Research Article

Genetic Diversity Analysis of Forty Maize Genotypes Using (SSR) Simple Sequence Repeat Markers: Implications for Germplasm Characterization

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Abstract

Maize (*Zea mays* L.) is the world's leading cereal by production volume, and sustained yield gains depend on the availability and efficient use of genetic diversity. We assessed molecular diversity among 40 maize genotypes (INV-1 to INV-40) using 25 simple sequence repeat (SSR) primers of the BNLG and UMC series. Amplification products were resolved on 3 % agarose gels, scored as binary data, and analysed in PAST to estimate marker informativeness and construct a similarity-based dendrogram. Of the 25 primers, 24 were polymorphic, and 1 (BNLG 1714) was monomorphic, with polymorphism information content (PIC) values of 0.58 - 0.74 (mean 0.67). Pairwise, genetic similarity ranged from approximately 0.69 to 0.93. Cluster analysis isolated INV-1 as the most divergent genotype and arranged the remaining 39 genotypes into six major groups (A–F); the most similar pair was INV-39 and INV-37 (≈0.93). These results provide a molecular basis for selecting divergent parents and for planning heterotic grouping and combining ability studies. They show that BNLG and UMC primers remain a cost-effective tool for diversity analysis in resource-limited breeding programs.

Keywords: maize; simple sequence repeats; genetic diversity; BNLG; UMC; cluster analysis; polymorphism information content

1. Introduction

Maize has been cultivated for roughly 9,000 years and is now the leading cereal in terms of global production, with a growing role in food, feed and fuel systems (Erenstein et al., 2022). In India, it is the third most important cereal after rice and wheat; national production reached about 42 million tonnes from roughly 12 million hectares in 2024–25, with demand rising quickly from the feed and ethanol sectors (ICAR–NIAP, 2025). Meeting this demand under climate variability, emerging pests and diseases, and limited arable land requires cultivars that combine high yield with stress resilience.

Genetic gains in maize have come largely from exploiting heterosis between divergent, complementary heterotic groups and from the continual recycling of elite germplasm (Duvick, 2005; Reif et al., 2005). Intensive selection within a narrow set of elite lines, however, erodes allelic diversity and limits future progress, while valuable alleles for adaptation and stress tolerance remain in under-characterised germplasm (Tanksley and McCouch, 1997; Prasanna, 2012). Reliable information on the extent and structure of genetic diversity is therefore a prerequisite for choosing parents, assigning lines to heterotic groups and managing genetic resources (Melchinger and Gumber, 1998).

Characterisation based on morphology and agronomic traits is slow, influenced by environment and developmental stage, and reveals little about underlying genetic relationships. DNA markers avoid these limitations by detecting variation directly at the sequence level. Restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), SSRs and single nucleotide polymorphisms (SNPs) have all been applied in maize, and comparisons of marker systems have repeatedly shown SSRs to be highly informative and discriminating per locus (Powell et al., 1996; Pejic et al., 1998).

SSRs, or microsatellites, are tandem repeats of short motifs (one to six nucleotides) that are abundant and dispersed throughout eukaryotic genomes (Weber and May, 1989). Length variation, generated mainly by replication slippage, is assayed by PCR with primers designed against the conserved flanking sequences. This yields co-dominant, multi-allelic and reproducible markers with broad genome coverage that require only PCR and a fragment-separation method (Gupta and Varshney, 2000; Kalia et al., 2011). Large sets of maize SSR primers have been developed and mapped (Senior and Heun, 1993; Taramino and Tingey, 1996; Sharopova et al., 2002) and are curated in MaizeGDB

(Portwood et al., 2019), including the BNLG and UMC series used here.

