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Research Article
Morphological Characteristics and Genetic Relations of the Star Apple Varieties (*Chrysophyllum cainito* L.)

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Abstract

Background and Objective: Identification of individual fruit plants is really necessary in the free trade context. Application of barcoding DNA is regarded as one of the innovations in plant sciences. This study aimed to identify four varieties of star apples: 'Lo Ren', butter, purple butter and purple by using some of their morphological characteristics and DNA markers. **Materials and Methods:** The morphological characteristics including leaves, fruits, flowers and seeds of four-star apple varieties were compared Six DNA barcodes including ITS, *matK*, *rbcL*, *atpF-atpH* and *ycf1b* were utilized for their genetic analysis. The ISSR marker with four primers (ISSR03, ISSR13, UBC841 and IISRS 3G) was then used to discriminate them. DNA sequences were aligned and clustered using Mega software and the ISSR gel images were converted to binary data and analyzed the similarity using NTSYSpc 2.1 program under the UPGMA algorithm. **Results:** The results showed that the color of the matured fruits was an efficient distinguishable morphological feature, while the sequences of examined regions did not differ. By analyzing PCR products, it could be inferred that ISSR03 primer played a key role in the identification of purple variety among the others and UBC841 could be used to distinguish purple butter and purple ones. Although the remaining primers (ISSR13 and IISRS 3G) could not identify individual varieties, they were capable of dividing them into two groups: 'Lo Ren', purple and purple butter, butter star apples. **Conclusion:** The findings indicated that there was a complete conservation in the DNA sequences of ITS, *matK, rbcL, atpF-atpH* and *ycf1b* regions.

Key words: *Chrysophyllum cainito* L., morphology, ISSR, 'Lo Ren' star apple, DNA barcode, DNA sequencing

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

INTRODUCTION

Star apples, whose scientific name is *Chrysophyllum cainito* L., belong to the family Sapotaceae; they originated in the tropical regions of America, mainly in Antilles and Mexico. They are imported and planted in Sri Lanka, India, Thailand, the Philippines and Vietnam. In Vietnam, they are grown widely in the Mekong Delta such as Tien Giang, Dong Thap, Ben Tre, Can Tho and Ca Mau provinces[1]. Star apples are very popular throughout the tropics as fruit and ornamental plant. Their peels and leaves are used as drugs to treat laryngitis, pneumonia, cough, rheumatism and diabetes. Besides, star apple leaves also contain some chemical compositions (alkaloids, flavonoids, phenols, sterols and triterpenes)[2,3].

The value of star apples has risen in recent times as they are being exported to the US. Therefore, the work of selecting breeds is being given more attention to overcome the susceptibility of plants to diseases, low productivity, short life expectancy and especially, the difficulty of identifying the different varieties. Thus, improving crop yield and quality as well as selecting good pure lines for genetic resource conservation and development is essential. However, this work requires a huge database based on the combination of breeding methods and molecular techniques.

Up until recently, these plant samples were often identified by external morphological characteristics, so the biological classification at the species level based on morphological characteristics is facing many difficulties, especially for groups of species with close genetic relationships that carry high homologous morphological characteristics. Around the world, many studies have used molecular biology techniques to identify fruit trees. In 2014, Jennifer *et al.*[4] used molecular markers to assess the domestication of fruit trees found in Central and South America to observe the level of genetic diversity or the use of sequences of barcode DNA to identify species of the Sapotaceae family from Atlantic forests[5]. Incorporating ISSR molecular markers has been used successfully to evaluate the genetic diversity of wild berries[6], Indian cashew nuts[7] and *Citrullus colocynthis*[8]. However, there have not been many studies on morphological characteristics of the genetic relationship between star apple varieties. This study was conducted to address these aspects. Moreover, the study aimed to gather information to create a seed management system based on molecular biology techniques.

MATERIALS AND METHODS

Study area: The study was conducted from September, 2019 to August, 2020.

Materials: Morphological research materials constituted samples of star apple varieties *Chrysophyllum cainito* L. collected from farms and gardens in Can Tho, Tien Giang, Vinh Long and Ben Tre provinces in Table 1.

Equipment and chemicals

Equipment: Eppendorf 5417C centrifuge, electronic scales, microwave oven, vacuum, electrophoresis, BioRad UV 2000 gel reading and imaging machine and Perkin Elmer 9700 PCR machine.

Chemicals: EB buffer solutions, SDS (sodium dodecyl sulfate), β-mercapto-ethanol, isopropanol, CTAB, chloroform, TE buffer, loading buffer, *Taq* polymerase, dNTPs and primers were used expressed in Table 2.

