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Research Article
Evaluation of Genetic Variations of *Mangifera* sp. in Southern Vietnam

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Abstract

Background and Objective: The genus *Mangifera* is a group of fruit trees with high economic and export value in Vietnam. However, the current management of the diversity of mango cultivars and selection of propagation materials is mainly based on morphological and agronomic characteristics. There is still a lack of molecular information for more accuracy and quick development of mangoes in Vietnam. In this study, the sequence-based barcoding method and PCR-based SCoT technique were used for the molecular identification of some mango cultivars in Vietnam. **Materials and Methods:** Nine mango cultivars of Southern Vietnam i.e., XoaiHoa Loc, Xoai Cat Chu, Xoai Buoi, Xoai Thanh Ca, Xoai Xiem Num, Xoai Tu Quy, Xoai Thai, Xoai Coc and Xoai Dai Loan were examined for identification using 46 SCoT markers and 4 barcoding markers including ITS, *mat*K, *trn*L-F and *rpl*20-*rps*12. **Results:** A total of 29 variable sites on the ITS sequence could clearly distinguish all 9 groups of mango cultivars. The appearance of SCoT fragments varied significantly due to the number and component of primers used. Twenty two over 46 SCoT primers gave 305 polymorphic bands that could not separate groups of cultivars but could identify studied samples at the individual level. **Conclusion:** The SCoT markers were potential for molecular identification at the individual level. The combination of ITS barcode and SCoT technique were recommended for diversity management and breed selection of mango cultivars in Vietnam.

Key words: *Mangifera*, mango, molecular identification, barcoding, ITS, SCoT (start codon target), phylogenetic, simple sequence repeat

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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 is a tropical fruit tree. Not only delicious but mango is also rich in protein, fibre, vitamins C, A, folic acid, bringing many health benefits. Vietnam ranks 13th in mango production in the world. In Vietnam, mangoes are grown from the South to the North and there are many different varieties of mangoes, including indigenous varieties such as XoaiHoa Loc, Xoai Thanh Ca, Xoai Cat Chu etc. and imported such as Xoai Thai, Xoai Dai Loan etc[1]. However, the management of mango varieties in Vietnam is still vague, inaccurate and incomplete. Besides, the selection and creation of precious mango varieties are necessary that serving the needs of domestic and foreign markets. Nevertheless, the current management and selection of varieties are mainly based on morphological and agronomic characteristics, which can lead to the omission of some value varieties and the selection process may take a long time to evaluate. Therefore, the development of molecular data for the management, rapid and efficient selection and breeding of mango varieties is essential.

Several works have also been performed to understand the molecular information of mango varieties for the identification and assessment of genetic diversity. Previously, 15 indigenous mango varieties (*Mangifera indica* L.) of Pakistan have been studied for Molecular diversity based on microsatellite markers. Twelve SSR primers gave 181 bands that could indicate the expression of inbreeding and their parents[2]. Simple Sequence Repeat (SSR) markers were also used for studying the difference among the subpopulation of mango in Eastern Kenya. Accordingly, indigenous varieties were well separated from the exotic[3]. Recently, Jena and Chand[4] evaluated the genetic diversity of 70 Indian mango varieties using multiple DNA markers including Random amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR), Directed Amplification of Minisatellite-region DNA (DAMD), Start Codon Targeted (SCoT), CAAT Box-derived Polymorphism (CBDP) and Simple Sequence Repeat (SSR) in single or in combinations. SSRs were reported to express the highest ability to examine genetic variability, followed by the two types of markers CBDPs and SCoTs[4]. Among PCR-based techniques, although also using unspecific primers for amplification reactions, primers in the SCoT technique is specifically designed around the start codon ATG to target the products of the DNA encoding traits. Thus, the genetic examination is directly related to the expressed characteristics rather than random DNA fragments in the genome[5-7]. Zhou *et al.*[8] determined the genetic relatedness among 168 mangoes (*Mangifera indica* L.) germplasm resources using this technique. From 337 DNA bands created by 45 SCoT primers, 34 seedling germplasm resources could be identified as potential parents or sister lines. Hence SCoT markers were recommended as potential assistance for the identification of mango cultivars.

In addition to the PCR-based techniques, the DNA barcoding method which uses a short DNA segment specific for species is an approach towards providing sequence information for species classification. different. The chloroplast genomic locus *mat*K was used for the efficient identification and phylogenetic analysis of 19 *Mangifera* species from Thailand and Indonesia[9]. The nuclear universal ITS region (Intergenic Transcribed Spacer) sequence was also used as a molecular marker to evaluate the genetic diversity of 13 cultivated mango samples collected from three countries Indonesia, Malaysia and Taiwan[10] and for the identification of *Mangifera* species in Sumatra[11]. The study also proposed that the *trn*L-F, *rbc*L gene regions assisted invalidation of the accuracy of mango classification via morphological characteristics. Another study suggested the use of sequence region in the chloroplast, *rpl*20-*rps*12, with high efficiency in the distinguishment of 19 mango cultivars grown at the subspecies level[12].

In this study, we sequenced some barcoded DNA regions and amplified some DNA regions using SCoT primers to investigate the characteristics and evaluate the genetic diversity of some mango cultivars in Southern Vietnam. The study of genetic differentiation of mango varieties not only helps in the control of mango resources in Vietnam but also supports the selection and conservation of diverse varieties, especially value cultivars.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Biotechnology, Nguyen Tat Thanh University, Vietnam from July, 2020-June, 2021).

DNA extraction and amplification of barcoding markers: Samples of mango from Southern Vietnam were collected. Total DNA was extracted using Isolate II Plant DNA kit BIO-52069 (TBR company, Ho Chi Minh City, Vietnam) and stored at -20°C for further study. The four regions ITS, *trn*L-F, *rpl*20-*rps*12 and *mat*K were amplified using referenced primers as in Table 1.

