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Genetic diversity of Guava varieties (*Psidium guajava* L.) based on morphology and ISSR molecular markers

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Abstract. *Hien PTT, Cham LTT, Hang VTT. 2024. Genetic diversity of Guava varieties (*Psidium guajava *L.) based on morphology and ISSR molecular markers. Biodiversitas 25: 1037-1045.* Guava (*Psidium guajava* L.) is a fleshy-fruited representative belonging to the Myrtaceae family that has attracted attention due to its economic benefits. In this study, seventeen guava varieties in the Mekong Delta and Hanoi were collected and genetically validated by both morphology and DNA markers. The results revealed that fruit shape is a powerful indicator of guava discrimination. There are 12 out of 15 Inter Simple Sequence Repeats (ISSR) markers that show informative band patterns and 93 amplicons are generated with the size in length 200-2000 bp. The average polymorphic band and PIC value are 97.85% and 36%, respectively. Based on the dendrogram constructed by the Unweighted Pair Group Method using arithmetic Averages (UPGMA) method, samples were grouped into two main groups and the similarity level ranged from 55-98%. Group 2 contains Oi Tim (Purple guava), a special variety that showed high differences in terms of morphology and DNA profiles. Such information reflects the diversity of guava germplasm in Viet Nam, which is useful for conservation, and breeding strategies. The result revealed that 11 varieties expressed genetic diversity through both phenotypic traits and DNA profiles. Such information illustrates the genetic diversity among *Psidium guajava*, contributing to germplasm resources and plant breeding programs.

Keywords: Cluster analysis, fruit genotyping, fruit shape, guava varieties, ISSR

INTRODUCTION

Guava (*Psidium guajava* L.), a member of the *Myrtaceae* family, is a tropical fruit tree native to South America (Rajan and Hudedamani 2019). It is commonly cultivated in tropical and subtropical countries, including Brazil, Thailand, Mexico, and Peru, with a focus on fruit exports (Rajan and Hudedamani 2019). Guava fruit is rich in ascorbic acid (vitamin C), vitamin A, and fiber, which contribute to strengthening the immune system and supporting digestive function (Jamieson et al. 2023). Guava also provides minerals such as phosphorus, calcium, iron, nicotinic acid (vitamin B3), and antioxidant compounds that are beneficial in preventing cardiovascular diseases, diabetes, and cancer (Rajan and Hudedamani 2019; Jamieson et al. 2023). With high nutritional properties, guava fruit was often used directly or processed into beverages (Manjusha et al. 2022). In addition, guava fruit was also used in the form of powder, jam, jelly, syrup, and many other guava products (Chiveu et al. 2019). Different parts of the guava tree such as leaves, roots, stems, and bark were used as traditional medicine in many countries around the world (Lok et al. 2023). In particular, guava leaves have been used for treating stomach diseases, diarrhea, and diabetes (Kumar et al. 2021). In research conducted on six guava cultivars grown in Vietnam, guava essential oil extract was used in pesticide activities, contributing to reducing diseases transmitted by mosquitoes and snails (Luu et al. 2023).

In Vietnam, many guava cultivars have been developed and widely planted in recent years, contributing to improving economic efficiency for growers. Depending on the geographical and climatic characteristics of the region, there are some famous guava cultivars such as “Dong Du” guava, Giant guava (“Xa Li”), and White guava No. 5 (“Bo”). Besides some popular guava cultivars such as Taiwan guava, Ruby red flesh guava, Queen guava, Pink flesh guava, and Pink Pearl guava (“Se”), there are also new seedless guava cultivars imported from Thailand and Malaysia. Guava fruits in these cultivars are mainly spherical or oval, the main differences are in fruit diameter, skin texture (rough or smooth), color (white or pink), and texture (soft, crispy, or thin) of fruit flesh, small or large number of seeds (Shukla et al. 2022). Many advanced processing methods are being applied to the guava planting and harvesting process to increase productivity as well as guide guava products to meet standards such as VietGAP, GlobalGAP, or OCOP. However, studies in assessing genetic diversity have not been conducted on guava cultivars in Vietnam.

