JOURNAL OF TROPICAL LIFE SCIENCE
2023, Vol. 13, No. 2, 319 – 334
http://dx.doi.org/10.11594/jtls.13.02.10

Research Article

Morphological and Genetic Analysis of *Momordica cochinchinensis* (Lour.) Spreng. (Gac) from Different Accessions in Malaysia

Nurin Nazihah binti Mohd Khairi *, Halimaton Saadiah binti Mohd Othman

Department of Biotechnology, Kulliyyah of Science, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia

Article history:
Submission June 2022
Revised June 2022
Accepted September 2022

**Corresponding author:*
E-mail: nurinkhairi07@gmail.com

ABSTRACT

Momordica cochinchinensis or gac fruit is a 'superfruit' that is well-known in Vietnam. Gac is an orange fruit that is ovoid in shape and has a soft spiny texture. In Malaysia, gac fruit is a new and less known plant. This study aimed to characterize gac fruit using morphological analysis involving both vegetative and reproductive parts and to characterize the genetic diversity in gac fruit by using Inter-simple sequence repeat (ISSR) analysis. Four different gac accessions were collected from different areas (Kota Damansara (Selangor), Melaka Tengah (Melaka), Hulu Langat (Selangor) and Kuantan (Pahang)) were cultivated under tropical conditions in Kuantan, Pahang. The gac accessions showed differences in morphological characters. Generally, the gac fruits were reddish-orange in colour, the leaf was dark green on the adaxial part and light green on the abaxial part, and the female and male flower was light yellow and white in color. The fruit weight ranged from 193.72 g (GD) to 334.70 g (GH) with varied shapes and spike density. DNA extraction was following the CTAB method. All 30 primers showed high levels of polymorphism (83%) and the polymorphism information content (PIC) with the mean of 0.48. Nei's genetic distance coefficient ranged between 0.27 and 0.6 with the mean value of 0.41. Dendrogram based on UPGMA analysis grouped the four gac accessions into two main groups. Cluster I consisted of accession GD, GM and GH while cluster II consisted of only GX. Results from both morphological and molecular analysis showed genetic diversities in all four gac fruits studied.

Keywords: Gac fruit, Genetic analysis, Inter-simpler Sequence Repeat (ISSR), Morphology, Phylogenetic tree

Introduction

Momordica cochinchinensis, also known as gac fruit, is a 'superfruit' popular in Vietnam [1]. Other names for gac fruit are baby jackfruit, sweet gourd and Cochinchin gourd. *Momordica* is the family of Cucurbitaceae [2], while *cochinchinensis* named from a region in northern Vietnam called Cochinchina [3]. Gac fruit has been widespread across Southeast Asia, Malaysia, and India [4].

Gac fruit is an orange-red fruit with an ovoid shape and has a soft spiny texture. Gac fruit is a climber plant that can climb up to 20 meters. It has tuberous roots and brown or grey-black seeds. The gac fruit tastes like papaya [5]. Traditionally, Vietnamese people use gac fruit in their rice, called *Xoi Gac*, as a red colorant, especially during their wedding ceremony. Furthermore, mature gac fruit is used as a cosmetics product in Thailand due to their photoprotection against UV radiation [6].

Moreover, gac fruit has been studied to have high carotenoids. Carotenoids are phytochemical properties containing nutrition values that give various health benefits [6]. Gac fruit has β-carotene and lycopene; these two phytochemical properties are antioxidant substances. The highest antioxidant activity of gac fruit can be seen during the immature stage of the fruit [6]. It also has the potential for antimicrobial properties. The lycopene concentration is five times greater than some famous fruits analyzed like tomato, papaya, guava

How to cite:
Khairi NNBM, Othman HSBM (2023) Morphological and Genetic Analysis of *Momordica cochinchinensis* (Lour.) Spreng. (Gac) from Different Accessions in Malaysia. Journal of Tropical Life Science 13 (2): 319 – 334. doi: 10.11594/jtls.13.02.10.

and watermelon since its gac fruit is abundant in carotenoids. Gac aril has the greatest β-carotene concentration of all fruits and vegetables. For instance, eight times greater than the level of carrots, which are classified as high in β-carotene [4].