The thermocycling conditions were Denaturation 94°C/10 min, followed by 30 cycles of denaturing 94°C/40 sec, annealing Ta°C/40 sec, extending 72°C/40 sec and final extension 72°C/7 min. Ta°C was different depending on the corresponding primer pairs (Table 1). Components of PCR

Table 1: Primers used for amplification of barcoding loci in the study

Locus	Primers name	Primer sequence (5'-3')	Temperature (°C)	Expected product length (bp)	References
ITS	5F	GGAAGTAAAAGTCGTAACAAGG	58	900	CBOL *et al.*[13]
	4R	TCCTCCGCTTATTGATATGC			
*trn*L-F	TrnL	GAGAGAAACATTTCTGGTCGG	62	600	Fitmawati *et al.*[11]
	TrnF	GGGCCAATCCTGACCAAATCC			
*rpl*20-*rps*12	rpl20-F	TTTGTTCTACGTCTCCGAGC	50	768	Khan and Azam[12]
	rps12-R	GTCGAGGAACATGTACTAGG			
*mat*K	TAA-09F	GGTTTTCCCATGAGTAGATTATCG	49	1200	Hidayat *et al.*[9]
	trnK-2R	AACTAGTCGGATGGAGTAG			
	TAA-09R	CGAAGTAGACGAAGCTCTTGG			
	TrnK-5F	TGGGTTGCTAACTCATGG			
	390F	CGATCTATT-CATTCAATATTTC	52	930	Cuenoud *et al.*[14]
	1326R	TCTAGCACACGAAAGTCGAAGT			

reaction included 10 µL Taq DNA pol 2X-preMix (TaqDNA pol 2X-preMix: 0.1 U mL^{-1} DNA Polymerase, 0.4 mM dATP, 0.4 mM dGTP, 0.4 mM dCTP, 0.4 mM dTTP, 0.4 mM $MgSO_4$, 20 mM KCl, 16 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl (pH8)), 1 µL forward primer 5 µM, 1 µL backward primer 5 µM, 1 µL total DNA and 12 µL H_2O.

Barcoding analysis: Raw sequences were visualized and adjusted using FinchTV software[15]. Forward and reverse bidirectional sequences were aligned to obtain highly reliable consensus sequences. Similarity search was applied using the BLAST tool to compare with sequences from NCBI. Sequences were aligned automatically using Sea view software[16] and then manually for more precise. Alignment data was input into MEGA 7.0 software[17] to analyze sequence characteristics including variation and phylogenetic relationship with Maximum Likelihood algorithm, Kimura 2-parameter model, bootstrap 1000 times.

Amplification of SCoT markers: Total 46 SCoT primers from previous studies (Table 2) which were reported to have high polymorphism were screened in this study. In contrast with the barcoding approach, in the SCoT technique, only one primer was added to each PCR reaction which plays the role of both forward and backward primers. Components of each amplification running consisted of 2 µL template DNA, 1 µL primer, 12.5 µL 2X GoTaq® G2 Green Master Mix (Taq DNA Polymerase, 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP and 3 mM $MgCl_2$), adding H_2O up to 25 µL/reaction. The thermocycling included initial Denaturation 94°C/3 min (1 cycle), followed by 40 cycles of denaturing 94°C/45 sec, annealing 52°C/60 sec, extending 72°C/60 sec and final extension 72°C/5 min (1 cycle). The PCR products were electrophoresed on gel agarose 2% in 1X TBE buffer stained with 6X GelRed™ for determining the presence or absence of DNA bands.

SCoT analysis: The presence or absence of PCR bands from each SCoT marker was recorded for all mango cultivars in the study, where 0 was without DNA band and 1 was with DNA band based on a size comparison with the standard (2000 bp DNA Ladder Plus). Only clear and reproducible bands were scored. NTSYS 2.1 software[21] was used to calculate genetic diversity including Simple Matching (SM) similarity coefficient and cluster analysis using the Unweighted Pair-Group Method with Arithmetic averages (UPGMA) algorithm.

RESULTS AND DISCUSSION

DNA sequence characteristic of *Mangifera* sp. from Southern Vietnam based on barcoding markers: Sixteen samples representing nine mango groups from different provinces of Southern Vietnam collected based on Vietnamese vernacular names (Table 3) were included in this barcoding study on 4 marker regions ITS, *trn*L-F, *rpl*20-*rps*12 and *mat*K. The three loci ITS, *trn*L-F and *rpl*20-*rps*12 were all successfully amplified and sequenced on all samples. Unexpectedly, the PCR products of *mat*K locus could still not be achieved after trying with different primer pairs as in Table 2. Hence *mat*K was excluded from our further study.

The BLAST results showed that all the sequences had high similarity (96-100%) to the *Mangifera* accessions from GenBank on all three corresponding regions ITS, *trn*L-F and *rpl*20-*rps*12. All obtained sequences were registered for GenBank accessions on the NCBI database (Table 3).

Nucleotide features of different mango taxa in Vietnam were completely similar in each of two regions *trn*L-F and *rpl*20-*rps*12. Particularly, the ITS data indicated diversity among different mango groups by 29 variable sites containing 27 informative parsimony sites and 3 singletons. Based on this information, the endemic valuable taxa Xoai Hoa Loc of Vietnam could be quickly identified by the characteristic variable nucleotide T at site 485 bp in their sequences, which

Table 2: Primers used for amplification of DNA fragments in the SCoT study

Primers name	Sequence (5'-3')	References
SCoT1	CAACAATGGCTACCACCA	Luo *et al.*[6] and Luo *et al.*[7]
SCoT2	CAACAATGGCTACCACCC	Gajera *et al.*[5]
SCoT3	CAACAATGGCTACCACCG	Luo *et al.*[6] and Luo *et al.*[7]
SCoT4	CAACAATGGCTACCACCT	Guo *et al.*[18]
SCoT5	CAACAATGGCTACCACGA	Gajera *et al.*[5]
SCoT6	CAACAATGGCTACCACGC	Gajera *et al.*[5]
SCoT7	CAACAATGGCTACCACGG	Guo *et al.*[18]
SCoT8	CAACAATGGCTACCACGT	Que *et al.*[19]
SCoT9	CAACAATGGCTACCAGCA	Luo *et al.*[6] and Luo *et al.*[7]
SCoT10	CAACAATGGCTACCAGCC	Guo *et al.*[18]
SCoT11	AAGCAATGGCTACCACCA	Que *et al.*[19]
SCoT12	ACGACATGGCGACCAACG	Que *et al.*[19]
SCoT13	ACGACATGGCGACCATCG	Guo *et al.*[18]
SCoT14	ACGACATGGCGACCACGC	Gajera *et al.*[5]
SCoT15	ACGACATGGCGACCGCGA	Que *et al.*[19]
SCoT16	ACCATGGCTACCACCGAC	Gajera *et al.*[5]
SCoT17	ACCATGGCTACCACCGAG	Que *et al.*[19]
SCoT18	ACCATGGCTACCACCGCC	Guo *et al.*[18]
SCoT19	ACCATGGCTACCACCGGC	Gajera *et al.*[5]
SCoT20	ACCATGGCTACCACCGCG	Luo *et al.*[6] and Luo *et al.*[7]
SCoT22	AACCATGGCTACCACCAC	Que *et al.*[19]
SCoT23	CACCATGGCTACCACCAG	Que *et al.*[19]
SCoT25	ACCATGGCTACCACCGGG	Luo *et al.*[6] and Luo *et al.*[7]
SCoT26	ACCATGGCTACCACCGTC	Gajera *et al.*[5]
SCoT27	ACCATGGCTACCACCGTG	Que *et al.*[19]
SCoT28	CCATGGCTACCACCGCCA	Que *et al.*[19]
SCoT30	CCATGGCTACCACCGGCG	Guo *et al.*[18]
SCoT31	CCATGGCTACCACCGCCT	Que *et al.*[19]
SCoT32	CCATGGCTACCACCGCAC	Collard and Mackill[20]
SCoT33	CCATGGCTACCACCGCAG	Gajera *et al.*[5]
SCoT34	ACCATGGCTACCACCGCA	Luo *et al.*[6] and Luo *et al.*[7]
SCoT35	CATGGCTACCACCGGCCC	Que *et al.*[19]
SCoT36	GCAACAATGGCTACCACC	Collard and Mackill[20]
SCoT40	CAATGGCTACCACTACAG	Gajera *et al.*[5]
SCoT44	CAATGGCTACCATTAGCC	Luo *et al.*[7]
SCoT45	ACAATGGCTACCACTGAC	Gajera *et al.*[5]
SCoT51	ACAATGGCTACCACTGTC	Gajera *et al.*[5]
SCoT60	ACAATGGCTACCACCACA	Luo *et al.*[7]
SCoT61	CAACAATGGCTACCACCG	Luo *et al.*[6]
SCoT63	ACCATGGCTACCACGGGC	Gajera *et al.*[5]
SCoT65	ACCATGGCTACCACGGCA	Gajera *et al.*[5]
SCoT66	ACCATGGCTACCAGCGAG	Gajera *et al.*[5]
SCoT70	ACCATGGCTACCAGCGCG	Gajera *et al.*[5]
SCoT73	CCATGGCTACCACCGGCT	Gajera *et al.*[5]
SCoT77	CCATGGCTACCACTACCC	Gajera *et al.*[5]
SCoT78	CCATGGCTACCACTAGCA	Gajera *et al.*[5]

