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RESEARCH ARTICLE

Genetic Relationships and Population Structure among Maize (*Zea mays* L.) Landraces as Revealed by Simple Sequence Repeat Markers

S.K. Singh, Shipra Deo, S.B. Chaudhary, Kuldeep Singh and Mukesh K. Rana*

Abstract

A set of 48 maize (*Zea mays* L.) genotypes, including landraces, some hybrids and inbreds, was characterized using 34 simple sequence repeat markers distributed throughout the genome. The 34 SSR primers produced bands in the range of 2 to 6 with an average of 3.2 bands per primer. The size range of these bands varied from 90 to 310 bp. Genetic similarity calculated using Jaccard's coefficient varied from 0.122 to 0.750 with a mean of 0.475. Gene diversity or expected heterozygosity varied from 0.208 to 0.805, averaging 0.502. The average PIC was 0.433, with a range of 0.078 to 0.776. The range of resolving power was from 0.17 to 2.15 and marker index ranged from 0.16 to 4.83. Cluster analysis using the unweighted pair group method with arithmetic mean showed two major clusters with minor sub-clusters. The first three principal coordinates accounted for 26.7% of the total variation. Principal coordinates analysis and population structure aided in further elucidation of the genetic relationships as well as differentiation of genotypes. Analysis of variance revealed 81.7% within population variation and 18.3% between population variation. The analysis also led to the identification of specific and highly informative SSR markers, namely BNLG 1182, BNLG 1175, UMC 1353, BNLG 1159, BNLG 1272 and BNLG 1045, which significantly contributed to the differentiation of the material.

Keywords: Genetic diversity, Landraces, Population structure, SSR markers, *Zea mays* L.

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Introduction

Maize (*Zea mays* L.) is one of India's most dynamic crops with wider adaptability under varied agro-climatic conditions with an area of 9.86 mha and productivity of 3195 kgha^{-1} during 2020-21 (GOI, 2021). In addition to staple food for human being and quality feed for animals, it serves as a basic raw material as an ingredient to thousands of industrial products that includes starch, oil, protein, alcoholic beverages, food sweeteners, pharmaceutical, cosmetic, textile, gum, package and paper industries *etc*. Maize landraces represent a rich source of variation and must be properly characterized for efficient management, particularly for efficient nutrient uptake and utilization, as well as for useful genes for adaptation to stressful environments. The morphological and physiological variation of maize is evidenced by its large number of landraces being grown in India. Landraces are dynamic populations with a historical origin, and distinct identity, often genetically diverse and locally adapted, and associated with a set of farmers' practices of seed selection and field management as well as with traditional knowledge.

Various kinds of marker techniques, such as morphological, biochemical and DNA-based, have been used to study genetic diversity and population structure

analysis in maize. Morphological assessment is usually the first step in this direction; however, DNA-based markers are nowadays preferred because these are not influenced by environmental factors or by the plant growth stage. DNA-based markers such as restriction fragment length polymorphisms (RFLPs) (Zehr *et al.*, 1992), random amplified polymorphic DNAs (RAPDs) (Lahane *et al.*, 2016; Berhitu *et al.*, 2019), amplified fragment length polymorphisms (AFLPs) (Roy and Kim, 2016), inter- simple sequence repeats (ISSRs) (Dar *et al.*, 2018) and single nucleotide polymorphism (SNPs) (Boakyewaa Adu *et al.*, 2019; Tomkowiak *et al.*, 2019) markers have been used extensively in various genetic analyses in maize.

SSR markers (Tautz, 1989) are the markers of choice due to their many advantages over the others because they are PCR based, abundant and dispersed throughout a genome; highly mutagenic, polymorphic, and informative; co-dominant, suitable for detecting heterozygotes, and multi-allelic; experimentally reproducible; transferable among related taxa; cost-effective and easy to detect; amplified from low quality and low quantity of DNAs; and presumably neutral (Abdurakhmonov, 2016). SSRs have been used in maize for various applications, including population structure and diversity analysis (Mahar *et al.*, 2009; Bracco *et al.*, 2016; Saiyad and Kumar, 2018; Malik *et al.*, 2020), DNA fingerprinting, discrimination, identification and DUS analysis of varieties (Wang *et al.*, 2002; Gunjaca *et al.*, 2008; Nguyen *et al.*, 2012) heterotic group construction and heterosis performance (Barbosa *et al.*, 2003; Hu *et al.*, 2017; Punya *et al.*, 2019) and core collection construction (Yao *et al.*, 2008). The use of microsatellite markers in maize landrace characterization has also been demonstrated (Qi-Lun *et al.*, 2008; Singode and Prasanna, 2010; Herrera-Saucedo *et al.*, 2019; Rathod *et al.*, 2020; Goyanka *et al.* 2021). In the present study, the SSR markers has been used to reveal the diversity and genetic structure in landraces of maize from Jharkhand.

Materials and Methods

Plant Material and DNA Extraction

In 37 landraces of maize were included in the present study (Table 1). In addition, four hybrids (DHM117, HQPM1, VL Baby Corn 1 and VL Amber Popcorn) and seven inbred lines (BML 7, CM 128, CM 141, CML 32, CM 144, LM 15 and PMC 6) were also studied. The 37 landraces represented four Jharkhand state districts: Jaspur, Korba, Koriya and Kunkuri, with 18, 7, 9 and 1 landraces, respectively. The 40 to 50 seeds of each genotype (sufficient to represent the variability) were raised in paper towels in the laboratory in replicates and 2 to 3 weeks old seedlings were used for DNA extraction. Equal amount of fresh leaf tissue from each seedling was pooled to make a representative sample for each genotype. Total genomic DNA was extracted by following CTAB method (Saghai-Maroof *et al.*, 1984) with minor modifications. The

