



Comparison of five wax apples (*Syzygium samarangense*) from Dong Thap Province, Vietnam based on morphological and molecular data

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Abstract. Wax apple (*Syzygium samarangense*) is a popular species with high economic value in Vietnam and other countries in Asia. This research analyzed and compared morphological and molecular characteristics (ITS and trnL-F sequences) of 5 varieties of wax apple in Dong Thap Province, Vietnam, including of An Phuoc, Xanh Duong, Hong Dao, Hoa An, and Sua wax apples. The results revealed that five varieties of wax apples had a board range of morphological characteristics, especially in shape, color, and size of fruits. Furthermore, molecular analysis results showed that ITS and trnL-F sequences of four varieties of wax apples, including of Xanh Duong, Hong Dao, Hoa An, and Sua, were identical with 100 % similarity whereas An Phuoc wax apple had differences in one and five positions in ITS and trnL-F sequences as compared to other varieties. The phylogenetic tree was established by comparison of ITS sequences of five varieties in this study and other varieties in Genbank (NCBI) and showed that four varieties of wax apples (Xanh Duong, Hong Dao, Hoa An, and Sua) were 100 % match with two varieties grown in China (KC815987, KC800610) and one variety planted in Sri Lanka (MN104146). On the other hand, ITS sequence of An Phuoc wax apple was high similarity with another wax apple variety planted in Sri Lanka (MN104142).

Keyword: Wax apple, ITS, trnL-F, Phylogeny.

Introduction

The *Syzygium* genus belongs to the Myrtaceae family with about 1100 species.

Some edible species of this genus are grown in tropical regions over the world with a wide variety of colors of fruits from the pink/red to green.

However, colors of fruits of one species also vary from bright to dark depending on environmental conditions and cultivation practices [SHU *et al.*, 2001].

Among members of *Syzygium* genus, *S. samarangense* is a popular species in Southeast Asia region and has the potentially high economic value [LITTLE *et al.*, 1989].

This species originated from Philippine and is popularly grown in Malaysia, Indonesia, Thailand, Cambodia, Laos, Vietnam, and Taiwan [SHU *et al.*, 2006; LIM, 2012; ROSNAH *et al.*, 2012].

In Vietnam, *S. samarangense* is grown from Northern to Southern regions, because it is easy to grown and does not

require much care as compared to other edible plants.

Depending on the environmental conditions of geographic regions, this plant also has a variety of qualities of fruits, plant morphologies as well as the different names.

Accordingly, some names of this plant can be listed, such as Sua, Xanh Duong, An Phuoc, Hong Dao, Dieu Do, Dao Hue, Huyet, Hoa An wax apple.

Among them, An Phuoc wax apple, a species originated from Thoongsamri wax apple of Thailand, is commonly planted in Mekong Delta region [TON, 2000].

In Dong Thap Province, there are many wax apple varieties, not only popular varieties such as An Phuoc, Xanh Duong, Sua, Hong Dao wax apple, but also the particular variety of wax apple in Hoa An commune, Cao Lanh city, "Hoa An" wax apple.

These varieties re planted in a broad range of geographic regions of Dong Thap Province with a variety of fruit qualities and plant morphologies.



In this study, we compared for the first-time morphologies and genetic characteristics (via the ITS and trnL-F regions) of five varieties of wax apples, including of An Phuoc, Xanh Duong, Sua, Hong Dao, Hoa An of Dong Thap Province. Moreover, this study also provide more information to improve economic value of *S. samarangense*.

Material and methods

Materials

Leaves of five wax apples were collected from Dong Thap Province (Figure 1).

The collected sites were described in Table 1.



Figure 1. Five studied samples in habitat. A. An Phuoc wax apple, B. Hoa An wax apple, C. Hong Dao wax apple, D. Sua wax apple, E. Xanh Duong wax apple.

Table 1.

