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Molecular Assessment of Mango (*Mangifera indica* L.) Genotypes Using Inter-Simple Sequence Repeat Markers

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Abstract

Molecular evaluation of mango (*Mangifera indica* L.) genotypes plays a vital role in shaping effective strategies for enhancing productivity, conserving genetic resources, and improving germplasm for future breeding efforts. In this context, a study was conducted to genetically characterize 18 mango genotypes (BARI Aam-1 to BARI Aam-18) using eleven ISSR (Inter Simple Sequence Repeat) markers. The ISSR analysis produced a total of 67 bands, of which 51 were polymorphic, indicating a high level of genetic variability with an average polymorphism rate of 76.11%. The overall genetic diversity and Shannon's information index were calculated at 0.446 and 0.636, respectively, further confirming substantial genetic variation among the genotypes. Genetic distance analysis revealed the closest relationship between BARI Aam-1 and BARI Aam-18 (0.008), while the most distant relationship was observed between BARI Aam-6 and BARI Aam-11 (0.836). Likewise, genotype similarity indices showed the highest similarity (0.991) between BARI Aam-17 and BARI Aam-18 and the lowest (0.443) between BARI Aam-6 and BARI Aam-11. Based on UPGMA cluster analysis, the genotypes were divided into two major groups: Cluster I comprised BARI Aam-11, BARI Aam-12, and BARI Aam-7, while Cluster II included the remaining fifteen genotypes. This comprehensive description of genetic variation and relationship among mango genotypes may provide great value for future studies, breeding initiatives, and the development of improved cultivars.

Introduction

Mango (*Mangifera indica* L.), a member of the family Anacardiaceae and the order Sapindales (Mukherjee 1950), stands as one of the most significant and extensively

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cultivated tropical fruits worldwide. Being renowned for its rich nutritional profile and appealing sensory attributes, mango serves a wide array of purposes, from fresh consumption to industrial processing. Believed to have originated in the Indian subcontinent and southern Asia, mango has been cultivated for over 4,000 years, underscoring its deep historical and cultural roots. Today, it is grown in more than 100 tropical and subtropical countries, with global production reaching approximately 61.1 million metric tons annually (FAO 2023). In Bangladesh, mango cultivation covers about 205,034 hectares, producing nearly 2.7 million metric tons each year (BBS 2023). Although mango is grown across nearly all districts, key production hubs include Rajshahi, Chapainawabgonj, and the greater Dinajpur region. Despite its considerable economic and agricultural value, research into mango genetics and genome characterization remains relatively underdeveloped, highlighting the need for deeper scientific exploration in this field. While traditional breeding approaches have played a key role in enhancing mango cultivars, the crop's perennial growth habit, extended juvenile phase and high genetic heterozygosity pose significant challenges to conventional breeding efforts. To overcome these constraints, DNA-based molecular marker technologies have increasingly been integrated into mango genetic research and breeding programs. These markers offer greater precision and reliability in evaluating genetic variation compared to morphological traits. In the last few decades, many crop species, especially fruit trees, have successfully used a variety of molecular markers, such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter-simple sequence repeat (ISSR), and simple sequence repeat (SSR) markers (Gupta et al. 1999, Khan et al. 2015, Rifat et al. 2019). Among them, ISSR markers have emerged as a preferred tool due to their ease of use, reproducibility, cost-efficiency, and strong capability to detect polymorphisms. ISSR markers have proven valuable for identifying cultivars, assessing genetic diversity, and validating genotypes in various fruit crops, including mango. Research conducted in India and other mango-producing regions has highlighted the effectiveness of ISSR markers in differentiating mango varieties (Uddin et al. 2014). Recognizing the limited molecular characterization of mango genotypes in Bangladesh, this study was designed to establish a robust genetic framework for identifying mango genotypes and analyzing their diversity using ISSR markers. The findings will support the genetic enhancement and conservation of mango varieties, laying the groundwork for future breeding initiatives.

Materials and Methods

Leaf samples from 18 mango genotypes (BARI Aam-1 - BARI Aam-18) were collected from BARI horticulture field. Collected fresh young leaves were used to isolate good quality genomic DNA. Some important morphological characteristics of BARI mango genotypes are presented in Table 1.

Table 1. Name of mango genotypes and their important characteristics (Barua et al. 2013).