Table 1: List of maize (*Zea mays* L.) genotypes (landraces, hybrids and inbreds) used for SSR analysis

S. No.	*National Identity/ Name*	*Collector No.*	*Village/Block/District*	*Biological Status*
1	IC624140	SKB/PM-5	Dipatoli/Jaspur/ Jaspur	Landrace
2	IC624141	SKB/PM-6	Dipatoli/Jaspur/ Jaspur	Landrace
3	IC624142	SKB/PM-7	Dipatoli/Jaspur/ Jaspur	Landrace
4	IC624143	SKB/PM-8	Dipatoli/Jaspur/ Jaspur	Landrace
5	IC624144	SKB/PM-9A	Khatanga/ Mahakultoli/Jaspur	Landrace
6	IC624145	SKB/PM-10	Khatanga/ Mahakultoli/Jaspur	Landrace
7	IC624146	SKB/PM-11	Khatanga/ Mahakultoli/Jaspur	Landrace
8	IC624147	SKB/PM-12	Khatanga/ Mahakultoli/Jaspur	Landrace
9	IC624148	SKB/PM-14	Bilaspur/Kunkuri/ Jaspur	Landrace
10	IC624149	SKB/PM-15	Matashi/Kunkuri/ Jaspur	Landrace
11	IC624150	SKB/PM-16	Matashi/Kunkuri/ Jaspur	Landrace
12	IC624151	SKB/PM-17	Deoratoli/Jaspur/ Kunkuri	Landrace
13	IC624153	SKB/PM-19	Rauni/Bagicha/ Jaspur	Landrace
14	IC624154	SKB/PM-21	Budhadand/ Gajupura/Jaspur	Landrace
15	IC624155	Not known	Not known	Landrace
16	IC624157	SKB/PM-28	Chikani pani/ Pathalgaon/Jaspur	Landrace
17	SKB/PM-30	Not known	Not known	Landrace
18	IC624158	SKB/PM-31	Sakdukala/Korba/ Korba	Landrace
19	IC624159	SKB/PM-35	Madanpur/Pali/ Korba	Landrace
20	IC624160	SKB/PM-36	Madanpur/Pali/ Korba	Landrace
21	IC624164	SKB/PM-46	Dubar muddha/Pali/ Korba	Landrace
22	IC624165	SKB/PM-47	Ranwa/Pondi/Korba	Landrace
23	IC624166	SKB/PM-48	Beran/Pondi/Korba	Landrace
24	IC624168	SKB/PM-54	Amjhar/ Pondipurpara/Korba	Landrace
25	IC624169	SKB/PM-56	Devadant/ Khadgaon/Koriya	Landrace

26	IC624170	SKB/PM-58	Pahadpara/Sonhat/Koriya	Landrace
27	IC624171	SKB/PM-61	Turi Pauni/Sonhat/Koriya	Landrace
28	IC624172	SKB/PM-63	Turi Pauni/Sonhat/Koriya	Landrace
29	IC624173	SKB/PM-66	Senghor/Sonhat/Koriya	Landrace
30	IC624174	SKB/PM-71	Chainpur/Manendergarh/Koriya	Landrace
31	IC624175	SKB/PM-73	Mauari/Manendergarh/Koriya	Landrace
32	IC624176	SKB/PM-75	Gutra/Manendergarh/Koriya	Landrace
33	IC624177	SKB/PM-76	Siroli/Manendergarh/Koriya	Landrace
34	IC624178	SKB/PM-77	Siroli/Manendergarh/Jaspur	Landrace
35	IC624179	SKB/PM-78	Rauni/Bagicha/Jaspur	Landrace
36	IC624180	SKB/PM-79	Rauni/Bagicha/Jaspur	Landrace
37	IC624181	SKB/PM-83	Songada/Manora/Jaspur	Landrace
38	DHM117	_	_	Hybrid
39	BML-7	_	_	Inbred line
40	CM128	_	_	Inbred line
41	CM141	_	_	Inbred line
42	CML32	_	_	Inbred line
43	CM144	_	_	Inbred line
44	LM15	_	_	Inbred line
45	PMC6	_	_	Inbred line
46	HQPM1	_	_	Hybrid
47	VL Baby Corn-1	_	_	Hybrid
48	VL Amber Popcorn	_	_	Hybrid

concentration and quality of the isolated genomic DNA was estimated using the Nano-drop spectrophotometer (ND-1000, USA) and a final working concentration of 10 ng μL^{-1} was made and stored at -4°C for further analysis.

PCR Amplification of SSR Markers

Initially, PCR conditions were optimized using different template DNA and $MgCl_2$ concentrations. Each optimized PCR reaction mixture consisted of 10.5 ng template DNA, 1X PCR buffer [10 mM Tris-HCl (pH.8.3), 50 mM KCl] 1.5 mM $MgCl_2$, 0.5 U of *Taq* polymerase, 200 µM of each dNTP (all chemicals from Sigma-Aldrich, USA) and 0.4 µM forward and reverse primers in a total volume of 15 µL. Amplifications were performed in a thermocycler (MJ Research, Model PTC-200) using the following cycling conditions: a denaturation step of 5 minutes at 95°C followed by 40 cycles each composed of 1-minute at 94°C, 1-minute at 55°C and 1-minute at 72°C. A final extension step of 8 minutes at 72°C was run at the end of the last PCR cycle.

Based on their high PIC value and representing the whole genome of maize, 34 SSR primers were used for diversity assessment (Table 2). At the time of electrophoresis, PCR products were mixed with 1-µL of gel loading dye (6x buffer: bromophenol blue, 0.25; xylene cyanol FF, 0.25; and glycerol in water 30%) and spun briefly in a microfuge before loading on to the gel. The amplification products were electrophoresed and visualized on a 3% metaphor-agarose (3:1) gel stained with ethidium bromide. The size of the amplified SSR fragments was estimated by running 100 bp DNA ladder (M/S BR Biochem Life Sciences) in the gel as a standard size marker. After electrophoresis, the gel was photographed in a gel documentation system under UV light.

