Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2023.302.308

Research Article
Correlation Between Molecular Markers and Sweetness and Peel Thickness of Mango (*Mangifera indica* L.)

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Abstract

Background and Objective: Mango is one of the most important fruits in the Mekong Delta. This study was conducted to investigate the SSR marker which showed a high linkage to peel thickness and sweetness of mango cultivars. **Materials and Methods:** Ten SSR molecular markers including SSR-18, SSR-20, SSR-23, SSR-28, SSR-41, SSR-51, SSR-52, SSR-59, SSR-62 and SSR-68 on 8 mango cultivars: Hoa Loc, Cat Chu, Aroma, Thanh Ca, Australia, Taiwan, Green Thai and Keo were collected and analyzed. **Results:** The procedure for extracting mango DNA by CTAB achieves good DNA quality. The PCR analysis on 10 standard markers of SSR showed polymorphisms. The PIC coefficients of the 10 molecular markers ranged from 0.56 (SSR-52) to 0.74 (SSR-23, SSR-41) for mango. The average PIC coefficient of the 10 molecular markers studied is 0.673. **Conclusion:** The study also showed that 4 standard molecular markers of SSR associated with genes regulating peel thickness and sweetness on 8 mango varieties were: SSR-20, SSR-41 (peel thickness), SSR-51 and SSR-68 (sweetness). Such data contribute to the molecular basis for mango breeding programs.

Key words: Mango, *Mangifera indica*, molecular markers, peel thickness, SSR marker, SSR-20, SSR-51, sweetness

Citation: Duyen, N.T.M., H.T.P. Nhi, T.G. Huy, N.T. Pha and D.T. Khang, 2023. Correlation between molecular markers and sweetness and peel thickness of mango (*Mangifera indica* L.). Asian J. Plant Sci., 22: 302-308.

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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.) is mainly cultivated in the Southern Region of Vietnam. The mango farming area increased considerably. The Mekong Delta is the center of fruit production in Vietnam, which accounts for the largest yields of mango. Owing to its appropriate natural conditions and cultivated technology effectively, the Mekong Delta became the highest mango-producing area, reaching nearly 5000 ha of the mango cultivated area (more than 25% of the total area of the country) and half of a million tons of mango yield (over 60% of the total area of the country) in 2019. This major tropical fruit contributes an appreciable source of income for farmers and gardeners. The largest mango granaries in Southern Vietnam are in the Mekong Delta and Dong Nai Province[1]. This fruit is considered the "king of fruits" due to its distinctive color, flavor and taste as well as ideal yield and diverse uses.

Within 6 to 7 days after harvest, mangoes ripe under the native climate and the overripe and spoiled stage is within 15 days[2]. The important determinants of postharvest mango quality are similar to other fruit plants including microbial contamination and excessive softening state[3]. Based on the variety of the mango, the peel color when ripe turns pale green combined with black spots[4,5]. The external appearance of the fruit is assessed through phenotypic properties, while the epicarp influences these characteristics and relates to resistance to pathogens and drought, the effectiveness of post-harvest treatments and shelf life[6]. Based on the physical removal, the peel consists of the epidermis, collenchyma and parenchyma cells evenly[7]. The cuticle is a hydrophobic layer synthesized by epidermal cells, consisting of two main components, cutin and wax[8]. The cuticles are between the aerial parts and the environmental conditions and act as an external barrier reducing water loss as well as gas change, preventing the accumulation of water and dust, aiding in the control of temperature changes and participating in plant-insect interactions[9]. Hence, the cuticle plays an important role in constituting commercial characteristics and influencing the postharvest shelf life of fruit. It is necessary to investigate more about the internal processes related to the changes in the cuticle, which may have a positive impact on strategies to improve fruit quality.

In addition, mangoes have many excellent biological compounds such as β-carotene, dietary fiber, phenolics and Vitamin C providing positive effects on lipid profiles and blood sugar stability. Mangoes have significant potential to develop into valuable products beyond being eaten raw, becoming a major competitor to cane sugar.

