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# Genetic diversity of rose apple (*Syzygium samarangense*) varieties based on ISSR molecular markers

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**Abstract.** *Hien PTT, Hang VTT, Cham LTT. 2024. Genetic diversity of Rose apple* (*Syzygium samarangense*) *varieties based on ISSR molecular markers. Biodiversitas 25: 2003-2010.* Rose apple (*Syzygium samarangense*) exhibits a rich diversity in its varieties, metabolic compounds, and biological activities. Genetic diversity studies are crucial in understanding plant species taxonomy and evolutionary history. They provide a roadmap for researchers to trace the relationships between different varieties and offer insights into the evolutionary processes that have shaped the diversity within the species. Many traditional or indigenous varieties of plants may harbor unique traits adapted to specific local conditions. This study, therefore, holds immense value as it aims to evaluate the genetic relationship among rose apple varieties in Vietnam using morphological characteristics and ISSR markers. Sixteen samples of 14 local varieties of rose apple were collected from provinces in Vietnam for this purpose. The morphological characteristics of leaves and fruits were compared among samples, and the genetic diversity was evaluated using ISSR markers. The results revealed significant morphological diversity in the leaf base, blade shape, leaf color, and fruit shape and size. Based on the DNA profiles of 15 ISSR markers, 102 amplicons were obtained, and the percentage of polymorphism ranged from 57 to 100%. The dendrogram constructed using the UPGMA method classified these samples into two main clusters with a genetic similarity of 64%. Such comprehensive information underscores the genetic diversity among *S. samarangense*, thereby contributing to the germplasm resources for future plant breeding programs.

**Keywords:** Genetic diversity, ISSR markers, rose apple, *Syzygium samarangense*

## INTRODUCTION

Fruit plants play a crucial role in the overall biodiversity of ecosystems. Understanding their genetic diversity is the key to conservation efforts, as it enables the identification and preservation of unique genetic resources, including wild relatives and landraces, which may hold valuable traits for future breeding endeavors. Genetic diversity is a cornerstone of sustainable agriculture. Diverse fruit plant populations exhibit greater resilience to environmental fluctuations and are less vulnerable to catastrophic crop failures triggered by pests or diseases. Therefore, by delving into genetic diversity, agricultural practices can be fine-tuned to uphold or enhance biodiversity while ensuring food security. The findings of this study on the genetic diversity of rose apple varieties in Vietnam using morphological characteristics and ISSR markers contribute to this broader understanding and offer practical insights for plant breeding programs.

Rose apple (*Syzygium samarangense*) is one of the tropical fruits of the *Myrtaceae* family, originating in Southeast Asia for a long time. Rose apple trees have an average height of about 8-10 m; flowers are yellowish-white or pinkish with leaves about 15 cm long and 10 cm wide. Rose apples have a characteristic bell shape, starting to change from green to purple, red, light pink, milky white, or remaining green when the fruit is ripe, depending on the rose apple variety (Ngan 2023). The flesh has a rose scent, crispy and succulent (Sonawane 2018). Rose apples are eaten fresh or made into jelly, syrup, juice, or rose apple wine (Ngan 2023), they contain a very rich of water (approximately 90%), making them a favorite cooling fruit during the summer months. The fiber in rose apples benefits the digestive system and helps prevent intestinal diseases (Yassir et al. 2022). The high contents of vitamins A and C, along with the presence of natural compounds and minerals such as calcium, potassium, iron, and zinc, help the body increase resistance, regulate blood sugar, reduce bad cholesterol, support cardiovascular prevention, antioxidant activity, and cancer prevention (Chua et al. 2019; Subbulakshmi et al. 2021). Different parts of the tree have diverse traditional uses, such as its blossoms are effective in treating fever and diarrhea; its bark decoction is effective in treating thrush; root preparations are used to relieve itching and reduce swelling (Sonawane 2018; Sirisha and Shreeja 2019). Despite the many benefits of rose apples, unfortunately, currently, farmers' interest in cultivating rose apples in Vietnam is decreasing; as a result, some rose apple varieties have been disappearing.

Studies on the genetic diversity of rose apples from Vietnam are still limited. Even though the rose apple's diversity in the country is very high, the genetic relationships of plant varieties can be identified through morphological and molecular marker methods. The genetic

