

TRENDS IN REGIONAL AND CHRONOLOGICAL DIVERSITY OF MAIZE (*ZEA MAYS* L.) GERMPLASM IN PAKISTAN

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Abstract

Maize germplasm was exclusively collected across Pakistan to probe the trends in genetic variability at regional levels and chronological periods. A total of 290 accessions were collected from 6 different regions. These were comprised of the following chronological order: 1980-1984, 1985-1990, 1991-2000, 2001-2007 and 2008-2016. These accessions represent maize growing provinces of Pakistan except Sindh. The performance of these entries were compared in the field and the data for different agronomic and yield related traits were subjected to statistical analysis. Mean comparison showed that Punjab region has highest mean grain yield followed by Azad Jammu & Kashmir (AJK). Regional differences in genetic diversity of maize germplasm were evident; AJK and Punjab possessed comparatively high genetic differences for various traits. Differences in the genetic variability across the region showed that various variability changing forces worked differently in different regions. Chronological studies showed that grain yield had been improving in Pakistan since 1980s. Highest grain yield was observed during the recent period (2008-2016) compared to earlier periods. Genetic diversity among available maize germplasm did not continuously reduce during the last 36 years based on the findings of this study. In the earlier years diversity showed increasing trend followed by declining trend. Highest genetic diversity was observed in the genotypes collected during 1980-1984 whereas, again there was boost in diversity during 1991-2000. It has been observed that addition of newly developed and better performing genotypes into informal seed system initially increased the diversity followed by substitution of traditional accessions which led to change in genetic variation in Pakistan. The gradual change in patterns of genetic variability in maize at regional and chronological levels in Pakistan emphasizes the importance of conserving genetic resources to avoid genetic vulnerability and depletion.

Key words: Post-domestication era, Biological diversity, Genetic erosion, Chronological periods, PCA biplots, Yield and yield components, Agronomic traits. Arrange them alphabetically.

Introduction

Continuous domestication and imposition of different selection methods has narrowed the genetic diversity which is described as "genetic erosion". Harlan (1970, 1975) described that the varietal wealth of crop plants is wiping out the genetic diversity before our eyes and we can't afford such losses. Genetic diversity losses due to domestication and dispersal have prevailed long ago in history and currently crops have been domesticated and dispersed the most. Whereas, modernization is still playing key role in genetic erosion of crop plants (van de Wouw *et al.*, 2009). Replacement of landraces with newly developed cultivars is casual factor for genetic erosion. This replacement is still continued in developing countries but completed in developed countries. In modern breeding, there is continuous process of selection which acts as second order modernization bottleneck due to increase in uniformity among the genotypes (van de Wouw *et al.*, 2009).

Genetic erosion can occur in particular region or over particular chronological time period (Frankel & Bennett, 1970). This loss of diversity in genetic makeup is attributed to loss of individual genes or group of genes present in outdated or older landraces, and this loss might be irreversible (Anon., 2002). To evaluate the replacement of landraces, genotypes could be grouped into chronological time periods (different time periods) and subjected to variability analysis (van de Wouw *et al.*, 2009).

Different types of seed systems are working in different countries whereas, informal seed system is very prevalent in developing countries. Initially, new genotypes were introduced while older ones were not fully eliminated because new ones were added in the prevailing informal seed system. The addition of new genotypes into informal seed system posed the problems in studying the genetic dilution because this addition increased the diversity (Louette & Smale, 2000). These challenges in the study of trends in genetic variability necessitate the establishment of standards for quantification of genetic diversity at field level. FAO/IPGRI (2002) suggested following parameters for quantification of trends of genetic variability;

- Number of different accessions in particular region or at particular time is considered as germplasm richness.
- Distribution of different accessions in particular region or at particular time is described as germplasm evenness.
- Extent of differences (variability) between the accessions.

Genetically diverse seed lots are potentially valuable for farmers and for representation of diversity in informal seed system (Heerwaarden *et al.*, 2009). So, these distinct seed lots are used as unit for genetic erosion studies. Maize dispersed from center of origin to North and South

America, Europe, Africa and Asia through complicated trajectories (Rebourg *et al.*, 2003). In Indo-Pak regions, stones carved in shape of maize ears were sculptured in 12th and 13th century A.D which showed the Pre-Columbian introduction of maize (Johannessen & Parker, 1989). The mass production and promotion of modern varieties in the 1960s and 70s in South Asia is believed to reduce genetic diversity and replacement of cereals landraces. Present study was planned with the key objectives of exploring the trends in genetic variability across the regions and chronological periods in Pakistan to understand the trends in maize.

Materials and Methods

To evaluate the regional genetic diversity, Pakistan was divided into five main regions *i.e.*, Balochistan, Gilgit-Baltistan, Punjab, Khyber Pakhtunkhwa (KPK) and Azad Jammu & Kashmir (AJK). Sindh province was excluded from study due to non-availability of germplasm collected from that region. The locally collected germplasm were compared with accessions sourced from China, Japan and Mexico and these were merged into one distinct group named as “exotic” in the current study. A total of 25 accessions from Balochistan, 18 from Gilgit-Baltistan, 48 from Punjab, 92 from KPK, 60 from AJK and 47 from exotic sources were included in this study. These genebank collections were provided by the Plant Genetic Resource Institute (PGRI), National Agricultural Research Council (NARC), Islamabad, Pakistan. The University of Agriculture Faisalabad (UAF) also coordinated in the collection maize germplasm from open market and supported this study. List of maize genotypes used in the study across the regions and time periods are given in Table (1 & 2).