Early evaluations showed that SSRs were more informative than RFLPs for distinguishing maize inbred lines (Smith et al., 1997) and could be scored on agarose gels (Senior et al., 1998). Later surveys used SSR panels to resolve genetic structure among diverse inbreds (Liu et al., 2003), to characterize CIMMYT and African germplasm (Warburton et al., 2002; Xia et al., 2004; Legesse et al., 2007) and to establish heterotic groups in subtropical maize (Reif et al., 2003). Indian studies of released and experimental inbreds have likewise used SSR profiles to estimate similarity and to cluster lines (Kumar et al., 2008; Sathua and Gayatonde, 2018). Although SNP platforms offer higher density and automated scoring, and genomic resources continue to expand (Hufford et al., 2021), empirical comparisons show that highly polymorphic SSRs remain highly informative for clustering germplasm and measuring relatedness (Hamblin et al., 2007; Jones et al., 2007), particularly when sample numbers are moderate, and budgets are limited.

Knowledge of the molecular relationships among the INV genotypes is needed to guide parental selection and heterotic grouping. The objectives of this study were therefore to (i) evaluate polymorphism and informativeness of 25 BNLG and UMC SSR primers across 40 maize genotypes, (ii) estimate genetic similarity and construct a dendrogram, and (iii) identify divergent genotypes and groups for use in hybrid breeding.

2.Materials and Methods

2.1Plant material

Fourty maize genotypes, designated INV-1 to INV-40, were used from gene bank of Department of Biotechnology, M.S Swaminathan School of Agriculture, Centurion university of Technology and management, Bhubaneshwar, Odisa.

2.2DNA extraction

Young leaf tissue was collected from the Research and Development Field. Genomic DNA was extracted from leaf tissue by the CTAB method (Doyle and Doyle, 1990), treated with RNase, checked for integrity on 0.8% agarose gels, quantified by spectrophotometry, and diluted to 20 ng μl^{-1} for PCR.

2.3SSR primers

Twenty-five SSR primers (16 BNLG and 9 UMC) were used (Table 1). Primer sequences were taken from the public maize SSR resources curated in MaizeGDB (Portwood et al., 2019; Sharopova et al., 2002).

Table 1. SSR primers used for genotyping 40 maize genotypes, with number of alleles and PIC per locus.

No.	Primer	Forward (5'→3')	Reverse (3'→5')	Alleles	PIC
1	BNLG-565	TAAGAACGACGAACGGTAACTG	GCTCACTGCACGCCAACAC	[6]	[0.69]
2	BNLG-1194	GCGTTATTAAGGCAAGCTG C	ACGTGAAGCAGAGGATCC AT	[5]	[0.67]
3	BNLG-1265	GGTTGTCCGTAAAGGCAA GA	TGTGAAGGCCAGACAGTC AG	[3]	[0.61]
4	UMC-200	TGACCTCAACTCAGAATGC TGTTG	CACAAAATCCTCGAGTTCT TGATTG	[6]	[0.70]

	2				
5	UMC-2225	TCGGCTGACATAATAAAAC CATAGC	ATGCGAATTTTACCGGGTT TTT	[4]	[0.71]
6	BNLG-1137	ATGAGCTCAGTCACACTGT AGTG	ACTGATGACTGGTCCATGC A	[5]	[0.62]
7	BNLG-1179	GCGATTCAGTCCGCAGTAG T	GTACTGAACAAACCGTGG GC	[4]	[0.66]
8	BNLG-2094	GGCTGACATAATAAAACCA TA	GCGAATTTTACCGGGTTTT AC	[4]	[0.68]
9	BNLG-1185	CGGTCCAGGCAGGTTAATT A	GACTCGAGGACACCGATTT C	[6]	[0.70]
10	BNLG-1523	GAGCACAGCTAGGCAAAA GG	CTCGCACGCTCTCTCTTCTT	[3]	[0.71]
11	BNLG-2162	GTCTGCTGCTAGTGGTGGT G	CACCGGCATTCGATATCTT T	[5]	[0.73]
12	UMC-1265	GCCTAGTCGCCTACCCTAC CAAT	TGTGTTCTTGATTGGGTGA GACAT	[1]	[0.64]
13	BNLG-2248	CCACCACATCCGTTACATC A	ACTTTGACACCGGCGAATA C	[4]	[0.61]
14	BNLG-1014	CACGCTGTTTCAGACAGGA A	CGCCTGTGATTGCACTACA C	[4]	[0.58]
15	BNLG-589	GGGTCGTTTAGGGAGGCA CCTTTGGT	GCGACAGACAGACAGACA AGCGCATTGT	[3]	[0.72]
16	BNLG-1484	GTAAAAGACGACGACATTC CG	GACGTGCACTCCGTTTAAC A	[4]	[0.64]
17	BNLG-1042	CGTTTAGGGAGGAAGAAC AT	TGTTCTTGATTGCAGGCAG TCC	[4]	[0.69]

