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Original Article

Agro-morphological characterization of Pakistani maize accessions using qualitative and quantitative traits

Caracterização agromorfológica de acessos de milho paquistanês usando características qualitativas e quantitativas

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Abstract

Agro-morphological characterizations offer robust and vigorous means for the precise characterization of germplasm to be used in breeding programs. Here, agro-morphological features-based analyses were considered to figure out the genetic variability within 99 maize accessions and five (5) check varieties including *Azam, W. Islamabad, Haq Nawaz, Ev-5* and *Evr$_1$97.* A total of 28 important agro-morphological traits were accounted in the field trails at Hazara University Mansehra Pakistan, during spring 2015. The maximum variation was detected in grain weight per cob (53.43), followed by number of kernels per row (38.64) and flag leaf area (cm^2), (38.09%). Cluster analysis divided the 99 maize accession with five check varieties of maize accessions into 7 clusters following the hierarchical clustering. Cluster II recorded 29 genotypes with maximum grain yield per cob, and highest flag leaf length, followed by cluster IV. Lowest grain yield per cob was noted for accessions of cluster I. Similarly, cluster VII consisted of accession with the longest cob length. Moreover, the investigations also revealed that the primary constituent among first five principal components with an eigen value about more than 0.98 in relation to 68.75% of the total variants. PCI accounted for 25.53%, PCII contributed 18.31%, and PCIII is 9.88% of the overall morphological variability were significant contributors were grain weight per cob, number of kernel per row, 1000 grain weight. The identification of a significant level of genetic diversity during the present investigation having implications for maize germplasm characterisation, conservation, and breeding programs aiming at developing improvement maize cultivars.

Keywords: maize, accessions, genetic diversity, principal component analysis, northern Pakistan.

Resumo

As caracterizações agromorfológicas oferecem meios robustos e vigorosos para a caracterização precisa do germoplasma a ser utilizado em programas de melhoramento. Aqui, análises baseadas em características agromorfológicas foram consideradas para descobrir a variabilidade genética dentro de 99 acessos de milho e cinco variedades de controle incluindo Azam, W. Islamabad, Haq Nawaz, Ev-5 e Evr197. Um total de 28 características agromorfológicas importantes foi contabilizado nas trilhas de campo na Universidade Hazara Mansehra, Paquistão, durante a primavera de 2015. A variação máxima foi detectada no peso de grãos por espiga (53,43), seguido pelo número de grãos por linha (38,64) e área da folha da bandeira (cm^2), (38,09%). A análise de cluster dividiu os 99 acessos de milho com cinco variedades de acessos de milho em 7 clusters seguindo o agrupamento hierárquico. O cluster II registrou 29 genótipos com máxima produtividade de grãos por espiga e maior comprimento de folha bandeira, seguido do cluster IV. O menor rendimento de grãos por espiga foi observado para os acessos do cacho I. Da mesma forma, o cacho VII consistiu no acesso com maior comprimento de espiga. Além disso, as investigações também revelaram que o constituinte primário entre os cinco primeiros componentes principais com um valor próprio de cerca de 0,98 em relação a 68,75% do total de variantes. PCI representou 25,53%, PCII contribuiu com 18,31% e PCIII é 9,88% da variabilidade morfológica geral, onde os contribuintes significativos foram peso de grãos por espiga, número de grãos por linha, peso de 1.000 grãos. A identificação de um nível significativo de diversidade genética durante a presente investigação tem implicações para a caracterização de germoplasma de milho, conservação e programas de melhoramento visando o desenvolvimento de cultivares melhoradas de milho.

Palavras-chave: milho, acessos, diversidade genética, análise de componentes principais, norte do Paquistão.

