

GENETIC DIVERGENCE IN MAIZE (*ZEA MAYS* L.) GERMPLASM USING QUANTITATIVE AND QUALITATIVE TRAITS

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Abstract

Agro-morphological characterization of germplasm is equally vital for the accurate conservation and successful breeding programs. During the present investigation the magnitude of genetic divergence was elucidated among 150 collections of maize of Chinese, Japanese and Pakistani origin. Three commercial varieties named Agaiti-2002, Sadaf and Sahiwal-2002 were used as check for comparison. All genotypes were evaluated for 24 quantitative and 10 qualitative agro-morphological traits under open field conditions at Plant Genetic Resources Institute (PGRI), National Agricultural Research Center (NARC) Islamabad, Pakistan during spring-2011. Basic statistical analysis showed maximum variability in grain yield per plant, plant height, 1000 kernel weight, ear height, leaf length and days to harvest among the tested genotypes. Simple correlation coefficient portrayed that some functionally related variables were significantly correlated. Cluster analysis of the data reflected high level of genetic divergence for most of the agro-morphological traits among the studied accessions such as maximum Euclidean genetic distance of 17.8 was observed between the accession 15232 and 15014 followed by 17.7 between 15329 and 15232. Accessions collected from the province of Khyber Pakhtunkhwa were noted with the highest genetic diversity followed by the accessions from Punjab, Baluchistan and Northern Areas. Principal component analysis based on quantitative agro-morphological traits further strengthened these findings. The present investigation revealed valuable amount of genetic variability among the tested maize genotypes which could be cashed for the maximum by further utilization in maize breeding strategies as a source of broad genetic base.

Key Words: Maize germplasm, Genetic diversity, Agro-morphological traits, Correlation, Multivariate analysis

Introduction

Maize (*Zea mays* L.) has tremendous productive potential. Among cereal crops it occupies the first position in production and consumption globally with 861 million tones and 864 million tones, followed by wheat and rice, while third in covered area after wheat and rice (Anon., 2014). In Pakistan it is 5th in importance among all crops and 3rd among cereals with the production of 4.63 million tones after wheat with 24.23 million tones and rice with 5.54 million tones. Its share in the value added crops is 2.2%, in the total cropped area under cereal is 8.98% and in the total cereal production is 13.46%. In terms of contribution by provinces, Punjab is the major producer with 79.3% of the total production by occupying 55.54 % of the total area, followed by Khyber Pakhtunkhwa (KP) with 20.46% of the total production and 43.71 % of the total area, while remaining 0.24 % of the production is the part of Sindh and Baluchistan by occupying 0.75 % of the total area under maize cultivation (Farooq, 2013). As compared to global yield, Pakistan is far behind with yield of 4268 kg/ha and occupies the position of 41st throughout the world (Anon., 2013). To fulfill the needs of food, feed, industrial raw material and energy, it is necessary to increase the maize production by exploiting all the available resources (Marwat *et al.*, 2007; Khan *et al.*, 2014). The development of high yielding, early varieties and hybrids locally can be proved the most possible approach, which will make easier even for low scale farmers to get productive varieties and hybrids cost effective. That is why the screening of new maize germplasm is necessary to get lines with more genetic diversity for the

development of varieties and hybrids with broad genetic base. Genetically more diverse species are capable to survive and flourish for more number of generations. They can withstand various kinds of biotic and abiotic stresses. The knowledge of genetic variability is therefore immensely needed to characterize the genetic diversity present in the plant genetic resources (Shinwari *et al.*, 2014) and its potential utilization by breeders, to identify possible genetic diversity erosion, to elucidate evidence of the evolutionary forces cause genetic diversity and to select elite genotypes for conservation (Thormann *et al.*, 1994). It mainly depends upon the study of all parameters related to the exploration of genetic diversity (Iannetta *et al.*, 2007). For the effective conservation, management and utilization of plant genetic resources, sufficient knowledge about the vital biological phenomena that occur in plants and magnitude of genetic diversity is necessary (Iqbal *et al.*, 2014). The wild relatives and traditional varieties are continuously studied to shape resistant and high yielding varieties (Mondini *et al.*, 2009; Shinwari *et al.*, 2013) and hybrids with more genetic diversity, which are supplied finally to farmers to get the economic return (Vaughan & Jackson, 1995). There are different methodologies i.e. morphological, biochemical and molecular markers to investigate the extent of genetic variability (Shengwu *et al.*, 2003; Iqbal *et al.*, 2014), but morphological characterization is the initial step (Smith & Smith, 1989; Sultan *et al.*, 2012; Ali *et al.*, 2015). During the present study 150 accessions and three commercial varieties were evaluated for 24 quantitative and 10 qualitative traits to estimate the level of genetic diversity in the tested maize germplasm and select elite lines rich in genetic diversity for breeding purpose.

Materials and Methods

A total of 150 accessions and three commercial varieties named Agaiti-2002, Sadaf and Sahiwal-2002 were obtained from national gene bank of Plant Genetic Resource Institute (PGRI), National Agricultural Research Center (NARC) Islamabad (Table 1). All the genotypes were characterized through 34 agromorphological traits according to the standard descriptors for maize (IBPGR, 1991) in open field at PGRI, NARC, Islamabad, during spring-2011. Among these 24 were quantitative traits (i.e. days to field germination (DFG), days to tassel (DT), days to pollen shedding (DPS), days to silk (DS), anthesis-silking

interval (ASI), plant height (PH), number of leaves per plant (NL/P), leaf length (LL), leaf width (LW), ear height (EH), number of ears per plant (NE/P), days to ear leaf senescence (DELS), days to harvest (DH), field weight per plot (FW/Pl), ear length (EL), ear diameter (ED), number of kernel rows (NKR), number of kernels per row (NK/R), kernel length (KL), kernel width (KW), cob diameter (CD), 1000 kernel weight (1000KW), harvest grain moisture (HGM), grain yield per plant (GY/P) and 10 qualitative traits (i.e. leaf orientation (LO), pubescence of culm (PbC), tassel type (TT), tassel size (TS), husk cover (HC), ear damage (EDa), kernel row arrangement (KRA), kernel type (KT), kernel color (KC) and cob color (CC).