The 290 collected accessions were grown in the field following randomized complete block design (RCBD) with two replications using standard agronomic practices. The differences in performance were attributed to the genetic diversity among the germplasm. Data were collected for total plants (TP), root lodging (RL), stem lodging (SL), days to 50% tasseling (DT), days to 50% silking (DS), anthesis silking interval (ASI), total cobs (TC), plant height (PH; cm), ear height (EH; cm), rows per cob (RPC), grains per row (GPR), yield per plant (YPP; g) and yield per hectare (YPH; monds/ha). TP are indicating the availability of seeds which were sown and based on those plants data were collected for all other traits. TP did not subject to germination differences. TC is indicating the total number of cobs on which inferences were made for traits. YPH was estimated based on field weight by converting the small samples into monds per ha.

The data of 290 accessions were divided into regional and chronological groups for statistical analysis. However, to evaluate the chronological trends in genetic diversity, the above mentioned accessions were arrayed into following chronological periods *i.e.*, 1st period, 1980-1984; 2nd period, 1985-1990; 3rd period, 1991-2000; 4th period, 2001-2007; and 5th period, 2008-2016. Exotic accessions were not included in the chronological diversity study because in this study these accessions were unable to maintain the independent group. Out of

total collection, 107 accessions belongs to 1st period, 49 belong to 2nd period, 46 belong to 3rd period, 17 belong to 4th period and 24 belong to 5th period. PGRI exclusively expedited the country for exclusive germplasm collections which is used in current study.

To conduct the regional and chronological diversity analysis, basic summary statistics and principal component biplot analysis were used. Means and standard deviation were estimated using GenStat software, 16th edition. Data collected for the maize accessions were also subjected to the analysis of variance (Steel *et al.*, 1997) using GenStat software.

Principal component analysis (PCA) is a multivariate analysis to study the patterns, trends, similarities or differences in the data. Data reduction is also key benefit of the PCA since data dimensions are reduced with minimum loss of information (Gabriel, 1971). Studied traits were depicted as vectors in the PCA biplots and accessions were presented as scattered points. Diversity is presented by convex hull or convex envelop which connected the distantly apart genotypes and generated hull like structure. Area of the convex hull reflected extent of genetic diversity in particular region or in particular chronological period. Larger the area of hull, higher is the genetic diversity and vice versa. PCA biplots with convex hull were generated by using the GenStat software.

Results and Discussion

Analysis of variance showed that accessions from six regions were significantly different for agronomic and yield related traits. Grouping of accessions into different regional groups and chronological periods, showed the significant differences among accessions across the regions and chronological periods (Table 3).

Regional diversity trends: Means and standard deviation for studied traits of maize for six locations were presented in Table 4. The RL (0.88) and SL (0.04) were lowest in germplasm of Balochistan (Table 4). Germplasm of Punjab has highest mean value for DT (58.17 days) and DS (60.57 days) compared to exotic germplasm DT (51.33 days) and DS (54.17 days). The ASI was highest for exotic germplasm (2.84 days) and lowest for germplasm of Punjab region (2.41 days). Plants of the Punjab germplasm had highest PH (180.5cm) and EH (87.28cm) whereas, Balochistan have lowest PH (153.3cm) and EH (70.61cm). Accessions of exotic germplasm have least RPC (11.74 rows per cob) whereas, accessions of Punjab germplasm have highest mean for RPC (13.87 rows per cob). Accessions of Balochistan germplasm have lowest GPR (17.2) and genotypes of Punjab have highest mean for this trait (28.17). The YPP was lowest for accessions of exotic germplasm (70.97g) and highest for Punjab germplasm (138.1g). The YPH was lowest for genotypes of exotic germplasm (145.3mond/ha) and highest for genotypes of Punjab (288.3mond/ha). Descending sequential array for yield per hectare for targeted regions was as following; Punjab>AJK>Balochistan>KPK>Gilgit-Baltistan>Exotic (Table 4) based on the inferences of studied 290 genotypes.

Table 1. List of maize genotypes collected across different regions of Pakistan and abroad.