Research methods

Observation of morphological characteristics: The sampling method was carried out according to Aguirre-Dugua *et al.*[14]. Three to four-year-old trees were chosen. Mature leaves from two different branches of a tree were collected, while fruits of

Table 1: List of collected samples

			Coordinates	
Name	Symbol	Location of sampling	N	E
'Lo Ren' star apple	'LO REN'1	Southern Horticultural Research Institute, Chau Thanh, Tien Giang, Vietnam	10°23'50.68"	106°16'47.15"
	'LO REN'2	Cai Be Garden, Tien Giang, Vietnam	10°22'18.79"	105°56'47.35"
	'LO REN'3	Chau Thanh Farm, Tien Giang, Vietnam	10°24'03.37"	106°13'43.25"
Butter star apple	BUTTER1	Chau Thanh Farm, Tien Giang, Vietnam	10°24'03.37"	106°13'43.25"
	BUTTER2	Phong Dien garden, Can Tho, Vietnam	9°59'48.36"	105°40'07.18"
	BUTTER3	Binh Minh garden, Vinh Long, Vietnam	10°03'22.16"	105°50'48.19"
Purple butter star apple	PURPLE BUTTER1	Chau Thanh Farm, Tien Giang, Vietnam	106°13'43.25"	106°13'43.25"
	PURPLE BUTTER2	Cai Be Garden, Tien Giang, Vietnam	10°22'18.79"	105°56'47.35"
Purple star apple	PURPLE	Phong Dien Garden, Can Tho, Vietnam	9°59'48.36"	105°40'07.18"

Table 2: Primers used in the study

Sequence region	Name of the primer	Sequences (5'-3')	References	Amplified product length (bp)
ITS	ITS1	TCCGTAGGTGAACCTGCGG	White *et al.*[9]	500-700
	ITS4	TCCTCCGCTTATTGATATGC		
matK	*matK*-390F	CGATCTATTCATTCAATATTTC	Sun *et al.*[10]	100-900
	matK-1326R	TCTAGCACACGAAAGTCGAAGT		
atpF-atpH	*atpF*	ACTCGCACACACTCCCTTTCC	Vijayan and Tsou[11]	196-573
	atpH	GCTTTTATGGAAGCTTTAACAAT		
rbcL	*rbcL*-aF	ATGTCACCACAAACAGAGACTAAAGC	Wang *et al.*[12]	550-600
	rbcL-aR	GTAAAATCAAGTCCACCRCG		
ycf1b	*ycf1b*-F	TCTCGACGAAAATCAGATTGTTGTGAAT	Dong *et al.*[13]	909-962
	ycf1b-R	ATACATGTCAAGTGATGGAAAA		

ITS: Internal transcribed spacer, bp: Base pair

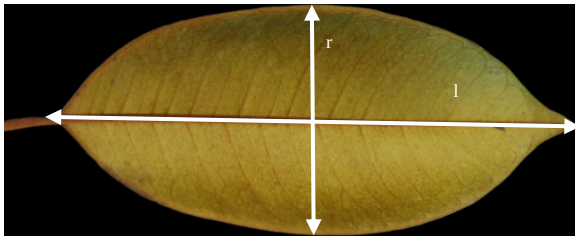

Fig. 1: Measuring the leaf size
l: Length of leaf, r: Width of leaf

Fig. 2: Measuring the diameter of the fruit and the thickness of the shell
d: Diameter of fruit, m: Thickness of skin

all different stages (from raw to ripe) were included. Description of plant morphology (leaves, fruits and seeds) was based on the method of Inyama *et al.*[15] with some modifications. Some characteristics were measured: The length from the tip of the blade to the tip and the width of the leaf (Fig. 1).

The outer shape of the fruits was photographed and cut in half to measure the diameter and thickness of the shell (Fig. 2). The sweetness of the fruit was measured by a refractometer. The seeds were photographed of old seeds taken from ripe fruits, the particle size was measured with a clip of the length of the grain calculated from the two longest ends of the grain, the width and thickness of the seeds were measured between particles.

DNA sequencing of ITS, *matK*, *rbcL*, *atpF-atpH* and *ycf1b* regions: With the help of silk leaves (about three to four from the buds), DNA of the varieties was extracted following the CTAB procedure of Rogers and Bendich[16]. DNA test after extraction was checked by electrophoresis on 0.8% agarose gel.

The sequence of ITS, *matK, rbcL, atpF-atpH* and *ycf1* were amplified by the PCR technique. DNA samples of the star apple varieties after extraction and electrophoresis to check the quality and purity of DNA were used to perform the PCR reaction as shown in Table 3. DNA sequences were compared for similarity with the BLAST program on the NCBI database. The sequences were checked and aligned with ClustalW Multiple Alignment algorithm[17] using the BioEdit software.

