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ASSESSMENT OF GENETIC DIVERSITY AND POPULATION STRUCTURE AMONG NORTH-EASTERN AND NORTH-WESTERN HIMALAYAN MAIZE LANDRACES USING MORPHOLOGICAL AND MOLECULAR MARKERS

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ABSTRACT
Genetic diversity among 60 maize genotypes was determined using morphological and SSR markers. Sufficient genetic variability was observed for all the twelve traits studied during present investigation. Estimates of phenotypic coefficient of variation (PCV) were higher than genotypic coefficient of variation (GCV) for all the traits. PCV and GCV was highest for grain yield per plant (42.76%, 34.37 %) followed by 100 gram weight (17.79%, 17.37%), respectively. The broad sense heritability was found to be high (>80%) for most of the traits except for grain yield per plant (64.60%). High PCV, GCV and GA were observed for grain yield per plant with moderate heritability. Grains per row revealed high direct contribution towards grain yield per plant. Cob placement height, cob length and cob girth contributed most towards genetic divergence. At molecular level, 20 SSR primers amplified a total of 52 polymorphic alleles with an average of 2.60 alleles per primer. Mean polymorphic information content was 0.31 showing a moderate level of SSR polymorphism. Cluster analysis differentiated 60 maize landraces into four major clusters. During present studies few genetically divergent landraces (LM-18-08, LM-19-07, LM-14-11, LM-01-08 and LM-11-11) could be employed for their systematic and efficient use in breeding programs.

INTRODUCTION

Assessment of genetic diversity of any given crop species is a suitable precursor for crop improvement as it provides information to guide the selection of parental lines and design of breeding programs. Among the cereals, maize (*Zea mays* L., 2n = 20) is the leading cereal worldwide, originated in Central America and Mexico but because of its wide adaptability and higher productivity potential, it is grown over a wide range of environments around the world. It belongs to the tribe Maydeae, of the grass family, Poaceae.

Maize is the important crop followed by wheat and rice and it accounts for 4.8% of the total cropped area and 3.5% of the value of the agricultural output and contributes more than 7% in national food basket. Maize is one of the most important *Kharif* crop of Himachal Pradesh occupying an area of 0.30 million ha with a total production and productivity of 0.68 million tonnes and 23.25 q/ha, respectively (Anonymous, 2015). There are many virgin pockets where a lot of variability for the traits of economic importance exists, which neither has been evaluated systematically nor exploited so far. Many primitive maize landraces cultivated in hilly areas possess useful characteristics like resistance to stalk rot, stem borer and can withstand water lodging and are sweet in taste. Despite the advent of hybrid varieties, about 70% areas are still under local landraces. These local cultivars are adapted to the agricultural system characterized by the limited use of chemical fertilizer and also to consumption preference by local people. Thus, knowledge about germplasm diversity and genetic relationships among breeding materials could be an invaluable aid in crop improvement strategies (Prasanna and Hoisington, 2003).

The genetic variability in maize landraces has been characterized by using morphological traits (Goodman and Bird, 1977) and isozymes (Revilla *et al.*, 1998). For effective utilization of genetic resources, it is important to evaluate the phenotypic variation for important agronomic attributes (Franco *et al.*, 2001, Meena *et al.*, 2016).The importance of phenotypic characterization of maize landraces has been highlighted by studies in various countries including Canada (Azar *et al.*, 1997), Ethiopia (Beyene *et al.*, 2006)), Italy (Hartings *et al.*, 2008), India (Prasanna and Sharma, 2005) and China (Wei *et al.*, 2009). However, variation at phenotypic level may not always guarantee the genetic constitution because of environmental influences and genetic heterogeneity (Smith and Smith, 1992). A combined approach of using phenotypic and molecular markers is required to analyze diversity in maize and develop the genetic resources (Hammer *et al.*, 1999, Soni and Khanorkar, 2013).

Molecular markers have significantly aided in various PCR based markers available for germplasm characterization, the microsatellites or simple sequence repeat (SSR) markers are widely preferred in maize for diversity analysis (Prasanna and Hoisington 2003, Dubreuil *et al.*, 2006, Shukla *et al.*, 2014, Thakur *et al.*, 2017). Due to their high allelic diversity and genetically codominant nature, the SSR loci are also well-suited for the study of population structure (Rana *et al.*, 2015, Thakur *et al.*, 2017, Malik *et al.*, 2020).

To meet the demands of the increasing population on a global front, it is important to screen the available germplasm of diverse origin. Since the maize landraces of north east and north western Himalayan region has not been fully exploited, documented and utilized systematically, so the screening of the available maize landraces of diverse origin along with checks and its genetic variability analysis using morphological and molecular markers will generate information on useful traits. Keeping all these considerations, the present investigation has been formulated with the aim to access genetic diversity among sixty genotypes of north east and north western Himalayan regions using SSR markers and agro morphological traits, which could be used as source populations for deriving inbred lines for utilization in the hybrid breeding program.

MATERIALS AND METHODS

Plant materials

Sixty maize genotypes which comprised of 56 diverse local landraces and four checks *viz.*, Bajaura Makka, Girija, Early Composite and Bajaura Popcorn were used during present study. The forty genotype of maize were obtained from Himachal Pradesh, four genotypes were obtained from Mizoram and twelve were obtained from Sikkim and were evaluated with four checks (Table 1).