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1. Introduction

Maize (*Zea mays* L. 2n = 2x = 20) is a staple cereal crop after wheat and rice (Malhotra, 2017). Maize is also known as 'miracle crop' and belongs to family Poaceae (Gramineae), which is a short-duration, fast-growing, cross pollinated and the only exceptional monoecious crop among the grasses. It is as a good source of food for human beings (45%), raw material in industries (20%) and fodder for livestock (35%) (Ullah et al., 2010; Tariq and Iqbal, 2010). In Pakistan maize was cultivated on an area of about 1,413 million hectares with an increase of 2.9% over last year i.e. 1,374 thousand hectares while, its productivity improved by 5.1 percent reaching to record highest 6.309 million tonns (Government of Pakistan, 2019). At the provincial level highest production (79.3%) comes from Punjab (55.5%), followed by Khyber Pakhtunkhwa (43.7%), Sindh (0.75%) and Baluchistan (0.24%) of overall maize cultivating land (Manzoor et al., 2019). The average yield of maize is 4.3 metric tons/ha that is relatively lower than other maize producing countries, However its average is almost double than that of the India (2.63 metric tons/hec) (Kashif et al., 2020). In Pakistan, major reasons of the low maize productivity include, terminal high temperature, poor crop management, high input rates, shoot fly infestation, maize borer, stalk rot infestation and use of low-quality seeds. Due to the extensive usage of maize crop or its products, the requirements are increasing day by day, which motivates farmers and agriculture departments to put all their efforts for improving the overall maize production (quality and quantity) (Ahmad et al., 2016; Yadava et al., 2017). Maize crop offer rich sources of essential amino acids and producing about 42 million tons of proteins each year, accounting for almost 15% of overall proteins acquired from food crops globally. This helps and contributes to various sectors including, food, feed and industrial potential and grain yield are highly profitable (Iqbal et al., 2014; Sulewska et al., 2014).

Maize is considered one of the most versatile crop species that can grow in a broad range of conditions due to genetic flexibility. Evaluation of genetic diversity is important for crop improvement and it can be achieved by morphological, biochemical and molecular markers. However, the morphological characterization is first and the most important step to study the genetic variability plants (Iqbal et al., 2014; Shinwari et al., 2014). The identification and subsequent production of new resistant and high yielding lines is a promising strategy that may benefits farmers to obtain supergiant, efficient and highly productive maize varieties.

Genetic diversity is more importance factor than rest of the factors for improving maize production (Hoxha et al., 2004). A significant improvement of maize has been reported from 3415 kg/ha to 4268 kg/ha, during 2008-09 and 2012-13, respectively (Farooq et al., 2015). Rahman et al. (2015) indicates that the genetic variability of maize plants are directly linked to the morphological features such as, days to field germination, plant height, number of leaf/plant, leaf length, flag leaf area, flag leaf length, stem diameter, number of cob/plant, ear height and ear diameter. However, the extent of genetic variability remained premeditated by several investigators in different yields, like *Oryza sativa* L. (Rabbani et al., 2010), *Sesamum indicum* L. (Fazal et al., 2011), *Zeya maize* L. (Iqbal et al., 2014), *Carthamus tinctorius* L. (Shinwari et al., 2014), and in *Brassica juncea* L. (Ali et al., 2015). The current research was carried out to find the extent of genetic diversity in maize accessions from Northern areas of Pakistan collected from NARC Islamabad for agro-morphological characterization and the identification of accessions with wider genetic base for breeding programs.

2. Materials and Methods

2.1. Experimental set-up

A total of ninety-nine (99) maize accessions with five (5) check varieties from the northern area (Khyber Pakhtunkhwa) of Pakistan were obtained from gene bank of Plant Genetic Resources Institute (PGRI), and National Agriculture Research Center (NARC) Islamabad. The collected varieties were investigated under field trials during spring 2015-2016, at Hazara University Mansehra, Pakistan. The mean annual temperature and rainfall of the experimental site are 18.5 °C and 1445 mm, respectively (Riaz et al., 2021). The soil characteristics were as, soil pH (7.2), electrical conductivity (0.281 dS/m), organic matter (0.91%), Potassium (112 ppm), nitrogen (0.0045%), and phosphorus (6 ppm).

2.2. Data collection

The data were noted for the 30 agro-morphological traits, i.e. 12 qualitative and 18 quantitative traits. The quantitative parameters include;

1. Days to field germination (DFG). For the determinations of Days to field germinations the number of days were noted from the day of sowing till 50% seeds of each germinated genotypes accordingly.
2. Plant height (PH). The plants heights were properly measured i.e. from ground level off to the tassel base, after milk stage.
3. Number of leaves/plant (NL/P). Leaves number were noted in each accession after flowering stage.
4. Leaf length (LL) (cm). For the leaf length measuring the leaf's subtended uppermost ear was noted, from ligule to the apex after flowering stage.
5. Flag leaf width (FLW) (cm). The width of the same leaf was noted at mid-way along its length.
6. Flag leaf length (FLL) (cm). The matured maize plant's flag leaf length was measured by using a ruler (scale).
7. Stem diameter (SD) (cm). The diameter of the maize mature plant's stem was measured manually for each accession.
8. Ear height (EH) (cm). The height of ear from the ground to the node position that possessing the uppermost ear was noted, after milk stage.
9. Ear diameter (ED) (cm). Using the Vernier caliper, the diameter (in mm and later on converted into cm) of the upper-most ear was monitored at the middle position.