Table 1. List of maize (*Zea mays* L.) germplasm and their passport data used in the present study.

No.	Accession	Collecting Organization	Year	Province Name	Altitude	Origin
01	14862	MSM/NARC	1.12.81	Punjab	1880	Pakistan
02	14878	PARC/SVP	1.05.81	Baluchistan	1880	Pakistan
03	14880	PARC/SVP	1.06.81	Baluchistan	2100	Pakistan
04	14883	PARC/SVP	1.06.81	Baluchistan	2100	Pakistan
05	14884	PARC/SVP	1.06.81	Baluchistan	2100	Pakistan
06	14886	PARC/SVP	1.06.81	Baluchistan	1600	Pakistan
07	14894	PARC/SVP	1.05.81	Baluchistan	1550	Pakistan
08	14895	PARC/SVP	1.05.81	Baluchistan	1940	Pakistan
09	14898	PARC/SVP	1.05.81	Baluchistan	1650	Pakistan
10	14899	PARC/SVP	1.05.81	Baluchistan	1730	Pakistan
11	14900	PARC/SVP	1.05.81	Baluchistan	1730	Pakistan
12	14903	PARC/SVP	1.05.81	Baluchistan	1840	Pakistan
13	14904	PARC/SVP	1.05.81	Baluchistan	1970	Pakistan
14	14906	PARC/SVP	1.05.81	Baluchistan	2200	Pakistan
15	14907	PARC/SVP	1.05.81	Baluchistan	2320	Pakistan
16	14909	PARC/SVP	1.05.81	Baluchistan	1850	Pakistan
17	14911	PARC/SVP	1.06.81	Baluchistan	2000	Pakistan
18	14912	PARC/SVP	1.06.81	Baluchistan	2370	Pakistan
19	14913	PARC/SVP	1.06.81	Baluchistan	2370	Pakistan
20	14914	PARC/SVP	1.06.81	Baluchistan	2050	Pakistan
21	14915	PARC/SVP	1.06.81	Baluchistan	2050	Pakistan
22	14924	PARC/SVP	1.06.81	Baluchistan	2280	Pakistan
23	14954	PARC/IBPGR	1.06.82	AJK	1050	Pakistan
24	14959	PARC/IBPGR	1.06.82	AJK	1550	Pakistan
25	14979	PARC/IBPGR	1.06.82	AJK	1400	Pakistan
26	14987	PARC/IBPGR	1.06.82	AJK	1500	Pakistan
27	14993	PARC/IBPGR	1.06.82	AJK	1360	Pakistan
28	15000	PARC/IBPGR	1.06.82	AJK	1700	Pakistan
29	15007	PARC/IBPGR	1.06.82	AJK	1125	Pakistan
30	15009	PARC/IBPGR	1.06.82	AJK	1650	Pakistan
31	15011	PARC/IBPGR	1.10.82	Punjab	0290	Pakistan
32	15012	PARC/IBPGR	1.10.82	Punjab	0300	Pakistan
33	15013	PARC/IBPGR	1.10.82	Punjab	0225	Pakistan
34	15014	PARC/IBPGR	1.10.82	Punjab	0130	Pakistan
35	15020	PARC/IBPGR	1.01.83	N.A.	1470	Pakistan
36	15024	PARC/IBPGR	1.01.83	N.A.	1550	Pakistan
37	15026	PARC/IBPGR	1.04.83	Punjab	0300	Pakistan
38	15027	PARC/IBPGR	1.04.83	Punjab	0280	Pakistan
39	15030	CHINA	1.09.83	Unknown	Unknown	China
40	15043	CHINA	1.09.83	Unknown	Unknown	China
41	15044	CHINA	1.09.83	Unknown	Unknown	China
42	15047	PARC/CHINA	1.09.83	Punjab	0350	Pakistan

Table 1. (Cont'd).

