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Exploitation of Germplasm for Grain Yield Improvement in Spring Wheat (*Triticum aestivum*)

M. SAJJAD[1]†, S.H. KHAN† AND ABDUS SALAM KHAN†
Western Regional Research Center, U. S. Department of Agriculture, 800 Buchanan Street, Albany, CA 94710
†*Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan*
[1]Corresponding author's e-mail: Muhammad.sajjad@ars.usda.gov; msajjadpbg@gmail.com

ABSTARCT

Diverse genetic resources are necessary for adequate food production in changing environments. The present study was designed for two consecutive years to find the diversity pattern among 500 genotypes and select diverse parents for future breeding programs. Twelve quantitative traits were phenotyped. The number of kernels per plant, numbers of spike per plant, spike dry weight, and spikelets per spike contributed towards significant prinicipal components (PCs) and were highly related with grain yield. The projection of genotypes on PC1 and PC2 showed population structure for both years. First year, the best diverse parents were QAFZAH-21) vs GOSHAWK'S'; HUBARA-3 vs BOLSENA'S'; OASIS AGA/3*YR, IZAZ-1, and ABADGAR-93 vs WEBELLI/KAMBI, KAMBARA-1, CROW'S'/BOW#1 and QAFZAH-18. For the second year the diverse parents were BLS/KLT'S' vs. KVZ/3/TOB/CFTN//BB/4/BLO'S'/5/VEE#5/6/BOW'S'/3/YDING'S'//BB/CHA, BOLSENA'S' vs. HUBARA-3, and (PARULA) vs. 307 (BUC'S'/BJY'S'/3CNDR'S'/ANA//CNDR'S'/MUS'S'). The results from this study are very useful for planning future wheat breeding programs especially in Pakistan.

Key Words: Germplasm; Grain yield; PCA; *Triticum aestivum* L

INTRODUCTION

The germplasm is often exploited to develop improved crop varieties for changing needs and environments. The variability for economic traits in the working germplasm is very important for rewarding utilization following recombination breeding and selection. The selection of genetically diverse parents for recombination breeding in a self pollinated species such as wheat to produce transgressive segregants has been repeatedly emphasized (Jatasara & Paroda, 1983; Franco *et al*., 2001; Kumar *et al.*, 2009). Assessment of the genetic diversity can also be invaluable for analysis of genetic variability in cultivars (Smith, 1984; Cox *et al.*, 1986) and introgression of desirable traits from diverse germplasm into the available genetic base (Thompson *et al.*, 1998).

The estimation of genetic diversity can be used on pedigree records (Cox *et al.*, 1986), morphological traits or molecular markers (Maric *et al.*, 1998). Genetic diversity based on morphological traits has been extensively estimated in different crops including wheat (Maric *et al.*, 2004; Masood *et al.*, 2005; Kumar *et al.*, 2009; Sajjad & Khan, 2009; Maqbool *et al.*, 2010) and used to plan successful breeding programs.

For large number of germplasm accessions, multivariate analytical techniques are commonly used in analysis of genetic diversity irrespective of the data set (biochemical, molecular markers or morphological data). Among these algorithms, cluster analysis, multidimensional scaling (MDS), principal component analysis (PCA) and principal coordinate analysis (PCoA) are, at present, commonly used (Melchinger, 1993; Thompson *et al.*, 1998; Brown-Guedira *et al.*, 2000).

Plant breeders are employing PCA and PCoA as a "pattern finding method" to complement cluster analysis (Kantety *et al.*, 1995; Schut *et al.*, 1997; Barrett & Kidwall, 1998; Thompson *et al.*, 1998). For nonhierarchical and reticular patterns of diversity, the hierarchical algorithms are somewhat limited in their usefulness to estimate the pattern of genetic diversity (Lessa, 1990). In such a case, ordination technique such as PCA, PCoA and MDS are more useful (Rendine *et al.*, 1986; Derish & Sokal, 1988). Cluster analysis is rather useful than PCA and PCoA when variable are nonlinearly related (Wartenberg *et al.*, 1987). Linkage disequilibrium and frequency data particularly when heterogeneous lead to unstable and unreliable patterns of genetic diversity (Lessa, 1990).