Serial No.	KPK	AJK	Punjab	Exotic	Balochistan	Gilgit-Baltistan
1.	015075	014959	015011	015257	014870	015135
2.	015076	014961	015015	015258	014874	015136
3.	015077	014965	015016	015260	014880	015137
4.	015079	014966	015017	015263	014882	015138
5.	015081	014967	015027	015264	014890	015139
6.	015084	014968	015046	015265	014897	015141
7.	015085	014969	015047	015266	014908	015149
8.	015086	014970	015048	015267	014910	015150
9.	015087	014971	015050	015268	014917	015152
10.	015088	014972	015054	015269	014918	024679
11.	015089	014973	015155	015270	014919	024680
12.	015090	014976	015157	015271	014921	024681
13.	015091	014977	015158	015272	014925	024682
14.	015092	014978	015159	015273	014927	024684
15.	015093	014979	015160	015274	015018	024677
16.	015095	014981	015162	015275	015019	015021
17.	015096	014982	015163	015276	015131	015023
18.	015097	014983	015164	015277	015132	015025
19.	015098	014984	015202	015278	015133	
20.	015099	014985	015253	015279	015134	
21.	015100	014987	019202	015280	015205	
22.	015101	014988	019203	015281	015207	
23.	015102	014990	019204	015282	024674	
24.	015103	014992	019205	015283	024675	
25.	015104	014996	Punj-001	015284	024676	
26.	015105	014997	Punj-002	015285		
27.	015106	024685	Punj-003	015311		
28.	015107	024686	Punj-004	015120		
29.	015108	024687	Punj-005	015121		
30.	015109	024688	Punj-006	015122		
31.	015110	024689	Punj-007	015123		
32.	015111	015167	Punj-008	015125		
33.	015113	015169	Punj-009	015126		
34.	015118	015170	Punj-010	015127		
35.	015119	015171	Punj-011	015128		
36.	015143	015172	Punj-012	015130		
37.	015144	015173	Punj-013	015032		
38.	015145	015174	Punj-014	015033		
39.	015146	015178	Punj-015	015035		
40.	015147	015182	Punj-016	015036		
41.	015148	015185	Punj-017	015037		
42.	015216	015186	Punj-018	015038		
43.	015218	015187	Punj-019	015039		
44.	015219	015188	Punj-020	015040		
45.	015220	015189	Punj-021	015041		

Table 1. (Cont'd.).

Serial No.	KPK	AJK	Punjab	Exotic	Balochistan	Gilgit-Baltistan
46.	015221	015190	Punj-022	015043		
47.	015222	015191	Punj-023	015044		
48.	015226	015192	Punj-024			
49.	015227	015153				
50.	015228	015154				
51.	015229	015355				
52.	015233	019201				
53.	015234	019206				
54.	015236	015051				
55.	019174	015052				
56.	019175	015053				
57.	019176	015055				
58.	019177	015056				
59.	019178	015005				
60.	019179	015010				
61.	019180					
62.	019181					
63.	019182					
64.	019183					
65.	019184					
66.	019185					
67.	019186					
68.	019187					
69.	019188					
70.	019189					
71.	019190					
72.	019191					
73.	019192					
74.	019194					
75.	019195					
76.	019196					
77.	019197					
78.	019198					
79.	019199					
80.	024669					
81.	024670					
82.	024673					
83.	015059					
84.	015060					
85.	015061					
86.	015063					
87.	015064					
88.	015065					
89.	015066					
90.	015067					
91.	015068					
92.	015069					

Table 2. List of maize genotypes collected across different chronological periods.

Serial No.	1980-1984	1985-1990	1991-2000	2001-2007	2008-2016
1.	014870	015131	015253	024674	Punj-001
2.	014874	015132	019202	024675	Punj-002
3.	014880	015133	019203	024676	Punj-003
4.	014882	015134	019204	024679	Punj-004
5.	014890	015205	019205	024680	Punj-005
6.	014897	015207	015216	024681	Punj-006
7.	014908	015135	015218	024682	Punj-007
8.	014910	015136	015219	024684	Punj-008
9.	014917	015137	015220	024677	Punj-009
10.	014918	015138	015221	024669	Punj-010
11.	014919	015139	015222	024670	Punj-011
12.	014921	015141	015226	024673	Punj-012
13.	014925	015149	015227	024685	Punj-013
14.	014927	015150	015228	024686	Punj-014
15.	015018	015152	015229	024687	Punj-015
16.	015019	015155	015233	024688	Punj-016
17.	015021	015157	015234	024689	Punj-017
18.	015023	015158	015236		Punj-018
19.	015025	015159	019174		Punj-019
20.	015011	015160	019175		Punj-020
21.	015015	015162	019176		Punj-021
22.	015016	015163	019177		Punj-022
23.	015017	015164	019178		Punj-023
24.	015027	015202	019179		Punj-024
25.	015046	015143	019180		
26.	015047	015144	019181		
27.	015048	015145	019182		
28.	015050	015146	019183		
29.	015054	015147	019184		
30.	015075	015148	019185		
31.	015076	015167	019186		
32.	015077	015169	019187		
33.	015079	015170	019188		
34.	015081	015171	019189		
35.	015084	015172	019190		
36.	015085	015173	019191		
37.	015086	015174	019192		
38.	015087	015178	019194		
39.	015088	015182	019195		
40.	015089	015185	019196		
41.	015090	015186	019197		
42.	015091	015187	019198		
43.	015092	015188	019199		
44.	015093	015189	015355		
45.	015095	015190	019201		
46.	015096	015191	019206		
47.	015097	015192			
48.	015098	015153			
49.	015099	015154			
50.	015100				
51.	015101				
52.	015102				
53.	015103				

Table 2. (Cont'd.).

