RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
48 (4) 518-527, 2016

COMPARATIVE ANALYSIS OF GENETIC DIVERSITY OF MAIZE INBRED LINES FROM KASHMIR VALLEY USING AGRO-MORPHOLOGICAL AND SSR MARKERS

M. MUSHTAQ[1], M.A. BHAT[2], J.A. BHAT[1]*, S. MUKHTAR[1] and A.A. SHAH[3]

[1]School for Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha, Jammu-180009Jammu & Kashmir, India
[2]Division of Plant Breeding and Genetics, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Jammu & Kashmir-190025, India
[3]Division of Plant Breeding and Genetics, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu-180009, India
*Corresponding author's email: javid.akhter69@gmail.com
Email addresses of co-authors: muntazirbt@gmail.com, mashrafbhat@skuastkashmir.ac.in, shaziamukhtar3@gmail.com, ashu.ashraf@gmail.com

SUMMARY

In India maize is emerging as third most important crop that contributes 2.5 billion dollar to Indian agriculture GDP. The maize productivity figures are low primarily because of the cultivation of landraces and composite varieties. Therefore, efforts are needed to develop hybrids for exploiting maximum heterosis for increased yield and quality traits. In this regard, 20 maize inbred lines suited to high altitude and plain areas of Kashmir Valley are comparatively evaluated for diversity analysis using both agro-morphological and SSR markers. Analysis of variance showed that all the characters except ear girth were significantly different ($P < 0.01$) among the genotypes. The first four principal components (PCs) of the PCA analysis contributed 97.9% of the variability. The dendrogram obtained through agro-morphological and SSR analysis separated the genotypes into three (I, II and III) and four (I, II, III and IV) major clusters, respectively. Out of 25 SSR markers tested only 10 primer pairs were found polymorphic and detected a total of 31 alleles with an average of 3.1 alleles per locus. The maximum and minimum polymorphic information content (PIC) values were found to be 0.78 and 0.29 for the primers Phi022 and Phi109188, respectively. The Mantel test revealed a non-significant low correlation ($r = 0.12$, $P < 0.148$) between the agro-morphological and SSR matrices. Both methods result in diverse clustering of genotypes suggesting considerable diversity among the studied genotypes. The diverse maize inbred lines identified can be used as parents in exploiting heterosis as well as to identify transgressive segregants for yield and quality traits of maize. Hence, both methods proved effective for the diversity analysis of maize inbreds, and their combined study provides useful information.

Keywords: Genetic diversity, maize inbreds, agro-morphological, SSR, dendrogram

Key findings: The combined genetic diversity analysis using both agro-morphological and SSR markers provides useful information and identified diverse maize inbred lines which could be effectively used as parents in heterosis and transgressive breeding.

Manuscript received: June 23, 2016; Decision on manuscript: October 19, 2016; Manuscript accepted: November 2, 2016.

Communicating Editor: Naqib Ullah Khan

INTRODUCTION

Maize (*Zea mays* L.) also known as corn, is the only cereal crop of American origin that is cultivated in tropical and subtropical regions throughout the world. Maize is currently produced on nearly 100 million hectares in 125 developing countries and is among the three most widely grown crops in 75 countries (FAO STAT, 2010). In India, maize is emerging as third most important crop after rice and wheat, and contributes 2.5 billion dollar to Indian agriculture GDP (Kumar *et al*., 2013). India occupies fifth place in average under maize in the world after US, Brazil, China and Mexico. As regards, Jammu & Kashmir it plays an important role in the livelihood of the people of this hilly and sub-mountainous state and occupies highest area in the state, but productivity figures are very low primarily because of the cultivation of landraces and composite varieties. Therefore, efforts are required to develop hybrids for exploiting maximum heterosis in order to increase production and productivity of maize.

The choice of parents is the initial step in plant breeding and directly benefit transgressive segregation and heterosis, which is considered to be high by the parents that are distantly related (Joshi *et al*., 2004). The diverse parents are observed to give progeny with higher heterosis (Joshi and Dhawan, 1966; Anand and Murrty, 1968). Thus, genetic diversity estimation of crop species determines its potential for improved efficiency and its use for breeding, which inevitably prompted increased food production. The genetic diversity among individuals/populations can be determined using different markers systems viz., morphological, biochemical and molecular.