SSR Data Scoring and Marker Statistics

Amplified fragments of different sizes were considered as different alleles. Only distinct and reproducible bands were scored as present (1) or absent (0) for each SSR primer pair. The scored data thus was recorded in an MS excel spreadsheet and the resultant data matrix was subjected to further analysis. Various marker statistics were calculated which consisted of total number of bands, total number of polymorphic bands, percent polymorphism, number of bands per marker, allelic frequency, gene diversity, heterozygosity, polymorphic information content (PIC) (Botstein *et al.*, 1980), effective multiplex ratio (EMR), resolving power (RP) (Prevost and Wilkinson, 1999), marker index (MI) (Powell *et al.*, 1996) and probability of identity (PI) (Paetkau *et al.*, 1995). Microsoft Excel spreadsheet was used to calculate all these indices.

Genetic Diversity and Genotype Clustering

Computer software NTSYS-pc version 2.2 (Rohlf, 2004) was used to calculate Jaccard's similarity coefficient values and generate a dendrogram to decipher the genetic relationship among the genotypes. For comparing any two genotypes, i_1 and i_2, Jaccard's coefficient was calculated as: a/ (a + b + c), where *a* denotes the number of positions with shared bands (1s) for both individuals; *b* the number of positions where

Table 2: SSR primers used for genetic diversity analysis in maize (*Zea mays* L.) landraces

S. no.	*Primer name*	*Size range (bp)*	*Forward sequence*	*Reverse sequence*
1	BNLG 128	170-200	CACCTGGAGGGACCCATTCC	AGGACCACAGGATCCATCATCCT
2	BNLG 238	160-200	CTTATTGCTTTCGTCATACACACACATTCAT	GAGCATGAGCTTGCATATTTCTTGTGG
3	BNGL 240	130-140	AAGAACAGAAGGCATTGATACATAA	TGCAGGTGTATGGGCAGCTA
4	BNLG 1346	160-210	CATCATGAAGCAATGAAGCC	CCGCGCCATTATCTAGTTGT
5	BNLG 105	120-150	GACCGCCCGGGACTGTAAGT	AGGAAAGAAGGTGACGCGCTTTTC
6	BNLG 615	190-220	CTTCCCTCTCCCCATCTCCTTTCCAA	GCAACCTGTCCATTCTCACCAGAGGATT
7	BNLG 1045	120-160	TCCCCGATAGCATATCGATC	GTGACTTTGGGGAGTTTGGA
8	BNLG 1159	90-190	GTGTGCCTATCCTTCCGAGA	AAGGACGTCAACAACGAACC
9	BNLG 490	100-190	GCCCTAGCTTGCTAATTAACTAACA	ACTGTAAGGGCAGTGGACCTATA
10	BNLG 589	150-160	GGGTCGTTTAGGGAGGCACCTTTGGT	GCGACAGACAGACAGACAAGCGCATTGT
11	BNLG 1154	170-190	GGGTGATCACATGGGTTAGG	AAATCAATGCTCCAAATCGC
12	BNLG 1178	250-310	GCTCATGTGCAAATGCAAGT	ACTACAGTTGAACGCCCCTG
13	BNLG 1182	110-180	AGCCGAGTCAGTTCGAGGTA	CAGGGGCTTGAGGTGAGTTA
14	BNLG 1272	180-250	ACCGAAGATGAGGTGTGACA	TCAGTGCAAGGGCAATTTAG
15	BNLG 1175	120-220	ACTTGCACGGTCTCGCTTAT	GCACTCCATCGCTATCTTCC
16	BNLG 1520	170-200	TCCTCTTGCTCTCCATGTCC	ACAGCTGCGTAGCTTCTTCC
17	BNLG 1190	110-180	ACCTTAGTTACACAGGCACACGGT	GGTGATGGGATTTTCGCATTATTA
18	BNLG 1325	160-180	CTAAATGCGCAGCAGTAGCA	TGCTCTGCAACAACTTGAGG
19	PHI 38920	100-110	ACCTTAGTTACACAGGCACACGGT	GGTGATGGGATTTTCGCATTATTA
20	PHI 116	170-180	GCATACGGCCATGGATGGGA	TCCCTGCCGGGACTCCTG
21	PHI 119	90-180	GGGCTCCAGTTTTCAGTCATTGG	ATCTTTCGTGCGGAGGAATGGTCA
22	UMC 2336	130	ATCTCACCGCACGTAACTGAGACT	CCTATGCTCTTGCTCTTCCTGGTA
23	UMC 2134	150-160	CAGGCGACGAAGATGAATTGAA	TAGTCTAGCGTCGACGAAAAATGC
24	UMC 2165	150-170	AGAACACCAAATGGTGACGTTATGT	CTAGCTCGTCTTCCCTGTGGTCT
25	UMC 1068	120-150	AGTCGTTTTCAAAGGCTGCTGATA	TGAGTCACCTCATTTCTTCTGGTTC
26	UMC 1353	180-230	AGACAGGATCATCGAAAACACACA	ACCTCAGCCTCCTCGTCAACTACT
27	UMC 2017	170-180	AGAGGTTACTACGGAGTGTGGCAG	GTCAGGGTACTGCTTCTCGAACTC
28	UMC 1690	110-120	ACCTTAGTTACACAGGCACACGGT	GGTGATGGGATTTTCGCATTATTA
29	UMC 2043	100-170	AGAGGTTACTACGGAGTGTGGCAG	GTCAGGGTACTGCTTCTCGAACTC
30	UMC 2258	100-110	AAGATTGTATAAATGGCAGCCACG	GAATAAGACCAGACAGCACCGAAC
31	UMC 2325	150-160	CTACGATATCCACCTCTACCACCG	CCTATGCTCTTGCTCTTCCTGGTA
32	UMC 2036	110	TCAATCAAGCCTCTCGTAAGGAAC	CTCTTGATCTCAACCGAAATCCTG
33	UMC 2190	190	GATCCGTTGAGGTCGATCCTTT	GAGGAGTTCCTGCAGTTTCTTGAC
34	UMC 2229	300	CGAAGAGCACGATGTTGACG	GAGAAGGGCGGGAGGAATAAC