Since the several limitations of the interpretation of genetic diversity by way of morphological characters comprise complex inheritance patterns, the limit on number and vulnerability to the states of the environment, the using of genetic markers for molecular studies is examined on identifying plant, conventional phenotypic diversity and appreciation of their genetic relatives. The advantages of simple sequence repeat are highly polymorphic, codominant and have multiple alleles compared with the other marker types. The SSR markers have been applied in many aspects including storing cultivars and determining related genes in plant breeding[10,11]. The SSR markers have many uniquely important applications in mango such as the identification of varieties (or domestication), determination of genetic variability, movement of germplasm and conservation of mango germplasm[12]. From the several mango germplasms, more than 100 SSR markers have been researched and developed[10,12]. Some practical applications of SSR markers in studies of the regional genetic diversity of mangoes include Florida mango varieties[13], Myanmar mango landraces[14] and Taiwanese mango varieties[15]. Therefore, this study aims to investigate the potential SSR markers which correlate with the peel thickness and sweetness of varieties of mango in the Mekong Delta.

MATERIALS AND METHODS

The study was conducted from April, 2022 to December, 2022 in An Giang, Vietnam.

Plant collection: Fresh leaves were collected from eight mango cultivars including Hoa Loc cultivar, Cat Chu cultivar, Thanh Ca cultivar, Vinh Hoa aroma cultivar, Taiwan cultivar, Green Thai cultivar, Keo cultivar and Australia cultivar. Such samples originated from elite mother trees in an Giang Province, which were verified by the Department of Agriculture and Natural Resources, An Giang University. For each mango, three ripen fruits were collected to measure the peel thickness and sweetness.

DNA isolation: Total DNA was extracted following the protocol from Rogers and Bendich[16] with appropriate modifications. The DNA concentration and purification were checked by a Nanodrop spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA). Additionally, DNA integrity was observed by 1% agarose gel electrophoresis.

Table 1: Nucleotide sequence of ten ISSR primers in this study

Primer	Sequence (5'-3')	Ta (°C)	Amplicon size
SSR-18	F: CGTCATCCTTTACAGCGAACT	56	100-115
	R: CATCTTTGATCATCCGAAAC		
SSR-20	F: CGCTCTGTGAGAATCAAATGGT	58	295-310
	R: GGACTCTTATTAGCCAATGGGATG		
SSR-23	F: AAACAAAGAATGGAGCA	50	240-270
	R: TGGACTGAATGTGGATAG		
SSR-28	F: GACCCAACAAATCCAA	52	160
	R: ACTGTGCAAACCAAAAG		
SSR-41	F: ATCCCCAGTAGCTTTGT	53	210-244
	R: TGAGAGTTGGCAGTGTT		
SSR-51	F: ATGGAGACTAGAATGTACAGAG	52	287
	R: ATTAAATCTCGTCCACAAGT		
SSR-52	F: AAAAACCTTACATAAGTGAATC	52	207
	R: CAGTTAACCTGTTACCTTTTT		
SSR-59	F: TTCTTTAGACTAAGAGCACATT	56	191
	R: AGTTACAGATCTTCTCCAATT		
SSR-62	F: CACAGCTCAATAAACTCTATG	53	172
	R: CATTATCCCTAATCTAATCATC		
SSR-68	F: GGTCAGCTGTGTGTGTGTG	56	158
	R: CAATTCAATGCTTTGGATGCT		

Amplification of SSR markers: Ten SSR markers were amplified by using the corresponding primers (Table 1). Each reaction was performed with a volume of 50 µL consisting of the following components: 25 µL H_2O, 20 µL Master Mix (Buffer, $MgCl_2$, dNTPs and *Taq* polymerase), 1 µL forward and reverse primer (20 µM), 3 µL template DNA. Primer information was listed in Table 1. The PCR reaction was carried out with a thermal cycle of 94°C-4 min, 35 cycles with 94°C, 1 min, 50°C, 45 sec and 72°C, 2 min, 72°C, 7 min. The PCR products were electrophoresed on 2% agarose gel at a potential difference of 50V for 40 min and checked by Gel Doc XR system (Bio-rad, USA).

