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DNA Profiling of Indonesian Maize Hybrids and their Parental Lines Using SSR Markers

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DNA fingerprinting is an effective genomic tool for hybrid/elite genotype identification. The objective of the research was to conduct DNA fingerprint characterisation of national maize hybrids and their parental lines using SSR markers. This research was conducted from April to July 2020. Moreover, 57 SSR markers were used for genotyping 10 maize genotypes of 4 national maize hybrids, three male and three female parents. SSR analysis in maize fingerprinting evaluates allele number, frequency, polymorphism, heterozygosity, PIC value, genetic similarity, distance, and relationships among samples. A dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Mean and Sequential Agglomerative Hierarchical Non-overlapping (SAHN) clustering. The results indicated that 230 SSR alleles were generated with molecular weights of 75.60 – 553.00bp. The polymorphism information content values varied between 0.10 – 0.85 (average = 0.59). A coefficient similarity of 42% classified the maize genotypes into 4 clusters, A, B, C, and D. JH 27 and two-parent lines (CY7 and MR14) made up Cluster A while B11209 was in Cluster B with 35% resemblance to Cluster A. Two pure lines and two hybrids comprised cluster C, C1, and C2 formed out of cluster C. The hybrid (JH 37) and its parental line (MAL03) had the most similarity (70%) in the C2 subcluster. A hybrid (NASA 29) and a single parental line (G1026-12) comprise cluster D.

Keywords: DNA fingerprinting, hybrid maize, parental lines, simple sequence repeats, genetic purity

Maize production technology development in Indonesia has recently accelerated with the availability of market-ready varieties and improved information access. As maize seeds are used frequently and the technique for seed production, seeds are easily contaminated and seeds genetic and physiological quality may deteriorate with time, resulting in a decline in plant yield. Maintaining the genetic purity of hybrid seeds is crucial and requires special care since it significantly impacts crop productivity. Even on ideal farms, the genetic purity of seeds can deteriorate over time because of different variables that occur during the production cycle. Hybrid seed maintenance must be carefully monitored because it significantly impacts plant productivity. Germplasm is the primary source of genes; consequently, characterisation of accessions, population genetic investigations, and selection of core assemblages are necessary for efficient application in crop development. DNA fingerprinting based on the reaction chain polymerase and relying on various types of molecular markers has been widely adopted to replace

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the traditional identification method. Additionally, this technique is not particular to a stage or network and is unaffected by the environment (Hammad *et al.* 2017; Patel *et al.* 2017; Jamil *et al.* 2020).

DNA fingerprinting enables the identification of hybrids at various stages of plant development and is considered an effective genomic tool for hybrid/elite genotype identification (Jehangir *et al.* 2018). DNA fingerprinting technology provides an approach that is fast, accurate and applicable in any setting. This method is used to differentiate between several species of maize (Huang *et al.* 2017). Crop variety DNA fingerprinting compares genetic material obtained from a sample with the most similar genetic profile in a reference database. The reference database should comprise released improved varieties likely to be present in the surveyed area for precise identification of enhanced varieties (Kosmowski *et al.* 2021). Comparisons of samples with true-type controls and their parental lines were performed as part of the fingerprint procedure. Simple sequence repeat (SSR) markers have become the markers of choice for genetic purity assays, such as co-dominant, polymorphism level, multi-allelic, and their distribution across genomes (Kumar *et al.* 2016). DNA fingerprinting technology prevents the falsification of an organism's identity. The evaluation of plant morphology is subjective and influenced by surrounding technical factors. Hence, combining with DNA fingerprinting enables a more accurate identification process. Numerous investigations have been dedicated to advancing and refining genotyping methodologies, with a specific emphasis on utilising molecular markers, including single-nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs), for DNA fingerprinting in maize (Setimela *et al.* 2016).

Simple sequence repeat (SSR) markers have been extensively employed in maize research to assess genetic diversity, variation, and population structure. These markers are highly informative and have proven effective in various analyses, including genetic diversity studies, evolutionary research, and genetic mapping (Lopes *et al.* 2015; Aci *et al.* 2018; Zebire 2020). SSR analysis gives an advantage in controlling the purity of maize hybrid varieties and their inbred lines (Fernandez *et al.* 2023), which displays the genetic relationship between various inbred lines. SSR markers have the potential to effectively characterise lines at the molecular level, hence aiding maize breeders in the efficient allocation of lines to diverse groups and facilitating the selection of parents for the development of new hybrids (Shete *et al.* 2023). It is possible that using SSR markers to identify a fixed pattern may assist in making better use of inbred lines, which will allow for the formation of genetically diverse source populations and the application of heterosis. The objective of this research was to conduct a DNA fingerprinting analysis on four well-known national maize hybrids in Indonesia: JH 27, JH 45, JH 37, and NASA 29, as well as the hybrids' parental lines, by using SSR markers.