diversity analysis technique helps identify polymorphic regions in the DNA molecule, thereby identifying genes and genetic polymorphisms (Khachtib et al. 2023). Several reports related to applying molecular markers to determine the genetic diversity of fruit plants. For instance, a study classified apple varieties in 14 Ardahan province (Turkey) through five pairs of ISSR primers to help classify apple varieties into five groups (Sevindik et al. 2018). From 11 ISSR markers, 55 superior durian varieties from five sites in Indonesia have been proven to be different (Angeliena et al. 2019). Genetic diversity analysis of 10 mango varieties collected in 10 different provinces of Vietnam was conducted using 10 ISSR primer pairs; the research results were classified into four groups of these ten genotypes (Ho and Tu 2019). Another study showed the effectiveness of 19 microsatellite primers and 10 ISSR primers in determining intraspecific and interspecific genetic diversity in 111 species of the genus *Diospyros* spp. (Samarina et al. 2021). Similar experiments on 70 accessions of mulberry in Lebanon, combined with evaluation using 27 morphological traits and selected SSR and ISSR primers, demonstrated a high level of genetic diversity in these mulberry varieties (Kadri et al. 2021). A study was conducted in 2022 on 21 selected watermelon seeds with different origins and production years in Iraq and using 12 ISSR primer pairs, the 21 selections were divided into two main clusters or five sub-clusters (Elias and Al-Jubouri 2022). The above studies have successfully determined the genetic relationships of fruit tree varieties, showing the potential of the ISSR molecular marker technique in analyzing the genetic diversity of rose apple varieties in Vietnam. This study aimed to evaluate the genetic relationship among rose apple varieties in Vietnam using morphological characteristics and ISSR markers.

## MATERIALS AND METHODS

### Sample collection

Leaf and fruit samples of some rose apple varieties of Vietnam, after being preserved in plastic bags with complete information recorded and stored at the appropriate temperature. The sample's identities are presented in Table 1.

Samples were collected on normal growth and development trees, not severely damaged by pests or too few fruits. Leaves were collected from a representative branch with all the young, mature, and old leaves (based on the color of an intact branch). Fruit samples were collected at the harvesting stage.

### Morphological analysis

After collection, the leaves and fruits of each rose apple variety were washed, observed with the naked eye, and photographed. The external morphology described according to Ba (2010) and compared with the description included leaves (shape, color, leaf margin, veins, tip, and base), fruit (shape, color, calyx lobe, seeds, and fruit pulp). Leaves were measured in length from leaf base to leaf tip, along the longest axis of the leaf using Toupview software (ToupTeck Inc, China) (Figure 1). Fruits' size, length, and width were measured using ToupView software (Figure 1).

**Table 1.** A list of 16 rose apple samples collected in Hanoi, Ben Tre, Tien Giang, Vinh Long, Can Tho and Soc Trang Provinces

| Code of samples | Varieties | Collected sites |
|---|---|---|
| TH | Tam Hoa | Phong Dien (Can Tho) |
| AP.1 | An Phuoc | Phong Dien (Can Tho) |
| AP.2 | An Phuoc | Cho Lach (Ben Tre) |
| AP.3 | An Phuoc | Thot Not (Can Tho) |
| X | Man Xanh | Phong Dien (Can Tho) |
| XDN | Xanh Da Nguoi | Phong Dien (Can Tho) |
| B | Man Bo | Phong Dien (Can Tho) |
| HDD | Hong Dao Da | Phong Dien (Can Tho) |
| D | Man Dai | Thot Not (Can Thơ) |
| HDH | Hong Dao Huyet | My Tho (Tien Giang) |
| AD | Man An Do | Cho Lach (Ben Tre) |
| XST | Xanh Soc Trang | Cu Lao Dung (Soc Trang) |
| Do | Man Do | Gia Lam (Ha Noi) |
| T | Man Thai | Tra On (Vinh Long) |
| TL | Trung Luong | Cai Lay (Tien Giang) |
| Bo | Man Bom | Ninh Kieu (Can Tho) |


**Figure 1.** Positions of leaf and fruit measurement

**Table 2.** List of ISSR primers (Shankar and Anjani 2023)

| Primer | Primer sequence 5'- 3' | Annealing temperature (Ta) |
|---|---|---|
| WA-ISSRK2 | GTGGTGGTGGTGAC | 50°C |
| WA-ISSR 13 | AGAGAGAGAGAGAGAGCA | 50°C |
| WA-ISSR818 | CACACACACACACACAG | 50°C |
| WA-ISSR825 | ACACACACACACACACT | 50°C |
| WA-ISSR827 | ACACACACACACACACG | 50°C |
| WA-UBC809 | AGAGAGAGAGAGAGAGG | 50°C |
| WA-UBC826 | ACACACACACACACACC | 50°C |
| WA-UBC829 | TGTGTGTGTGTGTGTGC | 50°C |
| WA-UBC840 | GAGAGAGAGAGAGAGACT | 50°C |
| WA-UBC848 | CACACACACACACACAAG | 50°C |
| WA-UBC856 | ACACACACACACACACCA | 50°C |
| WA-UBC866 | CTCCTCCTCCTCCTCCTC | 50°C |
| WA-UBC888 | ATGCACACACACACACA | 50°C |
| WA-UBC889 | ATGACACACACACACAC | 50°C |
| WA-UBC890 | GAAGTGTGTGTGTGTGT | 50°C |

**Determination of °Brix and pH of the fruit**

The soluble solid content (°Brix) and pH are important criteria to evaluate fruit quality (Mothina and Yapwattanaphun 2017). Each criterion was measured three times on three different fruits of the same variety. Brix degree (%): was analyzed using a handheld Brix meter (Atago N-1∝ Brix 0-32%, Japan). Fruit pH was measured using a Hanna HI2210 pH Meter.