were different to all the remaining taxa (Fig. 1). Xoai Buoi could be recognized by unique nucleotides G at sites 461 or 580 or 583 or by nucleotide C at sites 583. Xoai Thanh Ca was identified by nucleotides T at site 455 or 527. The three singletons C, C and A of the accession Xoai Tu Qui XTQ1-MN477191 at sites 461, 489 and 525 bp, corresponding were also unique features for distinguishing Xoai Tu Quy XTQ1. Nevertheless, the calculation of all variations could also definitely identify all the studied cultivars of mangoes, which was expressed into separated monophyletic branches on the ITS tree (Fig. 2). Meanwhile, taxa of the same groups were clustered together as expected. Hence the use of ITS sequence was strongly recommended for the identification of mango cultivars.

Genetic diversity of *Mangifera* sequences of Vietnam in comparison with mango accessions from GenBank: To evaluate the genetic diversity of Vietnam mango groups with

Accession	Variable Site																												
	43	46	59	428	453	455	461	484	485	489	514	520	525	527	536	539	543	566	575	577	580	583	587	595	601	603	607	610	627
Xoai Hoa Loc XHL9 - MN477178	T	G	G	C	G	C	T	C	T	G	G	G	C	C	G	A	G	G	T	G	C	T	G	G	G	G	T	G	A
Xoai Hoa Loc XHL10 - MN477179	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Xoai Hoa Loc XHL11 - MN477180	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Xoai Hoa Loc XHL12 - MN477181	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Xoai Cat Chu XCC1 - MN477182	C	.	T	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Xoai Cat Chu XCC2 - MN477183	C	.	T	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Xoai Dai Loan XDL1 - MN477184	.	C	T	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Xoai Dai Loan XDL2 - MN477185	.	C	T	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Xoai Coc XC2 - MN477186	C	C	T	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Xoai Xiem Num XXN1 - MN477187	C	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Xoai Thai XT2 - MN477188	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Xoai Thanh Ca XTC1 - MN477189	C	.	.	.	.	T	.	.	G	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Xoai Thanh Ca XTC2 - MN477190	C	.	.	.	.	T	.	.	G	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Xoai Tu Qui XTQ1 - MN477191	C	.	.	T	C	.	C	G	G	C	A	C	A	.	C	C	C	A	G	C	.	.	C	.	C	A	C	C	C
Xoai Buoi XB1 - MN477192	C	.	.	T	C	.	G	G	G	.	A	C	.	.	C	C	C	A	G	C	G	G	C	C	C	A	C	C	C
Xoai Buoi XB2 - MN477193	C	.	.	T	C	.	G	G	G	.	A	C	.	.	C	C	C	A	G	C	G	G	C	C	C	A	C	C	C

Fig. 1: Nucleotide variable sites of *Mangifera* sp. sequences in the study from Southern Vietnam based on ITS sequence data

Dote: Represented the same nucleotide with the nucleotide at the first row in the same column. Outside border cell: Unique variable nucleotide, grey filled cell: Singleton nucleotide

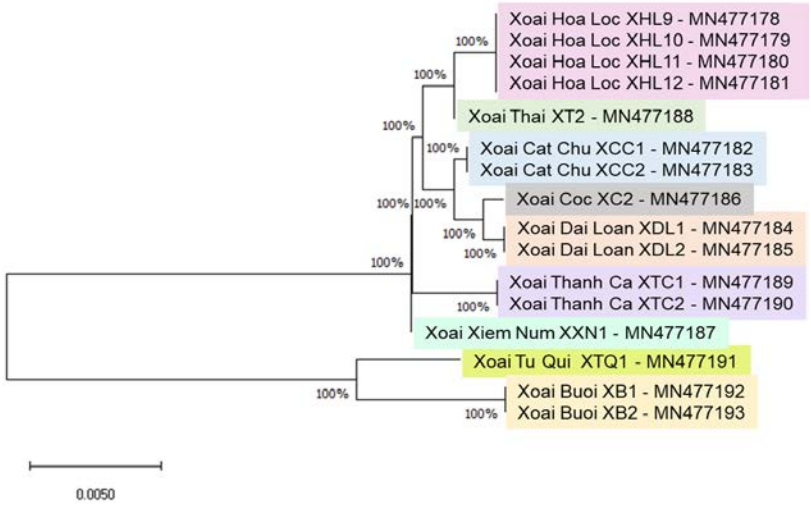

Fig. 2: Phylogenetic tree of *Mangifera* sp. sequences in the study from Southern Vietnam based on ITS sequence data

With (%): Bootstrap value, 0.0050: Genetic distance scale

Table 3: Sample voucher, GenBank accession and group of *Mangifera* sp. using in barcoding study