MATERIAL AND METHODS

Study Area

This research was conducted at the Indonesian Cereals Research Institute (ICERI) in Maros, Indonesia, from April to July 2020.

Plant Materials and DNA Extraction

This study used 10 maize genotypes, including four national hybrid varieties, three female parents, and three male parent lines (Table 1).

The DNA of young maize leaves was extracted by following the protocol of CIMMYT, where the fully developed leaves were randomly picked from 15 sheets per plant sample. The leaf was then sliced into small pieces, weighed at 0.4 g per sample, and the DNA was extracted. The DNA was extracted and PCR assembled following the Ramlah procedure (Ramlah *et al.* 2020). The quality of the extracted DNA was determined by using horizontal electrophoresis with 0.9% agarose gel and a nano spectrophotometer (Implen CmbH, Munich, Germany).

PCR Analysis

Following the step of Ramlah (Ramlah *et al.* 2020), a PCR MaxyGene II, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California, USA, was used to amplify DNA. Furthermore, 30 amplification cycles were performed per the CIMMYT protocol, which included denaturation for 2 minutes at 94°C, 30 seconds at the same temperature, 1 minute at 56°C, and 1 minute at 72°C. The

annealing temperature decreases by 1°C per two cycles until the annealing process is complete. The second stage was repeated 29 times and concluded with a cycle of elongation at 72°C and chilling at 4°C, after which the reaction was terminated. Using the Dual Mini-Verticals Complete System MGV-202-33 and 8% polyacrylamide gel, the PCR data were separated by vertical electrophoresis.

Data Analysis

The data were analysed based on the DNA band pattern scoring results in the electrophoresis results, the data were analysed. The data is scored as binary data. If a band shows up, it's written as one (1); if it doesn't, it's written as zero (0). The number 9 is used if bands are unclear or hard to score. Alleles are named based on where the DNA bands are concerning the oX174/Hin f1 fragment. The selection of SSR markers was informed by prior studies, considering factors such as chromosomal distribution, minor allele frequency (MAF), polymorphic information content (PIC), and repeat length (Abakemal *et al.* 2015). For dominant markers, the PIC value reflects the likelihood that a marker will be present or absent in two randomly chosen individuals within a population (Serrote *et al.* 2020). The level of polymorphism (PIC) for each SSR marker is calculated by the formula (Smith *et al.* 1997):

$$PIC = 1 - \sum_1^n f_i^2,\ i = 1, 2, 3, \ldots\ldots n$$

where: f_1^2 is the frequency of the i-th allele.

The data from the electrophoresis test is processed using NTSYS-2.1 PCs Rohlf (Rohlf 2015) and Power Marker software version 3.25. The genetic similarity matrix was clustered using the UPGMA (Unweighted Pair Group Using Arithmetic Average) technique and the Jaccard coefficient (Isnaini *et al.* 2020). The distance matrix and dendrogram were analysed using NTSYS-pc (Numerical Taxonomic System) version 2.1 to determine their relationships. Genetic similarity is the degree of similarity between characters, such as the band fragments shared by the identified genotypes. The confidence bounds of the UPGMA-based dendrogram were checked by bootstrapping 2,500 times using the IRRI- 'Winboot' computer program to determine the repetition of grouping. As a DNA fingerprint or profile, the amplification patterns of SSR primer pairs that generated double bands for a given hybrid combining each of its parental lines were chosen.

RESULTS

SSR Marker Characteristics

This study used SSR markers to evaluate four national maize hybrids and their male (P1) and female (P2) parents for polymorphism. Therefore, 57 SSR primer pairs were evaluated for their amplification patterns in four maize hybrids and six parental lines, and all of them produced amplified products.