Morphological evaluation

Sixty landraces along with checks were evaluated for different morphological and quality trait in α-RBD design during the year 2013-14 with plot size of 3.0 × 1.2 m^2 with row to row and plant to plant distance of 60 cm and 20 cm, respectively (having 2 rows/plot) with 2 replications, 12 blocks/replication and 5 entries/block carried out at the experimental farm of the Department of Crop Improvement, College of Agriculture, CSK HPKV, Palampur, situated at 1290.8 m amsl having latitude 32° 6′ N and longitude 76° 3′ E. To raise a healthy crop, recommended cultural practices were followed for the field experiments throughout the cropping season. The data were recorded on 11 quantitatively assessed and one qualitatively measured traits. The quantitatively assessed traits were plant height (cm), cob placement height (cm), 100-grain weight (g), cob length (cm), cob girth (cm), kernel rows per ear, grains per row, days to 50 per cent pollen shed, days to 50 per cent silking, days to 75 per cent maturity, grain yield and were recorded as per Thakur *et al.*, 2017. The crude protein content for each entry was calculated by Micro-Kjeldhal Method of AOAC (AOAC, 1970). Morphological traits were measured based on maize descriptors developed by the Bioversity International. The data recorded for various traits were subjected to statistical analysis using the softwares PROC GLM SAS (Falush *et al.*, 2007) and StatistiXL version 1.10. The genetic divergence of maize genotypes was estimated using Mahalanobis D^2-statistics (Mahalanobis, 1936).

Molecular analysis

DNA extraction

Young leaves of each landrace were used for DNA extraction following CTAB method (Murray and Thompson 1980) with some modifications. Preparation of DNA stocks in Tris (10 mM) EDTA (1 mM) buffer, quantification and dilutions were done according to Sharma *et al.*, 2015.

SSR genotyping

A total of 100 maize specific SSR primers were initially screened for polymorphism, of which 20 were selected based on polymorphism and reproducible amplification products. PCR reactions were carried out using these primers. For amplification of genomic DNA, the PCR reactions were carried out in 10.0 µl final volumes containing 4.65 µl sterilized distilled water, 1.0 µl template DNA (13ng/µl), 1.0 µl of dNTP mix (0.2 mM each of dATP, dCTP, dGTP, dTTP), 1.25 µl 10X PCR buffer (10 mM Tris–HCl, 50 mM KCl, pH 8.3), 1.0 µl of $MgCl_2$ (25 mM), 0.5 µl of each primer (5 µM) and 0.1 µl Taq polymerase (5U/µL). PCR amplification was carried out in S1000 TM Thermal Cycler (BIO-RAD) and PCR reactions were performed as per Kaur *et al.*, 2016. The amplified products were electrophoresed on 3% agarose gel and stained with ethidium bromide (0.5 µg/ml). The PCR products were visualized and photographed using the Gel-Documentation Unit (BIO-RAD). Sizing of alleles was done with the help of 50-bp DNA ladder (Fermentas, Lithuania).

Data analysis

All fragments were scored manually and converted into binary data, i.e., 1 for the presence of band and 0 for the absence of the band. For each primer pair, polymorphism information content (PIC) was calculated as per the formula provided by Botstein et al, 1980.

$$PICi = 1 - \sum_{j=i}^{n} 2ij$$

Where Pij is the frequency of the jth pattern for the marker, i and summation extend over n patterns. Marker index (MI) was calculated as per formula given by Prevost and Wilkinson 1999. Various genetic diversity estimates such as expected heterozygosity (He), observed heterozygosity (Ho) etc. was calculated as per Yeh *et al.*, 1997. Cluster analysis was performed using distance method and dendrogram based on the unweighted pair group method with arithmetic mean (UPGMA) was constructed with the help of NTSYS pc2.0 (Rohlf, 1993). Neighbor-joining (N-J) tree was constructed with the help of DARwin software (Perrier and Jacquemoud 2006). Bootstrapping with 1000 replicates was performed with DARwin software. The genetic structure was assessed at the population level with STRUCTURE software, version: 2.3.3 (Pritchard, 2000, Falush *et al.*, 2007) and setting of parameters was done as per Thakur *et al.*, 2017. K was explored between 1 and 10, with ten independent assessments of the log likelihood of the data. The highest value was shown at K = 4. Therefore, STRUCTURE analysis was conducted for K = 4.

Genetic differentiation (Fst) estimates for each cluster were also detected by STRUCTURE. Genetic relationships among the genotypes were analyzed by principal coordinate analysis (PCA) using the GenAlex 6.4 program (Peakall and Smouse, 2006). All the genotypes were plotted on the first two principal axes. An analysis of molecular variance (AMOVA) was performed using GenAlex 6.4 program (Peakall and Smouse, 2006).

