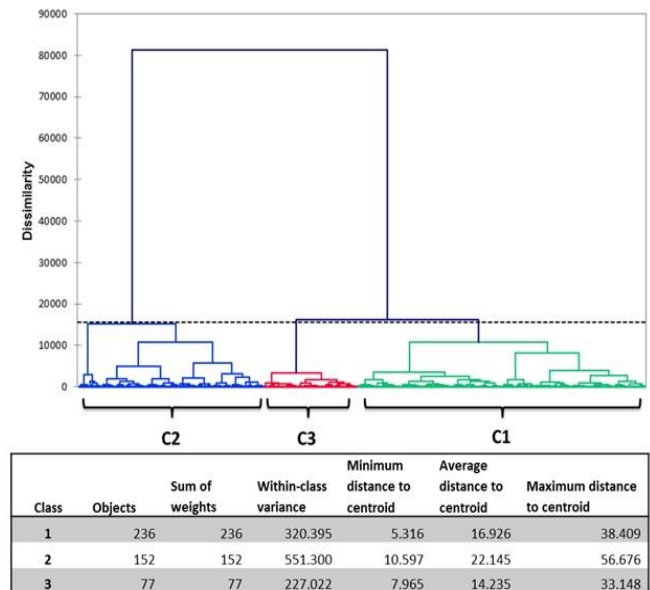


L333, L435 and L452. A total of 18 genotypes were present in group 2 for both years. Seventeen genotypes in group 3 performed consistently in both years while 32 genotypes in group 4 were similar for both years (2015-16 and 2016-17).

**Morphological Clustering of Wheat Genotypes:** Morphological clustering grouped the genotypes based on Euclidean distance by Ward's method. All 465 genotypes were divided into three clusters (C1, C2 and C3) for the year 2015-16 where 126 genotypes clustered in C1, C2 grouped 200 genotypes and C3 grouped 139 genotypes (Fig. 4). For the year 2016-17 all genotypes were again divided into three main clusters where C1 grouped 236 genotypes, C2 grouped 152 genotypes and C3 grouped 77 genotypes (Fig. 5).



**Figure 4. Cluster analysis of 465 Pakistani wheat genotypes for year 2015-16.**



**Figure 5. Cluster analysis of 465 Pakistani wheat genotypes for the year 2016-17.**

## DISCUSSION

Present study was carried out to genetically characterize the Pakistani spring wheat genotypes using multivariate analysis. Mean value of morphological traits indicated wide variation exhibited by genotypes for days to heading, days to maturity, plant height, peduncle length, tillers per plant, grains per spike, 1000 grain weight, yield per plant and harvest index. In recent times, descriptive statistics are becoming a norm for the characterization of morphological traits in wheat (Yaqoob, 2016; Shahid *et al.*, 2017; Iqbal *et al.*, 2017; Din *et al.*, 2018; Pooja *et al.*, 2018; Gulnaz *et al.*, 2019).

Previous studies are in line with our findings that yield per plant highly correlates with plant height, spikes per plant and 1000 grain weight (Dogan, 2009; Sajjad *et al.*, 2012). In our study, yield per plant had a positive correlation with tillers per plant, 1000 grain weight, spike length and plant height. Yield per plant positively correlates with 100-grain weight and plant height are in line with the findings of (Ajmal *et al.*, 2013). The positive correlation among grain per spike and spike length also exhibited in our study as in lined with Shah *et al.*, 2007; Khodadadi *et al.*, 2011; Ajmal *et al.*, 2013). The negative correlation among 1000-grain weight and grain per spikes was also evinced traditionally in bread wheat (Wu *et al.*, 2012; Gulnaz *et al.*, 2019).

Principal component analysis (PCA) precisely indicates the differences in wheat genotypes (Bhanupriya *et al.*, 2014; Mecha *et al.*, 2017). PCA is widely used for the evaluation of genotypes based on morphological traits and for their

grouping (Maqbool *et al.*, 2010; Sajjad *et al.*, 2011; Janmohammadi *et al.*, 2014). Eigenvalue helps in the selection of factors that have the highest contribution in variation. In the present study, first four factors (F1, F2, F3 and F4) had eigenvalue of >1 out of which the first two factors retain the highest information. F1 accounts 2.080 information about original variables while F2 retain 1.892. First principal factor explained the maximum variability than succeeding factors (Ajmal *et al.*, 2013). Plant height was the major contributing vector in F1 and accounted for diversity while in F2 yield per plant was the major contributing vector. Based on PCA, genotypes with the highest score and desirable characters can be selected for further breeding programs. Cluster analysis based on dissimilarity grouped the genotypes in three clusters for the two consecutive years 2015-16 and 2016-17. Pakistani genotypes exhibit great diversity that can be exploited in breeding programs to develop new enhanced varieties to combat with food challenges in the future.

**Conclusion:** On the basis of present findings, it can be concluded that considerable variation existed among the genotypes which can further be exploited in breeding programs aimed at improving wheat production in Pakistan.

**Acknowledgement:** We thank Wheat Research Institute, Ayub Agricultural Research Institute (AARI), Faisalabad for providing seeds for spring wheat genotypes used in this study. This work was supported by the Higher Education Commission (HEC), Government of Pakistan.

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[Received 17 Apr 2019; Accepted 10 Aug 2020; Published (online) 1 Sept 2020]