Gac fruit is also known as the 'fruit of heaven' as its acclaimed properties in enhancing lifespan, strength and vitality. Thus, gac fruit has many benefits in preventing severe diseases. The lycopene concentration has a cholesterol-reducing effect and can lower the risk of death from chronic diseases such as cancers. The apoptosis in human MCF-7 breast cancer cells can be induced by gac fruit aril extract [7].

Gac fruit is a new and less-known plant in Malaysia. Malaysians still unheard of gac fruit because gac fruit originated from Vietnam and is not well-introduced and common in Malaysia like any other fruit like avocados and peaches. Information on gac fruit regarding classification in morphology and genetic diversity study is still inadequate in Malaysia [8]. In the context of breeding development of gac fruit, it becomes a challenge as there is a lack of information on cultivation in Malaysia. The gac fruit itself is a dioecious plant where hand pollination or natural pollinator is required to increase the fruit set because gac fruit yield has always been the common cause of the requirement for pollination to occur and happen is low [9]. Plant sex cannot be determined from seed and can be defined only upon flowering. Insufficient information is known to assess gac fruit's market potential, growth potential, and post-harvest needs [10]. Even though gac fruit is commercially developed on a limited scale in Vietnam and Thailand, little is known about its yield capacity or the production parameters that influence yield and fruit quality, particularly if grown in Malaysia's tropical climate.

Identifying morphological and molecular variation is essential as these data complement each other. In many cases, clear molecular differentiation could confirm morphology-based species hypotheses. It is not enough to depend on morphology alone. Molecular evidence will continue to enhance the knowledge of phylogenetic relations for ever-increasing molecular datasets. Genetic diversity can be conducted using the ISSR marker to analyze gac fruit. ISSR is among the molecular markers that can be used despite foreknowledge of the DNA sequence to identify polymorphisms in microsatellites and inter-microsatellite loci. The ISSR marker is one of the markers that produce high polymorphism, robust, rapid, simple, reproducible, and inexpensive [11]. Through this project, molecular and morphological information will help identify the genetic variability of gac fruit. Higher variability in the initial breeding material thus ensures more significant chances of producing desired crop plant forms. This study aimed to characterize gac fruit using morphological analysis involving both vegetative and reproductive parts and characterize the genetic diversity in gac fruit by using Inter-simpler sequence repeat (ISSR) analysis.

Material and Methods

Sample collection

A total of four different gac accessions (seeds) were collected in Damasara (GD), Melaka (GM), Hulu Langat (GH) and Kuantan (GX). In August 2019, the gac fruit's seeds were sown by hand in plastic pots. They were transplanted into polybags at an experimental plot at Kulliyyah of Science International Islamic University Malaysia (IIUM) Kuantan, Pahang. A Completely Randomized Design (CRD) was used for the experimental design (Figure 1). The experimental units consisted of five replications of four different gac accessions with one meter spacing between rows and 50 cm spacing between plants within rows. Polyvinyl chloride (PVC) structures were used to function as the trellis to help support the plant twirl and climb up. The trellis was arranged along the rows. Organic chicken manure and Caviota 2000 Liquid Fertilizer were used every week. Natural fungicide was also applied when necessary.

Morphological evaluation

The morphological traits were studied and recorded. The fruits were sliced; the seeds were extracted from the arils for preservation at -20°C. The characteristics of the plant were studied, such as leaves, flowers, fruits and seeds.

Quantitative characters of gac fruit

Quantitative characteristics of gac fruit were characterized, including the fruit weight, fruit width, fruit length, seed weight, seed length, leaf length, and leaf width. All the characteristics were recorded and calculated, such as the minimum and maximum values and mean.