Genetic diversity assessment based on morphological data needs high precision of field experiment through design and analysis. Alpha lattice design is better than RCBD to provide smaller standard error of difference, coefficient of variation and error mean squares when the number of entries in the experiment is large (Wu & Dutilleul, 1999; Campbell & Bauer, 2007; Masood *et al.*, 2008).

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The present study was carried out, considering the importance of genetic diversity for wheat crop improvement and keeping in view the merits and demerits of different statistical tools and field designs being used for the assessment of morphological data based genetic diversity. In this study, a set of 500 cultivars and breeding lines of spring bread wheat was evaluated; (i) to determine the magnitude of variability in the germplasm for twelve quantitative traits; (ii) to determine the grouping patterns of genotypes; (iii) To identify genetically diverse and agronomically promising genotypes for their exploitation in breeding programs to improve grain yield potential of wheat.

MATERIALS AND METHODS

A panel of 500 landraces, cultivars and breeding lines of bread wheat (*Triticum aestivum* L.) was provided by Cereal Research Institute, Ayub Agriculture Research Institute Faisalabad, Pakistan. The germplasm was studied at the department of Plant Breeding and Genetics, University of Agriculture, Faisalabad (LATITUDE = 31 ^{O}- 26' N, LONGITUDE = 73 ^{O} - 06' E, ALTITUDE = 184.4 m) during the years 2008-2009 and 2009-2010. The first growth season was rainy in the years 2008-2009- receiving 83.4 mm of rain and the second crop season was dry in the years 2009-2010 receiving only 22.8 mm rain. (http://www.uaf.edu.pk/faculties/agri/depts/crop_physiology/agri_met_cell/met_bulletin.html). The experiment was laid out according to alpha lattice design with two replications, 500 entries and 25 blocks consisting of twenty entries in each block. The length of each plot consisting of one entry was 5 m. Plant to plant and plot to plot distance was 12 cm and 30 cm, respectively. The randomization of 500 genotypes was done with Crop Stat v7.2 software. Recommended package of agronomic practices was followed to raise the crop. For data recording 10 randomly selected plants from each plot were tagged. Twelve quantitative traits- grain yield (GY), kernels per plant (K/P), 1000-grain weight/kernel size (KS), number of spikes per plant (S/P), number of fertile spikelets per spike (NSpt/S), maximum fertile floret per spikelet (MFFl/Spt), spike dry weight (SDW), plant height (PH), spike length (SL), awn length (AL), spike density (SD) and chlorophyll contents (CC) were measured on each selected plant..

The data for all these attributes were subjected to analysis of variance following Steel *et al.* (1997) and Principal Component Analysis (Ogunbayo *et al.*, 2005) to determine the contribution of yield and yield traits to diversity and selection of diverse genotypes. The correlation matrix was used for principal component analysis. The eigen value significance criterion, as established by Kaiser (1960), was used to select statistically significant principal components (PCs).

RESULTS

The analysis of variance (ANOVA) revealed that the genotypes included in the study had significant variation for most of the traits. For the year 2008-2009, the spike density and chlorophyll contents had no workable diversity in the germplasm (Table I). The chlorophyll content was the only trait that showed no significant variation among the genotypes for the year 2009-2010 (Table II). The spike density was significant for the year 2009-2010 (Table III). The means of all traits were decreased from the year 2008-2009 to the year 2009-2010.