Serial No.	1980-1984	1985-1990	1991-2000	2001-2007	2008-2016
54.	015104				
55.	015105				
56.	015106				
57.	015107				
58.	015108				
59.	015109				
60.	015110				
61.	015111				
62.	015113				
63.	015118				
64.	015119				
65.	015059				
66.	015060				
67.	015061				
68.	015063				
69.	015064				
70.	015065				
71.	015066				
72.	015067				
73.	015068				
74.	015069				
75.	014959				
76.	014961				
77.	014965				
78.	014966				
79.	014967				
80.	014968				
81.	014969				
82.	014970				
83.	014971				
84.	014972				
85.	014973				
86.	014976				
87.	014977				
88.	014978				
89.	014979				
90.	014981				
91.	014982				
92.	014983				
93.	014984				
94.	014985				
95.	014987				
96.	014988				
97.	014990				
98.	014992				
99.	014996				
100.	014997				
101.	015051				
102.	015052				
103.	015053				
104.	015055				
105.	015056				
106.	015005				
107.	015010				

Ali *et al.*, (2007), Ahmad *et al.*, (2011), Hussain *et al.*, (2011), Mahmood *et al.*, (2004), Tahir *et al.*, (2008), Turi *et al.*, (2007) and Younas *et al.*, (2002) also reported the significant genetic diversity in yield and yield components of maize from different regions of Pakistan whereas, these researchers focused independently on one particular region. This extent of diversity among accessions used in current study belongs to different regions which may be due to different areas of origin. So adaptability and acquisition of traits in different environments is quite understandable in these results. Accessions with different areas of origin made changes differently during the process of adaptability and modernization which leads to differential level of regional variability in agronomic and yield related traits.

Accessions with origin from Punjab showed highest levels of standard deviation for RL (1.69), DT (6.24), DS (5.96), PH (32.36), RPC (2.508), GPR (12.88), YPP (75.35) and YPH (157.2). On the other hand, accessions from AJK had lowest standard deviation for PH (25.59) and EH (15.62); whereas, exotic germplasm had lowest standard deviation for DT (4.337), DS (4.262), YPP (43.59) and YPH (91.52) (Table4). Principally high value of standard deviation is the indicator of higher dispersion from mean values; therefore it explains higher genetic diversity. Standard deviation is also associated with central tendency of data so, lowest value for standard deviation depicts the condensation of data about mean value which explains more consistency, more uniformity and least diversity.

Change in trends of genetic diversity on regional basis might be due to poor resource management and adverse environmental factors (biotic and abiotic) across different locations. Several studies show that environmental causes like drought stress, floods, disease and pest epidemics among others attributes to reduction in germplasm variability and regional differences in trends of diversity (Worede & Mekbib, 1993; Stephen *et al.*, 2002). Wars, displacement of people, social disruption, global warming and pollution are also causes of regional and temporal changes in genetic variability (Myers, 1994) in recent times however there is no exception for these circumstances to cause changes in genetic variability for maize in Pakistan. Differential trends of genetic diversity in different regions of Pakistan could be attributed to variable imposition of the factors enhancing the changes in genetic variability. Socio-economic factors, environmental conditions, access to inputs, water shortage, land degradation, imbalance in production cost versus selling cost, modifications in land use practices, decreased soil quality, unsuitable cultivation pattern are the factors to accelerate the regional changes in genetic variability (Davari *et al.*, 2013). The basic contribution toward genetic variability trends is different in different regions of Pakistan therefore differences among accessions belonging to different regions are highly significant.

PCA followed by biplot graphical display is most effective statistical tool for studying the variability of different traits of crop plants (Aslam *et al.*, 2013a, b; Aslam *et al.*, 2014; Aslam *et al.*, 2015; Maqbool *et al.*, 2015a, b; Naveed *et al.*, 2014). PCA biplots were generated for each region separately. Accessions were independently and separately numbered for each region. PCA biplot for Balochistan region was most appropriate for explanation of multivariate parameters representing 98.9% variability of actual data. Accessions 22, 20, 2, 4, 14, 11 and 16 were distantly apart from origin and proved superior in performance (diversified) whereas, accession 23, 25 and 19 were clustered closely which showed that these had uniform performance (Fig. 1). PCA biplot for germplasm belongs to Gilgit-Baltistan represented 98.92% of total genetic diversity. Accession 14, 2, 16, 17, 7, 10, 11 and 1 were most diverse in Gilgit-Baltistan germplasm. All accessions were scattered which showed that accessions of this region were distinctive from each other (Fig. 1). PCA biplot for Punjab germplasm depicted 99.39% of total variability in data. Accessions 4 and 22 were most identical in performance (uniform) whereas, accessions 15, 44 and 35 were proved highly diverse among Punjab germplasm. PCA biplot distinctively classified the accessions into two groups. Group 1 comprised of poor performing (based on all traits used for biplot) accessions those were 9, 10, 7, 5, 21, 6, 14, 8, 24, 2, 12, 11, 4 and 22. Group 2 possessed superior performing accessions like; 35, 44, 34, 48, 25, 27, 29, 26, 28, 30, 33, 35, 31, 34, 32, 37, 39, 38, 40, 42, 44, 46, 48, 41, 43, 45 and 47 (Fig. 1). Superior performing accessions were actually F₁ hybrids whereas, poor performing accessions were open pollinated populations.

PCA biplot for accessions of KPK was representative of 98.96% of total genetic diversity. Accessions 76, 92, 48, 45, 56, 3, 26 and 33 had high variability among all other accessions from KPK region. So, only these superior performing accessions were represented by biplot as outliers whereas, others were uniform in performance regarding studied traits (Fig. 1).