Morphological traits have been already used to assess genetic diversity of maize genotypes by number of earlier studies (Kashiani *et al*., 2014; Azad *et al*., 2012; Syafii *et al*., 2015; Kumar *et al*, 2015). Various types of molecular markers (RFLP, RAPD, AFLP, ISSR and SSR etc.) are available for varietal identification and genetic characterization of crop germplasm, among them SSR are the marker of choice being co-dominant, multiple allelic, simple, reproducible and reliable in nature. These markers have been used for studying the genetic relationship in maize (Adeyemo *et al*., 2011; Ristic *et al*., 2013; Sserumaga *et al*., 2014; Salami *et al*., 2016), rice (Kunusoth*et al*., 2015), wheat (Arora *et al*., 2014), and many other crops including pea (Handerson *et al*., 2014), faba bean (Abid *et al*., 2015), and soybean (Bisen *et al*., 2015).

The combined analysis through morphological and molecular marker is the best option to characterize inbred lines giving an opportunity to comparatively analyze the phenotypes from field experiments with molecular phenotypes and genotypes from laboratory studies. Comparison of different methods in genetic studies provides researchers and plant breeders with more information in the screening and selection process. Keeping this in view, this study is undertaken to estimate the genetic distance of 20 maize inbred lines collected from Kashmir Valley of State of Jammu and Kashmir using a combination of morphological and SSR markers, which will allow us to study the relative efficacy of these two methods in cultivar differentiation as well as identification of diverse inbred lines that can be used as parents in exploiting heterosis for maximizing maize production and productivity.

MATERIALS AND METHODS

Plant material and experimental site

A set of 20 inbred lines of maize suited to high altitude and plain areas of Kashmir valley were selected for genetic divergence studies. These lines comprised of promising inbred lines from Dryland (Karewa) Agriculture Research Station, Budgam and High Altitude Maize Research, Sub-Station, Sagam, Anantnag. The genotypes included lines from CIMMYT, Mexico. All the lines were in the advanced stage of development. Details about the origin and pedigree of these genotypes are provided in Table 1. Some of the lines used in this study are also currently used as parental lines of two popular maize hybrids released in Kashmir. W3 and W5 are the parents of the first single cross hybrid viz., Shalimar Maize Hybrid-I, whereas KDM-500 is the male parent of Shalimar Maize Hybrid-II, which has

been proposed for release in recently held 31st ZREAC meeting.

The experimental material for the present research was laid out at the experimental area of the Dryland (Karewa) Agriculture Research Station, Budgam and Centre for Plant Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, J & K.

Morphological characterization

The data on ten agro-morphological traits are recorded from five randomly selected representative plants in all the genotypes in each replication. The standard method of DUS test (Distinctiveness, Uniformity and Stability, Govt. of India) was used for recording observation for each of the character which includes plant height, ear height, days to 50% pollen shed, days to 50% silking, ear length, ear girth, 100 grain weight, grain yield per plant, grain yield and shelling % (Biodiversity protocols: www.bioversity.org). The mean values of the data obtained were used for the various statistical analyses.

DNA extraction and SSR analysis

Total genomic DNA was isolated from young leaves at 5 leaf stage from 8-10 field grown plants of each inbred line (approximately 5-7g of fresh weight) using CTAB (CetylTrimethyl Ammonium Bromide) method as modified by Saghai-Maroof *et al*. (1984). Quantification of DNA samples was done by using Nanodrop (mySPEC, Scientific GmbH, Germany) and quality was estimated by using 0.8% agarose gel electrophoresis. High concentration of DNA samples was further diluted in 10:1 Tris-EDTA to a working concentration of 50ng/µl andstored at 4^{o}C for PCR based marker analysis. A total of 25 pairs of SSR primers flanking themicrosatellite region previously developed and published by Sharopova *et al*. (2002) wereselected. After testing the 25 primers, a total of 10 primers were found polymorphic and used for further analysis. Detailed description of the primers is available at Maize DB: http://www.agron.missouri.edu; http://www.gramene.org/markers/microsat/.
PCR reaction was prepared with 50 ng of rice genomic DNA, 0.2 µg of 3' and 5' end primers,

Table 1. List of the maize (*Zea mays* L.) genotypes used for morpho-molecular characterization with their name, source population, altitude and place of collection.

