



Biochemical characterization of Pakistani *Zea mays* landraces growing in the remote areas of Khyber Pakhtunkhwa

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Abstract

The landraces extensively cultivated in Malak and Division of Pakistan hold important adaptations to local environmental conditions, being expressed significant levels of genetic diversity. In the present investigation, the genetic divergence in 29 *zea maize* landraces was assessed using seed storage protein through SDS-PAGE. In total, eight loci were expressed which were entirely polymorphic and a high level of genetic diversity was observed in L-8 i.e. 10.34% followed by L-2 with 17.2% polymorphism. Among landraces, ST-2 was unique because of the expression of all loci. Genetic tree based on protein binary data matrix delineated landraces into two main groups. It was found that a high level of divergence exists within and between collection sites. In dendrogram, each group represented landraces of different collection sites, but group-1 was the diverse group and represented a high level of genetic diversity within landraces reported from Swat, Bajaur, Dir and Buner. In Group-2, majority of the landraces showing tight linkage, although originated from different agro-ecological zones like Malakand, Buner and Dir. This may be due to the utilization of a shared gene pool at different agro-ecological zones. Significant levels of genetic diversity within landraces will decrease the genetic vulnerability and will help in the selection of superior genotypes for future breeding programs.

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Introduction

One of the most important cereal crop in the world is Maize (*Zea mays*) and also a strategic food crop for the majority of developing countries (Lopes and Larkins, 1996). Which belongs Maize to family Poaceae (Gramineae), sub family Panicoideae, genus *Zea* and species *Zea mays*. Which is also asses at position third in the list of cereal crop after wheat and rice in the world and also in our country(Pakistan) (Chaudhry, 1994: Gerpacio and Pangali, 2007).*Mays* specie have the potential to grow in short duration, rapidly complete has life cycle and also to produce maximum quality grains in a unit area (Akbar *et al.*, 2008), while comparing it with other cereals it also need less effort. (Abdullah, 1999), 5.2 million metric tons production has been noted in 2016 in Pakistan (PCP, 2017).

The grain of *Zea mays* contains 10%, of proteins 72% starch, 4.8%, fibers 5.8 %, sugar 3.0%, vitamin A 3–5%, vitamin B, oil 4.8% and ash 1.7%. One hundred gram of fresh grain contains 361 calories of energy, 9.4g protein; 4.3g fat, 74.4g carbohydrate (Kozioł *et al.*, 1992).

The landraces, as an important source of the genetic diversity and potential material that could be used to widen the base for plant breeding, are among the main objects of these efforts (Bellon, 2004). Development of cultivars with better agronomic traits is most easily accomplished in genetically diverse species. Consequently, the presence and estimation of genetic variability within such species is important to plant breeding programs (Ghafoor *et al.*, 2005).

Genetic diversity within and between crop populations is determined using techniques such as morphological characterization, Gel electrophoresis, and DNA markers techniques (Nisar *et al.*, 2008: Ghafoor *et al.*, 2008). For the extraction of Proteins viable and notable technique in electrophoresis as SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) which was always commonly and abundantly used biochemical techniques to examine the genetic makeup of crop plants because of its

validity, simplicity and independence of environmental factors (Ghafoor *et al.*, 2005; wadood *et al.*, 2016).

The main focusing objective of present investigation is to asses to us mainly the correlations present in landraces and also to determine the level of genetic divergence in maize grown in the remote area of Khyber Pakhtunkhwa, Pakistan based on total seed protein profiling through SDS-PAGE techniques.

Materials and methods

Collection of maize landraces

For present investigation maize landraces were collected from Different parts of Malakand Division Including Swat, Buner, Dir, Bajaur and Malakand agency during 2016. A total of twenty nine (29) landraces were collected from the mention sites.

Biochemical characterization

Biochemical characterization was carried out using seed storage protein through SDS-PAGE. For protein Extraction 100g seeds of each landrace were ground into a fine powder with the help of pestle and mortar. 400µl of protein extrication buffer (0.5 M Tris-HCL pH 8.0, 0.2%SDS, 5 M Urea, 1%B-mercaptoethanol and BPB) was added to 0.02g of seed flour taken in 1.5 ml Eppendorf tube. The E-tube was vortexed and centrifuged at 18,000rpm for 20 minutes at 20 °C.

