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Genetic diversity assessment of wheat germplasm from Pakistan using agro-morphometric traits

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Abstract

In the present study, Genetic diversity was studied among 25 bread wheat genotypes using 13 agro-morphological traits. The data were analyzed by cluster analysis and principal component analysis. A significant level of variability was noticed for some agro-morphological traits. The largest variation was observed in the Harvest index (203.11), Flag leaf area (153.39) and plant height (103.92). The 25 bread wheat varieties in this study were grouped into four clusters based on the hierarchical clustering method. Most important PCI explained 31.96%, PCII contributed 21.23% and PCIII accounted for 11.88% of total morphological variability. Genotypes Bluesilver, Pari-73 and Haider-2000 have high thousand-grain weight, biological yield and the high number of tillers per plant. Bluesilver, Pari-73 and Haider-2000 are three elite genotypes identified based on important agro-morphological traits for the future breeding program under Mansehra conditions.

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Introduction

Genetic diversity assessment is the foundation for crop improvement in a wide range of crop species including wheat (Salem *et al.*, 2008). However, modern intensive breeding narrowed the genetic diversity in wheat (Wang *et al.*, 2007; Abouzied *et al.*, 2013). Narrow genetic base causes intolerance to biotic stresses like insects, pests and diseases and in case of any disaster (disease or pest), the entire crop could be lost hence threatening food supplies (Huang *et al.*, 2002; Mir *et al.*, 2006). Moreover, a narrow genetic base presents difficulties in breeding crops for adaptation to abiotic and biotic stresses (Dodig *et al.*, 2010). Genetic variability could assist in the development of resistant crops to biotic and abiotic stresses and also in the adaptation of crops to various agro-climatic conditions (Wang *et al.*, 2007; Nawaz *et al.*, 2009; Khodadadi *et al.*, 2011).

Different data sets are used for estimation of genetic diversity in crop plants including pedigree data (Van Hintum and Haalman, 1994), biochemical markers (Hamrick and Godt, 1997), agro-morphological traits (BarHen *et al.*, 1995), or molecular markers. Morphological traits have been effectively used for the estimation of genetic diversity and varietal development. Many researchers used agronomic traits to calculate genetic diversity (Aharizad *et al.*, 2012; Siahbidi *et al.*, 2013; Fahim, 2014; Sabaghnia *et al.*, 2014; Verma *et al.*, 2014). Genetic diversity between two individuals can be computed by several statistical methods depending upon data sets (Mohammadi and Prasanna, 2003). Khodadadi *et al.* (2011) carried out cluster analysis which grouped 36 winter wheat genotypes into seven groups. Aharizad *et al.* (2012) clustered 96 bread wheat lines into three groups based on agronomic traits.

The objective of this study was to use a large number of morphological and agronomic characters to assess genetic variability and diversity among advanced lines of spring bread wheat from different areas of Pakistan. Another objective was to propose some ideas for using diversity based on many agronomic traits, grain yield, yield components and biological yield to generate pairs of genotypes for hybridization programs to develop improved wheat varieties.

Materials and methods

The research work was done at the experimental field of Hazara University, Mansehra Pakistan during the year 2016. The experimental materials consisted of twenty-five germplasm of bread wheat collected from the National Gene bank, Plant Genetic Resources Institute (PGRI), National Agricultural Research Centre (NARC), Islamabad, Pakistan.

The germplasm was sown in Randomized Complete Block Design (RCBD) with three replications. The Data collected for 13 quantitative traits on the particular growth stages of the wheat plant. Data were recorded on 50% days to heading (DH), Days to maturity (DM), Plant height (PH), Leaf length (LL), Leaf width (LW), Flag leaf area (FLA), Number of tillers per plant (NTP), Biological yield (BY), 1000 grain weight (TGW), Harvest index (HI), Spike length (SL), Spikelet per spike (SPS) and Awn length (AL).