Groups	Sample voucher	Collection place	ITS accession	*rpl*20-*rps*12 accession	*trn*L-F accession
Xoai hoa Loc	XHL9	Long An	MN477178	MN597088	MN481483
	XHL10	Tien Giang	MN477179	MN597089	MN481484
	XHL11	Tien Giang	MN477180	MN597090	MN481485
	XHL12	Tien Giang	MN477181	MN597091	MN481486
Xoai Cat Chu	XCC1	Ben Tre	MN477182	MN597092	MN481487
	XCC2	Tien Giang	MN477183	MN597093	MN481488
Xoai Thanh Ca	XTC1	Tien Giang	MN477189	MN597099	MN481494
	XTC2	Dong Nai	MN477190	MN597100	MN481495
Xoai Buoi	XB1	Ho Chi Minh City	MN477192	MN597102	MN481497
	XB2	Ben Tre	MN477193	MN597103	MN481498
Xoai Coc	XC2	Ben Tre	MN477186	MN597096	MN481491
Xoai Xiem Num	XXN1	Vinh Long	MN477187	MN597097	MN481492
Xoai Tu Quy	XTQ1	Ben Tre	MN477191	MN597101	MN481496
Xoai Thai	XT2	Ho Chi Minh City	MN477188	MN597098	MN481493
Xoai Dai Loan	XDL1	Ho Chi Minh City	MN477184	MN597094	MN481489
	XDL2	Ho Chi Minh City	MN477185	MN597095	MN481490

other mango taxa on the world, a total of 126 sequences of *Mangifera* accessions from GenBank, including 57 of ITS, 32 of *trn*L-F and 37 of *rpl*20-*rps*12, were all collected and aligned with those from Vietnam, corresponding. As the sequences of the same regions *trn*L-F and *rpl*20-*rps*12 from Vietnam samples were similar, one sample of each region was used as representative for constructing a phylogenetic tree with GenBank sequences. On the *trn*L-F tree, Vietnam samples were grouped with the other six species i.e., *Mangifera indica*, *M. zeylanica*, *M. laurina*, *M. lalijiwa*, *M. griffithii* and *M. camptosperma*, in which accessions of *M. indica* were most (Fig. 3a). As for *rpl*20-*rps*12, there was only one registered species *M. indica*, which was also grouped with our sequences (Fig. 3b). Hence there is still a limitation of sequence information of *trn*L-F and *rpl*20-*rps*12 for more analysis.

The single ITS region had a number of published accessions belonging to 15 species of which the most were also *M. indica*. The varieties of Xoai Hoa Loc, Xoai Cat Chu, Xoai Thai, Xoai Coc, Xoai Dai Loan and Xoai Xiem Num were closely clustered with other accessions of *M. indica* (Fig. 3c). However, due to the divergence characteristic of the ITS region, the sequences of the same species *M. indica* from GenBank were not 100% similar but expressed many variations, which resulted in paraphyletic branches. The same also went for other species such as *M. laurina*, *M. keymanga*. This result confirmed that molecular identification information of mangoes is still limit and should be further elucidated to increase accuracy. Even though, Xoai Thanh Ca were divided into an independent branch. Two samples of Xoai Buoi were also definitely separated from other GenBank species and closely related to Xoai Tu Quy. Three other groups Xoai Thai, Xoai Coc and Xoai Dai Loan which were imported cultivars were closely related to Xoai Cat and in the same cluster group with *M. indica*. However, each of these cultivars still expressed private differences. Our results indicated that mango cultivars in Vietnam were diverse and contained many different distinct features.

The ITS was popularly known as high potential barcoding markers in many previous research[22-25]. In terms of mangoes, the ITS region supported identifying and separate Vietnamese mango samples into separate varieties. This result was similar to Hidayat *et al.*[9] when separating mango varieties collected from different countries Malaysia, India, Indonesia, Thailand, Taiwan into 3 different groups based on ITS region. The ITS sequence also exhibited high diversity in DNA sequence characteristics and can be effectively applied in the molecular identification of native mangoes in Southern Iran[26]. Even with high diversity, we found that the genetic similarity of intra-genetic samples based on ITS was not affected by the geographical sampling sites. This result was consistent with Ho *et al.*[27] in a study of DNA fingerprinting some mango cultivars in Vietnam recently. Furthermore, the Cat Hoa Loc sample of this study, registered as accession MN011936.1, was also clustered with our Hoa Loc cultivars in the same branch (Fig. 3c). However, in the study of Ho *et al.*[27], Xoai Thanh ca and Xoai Tu Quy was grouped. While Xoai Tu Quy is known as *M. indica*, Xoai Thanh Ca is reported as *M. mekongensis* (or synonym name *M. indica* var. *mekongensis*) which is not registered on GenBank. In our study, two samples of Xoai Thanh Ca were also closed to *M. indica* branches but still isolated into a different branch. Hence the phylogenetic relationship between these two cultivars in our study seemed to be more reasonable and the scientific name *M. indica* var. *mekongensis* of Xoai Thanh Ca was agreeable.

As for *mat*K marker, different primer pairs including the universal primer pair 390F/1326R[14] and 4 specific primers previously used on mangoes by Hidayat *et al.*[9] were tested with the corresponding recommended procedures. However, despite repeated reactions, the PCR product has not been obtained yet. Previously, *mat*K has been also recommended as a highly diverse sequence region that is difficult to amplify in some plant groups[28, 29]. Hence a primer pair that is *mat*K-specific and has good repeatability needs to be designed for more efficiently amplifying this sequence locus.

Genetic polymorphism of *Mangifera* sp. from Southern Vietnam based on SCoT markers: In addition to the above barcoding results which returned obvious classification of mango groups in Southern Vietnam and expressed the distinctive characteristics of Vietnam mango among the taxa of other places on the world, some expectation was that to recognize at the individual level for genetic selecting and breeding. In Vietnam, Xoai Hoa Loc is an endemic value fruit that has high commercial value and hence is highly concerned in the selection of the best individuals for breeding. The genetic analysis of the SCoT approach could show the relationship and variation among individuals of the same group taxon. Therefore, to expand the study for this SCoT analysis, 5 more samples of Xoai Hoa Loc were included and a total of 21 mango samples were analyzed. The visualization of DNA fragments using electrophoresis after SCoT synthesis allowed the recording of polymorphic and monomorphic bands for genetic diversity analysis (Fig. 4a-j). The monomorphic band was the DNA fragment that was amplified on all studied samples at the same size in comparison with the standard ladder. On the contrary, the