The electrophoretic procedure was carried out using 12.25% polyacrylamide gel, separation gel (3.0M Tris-HCl pH9.0, 0.4% SDS) and 4.5% stacking gel (0.4M Tris-HCl pH 7.0, 0.4% SDS). Electrode buffer (0.025 M Tris, 129 M Glycine, 0.125% SDS) was poured into the top pool of the apparatus. 10µl of the sample was loaded in each wells of the gel and electrophoresis were run at 120V.

The gels were then stained in staining solution containing 0.2% BPB dissolved in 10% glacial acetic acid, 40% methanol and water in the ratio of 10:40:50. Gels were de-stained in a solution containing 5% acetic acid and 20% methanol for whole night. The data were recorded '1' for present

and 'o' for absent of protein bands.

Data analysis

The Data were analyzed through MS Excel, PC-ORD, and SPSS.

Results

Genetic diversity

The protein profile of 29 Pakistani *Zea mays* landraces tested through SDS-PAGE revealed eight reproducible Loci (Figure 1). Loci one (L-1) showed 41.3% diversity, loci two (L-2) 17.2%, loci three (L-3) 48.27%, loci four (L-4) 44.82%, loci five (L-5) 51.72%, loci six (L-6) 41.37 %, loci seven (L-7) 34.48% and loci eight (L-8) showed 10.34% diversity.

Table 1. Passport information and Genetic distance for 29 Pakistani *Zea mays* landraces calculated through UPGMA amalgamation rules using percent dis-agreement.

Landraces	Origin	Code	Seed color	100Seed weight (g)	UPGMA value	Genetic diversity (%)
1	Swat	ST-1	Half white	0.016	0.00	0.00
2		ST-2	Half white	0.016	0.13	12.50
3		ST-3	White	0.016	0.17	16.67
4		ST-4	Light yellow	0.02	0.41	40.63
5		ST-5	Half white	0.016	0.23	22.50
6	Bunner	BN-1	White	0.014	0.23	22.92
7		BN-2	Creamy white	0.016	0.30	30.36
8		BN-3	Creamy white	0.012	0.22	21.88
9		BN-4	Light yellow	0.014	0.19	19.44
10		BN-5	Dark creamy	0.016	0.48	47.50
11		BN-6	Light yellow	0.012	0.43	43.18
12		BN-7	Yellow	0.01	0.51	51.04
13	Malakand	Mk-1	Extreme light red	0.032	0.38	37.50
14		Mk-2	Milky white	0.026	0.35	34.82
15		Mk-3	Yellow	0.014	0.33	32.50
16	Dir	DR-1	Extreme light yellow	0.014	0.30	30.47
17		DR-2	Pure yellow	0.024	0.32	31.62
18		DR-3	White	0.018	0.29	29.17
19		DR-4	Blackish red	0.016	0.53	52.63
20		DR-5	Yellow	0.022	0.48	47.50
21		DR-6	Dark orange	0.024	0.44	44.05
22		DR-7	Milky white	0.02	0.48	47.73
23		DR-8	Pure yellow	0.028	0.41	41.30
24	Bajur	BJ-1	Creamy	0.02	0.34	33.85
25		BJ-2	Creamy white	0.024	0.53	52.50
26		BJ-3	White	0.018	0.56	55.77
27		BJ-4	Creamy white	0.024	0.55	54.63
28		BJ-5	Half white	0.02	0.41	40.63
29		BJ-6	Milky white	0.03	0.38	38.36
Average %					0.36	35.64

The entire loci were polymorphic in electrophoregram and high degree of genetic divergence was calculated for L-8 10.34% followed by L-2 calculated 17.2% variation. Significant level of Genetic variation observed within Loci pattern of Pakistani *zea mays* landraces is an advantage for plant breeder's in future breeding activities.

Protein binary data matrix was also preceded through UPGMA %disagreement to calculate the genetic

indices (GI) among 29 landraces.