Evaluation of morphological characteristics was done using descriptors recommended by the International Board for Plant Genetic Resources (IBPGR, 1978). The data collected were subjected to statistical analysis using software SPSS v17.0. Cluster analysis using the UPGMA clustering implemented in NTSYS-PC version 2.2e was used to construct a dendrogram showing the relationship among the genotypes. Principal Components Analysis (PCA) of the traits was used to examine, the percent contribution, of each trait to the total genetic variation. For graphical representation, of the pattern of variation among all the varieties of wheat scatter plots of the first three principal components were formed using Statistica, version 7.0.

Results and discussion

Morphological traits

Descriptive statistics for agronomical characters are tabulated in (Table 1) showed a considerable level of coefficient of variation for Agro-morphological traits among bread wheat varieties used in this study. Seed

yield being the ultimate product of all traits is very important (Saleem *et al.,* 2010). The high coefficient of variation (CV) was observed for flag leaf area (51.26%), leaf width (35.71%), harvest index (32.32%), spike length (27.83%), biological yield (27.41%) and leaf length (19.42%).

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

Traits	Mean	Minimum	Maximum	Variance	SD	CV%
Awn length	6.4	5	10	1.75	1.32	20.66
Spikelet per plant	18.24	16	21	1.60	1.26	6.94
Spike length	14.12	9	21	15.44	3.92	27.83
Plant Height	77.56	54	97	103.92	10.19	13.14
Leaf length	22.44	16	33	19	4.35	19.42
Leaf width	1.4	1	2	0.25	0.5	35.71
Flag leaf area	24.16	12	49	153.39	12.38	51.26
Number of tillers per plant	5.36	3	7	0.99	0.99	18.56
Biological yield	22.44	11	37	37.84	6.15	27.41
1000 grain weight	39.08	20	55	43.66	6.60	16.90
Harvest index	62.12	26	90	203.11	20.07	32.32
Days to heading	124.52	116	134	28.17	5.30	4.26
Days to maturity	159.04	150	168	31.70	5.63	3.54

The flag leaf area is very important for the preparation of food. Similar CV values have been reported for agronomic traits of bread wheat genotypes by Sabaghnia *et al.,* (2014). Grain yield is the complex trait which is the result of yield components such as the number of spikelet's per spike, harvest index, thousand-grain weight, plant height and other traits and also it is influenced by genotype and climatic conditions (Drezner *et al.* 2007; Atkinson *et al.* 2008).

CV value observed for plant height was (12.12) and the minimum plant height recorded was 54 cm and a maximum 97cm with a mean value of 75.7 cm.

Table 2. Eigenvectors, eigenvalues, total variance and cumulative variance based on agro-morphological traits.

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	4.15	2.76	1.54	1.33	1.08
% Total variance	31.96	21.23	11.88	10.26	7.56
Cumulative Eigenvalue	4.15	6.91	8.46	9.79	10.77
Cumulative %	31.96	53.20	65.08	75.35	82.91
Traits	Eigenvectors				
AL	0.33	0.03	-0.60	-0.60	0.15
SPS	0.20	0.69	-0.37	-0.04	-0.22
SL	0.28	0.74	-0.14	-0.23	0.29
PH	0.73	0.10	0.06	0.40	-0.12
LL	0.82	-0.12	-0.18	0.23	-0.29
LW	0.88	0.02	-0.12	0.16	0.04
FLA	0.92	-0.08	-0.17	0.20	-0.06
NTP	0.08	-0.34	0.35	-0.41	-0.57
BY	0.05	-0.69	-0.32	0.05	0.39
1000GW	0.25	-0.51	-0.28	-0.51	-0.25
HI	0.05	0.88	0.23	-0.22	-0.11
DH	-0.71	0.11	-0.49	0.25	-0.24
DM	-0.68	0.08	-0.56	0.27	-0.28

Significant differences were observed in all measured traits. The results of the present investigation indicated a large extent of genetic diversity in all tested local Pakistani genotypes. This constancy should be considered of quantitative relevance, as it shows that the genotypes have been enriched by material different from the locally adapted cultivars, which resulted in the 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 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.