PCA biplot showed 99.28% of total genetic diversity for accessions collected from AJK. Accessions 3, 17, 9, 35, 43, 41 and 29 were proved as superior performers among germplasm belonging to AJK whereas, accessions 22, 5, 21 and 28 were poor performers (Fig. 1). PCA biplot for exotic germplasm depicted 96.72% of total genetic diversity from raw data. Extensive dispersion of accessions on biplot showed the diverse nature of accessions and high level of genetic diversity. Exotic accessions had entirely distinctive environmental conditions for their adaptability and emerged with high level of genetic diversity in the exotic group. Accessions 2, 38, 3, 40, 43, 16, 5, 15 and 1 were superior among exotic germplasm for studied maize traits (Fig. 1). PCA biplot exclusively visualized the superior and poor performing accessions for particular regions.

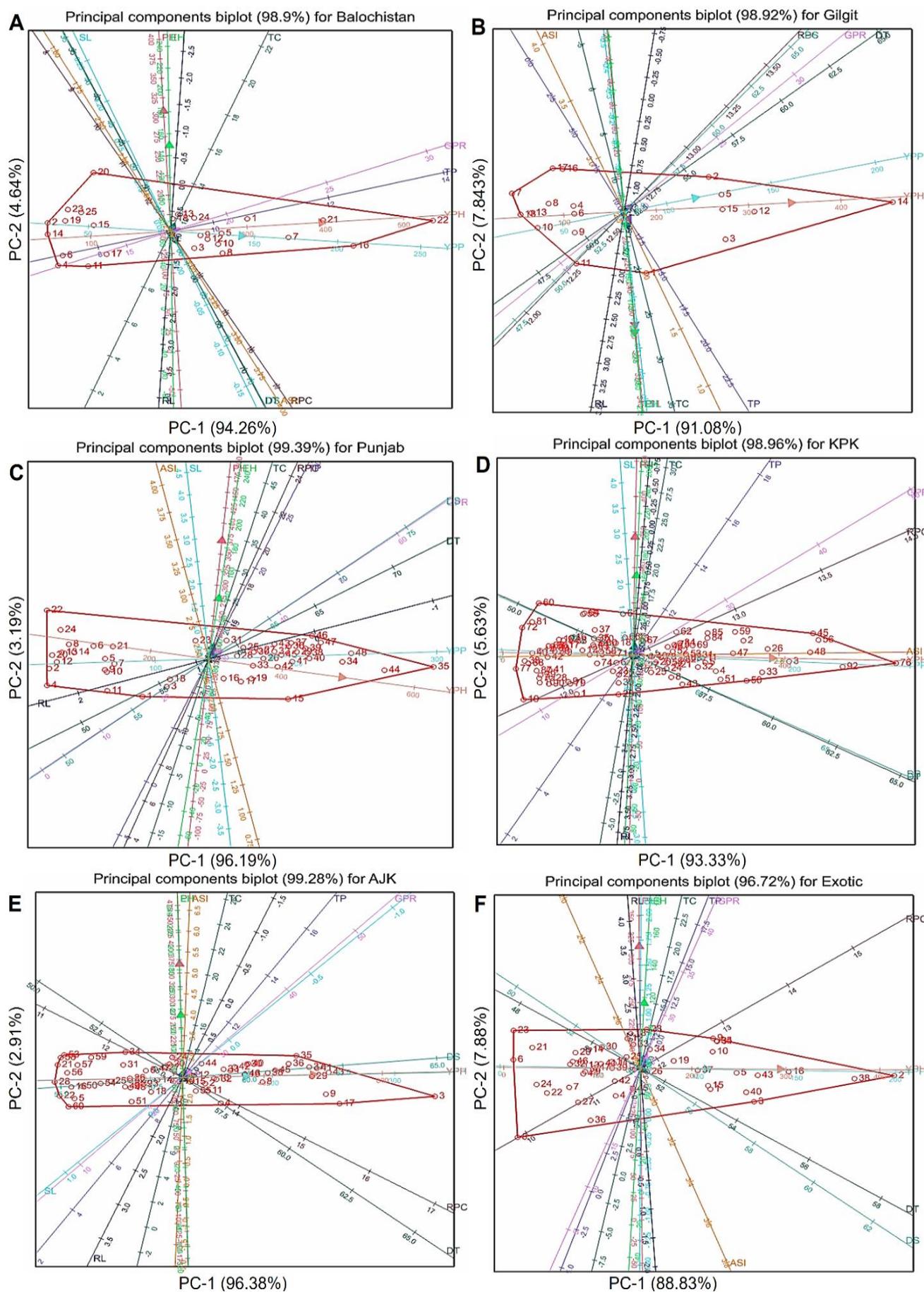


Fig. 1. PCA biplots for variability of different maize traits based on regional germplasm collection. (A) Balochistan, (B) Gilgit-Baltistan, (C) Punjab, (D) Khyber Pakhtunkhwa (KPK), (E) Azad Jammu & Kashmir (AJK), (F) Exotic.

Chronological diversity trends: Exotic accessions were not included for assessment of chronological trends of variability. More number of accessions were available from earlier periods whereas lesser were available in recent time periods, an indicator of genetic erosion (genotype richness). Conventionally genetic erosion is also perceived as loss of adapted set of landraces and substitution by small number of improved cultivars (Brush, 1999). This conventional concept was also verified in current research by the availability of large number of accessions in 1st chronological period (1980-1984) and less number of accessions in the recent chronological periods. Parallel to present study of maize, number of wheat genotypes cultivated in China was 10,000 in 1949 which was reduced to 1000 in 1970 (Thrupp, 1998). Number of upland rice genotypes reduced to 65 in 1994 whereas, it was 100 in 1980 (Hammer & Teklu, 2009).