A total of 35.64% average genetic diversity was calculated. Among landraces, ST-1, ST-2, ST-3, ST-5, BN-1, BN-3, BN-4 and DR-3 revealed low level of genetic diversity (average 18.13%). Individually ST-2 showed 12.50% genetic diversity, ST-3 (16.67), ST-1 (0.00%), ST-5 (22.50%), BN-1 (22.92%), BN-3 (21.88%), BN-4 (19.44%) and DR-3 showed total 29.17% genetic diversity. Furthermore 13.79%

Genotypes namely: BJ-3, followed by BJ-4, DR-4, BJ-2, and BN-7 revealed high level of average genetic diversity 53.31%. Individually BJ-3 showed 55.77% genetic diversity in population and hence selected as genetically diverse landraces in the population of maize. Similarly 55.17% genotypes were observed

with moderate genetic diversity from 30% to 47% (Table 1).

Bands frequency

The graphic representation of protein polypeptide bands revealed significant differences within bands frequency (Figure 1).

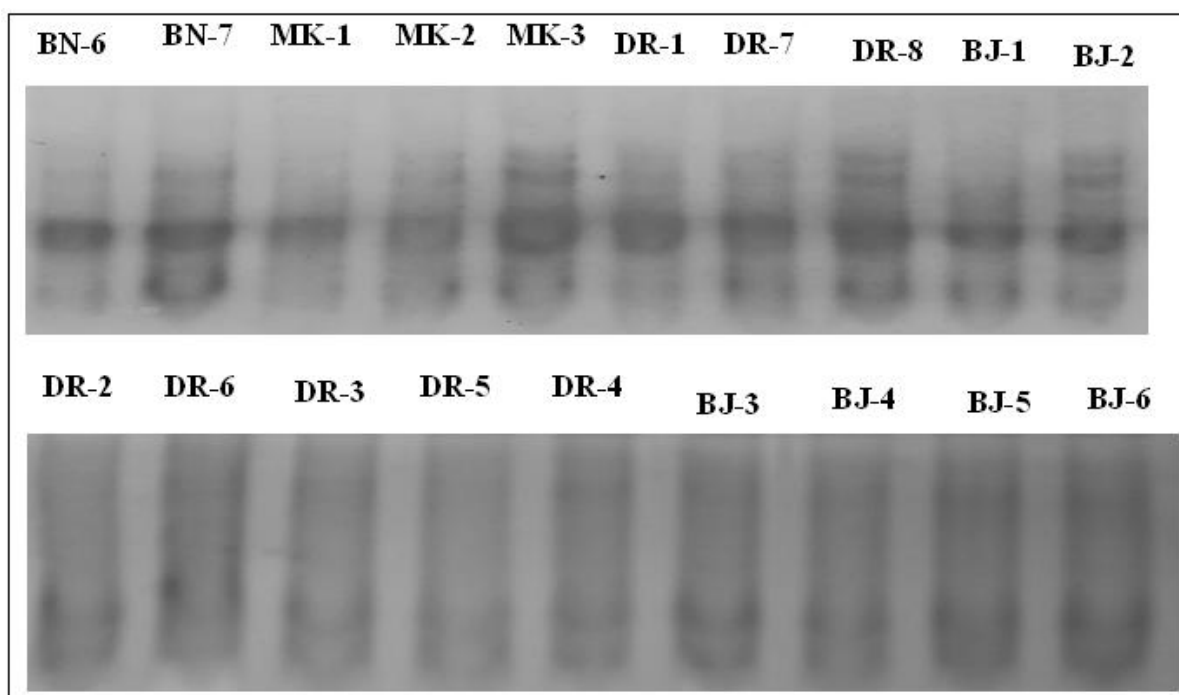


Fig. 1. Protein banding pattern of Pakistani maize landraces.

Band-8 (B-8) was abundantly present in landraces ($f=89.6\%$) followed by B-2 with 82.7% and B-7 with 65.5% frequency. Similarly B-5 was observed with low frequency ($f=48.2$), B-3 with 51.72%, B-4 with 55.1%, B-6 with 58.6% and B-1 with 58.6% frequency in population. The variation exists in protein bands frequency reflects the genetic diversity caused by genetic change, which expressed in seed storage protein.