The genetic diversity of crop cultivars is analyzed through morphological and genetic characteristics (Athinodorou et al. 2021). Among them, molecular markers are widely used in the world as one of the effective tools for planting variety identification. Studies on genetic diversity using ISSR (Inner Simple Sequence Repeat) markers have been successfully performed on many fruit tree cultivars. As a consequence of the 17 tested primers, the 36 grape (*Vitis vinifera*) cultivars grown in Palestine were clustered into eight main groups in

addition to an isolated genotype (Basheer-Salimia and Mujahed 2019). Research on the genetic diversity of 76 analyzed dragon fruit cultivars in Colombia was determined using eight ISSR primers, the results showed that the genetic characteristics of this yellow pitahaya or yellow dragon fruit genotypes were classified into three large groups (Morillo et al. 2022). The ISSR markers combined with the Random Amplified Polymorphism DNA (RAPD) markers contributed to identifying high genetic variation in 28 avocados (*Persea americana*) cultivars collected from Vietnam and other countries (Ninh et al. 2022). Another study was conducted on 66 *Arbutus unedo* trees from 11 natural populations with different climate and altitude conditions in Morocco, through 14 ISSR primers, these cultivars were analyzed into four independent groups of these natural conditions (Faida et al. 2023). In Eastern Kenya, the ISSR markers helped assess the genetic diversity of 64 tamarind (*Tamarindus indica*) cultivars and identified them into seven distinct tamarind groups (Kidaha et al. 2023). Through the above studies, the ISSR marker is a potential option for detecting the genetic relationship and diversity of available guava cultivars in Vietnam. Therefore, this study aims to investigate the genetic diversity among guava varieties by both morphological traits and ISSR markers.

MATERIALS AND METHODS

Sample collection

A total of 17 leaf samples of 11 guava varieties were collected in Hanoi and provinces in the Mekong Delta (Hau Giang, Ben Tre, Tra Vinh, Can Tho), Vietnam. The samples were selected based on the local names and certified by a local authority from the Agricultural Department. After collection, the samples were preserved in plastic bags with fully labeled information and refrigerated storage at the Molecular Biology Laboratory-Institute of Food and Biotechnology, Can Tho University. Sample collection locations and number of samples are presented in Table 1.

Morphological measurement

According to Vietnamese National Standards, samples were collected on trees with normal growth and development, contained no damage by pests or too few fruits, and fruits from trees at the end of the garden zone (trees located in the margin of two adjacted orchard) were not collected. For leaf samples, for each guava variety, a representative branch containing all the young leaves, mature leaves, and old leaves was selected (based on the color of the branch), the leaves are collected without pests or mechanical damage. For fruits, for each guava variety, fruit samples were from 1-3 trees, the number of fruits collected depends on each tree species; Trees with wide canopies and produce many or large fruits: 1-2 fruits from each tree were collected, trees with few or small fruits: 2-3 fruits from each tree were collected. The fruits must be uniformly ripe, and free of pests, rot, or deformities. Fruits are collected from 4 directions of 3 canopy levels (high, middle, and low), avoiding collecting fruits from the top layer or the bottom layer near the ground.

After collection, leaves, and fruits of each guava variety are washed, observed with the naked eye, photographed, and described the morphology and compared with the description of Methela et al. (2019) as follow:

Leaves: Shape, color, leaf margin, veins, tip, and base.

Leaf shape: Cordate - Heart shaped, with a sharp tip at the apex and the petiole coming out between the rounded parts of the heart at the leaf base; Elliptical - Longer than wide, but tapers at both ends; Lanceolate - Longer than wide, but tapers smaller at the apex; Linear - narrow and the same width at both ends; Ovate - Egg shaped and widest at the base.

Leaf margin: Cleft - rounded shapes with notches more than halfway to the midrib; Crenate - small, rounded teeth; Dentate - sharp “teeth” that point outward; Entire - a smooth margin with no teeth, notches or other textures along the edge of the leaf; Incised - margin cut with irregular teeth that may have deep notches toward the midrib; Lobed - rounded shape with notches less than halfway to the midrib; Serrate - teeth that point toward the apex; Sinuate - A wavy edge, larger than crenate and not as pronounced as lobed or cleft.