T a b l e 1

Characteristics of maize genotypes used in the study

Genotypes	Pedigree	Kernel color	Kernel type
JH 27	National maize hybrid	Yellow orange	Semi flint
JH 45	National maize hybrid	Yellow orange	Flint-semi flint
JH 37	National maize hybrid	Yellow	Flint
NASA 29	National maize hybrid	Yellow orange	Semi-flint semi-dent
CY-7	Female parent of JH 27	Orange yellow	Flint
MR-14	Male parent of JH 27	Yellow orange	Flint
B11-209	Female parent of JH 45	Yellow	Flint
CLYN-231	Male parent of JH 45 and JH 37	Orange	Flint
MAL-03	Female parent of JH 37 and NASA 29	Orange	Flint-semi flint
G1026-12	Mare parent of NASA 29	Yellow orange	Semi dent

Table 2 summarises the polymorphic informative ability parameters for the 57 SSR markers. In total, 230 alleles were found between the four hybrids and their two parental lines, and this was accomplished by using 57 polymorphic SSR primer pairs. The SSR loci had an average of 4 alleles per polymorphic main pair, with a range from 2 to 7. Sizes varied from 7.60 to 553.0 bp, and the number of alleles showed a wide range (0.10 to 0.85) with a mean of 0.59.

DNA Fingerprint for Maize Hybrids

As the fingerprint DNA profile of the specific hybrid, the amplification pattern of the SSR primer pair, resulting in two alleles for the hybrid containing each of the parental strains, was chosen. Figure

T a b l e 2

Information ability of polymorphic simple sequence repeats (SSR) markers

No.	Primary	Bin no.	Polymorphic information content (PIC)	Number of alleles	Size range
1	umc1292	1.00	0.65	7	118.00–157.99
2	umc1685	1.01	0.53	3	116.20–137.80
3	bnlg1614	1.02	0.65	4	179.00–212.20
4	bnlg1627	1.02	0.67	3	100.00–114.40
5	phi109275	1.03	0.62	5	135.60–192.99
6	bnlg1564	1.07	0.78	6	82.00–151.00
7	phi109642	2.00	0.56	4	124.60–151.00
8	phi96100	2.00–2.01	0.71	5	264.50–553.00
9	bnlg1537	2.03	0.58	3	186.00–196.50
10	umc1185	2.03	0.74	6	109.00–151.00
11	phi083	2.04	0.52	3	126.20–151.00
12	phi374118	3.03	0.71	6	208.16–311.00
13	phi102228	3.04–3.05	0.63	3	120.75–142.75
14	phi053	3.05	0.48	2	167.30–191.80
15	umc1136	3.10	0.53	3	140.00–189.11
16	phi072	4.01	0.41	3	143.66–156.50
17	phi079	4.05	0.32	2	185.90–192.50
18	phi093	4.08	0.51	3	280.00–427.00
19	nc013	5.00	0.83	6	103.60–120.20
20	nc130	5.00	0.75	5	131.20–249.00
21	phi109188	5.03	0.4	4	78.80–100.00
22	phi331888	5.04	0.67	5	126.60–151.00
23	bnlg1346	5.07	0.57	3	145.50–183.60
24	umc1153	5.09	0.71	4	109.00–126.80
25	phi126	6.00	0.77	6	124.60–192.99
26	umc1143	6.00	0.66	3	75.60–87.99
27	bnlg1165	6.01	0.73	5	137.99–160.80
28	bnlg1139	6.01	0.71	5	206.10–249.00
29	nc012	6.05	0.38	2	82.00–85.60
30	phi081	6.05	0.32	2	151.00–171.99
31	bnlg1740	6.07	0.66	5	109.00–151.00
32	phi299852	6.08	0.64	3	113.50–126.80
33	umc2059	6.08	0.63	4	122.88–140.00
34	umc1545	7.00	0.48	2	82.00–89.99
35	phi057	7.01	0.72	4	140.00–175.50
36	phi112	7.01	0.19	3	148.80–183.60
37	umc1066	7.01	0.80	6	100.00–171.99
38	phi034	7.02	0.82	5	120.70–200.00
39	umc1339	7.02	0.54	3	126.80–185.99
40	umc1401	7.02	0.66	3	126.80–145.25
41	phi114	7.03	0.72	6	137.55–185.99
42	phi328175	7.04	0.68	5	122.40–155.08
43	phi082	7.05	0.65	3	107.20–126.80
44	bnlg1327	8.01	0.85	7	100.00–151.00
45	phi420701	8.01	0.57	4	249.00–369.00
46	phi115	8.03	0.32	2	293.28–427.00
47	umc1858	8.04	0.42	2	151.00–154.49
48	bnlg666	8.05	0.69	6	118.00–157.50
49	umc1161	8.06	0.73	6	132.66–204.90
50	phi233376	8.09	0.6	5	140.00–189.49
51	umc1279	9.00	0.42	7	91.00–131.75
52	phi028	9.01	0.5	4	76.60–100.00
53	phi065	9.03	0.56	3	126.80–145.50
54	phi042	9.04	0.81	2	177.90–232.60
55	phi050	10.03	0.10	2	157.90–178.90
56	bnlg1547	10.03	0.34	3	172.00–193.00
57	umc1345	10.03	0.70	4	145.50–219.60
Total		–	33.84	230	–
Average		–	0.59	4	75.60–553.00