ISSR markers: The PCR reaction was performed with four ISSR primers synthesized by Phu Sa Biochemical Company. The primers have been listed in Table 4.

The reaction mixture with a volume of 25 µL included 12 µL BiH_2O, 10 µLPCR master mix, 1 µL primer (10 pmol) and 2 µL DNA template (20 ng μL^{-1})

The PCR reaction was performed with a thermal cycle of 94°C for 4 min; 35 cycles at 94°C for 1 min; 50°C for 4 sec, 7°C for 2 min; 72°C for 7 min. The PCR product was then electrophoresis on 2% agarose and photographed under UV light.

Table 3: PCR reaction cycles

Amplification sequence	Temperature, initial denaturation time	30 cycles			Final extension	Holding
		Denaturation	Annealing	Elongation		
ITS	95°C	95°C	55°C	72°C	72°C	10°C
	5 min	30 sec	30 sec	1 min	7 min	30 min
matK	94°C	94°C	50°C	72°C	72°C	
	1 min	30 sec	40 sec	40 sec	5 min	
atpF-atpH	94°C	94°C	51°C	72°C	72°C	
	4 min	30 sec	40 sec	40 sec	5 min	
rbcL	94°C	94°C	55°C	72°C	72°C	
	4 min	30 sec	30 sec	1 min	10 min	
ycf1b	94°C	94°C	52°C	72°C	72°C	
	4 min	30 sec	40 sec	1 min	10 min	

ITS: Internal transcribed spacer

Table 4: Names and sequences of ISSR primers used

Name of the primer	Sequence	References
ISSR03	(AG)8YT	Karuppanapandia *et al.*[18]
ISSR13	(CT)8RA	Karuppanapandia *et al.*[18]
UBC 841	(GA)8CTC	Samriti *et al.*[19]
IISRS 3G	(GTG)5	Samriti *et al.*[19]

Statistical analysis: The data of the study were recorded in binary form in which the appearance of DNA fragments and the absence of DNA fragments were observed. After that, these data were analyzed by subgroups based on Jaccard similarity coefficient, UPGMA type on NTSYS pc 2.10 software[20].

RESULTS AND DISCUSSION

Morphological characteristics: The analysis from the research samples of four-star apple varieties: 'Lo Ren', butter, purple butter and purple, the common characteristics of the research varieties was recorded as shown in Table 5.

Leaves including single, oval, staggered, entire margin, petiole, average length from 7.5-15.7 cm and the width 5.3-8.0 cm. There are variations in leaf shape and size on the same tree such as young leaves are closed, smaller and less fluffy than mature leaves; when mature leaves open, they are brown on the underside and are lighter than young leaves and the leaf veins. The leaves of star apple 'Lo Ren' are usually smaller in size than that of other star apple varieties (10-13 cm length and 5.3-6.6 cm width). The leaves of Butter star apple (Fig. 3a) and purple butter star apple (Fig. 3b) are more oval than those of 'Lo Ren' star apple (Fig. 3c). However, the size of the leaves also changes due to planting and tending conditions. If good care has been provided to the leaves, they will be bigger and on the contrary.

The diameter of star apple fruit ranges between 6.0 and 9.1 cm depending on the shape which ranges from round to that of an egg or a cone. 'Lo Ren' star apple has a round shape whereas butter star apple and purple star apple are round but slightly flattened and their diameter is bigger than that of 'Lo Ren' star apple; purple star apple is slightly round in the stem and its fruit is the smallest of all (6-7.1 cm) (Fig. 3d). The most distinguishable morphological feature in the studied samples is the color of the ripe fruit. 'Lo Ren' star apple is ripe when its fruit is pinkish or light purple, while butter star apple is milky or pink, purple butter star apple and purple star apple change from purple into charcoal purple. Usually, purple butter star apples ripen from the lower part of the fruit and finally to the stems; purple star apples ripen slowly changing the color of the whole fruit.

The thickness of the shell of the fruit also varies across varieties. When ripe, the star apple's shell is 0.3-1.1 cm thick; depending on the ripeness of the fruit, the thickness of the shell changes. In this study, the shell of 'Lo Ren' and purple butter skin was thicker than that of star apple butter, whereas the thinnest shell belonged to purple star apple.

Each fruit has three to 10 seeds that are flattened; each grain has a pointed tip, smooth, brown protein, white scar, length under the description of Inyama *et al.*[15]. The seeds were 1.7- 2.3 cm in length, 1.1-1.5 cm in width and 0.6-0.9 cm in thickness. The seeds of purple star apple are dark in color on the back and have a thin fringe. Additionally, its seeds are thicker but shorter, while the edges are full and the back is white. When cutting across the surface of the fruit, it is obvious that there was a star. The number of wings is just about 7-11 cm, thus, 'Lo Ren' and butter star apple had the same number of wings which were more than that of purple butter and purple stat apple in Table 5.