Line	Source population	Altitude	Place of collection
KDM-361A	F-7012	Low and mid	Dryland (Karewa) Agriculture Research Station, Budgam
KDM-343A	Seed Tech 3435	Low and mid	Dryland (Karewa) Agriculture Research Station, Budgam
KDM-332A	AAMH 204	Low and mid	Dryland (Karewa) Agriculture Research Station, Budgam
KDM-914A	AH-1139	Low and mid	Dryland (Karewa) Agriculture Research Station, Budgam
KDM-895A	C-170	Low and mid	Dryland (Karewa) Agriculture Research Station, Budgam
KDM-340A	PRO-349	Low and mid	Dryland (Karewa) Agriculture Research Station, Budgam
KDM-362A	DMR-6520	Low and mid	Dryland (Karewa) Agriculture Research Station, Budgam
KDM-916A	DMR-00RIBK114	Low and mid	Dryland (Karewa) Agriculture Research Station, Budgam
KDM-500A	CM-128	Low and mid	Dryland (Karewa) Agriculture Research Station, Budgam
CM-502	CM-502	Low and mid	Dryland (Karewa) Agriculture Research Station, Budgam
W5	CML-354	High	High Altitude Maize Research, Sub-Station, Sagam, Anantnag
462	-	High	High Altitude Maize Research, Sub-Station, Sagam, Anantnag
30	-	High	High Altitude Maize Research, Sub-Station, Sagam, Anantnag
53	-	High	High Altitude Maize Research, Sub-Station, Sagam, Anantnag
401	-	High	High Altitude Maize Research, Sub-Station, Sagam, Anantnag
W3	CML-349	High	High Altitude Maize Research, Sub-Station, Sagam, Anantnag
YI-1	-	High	High Altitude Maize Research, Sub-Station, Sagam, Anantnag
114-2	-	High	High Altitude Maize Research, Sub-Station, Sagam, Anantnag
460	-	High	High Altitude Maize Research, Sub-Station, Sagam, Anantnag
39	-	High	High Altitude Maize Research, Sub-Station, Sagam, Anantnag

200 mM of each dNTP, 1X PCR buffer containing 50 mM KCL, 10 mMTrisHCl (pH8.9), 2.0 mM $MgCl_2$ and one unit of *Taq* Polymerasein a total of 25 µL solution individually for all 14 primer pairs. PCR thermal cycler was programmed for 1 min at 94°C, 1 min and 30 seconds at 55°C, 1 min at 72°C and a final cycle of 10 min at 72°C.Amplification product was separated on 3.5% of agarose gel in 1X TBE buffer followed by staining with ethidium bromide.

Data analysis

Analysis of variance was performed for all agro-morphological traits in order to test the significance of variation among the genotypes using SPSS 16.0 software. Cluster analysis was done to yield a dendrogram depicting the morphological relatedness of the inbred lines. Principal component analysis (PCA) was also used to detect underlying sources of morphological variability, and to investigate patterns of genetic diversity (Mohammadi and Prasanna, 2003). Bray-Curtis distances and UPGMA (Unweighted pair group method with arithmetic mean) was the clustering method and all these analyses were done using the PAST software (Hammer *et al*., 2001).

For SSR data, the presence or absence of the band was scored as 1 or 0, respectively. In order to determine the utility of the SSR markers, Number of alleles per marker, Polymorphic Information Content (PIC), Effective multiplex ratio (EMR) and Marker Index (MI) were calculated. The Polymorphism Information Content (PIC) values of individual primers were calculated based on the formula PIC= 1- $\Sigma^{n}_{i=1}$ P2ij (Anderson *et al*., 1997). Marker Index, a product of information content, as measured by PIC, and Effective Multiplex Ratio (EMR), was calculated following (Powell *et al*., 1996). The Jaccard's similarity index was calculated using NTSYS-pc version 2.02e (Applied Bio-Statistics, Inc., Setauket, NY, USA) package to compute pair wise Jaccard's similarity coefficients (Jaccard, 1980) and this similarity matrix was used in cluster analysis using an unweighted pair-group method with arithmetic averages (UPGMA) and sequential, agglomerative, hierarchical and nested (SAHN) clustering algorithm to obtain a dendrogram.

Comparison between agro-morphological and SSR data was performed by calculating the correlation between the agro-morphological and SSR similarity matrices through mantel test (Mantel, 1967) with 1000 permutations using PASSaGE 2 software.

RESULTS

Agro-morphological analysis

The analysis of variance showed that mean squares due to genotypes were highly significant ($P \leq 0.01$) for all characters except ear girth which showed non-significant variation (Table 2). Principle component analysis (PCA) of the agro-morphological traits showed that the first four principal components together accounted for 97.929% of the total phenotypic variation (Table 3). The first principal component (PC_1) accounted the maximum portion of 83.364% of total variance, and characters that contribute more positively to this component were plant height, ear height, days to 50% silking, ear length, grain yield per plant, grain yield and shelling%. The second component (PC_2), which featured ear height as the principal trait, explained an additional 8.276% of the phenotypic variation. Finally, third and fourth principal component (PC_3 and PC_4) contributed around 3.762% and 2.527%, respectively of the variability present among the accessions for the traits used in this study. The PC_3 explained the pattern of variation in 100 grain weight, and for PC_4 the maximum variation is contributed by days to 50% pollen shed. The dendrogram obtained using phenotypic characters separated the genotypes into 3 major clusters (I, II and III) consisting of 8, 2 and 10 genotypes, respectively with Bray-Curtis distance ranging from 0.864 to 0.992 (Figure 1). The cluster I and II comprised of inbred lines from low and mid altitudes, while cluster III consists of all ten inbred lines from high altitude.