18	BNLG-1866	CCCAGCGCATGTCAACTCT	CCCCGGTAATTCAGTGGAT A	[3]	[0.70]

No.	Primer	Forward (5′→3′)	Reverse (3′→5′)	Alleles	PIC
19	BNLG-1714	CATCATGGAGGCATATGTC G	ACACATTTAGACCCACCCC A	[2]	[0.74]
20	UMC-1221	GCAACAGCAACTGGCAAC AG	AAACAGGCACAAAGCATG GATAG	[5]	[0.66]
21	UMC-1065	ACAAGGCCATCATGAAGA GCAGTA	CACGGTCTGGCACACTAAC CTTAT	[3]	[0.71]
22	UMC-1282	GCGAGGGTTCTTTCCATAG AGAAT	TACACTACACGACTCCCAA CAGGA	[2]	[0.69]
23	UMC-1292	TCACGGTTCAGACAGATAC AGCTC	GAAGTGGGGAACATGGTT AATGTC	[2]	[0.67]
24	UMC-1947	GGATCTCACCCCCTGCTGT C	ATCACGCGCTCACTCTCCT CT	[3]	[0.74]
25	UMC-1160	CGTTTGATATGATGTGGAG ATTCG	AAGCTTGTGAATGTTCTGG ATGTC	[4]	[0.72]

2.4PCR amplification and electrophoresis

PCR was carried out in 10 µl reactions containing template DNA, forward and reverse primers, dNTPs, Taq buffer with $MgCl_2$ and Taq DNA polymerase. Cycling comprised an initial denaturation at [94 °C for 5 min], [35] cycles of [94 °C for 45 s, primer-specific annealing for 45 s, 72 °C for 1 min] and a final extension at [72 °C for 5 min]. PCR Products were separated on 3% agarose gels containing ethidium bromide alongside a molecular-weight ladder (lane M) and visualized in a gel documentation system. Each gel carried the 40 genotypes in lanes 1–40.

2.5Scoring and data analysis

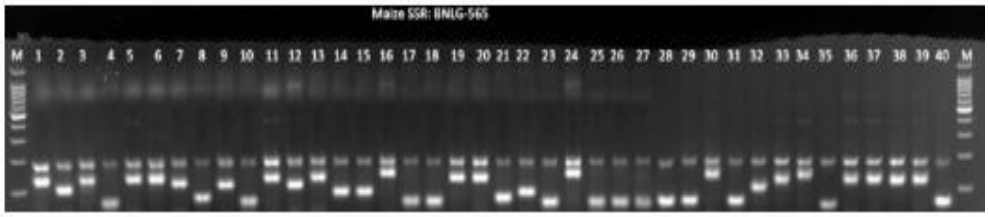
Amplified fragments were scored as present (1) or absent (0), with each distinct allele-size band treated as a separate allele. A primer was classed as polymorphic when it revealed more than one allele among the 40 genotypes. The percentage of polymorphic primers and the number of alleles per locus were calculated, and PIC for each locus was computed as $PIC = 1 - \Sigma p_i^2$, where p_i is the frequency of the ith allele (Botstein et al., 1980; Anderson et al., 1993). A genetic similarity matrix was generated using the [Jaccard / Dice / simple matching] coefficient, and genotypes were clustered by the unweighted pair group method with arithmetic mean (UPGMA; Sokal and Michener, 1958; Sneath and Sokal, 1973) in PAST 4.0 software.