The extent of range for grain yield was 10.5-71.0 g/plant during 2008-2009 and 10.3-66.8 g/plant during the year 2009-2010 (Table III). The number of kernels per plant ranged from 253 to 1812 for the year 2008-2009 and 246 to 1764 for the year 2009-2010. The minimum and maximum kernel size for the year 2008-2009 was 25.6 and 68.6 g/1000 grains, respectively. The range of kernel size for the year 2009-2010 was 24.7-66.4 g/1000 grains (Table III). The range of number of spikes per plant for the years 2008-2009 and 2009-2010 was 6.3-55.2 and 6.1-53.7, respectively. The range of the number of spikelets per spike, maximum fertile florets per spikelet, spike dry weight, plant height, spike length, awn length, spike density and chlorophyll content was 16.7-27.2, 3-508, 2.3-5.9 g, 62.1-129.4 cm, 8.1-16.8 cm, 0.0-13.6 cm, 1.5-2.2 spt/cm and 36.9-65.8, respectively for the year 2008-2009 (Table III). The range of the number of spikelets per spike, maximum fertile florets per spikelet, spike dry weight, plant height, spike length, awn length, spike density and chlorophyll content was 16.2-26.5, 2.9-5.6, 1.8-5.6 g, 60.9-126.8 cm, 7.8-16.0 cm, 0.0-13.2 cm, 1.4-4.7 spt/cm, and 35.5-63.2, respectively for the year 2009-2010 (Table III).

Out of 12 principal components (PCs), the first four exhibited eigenvalue (also called latent root) greater than one (significant) for the years 2008-2009 and 2009-2010 (Fig. 1 & 2). The rest eight PCs explained trivial (non-significant) amount of variation, and were not worth interpreting. For the years 2008-2009 and 2009-2010 the first four PCs showed 62.03% and 64.47% variation, respectively, in the germplasm (Fig. 1 & 2). The first PC accounted 27.01% of the variance, second 14.66%, third 11.77% and fourth 8.59% in the year 2008-2009 (Fig. 1). The first PC showed 27.76% of the total variance, second 16.28%, third 11.86% and fourth 8.57% in the year 2009-2010 (Fig. 2). The criterion developed by Johnson and Wichern (1988) was used to determine the importance of a trait coefficient for each significant principal component. The first PC was highly related to grain yield, number of kernels per plant, spike dry weight and spike length for both years (Table IV & V). This implies that PC1 is a weighted average of these four traits. The traits of significant importance in PC2 were the number of spikes per plant, 1000-grain weight and number of kernels per plant for the year 2008-2009 (Table IV). The important traits in PC2 for the year 2009-2010, were the number of spikes per plant, 1000-grain weight, number of kernels per plant and spike density (Table V). The third PC was related to the number of spikelets per spike and PC4 to 1000-grain weight and

Table I: Mean squares of the 12 trairs of bread wheat (*Triticum aestivum* L.) for the year 2008-2009

SoV	DF	GY	K/P	KS	S/P	NSpt/S	MFFl/Spt	SDW	PH	SL	AL	SD	CC
Reps	1	5	46743*	509**	112.1**	11.5*	0.02	5.73**	248**	9.3**	6.77**	0.015	92
Blocks	24	17	22808*	12	5.9	1.2*	0.16	0.48**	1043	1.4**	0.74	0.054**	194
Genotypes	499	183**	97553**	69**	19.4**	5.5**	0.27**	0.69**	62909**	3.0**	2.30**	0.032	185
Error	475	19	18662	8	12.0	0.9	0.11	0.21**	24688	0.5**	0.64	0.033	182
Total	999												

Table II: Mean squares of the 12 trairs of bread wheat (*Triticum aestivum* L.) for the year 2009-2010

SoV	DF	GY	K/P	KS	S/P	NSpt/S	MFFl/Spt	SDW	PH	SL	AL	SD	CC
Reps	1	143**	30749	572**	63.4**	8.2**	0.05	4.79**	459**	2.4**	4.58**	0.094	29
Blocks	24	25	21645	11	5.7	1.1	0.14	0.44**	41	1.3**	0.70	0.070**	179
Genotypes	499	165**	92486**	64**	18.5**	5.2**	0.24**	0.66**	120**	2.8**	2.17**	0.096**	171
Error	475	24	17691	8	11.4	0.9	0.10	0.21	50	0.5	0.61	0.041	168
Total	999												

Table III: Mean, minimum and maximum values of the quantitative traits for the years 2008-2009 and2009-2010