**Molecular analysis**

*DNA isolation*

DNA was extracted from leaf samples according to the adjusted CTAB procedure. The DNA concentration after leaf extraction was tested using a Nanodrop One spectrophotometer (Thermo Scientific) at 260 nm and 280 nm wavelengths with standard purity in the range $A_{260}/A_{280}$ = 1.8-2. The presence and integrity of DNA were detected by 1% agarose gel electrophoresis in 1X TAE solution. The gel is photographed under UV light, and the band's thickness and brightness reflect the DNA quality in the extraction solution.

*Amplification of ISSR markers*

PCR reaction was performed to amplify loci based on ISSR markers (Table 2). Basic ingredients for a 15 µL PCR reaction include 8 µL $BiH_2O$, 4 µL MyTaq mix 2X (Bioline, England), 1 µL Primer, and 2 µL DNA. An OptiMax PCR machine performed the amplification reaction with 40 cycles: initial denaturation phase 95°C in 4 minutes, denaturation phase 95°C in 30 seconds, primer annealing phase 50°C in 1 minute, extension phase 72°C in 2 minutes and final extension stage 72°C in 10 minutes.

**Data analysis**

Leaf and fruit indices were determined using Toupview software. Morphological data were statistically analyzed using Excel software 2019, ANOVA analysis, and Tukey's test using Minitab 16 software. Means followed by different letters are statistically significantly different ($p<0.05$). Electrophoresis results were recorded using a BioRad UV 2000 gel reader, and images were analyzed using Quantity One 4.6 software. Bands are encoded in binary form: 1-for cases where bands are present, 0-for cases where bands are not present. The encoding table is saved as an Excel file and transferred to NTSYSpc 2.1 software to draw a dendrogram and similarity matrix built based on the UPGMA method (Unweighted Pair Group Method with Arithmetic Average). Polymorphic Information Content (PIC) was determined using the web-based iMEC software. The Jaccard similarity coefficient is determined from the binary data matrix.

## RESULTS AND DISCUSSION

**Morphological characteristics**

The results described in Table 3 showed morphological diversity in the leaf base, blade shape, and leaf color. Except for the most common blade shape, oblong-lanceolate to elliptical, which was similar to that described by Idris et al. (2023). Rose apple varieties D (Man Dai) and XDN (Xanh Da Nguoi) had an additional ovate type (Figure 2). Differences in color during leaf maturation stages were also recorded and presented in Table 3. Leaf characteristic diversity is a fundamental tool in plant identification, taxonomic classification, ecological research, and botanical education, providing valuable insights into plant diversity, evolution, and adaptation. Leaf morphology can be highly specific to individual species or groups of closely related species. For example, subtle differences in leaf shape or venation pattern may distinguish between similar-looking species within a genus (Viacrucis III and Buot 2021; Qiao et al. 2022).

Statistical data on the leaf size characteristics of rose apple varieties at different developmental stages showed diversity in size in all three survey periods (Table 4). In general, the leaves of rose apple varieties ranged in size from 3.1-10.7 x 0.7-4.3 cm in the young leaf stage, 6-17.9 x 2.8-7.3 cm when mature, and 18-28.9 x 5.2-11.4 cm when the leaves are old. There were 4 among 14 rose apple varieties with the largest size in the old leaf stage recorded as XDN (Xanh Da Nguoi), X (Xanh), Do (Man Do), and B (Bo) (Table 4). Compared to the bright hues of flowers, leaves typically appear less remarkable since they are generally green due to chlorophyll. However, their appeal is found in their diverse shapes and sizes; such differences, resulting from various factors, are crucial in determining a plant species or variety. For instance, leaf size tends to reduce as altitude increases and rainfall and soil nutrient levels decrease. In comparison, smaller leaves are more suited to withstand hot or arid conditions (Nicotra et al. 2011).

