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Research Article

Molecular Authentication of Mango (*Mangifera indica* L.) Varieties in Asia Through SSR Markers

[1]Nguyen Thi My Duyen, [1]Cao Thi Luyen, [1]Trinh Thi Thu Hong, [1]Diep Nhut Thanh Hang and [2]Do Tan Khang

[1]An Giang University, Vietnam National University-HCMC, Ho Chi Minh City, Vietnam
[2]Institute of Food and Biotechnology, Can Tho University, Vietnam

Abstract

Background and Objective: Mango (*Mangifera indica* L.) is a major tropical fruit crop in Vietnam, yet accurate identification of varieties remains a challenge for breeding, germplasm conservation and sustainable agriculture. This study aimed to evaluate the genetic diversity of popular Vietnamese mango cultivars (Cat Hoa Loc, Cat Chu, Thom and Thanh Ca) and distinguish them from imported varieties (Thailand, Taiwan, Australia and Keo from Cambodia) using SSR molecular markers. **Materials and Methods:** Twenty SSR (Simple Sequence Repeat) markers were applied for genotyping, with PCR amplification and electrophoresis conducted to assess polymorphisms. Genetic data were analyzed using NTSYS-pc 2.1 software to determine diversity and relationships among cultivars. The polymorphic information content (PIC) was calculated for each marker. **Results:** The primer MiKVR_e295 showed the highest polymorphic discrimination (PIC = 0.61), followed by MiIIHR_n507 (0.51), MiKVR_a028 (0.50), MiKVR_d864 (0.50) and MiBNG_c268 (0.44). SSR markers successfully differentiated Vietnamese cultivars from foreign accessions, with Cat Chu notably distinct, demonstrating their utility for variety authentication and genetic resource management. **Conclusion:** The SSR markers provide an effective tool for the authentication and genetic characterization of mango varieties in Vietnam. These findings support germplasm conservation, breeding programs and future molecular studies on mango genetic resources.

Key words: Authentication, *Mangifera indica*, SSR marker, genetic characterization

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Corresponding Author: Do Tan Khang, Institute of Food and Biotechnology, Can Tho University, Vietnam

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mango (*Mangifera indica* L.) ranks among the five most extensively cultivated fruit crops in tropical zones, with annual global yields surpassing 58 million tons. In Vietnam, mango plays a vital role in fruit production, particularly in the Mekong Delta Region. High-value local varieties such as Cat Hoa Loc, Cat Chu and Thom Vinh Hoa not only play a crucial role in domestic markets but also contribute to Vietnam's fruit export sector. However, morphological traits alone are insufficient for accurate variety identification due to environmental influence and phenotypic similarity, leading to frequent mislabeling in commercial production[1].

With the increasing cultivation of foreign mango cultivars in Vietnam, including varieties from Taiwan, Australia, Thailand and Cambodia, there is a growing need for precise and reliable tools for variety authentication. DNA-based markers, particularly SSRs (microsatellites), have proven effective for cultivar identification due to their co-dominant inheritance, high polymorphism, reproducibility and ease of use[2,3].

Several international studies have demonstrated the utility of SSR markers in assessing mango genetic diversity, including in India[4], Thailand[5] and the Philippines[6]. However, in Vietnam, the application of SSR markers for mango variety identification, especially comparative analysis between native and introduced varieties, remains limited.

This study aims to analyze the genetic diversity of selected Vietnamese and imported mango varieties using SSR markers and evaluate the utility of SSR markers for mango variety identification in support of breeding, germplasm conservation and varietal management in Vietnam.

MATERIALS AND METHODS

Sample collection: Eight mango cultivars were collected from different sites in An Giang province as Cat Hoa Loc, Cat Chu, Thom Vinh Hoa, Thanh Ca and imported varieties from Thailand, Taiwan, Australia and Keo (Cambodia). Three leaf samples per cultivar were collected. The study was conducted from October, 2024 to June, 2025.

DNA extraction: Genomic DNA was extracted using the modified CTAB method[7]. DNA quality and quantity were assessed by 1% agarose gel electrophoresis and NanoDrop spectrophotometry.

PCR amplification and electrophoresis: Twenty SSR primer pairs were selected based on previous studies[2,3] (Table 1). The PCR was performed in 25 µL reactions including 12 µL BiH_2O, 10 µL PCR master mix, 1 µL primer (10 pmoL), 2 µL template DNA (20 ng/µL). The PCR reaction was performed with one step of 94°C for 4 min; then 35 cycles of 94°C in 1 min, 50°C in 45 sec, 72°C in 1 min; the final step of 72°C in 7 min. The PCR products were separated using 3% agarose gel electrophoresis with Safeview staining.