Star apple's sweetness ranged from 8-12 Brix°. Among these varieties, 'Lo Ren' star apple was the sweetest compared to the other three-star apple varieties; the purple star apple was the least sweet (Table 5).

Fig. 3(a-d): Leaves and fruit of star apple varieties
(a) Butter' star apple, (b) Butter purple star apple, (c) Loren' star apple and (d) Purple star apple

Table 5: Some morphological characteristics of four star apple varieties

Star apple varieties	Average measure	'Lo Ren'	Butter	Purple butter	Purple
Leaves (cm)					
Length	7.5-15.7	10-13	7.5-15.7	7.5-15.7	7.5-15.7
Width	5.3-8.0	5.3-6.6	5.5-8.0	5.5-7.9	5.5-7.9
Fruit (cm)					
Diameter	6.0-9.1	6.8-8.2	7.6-9.3	7.5-9.1	6.0-7.1
Thickness of shell	0.3-1.1	0.8-1.1	0.3-1.0	0.5-1.1	0.5-0.7
Seed (cm)					
Length	1.7-2.3	1.7-2.1	1.8-2.0	1.8-2.2	1.8-2.3
Width	1.1-1.5	1.1-1.3	1.2-1.5	1.2-1.5	1.2-1.4
Thickness	0.6-0.9	0.6-0.8	0.7-0.9	0.6-0.8	0.6-0.9
Number of wings	7-11	10-11	10-11	9-10	7-9
Sweetness (°Brix)	10-14	11-14	10-13	9-14	8-12

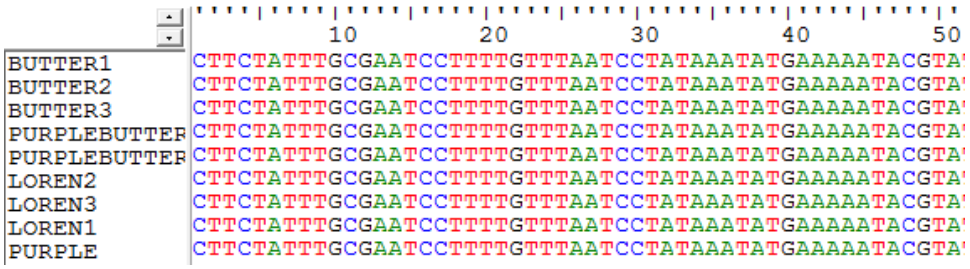

Fig 4: A sequence of nucleotide sequences of four-star apple varieties: butter purple, 'Lo Ren' and purple star apple varieties

Table 6: Ratios of four sequence regions

Sequence region	Size (bp)	Proportion (%)						Similar sequence
		Adenine	Thymine	Cytosine	Guanine	AT	GC	
ITS	678	20.21	22.57	27.73	29.50	42.77	57.23	100.00
matK	667	36.58	30.43	15.44	17.54	76.02	32.98	99.85
rbcL	534	27.15	28.65	21.54	22.66	55.81	44.19	100.00
atpF-atpH	551	30.85	37.39	13.43	18.33	68.24	31.76	99.07

Analysis result of the sequence regions including *ITS*, *matK*, *rbcL*, *atpF-atpH*

Genetic characteristics of star apple

Amplification and sequencing: The samples were amplified on five genomic regions including ITS, *matK, rbcL, atpF-atpH* and *ycf1b*. The amplification results reached 100% on the four regions such as ITS, *matK, rbcL* and *atpF-atpH*. In particular, the *ycf1b* region showed poor PCR results with many sub and weak bands. The product size of the sequence regions obtained including ITS 678bp, *matK* 667bp, *rbcL* 534 bp and *atpF-atpH* 551 bp.

All samples of each sequenced region show the same result. The length of these regions ranged from 534-678 bp. In the ITS region, AT proportion was lower than the GC proportion while there was an adverse trend in the remaining regions (*matK*, *rbcL* and *atpF-atpH*) (Table 6).