Statistical analysis: The data of peel thickness and sweetness were calculated and analyzed by Minitab version 16 software, Tukey's Test at $p<0.05$ was performed for statistical difference. The results were considered as the Mean±SE of the repeated experimental units. The amplification products of the ISSR markers of mango cultivars were analyzed for genetic diversity using NTSYSpc2.1 (Numerical Taxonomy System Personal Computer) software. The gel bands were obtained from the ISSR primer PCR product and imported into Excel software. The presence or absence of a certain band on the gel is recorded as 1 and 0. A phylogenetic tree was constructed by the UPGMA (Unweighted Pair Group Method using arithmetic averages) method to analyze the genetic relationship. The efficiency of each marker in giving polymorphic DNA bands was shown by polymorphism information contents (PICs) as:

$$PIC = 1-\sum P^2ij$$

where, Pij is the frequency of the jth allele for the ith marker.

RESULTS AND DISCUSSION

The Taiwan and Green Thai cultivar has the highest peel thicknesses of 0.28 and 0.27 mm. Hoa Loc and Cat Chu cultivars have the lowest peel thicknesses of 0.21 and 0.23 mm, respectively as shown in Table 2. The two groups with the highest and lowest peel thicknesses were selected for comparison on the gel row of molecular standards.

The selection of samples with a high difference in peel thickness will help the comparison process to have more obvious differences and better identify potentially relevant molecular markers. Compare each standard molecular marker as follows.

SSR-18: The Thai green mango sample has a high peel thickness, but the molecular standard marker cannot amplify the sample's DNA, so the molecular standard marker is not related to the peel thickness trait (Fig. 1).

SSR-20: Molecular Calibration Mark amplifies the DNA of all samples. Cat Hoa Loc and Cat Chu mango samples together with the band appeared at 200 pb size. The thick-peel Taiwanese and Thai green mangoes have no band present at that size. It is possible that the band appears to represent the thin peel trait in Cat Hoa Loc and Cat Chu mango samples (Fig. 2).

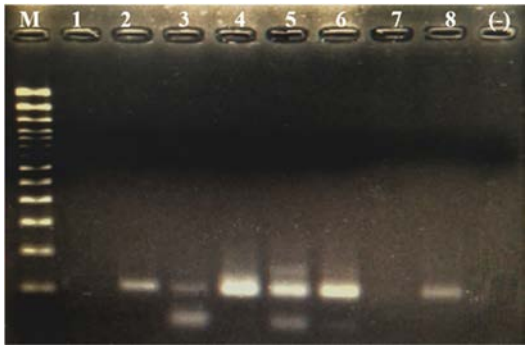

Fig. 1: Band pattern for SSR-18 marker under 2% agarose gel

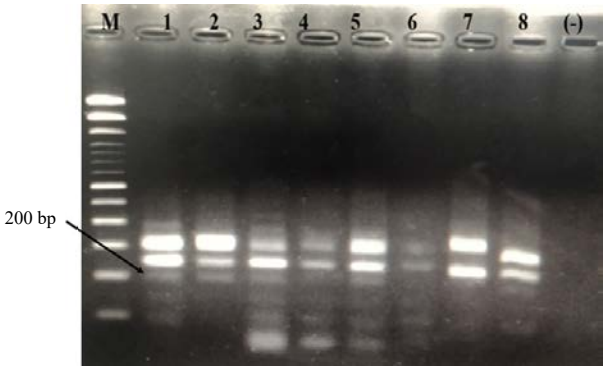

Fig. 2: Band pattern for SSR-20 marker under 2% agarose gel

Table 2: Phenotype analysis for peel thickness and sweetness

Cultivars	Peel thickness (mm)	Brix
Hoa Loc	0.21±0.01^{f}	16.31±0.19^{a}
Thanh Ca	0.24±0.02^{de}	23.43±0.76^{a}
Keo	0.24±0.01^{de}	18.30±0.49^{a}
Taiwan	0.28±0.01^{a}	14.52±0.39^{b}
Green Thai	0.27±0.006^{ab}	17.43±0.17^{b}
Cat Chu	0.23±0.006^{ef}	17.90±0.45^{a}
Vinh Hoa Aroma	0.25±0.01^{cd}	21.22±0.83^{a}
Australia	0.26±0.006^{bc}	13.82±0.25^{b}
p-value	0.0000	0.0000