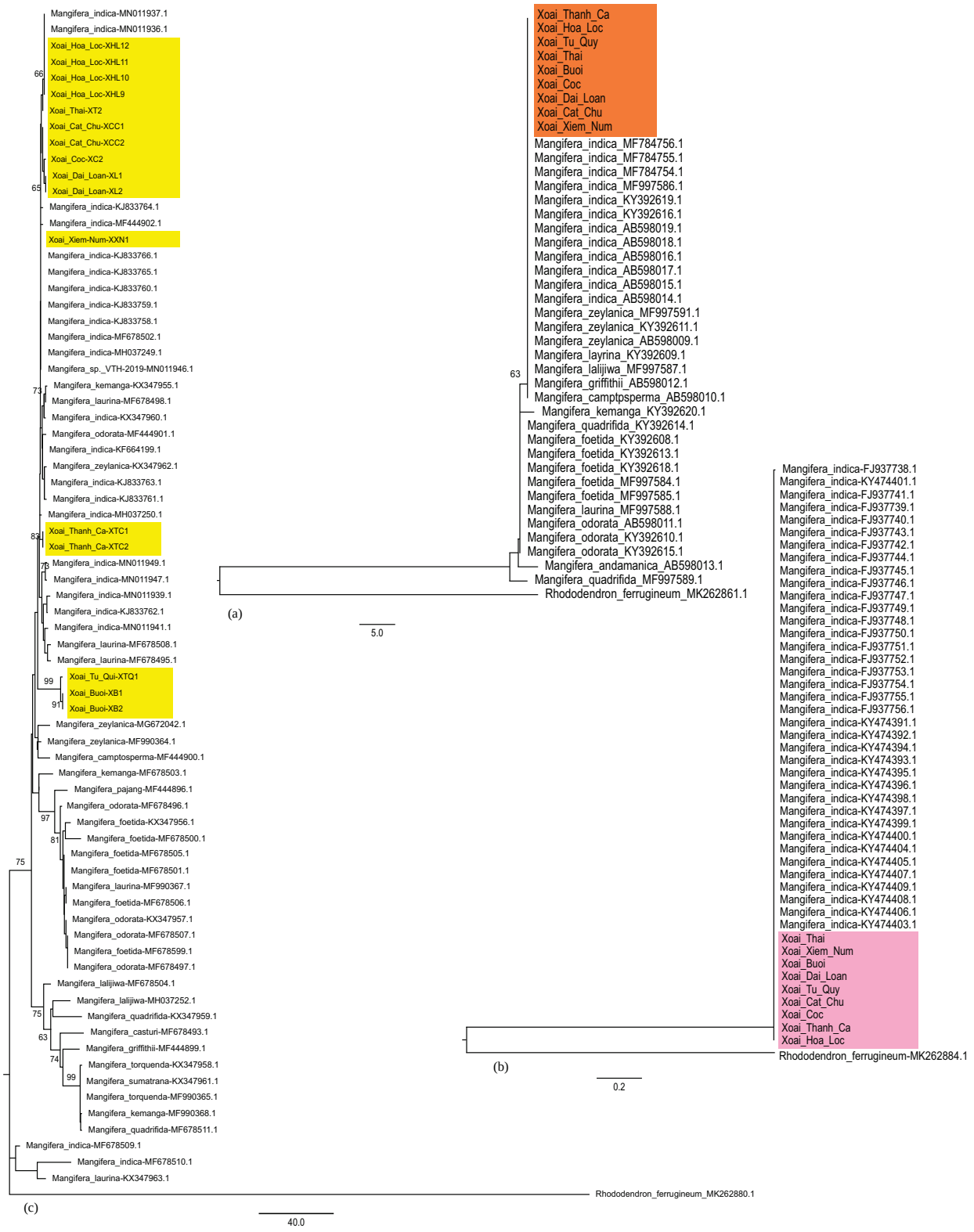

Fig. 3(a-c): Phylogenetic trees of studied *Mangifera* samples in comparison with GenBank accessions based on different sequence data, (a) *trn*L-F tree, (b) *rpl*20-*rps*12 tree and (c) ITS tree,

Coloured taxa: Accessions from Vietnam, Rhododendron ferrugineum: Outgroup species for rooting of the tree, number on branch: Bootstrap value, number under the line: Genetic distance scale

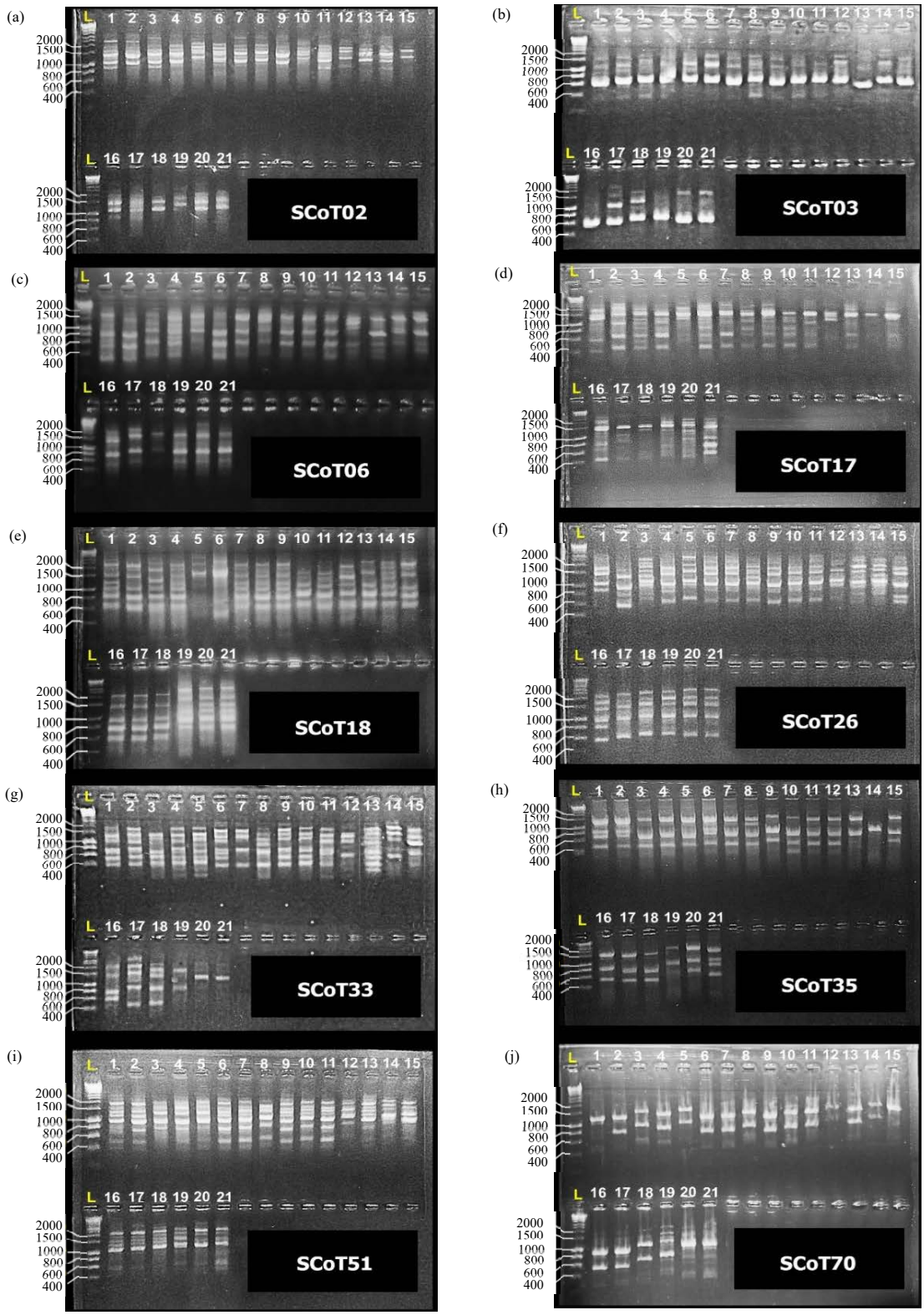

Fig. 4(a-j): Amplification results of some SCoT primers on 21 mango samples of Vietnam, (a) Primer SCoT2, (b) SCoT3, (c) SCoT6, (d) SCoT17, (e) SCoT18, (f) SCoT26, (g) SCoT33, (h) SCoT35, (i) SCoT51 and (j) SCoT70