Table 3. Elite genotypes are identified based on important agro-morphological traits for future breeding.

Traits of interest	Range	Accessions identified
Plant height	≥90	Pari73, SA42, Daman, Sariab97
Spike length	≥20	Khirman, Haider-2000
No of tillers/plant	≥7	Suleman96, Pari73
Thousand-grain weight	≥50	Blue Silver
Biological yield	≥33	SA42, Blue Silver, Daman
Spikelet per spike	≥21	Khirman
Awn length	≥ 9	Blue Silver, Haider 2000, Khirman

Cluster analysis

Cluster analysis includes objects in a group on basis of similarity. On basis of agronomic and morphologic characters, the arithmetic averages (UPGMA) is most commonly used (Mohammadi and Prasanna, 2003). The 25 bread wheat varieties in this study were grouped into four clusters based on hierarchical clustering (Fig. 1). Cluster I comprised of five genotypes, Cluster II includes six genotypes, Cluster Ill comprises of five genotypes, Cluster IV comprises of nine genotypes (Fig. 1).
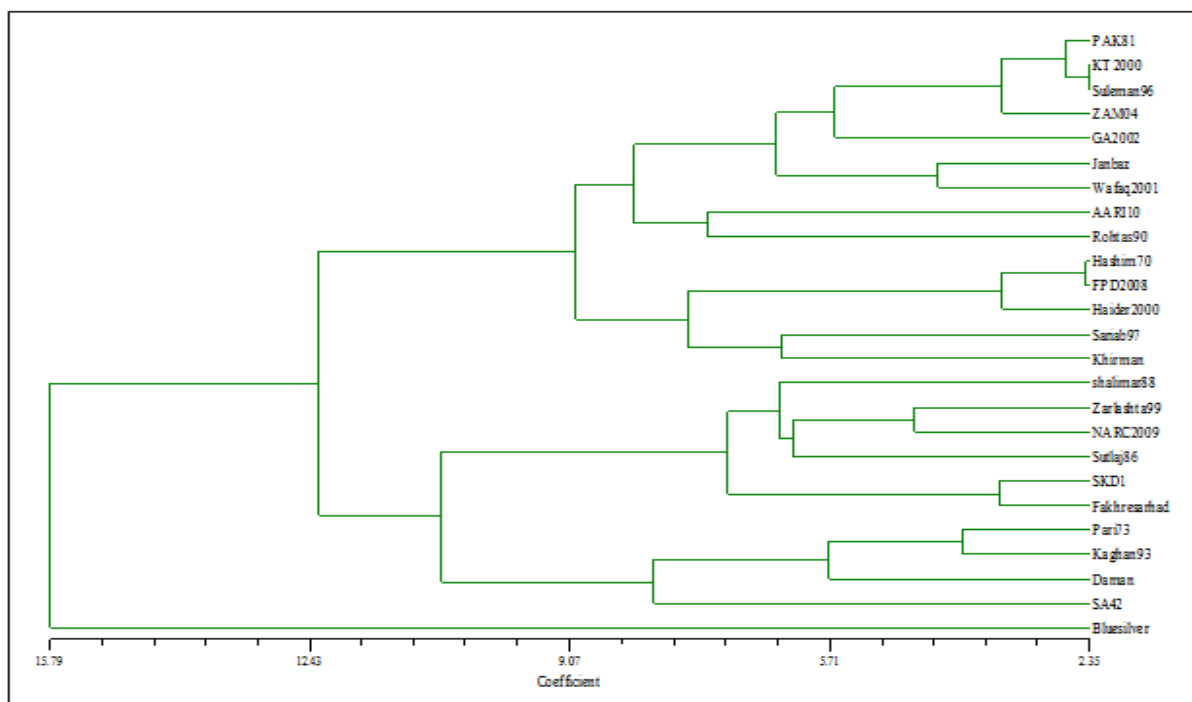

Fig. 1. Dendrogram showing the relationship among 25 varieties of wheat varieties based on 13 quantitative traits.