Table 2. Analysis of variance for yield and yield component traits in maize (*Zea mays* L.).

Source of variation	d. f.	Mean squares									
		Plant height (cm)	Days to 50% pollen shed	Days to 50% silking	Ear height (cm)	Ear girth (cm)	Ear length (cm)	100 Grain weight (g)	Grain yield plant^{-1}	Grain yield (q ha^{-1})	Shelling %age
Replication	1	119.75**	38.57**	41.75**	46.51**	0.38*	4.57**	0.01^{NS}	39.64**	8.44**	27.22**
Treatment	19	93.34**	222.59**	311.25**	318.66**	0.32^{NS}	15.26**	23.77**	105.04**	69.37**	150.75**
Error	19	0.63	3.18	0.34	0.35	0.07	0.11	0.02	0.14	0.49	0.96

Table 3. Eigenvectors, Eigen values, total and cumulative variability (%) for 20 maize genotypes based on ten agro-morphological traits.

Principal component (axes)	PC1	PC2	PC3	PC4
Eigen value	8.336	0.827	0.376	0.253
Variability (%)	83.364	8.276	3.762	2.527
Cumulative (%)	83.364	91.64	95.402	97.929
Traits	Eigenvectors			
Plant height (cm)	0.322	0.008	0.091	-0.699
Ear height (cm)	0.334	-0.117	0.075	0.303
Days to 50% pollen shed	0.3233	0.118	-0.347	0.478
Days to 50% silking	0.342	-0.049	-0.081	0.176
Ear length (cm)	0.332	-0.093	-0.228	-0.322
Ear girth (cm)	0.169	0.949	0.216	-0.004
100 Grain weight (g)	0.283	-0.233	0.848	0.169
Grain yield per plant (g)	0.341	-0.056	-0.143	-0.054
Grain yield (q ha^{-1})	0.339	-0.049	-0.072	-0.127
Shelling %	0.336	-0.035	-0.125	0.092

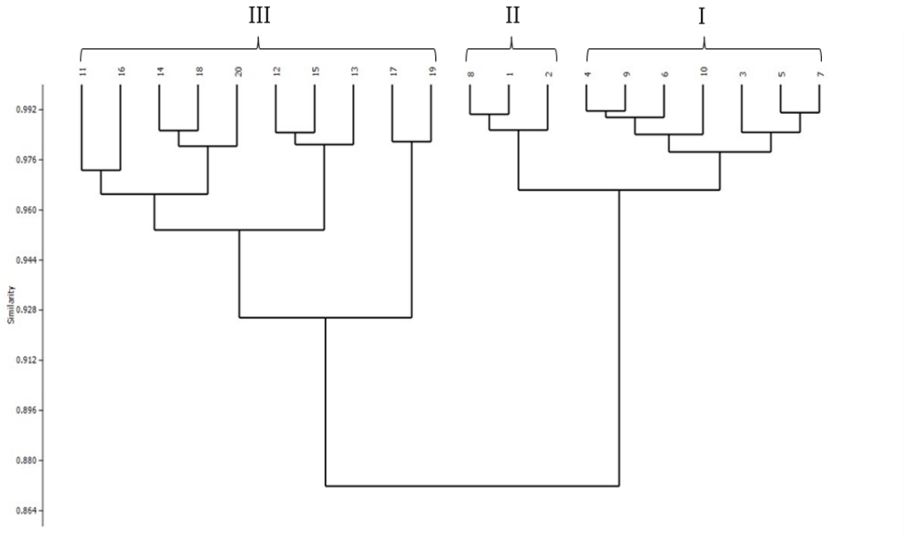

Figure 1. Dendrogram based on agro-morphological traits showing three clusters (I, II and III) of 20 maize genotypes using UPGAMA method and Bray-Curtis distance.

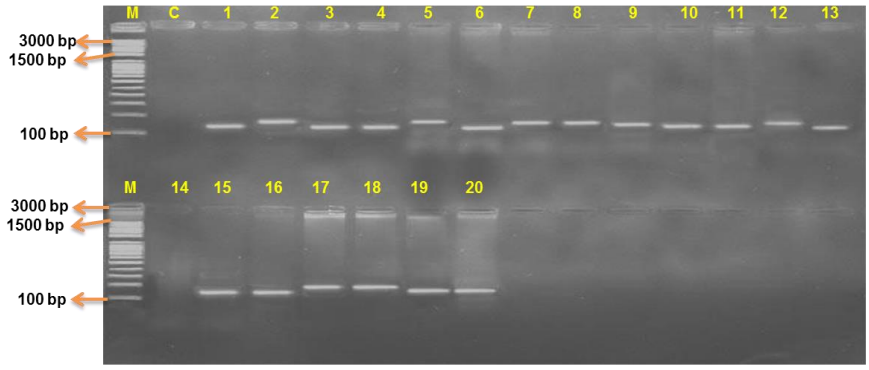

Figure 2. Gel picture showing banding pattern of 20 maize genotypes with phi022 marker.