Data analysis: Gel profiles were scored as binary data (presence/absence). Data matrices were analyzed using NTSYS-pc 2.1 software to compute genetic similarity and construct a dendrogram using the UPGMA method[2].

RESULTS

Results of SSR marker analysis: The PCR electrophoresis results with 20 SSR primers showed that 100% of the tested samples produced high-quality amplification products with clear, bright and intact DNA bands. Most observed DNA bands ranged from 200-300 bp in size (Fig. 1), consistent with the design characteristics of SSR primers. In particular, the study identified the primer pair MiIIHR_n507 that amplified specific DNA bands for the Taiwan variety, two bands (200 and 300 bp) and Vietnamese varieties with one band about 260 bp, respectively, indicating their potential use as molecular markers for cultivar identification.

These findings are significant for distinguishing Vietnamese native mango varieties from introduced ones, minimizing misidentification in production and trade. Furthermore, the discovery of specific SSR markers contributes to breeding new cultivars, conserving valuable genetic resources and establishing a standardized identification system for mango cultivar management in Vietnam. This provides a scientific basis for applying molecular biotechnology to improve the yield, quality and product diversity of mango in the context of international integration and competition. This demonstrates that SSR primers are not only effective in stable DNA amplification but also valuable for genetic diversity analysis and cultivar identification. The identification of specific and highly polymorphic primers provides an important foundation for developing a specialized SSR marker set to support germplasm conservation, breeding and mango cultivar management in Vietnam.

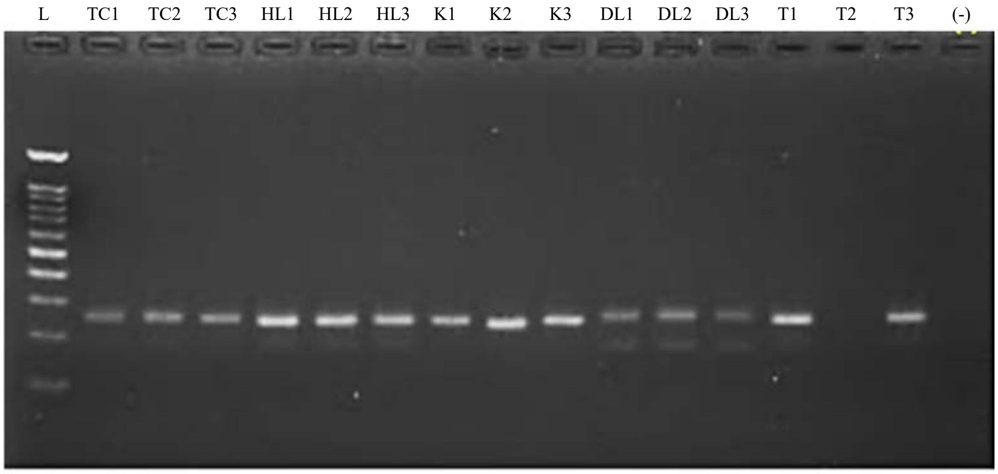

Fig. 1: Agarose gel image of SSR markers MiIIHR_n507

L: Ladder 100 bp (Bioline), TC1, TC2, TC3: Thanh Ca, HL1, HL2, HL3: Cat Hoa Loc, K1, K2, K3: Keo, DL1, DL2, DL3: Taiwan, T1, T2, T3: Thom and (-) Negative control