Detailed information of collected site of five wax apple in Cao Lanh City, Dong Thap Province

Code of Samples	Names	Collected sites
AP	<i>An Phuoc wax apple</i>	Ward 1
HD	<i>Hong Dao wax apple</i>	Phường Hòa Thuận ward
HA	<i>Hoa An wax apple</i>	Hòa An Commune
SU	<i>Sua wax apple</i>	Ward 11
XD	<i>Xanh Duong wax apple</i>	Tan Thuan Tay Commune,

Besides, ITS region of some species of *Syzygium* and other genus belonging to Myrtaceae from GenBank

were also used in phylogenetic analysis (Table 2).

Table 2.

Sequences from GenBank database used in this study

Taxa	Accession number	Taxa	Accession number	
<i>S. Samarangense</i>	KC815986	<i>Syzygium apodophyllum</i>	AY187173	
	KC815988	<i>Syzygium aqueum</i>	MN104136	
	KC815987	<i>Syzygium aromaticum</i>	MH101990	
	KC815989	<i>Syzygium campanulatum</i>	JF682811	
	KC815990	<i>Syzygium canicortex</i>	AY187183	
	KC800610	<i>Syzygium wilsonii subsp. cryptophlebium</i>	AY187217	
	MN104142	<i>Syzygium cumini</i>	MG730969	
	MN104146	<i>Syzygium diospyrifolium</i>	JF682813	
	MN104144	<i>Syzygium grande</i>	JF682814	
	MN104145	<i>Syzygium hancei</i>	KP093048	
	MN104143	<i>Syzygium nigrans</i>	JF682819	
	<i>Syzygium antisepticum</i>	JF682815	<i>Eugenia uniflora</i>	KM064994



Methods
Character coding method for phylogenetic analysis

In this research, we studied the morphological features and established the description for 5 varieties of wax apple.

Table 3.

Morphological characteristics of five wax apples varieties in this study

Characteristics	Hoa An	Xanh Duong	An Phuoc	Sua	Hong Dao
Leaf shape	Oblong	Oblong	Oval	Elliptical	Elliptical
Leaf apices	Acute	Acuminate	Acute	Acute	Acuminate
Leaf bases	Acute	Acute	Acute	Round	Round
Unripe fruit color	White–green	White– slightly green	Green–Red	White	White–green
Ripe fruit color	Slightly pink	Green	Red	White	Slightly pink
Fruit shape	Bell shaped	Triangle shaped	Bell shaped	Nearly round	Tri–oval shaped
Fruit weight	25–40 g	40–50 g	50–60 g	40–50 g	40–50 g
Fruit peel	Thin	Thick	Thin	Thick	Thick
Taste of ripe fruit	Sour and slightly sweet	Sweet	Sweet	Sweet and acid	Sweet
The number of seeds per fruit	Almost seedless, sometime 1–3 seeds/fruit	Seedless	Seedless	2–3 seed/fruit	Seedless
Seed shape	Oval	Seedless	Seedless	Nearly round	Seedless
Seed color	Dark brown	Seedless	Seedless	Brown	Seedless

In briefly, the morphological features were encoded with number 0 to 4, and established the matrix (Table 3 & 4).

The phylogenetic tree was subsequently established by PAUP* 4.0a146 [SWOFFORD 2002] using the neighbor–joining method.

Table 4.

Morphological codes of five wax apples varieties used in this study

Characteristics	Code of characteristics	Characteristics	Code of characteristics
Leaf shape	Oblong: 1; oval: 2, elliptical: 3	Fruit weight	30–40 gram: 1; 40–50 gram: 2; 50–60 gram: 3
Leaf apices	Acute: 1; acuminate: 2.	Peel	Thin: 1; thick: 2
Leaf bases	Acute: 1, round: 2	Taste of ripe fruit	Sour, slightly sweet: 1; sweet: 2; sweet and acid: 3
Unripe fruit color	White–green: 1, white–slightly green: 2; green–red: 3; white: 4	The number of seeds/fruits	Seedless: 1; Seedless but sometime 1–3 seeds/fruit: 2; 2–3 seeds/fruit: 3
Ripe fruit color	Slightly pink: 1; green: 2; red: 3; white: 4	Seed shape	Seedless: 1; oval: 2; nearly round: 3
Fruit shape	Bell–shaped: 1; triangle–shaped: 2; nearly round: 3; tri–oval shaped: 4	Seed color	Seedless: 1; dark brown: 2; brown: 3

Total genomic DNA extraction

Total genomic DNA was extracted from fresh leaf tissues via Gene Jet Plant Genomic DNA Purification Mini Kit (Thermo Fisher Scientific, USA) following the manufacture's instruction.