The apex represents the tip of the leaf blade. Apices can vary in form and structure. An acuminate apex features an elongated, slender, sharply pointed tip with an angle at the terminal end less than 45 degrees, and its sides are typically straight to convex. An acute apex presents a sharp-pointed tip with an angle at the terminal end ranging between 45 and 90 degrees, also with straight to convex sides. Mucronate apices terminate in a short, sharp, abrupt point. Cuspidate apices are sharply constricted into an elongated, sharp-pointed tip or cusp, resembling a sharp, rigid point. Obtuse apices have a blunt or rounded tip, with the sides forming an angle of more than 90 degrees, often straight to convex. Rounded apices curve to create a full, sweeping arc. Truncate apices appear as if they were cut off at nearly a right angle to the midrib, forming a flat or squared-off shape. Retuse apices exhibit a shallow notch in a rounded or obtuse apex. Emarginate tips have a broad, shallow notch at the apex. These descriptions offer a glimpse into the diverse array of forms leaf apices can adopt.

The lower portion of a leaf, known as the base, is where the lamina connects to the petiole or stem. Cuneate bases are characterized by sharp points, with an angle between opposite sides measuring less than 45 degrees, resulting in a wedge or triangular shape that tapers to a narrow point where the lamina attaches to the petiole. Acute bases feature a sharp-pointed base, with opposite sides forming an angle between 45 and 90 degrees at the juncture of the lamina and petiole. Obtuse bases have a blunt or narrowly rounded shape, with opposite sides forming an angle greater than 90 degrees at the connection of the lamina to the petiole. Rounded bases curve to create a full, sweeping arc. Truncate bases appear as if they were sliced off at nearly a right angle to the midrib, resulting in a flat or squared-off shape. Cordate bases resemble a valentine shape, with both the right and left margins forming broad arcs that converge in the middle at the junction of the lamina and petiole. Inequilateral bases exhibit asymmetry, with the left and right sides differing in size or shape.

Auriculate bases have lobes resembling ears where the lamina meets the petiole.

Fruit: Shape, color, peel, seeds, and pulp.

Leaves: Young and mature leaves were photographed on both sides, and then leaf size was measured by using Toupview software (ToupTeck Inc, China) according to the principles of Parnell et al. (2013) (Figure 1), the leaf length was measured along the longest axis of the leaf, from the base of the leaf to the tip of the leaf, and the leaf width was determined at the point of maximum width.

Fruit: The size of ripe fruits which were the representative samples was measured after collection by using ToupView software according to the principles of Methela et al. (2019) (Figure 2).

Brix and pH determination

Sweetness and pH are two important criteria to help evaluate the quality of fruit (Mothina and Yapwattanaphun 2017). Each criterion was evaluated by recording the measurement results of naturally ripened fruit extract, each criterion was repeated 3 times on 3 different fruits of the same guava variety. Degrees Brix (%) was measured by using a Brix meter (Atago N-1∝ Brix 0-32%, Japan). In terms of pH: by using Hanna HI2210 pH Meter.

DNA extraction

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

Table 1. Seventeen guava samples collected in Ha Noi, Hau Giang, Ben Tre, Tra Vinh, Can Tho

Sample code	Sample name	Location
HH	Hoang Hau	Phong Dien (Can Tho)
NH.1	Nu Hoang	Long Ho (Vinh Long)
NH.2	Nu Hoang	Cho Lach (Ben Tre)
NH.3	Nu Hoang	Thot Not (Can Tho)
L1	Oi Le	Phong Dien (Can Tho)
L2	Oi Le	Thot Not (Can Tho)
LDL	Le Dai Loan	Cho Lach (Ben Tre)
D	Oi Dai	Thot Not (Can Tho)
RH	Oi Hong	Cho Lach (Ben Tre)
S.1	Oi Se	Tieu Can (Tra Vinh)
S.2	Oi Se	Chau Thanh (Hau Giang)
S.3	Oi Se	Cho Lach (Ben Tre)
RB	Oi Ruby	Chau Thanh (Hau Giang)
TC	Tan Chau	Cho Lach (Ben Tre)
T	Oi Tim	Cho Lach (Ben Tre)
DD.1	Dong Du	Ha Noi
DD.2	Dong Du	Ha Noi