Chronological period 2008-2016 had highest mean value for DT (63.12), DS (65.33), PH (202.0), EH (101.2), RPC (15.58), GRP (38.98), YPP (200.3) and YPH (414). Mean values for studied parameters followed inconsistent trends across different chronological periods. YPH increased from 1980-1984 to 1985-1990 (193mond/ha to 236mond/ha) whereas, in 3rd period (1991-2000) yield was declined to 196mond/ha. In the 4th (2001-2007) and 5th (2008-2016) periods, yield again followed the increasing trend with means of 201 and 414 mond/ha, respectively (Table5). The increase in yield after first time period (1980-1984) might be due to improvement in the maize through different interventions. After third time period there was the time of introduction of F₁ hybrids for general cultivation along with introduction of spring season for maize. Increase in grain yield in recent years is attributed to overwhelming proportion of maize hybrids in the samples of that period. However, all of the genotypes collected from PGRI belong to previous time periods were open pollinated accessions.

Several other reports of genetic erosion in different crops during different time periods logistically validated our results. Hammer & Teklu (2009) reported 73% and 72% change in genetic variability in landraces of South Italy and Albania respectively. Hammer & Laghetti (2005) reported that relative to 1980s significantly higher changes in genetic variability occurred during 1920s to 1950s. Heerwaarden *et al.*, (2009) reported the changes of genetic diversity in maize germplasm of Mexico for vegetative, reproductive and molecular parameters. It was reported that genetic diversity of germplasm was changing time to time (Heerwaarden *et al.*, 2009) which was also in accordance to current study of maize germplasm in Pakistan.

In the last chronological period (2008-2016) most of the accessions were F₁ hybrids in nature which were developed by hybridization of inbred lines. Use of limited number of parents in heterosis breeding is also one of the factors contributing in genetic variability changes. There are small numbers of research institutes

working on maize which definitely have selected few parents those are continuously being used in breeding programs. Goodman (2005) reported that only six inbred lines were the parents of large number of commercial hybrids in USA. However, such chronological changes of genetic diversity and reduced variability in maize germplasm in Pakistan can be taken as a concern in case of unforeseen environmental changes or change in future breeding objectives should occur.

PCA biplots for the 1st chronological period (1980-1984), showed 99.11% of total genetic diversity of data. Accessions 77, 91, 83, 74 and 16 proved superior among studied 107 accessions and rest of the accessions were clustered into two group's i.e. superior and poor performers in terms of variability (Fig. 2). PCA biplot for 2nd chronological period (1985-1990) depicted 99.14% of total genetic variability for subjected traits. Accessions were well scattered and formed larger convex hull which depicted greater genetic diversity of selective germplasm in this chronological period. Accessions 6, 42, 40, 36, 34, 35, 44 and 20 have high variability whereas, accessions 27, 29, 28, 2 and 25 were found uniform among selected 49 accessions from 1985-1990 (Fig. 2). PCA biplot for 3rd chronological period (1991-2000) represented 99.05% of total diversity with contribution of 93.41% and 5.63% from PC-1 and PC-2 respectively. Out of total 46 accessions of 3rd period, accessions 40, 20, 9 and 12 were found to be highly diverse but accessions 41, 36, 3 and 24 were found uniform in nature. Accessions were scattered and enclosed in larger convex hull which reflected greater genetic diversity in maize germplasm for studied traits (Fig. 2). For 4th chronological period (2001-2007) PCA biplot showed 99.00% of total genetic variability in germplasm. Total 17 accessions were belonging to this period. Accessions 15, 8 and 16 were diverse whereas; accessions 14, 11 and 7 were uniform (Fig. 2). Total 24 accessions were collected in 5th chronological period (2008-2016) and subjected to PCA biplot which showed the 97.91% of total genetic diversity. All of the 24 accessions of this group were distinctive from each other but closely located on biplot and enclosed in convex hull of smaller area relative to other periods. Accessions 11, 20 and 24 were having high diversity whereas; accessions 7, 4 and 9 were relatively uniform among collected 24 genotypes (Fig. 2).

Chronological pattern in genetic diversity showed that there were significant changes in the variability during different chronological periods. Increase in diversity in earlier years showed that maize germplasm was improved through different interventions. Change in the genetic variability may be due to substitution of primitive genotypes with newly developed germplasm (Louette & Smale 2000). Genetic diversity changes are also reported in other crops and regions across different chronological periods. Ahmad *et al.* (2012) reported the loss of variability in cotton germplasm of Pakistan. They categorized the cotton genotypes into Pre-1975 and Post-1975 groups to evaluate the variability and found that post-1975 genotypes were more uniform in performance. During last 25 years, 40% changes in genetic variability of vegetables were reported in Iran (Davari *et al.*, 2013).

Table 3. Mean sum squares for yield and yield components of maize genotypes for subjected locations and chronological periods.