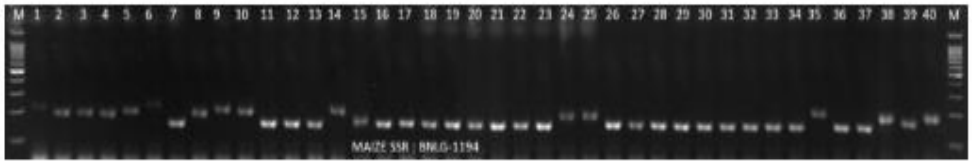
3.Results

3.1Amplification and polymorphism

All 25 primers amplified in the 40 genotypes. Of these, 24 were polymorphic and 1 was monomorphic. Together the primers detected alleles, ranging from 1 to 6 per locus (mean 3.8), and PIC values ranged from 0.58 (BNLG 1014) to 0.74 (UMC 1947) with a mean of 0.67 (Table 1). The gel profiles (Figure 1) show that informativeness varied markedly

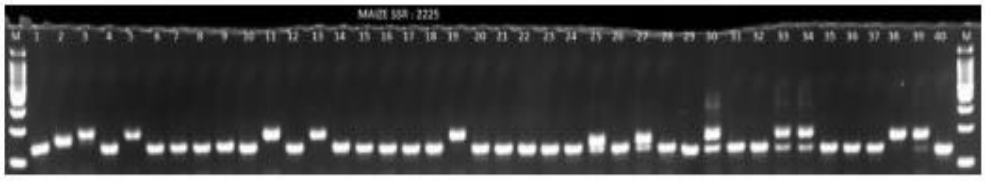
among primers. BNLG-565, BNLG-1194 and BNLG-1137 resolved clearly separated allele-size classes among the genotypes, and BNLG-1014 produced additional higher-molecular-weight bands in a subset of genotypes (for example INV-14 and INV-28). In contrast, BNLG-1042 showed only small-size shifts between genotypes, indicating lower discriminating power on agarose gels.

