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GENETIC DIVERSITY ASSESSMENT OF INDIGENOUS WHEAT GERMPLASM FROM DIFFERENT AREAS OF PAKISTAN USINGAGRO-MORPHOMETRIC TRAITS

KHATIBA BIBI[1], INAMULLAH[1], HABIB AHMAD[1], AYESHA BIBI[1], RABIA MASOOD[2],
HAKIM KHAN[1] AND AZIZ UD DIN[1]

[1]Department of Genetics, Hazara University, Dhodial Mansehra
[2] Department of Botany, Hazara University, DhodialMansehra
[*]Corresponding author's email: khatibanoor@gmail.com

Abstract

Genetic diversity was studied among 102 wheat genotypes using 19 agro-morphological characters. Multivariate analysis including cluster number and principal component analyses were carried out. Significant variation was found for all the characters. The largest variation was observed in awn length (44.10), number of tillers (38.57), biological yield (23.57), flag leaf area (20.19) and peduncle length (18.85). The 102 genotypes were grouped into five clusters based on hierarchical clustering method. PCI showed maximum genetic variability 18.15%, PCII contributed 13.33%, and PCIII accounted for 10.78% of total morphological diversity. Genotypes PS 2004, 011872 and 010742, have high thousand grain weight, highest spike length, high number of tillers per plant. PS 2004, 011872 and 010742 three elite genotypes identified on the basis of important agro- morphological traits for future breeding program under Mansehra conditions.

Key words: Genetic diversity, Morphometric traits, Principal component analysis

Introduction

Wheat (*Triticum aestivum* L.) is an important crop for the agriculture and economy of Pakistan.The development of high yielding genotypes with good quality could be achieved through genetic improvement (Knezevic *et al*., 2008; Sonmezoglu *et al*., 2012).The progress in crop breeding and improvement program could be achieved from the inclusion of diverse germplasm. (Mangova & Rachovska, 2004). Grain yield is a complex trait which is the result of yield components (number of plant per unit area, number of grain per spike, grain weight) as well as other yield related traits and agro ecological conditions (Drezner *et al.,*2007; Atkinson *et al*., 2008). Several morphological traits have been identified that influence grain yield (Goral *et al*., 2005; Knezevic *et al*., 2008; Aliu & Fetahu, 2010; Shinwari *et al.,* 2014). Morphological traits can be used to characterize genetic diversity and in plant breeding programs, it is desirable to have large genetic diversity for the creation of new genotypes (Gegas *et al*., 2010; Sonmezoglu *et al*., 2012). Genetic diversity can be assessed form pedigree analysis, morphological traits, biochemical or molecular markers and it is the material basis for crop improvement (Knezevic *et al*., 2008; Habash *et al*., 2009; Jan *et al.,* 2016). Genetic diversity analysis is used for estimating and establishing of genetic relationship in germplasm collection, identifying more promising diverse parental combinations to create segregating progenies with maximum genetic variability for further selection, and introgressing desirable genes, from diverse germplasm into the available genetic base (Smith, 1984; Thompson *et al*., 1998; Islam *et al.,* 2012,). In the current study we specifically explored wheat germplasm from different areas of Pakistan to assess the level of genetic diversity using multivariate analysis.

Materials and Methods

The experimental materials consisted of selected one hundred and two germplasm of common wheat collected from different regions of Pakistan and were characterized for genetic diversity. The germplasm were sown in Randomized Complete Block Design (RCBD) with three replications at the experimental field of Hazara University Khyber Pakhtunkhwa (KP), Pakistan Mansehra. Standard agronomic practices and plant protection measures were adopted. Data on 19 Morph - physiological parameters *viz*: plant height, peduncle length, stem diameter, leaf angle, number of nodes, spike length, awn length, number of tillers, leaf rolling, flag leaf area, days to 50% heading, number of days to maturity, Yield per plant, biological yield, thousand grain weight, number of grains per spike, number of spikelet per spike, spike density and harvest index were collected at appropriate stage of growth. The collected data was statistically analyzed for Principal Component Analysis (PCA) and cluster analysis using software R .