Molecular marker analysis

All the 20 maize inbred lines were genotyped with 10 polymorphic SSR markers; and are selected for their ability to produce amplified product at optimum concentration, polymorphism level among the genotypes and consistency of the pattern. Total 31 alleles were scored from these primer pairs, and 100 percent were found polymorphic. The gel picture showing a banding pattern of 20 maize inbred lines with phi022 marker is presented in Figure 2. The respective values of overall genetic variability for Polymorphism Information Content (PIC), Effective multiplex ratio (EMR), Number of alleles per locus and Marker Index (MI) across all the 20 genotypes are given in Table 4. Highest PIC value (0.78) was observed for the primer Phi022 and lowest PIC value (0.29) was recorded for the primer Phi109188 (Table 4), with an average 0.58. The MI values ranged from 1.19 to 0.01 with an average of 1.00. The EMR is a feature of marker that indicates the discriminatory potential of the primer, and ranged from 2.57 to 0.05 with an average of 1.06. The allele number per locus varied from 2 to 4, with an average of 3.1 alleles per locus(Table 4). The SSR data were also subjected to genetic cluster analysis to further elucidate the relationship among the genotypes and the dendrogram generated through UPGMA analysis have been presented in Figure3, which grouped all maize inbred lines into 4 major clusters I, II, III and IV comprising of 6, 7, 4 and 3, respectively with Jaccard's similarity coefficient ranging from 0.15 to 0.95. Cluster IV consist of 2 inbred lines from high altitude and one inbred line from low and mid altitude, while as cluster II consist of 4 genotypes from high altitude and 3 from low and mid altitude. The cluster III comprises mostly inbred lines from high altitude except inbred line 10 from low altitude, whereas cluster I consist of all inbred lines from low and mid altitude except inbred line 17 from high altitude (Figure 3).

Comparison of agro-morphological and SSR markers

The mantel test showed non-significant low correlation (r = 0.12, P < 0.148) between the agro-morphological and SSR data. Both agro-morphological and molecular analysis allowed separation of advanced maize inbred lines into different clusters of 3 and 4, respectively. The two methods showed considerable discrepancies between dendrograms as far as the grouping of genotypes is considered. For instance, the inbred lines from high altitude which are morphological clustered in cluster III were grouped into 4 separate clusters (I, II, III and IV) in SSR analysis (Figures 2 and 3). The range of agro-morphological data based genetic distance between pairs of genotypes was considerable narrow (0.864 to 0.992) as compared to that of SSR based data (0.15-0.95).

Table 4. List of markers used, chromosome number, number of alleles, PIC value, effective multiplex ratio (EMR) and marker index (MI).

Markers	Chromosome Number	Number of alleles	PIC	Effective multiplex ratio (EMR)	Marker index (MI)
Phi034	7	2	0.48	0.47	0.97
Phi022	3	3	0.78	2.57	1.19
Phi015	4	4	0.58	0.84	1.07
Phi006	4	4	0.71	1.92	1.10
Zcaa391	9	4	0.56	1.38	1.01
Phi101049	10	3	0.62	0.88	1.19
Phi109188	5	2	0.29	0.05	0.01
Phi063	10	3	0.43	0.20	0.85
Phi064	1	3	0.77	1.31	1.09
Phi053	3	3	0.59	0.94	1.11
Average		3.1	0.58	1.06	1.00