PCR amplification

The target ITS and trnL–F regions were amplified by polymerase chain reaction (PCR) using following barcoding primers (Table 5).

The PCR reactions were performed in an Mastercycler machine (Eppendorf, Germany) using a volume of 25 µL reaction mixture: 12.5 µL Go–Taq green master mix (Promega, USA), 1.25 µL of each forward and reverse primers (10 µM), 9.5 µL nuclease–free deionized water and 0.5 µL DNA template (25µg/ml).

PCR cycles consisted of an initial denaturation for 3 min at 95 °C; 35 cycles of denaturation (1 min at 94 °C),



annealing (1 min at 55 °C) and extension (2 min at 72 °C); and a final extension at

72 °C for 10 min.

Table 5.

Sequences of primers using in this study.

Primers	Sequences (5'– 3')	References
ITS1 (F)	TCCGTAGGTGAACCTGCGG	[WHITE <i>et al.</i> , 1990]
ITS4 (R)	TCCTCCGCTTATTGATATGC	[WHITE <i>et al.</i> , 1990]
trnL–F (F)	CGAAATCGGTAGACGCTACG	[TABERLET <i>et al.</i> , 1991]
trnL–F (R)	ATTTGAACTGGTGACACGAG	[TABERLET <i>et al.</i> , 1991]

Note: F, Forward primer; R, Reverse primer

Sequencing data analysis

Sequencing data of ITS and trnL–F regions of 5 wax apples were analyzed and established the consensus using FinchTV và Seaview.

For multiple alignments, the ClustalW and Bioedit software were used to recognize the homology between sequences.

Phylogenetic analysis was carried out with the software PAUP*4.0a146 [SWOFFORD, 2002] using the maximum parsimony and neighbor–joining methods with *Eugenia uniflora* as outgroup [SOH and PARNELL, 2011]. Bootstrap values of 50 % or

higher were performed to obtain cluster supports.

Results and discussion
Morphological phylogenetic analysis

After survey the morphological characteristics of 5 wax apple varieties planted in Cao Lanh city, Dong Thap Province, we encoded morphological characteristics with number 0 to 4 (Table 3 and 4) and established phylogenetic tree using PAUP* 4.0a146 software [SWOFFORD, 2002] and the neighbor–joining method.

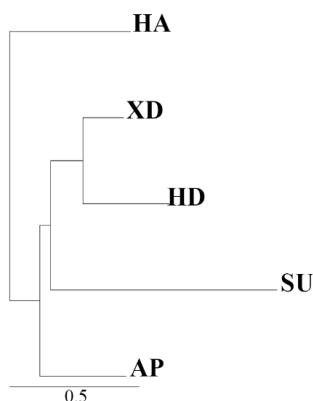


Figure 2. Phylogenetic tree established by morphological characteristics of 5 wax apple varieties

Note: HA. Hoa An wax apple; HD. Hong Dao wax apple; SU. Sua wax apple; XD. Xanh Duong wax apple; AP. An Phuoc wax apple.

Among five varieties, Hoa An wax apple was separated into one group, following by the group of An Phuoc and Sua wax apples, and other group of the Xanh Duong and the Hong Dao wax apples (Figure 2).

The categorization of five wax apple varieties showed that there were the significant differences in morphological characteristics in 5 varieties, especially in the fruit color, the shape, the taste the and seed, etc. (Table 3 and 4).

Molecular phylogenetic analysis

The PCR products of trnL–F and ITS sequences (Figure 3A & 3B) of five wax apple varieties were clear bands about 800 bps and 600b bps, respectively.

These bands had the similar sizes with predicted bands of Taberlet and collab., and White and collab., works, in which the authors used the same primers.

The PCR products were subsequently purified and analyzed sequences [TABERLET *et al.*, 1991; WHITE *et al.*, 1990].