10. Tassel height (TH) (cm). At the time of maturation, the tassel height was properly measured by means of rural (cm).
11. Node number (NN) (no.). The number of maize nodes were counted manually accordingly.
12. Days to maturity (DM) (no.). Number of maturity days was recorded from the sowing date to the 50% genotypes were harvested.
13. Number of cobs per plant (NC/p). Number of cobs per plant were counted for each accession after fully matured plant/before the harvest.
14. Number of kernel rows (NKR). Number of kernel rows were noted at the mid-point of the upper most ear.
15. Number of kernels per cob (NK/C) (no.). After the harvesting, the number of kernels were properly counted for each accession.
16. 1000 kernels weight (1000KW) (g). The 1000 kernels weight were properly calculated and adjusted to 10% moisture content.

The qualitative parameters included Leaf Color (LC), Leaf Orientation (LO), Pubescence of Culm (PbC), Husk Cover (HC), Tassel Types (TT), Tassel size (TS), Ear Damage (EDa), Kernel Color (KC), Kernel Type (KT), Cob Color (CC), Kernel Row Arrangement (KRA), and Silk Color (SC) were physically observed via standard descriptors for maize framed by IBPGR (IBPGR, 1991).

2.3. Statistical analysis

The agro-morphological data was analyzed by using Statistica software v 2.1 (Hayat et al., 2021). Similarly, cluster analysis was performed with the SAHN sub-program using the UPGMA clustering as applied in NTSYS-PC version 2.2e was used to construct a dendrogram showing the link among the accessions. Percentage of each trait to the total genetic distinction was observe by Principal Component Analysis (PCA) of traits. For graphical illustration of pattern for variation's between all maize accessions, scatter plots for first three PC were also designed by using the Statistica software v 2.1 (Ayesha et al., 2015).

3. Results

The morphological results showed high coefficient of variation within the diverse accessions of maize (Table 1). The highest coefficient of variance (CV) was noted for grain weight/cob (53.42%), followed by number of kernel/row (38.64%), ear height (37.17%), flag leaf area (38.09%) and number of kernel/cob (35.36%). The maximum variability was detected in the number of kernel per cob (10970.02) followed by plant height (2061.37), grain yield per cob (1896.72), 1000 grain weight (1062.7), ear height (302.7) and leaf length (160.01).

3.1. Cluster analysis

The 99 accessions and five checked varieties were placed in seven clusters based on hierarchical clustering at linkage distance 4.36 (Figure 1). Cluster I include the least number of accessions (2), cluster II contained the highest number of accessions (29), cluster III comprised of 22 accessions and cluster IV had 21 accessions. Similarly, cluster V included 5 accessions, 20 accessions were kept in VI cluster, 4 accessions were included in cluster VII

Table 1. Descriptive statistics for agro-morphological characters in the maize accessions.

Traits	Mean	Minimum	Maximum	Variance	SD	CV %
Days to field germination	13.0 I	9.0	20	3.8	2.01	15.44
Plant height	274.87	161.54	387.1	2061.37	45.4	16.51
Number of leaf/plant	12.6	9	19	3.81	1.95	15.47
Leaf length	81.59	60.96	106.68	160.01	12.64	15.49
Flag leaf area	3.15	0.1	6.1	1.44	1.2	38.09
Flag leaf length	18.61	14	29.1	8.32	2.88	15.47
Stem diameter	4.78	1.2	8.2	1.83	1.35	28.24
Number of cob/plant	1.8	1	3	0.39	0.62	34.44
Ear height	49.5	12.5	98.2	302.7	18.4	37.17
Ear diameter	3.2	2	4.5	0.2	0.5	15.62
Tassel height	42.93	30.48	57.91	66.13	8.13	18.93
Days to maturity	96.88	84	110	74.91	8.65	8.92
Node number	10.54	6	15	3.72	1.92	18.21
Cob length	12.17	6	19	6.64	2.57	21.11
Number of kernel/row	13.12	7	36	25.72	5.07	38.64
Number of kernel/cob	296.13	64	571	10970.02	104.73	35.36
Grain weight/cob	81.51	14.33	210	1896.72	43.55	53.42
1000 grain weight	216.5	143.7	340.4	1062.7	33.5	15.47