Group III varieties had the highest number of spikelets per spike, spike length and awn length (Fig. 2A). The leaf length was highest for the wheat varieties in Clusters I whileleaf width did not vary considerably (Fig. 2B). The harvest index was highest for members of group III. Genotypes belonging to

group I had the highest 1000 grain weight, while members of group I and III showed the highest and lowest biological yield respectively (Fig. 2C). Members of group I and III were tallest in plant height. The lowest plant height was shown by

members of group III. The genotypes in Cluster I was early maturing. The mean duration to days to maturity for the group I was 150, while late-maturing was showed by cluster II which is 168 days (Fig. 2D).
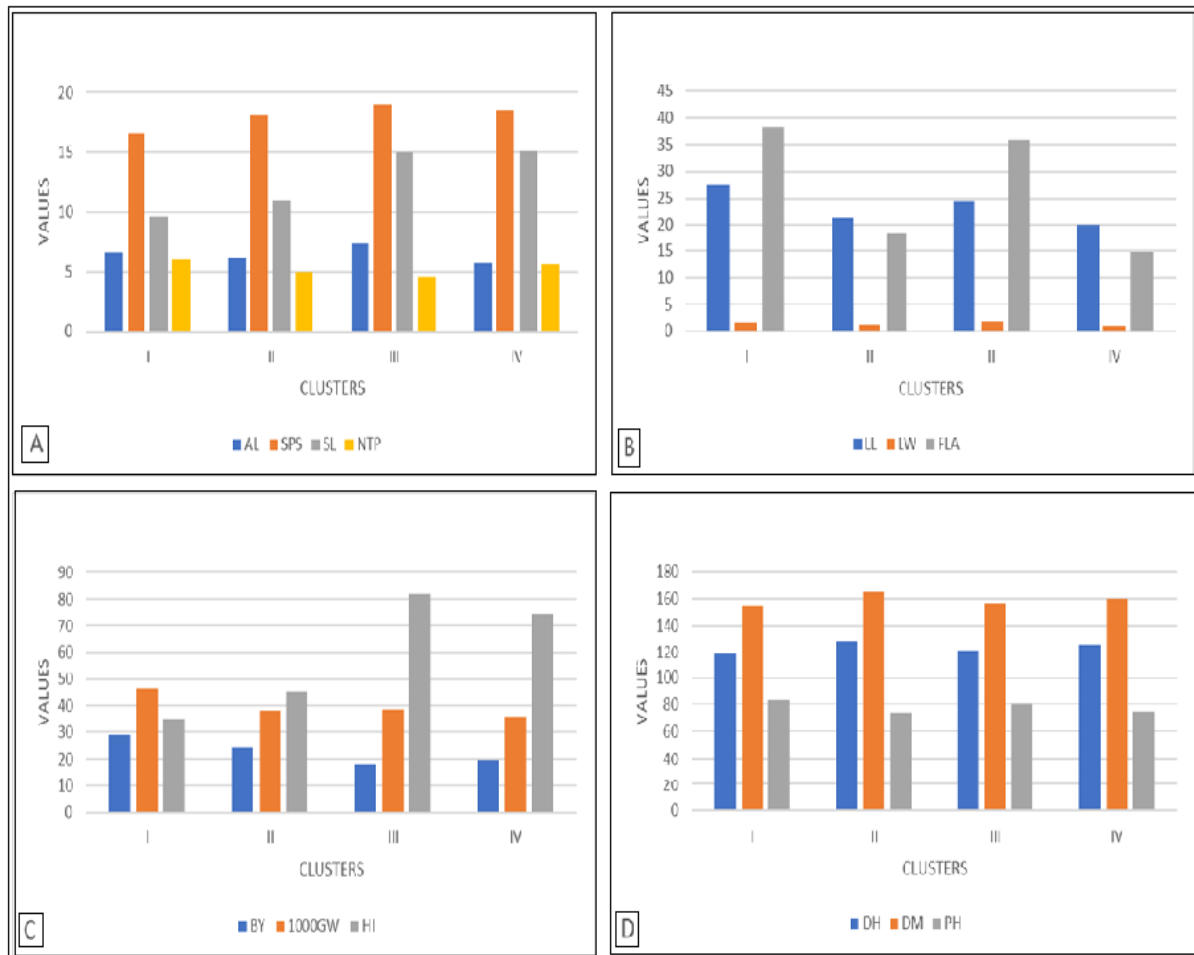

Fig. 2. (A, B, C, D). Characteristic means of eight similarity clusters generated by UPGMA based on the classification of 105 genotypes of *T. aestivum* L. using different agro-morphological traits.