No.	Accession	Collecting Organization	Year	Province Name	Altitude	Origin
43	15071	PARC/CHINA	1.09.83	Punjab	1180	Pakistan
44	15074	PARC/IBPGR	1.10.84	KP	1480	Pakistan
45	15090	PARC/IBPGR	1.10.84	KP	1980	Pakistan
46	15092	PARC/IBPGR	1.10.84	KP	1950	Pakistan
47	15096	PARC/IBPGR	1.10.84	KP	1550	Pakistan
48	15097	PARC/IBPGR	1.10.84	KP	1560	Pakistan
49	15106	PARC/IBPGR	1.10.84	KP	1300	Pakistan
50	15107	PARC/IBPGR	1.10.84	KP	0890	Pakistan
51	15108	PARC/IBPGR	1.10.84	KP	0680	Pakistan
52	15117	PARC/IBPGR	1.10.84	KP	2350	Pakistan
53	15118	PARC/IBPGR	1.10.84	KP	2350	Pakistan
54	15145	PARC/KUJ	1.09.87	KP	1480	Pakistan
55	15149	PARC/KUJ	1.09.87	N.A.	2070	Pakistan
56	15160	UAF/FAISALABAD	1.11.89	Punjab	Unknown	Pakistan
57	15162	UAF/FAISALABAD	1.11.89	Punjab	Unknown	Pakistan
58	15168	PARC/NIAR	1.10.89	KP	1920	Pakistan
59	15172	PARC/NIAR	1.10.89	KP	Unknown	Pakistan
60	15209	PARC/NIAR	1.11.89	Baluchistan	1650	Pakistan
61	15215	PARC/NIAR	1.10.91	KP	1380	Pakistan
62	15227	PARC/JICA	1.07.96	KP	2830	Pakistan
63	15228	PARC/JICA	1.07.96	KP	1725	Pakistan
64	15231	PARC/JICA	1.07.96	KP	1990	Pakistan
65	15232	PARC/JICA	1.07.96	KP	2175	Pakistan
66	15236	PARC/JICA	1.06.96	KP	1855	Pakistan
67	15244	PARC/JICA	1.07.96	N.A.	2275	Pakistan
68	15250	PARC/JICA	1.07.96	N.A.	2245	Pakistan
69	15258	KAES-MAFF	1.12.96	Unknown	Unknown	Japan
70	15262	KAES-MAFF	1.12.96	Unknown	Unknown	Japan
71	15263	KAES-MAFF	1.12.96	Unknown	Unknown	Japan
72	15264	KAES-MAFF	1.12.96	Unknown	Unknown	Japan
73	15265	KAES-MAFF	1.12.96	Unknown	Unknown	Japan
74	15276	KAES-MAFF	1.12.96	Unknown	Unknown	Japan
75	15277	KAES-MAFF	1.12.96	Unknown	Unknown	Japan
76	15278	KAES-MAFF	1.12.96	Unknown	Unknown	Japan
77	15279	KAES-MAFF	1.12.96	Unknown	Unknown	Japan
78	15280	KAES-MAFF	1.12.96	Unknown	Unknown	Japan
79	15290	KAES-MAFF	1.12.96	Unknown	Unknown	Japan
80	15296	KAES-MAFF	1.12.96	Unknown	Unknown	Japan
81	15302	KAES-MAFF	1.12.96	Unknown	Unknown	Japan
82	15304	KAES-MAFF	1.12.96	Unknown	Unknown	Japan
83	15306	KAES-MAFF	1.12.96	Unknown	Unknown	Japan
84	15308	KAES-MAFF	1.12.96	Unknown	Unknown	Japan
85	15310	KAES-MAFF	1.12.96	Unknown	Unknown	Japan
86	15311	KAES-MAFF	1.12.96	Unknown	Unknown	Japan
87	15317	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
88	15318	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
89	15319	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
90	15321	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
91	15325	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
92	15326	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
93	15327	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
94	15328	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
95	15329	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
96	15330	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
97	15331	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
98	15332	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
99	15333	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan

Table 1. (Cont'd).

No.	Accession	Collecting Organization	Year	Province Name	Altitude	Origin
100	15334	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
101	15336	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
102	15338	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
103	15339	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
104	15340	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
105	15341	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
106	15342	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
107	15343	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
108	15345	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
109	15346	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
110	15347	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
111	15348	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
112	15349	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
113	15350	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
114	15351	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
115	15352	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
116	15353	CRP-F/NARC	1.05.97	Punjab	Unknown	Pakistan
117	19188	PARC/JICA	12.10.97	KP	1490	Pakistan
118	19195	PARC/JICA	13.10.97	KP	2200	Pakistan
119	19198	PARC/JICA	14.10.97	KP	2750	Pakistan
120	24669	PGRI/NARC	5.11.07	KP	0520	Pakistan
121	24670	PGRI/NARC	5.11.07	KP	0630	Pakistan
122	24671	PGRI/NARC	5.11.07	KP	0600	Pakistan
123	24672	PGRI/NARC	5.11.07	KP	0600	Pakistan
124	24674	PGRI/NARC	6.11.07	N.A.	1180	Pakistan
125	24675	PGRI/NARC	6.11.07	N.A.	1140	Pakistan
126	24676	PGRI/NARC	6.11.07	N.A.	1140	Pakistan
127	24677	PGRI/NARC	7.11.07	N.A.	1560	Pakistan
128	24678	PGRI/NARC	7.11.07	N.A.	1500	Pakistan
129	24679	PGRI/NARC	7.11.07	N.A.	1500	Pakistan
130	24680	PGRI/NARC	8.11.07	N.A.	1540	Pakistan
131	24682	PGRI/NARC	8.11.07	N.A.	1950	Pakistan
132	24683	PGRI/NARC	9.11.07	N.A.	2433	Pakistan
133	24684	PGRI/NARC	9.11.07	N.A.	2433	Pakistan
134	24685	PGRI/NARC	20.11.07	AJK	0550	Pakistan
135	24686	PGRI/NARC	20.11.07	AJK	0550	Pakistan
136	24687	PGRI/NARC	20.11.07	AJK	0570	Pakistan
137	24688	PGRI/NARC	20.11.07	AJK	0610	Pakistan
138	24689	PGRI/NARC	20.11.07	AJK	0600	Pakistan
139	24690	PGRI/NARC	21.11.07	AJK	0520	Pakistan
140	24691	PGRI/NARC	21.11.07	AJK	0396	Pakistan
141	24692	PGRI/NARC	21.11.07	AJK	0680	Pakistan
142	24693	PGRI/NARC	21.11.07	AJK	0735	Pakistan
143	24694	PGRI/NARC	21.11.07	AJK	0735	Pakistan
144	24695	PGRI/NARC	22.11.07	AJK	0675	Pakistan
145	24696	PGRI/NARC	22.11.07	AJK	0710	Pakistan
146	24697	PGRI/NARC	22.11.07	AJK	0710	Pakistan
147	24698	PGRI/NARC	22.11.07	AJK	0770	Pakistan
148	24699	PGRI/NARC	22.11.07	AJK	1065	Pakistan
149	24700	PGRI/NARC	23.11.07	AJK	1670	Pakistan
150	24701	PGRI/NARC	23.11.07	AJK	1670	Pakistan

Check varieties used as standard for reference:

- Agaiti-2002 (MMRI, Yousafwala, Sahiwal).
- Sadaf (MMRI, Yousafwala, Sahiwal).
- Sahiwal-2002 (MMRI, Yousafwala, Sahiwal).