Results and Discussion

Morphological traits: Descriptive statistics was used to study different morphological traits among different wheat genotypes (Table 1). High coefficient of variation (CV) was observed for awn length (44.10), No. of tillers (38.57), biological yield (23.57), flag leaf area (20.19) and peduncle length (18.85), Plant height (11.44), tillers per plant (38.57). Similar CV values have been reported for these parameters of wheat genotypes by Sabaghnia *et al.,* (2014). Flag leaf area, plant height, peduncle length and number of tillers per plant are important morphological yield contributing traits and positively correlated with yield per plant (Inamullah *et al.,* 2006;

Nawaz *et al.,* 2013). Grain yield could be increased by improving yield components such as spike length, spikelets per spike, grains per spike and grain filling duration (Ashfaq *et al.,* 2003). Significant differences were observed in all measured traits. Results of the present investigation indicated large extent of genetic diversity in all tested local Pakistani genotypes. This constancy should be considered of qualitative relevance, as it shows that the genotypes has been enriched by material different from the local adapted cultivars, which resulted in broadening of the genetic background in different regions from Pakistan. Therefore, the classification obtained for these Pakistani wheat genotypes, based on morphological traits will be a useful tool for wheat breeders to plan crosses for positive agronomic traits by choosing genotypes with appropriate diversity. The current study confirmed the importance of morphological traits, to determine genetic variation among genotypes with the aim to select diverse parents in new crossing programs. Analysis of sample variance and standard deviation for these traits showed that variation was highly significant so it was concluded that a lot of variation existed among the germplasm

Cluster Analysis: Cluster analysis is a technique of grouping a set of object in such a way that objects in the same group are more similar to each other than to those in other groups. The unweighted pair group method using arithmetic averages (UPGMA) was used by others researchers to study different morpho-physiological traits of different crop species (Mohammadi & Prasanna, 2003, Shinwari *et al.,* 2013; Jan *et al.,* 2016$^{b, c}$). The 102 accessions in this study were grouped into five clusters based on hierarchical clustering (Fig. 1) Cluster I comprised of 4 genotypes, Cluster II included 3 genotypes, Cluster III comprised of 6 genotypes, Cluster IV comprised of 42 genotypes and Cluster V comprised of 47 genotypes. In this study we did not get any single cluster which may be appropriate for all agro morphological traits recorded. The overall objective of the study was utilization of the genotypes in wheat improvement work. Therefore accessions for important agronomical characters were identified (Table 3). No duplicates were identified among the studied accessions for quantitative traits in the cluster analysis (Fig.1), which means there is a high diversity among the accessions for these traits. Cluster analysis based on agro-morphological diversity assessment in wheat was also reported in several studies, such as Sabaghnia *et al.,* (2014).

Principal component analysis: Principal component analysis (PCA) is a statistical procedure that converts a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components. It is used as data reduction tool. Also to find the most important variables. The eigen values are often used to determine how many factors to retain (Xanthopoulos *et al.,* 2013). The sum of the eigen values is usually equal to the number of variables (Fujikoshi *et al.,* 2010). Principal component analyses help researchers to distinguish significant relationship among traits. Principal component analysis based on 19 agro-morphological traits showed that five of components with an eigen value higher than one accounted for 55.82% of the total variation among 102 wheat genotypes. PCI which is most contributed 18.15%, PCII contributed 13.33 %, and PCIII accounted for 10.78 % of total morphological variability (Table 2). First three PCs accounted for 42.66 % of total variability, traits that accounted for most of the observed variations of the first three principal components considering the coefficients with an absolute value equal or higher than 0.41, were plant height, peduncle length, spike length, flag leaf area, 50% heading, days to maturity, no of grains per spike and grain weight per plant. Whereas stem diameter, no of nodes, thousand grain weight, spike density and no of spikelet per spike had substantially negative weight. Our results show agreement with Sheykhi *et al.,* (2014), who reported that leaf, grain, spike and no of grains per spike were the major contributing factors to the total variation of some wheat (*Triticum aestivum* L.) genotypes. Leilah and Khateeb (2005) showed a significant positive relationship of plant height with grain yield.