individual i_1 has a band, but i_2 does not, and *c* the number of positions where individual i_2 has a band, but i_1 does not. Principal coordinate analysis (PCoA) was also computed using the same NTSYS-pc software.

Calculation of Discrimination Indices

The discrimination power (D_j) of a primer, which describes the probability that two randomly chosen individuals from a set of N individuals have different banding pattern or are distinguishable from one another, was calculated as described by Tessier *et al.* (1999). D_j was calculated from unity by subtracting Cj, i.e., the probability that two randomly chosen individuals have identical banding patterns. The confusion probability for i^{th} pattern with frequency of pi for the given j^{th} primer was calculated as: c_j = , and summing of all such c_js for *l* different patterns generated by a primer gives the confusion probability C_j of that primer. Hence, $D_j = 1 - C_j$ = 1 - . Estimated discrimination power (D_L) for each of the j primers was calculated as an extension of the polymorphism information content (PIC) described by Anderson *et al.* (1993) and is given as: D_L = 1 -Theoretically, the total number of non-differentiated pairs of individuals (x_j) for the j^{th} primer were calculated as: $x_j = (N(N-1)/2)C_j$. For a set of k primers, the total number of non-differentiating pairs of individuals (x_k),

under the hypothesis of independence of the considered primer patterns, were calculated as: $_j$.

AMOVA and Population Structure Analysis

AMOVA (Analysis of MOlecular VAriance) (Excoffier *et al*., 1992) was used to partition the total genetic variation to among- and within population variance components and to calculate genetic variation in each group, using the ARLEQUIN software ver. 3 (Excoffier & Lischer, 2010). Population structure was estimated using a Bayesian Markov Chain Monte Carlo model (MCMC) implemented in STRUCTURE v2.3.4 (Pritchard *et al*., 2000). Five runs were performed for each number of populations (k) set from 1 to 7. Burn-in time and MCMC replication numbers were set to 10,000 and 10,000, respectively for each run, respectively. The most probable K-value was determined by Structure Harvester (Earl and Vonholdt, 2012), using the log probability of the data [LnP(D)] and delta K (ΔK) based on the rate of change in [LnP(D)] between successive K-values. To create a graphical plot of structure, DISTRUCT software was used (Rosenberg, 2004).

Results and Discussion

The domestication of crop plants and modern plant breeding practices have resulted in the loss of crop diversity. In order to retain the allelic richness found in the wild, the resort to landraces is inevitable to sustain genetic diversity. Landraces are not only a valuable source of genetic diversity but are also useful to specific adaptations under local environmental situations, and these can serve as sources of various improved agronomical attributes (Lopes *et al*., 2015). The present study assessed the genetic diversity based on SSR markers in the maize landraces from mainly three districts: Jaspur, Korba and Koriya from the Jharkhand state of India. These landraces were obtained from the Regional Research Station of ICAR-National Bureau of Plant Genetic Resources, Ranchi. In addition, four hybrids and seven inbred lines were also included in the study to compare the genetic variability.

The Usefulness of SSR Markers for Polymorphism Detection

The 34 SSR primers, representing the whole genome of maize used to assess the genetic diversity in the maize landraces and to decipher their genetic relationships, produced very sharp and scorable bands in size range as low as 90 bp pairs (primers BNLG 1159 and PHI 119) to as high as 310 bp (primer BNLG 1178). The size range and the forward and reverse sequences of these 34 primers are provided in Table 2. A representative gel-based DNA fingerprint of maize landraces generated using SSR locus BNLG 1325 is provided in Figure 1. As is evident from Figure 1, the higher number of alleles per locus within landraces, as compared to hybrids and inbreds, is attributed to the genetic variation

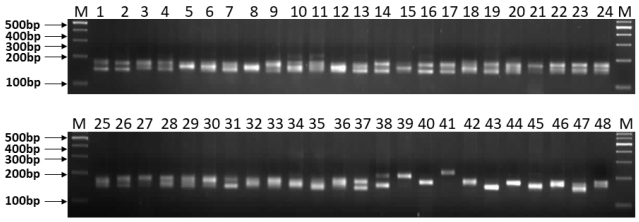

Figure 1: Representative DNA profile of maize (*Zea mays* L.) landraces generated using SSR locus BNLG 1325. Numbers (1-48) on top of the lanes denote the name of the landrace as depicted in Table 1. M is the 100 base pair molecular weight standard.