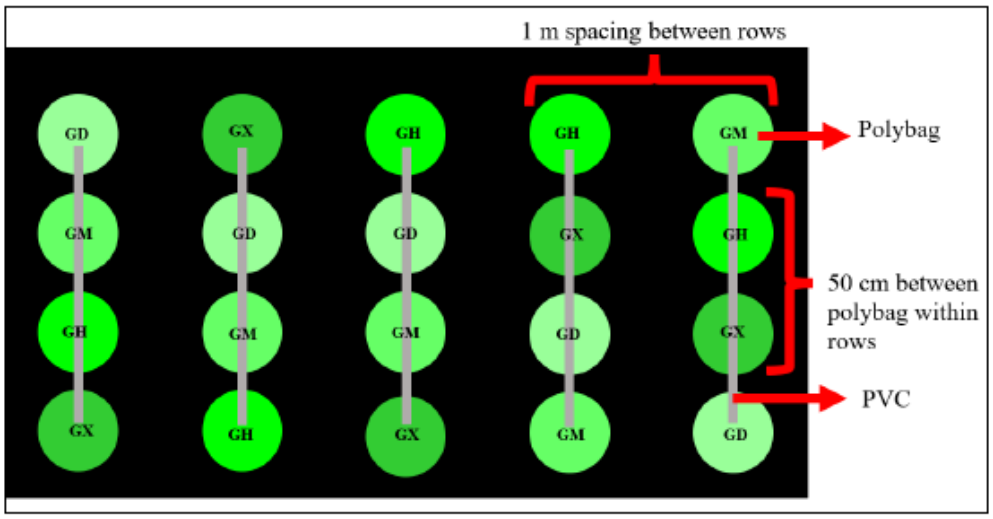

Figure 1. The Experimental Design Using Completely Randomised Design (CRD)

Morphological data analysis

The means of each of the quantitative characters were tested using R studio tools for significant differences via analysis of variance (ANOVA). The Tukey test was used to compare the mean of all the recorded parameters. Relationship evaluation between the examined variables and a correlation test was also performed [12].

DNA extraction

The DNA was extracted from the young leaves of each four accessions using the CTAB method according to the protocol described with a few modifications with single elution [13].

ISSR amplification

A total of 30 ISSR markers (Table 1) were used for application in the genetic diversity of gac. The composition of PCR reactions was performed as follows: 12.5 µL of 2X MyTAQ Red Mix (Bioline, UK), 250 ng of extracted DNA, 50 µM of primer and PCR for the final volume of 25 µL. PCR amplification. The amplification for primer was as follows: initial denaturation at 94°C for 5 minutes, followed by 40 cycles of 94°C for 1 minute, annealing for 1 minute (annealing temperature varied for each primer as stated in Table 1), and elongation at 72°C for 1 minute and final extension at 72°C for 7 minutes [9]. The amplified DNA was separated by electrophoresis in 1.2% agarose (Vivantis Inc., USA) gel in 1X TAE buffer and 5 µL of HyperLadderTM 1kb (Bioline Reagents Ltd, UK), which then was photographed over ultraviolet light in a Gel DocTM XR+ System with Image LabTM Software (Bio-Rad, Australia). Images were used to score amplification products and set up the binary data.

Scoring and molecular data analysis

Thirty ISSR primers were tested on the four different gac fruit accessions. The amplified fragments were scored manually for their presence (denoted as 1) and absence (marked as 0). The fragment data from the primers were pooled to generate a binary matrix, which was then used for phylogenetic analysis. Fragment size was compared to a molecular weight of the 1 kb DNA ladder. Software NTSYSpc version 2.1 was used [14], and the distance matrix was computed using Nei's genetic method [15] for phylogenetic analysis. The dendrogram was generated using the distance matrix output into the unweighted pair group method with arithmetic mean analysis (UPGMA). The average polymorphic information content (PIC) and marker index (MI) were calculated for each primer combination using the formula as:

$$PIC = 1 - i \quad = i=1 nfi2 \qquad \text{- Equation 1}$$

Where fi is the frequency (f) of the *ith* allele, the PIC value provides an estimate of a marker's discriminatory power by balancing the number and frequency of alleles at a given locus [16, 17]. The PIC product and the number of polymorphic bands per assay unit were calculated as marker indices [18]. For analysis, unique marker bands produced by each primer combination were considered genetically dominant. The average PIC value for each primer pair was obtained by averaging the PIC values for each marker band morphotype

Table 1. List of annealing temperatures and primer sequences of ISSR primers.