Principal component analysis of the wheat accessions revealed diverse grouping patterns which showing the contribution of 19 traits to total variation in first three PCs in a collection of T*riticum estivum* accession (Fig. 3). The first three principal components were plotted to detect the association between the accessions. The separation on the basis of PC1 and PC2 showed that the genotypes were scattered in all the quarters, which shows high level of genetic diversity in the evaluated genotypes (Fig. 4). Principal components 011872, 010794, Khyber 87, 010757, 010739, Gomal, 011871,010765, 010785, 010794, 010765 and 010785 showed greater genetic diversity. In the first and third principal components Khyber 87, 010777, 010763, 010798, 010804, 010789, 010757, 010777, 010765, 010785, 010765, 010742 and 010778 showed greater genetic diversity. Though principal component analysis organized accessions with more morphological similarities but cluster analysis also included the accessions from different or far by sites. All data presented in this study allowed the identification of elite genotypes that could be used as possible parents for wheat improvement program. Principal component analysis simplifies the complex data by transforming the number of correlated variables into a smaller number of variables called principal components. The first principal component accounts for maximum variability in the data with respect to succeeding components (Leilah & Al-Khateeb., 2005). Principal component analyses help researchers to distinguish significant relationship between traits. Multivariate analysis is used to explain the correlation between a large set of variables in terms of a small number of underlying independent factors (Beheshtizadeh *et al.,* 2013). This study was conducted in order to determine the dependence relationship between yield, yield components and some other morphological characters of bread wheat genotypes using principal component analysis. The principal component analysis had grouped the estimated wheat variables into three main components.