When comparing the sequence using BLAST (Basic Local Alignment Search Tool) on NCBI (National Center for Biotechnology Information), the ITS sequence area of the samples of the four varieties shows that the similarity in this sequence area is 100% with *Chrysophillum cainito* species. This result indicated that the ITS region is highly conservative between such *Chrysophillum* varieties in Viet Nam and other *C. cainito*. Furthermore, although it is highly inter specific; however, this loci is a failure to distinguish closely species[21,22]. In terms of matK gene, in comparison with the current Genebank database, the similarity of a nucleotide sequence of these four-star apple varieties to other *Chrysophillum* spp. is 99.85%. The sequences have the highest similarity with 99.85% with the sequences of *Chrysophyllum oliviforme* (MH621509.1), *Chrysophyllum argenteum* (JQ626548)[23], *Chrysophyllum brenesii* (JQ589176) and *Chrysophyllum cainito* (GQ981966)[24]. Based on such data, this chloroplast gene showed a great discriminatory power for species identification. Species resolution of the matK gene was also demonstrated in several land plants[25] and recently, matK was recorded for successful authentication of *Fritillariae* species from its adulterants[26]. Moreover, the *rbcL* sequences are 100% homologous to the sequences of *Chrysophyllum oliviforme* (MH549779), *Chrysophyllum argenteum* (FJ038161)[27], *Chrysophyllum inornatum* (MG718069)[28] and *Chrysophyllum splendens* (JQ413832)[29] on Genebank. Although rbcL was universal and high sequence quality, this loci was invaluable to identify *Chrysophillum* species. The performance of all rbcL was remarkably low in few species-rich clades, such as the Laureae and the Sapotaceae[27]. Therefore, a combination of matK and rbcL is a potential solution to increase species discrimination[29]. Similarly, the sequences of *atpF-atpH* regions have the highest similarity with 99.07% with the sequence code MN295595 of *Manilkara zapota* species on Genebank. Currently, there was no atpF-atpH sequence of star apple on the Genebank database, so it is impossible to compare with sequences.

Thus, the analysis results of five regions of ITS, *matK, rbcL, atpF-atpH* and *ycf1*b showed that the sequence of nucleotides among the four-star apple varieties was not different (Fig. 4). Figure 4 indicated that there was no variant found in the alignment result; thus, four samples were selected for further analysis using ISSR marker as an indicator.

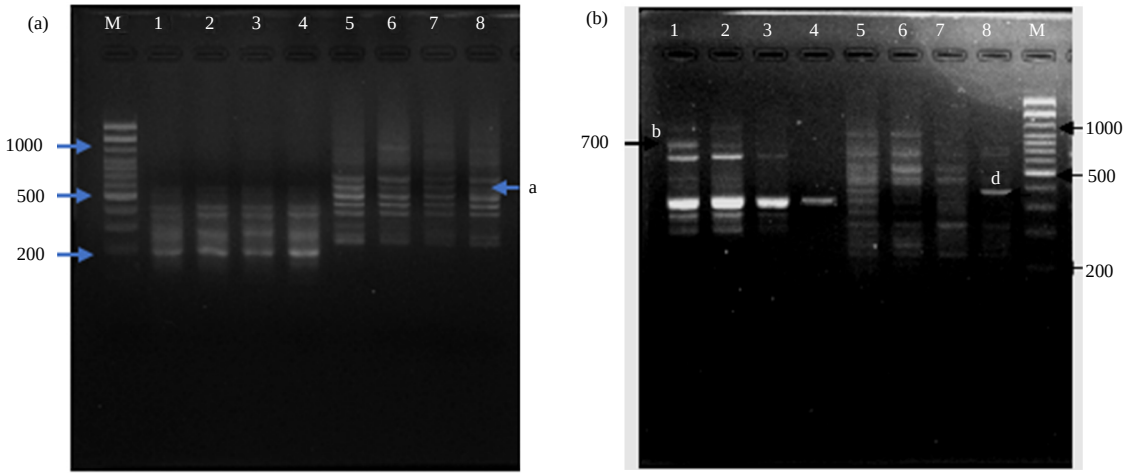

Fig. 5(a-b): (a) Electrophoresis of PCR product with ISSR03 primer and (b) Electrophoresis of PCR product with UBC841 primer
(a) Lane 1: 'Lo Ren', Lane 2: Butter, Lane 3: Purple butter, Lane 4: Purple star apple, Lane 5: Priming IISRS 3G on 'Lo Ren', Lane 6: Butter, Lane 7: Purple butter and Lane 8: Purple compared DNA standard scale 100 bp (lane M)
(b) Lane 1: 'Lo Ren' star apple, Lane 2: Butter star apple, Lane 3: Purple butter star apple, Lane 4: Purple star apple, Lane 5: ISSR13 on 'Lo Ren' star apple, Lane 6: Butter star apple, Lane 7: Purple butter star apple, Lane 8: Purple star apple compared to the standard 100 bp (lane M)

Table 7: Four primers and their amplification results

		Polymorphic bands		
Primers	Total bands	Number	Frequency (%)	Size (bp)
ISSR03	4	0	0.0	200-450
ISSR13	10	3	30.0	150-900
UBC841	13	11	84.6	200-700
IISRS 3G	9	2	22.2	250-800
Total	36	16	44.4	

Analysis results of ISSR indicator polymorphism: Results of analyzing the genetic relationship among star apple varieties. Through the analysis, it was found that all four primers were used for good amplification products. An anchor at 3'end of primers with non-motif nucleotides increased the band resolution as well as reduced non-specific amplicons[30,31]. A total of 36 bands were recorded with molecular fragment sizes ranging from 150-900 bp in Table 7.