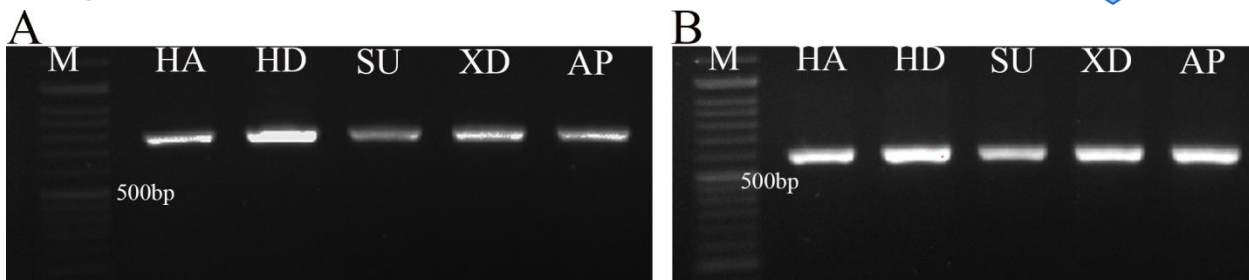


Figure 3. PCR products of trnL–F (A) and ITS (B) sequences of 5 wax apple varieties. Note: M. Maker; HA. Hoa An wax apple; HD. Hong Dao wax apple; SU. Sua wax apple; XD. Xanh Duong wax apple; AP. An Phuoc wax apple.

The ITS and trnL–F sequences of 5 wax apples after editing had the sizes about 531 and 726 bps, respectively.

These sequences were submitted to GenBank and the accession numbers were listed in [Table 6](#).

Table 6.

The GenBank accession numbers of ITS and trnL–F regions of 5 wax apples in this study

Taxa	Accession number (ITS/trnL–F regions)
Hoa An wax apple	MN689625/MN725089
Hong Dao wax apple	MN689626/MN725091
Sua wax apple	MN689627/MN725092
Xanh Duong wax apple	MN689628/MN725090
An Phuoc wax apple	MN689629/ MN725093

After comparing the ITS and trnL–F sequences of five wax apple varieties, we found that 4 wax apple varieties, including Hoa An, Hong Dao, Sua, and Xanh Duong wax apples, had the 100 % similarity in ITS and trnL–F sequences.

On the contrary, An Phuoc wax apple had differences at 5 positions (120, 161, 178, 405, and 446) in ITS sequence ([Figure 4A](#)) and 1 position (520) in trnL–F sequence ([Figure 4B](#)) as compared to other wax apple varieties.

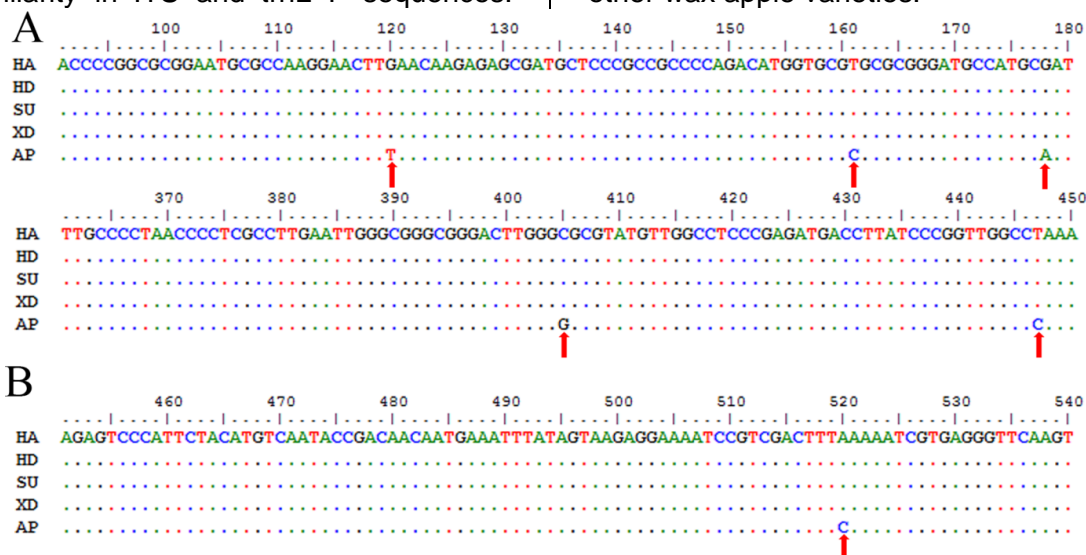


Figure 4. Comparison of ITS (A) and trnL–F sequences (B) of 5 wax apples in this study.