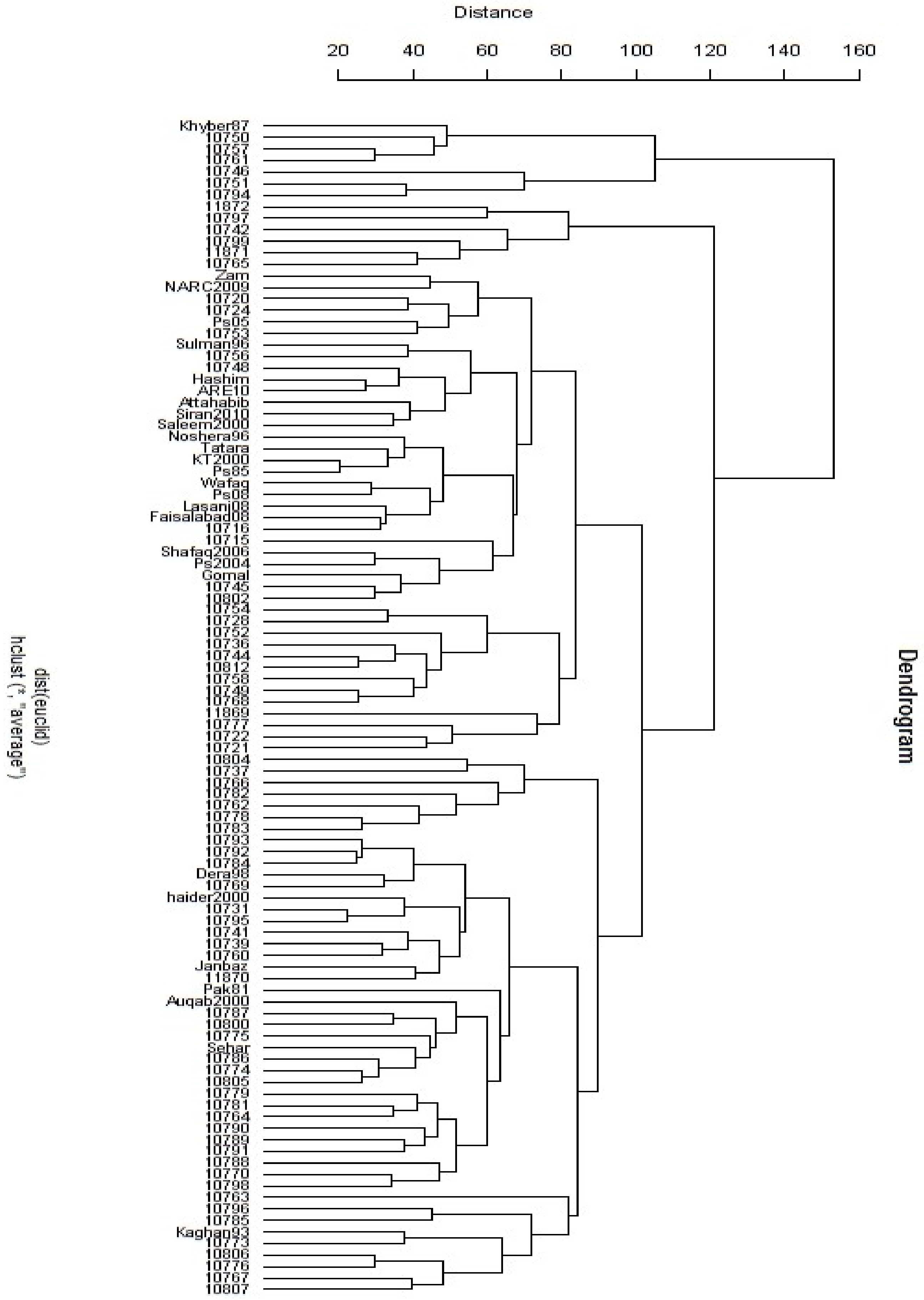

Fig.1 Dendrogram showing the relationship among 102 wheat genotypes based on 19 quantitative characters

Table 1. Descriptive statistics of agro-morphological traits in wheat germplasm.

Traits	Mean	Minimum	Maximum	Range	Variance	Standard Deviation.	Coefficient of Variation
Plant height	87.70	66.17	116.33	50.17	100.79	10.04	11.45
Peduncle length	33.05	21.53	50.83	29.30	38.84	6.23	18.86
Stem diameter	3.79	2.33	5.33	3.00	0.33	0.57	15.10
Leaf area	35.15	23.33	46.00	22.67	24.99	5.00	14.22
Number of nodes	3.68	2.33	5.00	2.67	0.22	0.47	12.66
Spike length	10.75	8.00	11.83	3.83	0.58	0.76	7.07
Awn length	4.04	0.00	5.73	5.73	3.17	1.78	44.10
Number of tillers	2.58	1.00	5.33	4.33	0.99	1.00	38.57
Leaf rolling	1.83	1.00	2.33	1.33	0.11	0.33	17.92
Flag leaf area	31.78	20.17	49.60	29.43	41.19	6.42	20.20
Days to 50% heading	146.09	132.00	161.00	29.00	43.59	6.60	4.52
Days to maturity	178.76	159.00	193.33	34.33	124.22	11.15	6.23
Yield per plant	3.29	2.11	4.12	2.04	0.17	0.42	12.68
Biological yield	23.30	11.44	36.46	25.77	30.16	5.49	23.57
Thousand grain weight	41.00	24.43	47.63	23.20	21.40	4.63	11.28
Number of grains /spike	50.07	31.00	57.67	26.67	19.77	4.45	8.88
Number of spikelets / spike	20.64	16.00	26.00	10.00	3.10	1.76	8.53
Spike density	1.93	1.50	2.56	1.06	0.05	0.21	11.13
Harvest index	43.07	12.49	50.47	15.69	26.76	5.17	12.01

Table 2. Principal components (PCs) for 19 morpho-physiological traits in 102 genotypes of wheat

	Component Matrix				
	PC1	PC2	PC3	PC4	PC5
Eigenvalue	3.15	2.55	2.03	1.51	1.37
Proportion of variation	18.15	13.33	10.78	7.95	7.21
Cumulative Eigenvalue	3.15	5.69	7.73	9.24	10.61
Cumulative Variance	18.15	29.97	40.66	48.61	55.83
Traits	**Eigen Factors**				
Plant height	0.654	0.453	0.060	-0.110	0.153
Peduncle length	0.654	0.406	0.162	-0.112	0.255
Stem diameter	-0.484	0.510	0.422	-0.176	0.084
Leaf area	0.081	0.020	0.004	-0.242	-0.454
Number of nodes	-0.439	0.441	0.501	0.063	0.032
Spike length	0.428	0.410	0.115	-0.478	-0.104
Awn length	0.341	-0.426	0.053	0.255	0.276
Number of tillers	0.021	0.129	-0.198	-0.026	-0.585
Leaf rolling	0.015	-0.217	-0.403	-0.108	0.118
Flag leaf area	0.471	-0.339	-0.445	-0.272	-0.122
Days to 50% heading	0.412	-0.013	-0.297	-0.364	0.273
Days to maturity	0.411	-0.384	0.637	0.128	-0.146
Yield per plant	0.676	0.214	-0.424	0.453	0.001
Biological yield	0.20	0.52	-0.09	-0.09	-0.36
Thousand grain weight	-0.130	0.317	0.174	0.254	-0.525
No of grains / spike	0.676	0.214	-0.424	0.453	0.001
No of spikelet / spike	-0.639	0.188	0.320	-0.064	0.087
Spike density	-0.783	-0.148	0.164	0.291	0.141
Harvest index	0.243	-0.162	0.194	0.363	0.304