Means with a different superscript letter in the same column indicate significant differences ($p < 0.05$)

Molecular markers SSR-23, SSR-28 and SSR-41 all show the similarity in number and position of the bands of Cat Hoa Loc and Cat Chu mango. But at molecular markers SSR-23 and SSR-28 Cat Hoa Loc and Cat Chu mangoes have band at the same sizes 195, 280 bp (SSR-23), 185, 465 and 765 bp (SSR-28) were similar to Thai green mango, so the similar bands of Cat Hoa Loc and Cat Chu mangoes are not significant. Besides, at the molecular standard SSR-41, it is different, Cat Hoa Loc and Cat Chu mangoes have a band at 150 bp size, which the 2 samples of thick-peeled mangoes do not have. Therefore, this could be a tape exhibiting the thin-shell trait (Fig. 3).

Markers SSR-51, SSR-52, SSR-59, SSR-62 and SSR-62: In these molecular markers, the group samples of high and low shell thickness had no position difference band position (Fig. 4).

Most of the molecular markers gave amplified DNA products on 4 selected samples (except SSR-18). The number and position of the band of the two samples of Cat Hoa Loc and Cat Chu mangoes are quite similar (molecular markers SSR-23, SSR-28 and SSR-41), possibly due to the genetic relationship between the two samples. The SSR molecular markers that can be related to the peel thickness trait are SSR-20 and SSR-41 because, through these two molecular markers, Cat Hoa Loc and Cat Chu mangoes appear to have the same size but not the same size with 2 samples of thick-skinned mango. Therefore, the band appearance of the same size as Cat Hoa Loc and Cat Chu mangoes may be related to the thin-skinned trait.

According to Table 2, the phenotypes of Thanh Ca mango and Vinh Hoa Thom mango has the highest sweetness at 23.43 and 21.22. Samples of Australian mango and Taiwanese mango have the lowest sweetness at 15.80 and 16.12. Similar to peel thickness, the two groups with the highest and lowest sweetness were selected for comparison on the gel row of molecular standards. Compare each standard molecular marker as follows.

Markers SSR-18, SSR-20, SSR-23, SSR-28, SSR-41, SSR-52, SSR-59 and SSR-62: In these molecular markers, the samples are grouped into low sweetness and high sweetness did not have a significant differences in term of band size (Fig. 1-4).

Marker SSR51: Australian and Taiwanese mango samples appeared with the same size at 245 bp that Thanh Ca mango and Vinh Hoa Thom mango did not. It is possible that the appearance of band is related to the low sweetness of Australian and Taiwanese mangoes (Fig. 3d).

Marker SSR68: The samples of Thanh Ca mango and Vinh Hoa fragrant mango appeared at a size of about 290 bp, while Australian and Taiwanese mangoes did not appear. Band in this size range may be related to the high sweetness of Thanh Ca mango and Vinh Hoa Thom mango (Fig. 4d).

Through the above analysis, all mango samples showed polymorphic amplified DNA products with 10 molecular markers (except SSR-52). The SSR molecular markers related to sweetness traits are SSR-51 low sweetness and SSR-68 high sweetness.