No.	Primer	Primer Sequence (5'-3')	Annealing temperature (°C)
1	ISSR1	5'-GAG AGA CAG ACA GAC A-3'	47
2	ISSR2	5'-GTG GTG GTG GTG GTG-3'	47
3	MISSR1	5'-GAG AGA GAG AGA GAG AC-3'	42
4	MISSR4	5'-GTG TGT GTG TGT GTG TC-3'	51
5	MISSR8_1	5'-GAG AGA GAG AGA GAG ACT-3'	51
6	MISSR8_2	5'-GAG AGA GAG AGA GAG ATT-3'	42
7	IS12	5'-AGA GAG AGA GAG AGA GT-3'	45
8	IS19	5'-CTC TCT CTC TCT CTC TT-3'	51
9	IS20	5'-CAC ACA CAC ACA CAC AA-3'	51
10	IS21	5'-CAC ACA CAC ACA CAC AG-3'	51
11	IS22	5'-GTG TGT GTG TGT GTG TA-3'	51
12	IS23	5'-GTG TGT GTG TGT GTG TC-3'	51
13	IS25	5'-TCT CTC TCT CTC TCT CA-3'	47
14	IS26	5'-GTG TGT GTG TGT GTG TGT-3'	56
15	IS30	5'-ACA CAC ACA CAC ACA CC-3'	55
16	IS34/1	5'-GAG AGA GAG AGA GAG ACT-3'	46
17	IS34/2	5'-GAG AGA GAG AGA GAG ATT-3'	51
18	IS42/2	5'-ACA CAC ACA CAC ACA CTG-3'	53
19	IS44/2	5'-ACA CAC ACA CAC ACA CTT-3'	53
20	IS45/1	5'-TGT GTG TGT GTG TGT GAT-3'	53
21	IS50	5'-GAA GAA GAA GAA GAA GAA-3'	54.2
22	IS54	5'-AGA GAG AGA GAG AGA GC-3'	55
23	IS55	5'-AGA GAG AGA GAG AGA GA-3'	51
24	IS56	5'-TCT CTC TCT CTC TCT CC-3'	51
25	IS57	5'-GAG AGA GAG AGA GAG ACT-3'	46
26	IS58/1	5'-GTG TGT GTG TGT GTG TCC-3'	55
27	IS58/2	5'-GTG TGT GTG TGT GTG TTC-3'	53
28	IS78	5'-AGA AGA AGA AGA AGA AGA AGA-3'	46
29	IS85	5'-CTC TCT CTC TCA CC-3'	46
30	IS90	5'-AGA GAG AGA GAG AGA GG-3'	51

(absence or presence) scored. In the same way, MI was determined as the product of the number of polymorphic bands and the corresponding PIC value per locus per primer pair [19].

Results and Discussion

Morphological characterization

Due to the limited source of gac seeds cultivated and found around Malaysia, 4 gac fruit accessions were recorded, consisting of GD, GM, GH and GX. These gac fruit accessions were collected from different localities i.e., Kota Damansara (Selangor), Melaka Tengah (Melaka), Hulu Langat (Selangor) and Kuantan (Pahang). Gac fruit showed significant morphological diversity depending on the country and location in which it is cultivated [9], with variations in both vegetative (leaves) and reproductive (fruits and seeds) components. Thus, morphological characterization of gac fruit was divided into 2 different characterizations, i.e., qualitative and quantitative.

Qualitative characteristics of Momordica cochinchinensis

Qualitative characteristics are characteristics that can be observed physically while conducting any study. Based on observation, the characteristics can be classified into 4 parts, e.g. the flower, seed, fruit and leaf (Table 2). All the leaves' results showed diverse variability in the leaf's characteristics.