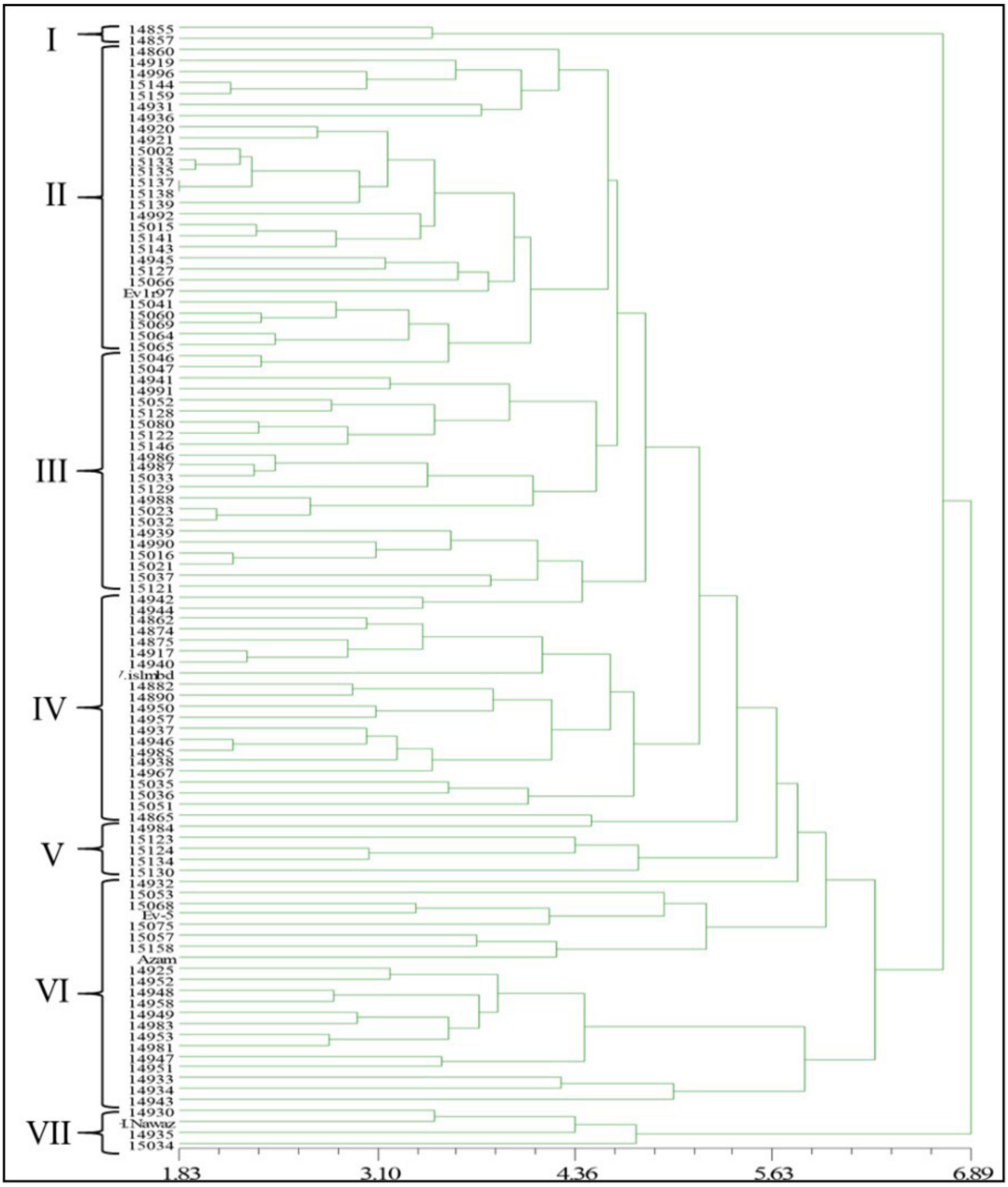

Figure 1. Dendrogram illustrating the genetic association between maize accessions divided into 7 cluster (I-VII) on the basis of agro-morphological traits during 2015.