SOV	Regions of provincial level											
	DF	RL	SL	DT	DS	ASI	PH	EH	RPC	GPR	YPP	YPH
Regions	5	9.83**	3.07**	516.2**	470.3**	2.23**	10260**	5758**	54.54**	1335**	67231**	301111**
Genotypes	289	3.65**	1.14**	50.34**	499.2**	2.85**	1521**	742**	10.23**	198**	7524**	32932**
Residual	285	0.084	0.054	0.173	0.050	0.075	0.786	0.698	0.142	0.3417	0.377	19.36
SOV	Chronological periods											
	DF	RL	SL	DT	DS	ASI	PH	EH	RPC	GPR	YPP	YPH
Chronological periods	4	28.93**	28.24**	832**	746**	4.83**	31537**	13825**	141**	42723**	118412**	511697**
Genotypes	242	3.57**	1.03**	46.16**	45.8**	2.94**	1224**	642**	9.2**	143**	7250**	31975**
Residual	239	0.137	0.213	0.273	0.234	0.114	0.956	1.001	0.476	1.987	2.1312	1.9787

RL: Root lodging, SL: Stem lodging, DT: Days to 50% tasseling, DS: Days to 50% silking, ASI: Anthesis silking interval, PH: Plant height, EH: Ear height, RPC: rows per cob, GPR: Grains per row, YPP: Yield per plant, YPH: Yield per hectare

Table 4. Summary statistics of yield and yield components of maize genotypes for six different regions.

Origin	TP	RL	SL	DT	DS	ASI	TC	PH	EH	RPC	GPR	YPP	YPH
Balochistan	9.26	0.88	0.04	54.86	57.62	2.76	11.24	153.3	70.61	12.84	17.2	99.51	202.5
Gilgit	10.61	1.28	0.167	51.92	54.44	2.53	13.64	173.5	88.61	12.61	19.08	79.61	165.4
Punjab	11.49	0.90	0.312	58.17	60.57	2.41	14.85	180.5	87.28	13.87	28.17	138.1	288.3
KPK	9.967	1.58	0.598	54.51	57.21	2.70	11.53	176.3	85.52	12.41	19.98	89.53	184.5
AJK	9.65	1.13	0.283	55.74	58.24	2.50	11.88	158.0	70.92	13.12	24.88	126.5	262.2
Exotic	8.09	1.45	0.468	51.33	54.17	2.84	9.362	174.3	76.49	11.74	23.81	70.97	145.3
Balochistan	4.194	0.971	0.200	5.890	6.009	1.530	5.015	27.83	20.71	2.050	7.34	63.06	133.3
Gilgit	4.71	1.127	0.383	4.924	4.853	1.510	5.575	26.36	27.92	1.568	5.56	48.97	114.2
Punjab	5.44	1.692	0.879	6.240	5.962	1.119	7.435	32.36	19.85	2.508	12.88	75.35	157.2
KPK	4.170	1.477	1.017	4.672	4.713	1.074	5.253	27.37	19.70	2.508	8.809	57.07	118.8
AJK	4.017	1.255	0.804	5.066	4.988	1.318	4.238	25.59	15.62	2.293	9.877	71.66	148.1
Exotic	4.274	1.501	0.654	4.337	4.262	1.340	5.381	27.22	18.37	2.095	11.99	43.59	91.52

TP: Total plants, RL: Root lodging, SL: Stem lodging, DT: Days to 50% tasseling, DS: Days to 50% silking, ASI: Anthesis silking interval, TC: Total cobs, PH: Plant height, EH: Ear height, RPC: Rows per cob, GPR: Grains per row, YPP: Yield per plant, YPH: Yield per hectare

Table 5. Summary statistics of yield and yield components of maize genotypes for different chronological periods.

Chronological period	TP	RL	SL	DT	DS	ASI	TC	PH	EH	RPC	GPR	YPP	YPH
1980 to 1984	9.07	1.682	0.15	55.00	57.60	2.590	10.90	155.2	71.64	12.87	18.64	94.38	193
1985 to 1990	11.11	1.184	0.184	53.95	56.60	2.653	12.86	167.6	77.11	12.63	23.51	112.6	236
1991 to 2000	9.315	0.978	1.37	53.84	56.68	2.848	11.02	186.8	91.74	11.58	23.30	94.79	196
2001 to 2007	10.21	0.941	0.059	55.12	57.29	2.176	13.62	180.3	93.03	14.12	17.85	93.68	201
2008 to 2016	14.71	0.083	0.083	63.12	65.33	2.208	19.92	202.0	101.2	15.58	38.98	200.3	414
1980 to 1984	4.46	1.45	0.492	4.688	4.676	1.343	5.22	26.90	17.41	2.130	8.047	60.25	125.9
1985 to 1990	3.47	1.69	0.441	5.976	5.875	1.267	3.768	22.91	21.56	2.099	9.909	71.3	149.7
1991 to 2000	3.909	1.043	1.388	4.794	4.922	1.032	5.200	28.47	19.11	2.639	9.682	58.92	122.2
2001 to 2007	5.013	1.144	0.243	4.686	4.437	1.310	5.647	18.28	15.98	1.90	7.064	63.69	142.5
2008 to 2016	4.027	0.282	0.282	2.610	2.681	0.721	5.610	13.76	11.02	1.442	5.409	34.48	71.30