Data Analysis: The data obtained from quantitative traits was made average and analyzed for simple statistical approaches such as mean, minimum, maximum, standard deviation and coefficient of variation to determine the magnitude of genetic variability among the maize genotypes. The data recorded from qualitative traits was converted into different classes and frequency percentage was calculated. Following the Steele & Torrie (1980) approach, the simple correlation coefficients for all pairs of quantitative traits were obtained using mean values. The whole agro-morphological data was analyzed by applying cluster and principal component analyses (PCA), through numerical taxonomic approaches following the Sneath & Sokal (1973). To avoid the scaling differences, the means of each trait were standardized using Z-scores prior to carry out the cluster analysis and PCA. Estimates of Euclidean distance coefficients were carried out for all pairs of genotypes. The resulted matrices of Euclidean dissimilarity coefficient were applied to assess the genetic relationships between maize genotypes with a cluster analysis through complete linkage way i.e. NTSYS-pc, version 2.1 (Applied Biostatistics Inc., USA). The same data matrices were utilized to carry out the PCA. Scatter plots of the first three principal components were made to determine the graphical display of the pattern of genetic diversity among the maize genotypes (Statistica, version 7.0).

Results

Descriptive statistics of the data recorded reflected high level of variation for the quantitative characters. All the traits were more or less, directly or indirectly, positively or negatively added to the yield and possessed key genetic status during the identification of productive

genotypes. The basic statistical data (mean, minimum, maximum, standard deviation, coefficient of variation and variance) for every quantitative character noted for all the genotypes during 2011 is given in Table 2. Maximum variability was observed in grain yield per plant, plant height, 1000 kernel weight, ear height, leaf length and days to harvest. The variances for the cited traits were 3394.8, 1243.3, 701.9, 534.3, 86.9 and 47.2, respectively.

The correlation study of the agro-morphological showed that important and functionally related characters presented significant level of correlation with each other (Table 3). The highest level of correlation ($r = 0.98^{**}$) was noted between the number of days to tassel and number of days to pollen shedding and between number of days to silking and number of days to pollen shedding, followed by 97 percent between number of days to tassel and number of days to silking. Similarly number of leaves per plant and plant height were highly significantly positively correlated with 82 percent. Days to harvest had highly significant positive correlation with several traits such as with number of days to tassel, days to pollen shedding, days to silking, number of leaves per plant and grain yield per plant. The anthesis-silking interval was highly significantly negatively correlated with grain yield per plant ($r = -0.53^{**}$). The plant height showed highly significant positive correlation with field weight per plot ($r = 0.52^{**}$) and number of leaves per plant ($r = 0.64^{**}$). Euclidean dissimilarity coefficient matrices were used to portray the relationship between maize accessions. The maximum Euclidean genetic distance of 17.8 was observed between the accession 15232 and 15014, indicating their diverse nature followed by 17.7 (between 15329 and 15232). Accession 24670 and 24669 were the most similar accessions having least genetic distance of 2.7.

Table 2. Basic statistical analysis of quantitative characters of maize during 2011.

Trait	Mean	Minimum	Maximum	SD	CV%	Variance
DFG	11.5	8.0	21.0	2.6	22.4	6.6
DT	63.0	46.0	74.0	6.5	10.3	42.0
DPS	66.9	50.0	78.0	6.5	9.7	42.0
DS	69.7	52.0	81.0	6.3	9.1	40.1
ASI	3.6	0.0	8.0	1.5	21.1	2.4
PH	153.6	71.9	226.1	35.3	23.0	1243.3
NL/P	11.1	6.4	15.2	2.1	18.9	4.4
LL	67.2	39.4	84.6	9.3	13.9	86.9
LW	7.4	4.4	10.3	1.3	17.5	1.7
EH	73.1	6.5	136.7	23.1	31.6	534.3
NE/P	2.0	1.0	5.3	0.6	30.2	0.4
DELS	95.2	79.0	117.0	7.2	7.5	51.5
DH	112.4	100.0	126.0	6.9	6.1	47.2
FW/PI	0.1	0.008	0.2	0.04	51.8	0.002
EL	8.7	2.7	15.5	2.5	29.0	6.4
ED	3.5	1.8	4.9	0.6	16.8	0.3
NKR	7.8	2.0	16.0	2.6	33.6	6.9
NK/R	8.6	1.7	25.0	4.3	50.0	18.5
KL	7.4	4.5	12.2	1.3	17.7	1.7
KW	7.2	4.0	9.2	0.9	12.8	0.9
CD	2.4	1.2	1.3	0.9	38.1	0.9
1000KW	229.3	159.0	326.0	26.5	20.5	701.9
HGM	24.0	9.0	35.6	4.6	19.2	21.2
GY/P	108.9	35.0	312.0	58.3	53.5	3394.8

Table 3. Correlation among quantitative agro-morphological traits of maize genotypes during 2011.