(Table 2). Similarly, the maximum leaf length and plant height was noted for the members of cluster VI, while cluster I accession belonging to cluster VII had highest grain yield per cob. Lowest grain yield per cob was noted for members of cluster I, and cluster VII was noted for the longest cob length. Similarly, it was also taken under consideration that, the representative of group III and IV possess the longest cob length. Lowest number of rows per cob was noted for members of group III. The dendrogram revealed that most reserved accessions were 14945, 14950, 15137, Haq Nawaz and 14932. The accessions identified for agronomic traits are listed in the (Table 2).

3.2. Principal component analysis

Principal components analysis based on 18 quantitative traits revealed that the first five PCs accounted with an eigenvalue were greater than one i.e (68.75%) of the total variant amongst accessions of maize (Table 3, Figure 2). The PC1 and PC2 determined that the accessions are scattered in each quarter and showed the highest genetic diversity in evaluating accessions (Figure 3). The greater variation among the accession made them appropriate for hybridization and breeding program to develop potential hybrid and synthetic varieties. The five check varieties out of 104 accessions were founded varyingly

Table 2. Number of genotypes per cluster, along with the percentage of other prominent characters that divided the maize germplasm distinctly into seven main clusters.

Cluster	Total genotypes	Percentage	Prominent Features
I	2	1.92	Highest Number of leaves, Nod numbers and maximum grain Weight per cob, medium plant height and Ear height and flag leaf length, broadest ear diameter, longest ears.
II	29	27.88	The highest flag leaf length, cob length, maximum grain yield per cob, number of nods, stem diameter and days to maturity, and the lowest row per cob.
III	22	21.15	Short leaf length, narrow flag leaf area, lowest row per cob, medium number of leaf, plant height, and 1000 grain weight.
IV	21	20.19	Most extended flag leaf length, cob length, maximum grain yield per cob, medium leaf length, nod number and plant height, short leaf length, tassel height, grain weight per cob and 1000 grain weight.
V	5	4.8	Shortest tassel height and cob number, leaf length, flag leaf area, number of row per cob, highest grain yield per cob, 1000 grain weight, grain weight per cob.
VI	20	19.23	Highest cob length and nod number and flag leaf length, lowest tassel height and cob number.
VII	4	2.88	Maximum number of grain yield per cob, cob length, medium 1000 grain weight, days to maturity and flag leaf area.

Table 3. Principal Component Analysis (PCA), eigenvectors, eigenvalues, cumulative variance and total variance, for quantitative traits of maize accessions during 2015.

Morphological traits	PC1	PC2	PC3	PC4	PC5
Eigenvalue	3.82	2.74	1.48	1.27	0.98
Cumulative	3.82	6.57	8.05	9.33	10.31
Total variance (%)	25.5	18.31	9.88	8.47	6.54
Cumulative (%)	25.5	43.85	53.73	62.2	68.75
Traits			**Eigenvectors**		
Days to field germination	-0.28	0.02	-0.10	0.18	-0.32
Plant height	-0.77	-0.15	-0.13	0.42	-0.02
Number of leaf/plant	-0.54	-0.21	-0.37	0.05	-0.16
Leaf length	-0.42	-0.19	0.54	0.13	0.38
Flag leaf area	-0.55	-0.02	0.35	-0.50	0.02
Flag leaf length	-0.60	0.11	0.47	-0.06	0.04
Stem diameter	-0.64	-0.15	0.14	-0.24	-0.09
Number of cob/plant	-0.52	-0.05	0.16	-0.32	-0.08
Ear height	-0.74	-0.02	-0.36	0.06	0.11
Ear diameter	-0.58	0.50	0.11	0.11	0.05
Tassel height	-0.32	-0.11	-0.59	-0.51	-0.14
Days to maturity	-0.12	0.21	0.34	0.33	-0.79
Node number	-0.67	-0.20	-0.20	0.51	0.13
Cob length	-0.11	0.89	-0.08	-0.01	0.03
Number of kernel /row	0.03	0.52	-0.05	0.14	0.36
Number of kernel/cob	-0.22	0.89	-0.06	0.03	0.03
1000 kernel weight	-0.24	0.11	-0.60	-0.24	-0.45

scattered on the plot. In 1st and 2nd PCs 14930, 14952, 15124, 15146, 15034, 15075, 15130, 15068, 15057, Haq Nawaz and 15124 show highest genetic diversity based on accessions number.