RESULTS AND DISCUSSIONS

Morphological traits

Among the 12 traits studied, a wider range of variation was observed for days to 75 percent maturity, plant height, cob placement height, grain yield per plot, 100-grain weight, grains per row and protein content (Table 2). Similar findings were observed by Rahman *et al.*, in 2008 and Saleem *et al.*, in 2008 for plant height, ear height, days to 50 per cent anthesis and days to 50 per cent silking, grain yield and protein content. The variation showed may be contributed to differences at the genotypic level and may be utilized in future maize breeding programs. Significant variation was observed for yield and morphological traits indicating enough variation among maize genotypes. Further, in this study based on the mean performance of genotypes, lines LM-18-08, LM-19-07 and LM-14-11 were found superior over the checks for seed yield per plant. Out of this LM-18-08 was found significantly superior for (days to 50 per cent pollen shed and cob girth). Whereas, grains per row was found to be significantly superior in genotype LM-14-11 as compared to best check(s). The statistically superior lines on the basis of overall mean performance for different traits can be exploited directly in the future breeding program(s) for genetic improvement in maize.

During present studies high PCV 42.76% (>30%) was observed for grain yield per plant, moderate (15-30%) for cob placement height and 100-grain weight whereas, it was low (<15%) for days to 50 per cent pollen shed, days to 50 per cent silking, days to 75 per cent maturity, plant height, cob length, cob girth, kernel rows per ear, grains per row and protein content. Meena et al., in 2016 reported high PCV for gain yield in newly developed maize genotype.

The genotypic coefficients of variability were high (>30%) also followed a similar pattern with respect to grain yield per plant. It was moderate (15-30%) for cob placement height and 100-grain weight and low (<15%) for days to 50 per cent pollen shed, days to 50 per cent silking, days to 75 per cent maturity, plant height, cob length, cob girth, kernel rows per ear, grains per row and protein content, thus indicating the true picture of phenotypic expression of these characters with their genotypic expression. Shanthi *et al.*, 2011 also observed high GCV for grain yield. Bello *et al.*, 2012 observed high values of PCV and GCV in grain yield and showed that PCVs were slightly higher than GCVs for all the characters, suggesting the influence of environment on the expression of these characters which was in confirmation with the present study. The high variability values for grain yield per plant among the genotypes could be beneficial for selection of high yielding superior lines. However, Rafiq *et al.*, 2010 observed high GCV for grain yield, cob length, cob placement height, 100-grain

Table 1: Details of the plant material used along with their source/ pedigree

Genotypes	Source/Pedigree
LM-01-08	BHATIYAT (CHAMBA)
LM-02-08	BHATIYAT (CHAMBA)
LM-04-08	SALOONI (CHAMBA)
LM-17-08	KANDWARI (KANGRA)
LM-18-08	RAKH (KANGRA)
LM-19-07	LOBER, KUKUMSERI (LAHAUL SPITI)
LM-27-07	PIMAL, KUKUMSERI (LAHAUL SPITI)
LM-28-03	PHALIA (KANGRA)
LM-33-06	DAROH (KANGRA)
LM-34-06	DARGELLA L_1 (KANGRA)
LM-35-06	DARGELLA L_2 (KANGRA)
LM-36-06	AMB (UNA)
LM-37-07	CHADIAR (KANGRA)
LM-40-07	KUTHAN, JAISINPUR (KANGRA)
MAHDHANU B	CHAMBA
LM-43-07	NAGPURI,JAISNPUR (KANGRA)
LM-01-11	BAROT L_1 (MANDI)
LM-02-11	BAROT L_2 (MANDI)
LM-03-11	CHAMBA
LM-04-11 A	RAMPUR,SATHOO (CHAMBA)
LM-05-11	MAHDHANU (CHAMBA)
LM-06-11	MIZORAM L_1
LM-07-11	MIZORAM L_2
LM-08-11	MIZORAM L_3
LM-09-11	MIZORAM L_4
LM-10-11	PRIUNGLE (CHAMBA)
LM-11-11	PRIUNGLE (CHAMBA)
LM-14-11	CHAMINU (CHAMBA)
LM-15-11	HAMIRPUR
JCR 2038	SIRMOUR
JCR 2039	SOLAN
JCR 2052	SHIMLA
JASINPUR	KANGRA
JCR 2058	SHIMLA
LOCAL WHITE	CHAMBA
JCR/DS 1034	CHAMBA
JCR/DS 1041	CHAMBA
JCR/DS 1062	CHAMBA
YP/JCR 4	KANGRA
YP/JCR 7	KANGRA
AB/JCR 4	KULLU
AB/JCR 12	KULLU
AS/JCR 1	KULLU
AS/JCR 7	KULLU
SETI MAKKI 1	SIKKIM
SETI MAKKI 3	SIKKIM
SETI MAKKI 4	SIKKIM
PAHENLO MAKKI 2	SIKKIM
PAHENLO MAKKI 4	SIKKIM
PAHENLO MAKKI 5	SIKKIM
PAHENLO MAKKI 6	SIKKIM
RATO MAKKI 2	SIKKIM
RATO MAKKI 4	SIKKIM
SIKKIM PRIMITIVE 2	SIKKIM
TEMPO RIMZING	SIKKIM
SEHRUNG	SIKKIM
BAJAURA MAKKA	PS 62/FH 3209/ FH 3198/ FH 3202/EC
GIRIJA	NAVJOT/PARVATI/ KH9405/ZC 2810/ MMH 81/MMH 60/PRO 306/ICI 736/ L 110/ ZC 2733/ JH 1136 /JH 1146
EARLY COMPOSITE	Kullu local/Abas Kajas/maize No. 8/ max-3c B/Bhodipur Yellow, JMI 603/ VL 1/YUZP SC-3, YUZP SC-4/YUZP SC-71C/YUZP DC-755/YUZP 1 SC-790/VL 2 and VL42
BAJAURA POPCORN	SELECTION FROM LOCALS (KULLU & CHAMBA)