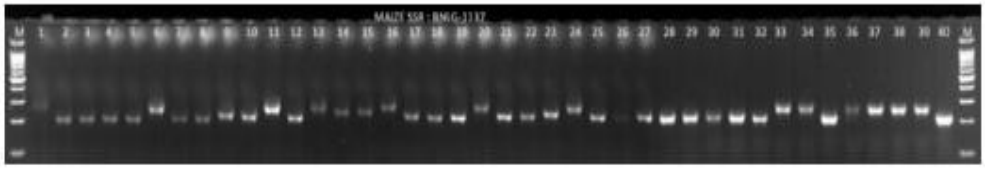

Maize SSR: BNLG-565
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 M

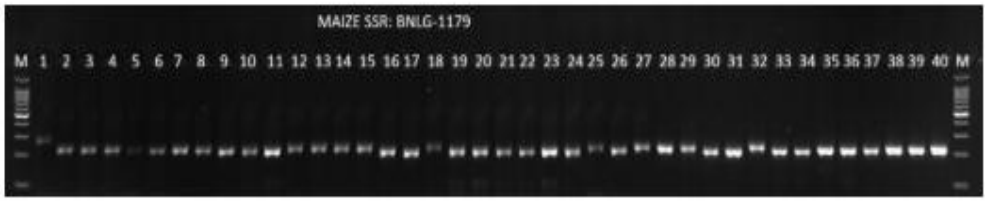

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 M
MAIZE SSR : BNLG-1194

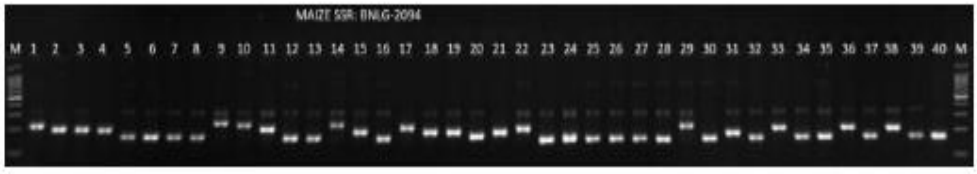

MAIZE SSR: BNLG-1265
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 M

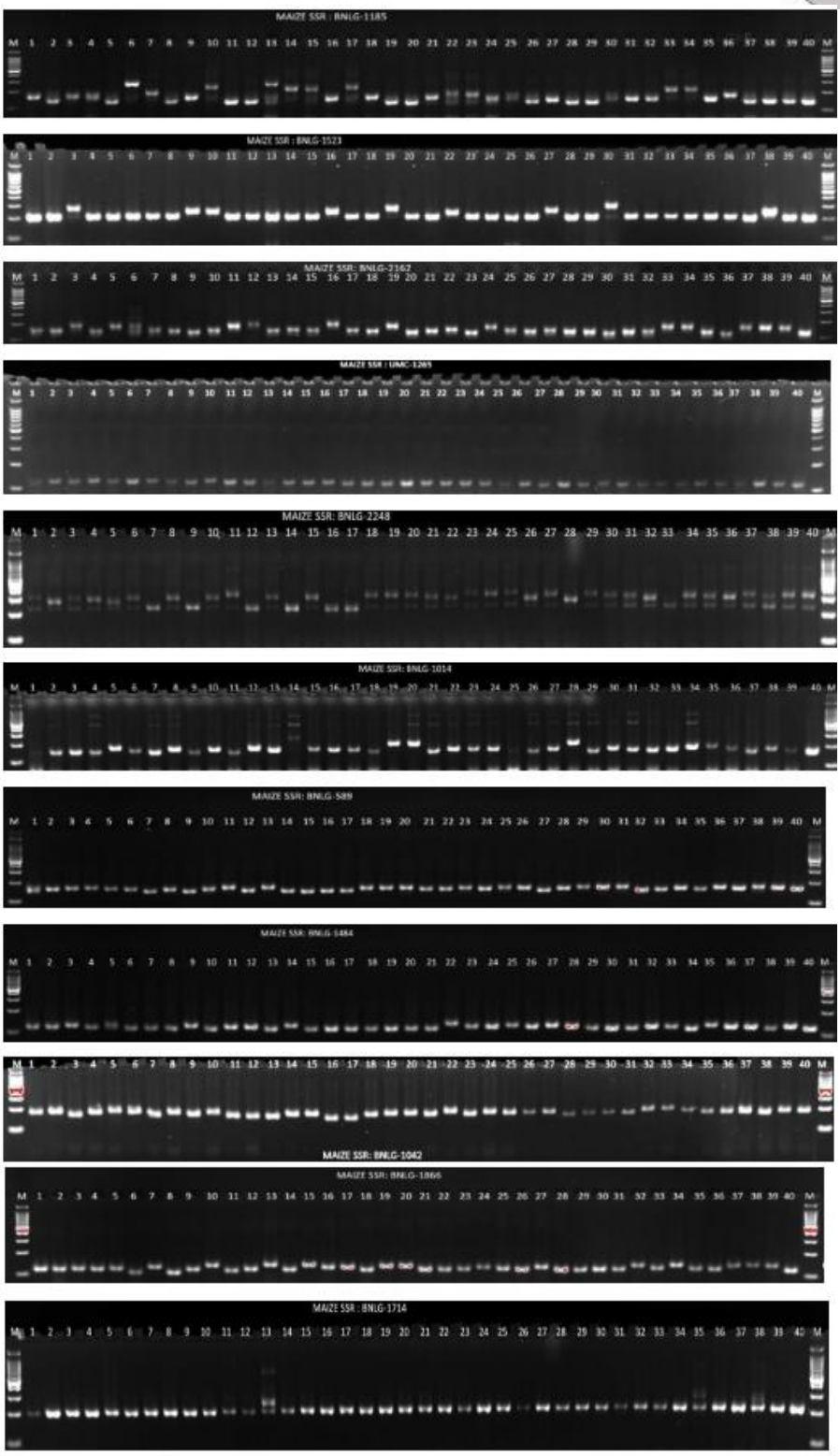

MAIZE SSR: UMC-2002
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 M


MAIZE SSR : 2225


MAIZE SSR : BNLG-1117


MAIZE SSR: BNLG-1179
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 M