Variables		GY	K/P	KS	S/P	NSpt/S	MFFl/Spt	SDW	PH	SL	AL	SD	CC
2008-2009	Means	37.8±0.33	889.8±7.9	42.5±0.20	13.2±0.13	22.8±0.05	4.4±0.01	3.9±0.02	97.2±0.3	12.9±0.04	7.4±0.04	1.8±0.005	48.8±0.42
	Min.	10.5	253	25.6	6.3	16.7	3.0	2.3	62.1	8.1	0.0	1.5	36.9
	Max.	71.0	1812	68.6	55.2	27.2	5.8	5.9	129.4	16.8	13.6	2.2	65.5
	SD	10.33	248.7	6.27	4.04	1.8	0.44	0.69	9.59	1.36	1.24	0.18	13.6
2009-2010	Mean	35.3±0.31	866.4±7.66	41.14±0.19	12.9±0.12	22.3±0.06	4.2±0.01	3.8±0.02	95.3±0.30	12.3±0.04	7.2±0.04	1.18±0.008	47.0±0.41
	Min.	10.3	246	24.7	6.1	16.2	2.9	1.8	60.9	7.8	0.0	1.4	35.5
	Max.	66.8	1764	66.4	53.7	26.5	5.6	5.6	126.8	16.0	13.2	4.7	63.2
	SD	10.00	242.13	6.08	3.94	1.76	0.42	0.68	9.38	1.30	1.20	0.26	13.04

maximum fertile florets per spikelet for the year 2008-2009 (Table IV). The significant trait in PC3 was number of spikelets per spike and significant traits in PC4 were 1000-grain weight and spike length (Table V). The projection of traits on PC1 and PC2 revealed that the number of kernels per plant, number of spike per plant and number of spikelets per spike are positively related to grain yield for both years (Fig. 3 & 4). For both years spike density was opposite to grain yield and other yield contribution trait on PC1, therefore, it had negative correlation with all other traits (Fig. 3 & 4). Since the chlorophyll content had non significant variation among genotypes (Table I & II), therefore, was not projected significantly on first two PCs by principal component analysis. The projection pattern of the traits on first two PCs for both years depicted that key yield contributing traits were the number of kernels per plant, spike dry weight, and spike length (Fig. 3 & 4).

The projection of genotypes on first two PCs was useful to identify divers groups of parents for better transgressive segregation. The projection of genotypes on PC1 and PC2 showed population structure for both years (Fig. 5 & 6). For the year 2008-2009 the following hetrotic groups were identified. The genotypes 192, 363 392, 414 and 488 (KAMBARA-1, KAUZ//TFAU/VEE#5, KVZ/3/TOB/CFTN//BB/4/BLO'S'/5/VEE#5/6/BOW'S'/3/YDING'S'//BB/CHA, V-97100 & JAUHAR-78) were opposite to 174, 297, 265 and 280 (CAR422/ANA//TRAP#1/3/KAUZ*2/TRAP//KAUZ, LOCAL WHITE (K)/4/V573.600/MRL'S'/3/BOW'S'//YR/TRF'S'/5/FURY/ANA PB 23744, CONDOR'S'/ANA75//CONDOR'S'/MUS'S'

Table IV: Principal components of twelve traits for the year 2008-2009

Variables	Eigen values			
	PC1	PC2	PC3	PC4
Grain yield	0.82	0.35	-0.22	0.16
Number of Kernels/plant	0.66	0.59	-0.10	-0.13
1000-grain weigh	0.32	-0.56	-0.22	0.51
Number of spikes/plant	0.39	0.62	-0.42	0.14
Number of spikelets/spike	0.42	0.24	0.69	-0.05
Maximum fertile florets/spikelet	0.48	-0.18	0.10	-0.50
Spike dry weight	0.77	-0.33	0.22	-0.06
Plant height	0.49	-0.17	0.23	0.36
Spike length	0.66	-0.35	0.09	-0.23
Awn length	0.40	-0.23	-0.16	0.30
Spike density	-0.23	0.42	0.54	0.40
Chlorophyl contents	0.03	-0.02	-0.47	-0.13