Genetic association

Genetic association within landraces based protein binary data matrix was tested via Ward method Two Way Cluster Analysis (TWCA) and Principal Component Analysis (PCA). The dendrogram sorted all the genotypes into 21 groups/clusters (Figure. 2). In tree-B, majority of the genotypes (6/29 or 20.6%) were genetic linked in G-17 (cluster-4) rather all these

landraces were collected from different areas two of these are related to Buner, three from Malak and one is related to Dir region. Similarly 3/29 or 10.3% genotypes were associated in G-16 (cluster-3) in these three, two landraces were collected from Buner and one from Swat, and 2/29 maize calculated 6.8% portion of population was grouped in G-13 (cluster-2), the group-13 (G-13) members one as collected from Bajaur and one from Dir. On the other hand each group out of G-1, G-2, G-3, G-4 up to G-21 except 17, 16 and 13 groups represent single maize landrace i.e. G-1 (group one) is consists of one (ST-1) which is related to swat region, G-2 contain (BJ-4) related with Bajaur agency, G-3 contain of (DR-4) related with Dir region, G-4 include of (BJ-3) which is related to Bajaur Agency, G-5 is consist of one genotype (BJ-5) it is also related to Bajaur Agency, G-6 include BJ-6 collected from Bajaur Agency, G-7

contain ST-2, G-8 contain BN-1, G-9 include DR-1 and G-10 contain DR-3 respectively.

These landraces represent low level of genetic association (3.44%) and high level of genetic divergence (96.55%).

The genetic association within landraces, found through TWCA was reconfirmed through Scatter Plots

and Principal Component Analysis (PCA). Similar pattern of genetic linkage among landraces were found that indicated the coordination of both these numerical techniques (i.e. TWCA and PCA) for exploring the genetic association and genetic diversity among 29 landraces collected from different agro ecological zones of Malak and Division, Pakistan.

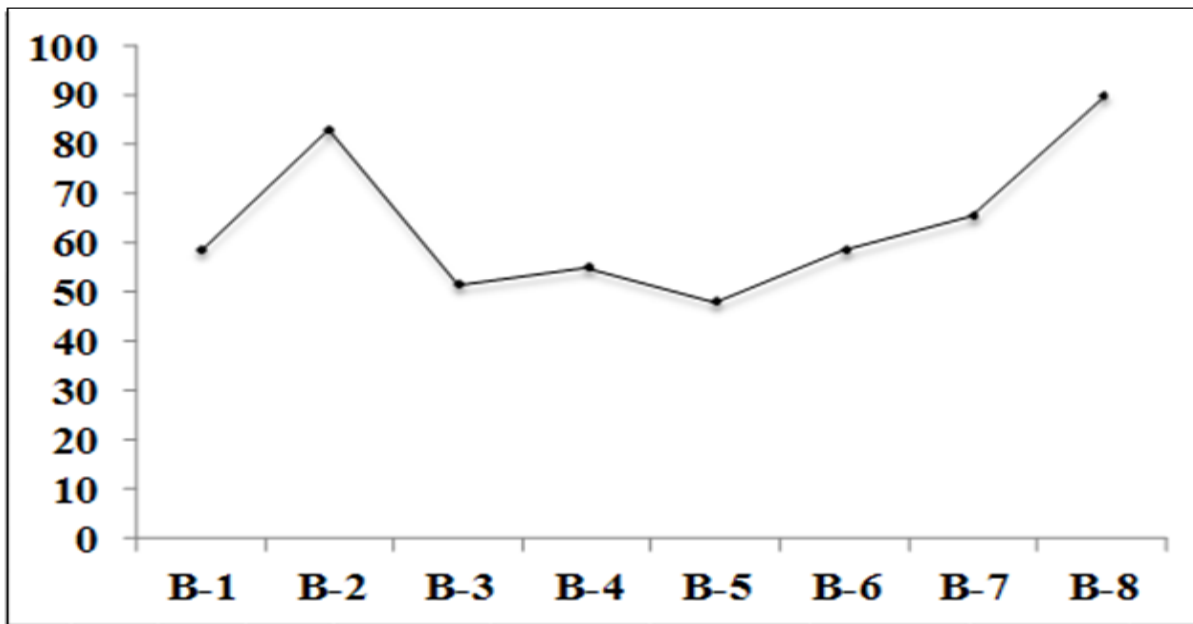


Fig. 2. Graphic representation of protein polypeptide bands, showing diversity within bands frequency in population of maize landraces.