Sl. No.	Mango Genotypes	Important Characteristics
1	BARI Aam-1	Earlier than most local mango varieties. Fiberless, soft, juicy, and sweet.
2	BARI Aam-2	Fiberless, smooth textured, very sweet, flavorful, and aromatic. Highly preferred by consumers.
3	BARI Aam-3	Smooth, juicy, fiberless, and sweet with rich aroma.
4	BARI Aam-4	Fiberless, juicy, smooth textured, and very sweet with a pleasant flavor. Excellent for fresh consumption.
5	BARI Aam-5	Oblong and slightly flattened shaped. Smooth, fiberless, juicy, and exceptionally sweet, with a strong, pleasant aroma.
6	BARI Aam-6	Late-season variety,. Smooth, soft, and fiberless.
7	BARI Aam-7	Turns deep yellow or golden when ripe. Fiberless, thick, soft, and juicy.
8	BARI Aam-8	Highest yield among BARI mango varieties.
9	BARI Aam-9	Known as Kacha-Mitha,. High yielding, early, and regular-bearing variety.
10	BARI Aam-10	Roundish shaped. Turns yellowish-green when ripe.
11	BARI Aam-11	Also known as the Baromasi Mango**.** The pulp is dense and yellow, with a slightly acidic taste.
12	BARI Aam-12	Also known as Gourmoti. Late-season fiberless variety and sweet in taste**.**
13	BARI Aam-13	Hybrid mango variety, with deep orange, and fiberless flesh. The skin turns maroon when ripe.
14	BARI Aam-14	Late-season variety and oblong in shape with a maroon color when ripe.
15	BARI Aam-15	High yielding, regular-bearing, and sweet in taste.
16	BARI Aam-16	High yielding, midseason, and regular-bearing variety. Attractive yellow fruit with a red tinge.
17	BARI Aam-17	Hybrid variety.
18	BARI Aam-18	High yielding, regular-bearing late season mango variety.

Fresh mango leaves were collected and processed for genomic DNA extraction using a modified CTAB protocol (Saclain et al. 2016). The extracted DNA was evaluated for purity through 0.8% agarose gel electrophoresis and its concentration was determined with a Thermo Scientific™ Nanodrop One UV-vis spectrophotometer.

Eleven arbitrary sequences (ISSR Primer) were chosen from a number of primers following the previous research papers (Table 2) and used for molecular analysis. On three DNA subsamples, eleven primers were tested for optimizing annealing temperature. PCR was carried out in a 10µl reaction volume containing 3 µl of template DNA, 1 µl primer, 1 µl dNTPs, 1unit Taq polymerase (TAKARA, Japan), 1 µl $MgCl_2$ and the required amount of sterile deionized water. The thermal cycler (Biometra, Germany) was programmed to initial denaturation for 3 min at 94°C, followed by 35 cycles consisting denaturing at 94°C, annealing at 52, 50 and 45°C for 1 min and extension at 72°C for 2 min.

To get the amplifiable end products, an electrophoretic separation was carried out on a 1% agarose gel. In 1X TBE buffer, agarose gel electrophoresis was carried out for 1.5 hrs at 120V. In the gel, a 100 bp DNA ladder made by BIONEER Corporation was run

concurrently. After electrophoresis, gel was carefully taken out of the electrophoresis chamber and put in a staining solution that had been prepared with ethidium bromide (10 mg/ml).

Table 2. ISSR primers for eighteen mango genotypes with corresponding polymorphic bands and an overall estimation of genetic variation.

Primer Code	Sequences (5'-3')	TB	P	PP	H	I
UBC880	GGAGAGGAGAGGAGA	7	4	57.14	0.371	0.558
UBC825	ACACACACACACACACT	9	7	77.78	0.464	0.657
UBC841	GAGAGAGAGAGAGAGACTC	3	3	100	0.496	0.689
UBC853	ACACACACACACACACCTT	5	5	100	0.488	0.681
UBC813	CTCTCTCTCTCTCTCTT	4	3	75	0.499	0.693
UBC811	TCTCTCTCTCTCTCTCRT	7	5	71.43	0.499	0.693
UBC810	AGAGAGAGAGAGAGAGG	7	4	57.14	0.441	0.632
UBC886	GAGAGAGAGAGAGAGAC	6	3	50	0.265	0.435
UBC855	GAGAGAGAGAGAGAGAT	8	8	100	0.499	0.692
UBC809	VDV CTC TCT CTC TCT CT	4	4	100	0.435	0.626
UBC876	GAT AGA TAG ACA GAC A	7	5	71.43	0.448	0.640
	Overall	67	51	76.11	0.446	0.636

TB = Total bands; P = Polymorphic bands; PP = % polymorphic bands; H = Nei's (1978) gene diversity; I = Shannon Information Index.