1a and 1b depict the amplification of typical profiles using SSR markers umc1161 and phi 109275. Characteristics found in ten test genotypes yielded 36 unique alleles at 28 SSR loci (Table 3).

The DNA fingerprint sizes varied from 109 bp (umc1185) to 553.00 bp (phi96100). Twelve unique DNA regions using 12 SSR markers were identified, which could be used as an identification mark or DNA fingerprint for JH 27. Maize hybrid JH 45 was identified with the help of 05 SSR markers, i.e., bnlg1564 (151.00), phi96100 (427.00), phi331888 (151.00), umc1066 (135.60), umc1161 (204.90), whereas maize hybrid JH 37 was identified with the help of 01 SSR marker phi126 (157.99). Maize hybrid NASA 29 was identified with the help of 04 SSR markers i.e., phi126 (192.99), phi420701 (311.00), phi233376 (140.00), and phi050 (178.90). G1026-12, which is the male parent line of NASA 29 (inbred line) was identified with the help of 05 SSR markers, i.e., umc1292 (127.77), bnlg1627 (114.40), phi072 (143.66), phi126 (178.99), and bnlg1740 (126.80). Similarly, male parent of JH 27 (inbred line) MR-14 was identified with the help of 06 SSR markers i.e., bnlg1614 (185.99), umc1153 (114.99), bnlg1165 (137.99), phi112 (159.10), bnlg666 (157.50), and umc1161 (132.66) whereas male parent line of JH 45 and JH 37 (inbred line) CLYN-231 was identified with help of phi109275 (175.50). B11-209 which is female parent line of JH 45 (inbred line) was identified with the help of 02 SSR markers i.e., bnlg1564 (118.00), and umc2059 (132.66).

Phylogenetic Analysis

In the present study, the genetic potential for purity in maize used the Jaccard similarity coefficients as input data for a cluster analysis performed in the NTSYS pc 2.1 program; the resulting dendrogram is displayed in Figure 2. Bootstrap Each cluster's *P* value can be found at the node corresponding to that cluster. When comparing the reliability of different groups, P values indicate the degree to which such claims can be trusted. At a similarity coefficient of 42%, all of the maize hybrids and parental lines were divided into four distinct groups.

Figure 2 illustrates the genetic relationships among ten maize genotypes, including four commercial hybrids and their respective parental

T a b l e 3

Allele-allele specific (fingerprint individual) detected 28 simple sequence repeats (SSR) loci

No.	Locus	Allele no.	Base pair	Genotype
1	umc1292	1.00	i3(127.77)	G1026-12
2	bnlg1614	1.02	g2(185.99)	Mr14
3	bnlg1627	1.02	j1(114.40)	G1026-12
4	phi109275	1.03	g3(175.50)	CLYN231
5	bnlg1564	1.07	h1(151.00)	JH45
–	–	–	j1(118.00)	B11-209
6	phi109642	2.00	h1(151.00)	JH27
7	phi96100	2.00 – 2.01	d1(427.00)	JH45
8	umc1185	2.03	h1(151.00)	JH27
–	–	–	j2(109.00)	JH27
9	phi374118	3.03	e3(280.00)	JH27
10	phi072	4.01	h2(143.66)	G1026-12
11	phi093	4.08	e1(311.00)	JH27
12	phi331888	5.04	h1(151.00)	JH45
13	umc1153	5.09	j1(114.99)	Mr14
14	phi126	6.00	g1(192.99)	NASA29
–	–	–	g2(178.99)	G1026-12
–	–	–	g2(157.99)	JH37
15	bnlg1165	6.01	i1(137.99)	Mr14
16	bnlg1740	6.07	i2(126.80)	G1026-12
17	umc2059	6.08	i3(132.66)	B11-209
18	phi112	7.01	g1(183.60)	JH27
–	–	–	g2(159.10)	Mr14
19	umc1066	7.01	i1(135.60)	JH45
20	phi034	7.02	g1(200.00)	JH27
21	phi114	7.03	g1(185.99)	JH27
–	–	–	h1(151.00)	JH27
22	bnlg1327	8.01	i1(140.00)	JH27
23	phi420701	8.01	e1(311.00)	NASA29
24	bnlg666	8.05	g2(157.50)	Mr14
25	umc1161	8.06	f1(204.90)	JH45
–	–	–	i19132.66)	Mr14
26	phi233376	8.09	g1(189.49)	JH27
–	–	–	i1(140.00)	NASA29
27	phi042	9.04	f1(232.60)	JH27
28	phi050	10.03	g1(178.90)	NASA29