The ISSR03 primer for the product at least amplified four DNA fragments of a molecular size between 200 and 450 bp with a DNA standard scale of 100 bp (Fig. 5a-lanes 1, 2, 3 and 4). All the analysis samples gave the same amplified product. Therefore, this primer cannot identify star apple varieties: 'Lo Ren', butter, purple butter and purple star apple.

The IISRS 3G primer provided the product with amplification of at least nine molecular-sized DNA fragments in the range of 250-800 bp with a DNA standard scale of 100 bp (Fig. 5a-lanes 5, 6, 7 and 8). The analytical samples exhibited polymorphism and recognized the difference between purple star apple (lane 8) and the other three varieties: 'Lo Ren', butter and purple butter star apple (lanes 5, 6, 7). Three-star apple varieties of 'Lo Ren', butter and purple butter gave the DNA amplification product at a molecular size of about 650 bp (symbol a), while purple star apple did not allow the DNA amplification product in position. Thereby, this primer can be used to distinguish purple star apple from the three-star apple varieties 'Lo Ren', butter and purple butter.

The primer UBC841 gave the product a minimum amplification of 13 DNA fragments of molecular size in the range of 200-700 bp with a DNA standard scale of 100 bp (Fig. 5b-lanes 1, 2, 3 and 4). The analyzed samples showed that four-star apple varieties were divided into two groups: 'Lo Ren' and butter (lanes 1, 2) were the same; different purple butter and purple breast groups (lanes 3, 4). Since then, this primer could not identify 'Lo Ren' and butter but could distinguish this star apple from purple butter and purple because the amplification of 'Lo Ren' and butter for the DNA amplification product in position, size of 700 bp (symbol b), while two-star apple varieties: butter and purple butter do not amplify products in this position. Besides, this primer was able to distinguish purple butter and purple because purple star apple only amplifies the product at a single position of about 300 bp (symbol c).

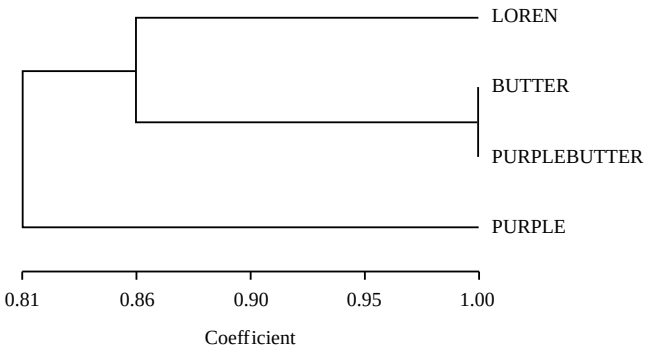

Fig. 6: Phylogenetic relation of four-star apple varieties: 'Lo Ren', butter, purple butter and purple

Similarly, the ISSR13 primer for the product amplifies a minimum of 10 DNA fragments of a molecular size between 150 and 900 bp with a DNA ladder of 100 bp (Fig. 5b-lanes 5, 6, 7 and 8). The samples analyzed were all amplified products divided into two groups: 'Lo Ren' and purple star apple (lanes 5 and 8), same butter and purple butter star apple group (lanes 6 and 7). Therefore, this primer could not identify the varieties of 'Lo Ren', butter, purple butter and purple star apple but could distinguish the varieties of 'Lo Ren' and purple from butter star apple and purple butter because 'Lo Ren' star apple and purple had the product which amplified at a position of about 390 bp (symbol d) whereas butter star apple and purple butter star apple did not have.

The primers in this study gave amplified products with a lower ratio of polymorphic bands than those of other primers on other plant species. The ISSR03 primer did not produce polymorphic bands, whereas in black gram studies of the polymorphic band was 100% and the ISSR13 primer gave polymorphic bands at 50%[18]. The ISSR UBC841 on Dendrobium cultivars has a polymorphic band ratio of 100%[21]; in this study, the percentage of polymorphic band is lower than 84.6%.