The gel was carefully taken out of the tray when the staining procedure was finished and set on the UVP BioDoc-ItTM imaging equipment, a high-performance ultraviolet light box, to visualize the DNA bands and capture gel images. The whole process was replicated for three times.

Each band was regarded as representing the phenotype at a single allelic locus because of the dominant nature of ISSR markers, following the assumption of Elo et al. (1997). Amplified product sizes were determined by comparing the migration distances of amplified fragments with those of molecular weight markers of known sizes. Each distinct band (ISSR marker) was given a unique identifier based on its gel position and scored visually as either present (1) or absent (0) for each individual and primer. Binary data from individual primers were consolidated into a single data matrix. This matrix was analyzed using POPGENE software (Version 3.5, Yeh et al. 1999) to evaluate genetic parameters including the proportion of polymorphic loci, Nei's gene diversity (Nei 1978), genetic distance and the Shannon information index. To explore genetic relationships among genotypes, the unweighted pair group method with arithmetic mean (UPGMA) was employed to generate a dendrogram using NTSYS-PC software (Version 2.11, Rohlf 2000). Pairwise genetic similarity indices (S) between individuals were calculated using the formula $S = 2Nxy / (Nx + Ny)$, where Nxy represents the number of shared bands and Nx and Ny denote the total number of bands in individuals x and y, respectively (Lynch 1990). The average similarity coefficient (Sij) was then determined across all genotype pairs (Lynch 1991).

Results and Discussion

Eleven ISSR primers were used to evaluate the genetic composition of 18 mango genotypes. A range of banding patterns was obtained by these tested primers (Fig. 1). Across 18 mango genotypes, the 11 primers produced a total of 67 bands. 76.11% polymorphism was found in the 67 bands, of which 51 were found to be polymorphic (Table 2). With a polymorphism rate of 100%, primer UBC825 generated the highest number of bands (9), while primer UBC855 generated the most polymorphic bands (8) (Table 2). On the other hand, primer UBC886 had the fewest polymorphic bands (3), having 50% of the total. The findings of the assessments of Nei's gene diversity (H) and Shannon Information Index (I) are likewise summarized in Table 2. The calculated values for overall genetic diversity across all genotypes and ISSR markers were 0.636 for the Shannon Information Index and 0.446 for Nei's gene diversity (Nei's 1978), indicating genetic variation within the population. Table 3 provided the predicted values for Nei's (1978) genetic distances and band sharing based inter-genotype similarity indices. Between BARI Aam-6 and BARI Aam-11, the Nei's (1978) genetic distance was the highest (0.836), while between BARI Aam-1 and BARI Aam-18, it was the lowest (0.008). Inter-genotypic similarity indices revealed a high band-sharing value of 0.991 between BARI Aam-17 and BARI Aam-18, whereas the lowest value of 0.443 was found between BARI Aam-6 and BARI Aam-11.

A dendrogram was created using Nei's (1978) genetic distance, which enabled the separation of 18 distinct mango genotypes into two primary clusters (Fig. 2). BARI Aam-7, BARI Aam-11, and BARI Aam-12 were found in cluster I and others 15 genotypes in Cluster II.

This study provides a thorough molecular assessment of 18 Bangladeshi mango (*Mangifera indica* L.) genotypes using ISSR markers, revealing substantial genetic diversity that has important implications for conservation and breeding programs. The analysis showed a polymorphism rate of 76.11% across 11 primers, underscoring the reliability of ISSR markers for distinguishing mango genotypes. These results are consistent with previous research conducted in other mango-producing regions (Pandit et al. 2007, Patil et al. 2019). The diversity observed here exceeds the polymorphism reported by Uddin et al. (2014) in Indian mango varieties, suggesting that Bangladeshi germplasm may contain richer genetic variation, possibly due to distinct environmental conditions or less intensive breeding practices.