TP: Total plants, RL: Root lodging, SL: Stem lodging, DT: Days to 50% tasseling, DS: Days to 50% silking, ASI: Anthesis silking interval, TC: Total cobs, PH: Plant height, EH: Ear height, RPC: Rows per cob, GPR: Grains per row, YPP: Yield per plant, YPH: Yield per hectare

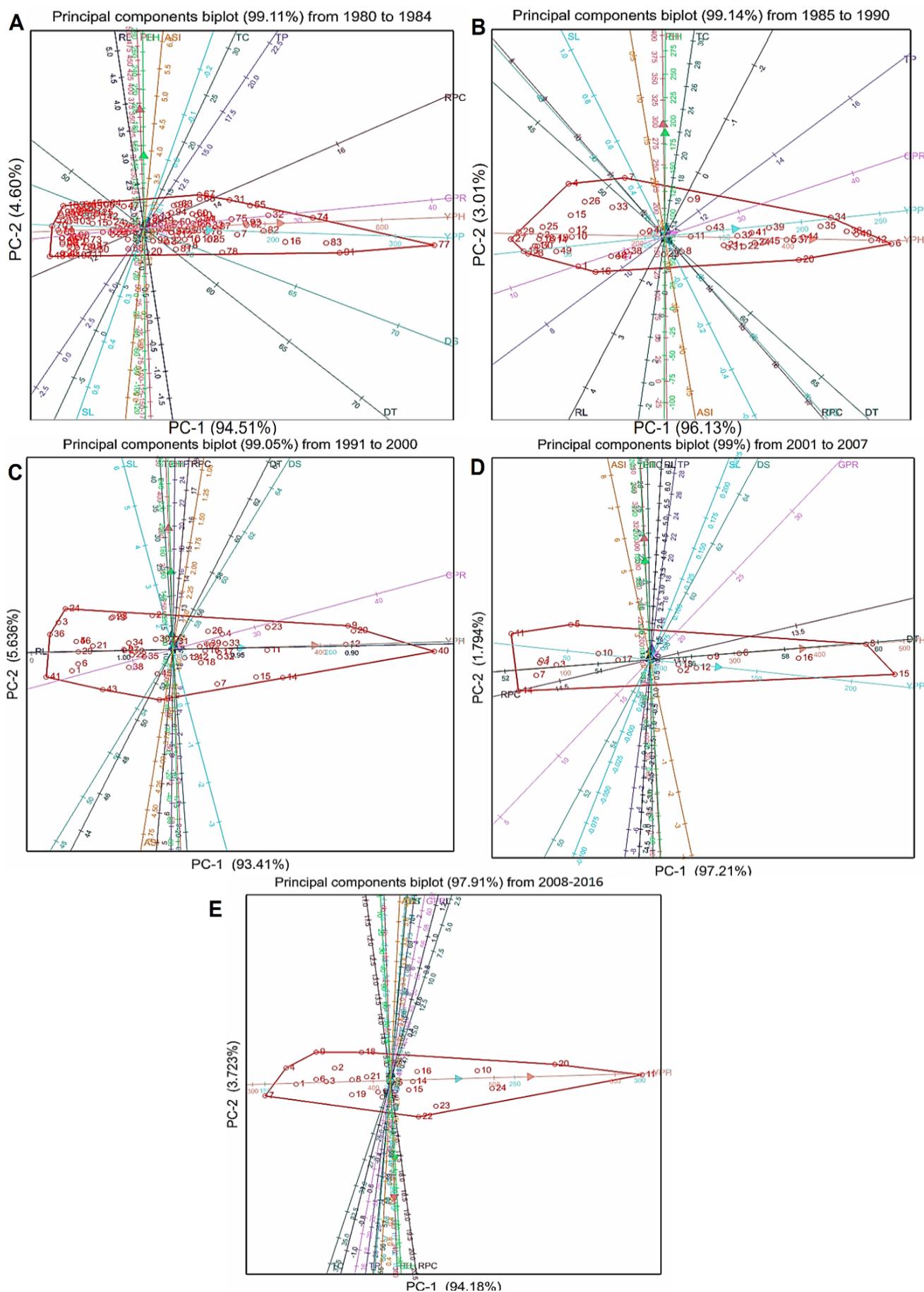


Fig. 2. PCA biplots for variability of different maize traits based on chronological periods of germplasm collection. (A) 1980-1984, (B) 1985-1990, (C) 1991-2000, (D) 2001-2007, (E) 2008-2016.

Conclusions

Significant genetic diversity in maize germplasm has been found across different regions of Pakistan. According to current study, mean yield of maize was highest in Punjab followed by AJK, Balochistan, KPK and Gilgit-Baltistan. Most diversity was observed in maize germplasm of AJK and Punjab relative to other regional groups. Yield of maize germplasm was greatly improved during the last 36 years. Highest mean yield for maize germplasm was observed during 2008-2016 followed by 1985-1990 and 2001-2007 respectively. Highest diversity was observed in 1st chronological period (1980-1984). Changes in variability are associated with changes in the use of germplasm in the maize farming system of Pakistan. To sustain the genetic variability of maize germplasm, participatory and evolutionary plant breeding practices should be initiated. Although high yielding genotypes should be adopted for higher economic gains, however, parallel efforts and initiatives should be in place or strengthened to retain the genetic variability of maize through indigenous germplasm collection, conservation and utilization to mitigate the impact of genetic erosion due to unforeseen environmental or manmade influences.

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