Note: The homologous positions (match) between 5 wax apples are shown by the dots (.) and mismatch position are shown by the arrows (↑). HA. Hoa An wax apple; HD. Hong Dao wax apple; SU. Sua wax apple; XD. Xanh Duong wax apple; AP. An Phuoc wax apple.



Four wax apple varieties, including of Hoa An, Hong Dao, Sua, and Xanh Duong wax apples, had significant differences in morphological characteristics: fruit color, seed, thickness of peel, leaf shape, the number of seeds per fruit, etc (Table 2).

However, the molecular data showed no difference in ITS and trnL-F sequences of 4 wax apple varieties. On the other hand, ITS and trnL-F sequences of An Phuoc wax apple were different with other varieties (Figure 4).

These results are reasonable because An Phuoc wax apple is imported Thoongsamri wax apple from Thailand while 4 wax apple varieties, including of Hoa An, Hong Dao, Sua, and Xanh Duong wax apples, are local varieties from Dong Thap Province.

Several taxonomy studies have shown the difference between molecular and morphological data. Some species

have identical morphological characteristics but differences in genetic characteristics. For instance, Bui and collab., indicated differences in ITS and trnL-F sequences of two species belonging to the Orchidaceae family, *Geodorum attenuatum* và *G. recurvum*, which had similar morphological characteristics [BUI et al., 2019].

Moreover, Ton and collab., also used trnL-F and matK sequences to distinguish *Rothmannia wittii* and *R. daweshanensis*, which were identical in morphological characteristics [TON et al., 2019].

To date, most of the studies about molecular data of *S. samarangense* were used the ITS sequence whereas the trnL-F sequence have not been used yet. Therefore, this study is the first study providing molecular data of trnL-F sequences of this species. There is only the sequence of ITS region of *S. samarangense* in Genbank (NCBI).

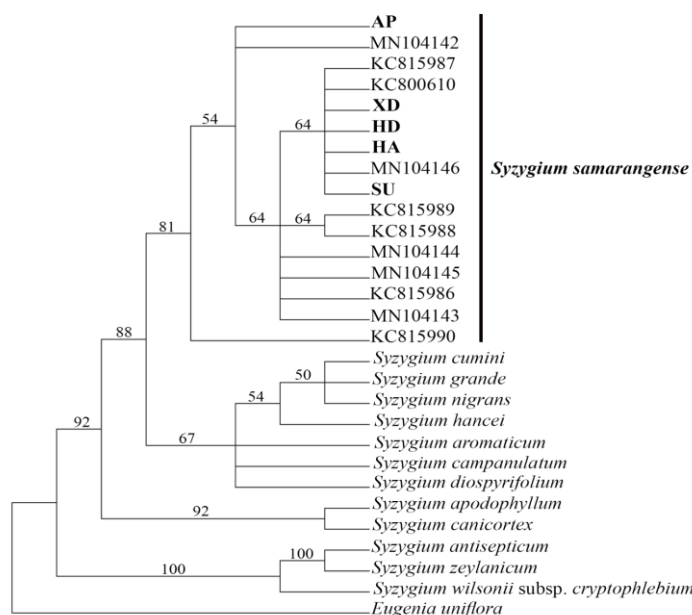


Figure 5. One of most-parsimonious tree obtained based on ITS region. Gaps treated as missing data. The bootstrap values of 50 % or more than from 1000 replicates are shown above the nodes.

After comparing the ITS sequence of this species and other species belonging to *Syzygium* genus of the Myrtaceae family in Genbank (NCBI), we established phylogenetic trees using neighbor joining and parsimony methods.

Both of phylogenetic trees using neighbor joining and parsimony methods showed the similar results.