Table 1: List of SSR primers used in this study

No.	Primer	Sequences	Motif	Size of PCR products (bp)
1	MiKVR_a009	F: CATGGATGCTTTAATGGTCA R: TCATTGACAATCTCGGTTTAAGAA	$(GT)_8$	162-208
2	MiKVR_ao28	F: AAAACTATATGCACCAATTTTGAA R: CATTATGAGTTCTTATTTTGGATGGA	(TA)	179-234
3	MiKVR_ao41	F: TCTAGAATTTTTGAAAAGATAATGTGA R: TTTCACATACAAGTTAGTTGGGG	$(TA)_7$	161-212
4	MiKVR_a187	F: TTTTTCTAAAATGGTAATGTGTCAA R: TGGGTTACAGTTTGAGTGATTTTC	$(TA)_8$	214-261
5	MiKVR_a230	F: TGTTGCATATGTAGTGCAATAGTTT R: AAACATCAAAATATCAAATTGGCA	$(AT)_7$	249-298
6	MiKVR_a257	F: CAACAAATCTACCCCTTTGGTT R: TGAGTGGCAAAAATCCATGA	$(TA)_9$	233-289
7	MiKVR_a394	F: AAACTTTAATGGATTTTATCGTGTAAT R: AAATACCCCACCCATTTTGC	$(TG)_7$	222-273
8	MiIIHR_n507	F: AAGGTTGTTGCGTGGTTAGG R: TCATGAAAATCACTGGGTGG	$(AT)_6$	223-273
9	MiBNG_c268	F: TATCGCCTACCTTTGAGGGA R: TTTTGTTTGTGGGTGCACAT	$(TC)_{12}$	160-220
10	MiBNG_a619	F: GCAAGGAAGCTGATTCTCCA R: TACCACTTTGTCCAAAGCCC	$(GA)_8$	142-186
11	MiKVR_b283	F: TCTTTTACGAGGATTTTTCTTTTT R: CGAATAAGGGTCAATCCGAA	$(TTC)_4$	134-204
12	MiKVR_d864	F: AAATTGGTGTTTTTACCTTGGG R: TGGAATTTGTTCCTCCTTCC	$(ATC)_4$	256-301
13	MiKVR_e295	F: AGGTTGCATGAGTTGATCCTC R: GAAAATTTGATGCCAGGTCG	$(TCT)_4$	299-301
14	MiKVR_f321	F: AACCACAATTTGCTTTTTGC R: TTCTACTTTCTTTTATCCTTTTGTTTC	$(ATA)_4$	285-294
15	MiKVR_f905	F: AATTGGGGTTCAAGTGACCT R: TGGTTTGGGTGAAACAAAGT	$(ATT)_6$	244-306
16	MiMRD_k987	F: CCATTACCGATGGATACGCT R: GATGGCACCAACAAGAAGGT	$(AATA)_4$	220-285
17	MiMRD_l202	F: TCCTGCAAATTCTTCATTTGG R: TTGGAGATCGTGGTAATTTCG	$(AATA)_4$	243-290
18	MiMRD_l656	F: GAGAAATGGGTTTCCAGCAA R: CACGCGAAGTAAACCAAATG0.	$(ATTT)_5$	257-308
19	MiMRD_l744	F: AAATCCCGTACCTTCATCCC R: TGCCAGAACTGCTCTCTTCA	$(AAAT)_4$	261-296
20	MiMRD_l896	F: AATGCGGAGTATTCCAGGTG R: CCTTCTCTCGTTTGATTGCAT	$(TTAA)_4$	206-219