Discussion

Maize is a large grain plant that evolved from its wild-grass ancestors by the direct intervention of human agriculture. Many varieties or “races” differ in physical properties (Goodman and Brown 1988), but generally maize is a single-stalk plant that grows to approximately 8 feet tall with about 20 long, narrow leaves growing individually from nodes along the stalk (Kiesselbach 1999). Several characteristics make it an attractive genetic system (Strable and Scanlon 2009). In addition to lower protein content maize is not a complete protein source and lacking two essential amino acids, tryptophan and lysine. In order to have a complete diet, humans and animals must consume protein sources which are complementary. For example, cereal grains and legumes are a complete protein source when mixed together and are

routinely used in grain rations for animal nutrition and human consumption (Grillenberger *et al.*, 2003). The landraces, as an important source of the genetic diversity and potential material that could be used to widen the base for plant breeding, are among the main objects of these efforts. The on-farm conservation is a way of conserving landraces that implies traditional maintenance of a more diverse, locally adopted plant population (Bellon 2004). Total 29 maize landraces were selected for this study and their seed storage proteins were characterized by SDS-PAGE which has been used for determination of genetic diversity.

Biochemical Assessment

Characterization of genetic diversity within population of crop plants is essential for selection of

elite germplasm with superior agronomic traits (Simon *et al.*, 2007). Protein electrophoresis using SDS-PAGE is one of the most reproducible, valuable techniques for describing genetic diversity at seed storage protein level within and among crop populations and the genetic structure of various grain legumes crop plants have been elaborated previously through electrophoresis of seed protein, for example

Chickpea (Ghafoor *et al.*, 2003; Nisar *et al.*, 2007; Hameed *et al.*, 2009); *Pisum sativa* (Nisar *et al.*, 2009) *Lens culinaris* (Sultan *et al.*, 2006; Wadood *et al.*, 2016), *Vigna unguiculata* (Win *et al.*, 2011) and Lima bean (Lioiet *et al.*, 1999) and Common bean (EI Fiky *et al.*, 2002; Sammour *et al.*, 2007; Adesoye and Ojobo 2012).