4. Discussion

Exploiting the genetic diversity for crop development is essential and it necessitates thorough examination of

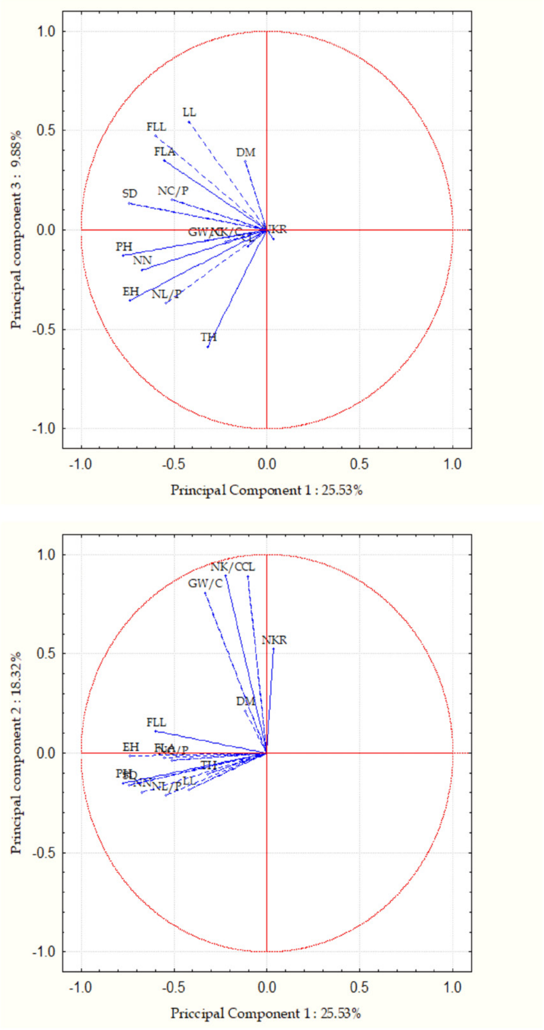

Figure 2. Contribution of agro-morphological characters in first three principal component analysis of maize during 2015.

all criteria relevant to genetic diversity (Iannetta et al., 2007). For this purpose, researchers are constantly studying wild and common varieties using cutting-edge breeding techniques. The degree of genetic variations is observed at some stage across both attempts to mount resistant and highly yield varieties (Mondini et al., 2009). One of the benchmarks to be reached is the conservation of crop genetic diversity, which is particularly important given the post-green revolution implications. Since germplasm is the only source to be used in order to explore new varieties during breeding programs, it is an evident that exploring the genetic variation is particularly important for the successful maintenance, assessment and use of accessions (Baranger et al., 2004). Due to the advancement in technologies, plant biologists are capable of inserting the more relevant genes into the genomes of plants and trying to silence or completely diminish the unwanted genes, in order to enhance the product as well as to create a suitable varieties (Narain et al., 2000). The purpose of germplasm collection is to conserve the genetic variability in cultivars as well as wild relatives (Williams et al., 1996). It is important to collect, manage and utilize the germplasm in effective way for the development and exploitations of new varieties.

Maize germplasm from China, Japan and various locations in Pakistan was analyzed using agro-morphological, biochemical and molecular markers (Iqbal et al., 2014). However, the biochemical markers included the calculation of total protein profiling deposited in their seeds, while DNA-based molecular markers included basic sequence repeats for the assessment of genetic diversity. Significant genetic diversity was predicted, which is in accordance with the findings of Aliu et al. (2013) and Shrestha et al. (2013), who reported a similar genetic diversity in maize accessions. The results of this study are more effective for identifying the genetic relation among the maize varieties, in terms of evaluation of genetic diversity, agro-morphological categorization and order to find out the relationship between trait and