Table V: Principal components of twelve traits for the year 2009-2010

Variables	Eigen values			
	PC1	PC2	PC3	PC4
Grain yield	-0.82	-0.37	0.22	0.22
Number of Kernels/plant	-0.67	-0.66	0.13	-0.07
1000-grain weigh	-0.33	0.55	0.17	0.57
Number of spikes/plant	-0.40	-0.59	0.45	-0.05
Number of spikelets/spike	-0.37	-0.30	-0.70	-0.20
Maximum fertile florets/spikelet	-0.47	0.13	-0.19	0.09
Spike dry weight	-0.74	0.27	-0.31	0.16
Plant height	-0.48	0.13	-0.25	0.23
Spike length	-0.69	0.39	-0.08	-0.53
Awn length	-0.40	0.22	0.11	0.10
Spike density	0.40	-0.57	-0.46	0.47
Chlorophyl contents	-0.05	0.03	0.47	0.07

& ABADGAR-93) Fig. 5. The genotype 338 (QAFZAH-21) contrasted to 259(GOSHAWK'S'). The genotype 374 (HUBARA-3) had maximum diversity from 352 (BOLSENA'S'). The genotypes 69, 385 and 485 (OASIS AGA/3*YR, IZAZ-1 & ABADGAR-93) were opposite to 124, 192, 334 and 377 (WEBELLI/KAMBI, KAMBARA-1, CROW'S'/BOW#1 & QAFZAH-18) Fig. 5. For the year 2009-2010 the most diverse parents were 280 (BLS/KLT'S') vs. 392 (KVZ/3/TOB/CFTN//BB/4/BLO'S'/5/VEE#5/6/BOW'S'/3/ YDING'S'//BB/CHA), 352 (BOLSENA'S') vs. 374 (HUBARA-3), and 302 (PARULA) vs. 307 (BUC'S'/BJY'S'/3CNDR'S'/ANA//CNDR'S'/MUS'S') etc. (Fig. 6).

DISCUSSION

Since, the second year (2009-2010) received very low precipitation as compared to the first year (2008-2009), the difference of analysis of variance (ANOVA) between two years might be because of seosonal deifference. The decrease in yield and yield contributing traits from the year 2008-2009 to 2009-2010 was because of low precipitation during the crop season of 2009-2010. The range of each traits give an immediate extent of diversity. The range of grain yield per plant was higher for both years (10.5-71 & 10.3-66.8 g/plant). The range is higher than those of West Bengal germplasm (6.23-13.76 g/plant) as determined by Kotal *et al.* (2010). The extent of variation in 1000-grain weight (25.6-68.6 & 24.7-66.4 g) was higher than the findings of Joshi *et al.* (2004) in Nepalian germplasm (34.8-56.1 g), Kumar *et al.* (2009) in Indian germplasm (33.40-51.70 g), Kotal *et al.* (2010) in West Bengal germplasm (27.33-56.66 g), Maqbool *et al.* (2010) in Pakistani germpalsm (45.23-58.77 g).

The variability extent of the number of spikes per plant for 2008-2009 and 2009-2010 (6.3-55.2 & 6.1-53.7, respectively) was higher than found in Indian germplasm (Kumar *et al.*, 2009), CIMMYT and Pakistani germplasm (Gulnaz *et al.*, 2011), West Bengal germplasm (Kotal *et al.*, 2010). Similarly, the range of number of spikelets per spike, spike length, and plant height was higher than the range found in different sets of germplasm (Joshi *et al.*, 2004; Kumar *et al.*, 2009; Kotal *et al.*, 2010; Maqbool *et al.*, 2010). The range of plant height, number of spikes per palnt, spike length, and number of spikelets per spike in Chinese germplasm was 55-86 cm, 6-36, 6.5-15.5, 20-52, respectively (Liu *et al.*, 2007). The range of plant height was almost equel as found by Gulanaz *et al.* (2011) in CIMMYT and Pakistani germplasm (62–134 cm). The range of number of spikelets per spike, spike length, plant height and chlorophyl content was lower than the range found by Masood *et al.* (2005) in the set of 298 Baluchistani landraces. This comparison emphasizes the inclusion of landraces in breeding programs to widen the genetic diversity lost after green revolution (Cooper *et al.*, 1992).