**Table 3.** Leaf morphological characteristics of rose apple varieties of Ha Noi, Ben Tre, Tien Giang, Vinh Long, Can Tho, and Soc Trang Provinces, Vietnam

| Varieties | Leaf shape | Leaf apex | Leaf base | Leaf color |
|---|---|---|---|---|
| Tam Hoa | Oblong-lanceolate | Cuspidate | Rounded | Young leaves are wine, mature ochre, and old are dark green |
| An Phuoc | Oblong-elliptical | Cuspidate | Rounded | Young leaves are wine, mature ochre, and old are dark green |
| Man Xanh | Oblong-lanceolate | Cuspidate | Orbiculate | Young leaves are wine, and mature and old are light green |
| Xanh Da Nguoi | Lanceolate | Cuspidate | Rounded, orbiculate | Young leaves are wine, and mature and old are dark green |
| Man Bo | Lanceolate | Accuminate | Orbiculate | Young leaves: sepia, mature: olive, old: dark green |
| Hong Dao Da | Elliptical | Cuspidate | Rounded | Young leaves are light wine, mature ochre, and old are light green |
| Man Dai | Ovate | Cuspidate | Rounded | Young leaves: light wine, mature: light green, old: dark green |
| Hong Dao Huyet | Oblong-lanceolate | Cuspidate | Cordate | Mature leaves: avocado-green |
| Man An Do | Oblong-lanceolate | Cuspidate | Rounded | Young leaves: wine, mature: avocado green, old: dark green |
| Xanh Soc Trang | Ovate | Accuminate | Cordate | Young leaves: light olive, mature: light green, old: dark green |
| Man Do | Lanceolate | Cuspidate | Cordate | Upper surface: dark green, under: little red |
| Man Thai | Oblong-lanceolate | Cuspidate | Rounded | Young and mature leaves: light green, old: dark green |
| Trung Luong | Elliptical, oblong at leaf apex | Cuspidate | Rounded | Young leaves: light ochre, mature: ochre, old: green |
| Man Bom | Lanceolate | Caudate | Rounded | Young leaves: marigold, old: dark green |