The genetic diversity metrics (Nei's H = 0.446; Shannon's I = 0.636) revealed considerable variation among the mango genotypes analyzed. These values closely mirror those reported for Indian mango germplasm by Ravishankar et al. (2019), yet fall short of the higher diversity levels observed in wild *Mangifera* species (H > 0.5) as noted by Tewodros et al. in 2019. This reduction likely reflects the narrowing of genetic variation due to domestication and the widespread use of clonal propagation in elite cultivars. Notably, primer UBC855 achieved 100% polymorphism, outperforming

markers used in comparable studies, such as ISSR-14 in Patil et al. (2019), which showed 89% polymorphism, highlighting its strong potential for future applications in mango genetic diversity research.

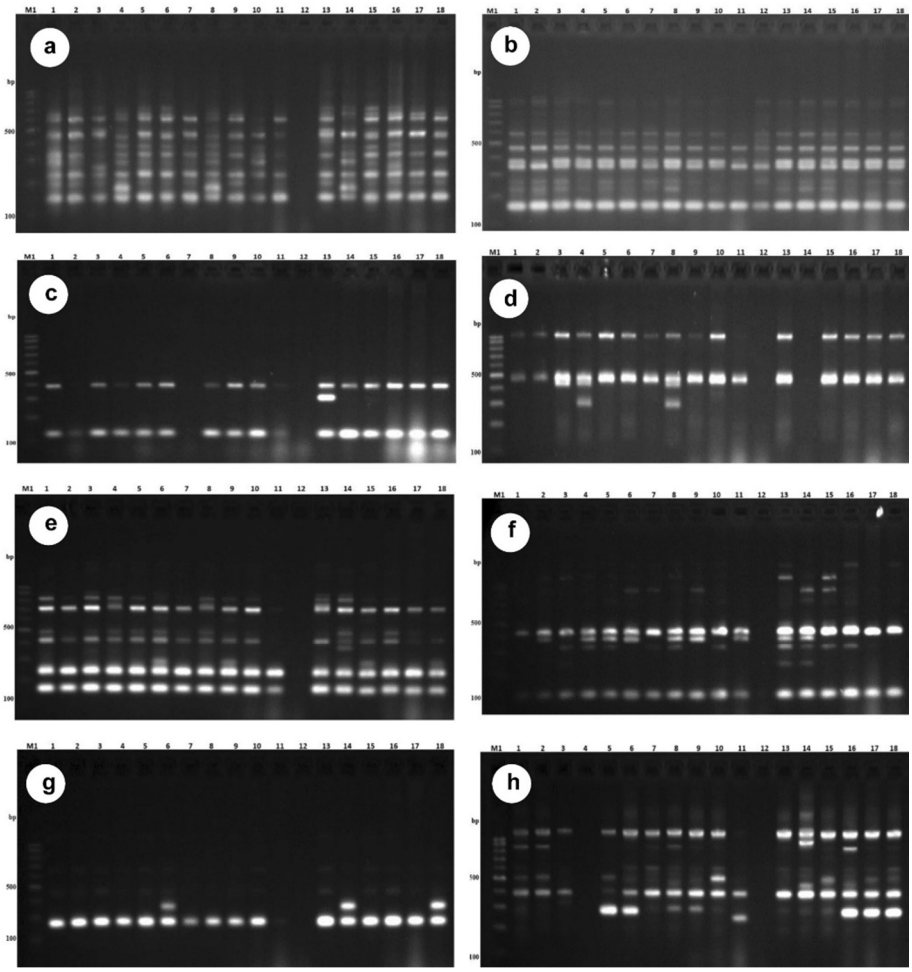

Fig. 1. ISSR profiles of 18 mango genotypes using primer UBC880 (a), UBC825 (b), UBC841 (c), UBC853 (d), UBC810 (e), UBC811 (f), UBC813 (g), and UBC855 (h). Lane 1: BARI Aam-1, Lane 2: BARI Aam-2, Lane 3: BARI Aam-3, Lane 4: BARI Aam-4, Lane 5: BARI Aam-5, Lane 6: BARI Aam-6, Lane 7: BARI Aam-7, Lane 8: BARI Aam-8, Lane 9: BARI Aam-9, Lane 10: BARI Aam-10, Lane 11: BARI Aam-11, Lane 12: BARI Aam-12, Lane 13: BARI Aam-13, Lane 14- BARI Aam-14, Lane 15: BARI Aam-15, Lane 16: BARI Aam-16, Lane 17: BARI Aam-17, Lane 18: BARI Aam-18, M1: 100 bp DNA ladder.