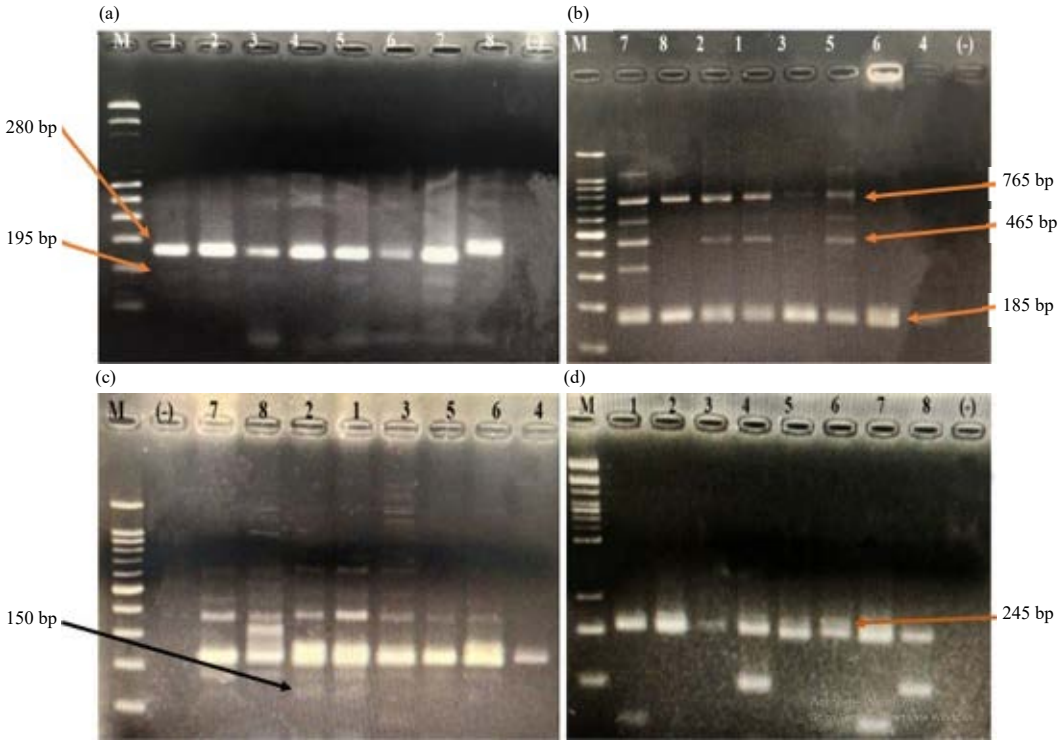

Fig. 3(a-d): Band pattern for SSR-23 (a), SSR-28 (b), SSR-41 (c), SSR 51 and (d) markers under 2% agarose gel
M: 100 bp DNA ladder, Lane 1: Hoa Loc, Lane 2: Cat Chu, Lane 3: Vinh Hoa Aromatic, Lane 4: Thanh Ca, Lane 5: Australia, Lane 6: Taiwan, Lane 7: Green Thai, Lane 8: Keo and Lane 9: Negative control