Table 3: Extent of polymorphism detected by SSR markers in maize (*Zea mays* L.) landraces

Primer	*TB*	*NP*	*GD*	*PIC*	*RP*	*EMR*	*MI*
BNL 128	3	3	0.532	0.439	1.71	3	1.60
BNL 238	4	4	0.502	0.495	1.36	4	2.01
BNL 240	2	2	0.269	0.233	0.64	2	0.54
BNL 1346	3	3	0.599	0.521	1.96	3	1.80
BNLG 105	2	2	0.489	0.369	1.70	2	0.98
BNLG 615	3	3	0.536	0.443	1.73	3	1.61
BNLG 1045	4	4	0.638	0.570	2.00	4	2.55
BNLG 1159	5	5	0.682	0.623	2.00	5	3.41
BNLG 490	5	5	0.534	0.496	1.40	5	2.67
BNLG 589	2	2	0.081	0.078	0.17	2	0.16
BNLG 1154	3	3	0.292	0.259	0.69	3	0.88
BNLG 1178	5	5	0.632	0.563	2.00	5	3.16
BNLG 1182	6	6	0.805	0.776	2.00	6	4.83
BNLG 1272	4	4	0.776	0.715	2.00	4	3.11
BNLG 1175	6	6	0.756	0.720	2.00	6	4.53
BNLG 1520	3	3	0.479	0.401	1.38	3	1.44
BNLG 1190	3	3	0.512	0.400	1.80	3	1.54
BNLG 1325	3	3	0.625	0.548	2.00	3	1.88
PHI 38920	2	2	0.249	0.218	0.58	2	0.50
PHI 116	2	2	0.464	0.356	1.46	2	0.93
PHI 119	4	4	0.548	0.446	2.00	4	2.19
UMC2134	2	2	0.371	0.302	0.98	2	0.74
UMC 2165	2	2	0.425	0.335	1.23	2	0.85
UMC 1068	3	3	0.371	0.323	0.93	3	1.11
UMC 1353	4	4	0.697	0.642	2.00	4	2.79
UMC 2017	2	2	0.487	0.368	1.68	2	0.97
UMC 1690	2	2	0.484	0.367	1.65	2	0.97
UMC 2043	3	3	0.534	0.417	2.15	3	1.60
UMC 2258	2	2	0.208	0.186	0.47	2	0.42
UMC 2325	2	2	0.488	0.369	1.68	2	0.98
Average	3.20	3.20	0.502	0.433	1.512	3.20	1.76

TB: total number of bands, NP: number of polymorphic bands, H: average heterozygosity, PIC: polymorphism information content, RP: resolving power; EMR: effective multiplex ratio, M: marker index

between individuals within an accession. Such multiple PCR amplification products can be ascribed to within-accession heterogeneity and segregation at a particular microsatellite locus (Dreisigacker *et al.*, 2005). High heterogeneity in landraces is an important adaptive trait under unfavorable environmental situations (Kyratzis *et al.*, 2019).

Various marker efficiency parameters for 30 polymorphic SSR primers such as total number of bands, polymorphic bands, gene diversity, PIC, resolving power, effective multiplex ratio and marker index were computed for diversity assessment and genetic relationships studies in maize landraces (Table 3). Total number of amplified bands varied from two for as many as ten primers to six for primers BNLG 1182 and BNLG 1175 with an average of 3.2 bands per primer. Dubey *et al.* (2009) also observed 3.2 mean number of alleles per locus in drought-tolerant and susceptible genotypes of maize. The mean number of bands (3.2) observed in our study is, however, low compared to other studies where mean number of bands was observed to vary from 3.8 to 11 (Sharma *et al.*, 2008; Arafayne *et al.*, 2018; Belalia *et al.*, 2019; Shyanowako *et al.*, 2018). This indicates that the allelic richness in the material in our study is comparatively low, which might be because the landraces in our study represent very limited geographical space of three districts of Jharkhand state of India. In addition, very high number of alleles in other studies could be the result of the inclusion of genetically very diverse material, which comprised landraces, open-pollinated varieties, inbreds, hybrids *etc*.

Gene diversity or expected heterozygosity, which is the probability that an individual is heterozygous for the locus in the population, varied from 0.208 (UMC 2258) to 0.805 (BNLG 1182) with an average value of 0.502. Tahir *et al.* (2016) observed the same extent of gene diversity (0.20-0.81) as did we. However, even higher levels of gene diversity have also been reported (Adu *et al.*, 2019). Polymorphism information content (PIC), a measure of polymorphism for a marker locus, ranged from 0.078 (BNLG 589) to 0.776 (BNLG 1182). The average PIC in the material was observed to be 0.433. More or less the same level of PIC has been reported earlier (Tahir *et al.*, 2016; Dubey *et al.*, 2009; Belalia *et al.* 2019). Resolving power is based on the distribution of alleles within the sampled genotypes and it correlates with the ability to distinguish analysed samples. The range of resolving power was from 0.17 (BNLG 589) to 2.15 (UMC 2043) with an average of 1.512 in the maize landraces evaluated in the present study. Effective multiplex ratio (EMR), the number of loci polymorphic in the material analysed for experiment fraction of polymorphic loci, ranged from 2 to 6. The product of EMR and gene diversity for polymorphic loci denoted as marker index was the highest for primer BNLG 1182 (4.83) and it was the lowest for primer BNLG 589 (0.16).

Genetic Diversity Among Maize Landraces

Average similarity, based on Jaccard's similarity coefficient, among 48 maize genotypes that included landraces, hybrids