In this study, we did not get any single cluster which may be appropriate for all agro-morphological traits recorded. Therefore, varieties of important agronomical characters were identified (Table 3). No duplicates were identified among the studied wheat varieties for quantitative traits in the cluster analysis (Fig.1), which means that there is a high diversity among the varieties for these traits.

Cluster analysis based on agro-morphological diversity assessment in wheat was also reported in several studies, such as (Singh and Dwivedi.,2002; Ali *et al*., 2008; Sabaghnia *et al.,* 2014).

PCA analysis

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 concerning succeeding components (Leilah and Al-Khateeb., 2005). Principal component analysis based on thirteen agro-morphological, traits during year 2016 showed that the first five of the 13 principal components with an eigenvalue was, higher than one accounted for 75.04 of the total variation among 25 bread wheat varieties (*Triticum aestivum* L.).
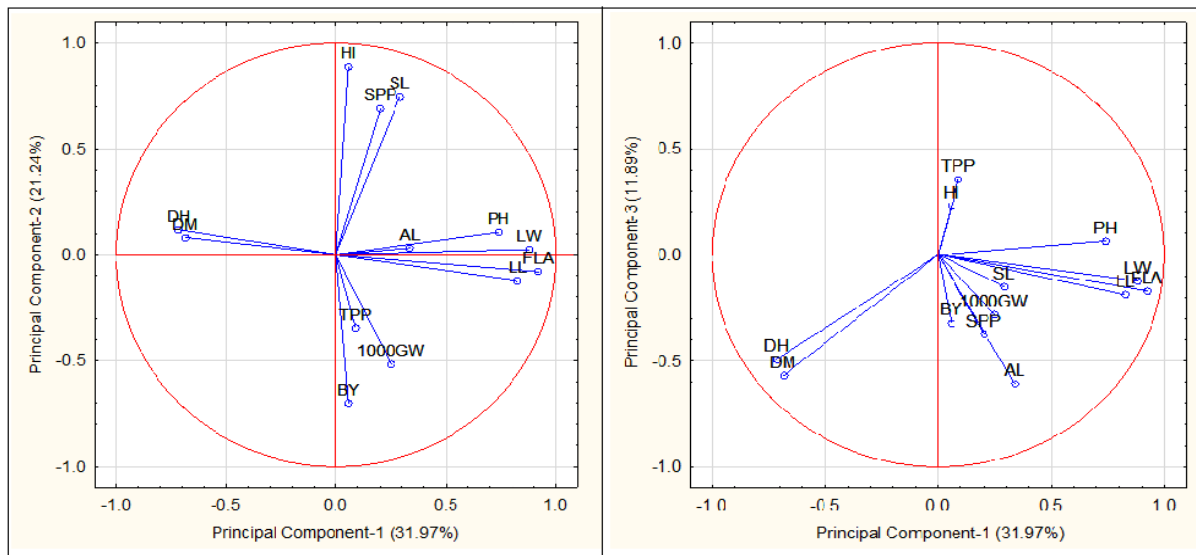
Fig. 3. Principal components analysis plot showing the contribution of 13 traits to the total variation in the first three PCs in a Collection of *T. aestivum L.* varieties.