Table 2. Fifteen ISSR primers were used in this study

Primer name	Sequence 5'- 3'	Annealing temperature (Ta)
G-ISSRK2	$(GTG)_4AC$	50°C
G-ISSR13	$(AG)_8CA$	50°C
G-ISSR818	$(CA)_8G$	50°C
G-ISSR825	$(AC)_8T$	50°C
G-ISSR827	$(AC)_8G$	50°C
G-UBC809	$(AG)_8G$	50°C
G-UBC826	$(AC)_8C$	50°C
G-UBC829	$(TG)_8C$	50°C
G-UBC840	$(GA)_8CT$	50°C
G-UBC848	$(CA)_8AG$	50°C
G-UBC856	$(AC)_8CA$	50°C
G-UBC866	$(CTC)_6$	50°C
G-UBC888	$ATG(CA)_7$	50°C
G-UBC889	$ATG(AC)_7$	50°C
G-UBC890	$GAA(GT)_7$	50°C

Source: He et al. (2005, 2007; Huang et al. (2009); Shankar and Anjani (2023)

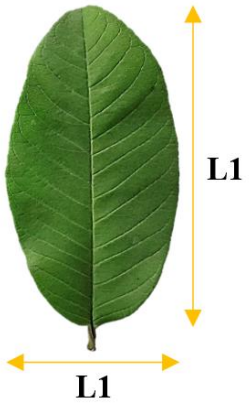

Figure 1. Guava leaf measurement method (wild-type guava). Note: L1: Length, L2: Diameter

Figure 2. Guava fruit measurement method (Ruot Hong). Note: L1: Length, L2: Diameter

Amplification of ISSR markers

PCR reaction was performed to amplify loci based on 15 ISSR markers presented in Table 2. Basic chemical components for a 15µL PCR reaction include 8µL BiH_2O, 4µL MyTaq mix, 1µL Primer, and 2µL DNA. The amplification reaction was performed by an OptiMax PCR

machine with 40 cycles: initial denaturation phase 95°C/4 minutes, denaturation phase 95°C/30 seconds, primer annealing phase 50°C/1 minute, elongation phase 72°C/2 minutes and final elongation stage 72°C/10 minutes.

Data analysis

Toupview software was used to determine leaves and fruit size. Morphological data were statistically analyzed using Excel software (2019), statistically analyzed 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 were encoded in binary form: 1 - for cases where bands are present, 0 - for cases where bands were not present. The encoding table was saved as an Excel file and transferred to NTSYSpc 2.1 software to construct the dendrogram and similarity matrix built based on the UPGMA method (Unweighted Pair Group Method with Arithmetic Average). Polymorphic Information Content (PIC) was determined by the iMEC software. The Jaccard similarity coefficient is determined from the binary data matrix.

RESULTS AND DISCUSSION

Morphological characteristics

Leaf morphology

The results of investigating the leaf dimensions characteristics of guava varieties presented in Table 3 showed that the RB (Ruby) variety was the guava variety with the largest leaf length and width with dimensions of 14.58 × 9.59 cm, and the smallest was the DD variety (Dong Du) with dimensions of 8.61 × 3.95 cm. The 3 guava varieties with the longest leaf length were S (Se), RH (Ruot Hong), NH (Nu Hoang), while the largest leaf widths belonged to the 4 varieties NH (Nu Hoang), LDL (Le Dai Loan), D (Dai), RH (Ruot Hong). Leaf length and width are some of the leading parameters that help classify leaves and identify species based on morphological characteristics.