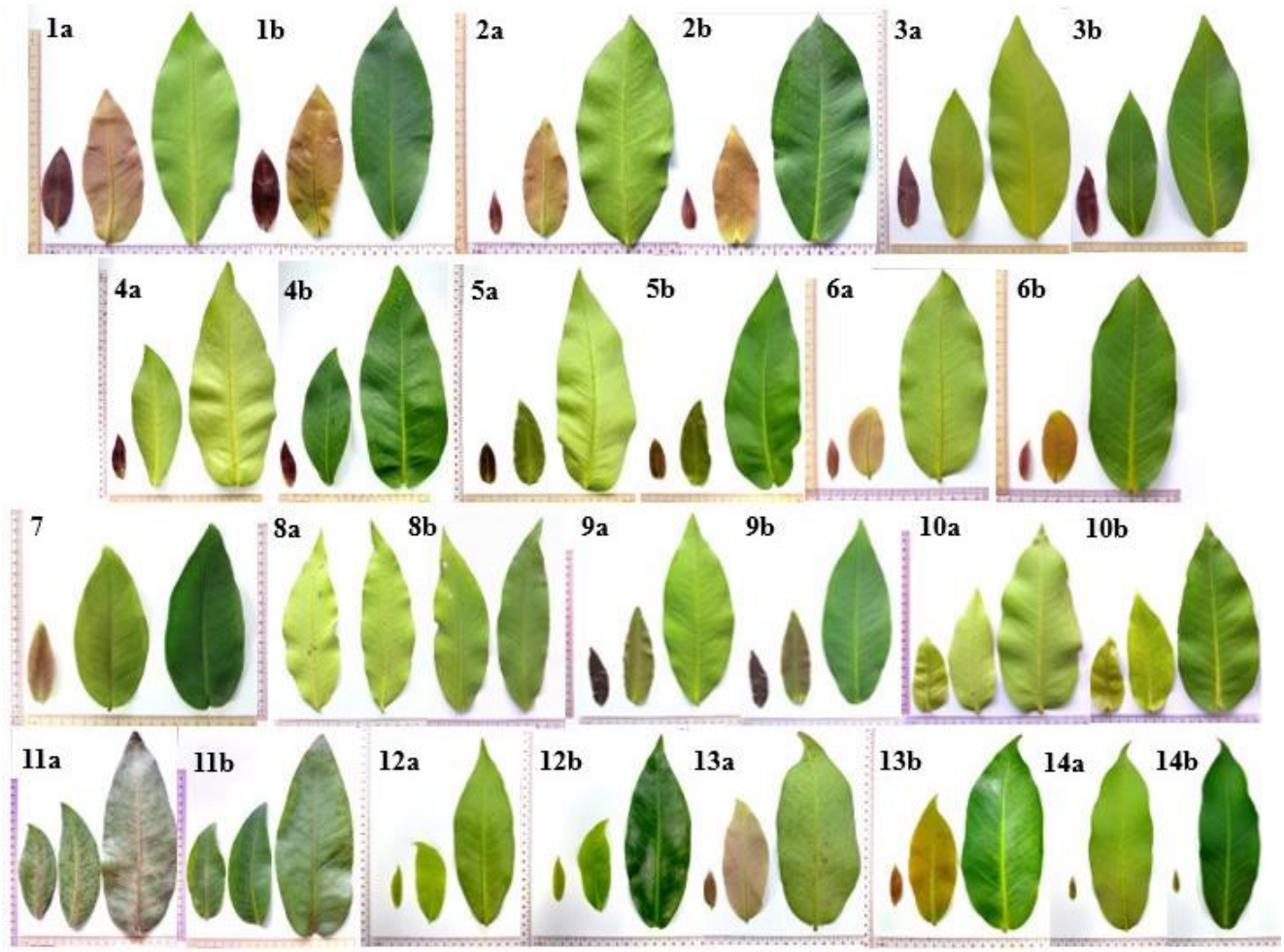


**Figure 2.** Leaf morphology of rose apple varieties of Hanoi, Ben Tre, Tien Giang, Vinh Long, Can Tho, and Soc Trang, Vietnam. Note: 1-Tam Hoa, 2-An Phuoc, 3-Man Xanh, 4-Xanh Da Nguoi, 5-Man Bo, 6-Hong Dao Da, 7-Man Dai, 8-Hong Dao Huyet, 9-Man An Do, 10-Xanh Soc Trang, 11-Man Do, 12-Man Thai, 13-Trung Luong, 14-Man Bom

**Fruit morphology**

Through the results in Figure 3 and Table 5, it is observed that there are various characteristics in color, shape, and fruit size among the rose apple varieties; this reflects the level of morphological diversity of varieties of the same species, similar to the observations of Galan (1989). Although the calyx lobes of different rose apple varieties do not differ greatly in number and shape, they differ in the degree of expansion when the fruit is ripe. The measurement values of Brix and pH of the fruit pulp extract differed among the eight rose apple varieties selected, ranging from 4-7%; the highest values were the samples HDD (Hong Dao Da), T (Man Thai), and B (Bo); The lowest pH values were TL (Trung Luong) and XDN (Xanh Da Nguoi). Diverse fruit morphology offers numerous combinations for future breeding programs to develop new lines or cultivars with desirable characteristics. Therefore, maintaining a diverse range of fruit appearances helps preserve genetic diversity within plant populations. Additionally, many people are attracted to different fruit appearances; some might prefer vibrant, colorful fruits, while others might be drawn to more subtle or unique appearances. Offering a diverse range of fruit appearances increases the likelihood of appealing to a wider consumer base, ultimately driving sales and consumption (Li et al. 2022).

**Table 4.** Leaf size characteristics of rose apple varieties of Ha Noi, Ben Tre, Tien Giang, Vinh Long, Can Tho, and Soc Trang, Vietnam

| Varieties | Young leaf | | Mature leaf | | Old leaf | |
|---|---|---|---|---|---|---|
| | Length (cm) | Width (cm) | Length (cm) | Width (cm) | Length (cm) | Width (cm) |
| TH | $7.8^{bc}$±0.2 | $2.6^{bc}$±0.1 | $13.3^{bc}$±0.9 | $4.5^{bcd}$±0.9 | $20.7^{efgh}$±1.0 | $7.7^{bc}$±1.0 |
| AP | $3.8^{fg}$±0.4 | $1.5^{bcd}$±0.4 | $11.0^{cd}$±1.2 | $4.5^{abcd}$±0,7 | $18.9^{ghi}$±0.3 | $8.3^{b}$ + 0.4 |
| X | $9.2^{ab}$±0.9 | $3.0^{ab}$±0.6 | $17.9^{a}$±0.5 | $6.0^{abc}$±0.1 | $26.1^{b}$±0.7 | $9.1^{ab}$ + 0.6 |
| XDN | $7.0^{bcd}$±1.1 | $2.2^{bcd}$±0.4 | $9.4^{d}$±0.8 | $7.2^{ab}$±0.8 | $28.9^{a}$ ±1.4 | $11.4^{a}$±1.0 |
| B | $4.8^{defg}$±0.2 | $1.7^{bcd}$±0.3 | $10.9^{cd}$±0.9 | $3.6^{cd}$±0.0 | $24.9^{bc}$±0.8 | $8.9^{ab}$±0.2 |
| HDD | $3.4^{g}$±0.01 | $0.7^{d}$ ±0.1 | $6.0^{e}$±0.5 | $2.8^{d}$±0.0 | $18.0^{i}$±0.2 | $6.6^{bc}$±0.2 |
| D | $6.5^{cde}$±0.7 | $3.1^{ab}$±0.3 | $16.3^{ab}$±0.9 | $7.3^{a}$±0.5 | $18.4^{hi}$±0.4 | $7.7^{bc}$±0.3 |
| HDH | * | * | $17.3^{a}$±1.5 | $4.6^{abcd}$±1.3 | $19.9^{fghi}$±0.2 | $5.2^{c}$±0.3 |
| AD | $7.6^{bc}$±0.6 | $2.2^{bcd}$±0.6 | $11.8^{cd}$±0.2 | $3.9^{cd}$±0.4 | $23.6^{bcd}$±0.6 | $8.8^{ab}$±0.3 |
| XST | $8.9^{ab}$±0.4 | $4.3^{a}$±0.5 | $13.9^{bc}$±0.5 | $4.8^{abcd}$±0.8 | $21.5^{defg}$±0.7 | $8.8^{ab}$±0.8 |
| Do | $10.71^{a}$±0.5 | $4.3^{a\pm}$0.7 | $13.8^{bc}$±0.3 | $7.3^{a}$±0.5 | $25.7^{bc}$±0.9 | $8.5^{b}$±0.6 |
| T | $5.9^{cdef}$±1.1 | $1.7^{bcd}$±0.6 | $11.1^{cd}$ ±1.1 | $4.0^{cd}$±0.5 | $22.0^{def}$±0.5 | $8.1^{b}$±1.3 |
| TL | $4.2^{efg}$±0.2 | $1.2^{cd}$±0.2 | $13.9^{bc}$±0.1 | $5.4^{abcd}$±0.4 | $20.0^{fghi\pm}$0.3 | $8.0^{b}$±0.8 |
| Bo | $3.1^{g}$±0.2 | $0.9^{cd}$±0.2 | | | $23.1^{cde}$±0.5 | $7.8^{bc}$±0.3 |