inbred lines, using UPGMA cluster analysis based on Jaccard similarity coefficients. The dendrogram delineates four main clusters (A–D), each reflecting varying levels of genetic similarity. Cluster A comprises inbred lines JH27, CY7 and Mr14, which exhibit high genetic similarity, suggesting close genetic relatedness that may limit heterotic potential if combined. Cluster B, consisting solely of hybrid B11-209, is genetically distinct, indicating its unique allelic composition and potential value for maintaining diversity in breeding pools. Cluster C is subdivided into C1 (JH45 and CLYN231) and C2 (JH37 and MAL03), both showing high internal similarity, particularly C2 (98.3%), pointing to near-identical genetic makeup likely derived from the same pedigree. Cluster D includes hybrids NASA29

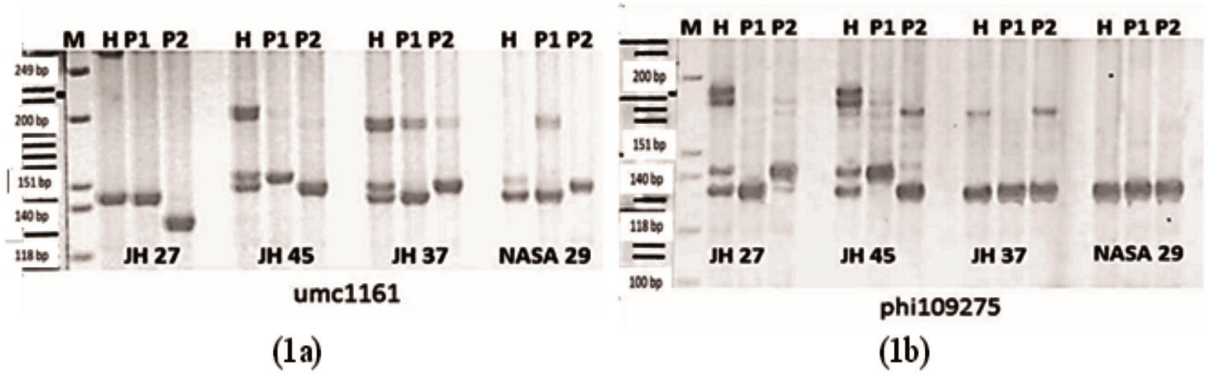

Figure 1. Example of two SSR marker amplification profiles assayed in four national maize hybrids (H) and their male (P1) and female (P2) parental lines; M: 100-249 bp DNA marker with SSR marker (a) umc1161 and (b) phi109275.