The results of investigating the external morphological characteristics of the leaves showed that most of the guava varieties examined had elliptic leaves, rounded or cuneate leaf bases, and entire or serrate leaf margins (Table 4), which were similar to the results described by Sharma et al. (2010) and differed from Methela et al. (2019). However, some guava varieties had different characteristics compared to other varieties such as oval leaf shape (Tan Chau) or oblong-lanceolate (Oi Tim). Some of the guava varieties with the most different leaf morphology were RB (Ruby) and T (Tim). In addition, Oi Tim guava can also be identified through the special purple color in the leaves and fruit similar to the description of Sohi et al. (2022).

Fruit morphology

Morphological characteristics such as shape, color, and fruit size presented in Table 5 showed the diversity among guava varieties. Regarding the fruit shape, there were 3 different shapes such as pear-shaped (LDL), globose (S and DD), and Oblate (NH, TC) as described by Kareem et al. (2018). The fruit pulp also had differences in color such as white (LDL), pink (S, RB, TC), yellow (DD), and purple (T), which were different from the description of Kareem et al. (2018). Although there were similarities in the color of the skin and the fruit pulp (Figure 5), relying on differences in shape and size could be used to distinguish guava varieties from each other, showing that these are representative characteristics of each guava variety.

Sweetness is considered one of the indicators of fruit quality (Mothina and Yapwattanaphun 2017). Through the investigation results in Table 5, the guava varieties in the study had a sweetness level of 5-10%, the highest is the DD sample (Dong Du) and the lowest is the S sample (Oi Se). The pH index was generally highly acidic, ranging from 4.05 - 4.74, the lowest is Dong Du guava.

DNA profiles of the guava population

A total of 15 ISSR molecular markers were used to analyze genetic diversity in 17 guava samples collected in Ha Noi, Hau Giang, Ben Tre, Tra Vinh, and Can Tho. Among them, primer G-UBC888 was effective in successfully amplifying 17 guava samples (Figure 6). The results of PCR product analysis on 2% agarose gel showed that 12 of the 15 markers successfully amplified 93 DNA bands with sizes from 200 bp to 2000 bp, with the number of polymorphic bands accounting for 97.85% (Table 6). This shows that the level of genetic diversity of guava varieties in the study is quite high.

The results presented in Table 6 also showed that the number of DNA bands generated by each marker ranges from 5 to 13 bands, the least was primer G-ISSR818 and the most was G-UBC890. The rate of polymorphic bands reaching 100% was recorded in 11 of 12 primers, except for primer G-UBC888 which had a polymorphic rate of 70%.

According to Botstein et al. (1980), the polymorphic ability of a molecular marker is reflected through the value of the PIC coefficient. Primers with high polymorphism level are primers with PIC coefficient>0.5, primers with medium polymorphism level have PIC within the range of $0.25 \leq PIC \leq 0.5$, and primers with low polymorphism level with PIC<0.25. The PIC coefficient value of the 12 primers used in analyzing the genetic diversity of 17 guava samples reached an average polymorphism level, which lay in the range of 0.33-0.37, the highest level belonged to the 3 primers: G-UBC888, G-UBC889, G-UBC890 and the lowest was G-UBC840 (Table 7).

The average diversity index of the polymorphic bands was expressed through the MI value (Marker Index). The results presented in Table 7 show that the MI coefficient value of the 12 primers was in the range of 0-0.02, with most primers having an MI coefficient of 0.01. The primer that had the lowest MI value was G-UBC840 (MI = 0) and the primer that had the highest MI value was G-ISSR818 (MI = 0.02).

Table 3. Leaves dimensions collected from Ha Noi, Hau Giang, Ben Tre, Tra Vinh, Can Tho

Sample	Morphology		Length (cm)	Width (cm)
	Upper surface	Under surface		
NH			12.39^{a}±0.49	6.43^{bc}±0.44
LDL			12.26^{b}±0.35	5.78^{bcd}±0.12
D			12.18^{b}±0.36	6.82^{b}±0.30
RH			14.81^{a}±0.91	6.08^{bcd}±0.22
S			13.38^{ab}±0.61	5.01^{de}±0.47
RB			14.58^{a}±0.15	9.59^{a}±0.28
TC			12.33^{b}±0.50	5.24^{cde}±0.31