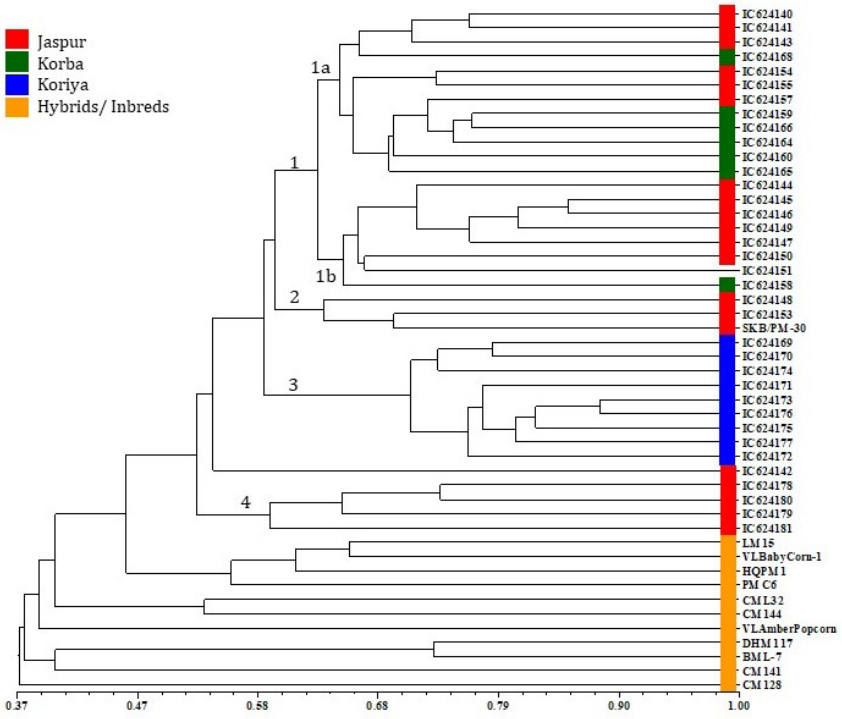

Figure 2: UPGMA based dendrogram generated using SSR markers depicting genetic relationships among maize (*Zea mays* L.) landraces and select hybrids and inbreds

Table 4: AMOVA analysis of maize landraces

Source of variation	*Degrees of freedom*	*Sum of squares*	*Variance components*	*Percentage of variation*
Among populations	2	67.10	2.29Va	18.33
Within populations	30	306.97	10.23Vb	81.67
Total	32	374.06	12.53	

Fst: 0.18331, Significance tests (10000 permutations) [Va and F_{ST} : P(rand. value > obs. value) = 0.00; P (rand. value = obs. value) = 0.000; p-value = 0.00 ± 0.00]

and inbreds, was found to be 0.475 with a minimum value of 0.122 (between landraces IC624173 and IC624176) and maximum value of 0.750 between landrace IC624178 and inbred CML32. Similarity among the 20 landraces from district Jaspur was found to be 0.401. A minimum similarity value of 0.149 (between landraces IC625145 and IC624146) and a maximum of 0.635 (between landraces IC624142 and IC624181) was observed in the landraces from Jaspur district. Among landraces from the district Korba, average similarity was observed to be 0.333 with a minimum similarity of 0.233 between the landraces IC624159 and IC624160 and a maximum similarity of 0.438 between the landraces IC624158 and IC624160. A minimum similarity of 0.122 between the landraces IC624173 and IC624176 and maximum similarity of 0.380 between the landraces IC624170 and IC624176 was observed among the landraces from district Koriya. However, the average similarity among the landraces from Koriya district was observed to be 0.251.

In earlier studies with maize landraces or inbred lines, Dubey *et al.* (2009) observed more or less similar genetic similarity (0.14–0.74) with an average of 0.31. Likewise, Saiyad and Kumar (2018) also reported the same level of genetic similarity between genotypes, ranging between 0.16 and 0.75 with an average of 0.49. A high level of genetic dissimilarity (72%) was observed in 124 landraces from Wuling mountain region in (Yao *et al.*, 2008) which might be true due to the diverse range of material in the study.

Population Structure and ANOVA Analysis

Cluster analysis of the SSR-based genetic similarity estimates provided substantial separation of maize landraces on the basis of their source district of the Jharkhand state. The UPGMA-based dendrogram (Figure 2) grouped all 37 maize landraces into four clusters. Hybrids and inbred lines included in the study were separated out from the landraces. Cluster 1 contained 20 maize landraces, all from Jaspur district or the Korba district of Jharkhand state. Although Jaspur and Korba landraces were intermixed in this cluster, within sub-cluster 1a Korba landraces, except IC624168 and IC624158 were having tendency to be grouped together. Sub-cluster 1b and cluster 4 contained all Jaspur landraces alone. All the maize landraces from Koriya district were grouped separately in cluster 3.

Principal coordinate analysis (PCoA) was undertaken in order to further substantiate the genetic relationship patterns observed using UPGMA clustering (Figure 3). The first three principal coordinates accounted for 26.7% of the total variation with first, second and third principal coordinates accounting for 11, 9.7 and 6.0% of the variability, respectively. Although very distinct patterns were not observed concerning the grouping of maize landraces, hybrids and inbreds (Figure 3A) were separately visible from the landraces (Figure 3B) in the three-dimensional plot. Among the maize landraces, four landraces IC624178, IC624179, IC624180 and IC624181 all from Jaspur district of Jharkhand tended to be differentiating from the rest (Figure 3C).

To further test these genetic relationships, a model-based clustering was done using the program STRUCTURE (Pritchard *et al.*, 2000). With no prior information about the populations and using an admixed model. STRUCTURE calculated that the estimate of the likelihood of the data (LnP(D)) was greatest when K=2 (Figure 4), suggesting that all maize genotypes in the study fell into one of the two clusters. The optimal K-value indicated that these two genetically distinct clusters primarily correspond to hybrids,

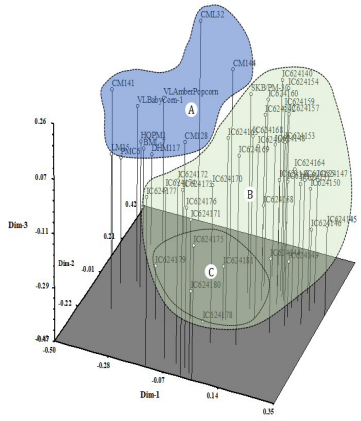

Figure 3: Principal coordinate analysis of maize (*Zea mays* L.) landraces generated using SSR markers