Trait	FGD	DT	DPS	DS	ASI	PH	NL/P	LL	LW	EH	NE/P	DELS	DH	FW/PI	EL	ED	NKR	NK/R	KL	KW	CD	1000KW	HGM	GYP	
FGD	1.00																								
DT	0.08	1.00																							
DPS	0.10	0.98**	1.00																						
DS	0.11	0.97**	0.98**	1.00																					
ASI	0.12	-0.20*	-0.10	0.02	1.00																				
PH	0.13	0.54**	0.56**	0.53**	-0.06	1.00																			
NL/P	0.25**	0.48**	0.51**	0.49**	-0.03	0.82**	1.00																		
LL	0.27**	0.34**	0.35**	0.34**	-0.05	0.78**	0.70**	1.00																	
LW	0.21**	0.35**	0.38**	0.35**	-0.06	0.64**	0.79**	0.71**	1.00																
EH	0.18*	0.48**	0.49**	0.48**	0.00	0.92**	0.80**	0.73**	0.62**	1.00															
NE/P	-0.04	0.08	0.08	0.08	-0.02	0.25**	0.21**	0.16*	0.16*	0.25**	1.00														
DELS	0.11	0.64**	0.66**	0.63**	-0.09	0.71**	0.65**	0.59**	0.52**	0.61**	0.10	1.00													
DH	0.20*	0.53**	0.55**	0.52**	-0.08	0.70**	0.68**	0.56**	0.47**	0.63**	-0.01	0.68**	1.00												
FW/PI	0.23**	0.32**	0.33**	0.30**	-0.12	0.52**	0.64**	0.59**	0.73**	0.53**	0.00	0.48**	0.42**	1.00											
EL	0.14	0.19*	0.19*	0.18*	-0.07	0.37**	0.41**	0.43**	0.49**	0.38**	-0.01	0.32**	0.19*	0.73**	1.00										
ED	0.20*	0.29**	0.30**	0.28**	-0.08	0.57**	0.66**	0.62**	0.76**	0.53**	0.03	0.51**	0.38**	0.84**	0.61**	1.00									
NKR	0.03	-0.02	-0.02	-0.01	0.06	0.17*	0.15	0.25**	0.10	-0.18*	0.07	-0.05	0.57**	0.61**	0.53**	1.00									
NK/R	0.11	0.05	0.08	0.05	-0.05	0.18*	0.22**	0.29**	0.33**	0.17*	-0.01	0.11	0.12	0.32**	0.31**	0.28**	0.22**	1.00							
KL	0.13	0.23**	0.25**	0.21**	-0.12	0.48**	0.59**	0.54**	0.66**	0.44**	0.06	0.39**	0.39**	0.78**	0.59**	0.77**	0.57**	0.37**	1.00						
KW	0.11	0.17*	0.17*	0.18*	0.00	0.24**	0.16*	0.16*	0.09	0.20*	0.17*	0.16*	0.14	-0.11	-0.09	-0.05	-0.36**	-0.20*	-0.19*	1.00					
CD	0.09	-0.09	-0.09	-0.12	-0.11	0.11	0.11	0.11	0.14	0.11	0.02	0.12	0.05	0.19*	0.10	0.24**	0.16*	0.09	0.14	-0.07	1.00				
1000KW	0.01	0.07	0.09	0.07	-0.05	0.23**	0.22**	0.12	0.27**	0.22**	0.20*	0.24**	0.03	0.22**	0.15	0.29**	0.03	0.04	0.21**	0.09	0.41**	1.00			
HGM	0.19*	0.38**	0.39**	0.36**	-0.11	0.39**	0.43**	0.42**	0.43**	0.34**	-0.01	0.30**	0.41**	0.53**	0.37**	0.43**	0.18*	0.22**	0.49**	0.19*	-0.14	-0.23**	1.00		
GYP	0.12	0.26**	0.23**	0.15	-0.53**	0.31**	0.32**	0.25**	0.33**	0.26**	0.07	0.28**	0.29**	0.32**	0.29**	0.35**	0.41**	0.43**	0.30**	0.07	0.13	0.31**	0.21**	1.00	

*Significant at 0.05 (5%) probability level; ** Highly significant at 0.01 (1%) probability level.

To elucidate the patterns of genetic variability, principal component analysis was carried out on the basis of quantitative agro-morphological traits for all genotypes by taking into account simultaneously all the variables. It was detected that 6 of the principal components with an Eigenvalue more than 1.0, accounted for 73.89 percent of the overall variations (Table 4, Figs. 2 and 3). The first principal component (PC1) accounted for 36.97 percent of the total which determined primarily the genetic variability in anthesis-silking interval (0.137) which was positive load and in the number of leaves per plant (-0.876) was negative load on PC1, PC2 for 12.89 percent mainly portrayed the variations in number of kernel rows (0.698) was positive load and in days to silking (-0.582) was negative load on PC2, PC3 for 7.56 percent mainly in harvest grain moisture (0.407) positive and in 1000KW (-0.608) was negative load on PC3, PC4 comprised of 6.38 percent mainly enlightened the variability in anthesis-silking interval (0.799) was positive load and grain yield per plant (-0.598) was negative load on PC4, PC5 accounted for 5.62 percent of the total genetic divergence mainly depicted the variations in grain yield per plant (0.444) was positive load and 1000KW (-0.457) was negative load on PC5 and PC6 accounted for 4.47 percent of the total variations mainly illuminated the divergence in days to field germination of seeds (0.663) was positive load and number of ears per plant (-0.597) was negative load on PC6. Hierarchical cluster analysis on the basis of 34 agro-morphological traits divided the tested genotypes into main

clusters, I, II, III, IV and V by using complete linkage method at dissimilarity coefficient value of 12.6 during 2011 (Fig. 1). The mean and standard deviation calculated for each agro-morphological trait included which resulted in 5 clusters presented in Table 5, while prominent features of the genotypes are portrayed in Table 6. Cluster-I consisted of 64 genotypes grouped together because of the presence of maximum plant height, ear height, leaf length, leaf width, ear diameter, number of days to tassel and silking, number of leaves per plant and grain yield per plant. Cluster-II comprised of 63 genotypes having the largest number of ears per plant, maximum kernel width and 1000 kernels weight. Cluster-III possessed 24 genotypes with distinguishing characters of the longest anthesis-silking interval, the lowest plant height, ear height, the shortest leaf length, the least number of days to ear leaf senescence and the lowest grain yield per plant. Cluster-IV consisted of only one accession with the shortest anthesis-silking interval, the least number of ears per plant, number of kernel rows and number of kernels per row, the shortest ear diameter, kernel length and kernel width. Similarly cluster-V also comprised of a single accession with the minimum number of days to tassel and silking, number of leaves per plant, leaf width, cob diameter and minimum grain yield per plant. Genotypes collected from the province of KP shared maximum 4 clusters I, II, III and V, followed by the accessions from the Punjab, Baluchistan and Northern Areas, while the Chinese accessions converged in only one cluster II (Table 7.).

Table 4. Principal component analysis for quantitative agro-morphological traits in maize during 2011.