The clustering analysis distinguished two distinct genetic groups, with Cluster I comprising only three genotypes; BARI Aam-7, BARI Aam-11, and BARI Aam-12. This grouping is particularly notable, as these genotypes share late-ripening traits and distinctive flavor profiles, including the pronounced acidity of BARI Aam-11. Similar

Table 3. Summary of band sharing based inter-cultivar similarity indices (above diagonal) and Nei's (1978) genetic distance (below diagonal) values between 18 mango genotypes.

Genotypes	BARI Aam-1	BARI Aam-2	BARI Aam-3	BARI Aam-4	BARI Aam-5	BARI Aam-6	BARI Aam-7	BARI Aam-8	BARI Aam-9	BARI Aam-10	BARI Aam-11	BARI Aam-12	BARI Aam-13	BARI Aam-14	BARI Aam-15	BARI Aam-16	BARI Aam-17	BARI Aam-18
BARI Aam-1	****	0.944	0.949	0.914	0.892	0.753	0.8864	0.9097	0.932	0.696	0.636	0.766	0.821	0.838	0.974	0.974	0.901	0.913
BARI Aam-2	0.057	****	0.927	0.844	0.944	0.790	0.766	0.858	0.822	0.859	0.700	0.694	0.671	0.811	0.863	0.912	0.836	0.850
BARI Aam-3	0.051	0.075	****	0.974	0.874	0.697	0.919	0.861	0.870	0.633	0.588	o.788	0.858	0.828	0.914	0.818	0.8523	0.852
BARI Aam-4	0.089	0.168	0.116	****	0.863	0.841	0.753	0.841	0.907	0.914	0.739	0.618	0.716	0.744	0.720	0.932	0.896	0.894
BARI Aam-5	0.0367	0.057	0.025	0.146	****	0.874	0.704	0.914	0.861	0.861	0.620	0.574	0.775	0.870	0.861	0.948	0.832	0.861
BARI Aam-6	0.114	0.235	0.134	0.172	0.134	****	0.624	0.769	0.837	0.838	0.433	0.565	0.741	0.745	0.674	0.885	0.800	0.853
BARI Aam-7	0.283	0.265	0.360	0.282	0.350	0.470	****	0.800	0.863	0.876	0.975	0.924	0.635	0.833	0.829	0.764	0.880	0.871
BARI Aam-8	0.120	0.152	0.083	0.173	0.089	0.262	0.222	****	0.918	0.909	0.707	0.638	0.718	0.921	0.870	0.883	0.870	0.888
BARI Aam-9	0.094	0.195	0.148	0.097	0.149	0.177	0.146	0.084	****	0.978	0.818	0.691	0.709	0.893	0.817	0.930	0.965	0.966
BARI Aam-10	0.070	0.151	0.139	0.089	0.148	0.176	0.132	0.094	0.021	****	0.837	0.735	0.710	0.857	0.844	0.932	0.980	0.981
BARI Aam-11	0.361	0.356	0.457	0.302	0.477	0.836	0.024	0.346	0.200	0.177	****	0.956	0.624	0.754	0.748	0.713	0.859	0.839
BARI Aam-12	0.451	0.364	0.529	0.480	0.554	0.569	0.078	0.447	0.369	0.307	0.044	****	0.610	0.687	0.735	0.619	0.753	0.731
BARI Aam-13	0.266	0.399	0.237	0.332	0.254	0.299	0.453	0.331	0.342	0.342	0.470	0.494	****	0.666	0.641	0.769	0.690	0.711
BARI Aam-14	0.196	0.208	0.152	0.295	0.138	0.293	0.182	0.082	0.113	0.154	0.282	0.374	0.405	****	0.923	0.820	0.837	0.862
BARI Aam-15	0.176	0.146	0.188	0.327	0.149	0.393	0.187	0.138	0.201	0.169	0.289	0.307	0.444	0.079	****	0.812	0.824	0.830
BARI Aam-16	0.026	0.092	0.089	0.070	0.053	0.121	0.269	0.123	0.071	0.069	0.337	0.478	0.262	0.197	0.207	****	0.936	0.940
BARI Aam-17	0.103	0.178	0.200	0.109	0.183	0.222	0.127	0.139	0.034	0.019	0.151	0.283	0.370	0.177	0.193	0.065	****	0.991
BARI Aam-18	0.008	0.162	0.159	0.111	0.149	0.158	0.138	0.118	0.034	0.018	0.174	0.312	0.340	0.147	0.185	0.060	0.090	****