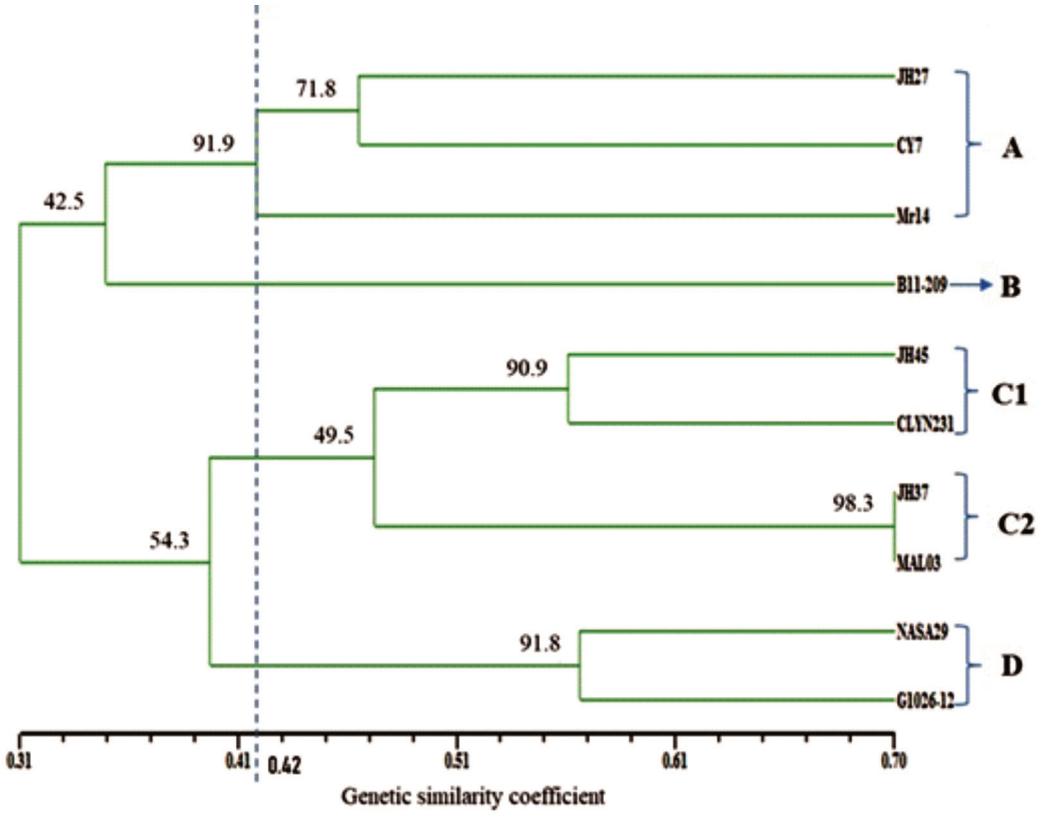

Figure 2. Dendrogram showing the relationship of four popular national maize hybrids along with parental lines based on UPGMA cluster analysis. The number at the nodes indicates the corresponding bootstrap. Note: A–D – four main clusters.

and G1026-12, which also display a strong genetic resemblance, possibly due to overlapping parentage (See Figure 2). Crop variety protection and hybrid performance prediction can benefit from genetic fingerprinting. Therefore, with the help of SSR markers, the genetic potential for purity in maize can be analysed by identifying its genetic diversity.

DISCUSSION

Genetic uniformity is crucial for producing high-quality hybrid maize seeds. Conventional field purity tests examine plant morphology, which is time-consuming, complex, and often affected by the environment. SSR is based on polymerase chain reactions, and polymorphisms can be easily found using agarose gel techniques (Giri Putra *et al.* 2020). They used SSR markers in this study to evaluate four national maize hybrids and their male (P1) and female (P2) parents for polymorphism. Therefore, 57 primers out of the 100 SSR markers examined were polymorphic, whereas monomorphic samples or included only one band were excluded from further study. SSR markers have proven effective and efficient in evaluating genetic diversity in maize. Certainly, it is possible to utilise SSR markers to characterise lines at the molecular level properly. These markers can also assist maize breeders in efficiently assigning lines to heterotic groups and guiding them in selecting parents to generate new hybrids. At the same time, SSR markers offer a method for unequivocally distinguishing individual genotypes based on the allelic pattern that is particular to them; this is an application that is becoming increasingly relevant in varietal protection (Kumari *et al.* 2018). Only polymorphic genotypes can be evaluated at a high level to obtain data with a high level of validity (Andayani *et al.* 2020).

Moreover, considering the number of alleles at a locus and the relative frequency of alleles in the genotype studied, PIC values, which range from zero for monomorphic to one for highly discriminatory markers, indicate the informativeness and discriminatory power of the SSR locus and its potential for detecting genetic diversity (Sathua *et al.* 2018). It was determined that the optimal marker for gauging genetic diversity in a population had a PIC value between 0.10 and 0.85, which indicates that genotypes are highly polymorphic. A similar study in maize utilising 18 SSR markers reported polymorphic information content (PIC) values ranging from 0.00 to 0.87, with an average value of 0.65, indicating a moderate to high level of genetic diversity among the genotypes analysed (Madhav *et al.* 2016). A correlation was identified between a few SSR markers and various characteristics, which points to the possibility of pleiotropy or the close connection of genes (Sa *et al.* 2018). SSR markers have the potential to effectively characterise lines at the molecular level, hence aiding maize breeders in the efficient allocation of lines to diverse groups and facilitating the selection of parents for the development of new hybrids (Shete *et al.* 2023). SSR markers were also utilised in this study, and their results showed that they were useful for distinguishing between the four hybrid maize varieties, meaning that they might be used as a standard for determining each variety's genetic purity.