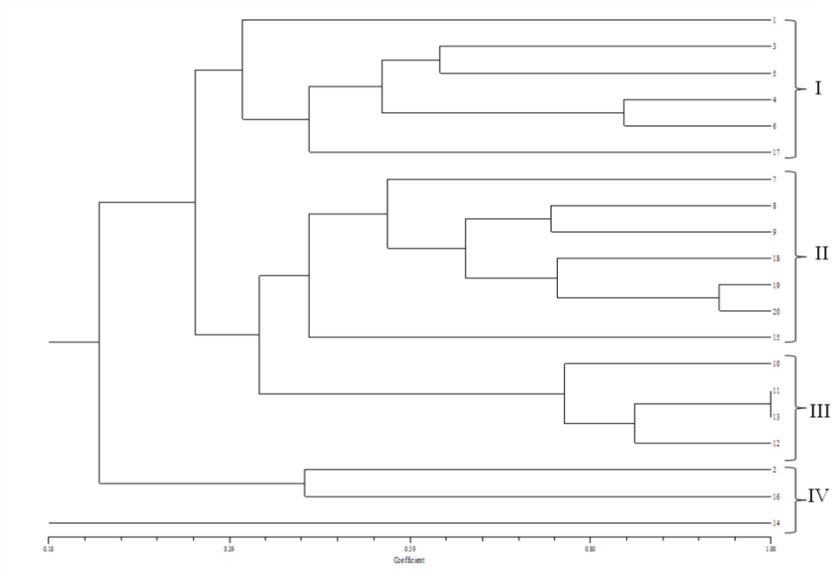

Figure 3. UPGMA dendrogram based on SSR data showing four clusters (I, II, III and IV) of 20 maize genotypes.

DISCUSSION

The analysis of genetic diversity and relationship among the elite breeding materials can significantly aid in crop improvement (Hallauer *et al*., 1988). In maize, this information is useful in planning for hybrid and line development, assigning lines to heterotic groups and in plant variety protection (Yuan *et al*., 2002). There exists an urgent need to promote maize breeding to meet the increasing demands for maize grain and its products. In this context, maize hybrid breeding remains the choice of methods considering its success over years. A logical way to start any breeding programme is to survey the variation present in the available germplam resources. In this regard, 20 maize inbred lines collected from low, mid and high altitudes of Kashmir Valley are characterized using both agro-morphological and molecular markers. The aim of our study was to identify the divergent inbred lines that will be subsequently used in maize breeding to exploit heterosis for yield and other quality traits of maize. The genetic

diversity estimates are often biased by the choice of data viz., phenotypic and molecular marker. Therefore, in the current study both types of data have been used to measure unbiased diversity estimation. In this study, all the agro-morphological traits except ear girth revealed significant (≤ 0.01) variations indicating the presence of sufficient amount of genetic variability among the maize inbred lines for all the traits. In maize significant variations was also reported earlier by other researchers for various morphological traits (Saleem *et al*., 2002; Ishaq *et al*., 2015; Hussain *et al*., 2014). PCA is an effective technique giving information about traits that are more important for the breeder to conduct specific breeding programs Salimi *et al*. (2012). In our study, first four PCs explained 97.929% of variation among 20 maize inbred lines and these results were supported by the finding of Ristic *et al*. (2013); Azad *et al*. (2012), who studied maize genotypes of Serbia and Bangladesh, respectively. The morphological cluster analysis divided the 20 maize inbred lines into 3 major 4 clusters with all the inbred lines from low and mid altitude clustered into cluster I and II, and inbred from high altitude into separate cluster III. The morphological clustering of breeding lines is to some extent in good agreement with their place of collection and altitude.

In this study, a total of 31 alleles were detected by 10 polymorphic SSR markers among 20 maize inbred lines with an average number of 3.1 alleles per locus and average PIC value of 0.58, which was also observed in maize by earlier studies (Adeyemo *et al*., 2011; Sserumaga *et al*., 2014). The UPGAMA analysis based on SSR data divided the genotypes into 4 major clusters I, II, III and IV. The inbred lines from plain areas (low and mid altitude) and high altitude do not form separate clusters as in morphological analysis but are clustered together in all the 4 major clusters. It is because SSR provides more information than morphological analysis distinguishing some genotypes that are not morphologically distinguished.

The Mantel test revealed a non-significant low correlation between the agro-morphological and SSR matrices (r = 0.12, P < 0.148) of the 20 maize inbred lines, showing that these methods discriminated very differently among the genotypes. As the two methods are based on different criteria, agro-morphological dendrogram are constructed based on phenotypic data, Bray-Curtis distances and UPGMA, whereas molecular phylogenetic tree is based on SSR data, Jaccard's similarity coefficient and UPGMA. Low correlation between morphological and molecular markers has been reported in many crops (Koehler-Santos *et al*., 2003; Ferriol *et al*., 2004; Bushehri *et al*., 2005) and these authors suggest that it could be as a result of the independent nature of morphological and molecular variations. The other reason may be that a large portion of variation detected by molecular markers is non-adaptive and is therefore not subject to either natural or artificial selection as compared with phenotypic characters, which in addition to selection pressure are influenced by the environment (Vieira *et al*., 2007).