MAIZE SSR: BNLG-2094
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 M


MAIZE SSR : BNLG-1185
MAIZE SSR : BNLG-1523
MAIZE SSR: BNLG-2162
MAIZE SSR : UMC-1265
MAIZE SSR: BNLG-2248
MAIZE SSR: BNLG-1014
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MAIZE SSR: BNLG-1484
MAIZE SSR: BNLG-1042
MAIZE SSR: BNLG-1866
MAIZE SSR : BNLG-1714

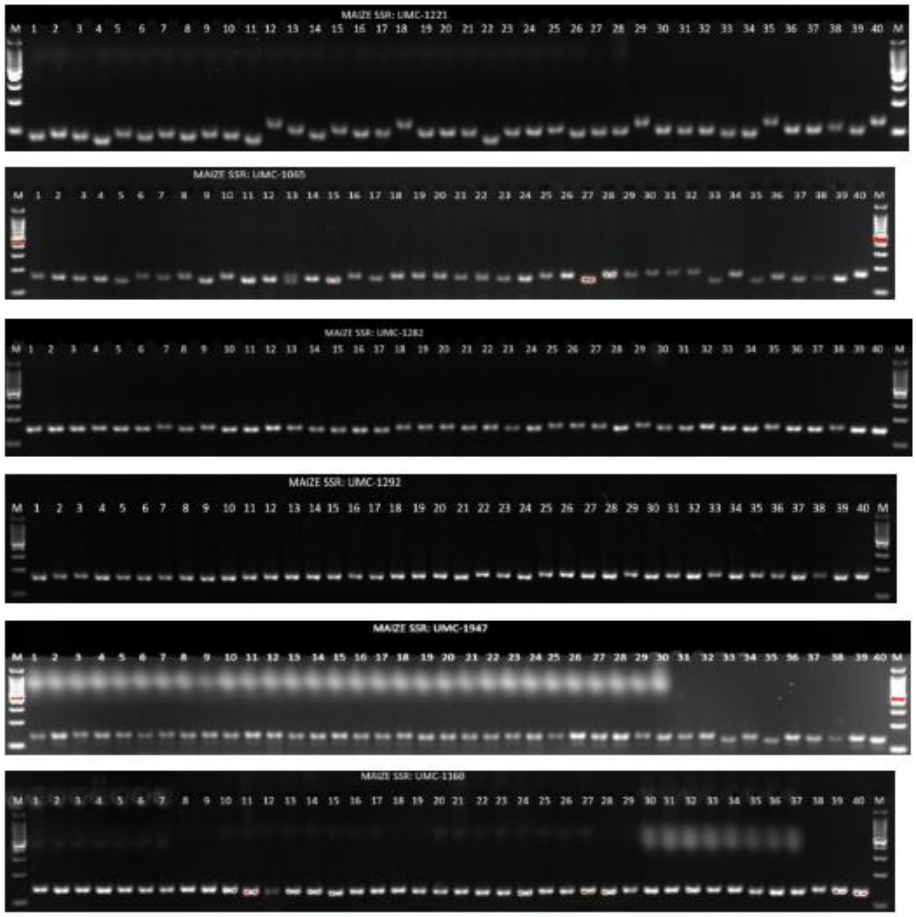

Figure 1. SSR amplification profiles of 40 maize genotypes (lanes 1–40 = INV-1 to INV-40; M = 100 Base Pair molecular-weight ladder) with primers BNLG-565 to BNLG-1160.