Table 2: Estimates of parameters of variability for various traits in maize genotype

Traits	Mean ± S.E(m)	Range	PCV (%)	GCV (%)	ECV (%)	Heritability h^2bs (%)	Expected GA
Days to 50% pollen shed	61.90 ± 1.26	52.00-83.00	8.51	8.01	2.87	88.60	15.54
Days to 50% silking	67.46 ± 1.19	55.00-85.00	7.43	7.00	2.49	88.80	13.60
Days to 75% maturity	110.31 ± 1.53	94.50-117.00	4.27	3.80	1.96	79.00	6.95
Plant height (cm)	251.71 ± 7.42	185.80-320.00	11.85	11.10	4.17	87.60	21.40
Cob placement height (cm)	142.44 ± 2.45	99.15-200.05	16.88	16.71	2.43	97.90	34.06
Grain yield/plant(g)	91.46 ± 16.45	30.03-192.91	42.76	34.37	25.43	64.60	56.92
100-grain weight (g)	30.22 ± 0.82	15.62-39.94	17.79	17.37	3.85	95.30	34.93
Cob length (cm)	16.83 ± 0.63	12.75-20.30	11.37	10.09	5.25	78.70	18.43
Cob girth (cm)	13.84 ± 0.19	9.31-15.67	9.35	9.14	1.99	95.50	18.40
Kernel rows/ear	12.86 ± 0.34	10.00-16.00	10.58	9.91	3.70	87.80	19.12
Grains/row	33.64 ± 0.94	20.00-42.70	12.97	12.35	3.95	90.70	24.24
Protein content (%)	9.73 ± 0.23	8.71-11.45	7.52	6.76	3.31	80.70	12.50

S.E. - Standard error, PCV - Phenotypic coefficient of variation, GCV - Genotypic coefficient of variation, ECV - Environmental coefficient of variation, h^2bs - Heritability due to broad sense, GA - Genetic advance

Table 3: Estimates of genotypic correlation coefficient among various yield, morphological and quality traits in maize

Traits	Days to 50% silking	Days to 75% maturity	Plant height (cm)	Cob placement height (cm)	Grain yield/ plant (g)	100-grain weight (g)	Cob length (cm)	Cob girth (cm)	Kernel rows/ ear	Grains/ row	Protein content (%)
Days to 50% pollen shed	0.944*	0.665*	0.244*	0.357*	-0.067	-0.071	-0.057	0.052	-0.119	0.101	-0.080
Days to 50% silking		0.694*	0.344*	0.465*	-0.103	-0.011	-0.094	0.103	-0.182*	-0.017	-0.029
Days to 75% maturity			0.476*	0.470*	0.310*	0.253*	0.367*	0.364*	-0.143	0.426*	-0.079
Plant height (cm)				0.923*	0.154	0.352*	0.305*	0.227*	-0.546*	0.177	-0.035
Cob placement height (cm)					0.037	0.243*	0.183*	0.106	-0.530*	0.135	-0.071
Grain yield/plant (g)						0.548*	0.746*	0.651*	-0.001	0.703*	-0.206*
100-grain weight (g)							0.556*	0.664*	-0.306*	0.150	0.130
Cob length (cm)								0.649*	0.017	0.746*	0.081
Cob girth (cm)									0.077	0.399*	0.089
Kernel rows/ear										0.058	-0.095
Grains/row											-0.145

*Significant at 5 per cent level of significance

Table 4: Eigenvectors for the first four components of quantitatively measured traits in maize

Variable	PC 1	PC 2	PC 3	PC 4
Days to 50% Pollen shed	0.450	-0.662	0.498	0.161
Days to 50% silking	0.480	-0.717	0.374	0.208
Days to 75% maturity	0.748	-0.301	0.348	0.083
Plant height	0.698	-0.329	-0.454	-0.229
Cob placement height	0.655	-0.475	-0.388	-0.257
Cob length	0.678	0.588	0.048	0.008
Cob width	0.651	0.473	0.077	0.329
Kernel rows/ear	-0.329	0.350	0.647	0.071
Grains/row	0.586	0.446	0.322	-0.294
Seed weight	0.600	0.348	-0.382	0.314
Yield/plant	0.573	0.607	0.114	-0.146
Protein (%)	-0.044	0.011	-0.287	0.803
Total variance explained (%)	32.796	22.971	13.654	9.658

weight and cob girth.

Heritability in a broad sense was high (>80%) for most of the traits studied, i.e., days to 50 per cent pollen shed, days to 50 per cent silking, plant height, cob placement height, 100-grain weight, cob girth, kernel row per ear, grains per row and protein content. Moderate heritability (50-80%) was observed for days to 75 per cent maturity, grain yield per plant and cob length. Similar results *w.r.t.* heritability for various traits was reported by different workers (Mahmood *et al.*, 2004, Hemavathy *et al.*, 2008, Rafiq *et al.*, 2010, Thakur *et al.*, 2017). These results suggested that the yield components in maize are less influenced by environmental conditions. The characters showing high GCV and high heritability can be considered for selection. Genetic advance has an added edge over heritability as a guiding factor to breeders in various selection programmes. Genetic advance expressed as a percentage of the mean was observed to be high (>50%) for grain yield per plant. However, it was moderate (25-50%) for cob placement height and 100-grain weight. It was low (<25%) for days to 50 per cent pollen shed, days to 50 per cent silking, days to 75 per cent maturity, plant height, cob length, cob girth, kernel rows per ear, grains per row and protein content.