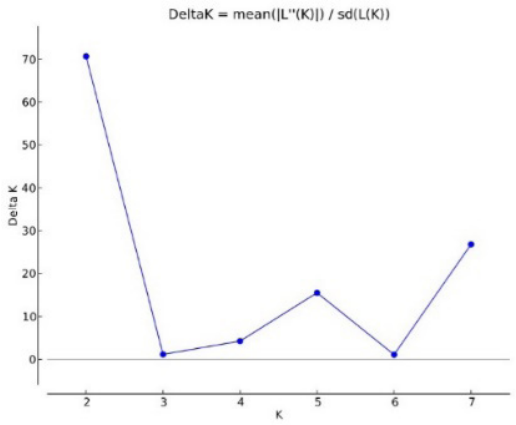

Figure 4: Delta K values for different groups assumed (K) in the structure analysis

inbred lines (Figure 5A), and landraces (Figure 5B). Among the group of landraces, four landraces IC624178, IC624179, IC624180 and IC624181 all from Jaspur district of Jharkhand tended to be differentiating from the rest as these were shown to be in the PCoA analysis as well.

Estimating the strength of the genetic population structure is usually done using F-statistics that decompose the genetic variance into within-population and among or (between) population components (Kempthorne and Wright, 1971). Fst, which quantifies the degree of population differentiation, depends on the balance among migration, mutation, and genetic drift. Analysis of Molecular Variance (AMOVA) performed using software Arlequin (Excoffier & Lischer, 2010) indicated that majority of genetic variation (81.67%) occurred within populations, while the variation between the populations (Fst) was 18.33% (Table 4). As we observed in our study, a similar level of population differentiation has been observed in Iranian maize genotypes using 18 SSR primers, revealing 17 and 83% of the total variation between and within populations, respectively (Tahir *et al.*, 2016). Higher levels of between population differentiation (24.4%) in maize landraces from northern Mexico (Herrera-Saucedo *et al.*, 2019) and in Algerian populations (22%) has been observed (Belalia *et al.*, 2019) as compared to our study which might be due to larger populations sizes in these studies.

DNA Fingerprinting for Identification of Maize Landraces

For the purpose of identification of maize landraces, discrimination power by which the efficiency of a primer alone or in combination with other primers can be tested was computed. The discrimination power, along with other parameters used to discriminate the maize landraces such as the probability of identity, confusion probability, and estimated discrimination power under the hypothesis when the sample size N tends to infinity and total number of undifferentiated landrace pairs are presented in Table 5. The probability of identity was found to be the lowest for primer BNLG 1272 (0.062) followed by for primers BNLG 1182 and BNLG 1175. However, this probability was the highest for primer BNLG 589 (0.847). The other two indices, namely, confusion probability and discrimination power, are interrelated. Subtracting confusion probability from unity gives discrimination power which was found to be the highest for primers BNLG 1182, BNLG 1175, UMC 1353, BNLG 1159 and BNLG 1272 in that order. Likewise, the estimated discrimination power was found to be highest in that order for these same five primers in the present study. More the number of undifferentiated landrace pairs by a primer, less efficient the primer is. These same five primers in the above order left 201, 257, 325, 342 and 362 pairs, respectively, and were more useful than the rest of the primers.

The expected number of indistinguishable pairs under the independence hypothesis are given in Table 6. From 48 genotypes in the study, 1128 pair-wise comparisons are possible. The theoretical total number of non-differentiating pairs with a particular primer is calculated by multiplying total pairs compared with the confusion probability of that particular primer. Out of 1128 pairs, primer BNLG 1182, selected based on its highest discrimination power, left 201 indistinguishable pairs. By adding primer BNLG 1175, with the second-highest discrimination power, the number of in-distinguishable pairs was reduced to 46. Similarly, in that order, the total number of indistinguishable pairs were reduced by adding primers UMC 1353, BNLG 1159 and BNLG 1272 in that order the total number of indistinguishable pairs were reduced to 13, four and one, respectively. Finally adding the primer BNLG 1045 all maize landrace pairs were distinguished. So, this set of six primers, namely, BNLG 1182, BNLG 1175, UMC 1353, BNLG 1159, BNLG 1272 and BNLG 1045 was found to discriminate all the landraces included in the study. Additional primers, if needed for discriminating larger sets of maize material, can be selected based on the discrimination power of the primers from Table 6.

Methods that allow varieties to be identified unequivocally are essential not only for variety identification but also to protect breeder and farmers rights. DNA markers have now reached a level of development that makes them suitable for this purpose, and when these are combined with conventional descriptors, the breeding stock can be unequivocally identified and any existing genetic diversity can be detected. As more and more closely related genotypes are being developed and need testing, the regular use of such markers is a fundamental requirement in the case of maize. DNA fingerprints of maize landraces can be generated, allowing easy identification

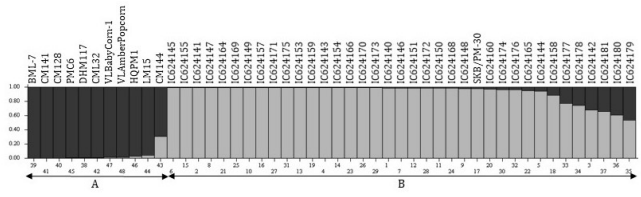

Figure 5: Model-based clustering of maize (*Zea mays* L.) landraces using STRUCTURE v2.3.4. Each landrace genotype is represented by a single row, which is partitioned into segments in proportion to the estimated membership in the two subpopulations. Numbers on the Y-axis show the subgroup membership and the X-axis shows the different genotypes as listed in Table 1