The most important PCI explained 31.97%, PCII contributed 21.24%, PCIII accounted for 11.89% of total morphological variability (Table 2). The first five PCs accounted for 82.91% of the total variance, 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 0.34, were plant height, leaf length, leaf width, 50% days to heading and days to maturity.
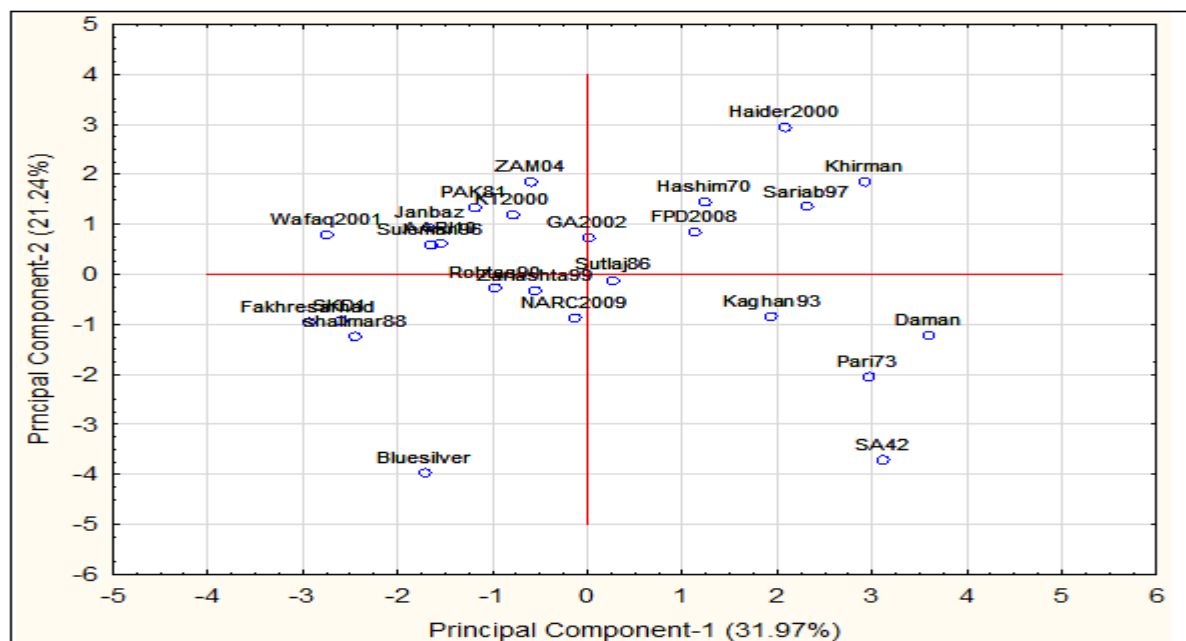

Fig. 4. Two-dimensional scatter plot of the genetic relationship among 25 *T. aestivum* L. varieties as revealed in PC1 and PC2 principal components.

Whereas biological yield, days to heading, days to maturity and spikelet per spike had substantially negative weight. Our results also agreed with Batiseba *et al.* (2018) who reported that leaf length, plant height, 50% days to heading, days to maturity, yield and its contributing factors were the major factors contributing to the total variation of parental lines of South Africa. One hundred and fifty wheat genotypes

were used in this study.Principal component analysis of the bread wheat varieties revealed a diverse grouping pattern which shows the contribution of 13 traits to the total variation in the first five PCs in a collection of 25 *Triticum aestivum L.* varieties (Fig. 3). The first 3 principal components were plotted to

detect the associations between the wheat varieties.