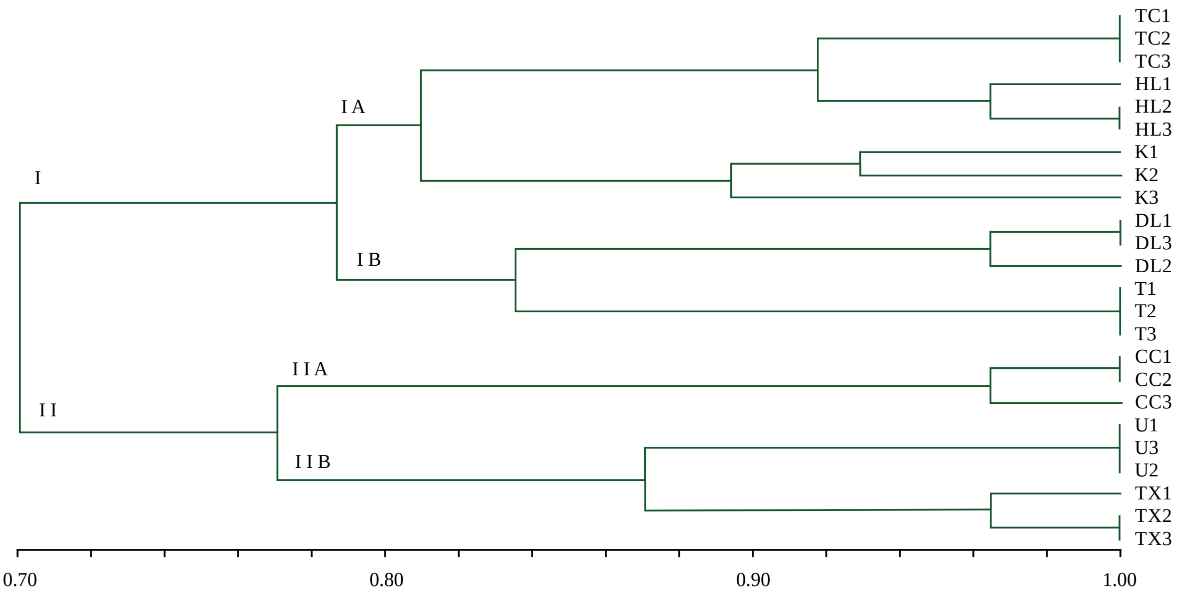

Fig. 2: Clustal dendrogram of mango cultivars based on SSR markers

TC1, TC2, TC3: Thanh Ca, HL1, HL2, HL3: Cat Hoa Loc, K1, K2, K3: Keo, DL1, DL2, DL3: Taiwan, U1, U2, U3: Australia, T1, T2, T3: Thom. TX1, TX2, TX3: Thailand and CC1, CC2, CC3: Cat Chu

Table 2: Polymorphism analysis of primers showing total bands, number and percentage of polymorphic bands and polymorphism information content (PIC) values

No.	Primer	Size	Total bands	Polymorphic bands	Polymorphic percentage	PIC
1	MiKVR_a009	200	1	0	0	0
2	MiKVR_a028	200-250	2	2	100	0.5
3	MiKVR_a041	200	1	1	100	0
4	MiKVR_a187	250	1	0	0	0
5	MiKVR_a230	200-300	2	1	50	0.2
6	MiKVR_a257	250	1	0	0	0
7	MiKVR_a394	250	1	0	0	0
8	MiIIHR_n507	200-300	3	3	100	0.51
9	MiBNG_c268	150-200	2	1	50	0.44
10	MiKVR_b283	150	1	1	100	0
11	MiKVR_d864	250-300	2	2	100	0.5
12	MiKVR_e295	250-550	3	2	67	0.61
13	MiKVR_f321	250	1	1	100	0
14	MiKVR_f905	700	1	1	100	0
15	MiMRD_k987	250	1	0	0	0
16	MiMRD_l202	250	1	0	0	0
17	MiMRD_1656	250	1	0	0	0
18	MiMRD_1744	250	1	0	0	0
19	MiMRD_1896	150	1	0	0	0
20	MiBNG_a619	150	1	0	0	0

Pimers MiKVR_e295, MiIIHR_n507, MiKVR_a028, MiKVR_d864 and MiBNG_c268 are promising candidates and should be prioritized in future studies

Results of PIC polymorphism analysis: The analysis showed that among the 20 primers tested, only six exhibited polymorphism (PIC>0), while the majority had PIC values of 0, indicating no polymorphic discrimination. The PIC values ranged from 0 to 0.61 (Table 2). Among these, the primer MiKVR_e295 (PIC = 0.61) exhibited the highest polymorphic discrimination, followed by MiIIHR_n507 (0.51), MiKVR_a028 (0.50), MiKVR_d864 (0.50) and MiBNG_c268 (0.44). These primers produced 2-3 bands each, demonstrating a high degree of polymorphism, reflecting strong discriminatory power among samples and are suitable for genetic diversity assessment. Conversely, primers with low PIC values (<0.25) or 0 are of limited analytical value and should be considered for replacement.

Results of clusterin: The dendrogram analysis revealed that Vietnamese mango cultivars (Thanh Ca, Cat Hoa Loc, Thom and Cat Chu) were distributed across multiple clusters, indicating high genetic diversity. Among them, Cat Hoa Loc and Thanh Ca were closely related to the Keo variety (originating from Cambodia), while the Thom variety clustered with the Taiwan mango. Notably, the Cat Chu variety formed a distinct branch, suggesting unique genetic characteristics with minimal influence from other germplasm sources (Fig. 2).

In the group of introduced mangoes, Keo and Taiwan cultivars showed relatively close relationships with certain Vietnamese cultivars, reflecting shared genetic backgrounds in Southeast Asia (Group I). By contrast, Australian and Thailand mangoes grouped into a separate cluster, clearly distinct from native cultivars Cat Chu, highlighting significant genetic divergence due to modern breeding and selection practices (Group II). In the sub-group IA, Thanh Ca and Cat Hoa Loc cultivars (local cultivars) were separated from Keo mango (originally from Cambodia). In the sub-group IB, the Thom cultivar was also distinguished from the Taiwan variety. Overall, Vietnamese mangoes exhibited not only inter-varietal diversity but also genetic similarities with some regional introductions, while retaining unique genetic resources, exemplified by the Cat Chu variety.