DNA fingerprint identification of hybrid maize with its parental line using SSR yielded similar findings, as reported by many researchers (Jhansi *et al.* 2015; Surender *et al.* 2015; Sharma *et al.* 2020). SSR markers exhibited higher polymorphism, which caused some parental lines with similar genetic backgrounds from pedigree data to be grouped into different clusters (Mahalingam *et al.* 2013). DNA fingerprints will be helpful in a variety of protection and registration processes. Following prior research, it has been reported that SSR markers i.e., umc087, umc1088, umc1389, umc2281, p-Phi022, p-Phi112, and p-Phi114, could be used for the identification of KH9374, KH 404, KH 2005, KH 9452, POLO, KH 789, and KH 717 hybrids, respectively (Jhansi *et al.* 2015; Sharma *et al.* 2020)

Utilising molecular markers is a crucial and supplementary approach to assessing genetic diversity and interactions among different maize genotypes. This method is precious when combined with the evaluation of phenotypic features (Mushtaq *et al.* 2016). By employing molecular markers, breeding programs can efficiently identify suitable parents for their breeding initiatives (Nyaligwa *et al.* 2015). Several reported a strong correlation between the morphological qualities of the landraces and their pattern of clustering based on SSR molecular analy-

sis, which was shown to be useful in identifying acceptable landraces as promising parents for a future breeding program (Gazal *et al.* 2016; Lakshman *et al.* 2019; Kamara *et al.* 2020). This was found to be the case because SSR molecular analysis clustered the landraces in a pattern that was found to be effective. This was consistent with a study that found that parental lines from separate clusters have a greater ability to combine for hybrid seed production (Sharma *et al.* 2020).

Success in employing SSR markers to genotype four popular national maize hybrids and their parental lines in this work demonstrates their use for determining a plant's genetic makeup. The utilisation of a collection of exceedingly informative molecular markers in the molecular characterisation of hybrids and their respective parental lines is accompanied by a multitude of noteworthy benefits when compared to the utilisation of morphological and biochemical markers. This approach allows for the revelation of the genetic composition of various genotypes originating from diverse sources (Fernandez *et al.* 2023). SSR markers are a valuable technique for studying the molecular variety among the maize genotypes since the environment does not influence them (Guevarra *et al.* 2022). This can help breeders select varied parental lines, which is useful for hybridisation programmes in heterosis breeding to improve maize production. The capacity to categorise inbred organisms into a recognized heterotic assemblage confers upon the maize breeders the opportunity to exercise informed assessments concerning the admissible amalgamations of hybrids, thereby mitigating the condition of ignorance in the identification of suitable progenitors for crossbreeding and amplifying the efficacy of the breeding process (Elec *et al.* 2022). Therefore, it is necessary to demonstrate the effectiveness of such markers in various genetic backgrounds and their value in breeding programmes for creating new hybrid maize varieties (Bocianowski *et al.* 2021). Furthermore, DNA fingerprints may be used as a quick reference for verifying the genetic purity of different sample sizes. In addition, SSRs with a distinctive amplification pattern can be utilised as diagnostic genetic markers for certain hybrids. The outcome may also be effectively evaluated in hybrids with unknown parental lines or additional SSR loci.

CONCLUSIONS

This study successfully delineated the genetic relationships among ten key maize genotypes using SSR markers, revealing a robust level of polymorphism and a clear population structure. The clustering analysis grouped the genotypes into four distinct heterotic groups, providing critical empirical validation for their genetic distinctness. Notably, the clustering of hybrids with their parental lines, such as JH 27 with CY 7 and MR 14, confirms the fidelity of the genetic data and the method's precision. The distinct separation of line B11-209 into its own cluster highlights its potential as a unique genetic resource for broadening the genetic base in breeding programs. These findings provide maize breeders with a genetically-informed framework for parent selection, enabling the strategic design of crosses to maximize heterosis and accelerate the development of superior hybrids. Furthermore, the SSR profiles established herein serve as a definitive reference for ensuring genetic purity in foundational seed stock production, thereby enhancing the efficiency and reliability of the entire maize breeding pipeline.

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