T		11.69^{b}±0.49	4.90^{de}±0.59
DD		8.61^{c}±0.50	3.95^{e}±0.12

Figure 5. Fruits morphological characteristics of guava varieties. Note: 1: Nu Hoang, 2: Le Dai Loan, 3: Oi Se, 4: Tan Chau, 5: Ruby, 6: Dong Du, 7: Oi Tim

Table 4. Morphological characteristics of guava variety leaves collected in Ha Noi, Hau Giang, Ben Tre, Tra Vinh, Can Tho

Sample	Leaf shape	Leaf apex	Leaf base	Leaf margin
NH	Elliptic	Obtuse	Rounded	Entire
LDL	Elliptic	Apiculate	Rounded	Entire
D	Elliptic	Obtuse	Rounded	Serrate
RH	Elliptic	Obtuse	Rounded	Entire
S	Oblong-Elliptic	Acute	Cuneate	Entire
RB	Elliptic	Obtuse	Rounded	Serrate
TC	Oval	Obtuse	Rounded	Serrate
T	Oblong-Lanceolate	Apiculate	Cuneate	Entire
DD	Elliptic	Obtuse	Rounded	Serrate

Cluster analysis

The results on the pedigree dendrogram showed that if the genetic similarity of 17 guava samples (11 varieties) is considered at 55%, it will be divided into 2 main clusters and several subclusters:

- Cluster A: includes samples HH, NH1, NH2, NH3, L1, L2, D, NH, S1, S2, RB, S3, DD1, DD2 with similarity ranging from 58.1% to 98%. In this cluster, the HH sample stands alone in a branch, with approximately 58.1% similarity to other samples, proving that the HH sample is less closely related in the same group. Cluster A is divided into many subclusters:

+ Subcluster A1: includes samples NH1 and NH2, the two samples have 90.3% similarity.

+ Subcluster A2: includes samples L1 and L2, the two samples have 87% similarity.

+ Subcluster A3: includes subcluster A1 and subcluster A2, the subclusters have 79.5% similarity.

+ Subcluster A4: includes subcluster A3 and sample D with 71.8% similarity.

+ Subcluster A5: includes subcluster A4 and sample NH3 with 67.7% similarity.

+ Subcluster A6: includes samples S1, S2, RB. In which, two samples S1 and S2 have a high similarity of 98%.

+ Subcluster A7: includes samples S3, DD1, DD2. In which, 2 samples DD1 and DD2 have 79% similarity.

+ Subcluster A8: includes subcluster A5 and subcluster A6, with 67% similarity.

+ Subcluster A9: includes subcluster A7 and A8, with 66.6% similarity.

- Cluster B: includes LDL, RH, T, and TC samples. The cluster has similarity from 69.9% to 84.9%. Which, the two samples RH and T have 84.9% similarity to each other and 82.8% similarity to the TC sample. The LDL sample stands alone in a branch and has 69.9% similarity to the other samples in the cluster.

Table 5. Differences in morphological characteristics between guava varieties

Sample	Brix (%)	pH	Length (cm)	Width (cm)	Morphology characteristic
NH	6	4.74	8.09	9.25	- Shape: Oblate; peel: pale green, rough; flesh: white, spongy; little seeds.
LDL	8	4.30	10.78	9.0	- Shape: Pear - shape; peel: pale green, moderately smooth; flesh: white, spongy; little seeds.
S	5	4.12	6.29	6.34	- Shape: globose, peel: pale green, moderately smooth; flesh: pink.
RB	7	4.45	7.56	8.57	Shape: Oblong globose; peel: green, smooth; flesh: pink.
TC	6	4.74	9.05	7.76	- Shape: Oblate; peel: green, moderately smooth; flesh: pink; little seeds.
DD	10	4.05	4.71	4.40	- Shape: globose; peel: yellow-green, very smooth; flesh: pale yellow; multi seeds.
T					- Peel and flesh: purple.