In conclusion, this study showed that both morphological and molecular analysis results in the diverse clustering of 20 maize inbred lines with the latter showed more diversity among the studied genotypes as compared to morphological analysis as indicated by similarity coefficient distribution. Therefore, it is evident that the studied maize inbred lines from Kashmir Valley possess fair amount of genetic diversity, and the diverse lines identified can be effectively utilized as parents in exploiting heterosis as well as to identify transgressivesegregants for higher yield and quality traits of maize. This will lead to increased maize production and income of resource poor farmers of state Jammu & Kashmir to make them self-sufficient. In addition, these inbred can be potentially used as donors in breeding programme as well as in the development of bi-parental and multi-parent mapping populations for identification of genes/QTLs for yield, yield contributing and quality traits of maize. Hence both agro-morphological and SSR markers proved to be useful in genetic diversity analysis of maize inbreds.

ACKNOWLEDGEMENTS

We thank the Vice Chancellor and Centre for Plant Biotechnology of SKUAST-K for providing the lab facilities and financial assistance for carrying this work.
Conflict of Interest: The authors declare that they have no conflict of interest.

REFERENCES

Abid G, Mingeot D, Udupa SM, Muhovski Y, Watillon B, Sassi K, M'hamdi M, Souissi F, Mannai K, Barhoumi F, Jebara M (2015). Genetic relationship and diversity analysis of faba bean (*Viciafaba* L. var. Minor) genetic resources using morphological and microsatellite molecular markers. *Plant Mol. Biol. Rep.* 33: 1755-1767.

Adeyemo O, Menkir A, Melaku G, Omidiji O (2012). Genetic diversity assessment and relationship among tropical yellow endosperm maize inbred lines using SSR markers. *Maydica* 56: 1-7.

Anand IJ, Murrty BR (1968). Genetic divergence and hybrid performance in linseed.*Ind. J. Genet.Plant Breed.* 28: 178-185.

Anderson JA, Churchill JE, Autrique SD, Tanksley S, Sorrells ME (1997).Optimizing parental selection for genetic linkage maps.*Genome* 36: 181-188.

Arora A, Kundu S, Dilbaghi N, Sharma I, Tiwari R (2014). Population structure and genetic diversity among Indian wheat varieties using microsatellite (SSR) markers.*Aust. J. Crop Sci.* 8: 1281-1289.

Azad MAK, Biswas BK, Alam N, Alam SS (2012). Genetic diversity in maize (*Zea mays* L.) inbred lines. *The Agriculturists* 10(1): 64-70.

Bisen A, Khare D, Nair P, Tripathi N (2015). SSR analysis of 38 genotypes of soybean (*Glycine max* (L.)Merr.)genetic diversity in India. *Physiol. Mol. Biol. Plants* 21: 109-115.

Bushehri A, Torabi S, Omidi M, Ghannadha M (2005). Comparison of genetic and morphological distance with heterosis with RAPD markers in hybrids of barley.*Int. J. Agric. Biol.* 7: 592–595

FAOSTAT (2010).Statistical databases and data-sets of the Food and Agriculture Organization of the United Nations (http://faostat.fao.org/default.aspx).

Ferriol M, Pico B, Fernandez D, Nuez F (2004). Molecular diversity of germplasm collection of squash (*Cucurbitamoschata*) determined by SRAP and AFLP markers. *Crop Sci.* 44: 653–664.

Hallauer AR, Russell WA, Lamkey KR (1988).Corn breeding. In: G.F. Sprague, J.W. Dudley, eds., Corn and corn improvement. 3rd edition, ASA-CSSA-SSSA, Madison, Wisconsin, WI. Pp. 469-564

Hammer Ø, Harper DAT, Ryan PD (2001). PAST: paleontological statistics software package for education and data analysis. http://palaeo-electronica.org/2001_1/past/issue1_01.htm, Accessed August 20, 2009.

Handerson C, Noren SK, Wricha T, Meetei NT, Khanna VK, Pattanayak A, Datt S, Choudhury P, Kumar M (2014). Assessment of genetic diversity in pea (*Pisumsativum* L.) using morphological and molecular markers.*Indian J. Genet.* 74: 205-212.

Hussain M, Shah KN, Ghafoor A, Kiani TT, Mahmood T (2014). Genetic analysis for grain yield and various morphological traits in maize (*Zea mays* L.) under normal and water stress environments. *J. Anim. Plant Sci*. 24: 1230-1240.

Ishaq M, Rahman H, Hassan G (2015). Genetic potential, variability and heritability of various morphological and yield traits among maize synthetics. *Electr. J. Biol.*11(4).

Jaccard P (1980). Nouvelles recherches sur la distribution florale. *Bull. Soc. Vaud. Sci. Nat.* 44: 223-270.

Joshi AB, Dhawan NL (1966). Genetic improvement of yield with special reference to self-fertilizing crops.*Indian J. Genet.Plant Breed.* 26: 101-113.