1: Xoai Tu Quy XTQ1, 2-3: Xoai Cat Chu XCC1-XCC2, 4-5: Xoai Dai Loan XDL1-XDL2, 6-14: XoaiHoa Loc XHL9-XHL17, 15: Xoai Thai XT1, 16: Xoai Coc XC2, 17-18: Xoai Buoi XB1-XB2, 19: XXN, 20-21: Xoai Thanh Ca XTC1-XTC2, L: DNA ladder

Table 4: SCoT primers that gave polymorphic bands on 21 mango samples of Vietnam

Primers name	Primer sequence (5'-3')	Number of total bands	Number of polymorphic bands	Portion of polymorphic bands (%)
SCoT02	CAACAATGGCTACCACCC	14	14	100
SCoT03	CAACAATGGCTACCACCG	13	13	100
SCoT05	CAACAATGGCTACCACGA	9	9	100
SCoT06	CAACAATGGCTACCACGC	22	22	100
SCoT07	CAACAATGGCTACCACGG	14	14	100
SCoT11	AAGCAATGGCTACCACCA	14	14	100
SCoT12	ACGACATGGCGACCAACG	16	16	100
SCoT13	ACGACATGGCGACCATCG	15	15	100
SCoT15	ACGACATGGCGACCGCGA	15	15	100
SCoT17	ACCATGGCTACCACCGAG	11	11	100
SCoT18	ACCATGGCTACCACCGCC	13	13	100
SCoT20	ACCATGGCTACCACCGCG	10	10	100
SCoT26	ACCATGGCTACCACCGTC	19	19	100
SCoT30	CCATGGCTACCACCGGCG	12	12	100
SCoT33	CCATGGCTACCACCGCAG	15	15	100
SCoT35	CATGGCTACCACCGGCCC	15	15	100
SCoT40	CAATGGCTACCACTACAG	12	12	100
SCoT45	ACAATGGCTACCACTGAC	11	11	100
SCoT51	ACAATGGCTACCACTGTC	12	12	100
SCoT65	ACCATGGCTACCACGGCA	13	13	100
SCoT70	ACCATGGCTACCAGCGCG	13	13	100
SCoT77	CCATGGCTACCACTACCC	17	17	100
Total number of bands		305	305	
Min number of bands		9	9	
Max number of bands		22	22	
Average number of bands		13.86	13.86	

DNA fragment that appeared at one size on one or some but not all samples were considered as a polymorphic band.

Among 46 screened primers, 5 primers gave monomorphic bands and 19 primers showed unclear or unstable results were excluded from the study. Data of the 22 remain primers which gave clear and reproducible polymorphic bands (Table 4) were put into the next analysis. The total band was calculated by the sum of all bands created by one primer reaction on all studied samples. From these 22 primers, all appeared bands were polymorphic (100%). The SCoT06 gave the most bands (22) and the SCoT05 gave the least (9 bands). The total number of bands was 305 and the average was 13.86.

The recording of produced specific unique DNA bands showed that many individuals could be quickly and direct identified by single primers (Table 5). For instance, the cultivar Xoai Buoi XB1 could be identified by one of four primers SCoT02, SCoT03, SCoT12 or SCoT26 based on specific fragments at 800, 2250, 1200 and 2250 bp, corresponding. Among 9 Xoai Hoa Loc samples, 7 samples including XHL9, XHL11, XHL12, XHL13, XHL15, XHL16 and XHL17were identified based on unique SCoT fragments from primers SCoT15, 18, 70, 65, 35, 26, 13, 30, 15 as in Table 5. Other 10 samples i.e., XB2, XC2, XCC1, XDL1, XDL2, XT1, XTC1, XTC2, XTQ1, XXN1 over 21 mango samples were also direct recognized.

Polymorphism data of all 22 primers were combined as single information for calculation of similarity using the UPGMA algorithm in NTSYS software. As expected, all cultivars, even taxa of the same group, were separated into single branches (Fig. 5). However, in contrast with the ITS barcode tree, the two taxa of each cultivar Xoai Cat Chu (XCC1 and XCC2), Xoai Dai Loan (XDL1, XDL2), Xoai Buoi (XB1, XB2) and Xoai Thanh Ca (XTC1, XTC2) were still separated into two mini-clusters. The same thing happened for all 9 samples of Xoai Hoa Loc, even the two samples XHL13 and XHL14 which only had a similarity coefficient of about 0.97.

A comparison of barcoding and SCoT technique: In the barcode matrix, the intra-specific genetic distance was zero (Fig. 6). Nevertheless, the maximum similarity coefficient (0.9656) among individuals of the same groups was all less than 1 on the SCoT matrix (Fig. 7). This means that the same cultivars still had certain differences. This result had significance in separating individuals of the same species or the same taxonomic group, which was still limited in the barcoding technique.

However, the interesting thing was that while Xoai Cat Chu and Xoai Dai Loan were in a group with Xoai Coc in the ITS sequence data (Fig. 2), they grouped with Xoai Tu Quy in the SCoT data (Fig. 5). Moreover, for SCoT, Xoai Buoi grouped with Xoai Coc instead of Xoai Tu Quy as in the ITS tree. Xoai