DISCUSSION

This study demonstrates the successful use of SSR markers for the identification and differentiation of mango varieties cultivated in Vietnam. The selected SSR primers showed high polymorphism, consistent with earlier findings in other countries[4,5] and provided sufficient resolution to distinguish between local and foreign cultivars.

The dendrogram generated from UPGMA clustering clearly separated the Vietnamese cultivars into one group (Cat Hoa Loc, Cat Chu, Thom and Thanh Ca) and grouped the foreign accessions (Taiwan, Thailand, Australia and Keo) into another. This clustering pattern reflects both genetic divergence and distinct origin, thereby supporting the effectiveness of SSR markers for mango cultivar authentication.

The observed high genetic diversity among Vietnamese mango varieties indicates a rich and underutilized germplasm base. This aligns with findings by Lin *et al.*[8], who reported considerable intra-varietal diversity within Vietnamese mango accessions. Such diversity is a valuable resource for mango breeding programs aiming to improve fruit quality, disease resistance and climate adaptability.

Beyond breeding, accurate variety identification is crucial in controlling varietal purity in nurseries and commercial orchards, where mislabeling remains a persistent issue. The SSR profiles established in this study can serve as a “genetic passport” for each variety, facilitating intellectual property protection and traceability in the mango value chain[9].

From a conservation standpoint, the use of molecular markers aids in the construction of a scientifically sound germplasm repository. Properly documented accessions allow for targeted conservation strategies, particularly for landraces that are at risk of being replaced by commercial hybrids.

Results reinforce the global trend of integrating molecular tools into horticultural crop management. Studies from diverse agroecological zones have highlighted the efficiency of SSR markers in genetic fingerprinting of mango cultivars, including those in Bangladesh[10], Indonesia[11] and Brazil[12]. The data obtained in this study add to this body of knowledge by providing specific insights into the Vietnamese mango germplasm and its relation to imported cultivars.

It is worth noting that while SSRs provide high discriminatory power, combining them with other marker systems such as SNPs or using next-generation sequencing could further enhance resolution, especially in closely related cultivars[13]. Future studies could also integrate morphological and biochemical data to develop a multi-trait identification system for mango germplasm.

This study confirms the efficiency of SSR markers in distinguishing mango cultivars grown in Vietnam, in line with earlier studies in Asia[4-6]. The UPGMA dendrogram clearly separated Vietnamese cultivars (Cat Hoa Loc, Cat Chu, Thom, Thanh Ca) from imported ones (Thailand, Taiwan, Australia, Keo), indicating genetic divergence due to geographical origin and selective breeding.

Genetic diversity is a critical component for mango improvement programs. Our results showed high allelic variation among native cultivars, suggesting that local germplasm remains an untapped reservoir of useful traits[8]. The use of SSR markers allows precise genetic fingerprinting for each variety, serving as a foundation for legal protection and plant variety registration[9].

Moreover, SSR-based diversity assessment aids germplasm conservation by enabling the construction of core collections and guiding *ex-situ* conservation strategies. Combining molecular and morphological data is a global trend in horticultural crop management[10,13]. However, as molecular techniques evolve, incorporating high-resolution markers such as SNPs or genome-wide sequencing may further enhance cultivar discrimination.

CONCLUSION

The SSRs remain a reliable and cost-effective option for mango genetic analysis in developing countries, especially when integrated into national variety protection and breeding schemes. The SSR molecular markers effectively discriminated among mango cultivars in An Giang, Vietnam, highlighting clear genetic differences between local and imported varieties. The study provides a reliable approach for accurate variety authentication and supports the conservation of mango genetic resources. These findings can guide breeding programs and help maintain cultivar purity. Moreover, the results promote sustainable mango production by enabling informed management of genetic diversity.

SIGNIFICANCE STATEMENT

This study discovered the genetic variability and unique molecular signatures among Vietnamese and imported mango (*Mangifera indica* L.) cultivars that can be beneficial for accurate variety authentication, germplasm conservation and breeding program optimization. By applying SSR molecular markers, the research highlighted distinct genetic differences, particularly among popular local cultivars such as Cat Chu and Cat Hoa Loc, which are critical for maintaining cultivar purity and enhancing sustainable mango production. The findings provide a scientific foundation for managing mango genetic resources effectively and improving cultivar selection strategies. This study will help researchers uncover the critical areas of mango genetic diversity that many researchers were not able to explore. Thus, a new theory on molecular-assisted mango breeding may be arrived at.

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