Table 5: Number of scorable and polymorphic SSR bands along with their fragment size generated by 20 primers

Primers	Number of fragments	Number of polymorphic fragments	Polymorphic bands (%)	PIC value	Effective multiplex ratio (EMR)	Marker index (MI)	HObs	HExp	Fragments size (bp)
bnlg1113	3	2	66.67	0.37	3	1.12	0.95	0.50	50-150
bnlg420	3	2	66.67	0.29	3	0.89	0.00	0.37	50-150
umc1831	2	2	100.00	0.37	2	0.73	0.78	0.49	50-150
umc1710	2	2	100.00	0.05	2	0.09	0.05	0.05	100-150
umc2331	3	2	66.67	0.37	3	1.11	0.00	0.49	125-150
umc2358	3	2	66.67	0.29	3	0.89	0.48	0.37	50-150
umc2043	4	2	50.00	0.35	4	1.38	0.00	0.45	130-140
umc2258	2	2	100.00	0.33	2	0.66	0.59	0.42	50-150
umc1456	3	2	66.67	0.33	3	0.98	0.00	0.41	125-150
bnlg240	2	2	100.00	0.36	2	0.72	0.75	0.47	50-150
umc1056	3	2	66.67	0.31	3	0.92	0.43	0.38	100-150
umc2240	2	2	100.00	0.23	2	0.47	0.29	0.27	50-150
phi087	3	2	66.67	0.36	3	1.07	0.49	0.47	100-125
umc2332	3	2	66.67	0.19	3	0.57	0.21	0.21	125-150
umc2334	2	2	100.00	0.37	2	0.75	0.85	0.50	50-150
umc1872	3	2	66.67	0.32	3	0.95	0.00	0.40	140-150
umc 2371	3	2	66.67	0.37	3	1.11	0.00	0.49	140-150
phi034	2	2	100.00	0.35	2	0.69	0.00	0.45	125-150
bnlg657	2	2	100.00	0.26	2	0.51	0.34	0.31	50-100
umc1808	2	2	100.00	0.38	2	0.75	0.98	0.50	50-100
TOTAL	52	40	1616.80	6.25	52	16.36	7.19	8.00	
MEAN	2.60	2.00	80.84	0.31	2.60	0.82	0.36	0.40	50-150

Table 6: Analysis of molecular variance (AMOVA)

Source	df	SS	MS	Variation	% of total Variation	P value
Among populations	1	22.652	22.652	0.656	8%	0.083
Within Population	58	420.932	7.257	7.257	92%	0.083
Total	59	443.583		7.913	100%	0.083

df - Degree of freedom, SS - Sum of square, MS - Mean sum of square, Est. var. - Estimated variance, Pops - populations

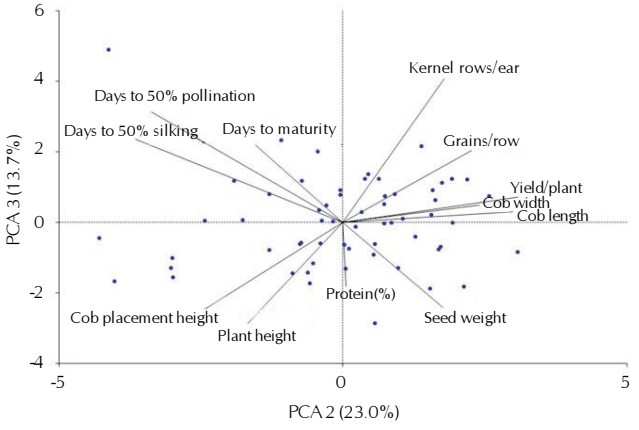


Figure 1: Biplot of different variables loaded on PC 2 and PC 3

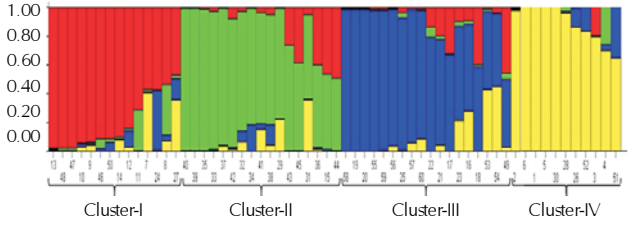


Figure 3: Genetic structure of 60 maize genotypes as inferred by STRUCTURE v2.3.3 with 20 SSR markers data set