Fig. 1: Scree plot between eigen values and number of principal components for the year 2008-2009

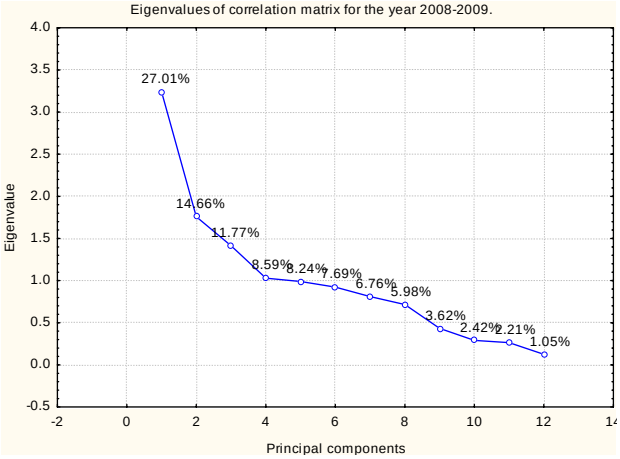


Fig. 2: Scree plot between eigen values and number of principal components for the year 2009-2010

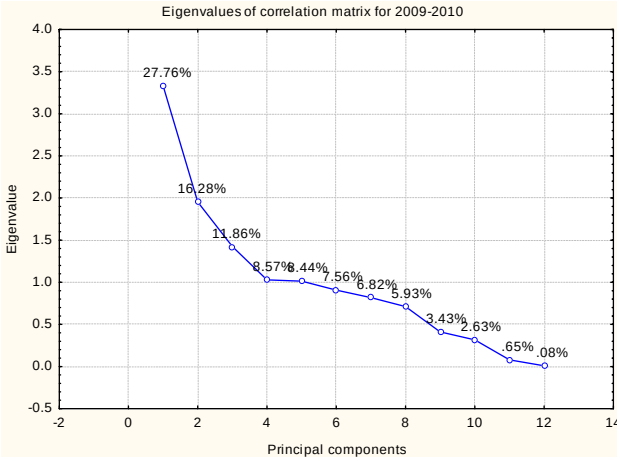


Fig. 3: Principal component biplot of yield trairs for the year 2008-2009

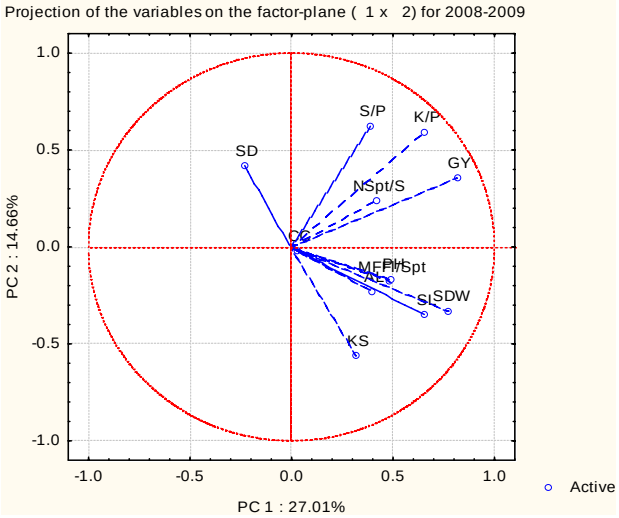


Fig. 4: Principal component biplot of yield trairs for the year 2009-2010

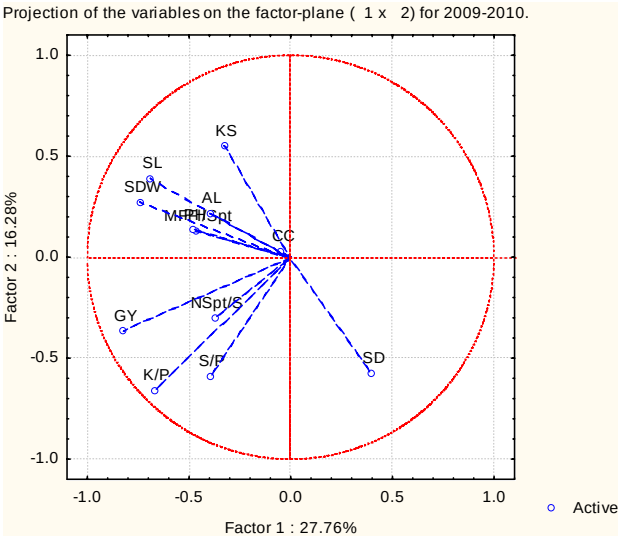
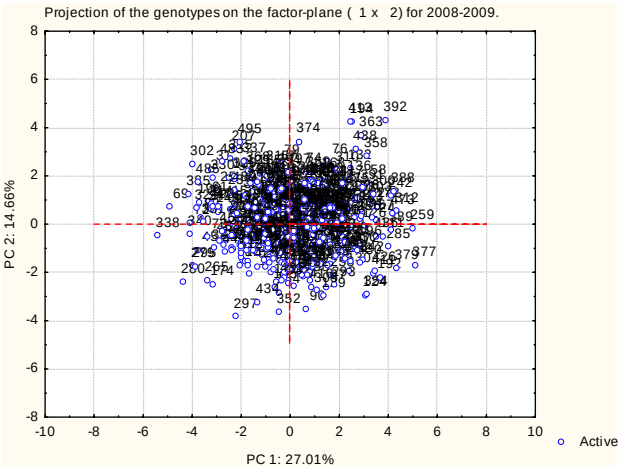


Fig. 5: Two dimensional ordinations of 500 wheat genotypes on PC1 and PC2 for the year 2008-2009

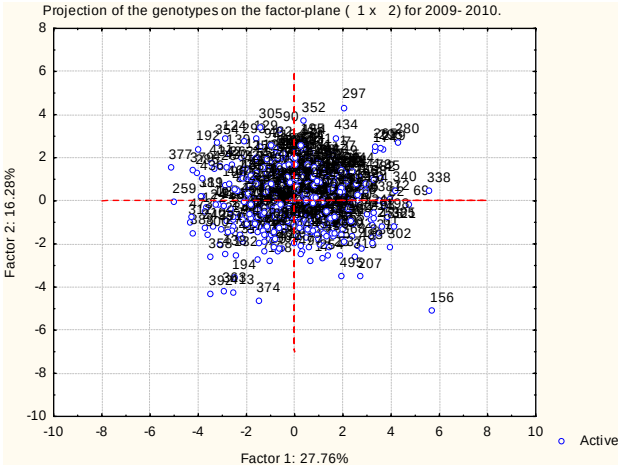


The maximum fertile florets per spikelet, spike dry weight, and awn length were not phenotyped before to assess genetic diversity. The spike dry weight appeared to be important trait with respect to diversity and yield contribution.

The spike dry weight, number of kernels per plant, numbers of spike per plant, and spikelets per spike contributed towards genetic divergence and were highly related with grain yield. Therefore, in line with the suggestions of Kumar *et al.* (2009), these traits could be used as criteria for single plant selection in the early segregation generations derived from the multiple crosses among the selected genotypes.

Despite the higher range of the studied traits in germplasm, the genotypes were structured (Fig. 5 & 6). The similar structured pattern was determined using principal component analysis in the set of hundred Pakistani wheat

Fig. 6: Two dimensional ordinations of 500 wheat genotypes on PC1 and PC2 for the year 2009-2010



varieties (Maqbool *et al.*, 2010). This structured pattern of the germplasm used in Pakistani wheat breeding programs reflects the fact that 77% of the spring bread wheat area in the developing world is sown with CIMMYT-relaesed wheats (Smale *et al.*, 2002). This population structure indicates the non-rondom crossings, and reccurrent use of limited number of parents. The inclusion of land races, noval genotypes to the germplasm and programs of rondom matings among multiple diverse parents can make the breeding material free of population structure. The hybridization between the divergent genotypes selected from this study will be carried out for developing promising line/varieties and create variability for future wheat breeding programs.

In conclusion, the number of kernels per plant, spike dry weight, numbers of spike per plant, and spikelets per spike were key traits for improving grain yield. The range of yield and yield traits was higher in the germplasm and could be expoited for transgressive segregation but the genotypes included in the study were structured.

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