Note: *: There were no young leaves at the time of; means with different letters in the same column are significantly different

**Table 5.** Differences in morphological characteristics between rose apple varieties

| Varieties | Brix (%) | pH | Length (cm) | Width (cm) | Morphological characteristics |
|---|---|---|---|---|---|
| AP | 5 | 4.33 | 8.3 | 6.0 | Shape: bell-shaped, oblong; peel: dark red, blurred longitudinal veins, no green veins; flesh: consistency, thick white; little or no seeds |
| X | 4 | 3.60 | 3.7 | 5.8 | Shape: globose, slightly protruding pedicel; peel: light green; flesh: white spongy; little small |
| HDD | 7 | 4.02 | 5.6 | 6.2 | Shape: cone, indenting pedicel; peel: pink, veins along the fruit; flesh: white spongy; little seeds |
| T | 7 | 4.10 | 6.2 | 4.0 | Shape: short-bell; peel: pale green, smooth; flesh: white spongy; small seeds |
| TL | 4 | 3.65 | 5.5 | 6.5 | Shape: globose and horizontally flattened; peel: light pink vein, smooth and shiny; flesh: white, thin; seed: big, globose |
| Bo | 7 | 3.62 | 7.5 | 7.8 | Shape: bell-shaped, oblong; peel: white green, no green veins; flesh: consistency, thick white; little or no seeds |


**Figure 3.** Morphological characteristics of rose apple varieties of Vietnam. Note: 1-An Phuoc, 2-Man Xanh, 3-Hong Dao Da, 4-Man Thai, 5-Trung Luong

**ISSR molecular marker**

The results of PCR product analysis showed that 12/15 ISSR markers successfully amplified 102 DNA bands with sizes ranging from 200-2500 bp (Table 6). Of which 11 primers achieved 100% amplification efficiency, primer WA-ISSR818 alone could only amplify 15/16 samples except for sample TL (Trung Luong). ISSR markers target the regions between adjacent and inversely oriented microsatellites, utilizing nucleotide repeats. Each band represents a DNA sequence bordered by two inverted microsatellites (Haritha et al. 2016). The higher number of amplified bands indicates the efficiency of the primer. In this study, the average band of each primer was around 8.5 bands. Therefore, the primers were good enough to analyze the genetic diversity.

The recorded polymorphic band results were quite high, accounting for 84.33%. The lowest number of polymorphic alleles was 3 (WA-ISSR818), and the highest was 14 (WA-UBC890) (Figure 4, Table

6). Primers with a 100% polymorphic band ratio included WA-ISSRK2, WA-ISSR827, WA-UBC888, WA-UBC889, and WA-UCB890. Additionally, the genetic differences between varieties can be determined based on the differences in DNA bands.

The PIC coefficient reflects the polymorphic ability of ISSR molecular markers. According to Zatybekov et al. (2023), if the PIC index is >0.5, the primer used gives high polymorphic results; on the contrary, if the PIC index <0.25 indicates low polymorphic results, within the range of 0.25≤PIC≤0.5 gives moderate polymorphic results. The results showed that the primers used were all capable of giving polymorphism. The PIC coefficients of the 12 primers all showed polymorphic results ranging from 0.32 to 0.37. They were all less than 0.5, showing that the 12 molecular markers used in the study gave moderate polymorphic results (Table 7).

The MI value (Marker index-average diversity index of polymorphic bands) of all 12 primers ranged from 0.01-0.02. Although it is impossible to evaluate primers' ability or effectiveness, the MI value reflects the effectiveness between different techniques.