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	8.87	3.09	1.82	1.53	1.35	1.07
Cumulative Eigenvalue	8.87	11.97	13.78	15.31	16.66	17.73
Total Variance (percent)	36.97	12.89	7.56	6.38	5.62	4.47
Cumulative Variance (percent)	36.97	49.86	57.42	63.81	69.42	73.89
Trait	Eigenvectors					
Days to field germination (FGD)	-0.254	0.048	-0.037	0.313	0.211	0.663
Day to tassel (DT)	-0.657	-0.565	0.311	-0.254	-0.219	0.005
Days to pollen shedding (DPS)	-0.673	-0.564	0.303	-0.172	-0.261	0.017
Days to silk (DS)	-0.640	-0.582	0.319	-0.084	-0.316	0.019
Anthesis-silk interval (ASI)	0.137	-0.021	0.020	0.799	-0.405	0.056
Plant height (PH)	-0.859	-0.199	-0.240	0.122	0.036	-0.095
Number of leaves per plant (NL/P)	-0.876	-0.044	-0.170	0.153	0.054	-0.022
Leaf length (LL)	-0.791	0.052	-0.179	0.242	0.180	-0.011
Leaf width (LW)	-0.818	0.198	-0.147	0.097	0.078	-0.070
Ear height (EH)	-0.812	-0.147	-0.249	0.193	0.010	-0.086
Number of ears per plant (NE/P)	-0.151	-0.174	-0.486	0.057	0.051	-0.597
Days to ear leaf senescence (DELS)	-0.773	-0.275	-0.054	-0.067	-0.176	0.073
Days to harvest (DH)	-0.711	-0.295	0.013	0.060	0.103	0.194
Field weight per plot (FW/P)	-0.797	0.449	0.121	-0.022	-0.012	0.011
Ear length (EL)	-0.587	0.495	0.185	0.009	-0.065	-0.090
Ear diameter (ED)	-0.793	0.423	-0.025	-0.022	-0.043	0.020
Number of kernel rows (NKR)	-0.301	0.698	0.336	-0.045	-0.253	-0.046
Number of kernels per row (NK/R)	-0.312	0.353	0.128	0.053	0.042	-0.074
Kernel length (KL)	-0.720	0.488	0.103	-0.037	0.013	-0.122
Kernel width (KW)	-0.126	-0.479	-0.306	0.176	0.328	0.070
Cob diameter (CD)	-0.139	0.313	-0.499	-0.265	-0.280	0.392
1000 kernel weight (1000KW)	-0.246	0.117	-0.608	-0.240	-0.457	0.057
Harvest grain moisture (HGM)	-0.571	0.010	0.407	0.130	0.416	-0.046
Grain yield per plant (GY/P)	-0.413	0.020	-0.122	-0.598	0.444	0.114

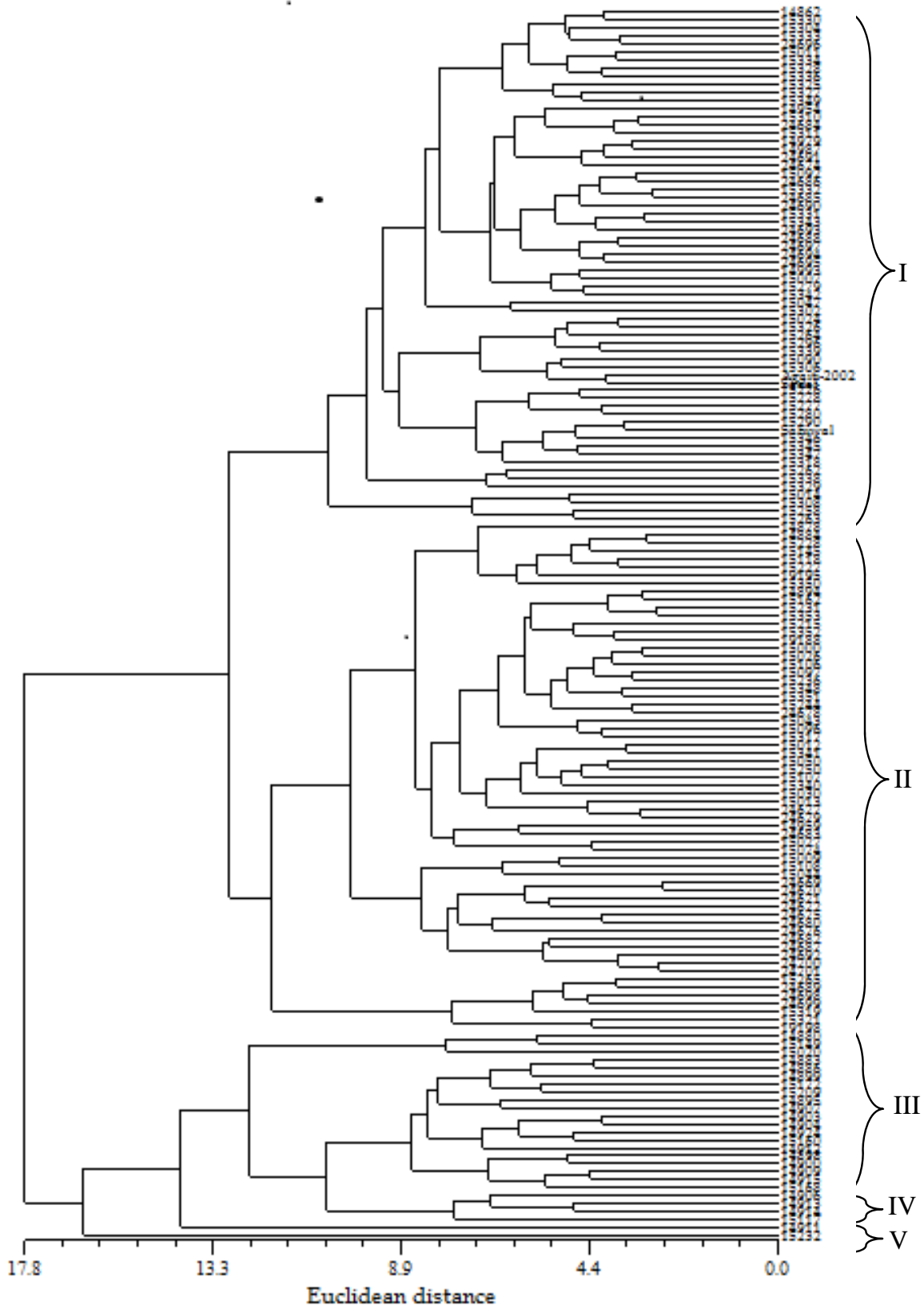


Fig.1. Dendrogram portraying the genetic relationship among maize genotypes on the basis of 34 agro-morphological traits during 2011.