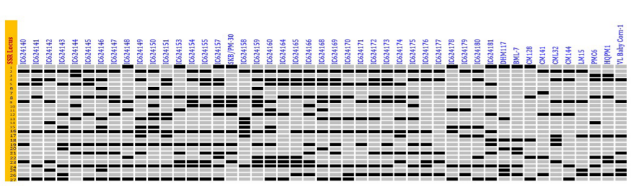

Figure 6: DNA fingerprints of maize (*Zea mays* L.) landraces represented as barcode generated using six most discriminating SSR primers (BNLG 1182, BNLG 1175, UMC 1353, BNLG 1159, BNLG 1272 and BNLG 1045)

Table 5: Discrimination indices among maize (*Zea mays* L.) landraces calculated using SSR markers

Primer	*PI*	*C*	*D*	*Order of D*	D_L	*X*
BNL 128	0.312	0.456	0.544	14	0.532	515
BNL 238	0.304	0.488	0.512	16	0.502	550
BNL 240	0.571	0.725	0.275	27	0.269	818
BNL 1346	0.239	0.388	0.612	9	0.599	438
BNLG 105	0.381	0.501	0.499	17	0.489	565
BNLG 615	0.309	0.453	0.547	12	0.536	511
BNLG 1045	0.199	0.348	0.652	6	0.638	393
BNLG 1159	0.161	0.303	0.697	4	0.682	342
BNLG 490	0.255	0.455	0.545	13	0.534	513
BNLG 589	0.847	0.917	0.083	30	0.081	1034
BNLG 1154	0.534	0.702	0.298	26	0.292	791
BNLG 1178	0.205	0.354	0.646	7	0.632	399
BNLG 1182	0.067	0.178	0.822	1	0.805	201
BNLG 1272	0.062	0.321	0.679	5	0.665	362
BNLG 1175	0.095	0.228	0.772	2	0.756	257
BNLG 1520	0.350	0.511	0.489	21	0.479	576
BNLG 1190	0.351	0.477	0.523	15	0.512	538
BNLG 1325	0.217	0.362	0.638	8	0.625	408
PHI 38920	0.595	0.746	0.254	28	0.249	841
PHI 116	0.395	0.527	0.473	22	0.464	594
PHI 119	0.306	0.440	0.560	10	0.548	497
UMC2134	0.465	0.621	0.379	25	0.371	701
UMC 2165	0.421	0.566	0.434	23	0.425	638
UMC 1068	0.444	0.621	0.379	24	0.371	700
UMC 1353	0.147	0.288	0.712	3	0.697	325
UMC 2017	0.382	0.503	0.497	19	0.487	567
UMC 1690	0.383	0.505	0.495	20	0.484	570
UMC 2043	0.334	0.453	0.547	11	0.534	511
UMC 2258	0.649	0.788	0.212	29	0.208	889
UMC 2325	0.381	0.502	0.498	18	0.488	566
Average	0.345	0.491	0.509		0.498	553.74

PI: probability of identity, C: confusion probability, D: discrimination power, D_L: Estimated discrimination power as N tends toward infinity, X: total number of undifferentiated pairs

with high precision. Using Microsoft Office Excel 2019, a DNA fingerprint barcode was constructed using 27 SSR allelic molecular weights (Figure 6). Six primers, namely, BNLG 1182, BNLG 1175, UMC 1353, BNLG 1159, BNLG 1272 and BNLG 1045 were identified for generating the DNA fingerprint barcode based on the high discrimination power of these markers. These SSR markers, therefore, can be used as alternative descriptors for landrace protection.

The study of genetic diversity in landraces is important in the light of narrowed genetic base owing to use of improved varieties and hybrids in maize. In addition to using modern tools to estimate diversity, traditional knowledge also needs to be exploited to augment the diversity patterns and optimize sampling of sub-samples in each particular landrace. Native edapho-climatic conditions to assess the variability also need consideration for agronomic evaluation of the landraces. *Ex-situ* conservation and strategies for characterization of within landrace variability needs to be developed. The analysis has led to the identification of specific, highly informative SSR primers, namely BNLG 1182, BNLG 1175, UMC 1353, BNLG 1159, BNLG 1272 and BNLG 1045, which significantly contributed in discrimination and diversity analysed in the study. Cluster analysis of molecular markers distinguished groups and identified landraces of maize that would be useful for the conservation and management of genebank collection and for possible utilization in maize breeding programs. SSR markers have a strong potential as a tool for complementary analysis of distinguishability, uniformity and stability required for cultivar registration and protection of plant breeders' rights. Strategies to retain diversity in landraces would include the development of core collections, allele mining and finding allelic variants for functional genes and genome-wide association studies (GWAS) for identifying marker-trait associations.

Table 6: Expected number of indistinguishable pairs under the independence hypothesis

Primer Combination	*Indistinguishable pairs per combination*
BNLG1182	201
BNLG1182 + BNLG1175	46
BNLG1182 + BNLG1175 + UMC1353	13
BNLG1182 + BNLG1175 + UMC1353 + BNLG1159	4
BNLG1182 + BNLG1175 + UMC1353 + BNLG1159 + BNLG1272	1
BNLG1182 + BNLG1175 + UMC1353 + BNLG1159 + BNLG1272 + BNLG1045	0
BNLG1182 + BNLG1175 + UMC1353 + BNLG1159 + BNLG1272 + BNLG1045 + BNLG1178	0
BNLG1182 + BNLG1175 + UMC1353 + BNLG1159 + BNLG1272 + BNLG1045 + BNLG1178 + BNLG 1325	0
BNLG 1182 + BNLG1175 + UMC1353 + BNLG1159 + BNLG1272 + BNLG1045 + BNLG1178 + BNLG1325 + BNL1346	0
BNLG1182 + BNLG1175 + UMC1353 + BNLG1159 + BNLG1272 + BNLG1045 + BNLG1178 + BNLG1325 + BNL1346 + PHI119	0

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