The separation on the basis of PC1 and PC2 showed that the genotypes were scattered in all the quarters, which show the high level of genetic diversity in the evaluated genotypes (Fig. 3).
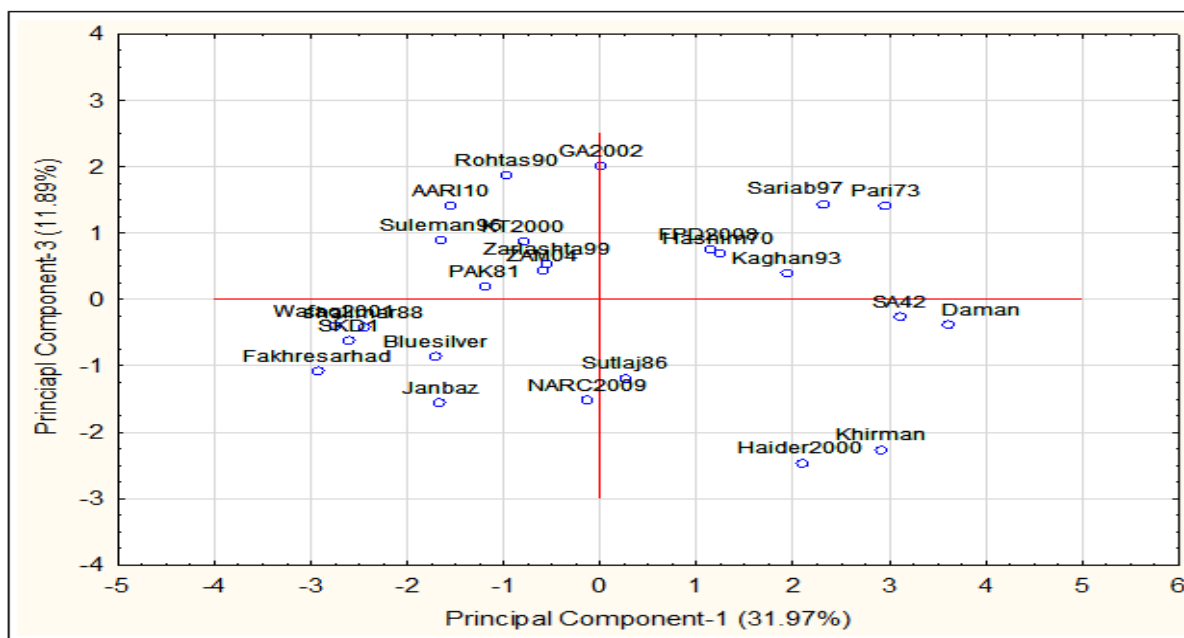

Fig. 5. Two-dimensional scatter plot of the genetic relationship among 25 *T. aestivum L.* varieties as revealed in PC1 and PC2 principal components.

In the first and second principal components Haider-2000, wafaq-2000, Bluesilver and SA-42 showed greater genetic diversity (Fig 4). In the first and third principal components Sariab-97, Pari-73, Khirman, Haider-2000 and Rohtas-90 showed greater genetic diversity (Fig. 5). All data presented in this study allowed the identification of varieties with diverse morphological traits that could be used as possible parents for the wheat improvement program.

Conclusion

For most of the important traits, highly significant differences among genotypes were showed by cluster analysis and the presence of diversity among *T.aestivum* L. genotypes. Genotype Bluesilver, SA-42, Daman Suleman-96 and Pari-73 have high thousand-grain weight, biological yield and the high number of tillers per plant. The highest spike length and plant height were showed by Khirman, Haider-2000, Pari-73, Sariab-97 and Daman. From the above

conclusions, it was recommended that: Genotypes Bluesilver, Pari-73 and Haider-2000 can be used for general cultivation under Mansehra conditions.

References
Abouzied HM, Samah M, Eldemery M, Abdellatif KF. 2013. SSR- based genetic diversity assessment in tetraploid and hexaploid wheat populations. British Biotechnology Journal **3**, 390-404.

Aharizad S, Sabzi M, Mohammadi SA, Khodadadi E. 2012. Multivariate analysis of genetic

diversity in wheat (*Triticum aestivum* L.) recombinant inbred lines using agronomic traits. Annals of Biological Research **3,** 2118–2126.

Ali Y, Atta BM, Akhter J, Monneveux P, Lateef Z. 2008. Genetic variability, association and diversity studies in wheat (*Triticum aestivum* L.) germplasm. Pakistan Journal of Botany **40,** 2087-2097.

Atkinson M, Kettlewell PS, Poulton PR, Hollings PD. 2008. Grain quality in the Broadbalk wheat experiment and the winter North Atlantic oscillation. Journal of Agricultural Science **146,** 541–549.