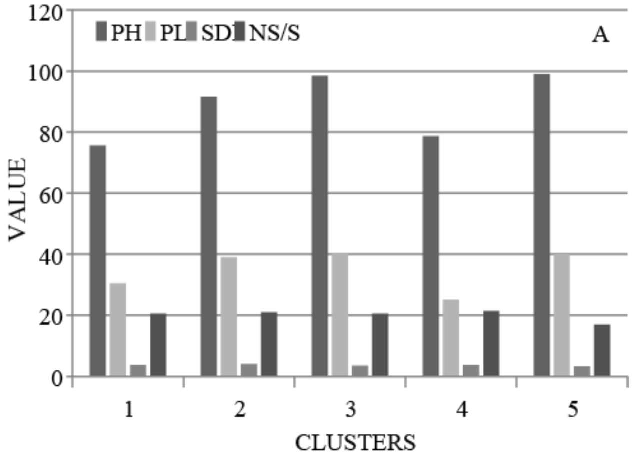

Fig. 2A Genetic variation for Plant height(PH), Peduncle length(PL), Stem diameter (SDI) ,and Number of spikelets per spike (NS/S) with in Five clusters

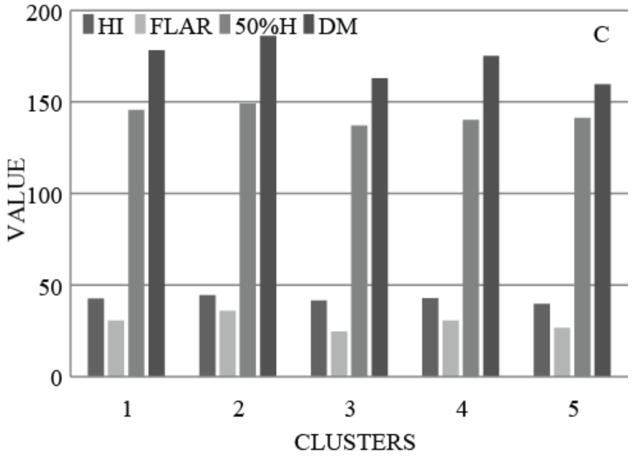

Fig. 2C Genetic variation for Harvest index(HI), Flag leaf area(FLAR), days to 50% heading(50%H), and days to maturity (DM)with in five clusters

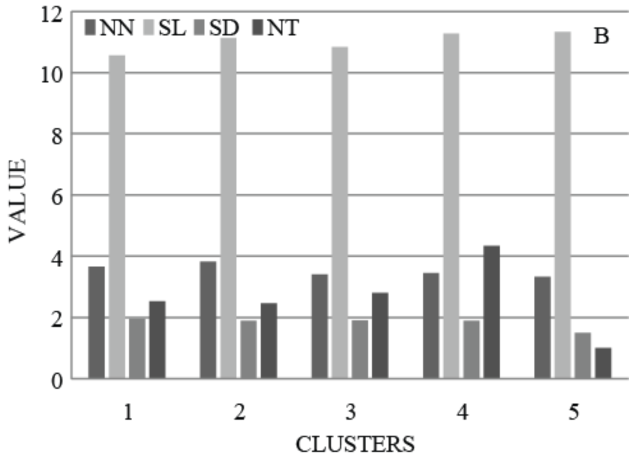

Fig. 2B Genetic variation for Number of Nodes(NN), Spike length(SL), Spike density(SD), and Number of tillers per plant(NT) with in Five clusters