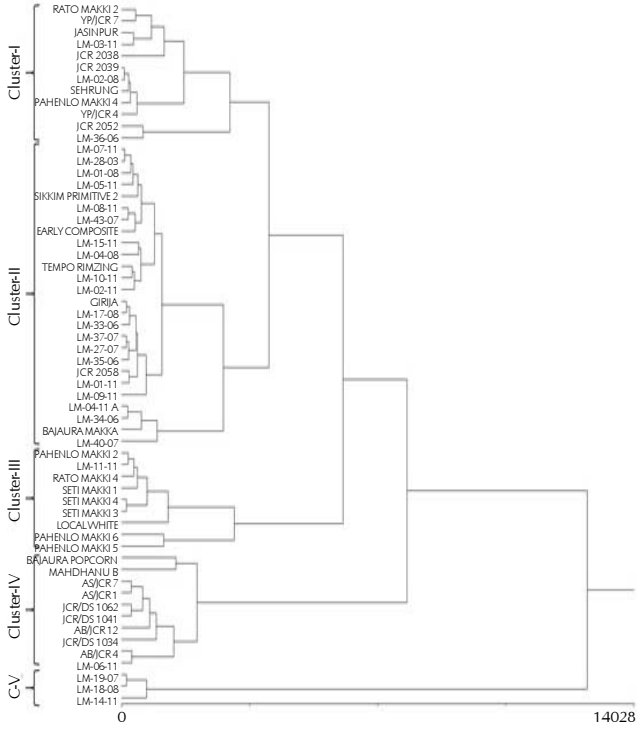


Figure 2: Dendrogram based on morphological traits constructed using squared Euclidean distance and group average clustering method

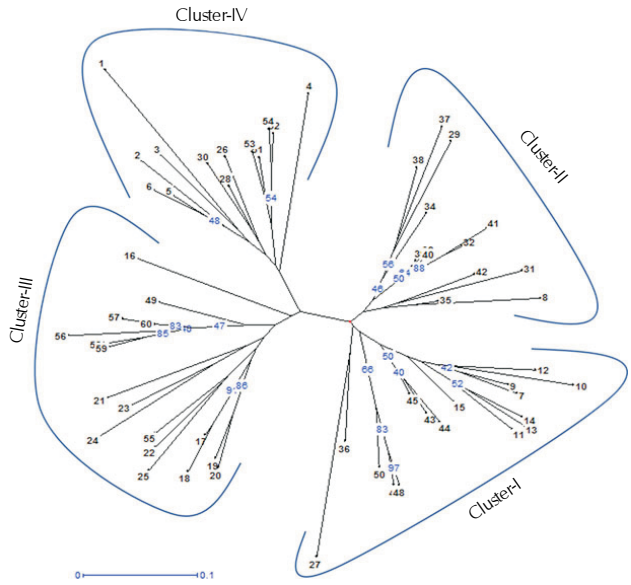
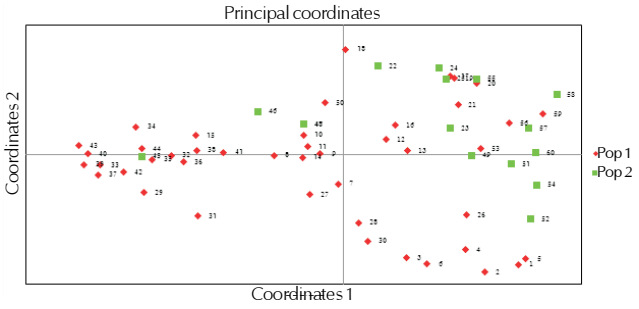


Figure 4: Neighbor-joining tree of maize genotypes using SSR markers generated by DARwin software



Pop1 - Himachal Pradesh genotypes, Pop2 – North-East states genotypes

Figure 5: Principal Coordinate Analysis (PCoA) of maize genotypes using GenAlex 6.4

For predicting reliable estimates of additive and non-additive effects, heritability should be considered in conjugation with genetic advance (Johnson *et al.*, 1955). On this consideration, high heritability with high genetic advance was found for none of the traits studied indicating the absence of high additive gene effects. However, high heritability with moderate genetic advance was observed for cob placement height and 100-grain weight. This indicated the presence of additive and non-additive gene action in the inheritance of these traits and thus providing scope for the improvement of these traits through hybridization and selection. Further, high heritability with low genetic advance was observed for 50 per cent pollen shed, days to 50 per cent silking, plant height, cob length, cob girth, kernel rows per ear, grains per row and protein content which indicated the role of non-additive gene action in the inheritance of these traits and thus revealed the importance of dominance and epistatic effects in the inheritance of these traits and selection would be less effective. Similarly, Mahmood *et al.*, 2004 reported high heritability coupled with the moderate genetic advance for grain yield per plant and high heritability coupled with the low genetic advance for a number of kernel rows per ear.