The genetic similarity of the four-star apple varieties was recorded based on the polymorphic polymorphism amplified by four primers and had similar ratios ranging from 0.71-0.85 (Fig. 6). The phylogenetic tree showed that all four-star apple varieties had a very close genetic relationship. Based on the tree diagram showing the DNA polymorphism among the four-star apple varieties in these studies, found that the survey of star apple varieties was divided into two large groups: the first group (the cluster at 0.81) had only one of the purple star apple varieties, whereas the second (the cluster at 0.86) is the remaining varieties divided into two subgroups at step I. The first subgroup at step I is the 'Lo Ren' star apple. The second subgroup at step I is two varieties: butter star apple and purple star apple that will help us manage our genetic resources and make the most of this resource and have a sufficient scientific basis. ISSR marker war a useful and valuable tool for genetic variation and genotyping; this technique may be used to obtain reasonable information on the genetic relationship among plant genotypes[31]. This will further help to prove the national ownership of biological resources when Vietnam joined AFTA and international trade organizations.

CONCLUSION

On analyzing the research samples of the star apple varieties, it has been observed that the biggest difference among the four varieties ('Lo Ren', butter, purple butter and purple star apple) was the various colors of fruits when they ripen. There was complete conservation in the DNA sequences of ITS, *matK, rbcL, atpF-atpH* and *ycf1b* regions. ISSR markers showed some differences in DNA profiles among star apple varieties.

SIGNIFICANCE STATEMENT

This study discovered the morphological and genetic characteristics of Vietnamese star apple varieties that can be beneficial for original identification. This study will help the researchers to uncover the critical areas of plant barcoding DNA that many researchers were not able to explore. Thus a novel method may be arrived at.

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REFERENCES

1. Hau, V.D. and T.P. Le, 2020. *Chrysophyllum cainito*: A tropical fruit with multiple health benefits. Evid. Based Complementary Altern. Med., Vol. 2020. 10.1155/2020/7259267.
2. Shailajan, S. and D. Gurjar, 2014. Pharmacognostic and phytochemical evaluation of *Chrysophyllum cainito* Linn. leaves. Int. J. Pharm. Sci. Rev. Res., 26: 106-111.
3. Oranusi, S.U., W. Braide and R.U. Umeze, 2015. Antimicrobial activities and chemical compositions of *Chrysophyllum cainito* (star apple) fruit. Microbiol. Res. Int., 3: 41-50.
4. Petersen, J.J., I.M. Parker and D. Potter, 2014. Ten polymorphic microsatellite primers in the tropical tree caimito, *Chrysophyllum cainito* (Sapotaceae). Appl. Plant Sci., Vol. 2. 10.3732/apps.1300079.
5. Vivas, C.V., R.C.S. Moraes, A.A. Araújo, M. Alves, E.M. Neto, C. Van den Berg and F.A. Gaiotto, 2014. DNA barcoding in Atlantic forest plants: What is the best marker for *Sapotaceae* species identification? Genet. Mol. Biol., 37: 662-670.