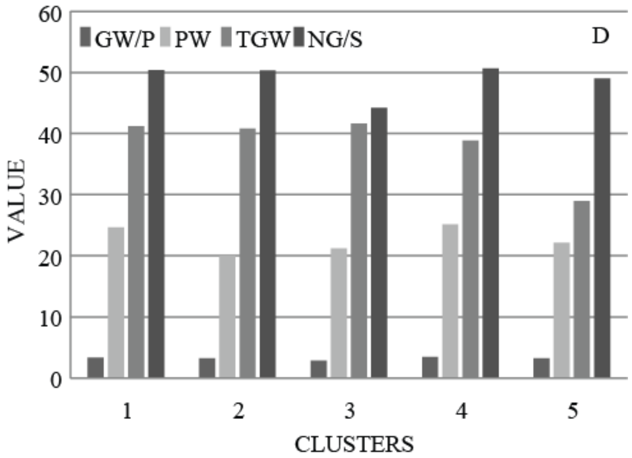

Fig. 2D Genetic variation for Grain weight per plant(GW/P), Plant weight(PW), Thousand grain weight(TGW) and Number of grains per spike(NG/S) with in Five clusters

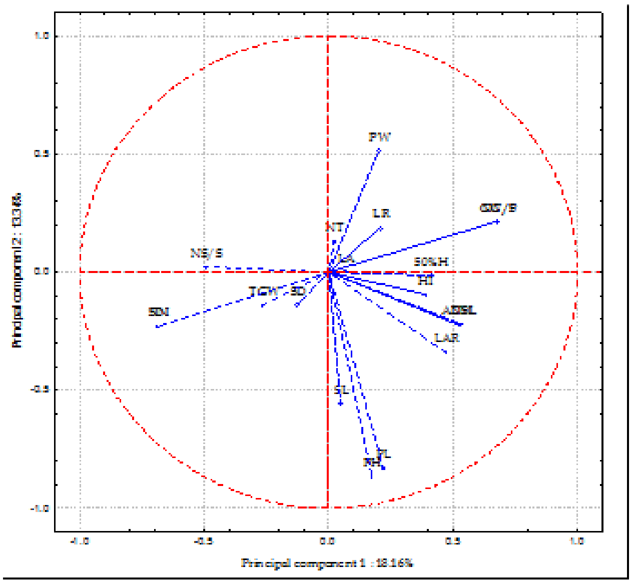

Fig.3A Principal components analysis plot showing the contribution of 19 traits to total variation in PC1 and PC2 in a collection of T. Estivum accessions

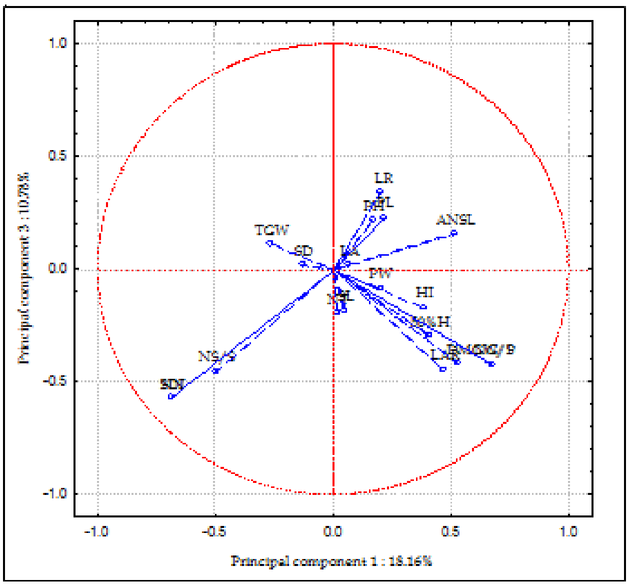

Fig.3B Principal components analysis plot showing the contribution of 19 traits to total variation in PC1 and PC3 in a collection of T. Estivum accessions