Correlation, principal component and cluster analysis

Association study was carried out among the quality parameters and agro-morphological traits including yield components to understand their inter-relationships that may help in chalking out effective breeding strategies. In the present study, bivariate correlation coefficients showed the significant positive correlation among 12 measured traits (Table 3). Grain yield showed significant positive correlation with days to 75 per cent maturity, 100-grain weight, cob length, cob girth and grains per row indicating that selection through these traits would be effective. Further, grain yield was positively correlated with plant height and cob placement height however it was negatively correlated with days to 50 per cent pollen shed, days to 50 per cent silking, kernel rows per ear and protein content. This could be attributed to the high dry matter accumulation function carried out by the high number of leaves possessed in the case of tall plants (Thakur *et al.*, 2017). Hence, these traits could be utilized in indirect selection to improve grain yield per plant. In contrasting to this, Rafiq *et al.*, 2004 and Hemavathy *et al.*, 2008 reported a positive correlation of grain yield with kernels per row, however in the present study, this trait was negatively correlated. In addition to this, Barros *et al.*, 2010 observed a negative correlation between grain yield and days to silking which was in accordance with the present study. Rafiq *et al.*, 2010 also reported a positive correlation of grain yield with plant height, ear diameter, 100-grain weight, ear length, ear diameter, grain rows per ear and grains per row, however, plant height was not positively significant correlated with grain yield per plant in the present study. Reddy *et al.*, 2013 also observed a positive correlation of grain yield per plant with 100-grain weight, ear girth and ear length and inter-correlation among yield components revealed that days to 50 per cent tasselling was significantly and positively correlated with days to 50 per cent silking, days to maturity and ear height.

Plant height exhibited significant positive correlation with cob placement height, 100-grain weight, cob length, cob girth, whereas, a significant negative correlation with kernel rows per ear indicating that an increase in plant height would lower kernel rows per ear. Cob placement height also showed significant and positive association with 100-grain weight, whereas, a negative significant association with kernel rows per ear indicating that due consideration must be given for above-mentioned characters. Similarly, significant positive correlation was observed for 100-grain weight with cob length and cob girth, whereas, a negative correlation with kernel rows per ear. The estimates of genotypic correlations, in general, were comparatively higher than the respective phenotypic correlations for most of the traits, whereas, the estimates of environmental correlations were generally lower than the respective genotypic and phenotypic correlations, revealing that there is strong inherent association between various characters and the genotypes were not super-imposed by the environmental conditions. Therefore, improvement in yield would be effective for the traits *viz.*, days to 75 per cent maturity, 100-grain weight, cob length, cob girth and grains per row which were significantly and positively correlated.

Principal component analysis (PCA) is multivariate data reduction technique which helps in visualizing the total variation in multiple variables into few components. In the PCA (Figure 1), the first principal component (PC1), explained 32.79% of the total variance, contributed mainly by days to maturity, plant height, cob length, cob placement height, cob width, seed weight, grains per row and days to 50 per cent silking. PC2 accounted for 22.97% variation through yield per plant, whereas kernel rows per ear, days to 50 per cent pollination and protein content contributed 13.65 and 9.65% variation to PC3 and PC4, respectively (Table 4). In PCA cob length and cob width resulted in highest positive values in all components, indicating that these traits contribute maximum to genetic divergence. Hence, selection based on these characters would be effective for yield improvement in maize.

Dendrogram constructed based on morphological traits using squared Euclidean distance and group average clustering method clustered landraces into five different clusters. The landraces analyzed in this study, clustered together to some extent despite their pedigree/location, and revealed higher levels of diversity. Further local landraces of Himachal Pradesh and Sikkim fall among all the clusters indicating the presence of different gene pools (Figure 2).

Molecular analysis

SSR polymorphism and diversity studies

Twenty polymorphic SSR primers pairs amplified a total of 52 alleles (size varying between 50 and 150 bp), and each primer exhibited polymorphism with an average of 2.00 polymorphic alleles per primer (Table 5). Such considerable differences in the number of alleles detected may arise from the difference in (i) the diversity of the landraces used, (ii) the number of landraces examined, and (iii) the genotyping method used (Thakur *et al.*, 2017). The markers exhibited 80% polymorphism, depicted that the population under study is genetically diverse, attributable to alien introgression and/or genetic recombinations. Bantte and Prasanna 2003 characterized 23 tropical maize lines using 36 SSRs, and found a mean of 3.25 alleles per locus. However, Legesse *et al.*, (2007) observed a mean of 3.85 average alleles per locus. Singode and Prassana (2010) characterized 48 maize landraces using 41 SSR markers and found a high mean number of alleles per locus (13.8), and Polymorphic Information Content (PIC) of 0.63 which reflects the extent of diversity. Babu *et al.*, (2014) studied diversity among 48 maize accessions including Indian and exotic germplasm using 75 simple sequence repeat (SSR) markers that yielded 258 scorable alleles, out of which 251 alleles were polymorphic with an average of 3.35 alleles per locus.