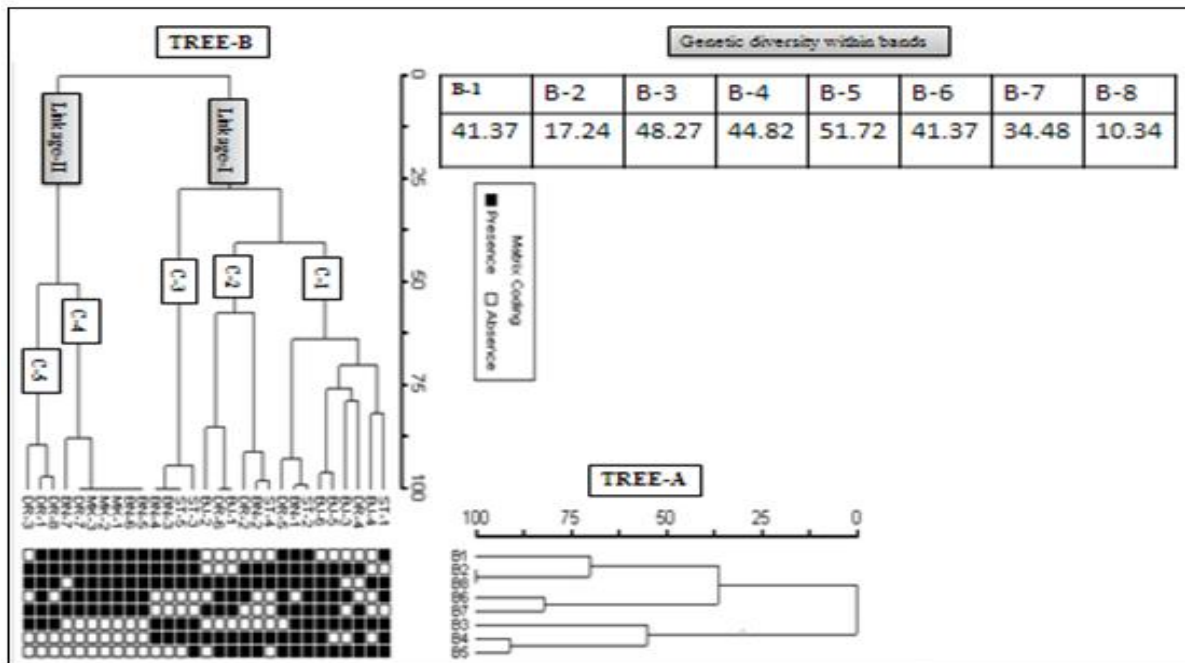


Fig. 3. Two-way Clusters Analysis based on seed storage proteins profile in 29 landraces. TREE-B indicates genetic relationship of 29 maize landraces based on 8 protein bands (B-1 to B-8).

The present study is the first documented report about estimation of genetic diversity of Pakistani local maize Landraces based on electrophoresis of seed storage protein. The electrophoretic profile exhibited variation with major differences within landraces, all proteins polypeptide band were polymorphic in electrophogram.

The average genetic diversity within bands was 36.15%. Among protein bands, high level of genetic diversity was 10.34% and 17.24%, calculated B-8 and B-2.

The variation in protein bands reflects the genetic diversity caused by genetic change/structure, which expressed in seed storage protein. The chances of improvement in crops are directly proportional to the

presence of genetic diversity.

Usually Cluster analysis (CA) and Principle Component analysis (PCA) are widely used for separating population based on genetic diversity or genetic association exists within landraces. CA is used to produce a dendrogram of the population, resulting various landraces which are genetically different from each other discriminated into different groups or genetically similar genotypes occupied by a separate cluster in a tree (Govindaraj *et al.*, 2015).

PCA is a multivariate approach used to complement the cluster analysis information and to produce a dimensional scatter plot of the germplasm which is more informative regarding distances among the genotypes (Hassan *et al.*, 2016).