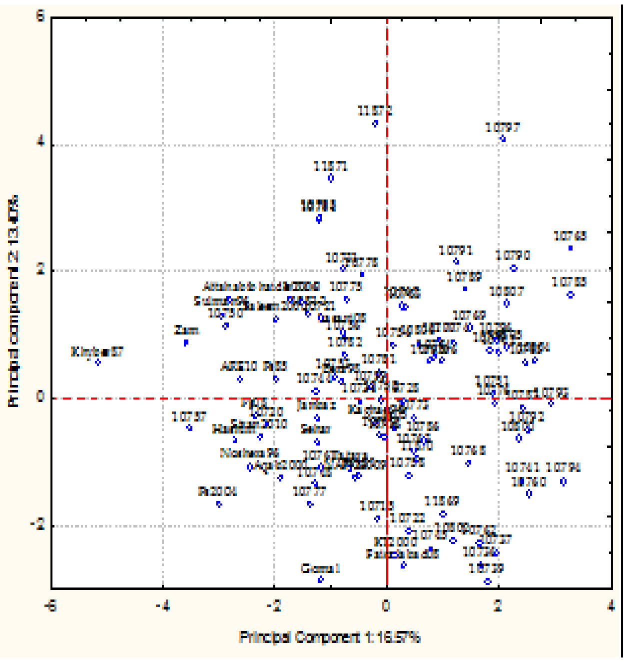

Fig. 4A Two dimensional scatter plot of the genetic relationship among 102 T. Estivum accessions as revealed in PC1 and PC2 principal components

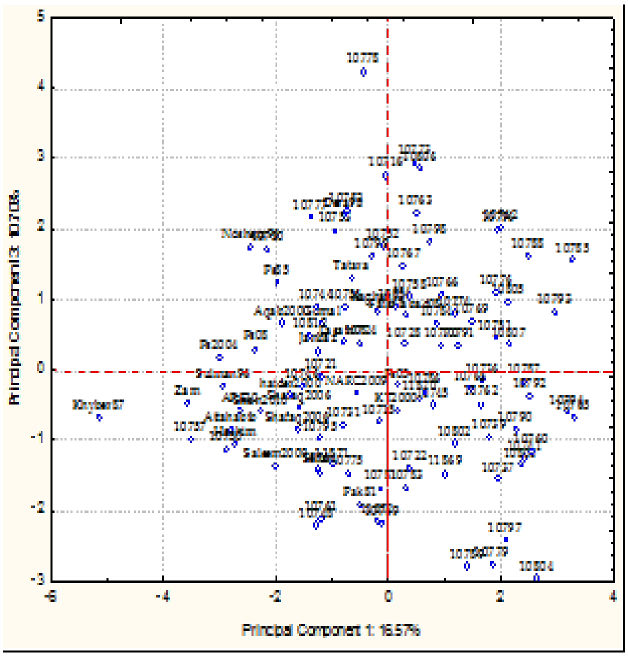

Fig. 4B Two dimensional scatter plot of the genetic relationship among 102 T. Estivum accessions as revealed in PC1 and PC3 principal components

Table.3 Elite genotypes identified on the basis of important agro-morphological traits for future breading

Traits of interest	Range	Accessions identified
Plant height	≥76	Khyber 87, Saleem 2000,Shafaq2006,Hashim,Gomal
Spike length	≥11.5	Atta habib,011872,010812,010716,010742
No of tillers	≥4	PS 2004,Gomal,010781,010716,010812
Days to maturity	≤132	Wafaq, Zam, Sulman 96, 010781, 011872
Grain weight /plant	≥4.12	Wafaq, Sulman 96,010716
Thousand grain weight	≥45.63	PS 2004, Siran 2010, 010742, 011872, Haider 2000, Sulman 96,010781
No of grains/spike	≥54.67	PS 2004,Noshera 96, PS 08,ARE 10,Gomal
No of spikelet/spike	≥23	Khyber87,Zam,Janbaz,Attahabib
Spike density	≥7.3	Shafaq 2006, PS 2004,Siran 2010, Noshera 96, Khyber 87, Wafaq, Hashim, Zam, Janbaz,
Harvest index	≥49	Hashim, Gomal, 010742

Conclusion

For most of the important traits highly significant differences among genotypes were observed by cluster analysis. Genotypes from cluster I and V, like Khyber 87and 10807 are more distantly related. The genotypes PS 2004,011872 and 010742 showed good performance for thousand grain weight, spike length, number of tillers per plant under Mansehra condition. Out of 102 lines, a sub-set of 18 accessions have been confirmed having quality traits. We are to analyze the same sub-set with DNA based markers and eliminate possible duplications for a crisper breeding ready material. PC1 and PC2 contributed 18.16% and 13.34% to the total variation. The most important characters identified through PCA were Plant height, Spike length, Number of grains per spike and grain yield per plant.

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