Polymorphic information content (PIC) value, a parameter associated with the discriminating power of markers, ranging from 0.19 for umc2332 to 0.38 for umc1808 with an average of 0.31 per primer. One reason could be a high proportion of closely related cultivars used in this study, resulting in the lower PIC values. These results coincide with the findings of Gurung *et al.*, (2010) which observed average PIC value 0.50 for polymorphic SSR markers. However the high average PIC value (0.81 and 0.82) was reported by Ignjatovic *et al.*, (2015) and Vivodik *et al.*, (2017), respectively. During present studies highest (0.50) and lowest (0.21) expected heterozygosity (He) values were obtained for primers *umc2334, bnlg1113, umc1808* and *umc*2265, respectively, with an average of 0.40. This value is similar to that reported by Salami *et al,* in 2016 (He = 0.46) and lower than the studies of Chen *et al,* in 2016 (He = 0.690) . Whereas highest (0.98) observed heterozygosity (HO) value was obtained for primers *umc1808* and lowest (0.00) for *umc2371, umc1872, umc2331, phi034, bnlg240, umc2043* and *bnlg420* with an average of 0.36. The expected and observed heterozygosity in genotypes revealed the deviations from Hardy-Weinberg expectations, indicating heterozygote deficiency. The expected heterozygosity (He) ranged from 0.01 to 0.73 with a mean of 0.51. Morales *et al.*, (2010) also found the high value of 0.68 for expected heterozygosity. These high values indicated high genetic variability among the population and considering the set of SSR primers used. These high values are normal for maize which is an allogamous or outbreeding crop. Similarly, in earlier study by Wasala and Prasanna in 2013 reported heterozygosity values ranging from 0.13 to 0.75 with an average of 0.63 Effective multiplex ratio (EMR) ranged from 2 to 4 with an average of 2.60 per primer, while Marker Index (MI) ranged from 0.09 to 1.12 with an average of 0.82 per primer. The high value of MI, which is considered to be over all measure of efficiency to detect polymorphism for markers, is derived from its high polymorphism, EMR and PIC values.

Bayesian genetic structure

STRUCTURE harvester was used to compute Delta K, which is used to determine the best fit value of K, for the given range, i.e., 1-10 and the highest value was shown at K = 4 indicates the presence of four gene pools among the landraces under study (Figure 3). Thus, STRUCTURE analysis was done at K = 4. These results corresponds to morphological studies where no specific pattern of clustering was found and showed that genetic diversity is not necessarily directly related to geographical distribution (Earl and Vonholdt, 2011). The landraces within the same cluster were originated from different geographical regions; this indicated that the geographical distribution and genetic divergence did not follow the same trend which might be due to the continuous exchange of genetic material among different states of the country. In addition to this, population structure also quantifies the extent of admixture within accessions. Similar studies have been done by Yang *et al.*, in 2010 on 155 maize inbred lines using 82 SSRs and found two main groups with further subdivision into eight subgroups.

Further, for assessing molecular variation among maize genotypes, AMOVA was computed assuming two models *viz.,* Model I containing 44 genotypes from Himachal Pradesh as a population I and Model II containing 16 genotypes from North-East states as population II. AMOVA based on SSR data in the model I showed 8% of the genetic variation among populations. While the variation within populations accounted for 92% of the total variation (Table 6). The results from AMOVA depicts that there is not much variation among populations, whereas, there is a high proportion of variability within populations. This may be due to the unconscious selection of ears from most heterozygous plants by farmers which prevents genetic drift among landraces and maintains a high level of genetic diversity within landraces. Grouping a large number

of maize genotypes into few homogenous clusters facilitate the selection of diverse parents for crossing program.

Cluster and PCA analyses

A neighbour-joining (N-J) tree was constructed; largely separated accessions into four groups (Figure 4). The grouping patterns of N-J tree and STRUCTURE were almost consistent i.e. about 85% of genotypes which fall under four clusters of STRUCTURE were also fallen under same clusters in NJ tree. This showed a high degree of correlation and association between the accessions falling in same groups. Further, chamba landraces were found to be more diverse than all the other landraces under study as these were present in all clusters obtained. This was further confirmed by the minimum genetic similarity value of 0.36 between LM-01-08 and LM-11-11 (local genotype from Chamba, HP) showing them the most distant accessions and it further supports the findings of Thakur *et al.*, (2017) regarding the existence of two gene pools in Himachal Pradesh.

Principal Coordinate Analysis (PCoA) was used to access structure of maize genotypes belonging to Himachal Pradesh and North-East states (Figure 5). Using SSR markers, the first two principal components explained 26.67% and 21.48% of the total phenotypic variance. The grouping patterns in PCA were in line with clustering pattern of both UPGMA tree and the STRUCTURE. The PCA and population structure has been used to complement the clustering method analysis as different combinations of genetic distance matrices and algorithms can give rise to different grouping patterns (Reif *et al.*, 2006). The present studies revealed high concordance in identifying principal groups that corresponded to a previous report (Reif *et al.*, 2006).

Clustering of the population into four distinct groups by SSR markers indicates the diversity between populations. In the sub-clusters, several local populations with different regional origin were classified into the same cluster, indicating the genetic difference between these populations. It may be due to that these populations may have diverged from the original ones due to natural selection in maintaining the environment, genetic drift, unintentional outcrossing and mutations. In populations outcrossing and mutations generate intra-population diversity, whereas direct natural or artificial selection and bottleneck effects lead to an increase in inter-population diversity (Dreisigacker *et al.*, 2005).

CONCLUSIONS

It may be concluded that it is urgent and imperative to enrich the north western-Himalayan maize gene pool with diverse alleles. Utilization and introgression of desirable traits from crossable wild relatives and landraces could be an alternative and good source of new allelic diversity for present commercial maize cultivars. Further, based upon mean performance and diversity analysis at molecular level LM-18-08, LM-19-07, LM-14-11, LM-01-08 and LM-11-11 have been observed as potential parents for future hybridization programme. In conclusion, the present study has revealed wide genetic diversity in the selected panel of maize germplasm both on morphometric and molecular marker-based analysis. Supplementing the existing morphological descriptors with reliable and repeatable DNA based marker profiles is a must considering the ramifications in the future maize breeding in India.

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