Bar-Hen, A, Charcosset A, Bourgoin M, Cuiard J. 1995. Relationships between genetic markers and morphological traits in maize inbred lines collection. Euphytica **84,** 145–154.

Batiseba Tembo, Julia Sibiya, Pangirayi Tongoona. 2018. Assessing genetic diversity in wheat using agro-morphological traits and the association between traits. World Journal of Agricultural Sciences **14,** 98-107.

Drezner G, Dvojkovic K, Horvat D, Novoselovic D, Lalic A. 2007. Environmental impacts on wheat agronomics quality traits. Cereal Research **35**, 357–360.

Fahim MG. 2014. Grouping of promising agronomic traits of genotypes and lines of bread wheat using cluster analysis and detection function. Indian journal of Fundamental and Applied Life Science **4,** 182–185.

Hamrick JL, Godt MJW. 1997. Allozyme diversity in cultivated crops. Crop Science **37,** 26–30.

Huang XQ, Börner A, Röder MS, Ganal MW. 2002. Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. Theoretical and Applied Genetics **105,** 699-707.

IBPGR. 1978. Descriptors for wheat and Aegilops International Board for Plant Genetic Resources (IBPGR) Rome, Italy, 25.

Khodadadi M, Fotokian MH, Miransari M, 2011. Genetic diversity of wheat (*Triticum aestivum* L.) genotypes based on cluster and principal component analyses for breeding strategies. Australian Journal of Crop Science **5,** 17-24.

Leilah AA, Al-Khateeb SA. 2005. Statistical analysis of wheat yield under drought conditions. Journal of Arid Environments **61,** 483-496.

Mir RR, Kumar J, Balyan HS, Gupta PKA. 2006. Study of genetic diversity among Indian bread wheat (*Triticum aestivum* L.) cultivars released during last 100 years. Genetic Resources and Crop Evolution an International Journal **53,** 1-10.

Mohammadi SA, Prasanna BM. 2003. Analysis of genetic diversity in crop plants—salient statistical tools and considerations. Crop Science **43,** 1235–1248.

Nawaz M, Hussain SA, Ullah I, Younus M, Iqbal MZ, Rana SM. 2009. Estimation of genetic diversity in wheat using DNA Markers. American-Eurasian Journal of Sustainable Agriculture **3,** 507-511.

Sabaghnia N, MohammadI MJ, Bashiri A, AsghariShirghan R. 2014. Genetic variation of several bread wheat (*Triticum aestivum* L.) genotypes based on some morphological traits. Annales universitatis mariae curiesкłodowska Lublin-polonia **1,** 43-54.

Salem KFM, El-Zanaty AM, Esmail RM, 2008. Assessing wheat (*Triticum aestivum* L.) genetic diversity using morphological characters and microsatellite markers. World Journal of Agricultural Sciences **4,** 538-544.

Siahbidi MMP, Aboughadareh AP, Tahmasebi

GR, Teymoori M, Jasemi M. 2013. Evaluation of genetic diversity and inter relationships of agro-morphological characters in durum wheat (*Triticum durum desf.*) lines using multivariate analysis. International Journal of Agriculture Research and Reviews **3,** 184–194.

Singh SP, Dwivedi VK. 2002. Genetic divergence in wheat (*Triticum aestivum* L.). New Agriculture **13,** 5-7.

Van Hintum TJL, Haalman D. 1994. Pedigree analysis for composing a core collection of modern cultivars, with examples from barley (*Hordeum vulgare*). Theoretical and Applied Genetics **88,** 70–74.

Verma PN, Singh BN, Singh G, Singh MK, Setter TL. 2014. Genetic diversity analysis for yield and other agronomic traits in bread wheat under water logged sodic soil condition. Journal of Wheat Research **6,** 51–58.

Wang H, Wang X, Chen P, Liu D. 2007. Assessment of genetic diversity of Yunnan, Tibetan and Xinjiang wheat using SSR Markers. Journal of Genetics and Genomics: Formerly Acta Genetica Sinica **34,** 623-633.