Table 6. Polymorphism evaluation indexes of 17 guava population samples amplified by 12 ISSR primers

ISSR marker	Total band	Poly morphic alleles	Mono morphic	Polymorphism range (%)	DNA ranges (bp)
G-ISSRK2	7	7	0	100%	250 - 1500
G-ISSR818	5	5	0	100%	300 - 700
G-ISSR825	9	9	0	100%	250 - 1500
G-ISSR827	8	8	0	100%	200 - 1000
G-UBC826	8	8	0	100%	250 - 1000
G-UBC840	4	4	0	100%	350 - 900
G-UBC848	5	5	0	100%	200 - 900
G-UBC856	11	11	0	100%	300 - 2000
G-UBC866	7	7	0	100%	300 - 900
G-UBC888	7	5	2	71%	250 - 1000
G-UBC889	9	9	0	100%	250 - 1000
G-UBC890	13	13	0	100%	200 - 1700

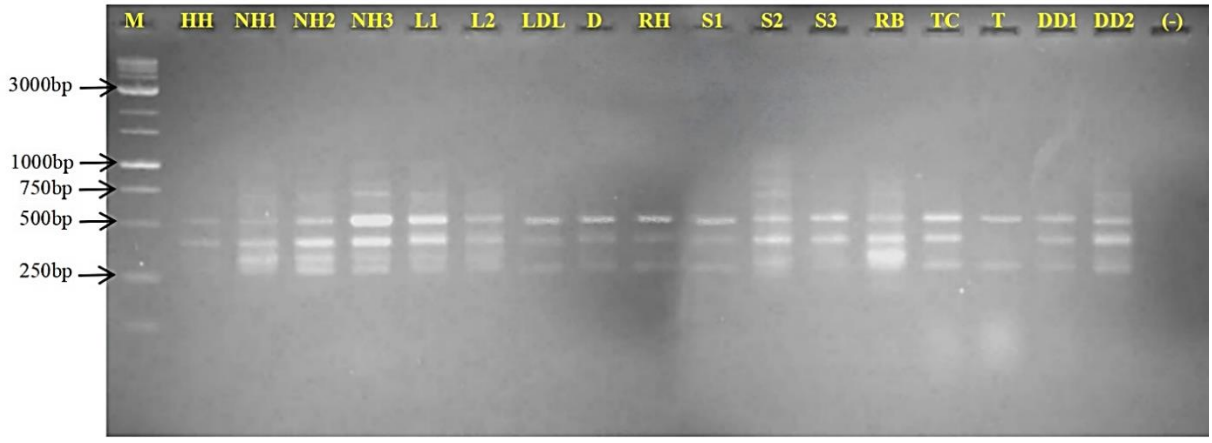

Figure 6. Electrophoresis results of using primer G-UBC888 on 2% agarose gel. Note: M: Marker, NH: Nu Hoang, L: Le, LDL: Le Dai Loan, D: Dai, S: Se, RB: Ruby, TC: Tan Chau, T: Tim, DD: Dong Du, -: Negative control