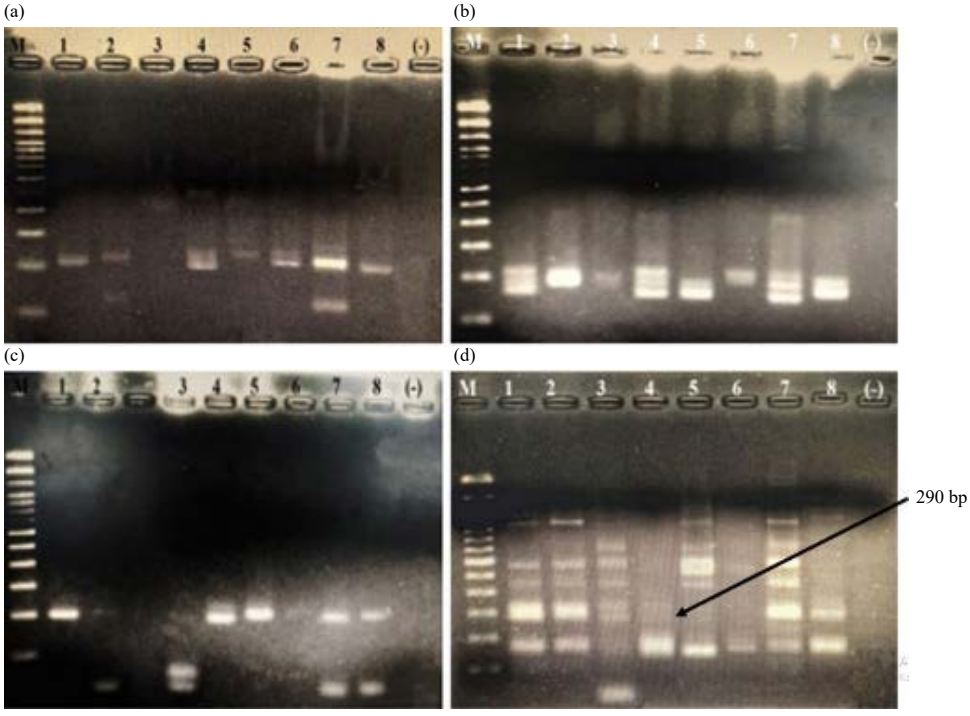

Fig. 4(a-d): Band pattern for (a) SSR-52, (b) SSR-59, (c) SSR-62 and (d) SSR-64 marker under 2% agarose gel

The sweetness of mango fruit is determined by the concentration of sugars, such as fructose and glucose, in the fruit[4,17,18]. The genetic basis of fruit sweetness in mango is complex and involves multiple genes[19]. Some of the genes that have been implicated in the regulation of mango fruit sweetness include the MADS-box transcription factor gene, MdMDBF1, which has been shown to regulate sugar metabolism in mango fruit and other genes involved in the biosynthesis and transport of sugars[19].

The sweetness of mango fruit is also influenced by environmental factors, such as temperature, light and water availability, which can modify gene expression and impact fruit development and sugar metabolism[5,17,20,21].

The correlation between the sweetness property and the genes of mango is complex and involves the interplay of multiple genetic and environmental factors. Simple Sequence Repeat (SSR) markers are short, repeated sequences of DNA that are widely used in molecular genetics and genomics. In mango, SSR markers have been used to study the genetic diversity and relationships among mango varieties, as well as to identify markers associated with important traits, such as fruit size, color, peel thickness and sweetness[12,15,22].

The correlation between sweetness property and SSR markers in mango has been studied in some research efforts. The findings have shown that SSR markers can be used to predict the sweetness of mango fruit, as well as to identify specific genetic regions associated with sweet fruit[23]. For example, one study reported that SSR markers on chromosome 4 were significantly associated with the sweetness of mango fruit and that these markers could be used to predict sweetness in new mango varieties[24].

The data revealed that 9 SSRs were polymorphic for 8 mango cultivars. The PIC value of the 10 pairs of molecular markers ranged from 0.56 (SSR-52) to 0.74 (SSR-23, SSR-41).

Four molecular markers of SSR can be related to two traits of peel thickness and sweetness in 8 mango varieties such as SSR-20, SSR-41 can be related to skin thickness trait and SSR-51 and SSR-68 molecules may be related to sweetness trait. This result showed the applicability of SSR molecular markers in the analysis of loci that regulate skin thickness and sweetness of mango for hybridization.

The SSR markers have been shown to be useful in identifying markers associated with the sweetness of mango fruit. However, the correlation between sweetness and SSR markers is complex and likely influenced by multiple genetic and environmental factors. Further research is needed to fully understand the genetic basis of mango fruit sweetness and to develop new strategies for breeding sweet mango varieties using SSR markers using sweet mango varieties.

CONCLUSION

The relationship of SSR markers and appearance properties consisting of peel thickness and sweetness of mango was estimated. The markes SSR-20 and SSR-41 could be related to skin thickness characteristic, while the markers SSR-51 and SSR-68 may be responsible for sweetness trait of mango. The findings support for mango breeding programs applying marker-assisted selection approach.

SIGNIFICANCE STATEMENT

The study was conducted with the aim of determining the correlation between the sweetness and peel thickness of mango and SSR markers. The co-efficiency of molecular markers being responsible for gene locations and phenotypic characteristics including sweetness and peel thickness in mango was determined. The markers SSR-20 and SSR-41 might allocate near genes encoding peel thickness and the markers SSR-51 and SSR-68 might relate to the sweetness gene relationship. The findings are really important in mango breeding and selection based on molecular markers.

ACKNOWLEDGMENT

The research fund was provided by Vietnam National University-HCMC in project level C, code C2022-16-02.

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