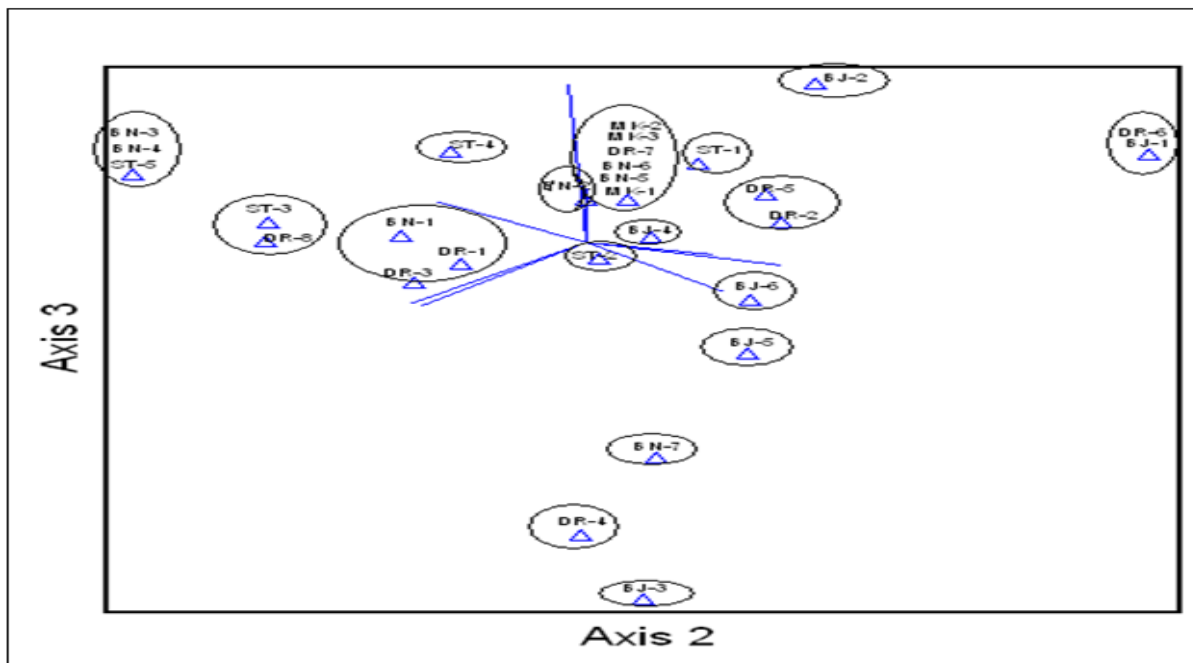


Fig. 4. Confirmation of Cluster Analysis by scattered plot detected through principle components in 29 maize landraces. The overall population show high level of genetic diversity rather than genetic association and split into 17 groups. Each group represents landrace with similar banding pattern.

In the present study, the same statistical analyses were used to understand the genetic association/genetic diversity exist among the landraces. The analysis for 29 Maize landraces separated the landraces into two distinct lineages at 0.00% similarity level. Three clusters namely C-1, C-2 and C-3 were interring spread into sixteen groups in linkage-II (L-II). The two clusters in linkage-I (L-I) were inter spread into five groups. The dendrogram sorted all the landraces into 21 groups (Fig. 4). In tree-B, majority of the landraces (6/29 or 20.6%) were genetic linked in G-17 (cluster-4) rather all these were collected from different areas two of these are related to Buner, three from Malakand and one is related to Dir region. Similarly 3/29 or 10.3% landraces were associated in G-16 (cluster-3) in these three, two were collected from Buner and one from Swat, and 2/29 maize calculated 6.8% portion of population was grouped in G-13 (cluster-2), the group-13 (G-13) members one was collected from Bajaur and one from Dir. On the other hand each group out of G-1, G-2, G-3, G-4 up to G-21 except 17, 16 and 13 groups represent single maize landrace i.e. G-1 (group one) is consists of one landrace ST-1 which is related to swat region, G-2 contain (BJ-4) related

to Bajawr region, G-3 contain of (DR-4) related with Dir region, G-4 include of (BJ-3) which is related to Bajaur Agency, G-5 is consist of one landrace (BJ-5) it is also related to Bajaur Agency, G-6 include BJ-6 collected from Bajaur Agency, G-7 contain ST-2, G-8 contain BN-1, G-9 include DR-1 and G-10 contain DR-3 respectively. These landraces show low level of genetic association (3.44%) and high level of genetic diversity (96.55%).

Interestingly 6 landraces, associated in G-17 were reported from different agro-ecological zones but represent 100% similarity in their protein profile. This may be due to the exchange of seeds among formers.

The protein binary data matrix was also proceeded through Un-weighted Pair Group of Arithmetic Means (UPGMA) described by Nie & Li in (1979) revealed that Average genetic diversity within landraces, calculated 35.64%. The landraces BJ-3, followed by BJ-4, DR-4, BJ-2, and BN-7 revealed high level of average genetic diversity 53.31%GD and hence selected as genetically diverse genotypes in the population of bean landraces.

Conclusion

Our study concluded that, The SDS-PAGE technology indicate considerable level of genetic divergence within protein profile of maize landraces. The diverse population provides plenteous opportunity to breeders to select genetically persistent genotypes/landrace (Ahmad *et al.*, 2012). ST-2 was unique because of the expression of all loci, followed by BJ-3, BJ-4, DR-4, BJ-2, and BN-7 revealed high level of average genetic diversity 53.31%. Individually BJ-3 showed 55.77% genetic diversity in population and hence selected as genetically diverse landraces in the population of maize. Present study also revealed that alone SDS-PAGE is not helpful for assessment of genetic diversity. DNA and other biochemical and molecular markers (SSR, EST-SSR, AFLP, and SNP etc.) are recommended to find and enhance genetic diversity for crop improvement and Germplasm conservation.

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