3.2Genetic similarity and cluster analysis

The UPGMA dendrogram (Figure 2) showed genetic similarity between genotypes ranging from approximately 0.69 to 0.93. The first split separated INV-1 from all other genotypes at a similarity of about 0.69, marking it as the most divergent genotype. The remaining 39 genotypes separated into six major groups (A–F; Table 2). Group A split from the rest at about 0.73 and Group B at about 0.75.

Groups C to F then formed a larger assemblage in which Group C separated at about 0.77 from a lineage comprising Groups D, E and F, which joined at about 0.78.

The closest relationships were found within Group C: INV-39 and INV-37 (≈0.93), INV-20 and INV-19 (≈0.91) and INV-13 and INV-11 (≈0.90). Other close pairs were INV-4 and INV-2 (≈0.90, Group F), INV-31 and INV-26 (≈0.88), INV-27 and INV-25 (≈0.88) and INV-21 and INV-8 (≈0.88). Within Group A, INV-17 and INV-14 were the most similar (≈0.87), with INV-33, INV-10 and INV-15 joining progressively at lower similarity. Similarity values were read from the dendrogram scale and are approximate.

Table 2. Grouping of the 40 maize genotypes in the UPGMA dendrogram.

Group	Genotypes	n	Remarks
Singleton	INV-1	1	Most divergent; joins all others at ≈0.69
A	INV-15, INV-33, INV-17, INV-14, INV-10	5	Joins the remainder at ≈0.73
B	INV-38, INV-36, INV-16, INV-9	4	Sub-pairs INV-38/INV-36 and INV-16/INV-9
C	INV-34, INV-30, INV-23, INV-20, INV-19, INV-39, INV-37, INV-24, INV-13, INV-11	10	Largest group; contains the most similar pairs
D	INV-22, INV-35, INV-40, INV-12	4	INV-22 joins the other three last
E	INV-27, INV-25, INV-28, INV-31, INV-26, INV-29, INV-18, INV-32, INV-7	9	INV-32 and INV-7 form a sub-pair
F	INV-21, INV-8, INV-6, INV-5, INV-3, INV-4, INV-2	7	Two sub-clusters joining at ≈0.81