Joshi BK, Mudwari A, Bhatta MR, Ferrara GO (2004). Genetic diversity in Nepalese wheat cultivars based on agro-morphological traits and coefficients of parentage. *Nepal Agric. Res. J.* 5: 7-17.

Kashiani P, Saleh G, Abdulla NAP, Sin MA (2014). Evaluation of genetic variation and relationships among tropical sweet corn inbred lines using agronomic traits. *Maydica* 59: 275-282.

Koehler-Santos P, Dornelles AL, de Freitas LB (2003). Characterization of mandarin citrus germplasm from southern Brazil by morphological and molecular analysis.*Pesq.Agropec.Bras.* 38: 797–806.

Kumar A, Kumari J, Rana JC, Chaudhary DP, Kumar R, Singh H, Singh TP, Dutta M (2015). Diversity among maize landraces in North West Himalayan region of India assessed by

agro-morphological and quality traits. *Indian J. Genet. Plant Breed.* 75: 188-195.

Kumar R, Srinivas K, Sivaramane N (2013). Assessment of the maize situation, outlook and investment opportunities in India.Country Report – Regional Assessment Asia (MAIZE-CRP), National Academy of Agricultural Research Management, Hyderabad, India.

Kunusoth K, Vadivel K, Sundaram RM, Sultana R, Rajendrakumar P, Maganti S, Subbarao LV, Chin-Wo SR (2015). Assessment of genetic diversity of elite indian rice varieties using agro-morphological traits and SSR Markers. *Am. J. Exp. Agric.* 6: 384.

Mantel N (1967). The detection of disease clustering and a generalized regression approach. *Cancer Res.* 27: 209-220.

Mohammadi SA, Prasanna BM (2003). Analysis of genetic diversity in crop plants - salient statistical tools and considerations. *Crop Sci.* 43: 1235-1248.

Powell W, Marchray GC, Provan J (1996). Polymorphism revealed by simple sequence repeats.*Trends Plant Sci.* 1: 215-222.

Ristić D, Babić V, Anđelković V, Vančetović J, Mladenović-Drinić S, Ignjatović-Micić D (2013). Genetic diversity in maize dent landraces assessed by morphological and molecular markers. *Genetika* 45: 811-824.

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984). Ribosomal DNA spacer-length polymorphisms in barlymendelian inheritance, chromosomal location and population dynamics. *Proc. Natl. Acad. Sci. USA* 81: 8014-8018.

Salami HA, Sika KC, Padonou W, Aly D, Yallou C, Adjanohoun A, Kotchoni S, Baba-Moussa L (2016). Genetic diversity of maize accessions (*Zea mays* l.) cultivated from benin using microsatellites markers. *Am. J. Mol. Biol.* 6: 12.

Saleem M, Shahzad K, Javid M, Ahmed A (2002). Genetic analysis for various quantitative traits in maize (*Zea mays* L.) inbred lines. *Int. J. Agric. Biol.* 4(3): 379-382.

Salimi S, Lahiji HS, Abadi GM, Salimi S, Moradi S (2012). Genetic diversity in soybean genotypes under drought stress condition using factor analysis and cluster analysis. *Word. Appl. Sci. J.* 16: 474-478.

Sharopova N, McMullen MD, Schultz L, Schroeder S, Sanchez-Villeda H, Gardiner J, Bergstrom D, Houchins K, Melia-Hancock S, Musket T, Duru N (2002). Development and mapping of SSR markers for maize. *Plant Mol. Biol.* 48: 463-481.

Sserumaga JP, Makumbi D, Ji H, Njoroge K, Muthomi JW, Chemining'wa GN, Kim H (2014). Molecular characterization of tropical maize inbred lines using microsatellite DNA markers. *Maydica* 59: 267-274.

Syafii M, Cartika I, Ruswandi D (2015). Multivariate analysis of genetic diversity among some maize genotypes under maize-albizia cropping system in Indonesia. *Asian J. Crop Sci.* 7(4): 244.

Vieira E, Carvalho F, Bertan I, Kopp M, Zimmer P, Benin G, da Silva J, Hartwig I, Malone G, de Oliviera A (2007). Association between genetic distances in wheat (*T. aestivum*L.) as estimated by AFLP and morphological markers. *Gen. Mol. Biol.* 30: 392-399.

Yuan LX, Zhang SH, Warburton M, Li XH, Fu JH, Li MS (2002). Assessment of genetic similarities among maize inbred lines using SSR markers. In: G. Srinivasan, P.H. Zaid, B.M. Prasanna, F. Gonzalez, K. Lesnick, eds., Proc. 8th Asian Regional Maize Workshop, August 5-8, 2002, Bangkok, Thailand.pp. 50-58.