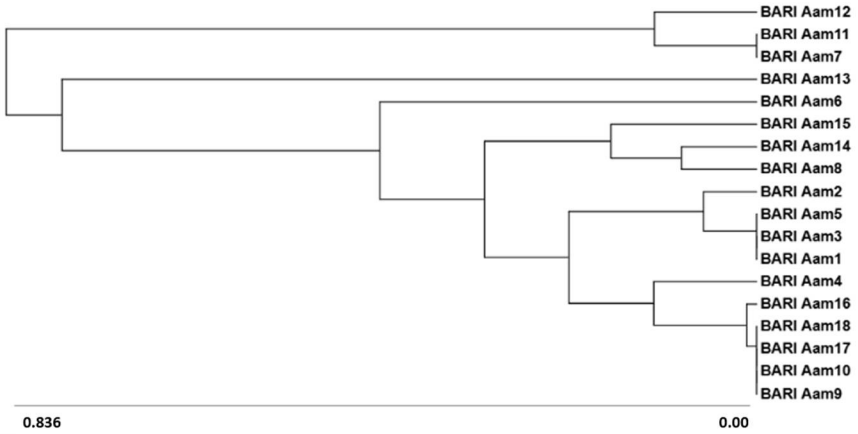

Fig. 2. UPGMA dendrogram based on Nei's (1978) genetic distance summarizing the data on differentiation among eighteen mango genotypes according to ISSR analysis.

patterns were reported by Pandit et al. in 2007, where late-maturing Indian mangovarieties formed genetically distinct clusters. Additionally, the minimal genetic distance between BARI Aam-1 and BARI Aam-18 (0.008) suggests a close genetic relationship, possibly due to shared ancestry or recent selection, which echoes the findings of Molla et al. (2019) in commercial cultivars with similar genetic backgrounds.

The highest genetic distance observed in this study was 0.836 between BARI Aam-6 and BARI Aam-11. This pronounced divergence likely reflects their distinct phenotypic characteristics: BARI Aam-6 is a fiberless, late-season cultivar, whereas BARI Aam-11 (Baromasi) features dense, acidic pulp. Such genetic contrast offers promising potential for heterosis breeding, as illustrated by Garcia et al. (2025) through the use of genetically distant parental lines.

In comparison with other molecular marker systems, the ISSR markers employed in this study demonstrated comparable discriminatory capacity to SSRs (Patil et al. 2019), though they fall short of the finer resolution provided by SNP-based approaches (Tewodros et al. 2019). Nonetheless, the affordability and ease of use of ISSRs make them especially advantageous for initial germplasm screening in settings with limited resources.

The results of this study carry several practical implications for mango breeding and conservation. Firstly, the highly polymorphic markers identified, particularly UBC825 and UBC855, offer strong potential for marker-assisted selection targeting traits such as fiberlessness (noted in BARI Aam-1 through -6) and high yield (as seen in BARI Aam-8). Secondly, the distinct late-maturing genotypes grouped in Cluster I present valuable genetic resources for breeding programs focused on extending the harvest period. Thirdly, conservation strategies should prioritize genetically divergent genotypes like BARI Aam-6 and BARI Aam-11 to preserve the breadth of genetic diversity within the germplasm.

Demonstrating ISSR markers' efficacy in diversity assessment, the molecular data generated in this study establish a foundational genetic framework that can inform strategic selection of parental lines for hybridization programs and guide prioritization of genotypes for ex situ and in situ conservation. Future research ought to explore trait-marker associations and broaden germplasm sampling to include wild relatives and international cultivars. This study provides an ISSR-based molecular analysis of 18 Bangladeshi mango genotypes, generating substantial knowledge on genetic diversity with potential implications for conservation and breeding programs of mango.

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