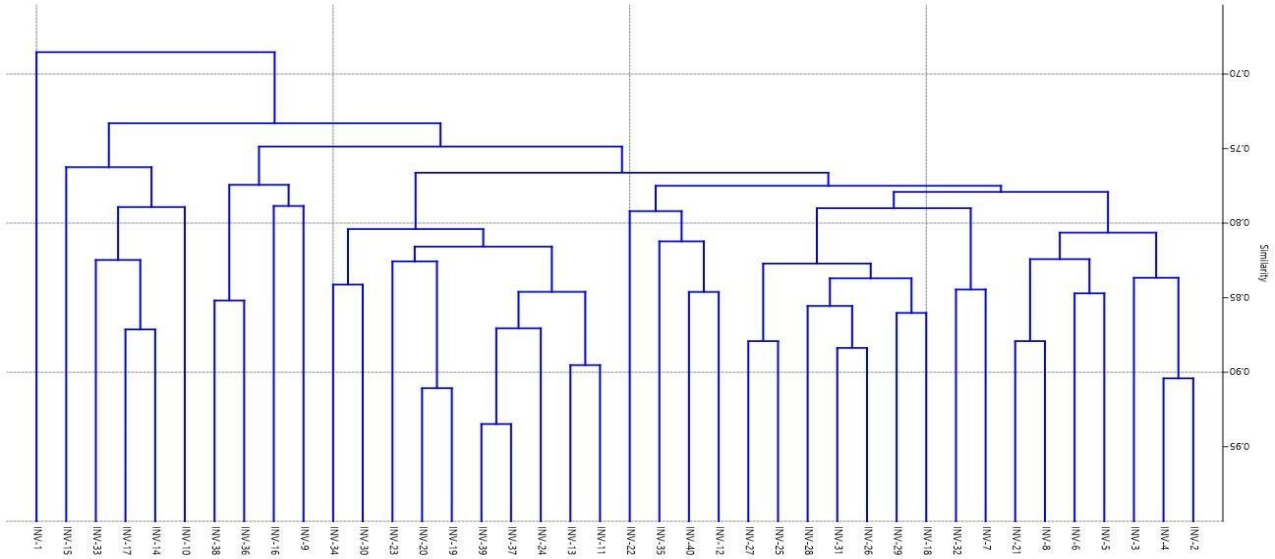

Figure 2. UPGMA dendrogram of 40 maize genotypes based on 25 SSR primers (PAST 4.0 software)

4.Discussion

The BNLG and UMC primers used here differed in informativeness, as expected for a panel drawn from public SSR resources. Comparable Indian studies have also found that only part of a primer set is informative. Sathua and Gayatonde (2018) found 20 of 40 SSRs polymorphic among 25 inbreds, while Kumar et al. (2008) reported a mean PIC of 0.39 and about three alleles per locus among 16 early-maturing inbreds. Primers that resolved several allele-size classes, such as BNLG-565, BNLG-1194 and BNLG-1137, are strong candidates for a core fingerprinting set,

whereas primers with small-size shifts, such as BNLG-1042, may need higher-resolution detection.

The similarity range of 0.69–0.93 indicates that the genotypes share a large proportion of alleles. This may reflect related pedigrees or a moderately narrow genetic base, but absolute similarity values depend on the coefficient used and cannot be compared directly across studies that used different coefficients. Of more practical value is the relative structure. INV-1 stood apart from all other genotypes, and Group A and Group B were the next most divergent lineages. In contrast, the tight pairs in Group C, especially INV-39 and INV-37, are candidates for pedigree checking, as they may be near-duplicates and redundant in a crossing programme or collection. Kumar et al. (2008) noted that SSR-based groupings did not match available pedigree records well, so without pedigree or phenotypic data the groups in Table 2 should be treated as hypotheses.

For hybrid breeding, crosses between genotypes from distinct groups, for example INV-1 or Group A with Groups C to F, are expected to be more likely to produce heterotic combinations than crosses within groups. Marker-based assignment is most reliable when combined with combining ability data, and the relationship between SSR distance and hybrid performance is often moderate and context-dependent (Melchinger, 1999; Reif et al., 2003, 2005). The groups should therefore guide the choice of crosses for testcross evaluation rather than replace it.

Several limitations apply. Agarose gels can be scored for SSRs (Senior et al., 1998) but have limited resolving power, so alleles differing by a few base pairs may merge and allele numbers and PIC may be underestimated. Twenty-five primers and a single set of 40 genotypes give limited genome coverage, and no population structure analysis was performed. Re-genotyping informative primers on polyacrylamide gels or capillary systems, adding loci, and integrating SSR data with phenotypic, combining ability and SNP data would give a more dependable basis for breeding decisions (Hamblin et al., 2007; Jones et al., 2007; Hufford et al., 2021).

5.Conclusion

The 25 BNLG and UMC SSR primers detected 6 polymorphic loci among 40 maize genotypes and separated them into one divergent genotype (INV-1) and six major groups. INV-1 and Groups A and B represent the most divergent material, whereas several tightly clustered pairs in Group C may be redundant. These results identify candidate parents for divergent crosses and a set of informative primers for fingerprinting, and they support the continued use of SSRs for focused diversity assessment in programmes where cost and sample numbers are limiting. Validation with higher-resolution genotyping, phenotypic evaluation and combining ability data is recommended before heterotic groups are finalised.

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