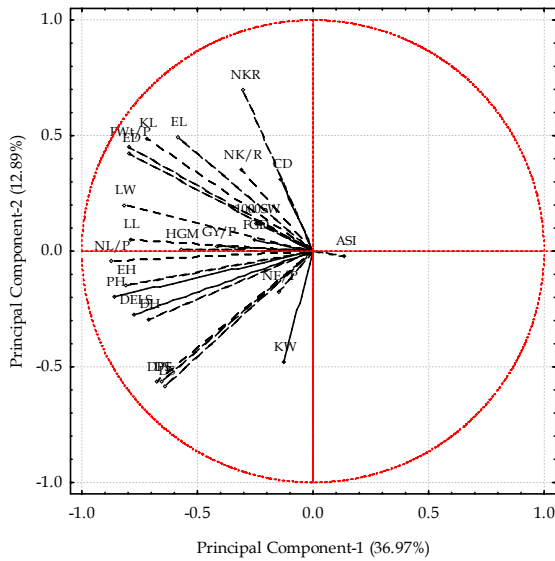


Fig.2. Contribution of quantitative agro-morphological traits in 1st and 2nd principal components in maize during 2011.

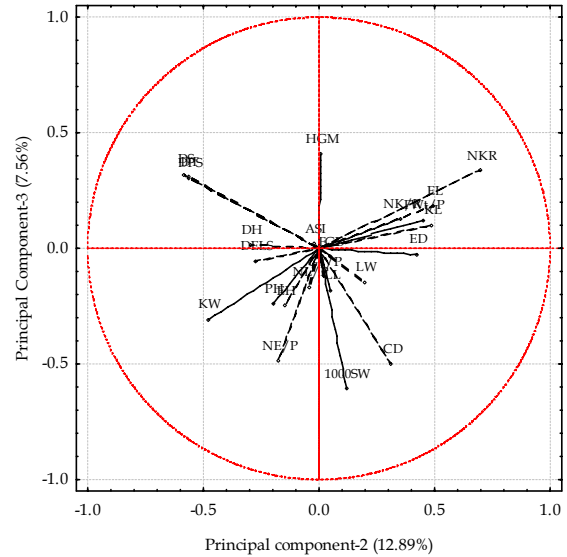


Fig.3. Contribution of quantitative agro-morphological traits in 1st and 3rd principal components in maize during 2011.

Table 5. Mean and standard deviation of five main clusters based on agro-morphological traits during 2011.

Trait	Cluster-I	Cluster-II	Cluster-III	Cluster-IV	Cluster-V
DFG	11.84±2.42	11.44±2.76	10.58±2.28	8.00	12.00
DT	65.94±5.11	62.37±5.90	58.00±6.58	51.00	47.00
DPS	69.84±5.09	66.40±5.77	61.54±6.41	53.00	50.00
DS	72.47±5.00	69.20±5.79	65.08±6.38	56.00	52.00
ASI	6.50±1.35	6.78±1.34	6.96±1.71	5.00	5.00
PH	174.63±20.19	155.04±27.44	97.54±18.64	97.92	112.24
NL/P	12.46±1.35	10.97±1.49	7.98±1.10	7.20	7.20
LL	73.05±5.82	66.31±6.97	54.55±8.89	59.56	55.52
LW	8.30±0.85	7.14±0.94	5.63±0.69	5.64	4.98
EH	86.27±15.51	73.52±18.64	39.44±14.43	46.20	44.52
NE/P	1.93±0.51	2.23±0.72	1.76±0.38	2.00	2.00
DELS	98.89±6.00	95.48±5.57	85.17±3.51	87.00	89.00
DH	116.14±5.18	111.54±6.31	105.17±5.55	103.00	105.00
FW/PI	0.12±0.03	0.06±0.02	0.03±0.02	0.02	0.02
EL	10.34±2.02	7.75±2.16	7.01±2.10	4.90	5.62
ED	3.91±0.35	3.33±0.39	2.73±0.45	2.21	2.60
NKR	8.96±2.45	6.76±2.41	7.87±2.45	4.80	6.33
NK/R	10.07±4.75	7.64±3.98	7.30±2.78	4.80	9.00
KL	8.39±1.01	6.94±0.71	6.24±1.41	5.30	5.40
KW	7.17±1.03	7.49±0.69	6.73±0.97	6.18	6.30
CD	2.63±0.33	2.37±0.32	1.98±0.35	1.62	1.30
1000KW	229.73±19.22	235.43±26.26	210.15±29.03	184.00	320.80
HGM	27.07±3.63	22.12±3.54	21.35±4.08	20.00	9.00
GY/P	130.94±69.19	98.32±39.88	78.00±48.64	104.00	109.00

Table 6. Total number of genotypes per cluster, percentage and prominent features of maize germplasm separated into five main clusters during 2011.

Cluster	Total Genotypes	Percentage	Prominent Features
I	64	41.8	Maximum plant height, ear height, leaf length, width, ear diameter, number of days to tassel and silking, and grain yield per plant.
II	63	41.1	Largest number of ears per plant, maximum kernel width and 1000 kernels weight.
III	24	15.7	Longest anthesis-silking interval, the lowest plant height, ear height, shortest leaf length, the least number of days to ear leaf senescence and grain yield per plant.
IV	01	0.7	Shortest anthesis-silking interval, number of ears per plant, ear diameter, number of kernel rows and number of kernel per row, kernel length and kernel width.
V	01	0.7	Least number of days to tassel and silking, number of leaves per plant, leaf width, cob diameter and minimum grain yield per plant.