Briefly, 5 wax apple varieties, Hoa An, An Phuoc, Xanh Duong, Sua, and Hong Dao wax apples, were classified in the same group with 11 wax apple



specimens from the Genbank (NCBI), these results proved that the sequencing data were reliable.

Among 11 wax apple specimens from the Genbank (NCBI), there were 6 specimens collected from China (KC815986, KC815988, KC815987, KC815989, KC815990, and KC800610) and 5 specimens grown in Sri Lanka (MN104142, MN104146, MN104144, MN104145, and MN104143).

The phylogenetic trees showed that classification of these species were not

related with geographic regions. For instance, 4 wax apple varieties, Xanh Duong, Hong Dao, Hoa An, and Sua wax apples, as well as 2 wax apple specimens from China (KC815987, KC800610), 1 wax apple from Sri Lanka (MN104146) were classified into the same group.

Furthermore, the genetic distance of 7 varieties was 0 (Table 7), which suggested that there was no difference in ITS sequences of 7 varieties.

Table 7.

Mean pairwise genetic distances between wax apples based on the ITS region

Samples	1	2	3	4	5	7	8	9	10	11	12	13	14	15	16
An Phuoc															
MN104142	0.003														
KC815987	0.005	0.004													
KC800610	0.007	0.006	0.002												
Xanh Duong	0.005	0.004	0.000	0.002											
Hong Dao	0.005	0.004	0.000	0.002	0.000										
Hoa An	0.005	0.004	0.000	0.002	0.000	0.000									
MN104146	0.005	0.004	0.000	0.002	0.000	0.000	0.000								
SUA	0.005	0.004	0.000	0.002	0.000	0.000	0.000	0.000							
KC815989	0.005	0.004	0.002	0.004	0.002	0.002	0.002	0.002	0.002						
KC815988	0.005	0.004	0.002	0.004	0.002	0.002	0.002	0.002	0.002	0.000					
MN104144	0.005	0.004	0.002	0.004	0.002	0.002	0.002	0.002	0.002	0.002	0.002				
MN104145	0.004	0.003	0.001	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001			
KC815986	0.004	0.003	0.001	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000		
MN104143	0.004	0.003	0.001	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	
KC815990	0.005	0.004	0.004	0.006	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.003	0.003	0.003

On the contrary, An Phuoc wax apple had 5 different position as compared to Xanh Duong, Hong Dao, Hoa An, and Sua wax apples, and the genetic distance between 2 groups was 0.005. Moreover, the genetic distance between An Phuoc wax apple and one wax apple variety collected from Sri Lanka (MN104142) was smaller (0.002). Hence, there is no relationship between classification and geographic regions in the phylogenetic trees (Figure 5).

The varieties of popular crops, especially in edible fruit plants, usually are hybridized between together and/or import from different geographic regions, thus explaining for these results.

An Phuoc wax apple variety is an imported variety from Thailand; therefore, this variety tends to separate from other wax apple varieties in this study, consisted of Xanh Duong, Hong Dao, Hoa An, and Sua wax apples.

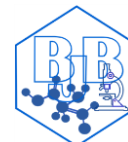
Furthermore, Lai and collab., also used 20 microsatellite markers to prove the genetic diversity among 14 wax

apples (*S. samarangense*) planted at Agricultural Research and Extension Station (KDARES), Kaohsiung District, Taiwan [LAI *et al.*, 2015].

Conclusions

The results revealed that five varieties of wax apples had a board range of morphological characteristics, especially in shape, color, and size of fruits. Furthermore, molecular analysis results showed that ITS and trnL-F sequences of four varieties of wax apples, including of Xanh Duong, Hong Dao, Hoa An, and Sua, were identical with 100 % similarity whereas An Phuoc wax apple had differences in one and five positions in ITS and trnL-F sequences as compared to other varieties.

The phylogenetic tree which was established by comparison of ITS sequences of five varieties in this study and other varieties in Genbank (NCBI) suggested that four varieties of wax apples (Xanh Duong, Hong Dao, Hoa An, and Sua) were 100 % match with two varieties grown in China (KC815987,



KC800610) and one variety planted in Sri Lanka (MN104146) whereas An Phuoc wax apple was high similarity with other wax apple variety planted in Sri Lanka (MN104142).

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