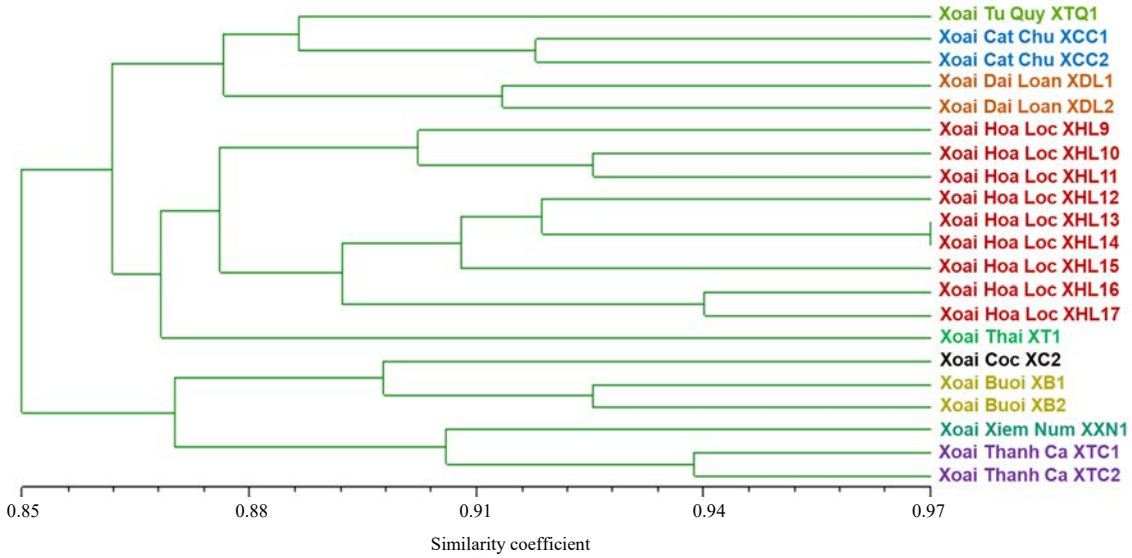

Fig. 5: Phylogenetic tree of 21 *Mangifera* sp. samples in the study based on SCoT data

Table 5: Specific unique bands of SCoT primers supported indirect identification of studied mango cultivars

Cultivar	Primer producing specific DNA band	Size of specific DNA band (bp)
Xoai Buoi XB1	SCoT02	800
	SCoT03	2250
	SCoT12	1200
	SCoT26	2250
Xoai Buoi XB2	SCoT07	900
	SCoT11	500
	SCoT15	1900
	SCoT33	2500
Xoai Coc XC2	SCoT20	1200
	SCoT40	1200
Xoai Cat Chu XCC1	SCoT06	1900
	SCoT26	550
	SCoT77	800
Xoai Dai Loan XDL1	SCoT30	400
	SCoT40	900
Xoai Dai Loan XDL2	SCoT06	1100
Xoai Hoa Loc XHL9	SCoT15	400
	SCoT18	500
Xoai Hoa Loc XHL11	SCoT70	700
Xoai Hoa Loc XHL12	SCoT65	1700
Xoai Hoa Loc XHL13	SCoT35	400
Xoai Hoa Loc XHL15	SCoT26	1200
Xoai Hoa Loc XHL16	SCoT13	1700
	SCoT30	950
Xoai Hoa Loc XHL17	SCoT15	370
Xoai Thai XT1	SCoT07	1300
	SCoT13	650
	SCoT65	1400
Xoai Thanh Ca XTC1	SCoT06	1600
	SCoT07	570
Xoai Thanh Ca XTC2	SCoT20	1400
	SCoT30	200
Xoai Tu Quy XTQ1	SCoT06	4000
	SCoT11	1400
	SCoT17	400
Xoai Xiem Num XXN1	SCoT18	1700
	SCoT35	2000

	Accession	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	Xoai Hoa Loc XHL9 - MN477178																
2	Xoai Hoa Loc XHL10 - MN477179	0.000000															
3	Xoai Hoa Loc XHL11 - MN477180	0.000000	0.000000														
4	Xoai Hoa Loc XHL12 - MN477181	0.000000	0.000000	0.000000													
5	Xoai Cat Chu XCC1 - MN477182	0.004792	0.004792	0.004792	0.004792												
6	Xoai Cat Chu XCC2 - MN477183	0.004792	0.004792	0.004792	0.004792	0.000000											
7	Xoai Dai Loan XDL1 - MN477184	0.004794	0.004794	0.004794	0.004794	0.003192	0.003192										
8	Xoai Dai Loan XDL2 - MN477185	0.004794	0.004794	0.004794	0.004794	0.003192	0.003192	0.000000									
9	Xoai Coc XC2 - MN477186	0.006397	0.006397	0.006397	0.006397	0.001594	0.001594	0.001595	0.001595								
10	Xoai Xiem Num XXN1 - MN477187	0.003192	0.003192	0.003192	0.003192	0.001594	0.001594	0.004792	0.004792	0.003192							
11	Xoai Thai XT2 - MN477188	0.001594	0.001594	0.001594	0.001594	0.003192	0.003192	0.003192	0.003192	0.004792	0.001595						
12	Xoai Thanh Ca XTC1 - MN477189	0.006402	0.006402	0.006402	0.006402	0.004794	0.004794	0.008008	0.008008	0.006397	0.003195	0.004800					
13	Xoai Thanh Ca XTC2 - MN477190	0.006402	0.006402	0.006402	0.006402	0.004794	0.004794	0.008008	0.008008	0.006397	0.003195	0.004800	0.000000				
14	Xoai Tu Qui XTQ1 - MN477191	0.035935	0.035935	0.035935	0.035935	0.034266	0.034266	0.037611	0.037611	0.035939	0.032597	0.034264	0.035936	0.035936			
15	Xoai Buoi XB1 - MN477192	0.037616	0.037616	0.037616	0.037616	0.035946	0.035946	0.039297	0.039297	0.037625	0.034272	0.035939	0.037611	0.037611	0.009639		
16	Xoai Buoi XB2 - MN477193	0.037616	0.037616	0.037616	0.037616	0.035946	0.035946	0.039297	0.039297	0.037625	0.034272	0.035939	0.037611	0.037611	0.009639	0.000000	

Fig. 6: Intra and inter-specific genetic distances among 16 accessions of *Mangifera* sp. sequences based on ITS sequence data

	Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	Xoai Tu Quy XTQ1	1.0000																				
2	Xoai Cat Chu XCC1	0.8871	1.0000																			
3	Xoai cat Chu XCC2	0.8898	0.9174	1.0000																		
4	Xoai Dai Loan XDL1	0.8788	0.8815	0.9008	1.0000											max intra-similarity:			0.9656			
5	Xoai Dai Loan XDL2	0.8691	0.8609	0.8829	0.9132	1.0000																
6	Xoai Hoa Loc XHL9	0.8678	0.8430	0.8567	0.8650	0.8802	1.0000															
7	Xoai Hoa Loc XHL10	0.8678	0.8595	0.8678	0.8843	0.8774	0.9174	1.0000														
8	Xoai Hoa Loc XHL11	0.8567	0.8512	0.8815	0.8733	0.8747	0.8678	0.9036	1.0000													
9	Xoai Hoa Loc XHL12	0.8774	0.8747	0.8664	0.8829	0.8733	0.8884	0.9242	0.8967	1.0000												
10	Xoai Hoa Loc XHL13	0.8733	0.8567	0.8815	0.8788	0.8829	0.8788	0.8898	0.9201	0.8967	1.0000											
11	Xoai Hoa Loc XHL14	0.8802	0.8609	0.8994	0.8802	0.8788	0.8664	0.8774	0.9160	0.8843	0.9656	1.0000										
12	Xoai Hoa Loc XHL15	0.8774	0.8306	0.8664	0.8636	0.8705	0.8719	0.8774	0.9022	0.8926	0.9050	0.9174	1.0000									
13	Xoai Hoa Loc XHL16	0.8691	0.8361	0.8581	0.8636	0.8512	0.8554	0.8609	0.8829	0.8760	0.8884	0.8981	0.9118	1.0000								
14	Xoai Hoa Loc XHL17	0.8623	0.8430	0.8650	0.8650	0.8526	0.8650	0.8760	0.8926	0.8802	0.8898	0.8912	0.8939	0.9380	1.0000							
15	Xoai Thai XT1	0.8402	0.8347	0.8512	0.8567	0.8636	0.8540	0.8512	0.8678	0.8774	0.8815	0.8884	0.8774	0.8719	0.8733	1.0000						
16	Xoai Coc XC2	0.8650	0.8457	0.8595	0.8678	0.8554	0.8485	0.8595	0.8540	0.8609	0.8650	0.8691	0.8719	0.8499	0.8623	0.8540	1.0000					
17	Xoai Buoi XB1	0.8526	0.8416	0.8581	0.8526	0.8623	0.8581	0.8526	0.8664	0.8567	0.8664	0.8650	0.8871	0.8485	0.8554	0.8581	0.8994	1.0000				
18	Xoai Buoi XB2	0.8595	0.8457	0.8595	0.8623	0.8747	0.8512	0.8595	0.8623	0.8554	0.8760	0.8774	0.8967	0.8609	0.8595	0.8595	0.8981	0.9242	1.0000			
19	Xoai Xiem Num XXN1	0.8457	0.8264	0.8375	0.8320	0.8581	0.8430	0.8512	0.8595	0.8526	0.8457	0.8471	0.8636	0.8581	0.8512	0.8320	0.8650	0.8774	0.8871	1.0000		
20	Xoai Thanh Ca XTC1	0.8457	0.8402	0.8457	0.8347	0.8609	0.8540	0.8623	0.8540	0.8636	0.8595	0.8609	0.8802	0.8499	0.8567	0.8375	0.8595	0.8774	0.8815	0.9118	1.0000	
21	Xoai Thanh Ca XTC2	0.8485	0.8320	0.8430	0.8347	0.8444	0.8320	0.8485	0.8375	0.8526	0.8512	0.8526	0.8609	0.8499	0.8512	0.8320	0.8540	0.8691	0.8871	0.9008	0.9366	1.0000

Fig. 7: Similarity matrix of intra and inter-group among 21 samples of *Mangifera* sp. in the study from Southern Vietnam based on data of 22 studied SCoT primers

Number in red colour: Intra-similarity coefficient

Xiem Num and Xoai Thanh Ca were on separately branches using ITS analysis while they were clustered in the SCoT phylogenetic relationship. The significant difference between these two phylogenetic trees inferred from two molecular methods raised important questions about the reason for this contradiction and which method we should use for mango identification. For better understanding, we performed further tests in which some primers were randomly excluded from the SCoT primer set during the analysis. The outcomes showed completely different results in structure and arrangement of relationship branches on different SCoT trees using different primer sets. This result indicated the dependence of SCoT analysis on the number and component of SCoT primers used in different research.

The similarity analysis of the genetic distance between taxa is often inferred from examining certain characters. The higher the number and the more stable of characters, the higher reliability of the analysis. Genetic relationships are inferred from molecular markers in the genome. Like other PCR-based techniques, in SCoT, each DNA band locus is a character[1,5,7]. In the analysis of 22 primers, a total of 305 bands could be considered as 305 characters for similarity comparison among individuals. However, these band characters changed when the primer sets changed as mentioned above. Meanwhile, for the barcoding technique, since a short specific DNA sequence of an organism is examined, each type of nucleotide is one type of character. Moreover, to some researchers, the insertion and deletion in the DNA sequence are also considered as the fifth type of

character[29]. Hence every nucleotide site is a character, the length of the studied sequence contains the number of analyzed characters with five different states for each character. In this study, the obtained ITS fragment contained 664 bp of nucleotide sequence, which was corresponding to 664 analyzed characters. The ITS alone might create more considerable characters than a combination of 22 SCoT primer markers.

Besides, since the amplification of the barcoding region is performed by a primer pair from two ends of a determined target sequence, both repeatability and stability are very high, even when different primer pairs are used[31,30]. Due to both high genetic diversity and consistent amplification, ITS is a barcoding region that has been selected and used by many previous studies on the identification and investigation of the relationship among a group of taxa on many different subjects[31-33]. On the contrary, the SCoT product is amplified from a non-specific single primer[4-8], so the appearance of bands will also vary significantly among other taxa and components of primer sets. In another way, from the practical study, we also found that the reliability of the SCoT technique also depended on subjectively reading the bands. Although only clear and repeatable bands were recorded, the ignoring of fuzzy bands also affected character analysis. Hence relatedness among cultivars based on SCoT data was not stable.

Even though, it is worth noting that, despite the changing of primer set and the phylogenetic tree arrangement, all the samples of the same cultivar were always clustered together, while still having certain intra-differences as mentioned above. Moreover, the SCoT primers are designed around the start codon ATG, amplification products are expected to contain coding fragments of the genome and hence directly concern with the quality and quantity traits of organisms[34-36]. We suggested the combined use of both barcoding and SCoT techniques in the discrimination of taxa at both group-level and individual-level. Besides, while barcoding can identify individuals even from unknown samples, SCoT as some other PCR-based techniques, can only identify taxa in a certain known group of taxonomy. Hence their consonance may give better effects on optimal molecular identification, especially when combined with morphological studies in searching for individuals with desirable traits.

CONCLUSION

Our study provided useful molecular information for the classification of mango groups in the world sequence library, which is still limit and should be further elucidated to increase accuracy. Among studied barcoding regions, ITS was the best with high nucleotide polymorphisms and had a high potential for the identification of mango cultivars. Based on this sequence, many cultivars could be quickly recognized using specific single nucleotide polymorphisms. As for the SCoT technique, it should not be used as species identification but as a marker for the separation of taxa at the individual level. For the optimal applications, SCoT should be used in combination with a sequence-based marker, such as ITS, for the management of diversity as well as conservation and development of sources of delicious, nutritious and high-value mango varieties.

SIGNIFICANCE STATEMENT

This study provides useful molecular information that can be beneficial for the identification of mango cultivars in Vietnam. This study will help the researcher to uncover the advantages and disadvantages of the SCoT technique. Thus, a new theory on the combination of SCoT and barcoding technique may be arrived at.

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