Table 7. Maize germplasm collected from seven places distributed into various clusters in 2011.

Place of collection	No.	Percentage	Cluster-I	Cluster-II	Cluster-III	Cluster-IV	Cluster-V
KP	26	17.30	2	20	3	0	1
Punjab	41	27.30	24	16	1	0	0
Baluchistan	22	14.70	0	3	18	1	0
NA	15	10	3	10	2	0	0
AJK	25	16.70	15	10	0	0	0
Japan	18	12	17	1	0	0	0
China	3	2	0	3	0	0	0

KP= Khyber Pakhtunkhwa, NA= Northern Areas, AJK= Azad Jammu and Kashmir

Discussion

During the present investigation a high level of genetic variability was detected which is congruent with the finding of Aliu *et al.*, (2013); Maruthi *et al.*, (2013); and Shrestha, (2013). Most of the qualitative traits showed low level of variability as compared to quantitative traits. Descriptive statistics of the data reflected high level of variation for the quantitative characters. The number of days to tassel ranged from 46 to 74 days with mean value of 63 days and the number of days to silking ranged from 52 to 81 days with mean value of 69.71 days. Several genotypes took less number of days to tassel and silking as compared to check varieties which may prove valuable for producing varieties with early maturity. Similar findings were reported by Rahman *et al.*, (2008). Our findings are also in conformity with the results of Shah *et al.*, (2000); Beyene *et al.*, (2005); Ihsan *et al.*, (2005); Ranatunga *et al.*, (2009); Ali *et al.*, (2011); and Shrestha, (2013). Considerable amount of variation was found in anthesis-silking interval which ranged from 0 to 8 days with mean value of 3.6 days which is in conformity with the findings of Shrestha, (2013). The plant height varied between 71.9 and 226.1 cm with mean value of 153.6 cm. High level of variation in this trait may be due to the difference in the genotypes used, their genetic origin and diverse environments. These findings were closer to the findings of Ihsan *et al.*, (2005), Aliu *et al.*, (2013) and Shrestha (2013). The number of leaves varied from 6.4 to 15.2 with mean value of 11.1 which is congruent with the findings of Beyene *et al.*, (2005). High level of variation was found in plant height and ear height which are in line with

the findings of Dijak *et al.*, (1999). The variability detected in ear length and ear diameter which are almost similar to the findings of Galarreta & Alvarez (1990). High level of diversity was also detected in the number of rows of kernels per ear, number of kernels per row, kernel length, kernel width, 1000 kernel weight and grain yield per plant which are concordant with the findings of Galarreta & Alvarez (1990), Miguel *et al.*, (2008), Ranatunga *et al.*, (2009) and Aliu *et al.*, (2013). The slight variation from the previously reported findings may be due to difference in plant material used in experiment and possible variation in climatic conditions.

Correlation plays pivotal role in the selection of right traits for breeding purposes. Sometimes the role of environment for both the traits is direct and synchronized in the same direction, while in other cases is in opposite or different directions (Yucel *et al.*, 2009). During the present investigation significant level of correlation was detected among important agro-morphological characters, such as the number of days to tassel was highly significantly positively correlated with the number of days to pollen shedding ($r = 0.98^{**}$) and number of days to silking ($r = 0.97^{**}$) which is congruent with the findings of Galarreta & Alvarez, (1990). The number of leaves per plant and plant height were highly positively correlated ($r = 0.82^{**}$), which is supported by the findings of Cortes *et al.*, (2007). Correlation between the anthesis-silking interval and grain yield per plant was highly negative i.e. ($r = -0.53^{**}$), which is in agreement with the findings of Betran *et al.*, (2003). Grain yield per plant was significantly correlated with ear length, number of kernel rows and number of kernels per row. Over all the plant traits expressed higher values of correlation as compared

to that of ear traits which is congruent to the findings of Galarreta & Alvarez, (1990) and Homayoun, (2011). For the selection of right trait(s) for crop improvement, the characterization of correlation among vital yield contributing traits is necessary.

Upon cluster analysis the maximum genetic diversity was shown by the accessions collected from the province of Khyber Pakhtunkhwa, which shared four clusters, followed by the accessions from Punjab and Northern Areas. Similarly, PCA grouped together the maize accessions with similar morphology but not because of their geographical background. PCAs displayed that the total genetic diversity was clearly distributed throughout the agro-morphological traits. It is important to point out that Pakistan possesses a variety of environmental and ecological conditions which effectively contribute to the genetic diversity of maize. The genetic diversity in an area has a direct relationship with the ecological and environmental conditions instead of affected by the geographical distribution (Li & Rutger, 2000). The selection process promotes the development of cultivars on one hand but on the other hand results in decrease in genetic diversity, which is immensely necessary to conserve and utilize in future (Rabbani *et al.*, 1998). It is clear from the cluster and principal component analysis that there is no geographical relationship among the studied genotypes supported by the findings of Gupta *et al.*, (1991) and Amurrio *et al.*, (1995). The findings will be fruitful for the agro-morphological characterization and finding the association between trait and gene(s). The overall distribution of the studied germplasm on the basis of agro-morphological traits, it can be inferred that the tested germplasm in Pakistan possess significant amount of genetic variability, which can be a valuable source for the development of broad based productive varieties and hybrids in future.

Conclusion

The current investigation reflected valuable amount of genetic variability among the tested maize genotypes. Valuable ago-morphological traits were grain yield per plant, plant height, days to anthesis, days to silking and 1000 kernel weight which served as selection criteria for the identification of elite genotypes. Our findings could be cashed for the maximum by further utilization of diverse collections of maize with additional reliable techniques like isozymes, seed storage proteins and DNA based markers to provide broad genetic base for breeding strategies.

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