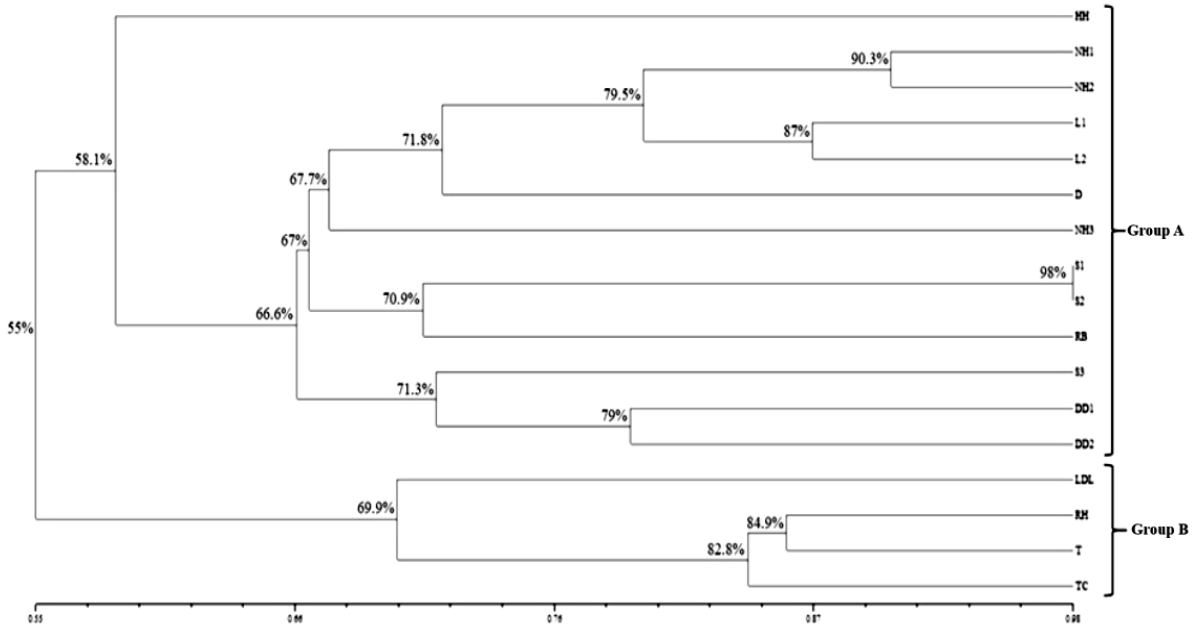

Figure 7. Pedigree dendrogram showing genetic correlation among guava samples. Note: NH: Nu Hoang, L: Le, LDL: Le Dai Loan, D: Dai, S: Se, RB: Ruby, TC: Tan Chau, T: Tim, DD: Dong Du

Table 7. Genetic diversity coefficient analysis of investigated guava samples

ISSR marker	H	PIC	E	H.av	MI	D	R
G-ISSRK2	0.48	0.36	2.94	0.00	0.01	0.82	4.11
G-ISSR818	0.47	0.36	3.11	0.01	0.02	0.61	2.35
G-ISSR825	0.47	0.36	3.47	0.00	0.01	0.85	4.94
G-ISSR827	0.45	0.35	2.82	0.00	0.01	0.87	4.35
G-UBC826	0.47	0.36	3.05	0.00	0.01	0.85	4.58
G-UBC840	0.42	0.33	1.23	0.00	0.00	0.9	2.23
G-UBC848	0.48	0.36	2.00	0.00	0.01	0.84	2.35
G-UBC856	0.48	0.36	4.58	0.00	0.01	0.82	4.58
G-UBC866	0.45	0.35	4.58	0.00	0.01	0.57	3.41
G-UBC888	0.49	0.37	3.82	0.00	0.01	0.7	3.17
G-UBC889	0.49	0.37	5.00	0.00	0.01	0.69	5.05
G-UBC890	0.49	0.37	5.76	0.00	0.01	0.8	7.05

Through analysis with 12 primers, 17 guava samples were classified into 2 main clusters and many subclusters in which the level of genetic correlation between samples ranged from 55% to 98% (Figure 7), showing that the samples were highly genetic variation. This also indicated that the molecular marker ISSRs were used effectively in genetic diversity analysis research.

Understanding the genetic diversity and the relatedness between fruit plant cultivars and plant ecotypes is a vital step in the conservation of genetic resources. As a result of various breeding programs, there are a lot of plant varieties in the market. Morphological and agronomical traits are the first characteristics that are easy to visualize. Fruit shape is a valuable descriptor that can distinguish guava varieties, this trait is also suggested by Valera-Montero et al. (2016) when analyzing the genetic diversity of guava varieties in Mexico. Moreover, DNA markers play an important role in identifying genetic diversity at the molecular level. Zabet et al. (2023) applied the ISSR markers to classify guava genotypes into three main groups while the clustering result from RAPD markers is two. Gangappa et al. (2022) utilized 33 morpho-biochemical characteristics that enabled an assessment of the genetic variation, diversity, and structure of 28 guava varieties. That data illustrates that there is highly significant genetic diversity in those traits in the guava germplasm.

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