**Table 6.** Polymorphic assessment indices of the population of 12 rose apple samples amplified by 12 ISSR primers

| ISSR marker | Total band | Polymorphic alleles | Monomorphic | Polymorphism range (%) | Amplicon size range (bp) |
|---|---|---|---|---|---|
| WA-ISSRK2 | 6 | 6 | 0 | 100% | 250-2500 |
| WA-ISSR818 | 5 | 3 | 2 | 60% | 250-1225 |
| WA-ISSR825 | 6 | 5 | 1 | 83% | 250-1200 |
| WA-ISSR827 | 9 | 9 | 0 | 100% | 300-1200 |
| WA-UBC826 | 13 | 12 | 1 | 92% | 200-1500 |
| WA-UBC840 | 7 | 6 | 1 | 86% | 250-1000 |
| WA-UBC848 | 6 | 4 | 2 | 67% | 200-1200 |
| WA-UBC856 | 7 | 4 | 3 | 57% | 200-1500 |
| WA-UBC866 | 6 | 4 | 2 | 67% | 250-900 |
| WA-UBC888 | 12 | 12 | 0 | 100% | 200-1500 |
| WA-UBC889 | 11 | 11 | 0 | 100% | 250-1500 |
| WA-UBC890 | 14 | 14 | 0 | 100% | 200-1500 |

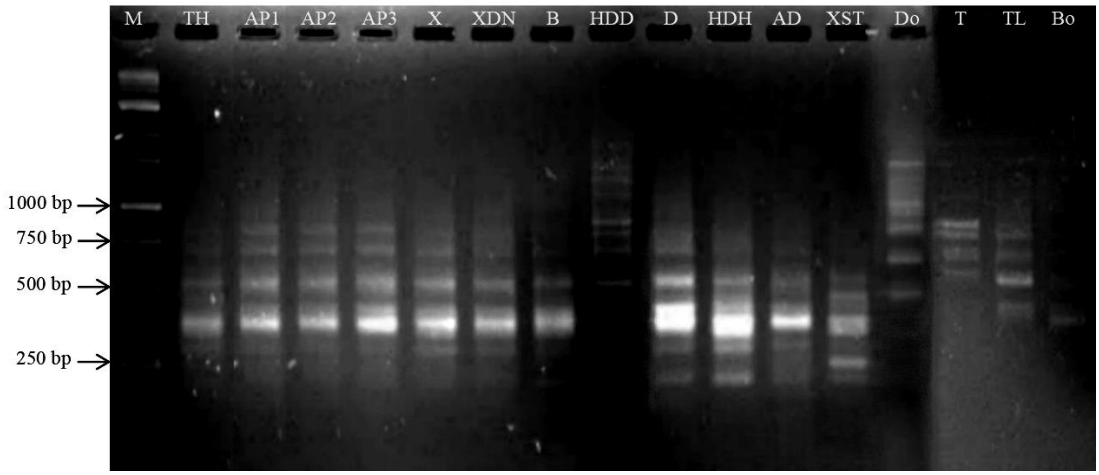


**Figure 4.** Results of WA-UBC890 primer electrophoresis on 2% agarose gel. Note: TH: Tam Hoa; AP: An Phuoc; X: Xanh; XDN: Xanh Da Nguoi; B: Bo; HDD: Hong Dao Da; D: Dai; HDH: Hong Dao Huyet; AD: An Do; XST: Xanh Soc Trang; Do: Man Do; T: Man Thai; TL: Trung Luong; Bo: Man Bom