6. Cekic, C., N.H. Battey and M.J. Wilkinson, 2001. The potential of ISSR-PCR primer-pair combinations for genetic linkage analysis using the seasonal flowering locus in Fragaria as a model. Theor. Appl. Genet., 103: 540-546.
7. Archak, S., A.B. Gaikwad, D. Gautam, E.V.V.B. Rao and K.R.M. Swamy *et al*., 2003. DNA fingerprinting of Indian cashew (*Anacardium occidentale* L.) varieties using RAPD and ISSR techniques. Euphytica, 130: 397-404.
8. Verma, K.S., S. Ul-Haq, S. Kachhwaha and S.L. Kothari, 2017. RAPD and ISSR marker assessment of genetic diversity in *Citrullus colocynthis* (L.) Schrad: a unique source of germplasm highly adapted to drought and high-temperature stress. 3 Biotech., 10.1007/s13205-017-0918-z.
9. White, T.J., T.D. Bruns, S.B. Lee and J.W. Taylor, 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: PCR Protocols: A Guide to Methods and Applications, Innis, M.A., D.H. Gelfand, J.J. Sninsky and T.J. White (Eds.). Academic Press, San Diego, CA., USA., ISBN-13: 9780123721808, pp: 315-322.
10. Sun, H., W. McLewin and M.F. Fay, 2001. Molecular phylogeny of *Helleborus* (*Ranunculaceae*), with an emphasis on the East Asian Mediterranean disjunction. Taxon, 50: 1001-1018.
11. Vijayan, K. and C.H. Tsou, 2010. DNA barcoding in plants: Taxonomy in a new perspective. Curr. Sci., 99: 1530-1541.
12. Wang, Q., Q.S. Yu and J.Q. Liu, 2011. Are nuclear loci ideal for barcoding plants? A case study of genetic delimitation of two sister species using multiple loci and multiple intraspecific individuals. J. Syst. Evol., 49: 182-188.
13. Dong, W.P., C. Xu, C.H. Li, J.H. Sun and Y.J. Zuo *et al*., 2015. *ycf1*, the most promising plastid DNA barcode of land plants. Scient. Rep. 10.1038/srep08348.
14. Aguirre-Dugua, X., E. Pérez-Negrón and A. Casas, 2013. Phenotypic differentiation between wild and domesticated varieties of *Crescentia cujete* L. and culturally relevant uses of their fruits as bowls in the Yucatan Peninsula, Mexico. J. Ethnobiol. Ethnomed., Vol. 9. 10.1186/1746-4269-9-76.
15. Inyama, C.N., F.N. Mbagwu and C.M. Duru, 2015. Morphological relationship among three *Chrysophyllum* species and their taxonomic implication. Med. Aromat. Plants, Vol. 4. 10.4172/2167-0412.1000201.
16. Rogers, S.O. and A.J. Bendich, 1988. Extraction of DNA from Plant Tissues. In: Plant Molecular Biology Manual A6, Gelvin, S.B. and R.A. Schilperoort (Eds.). Kluwer Academic Press, Dordrecht, The Netherlands, pp: 1-11.
17. Thompson, J.D., D.G. Higgins and T.J. Gibson, 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res., 22: 4673-4680.
18. Karuppanapandian, T., H.W. Wang, T. Karuppudurai, J. Rajendhran and M. Kwon *et al*., 2010. Efficiency of RAPD and ISSR markers in assessing genetic diversity and relationships in black gram (*Vigna mungo* L. Hepper) vari. Can. J. Plant Sci., 90: 443-452.
19. Samriti, R. Kaur, Shilpa, E.V. Malhotra, Poonam, D. Thakur and K. Kumar, 2017. Assessment of genetic diversity in *Rubusellipticus* (Smith) using molecular markers. Proc. Indian Natn. Sci. Acad., 83: 669-679.
20. Rohlf, F.J., 2000. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System. Exerter Publishing Ltd., New York.
21. Dharmarathna, T.D., P.A. Weerasinghe, H. Dinum and H. Venura, 2018. Simple sequence repeat markers for analysis of molecular diversity and genetic structure of eighteen *Dendrobium cultivars* in Sri Lanka. Inter. J. Inno. Edu. and Res., 6: 91-102.
22. Zhu, S., Z. Niu, Q. Xue, H. Wang, X. Xie and X. Ding, 2018. Accurate authentication of *Dendrobium officinale* and its closely related species by comparative analysis of complete plastomes. Acta Pharm. Sin. B, 8: 969-980.
23. Baraloto, C., O.J. Hardy, C.E.T. Paine, K.G. Dexter and C. Cruaud *et al*., 2012. Using functional traits and phylogenetic trees to examine the assembly of tropical tree communities. J. Ecol., 100: 690-701.
24. Kress, W.J., D.L. Erickson, F.A. Jones, N.G. Swenson, R. Perez, O. Sanjur and E. Bermingham, 2009. Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. Proc. Natl. Acad. Sci., 106: 18621-18626.
25. More, R.P., R.C. Mane and H.J. Purohit, 2016. matK-QR classifier: A patterns based approach for plant species identification. BioData Min., Vol. 9. 10.1186/s13040-016-0120-6.
26. Chen, Q., X. Wu and D. Zhang, 2020. Comparison of the abilities of universal, super and specific DNA barcodes to discriminate among the original species of Fritillariae cirrhosae bulbus and its adulterants. PLoS ONE, Vol. 15. 10.1371/journal.pone.0229181.
27. Gonzalez, M.A., C. Baraloto, J. Engel, S.A. Mori and P. Petronelli *et al*., 2009. Identification of Amazonian trees with DNA barcodes. PLoS ONE, Vol. 4. 10.1371/journal.pone.0007483.
28. de Lima, R.A.F., A.A. de Oliveira, G.D. Colletta, T.B. Flores and R.L.G. Coelho *et al*., 2018. Can plant DNA barcoding be implemented in species-rich tropical regions? A perspective from São Paulo State, Brazil. Genet. Mol. Biol., 41: 661-670.
29. Saadullah, Z.U.D. Khan, M. AShfaq and Z.U. Nisa, 2016. Evaluation of the discriminatory power of plant DNA barcodes rbcL & matK between species of Fabaceae. Int. J. Biosci., 8: 75-86.
30. Ng, W.L. and S.G. Tan, 2015. Inter-simple sequence repeat (ISSR) markers: Are we doing it right. ASM Sci. J., 9: 30-39.
31. Gelotar, M.J., D.T. Dharajiya, S.D. Solanki, N.N. Prajapati and K.K. Tiwari, 2019. Genetic diversity analysis and molecular characterization of grain amaranth genotypes using inter simple sequence repeat (ISSR) markers. Doc. Bull. Natl. Res. Cent., Vol. 43. 10.1186/s42269-019-0146-2.