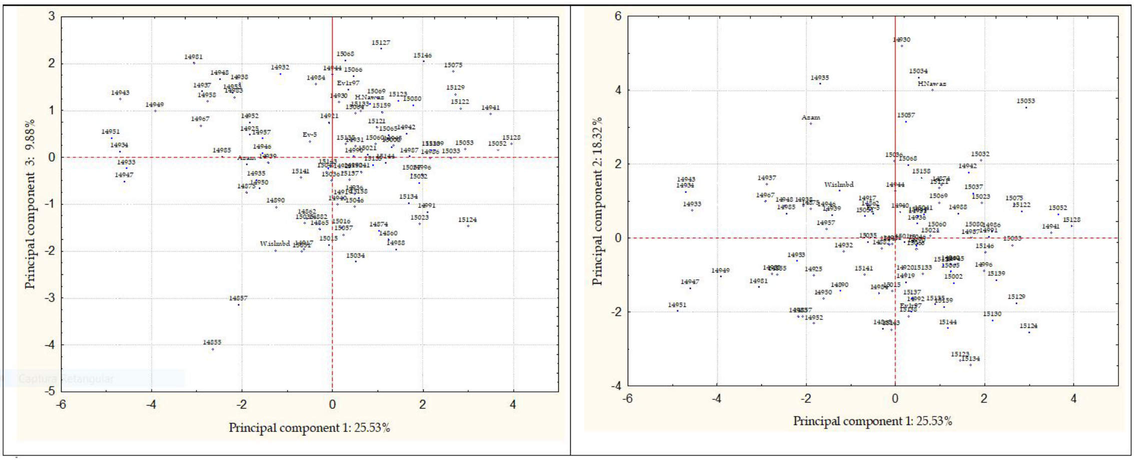

Figure 3. Two dimensional scatter plots (PC-I and PC-II) of the genetic relationship among 104 *Zea mays L.* accessions as revealed by first three principal components.

genes similar to Pervaiz et al. (2010b). The influence of each component on overall variance is measured, using principal component analysis. Higher the value of coefficients with respect to the direction (positive or negative), the more efficient they will be in distinguishing between accessions (Vishnu et al., 2020). In the present study, Guei et al. (2004) amalgamations method was imploded, and it was found a high significant discriminating variation patterns among the first three principal components of maize accessions and traits related with these are more appropriate in distinguishing germplasm.

Quantitative traits such as ear diameter, ear length, number of ears per plant, ear height, number of kernels per row, number of kernel rows, 1000 kernel weight, number of days to tasseling, and anthesis silking interval can be useful for breeding programs when used in combination with a few or more others. The dendrogram divided all genotypes into five clusters based on their similarity; A, B, C, D, and E. The cross between accessions from clusters A and cluster E will be more fruitful, that is consistent with Sherma et al. (2013). The presence of genetic divergence among genotypes was equated to their distribution into several clusters. As a result, the germplasm from northern Pakistan was divided into four different clusters based on agro-morphological performance, indicating a broad genetic base. Based on the general distribution of the examined germplasm based on agro-morphological features, it can be stated that maize landraces in Pakistan have a large degree of genetic variability that can be utilized to generate profitable varieties. Because some accessions evaluated with shorter life cycles than the control varieties, it is necessary to capitalize on this enormous amount of genetic variety by generating varieties with early maturity. The accessions collected from areas with severe environmental situations like low quality soils, drought, and higher radiations, as well as variation traits such as early flowering, short stature, and tapered leaves, provide the basis and opportunity for development of drought resistant varieties for those areas of country that having water shortage. Various agro-morphological parameters showed considerable levels of variation throughout the current study, which can be utilized to design high yielding cultivars effectively. On the one hand, the selection process encourages the growth of cultivars, but it also reduces the genetic variability in gene pool, that is crucial for preservation and utilization in future (Pervaiz et al., 2010a). The small variation in prior studies results may be due to differences in the genetic makeup of investigational material and environmental factors (Iqbal et al., 2014). In current research work all presented data allowed the identification of accessions with diverse morphological traits that might be used as promising parents for maize. However principal component analysis organized the accessions with more morphological comparisons but clusters also comprised the accessions from different or far by sites for maize improvement program.

5. Conclusion

Notable genetic diversity was found in these maize accessions for agro-morphological traits i.e. plant height, grain yield per plant, 1000 grain weight in the studied germplasm. Similarly, high yielding accessions with broader genetic base were identified on the basis of their field performance. This higher genetic diversity during present research can be translated for maize germplasm conservation, categorization and future breeding as well as improvement programs of maize cultivars.

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