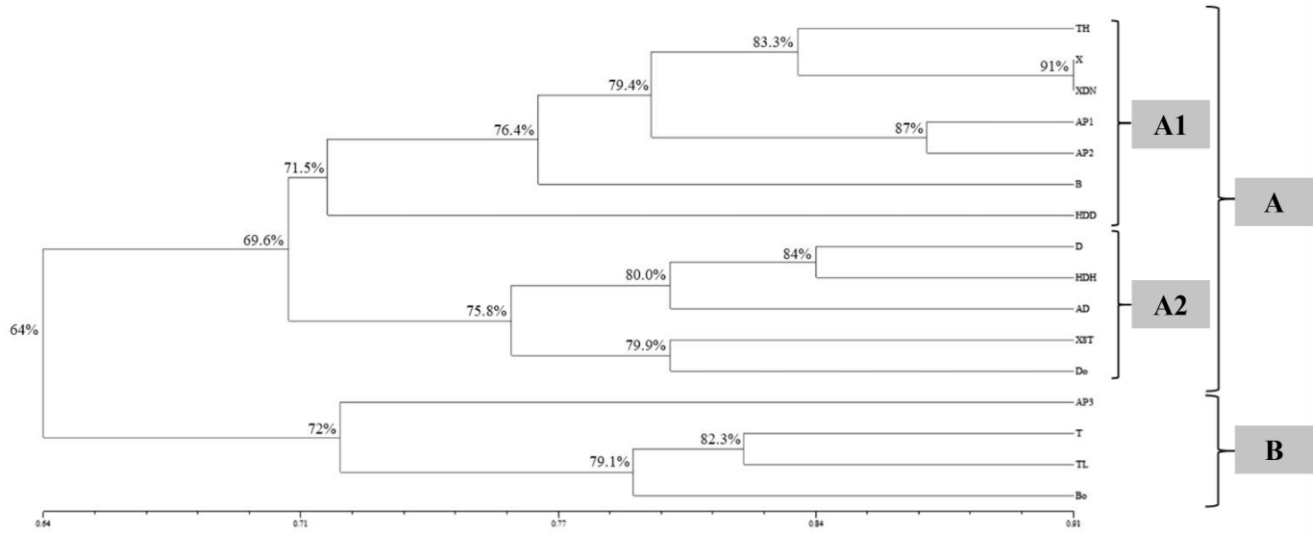


**Figure 5.** The dendrogram shows the genetic correlation between samples. Note: TH: Tam Hoa; AP: An Phuoc; X: Xanh; XDN: Xanh Da Nguoi; B: Bo; HDD: Hong Dao Da; D: Dai; HDH: Hong Dao Huyet; AD: An Do; XST: Xanh Soc Trang; Do: Man Do; T: Man Thai; TL: Trung Luong; Bo: Man Bom.

**Table 7.** Analysis of genetic diversity coefficient of researched rose apple samples

| ISSR marker | H | PIC | E | H.av | MI | D | R |
|---|---|---|---|---|---|---|---|
| WA-ISSRK2 | 0.49 | 0.37 | 2.56 | 0.01 | 0.01 | 0.82 | 3.63 |
| WA-ISSR818 | 0.47 | 0.36 | 3.13 | 0.01 | 0.02 | 0.61 | 2.25 |
| WA-ISSR825 | 0.39 | 0.32 | 4.38 | 0.00 | 0.02 | 0.47 | 2.5 |
| WA-ISSR827 | 0.44 | 0.35 | 3.00 | 0.00 | 0.01 | 0.89 | 5.00 |
| WA-UBC826 | 0.42 | 0.33 | 3.88 | 0.00 | 0.01 | 0.91 | 7.25 |
| WA-UBC840 | 0.41 | 0.32 | 2.00 | 0.00 | 0.01 | 0.92 | 2.25 |
| WA-UBC848 | 0.46 | 0.36 | 3.81 | 0.00 | 0.02 | 0.6 | 1.63 |
| WA-UBC856 | 0.45 | 0.35 | 4.63 | 0.00 | 0.02 | 0.57 | 2.00 |
| WA-UBC866 | 0.46 | 0.36 | 3.81 | 0.00 | 0.02 | 0.6 | 2.13 |
| WA-UBC888 | 0.43 | 0.34 | 3.81 | 0.00 | 0.01 | 0.9 | 4.38 |
| WA-UBC889 | 0.49 | 0.37 | 4.81 | 0.00 | 0.01 | 0.81 | 3.38 |
| WA-UBC890 | 0.47 | 0.36 | 5.25 | 0.00 | 0.01 | 0.86 | 5.5 |

**Cluster analysis**

Based on the dendrogram, the genetic diversity of 16 samples (14 varieties) was shown by a similarity coefficient of 64%, which could be divided into two main groups and many subgroups. Group A included samples TH, X, XDN, AP1, AP2, B, HDD, D, HDH, AD, XST, Do. The group has 69.6% similarity. This group was divided into two subgroups, including A1 group, with similarities of 71.5%, and A2 group, with 75.8% similarity (Figure 5). In addition, Group B included samples AP3, T, TL, and Bo; this group had a similarity of about 72% to 82.3%. The dendrogram shows that the molecular marker ISSR is effective for genetic diversity analysis research.

There were genetic differences between AP1, AP2, and AP3 samples, possibly because the samples were collected in different locations. According to Merrick et al. (2016), the genetic variation between individuals in populations and between crop varieties could occur due to mutation, gene transfer, recombination, adaptation to new environments, and continuous selection. Therefore, the difference between samples AP1, AP2, and AP3 might be due to one of the above reasons. And the changes in the genome might not fall into genes involving morphological characteristics.

Moreover, knowledge about the genetic diversity of rose apple varieties is valuable for plant breeding programs. It can help breeders select parent plants with diverse genetic backgrounds, developing new varieties that may possess improved traits such as disease resistance, better yield, or enhanced nutritional content. This study revealed that 16 varieties expressed genetic diversity through phenotypic traits and DNA profiles. The effectiveness of ISSR markers and morphological characters in evaluating the genetic diversity was also observed in the previous study of Cheong and Ranghoo-Sanmukhiya (2013) that elucidated the phylogenetic between 6 species of the *Syzygium* genus consisting of *S. commersonii*, *S. coriaceum*, *S. glomeratum*, *S. petrinense*, *S. Samarangense* and *S. venosum* applying morphological data, RAPD, and ISSR markers.

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