GD, GM and GH shared the same leaf morphology regarding the color and leaf parts. Dark green adaxial and light green abaxial parts characterized 3 accessions. However, for accession GX the adaxial part was deep green in color, while the other 3 accessions had broadly acuminate apices. GH was distinctively different from the

Table 2. Summary of qualitative data on 4 gac accessions

Accessions/Morphology				Damansara (GD)	Melaka (GM)	Hulu Langat (GH)	Kuantan (GX)
Leaf	Color	Adaxial (upper part)		Dark green	Dark green	Dark green	Deep green
		Abaxial (bottom part)		Light green	Light green	Light green	Light green
	Shape			Palmatipartite	Palmatipartite	Palmatipartite	Palmatipartite
	Arrangement			Alternate	Alternate	Alternate	Alternate
	Venation			Palmate	Palmate	Palmate	Palmate
	Apices			Broadly acuminate	Broadly acuminate	Abruptly acuminate	Broadly acuminate
	Bases			Auriculate	Auriculate	Auriculate	Cordate
	Surface			Glabrous	Glabrous	Glabrous	Glabrous, smooth, shiny
	Margin			Dentate	Dentate	Dentate	Doubly serrate
	Tendril			Presence	Presence	Presence	Presence
	Petiole			Presence	Presence	Presence	Presence
Flower	Petal	Color	Male	Light yellow and white	Light yellow and white	Light yellow and white	Light yellow and white
			Female	Light yellow and white	Light yellow and white	Creamy	White
		Aestivation	Male & Female	Vexillary	Vexillary	Vexillary	Vexillary
		Corolla	Male & Female	Rotate, wheel-shaped	Rotate, wheel-shaped	Rotate, wheel-shaped	Rotate, wheel-shaped
	Sepal	Surface	Male & Female	Coriaceous	Coriaceous	Coriaceous	Coriaceous
		Shape	Male	Ovate-oblong	Ovate-oblong	Ovate-oblong	Ovate-oblong
			Female	Linear oblong	Linear oblong	Linear oblong	Linear oblong
	Bract	Shape	Male	Subapical	Subapical	Subapical	Subapical
			Female	Small and subapical	Small and subapical	Small and subapical	Small and subapical
Fruit	Stage			Ripe	Unripe	Fully ripe	Fully ripe
	Color			Reddish-orange	Yellowish-orange	Orange	Orange
	Shape			Globose-oval	Globose-oval	Tapered	Globose-oval
	Spike density			Medium	Dense	Sparse	No spikes
Seed	Shape			Round	Round	Round	Round
	Color		Mature	Dark brown	No mature seeds	Black	Brown
			Immature	Creamy-yellow	Creamy-yellow	No immature seeds	No immature seeds
	Surface			Wrinkled	Wrinkled	Wrinkled	Wrinkled

rest of the with its abruptly acuminate apices (Table 2).

Furthermore, GX was the only accession with a distinct leaf base which was cordate and doubly serrate edges (Figure 3). On the other hand, the other 3 accessions (GD, GM and GH) had an auriculate base and dentate edges (Table 2). This suggested that the character was common from the rest, indicating that the gene is responsible for the auriculate base and dentate edges being expressed more phenotypically than cordate and doubly serrate edges. As for the leaf shape, the four accessions also showed similar characteristics regarding the shape of the leaf (palmatipartite), alternate leaf arrangement, palmate leaf's venation and tendrils [20]. This indicates the control from heterozygous alleles for both the characters. All 4-accession had glabrous surfaces except for GX, which had a smoother and shinier surface. Figure 3 displays the morphology of gac fruit leaves. It is unknown whether the variations in leaf diversity are due to genetic or environmental factors. Likewise, whether the diversity of gac vegetative components correlates with economically significant characteristics such as increased fruit yield (number of fruits per plant and size) or nutritional and phytochemical content.

The flower is one of the reproductive components that has been studied. The male and female flowers of the gac fruit had the same color: light yellow. However, the female flower of GH had a slightly different color which was creamy yellow similar to GX accessions (Table 2). In another reported study, the flowers were white to ivory yellow [21, 22]. Figure 2 shows the morphology of the gac fruit flowers.

Fruit is a reproductive component that shows wide variations. The fruit characteristics for GD accession were reddish-orange in color, globose-oval in shape with medium spike density. The

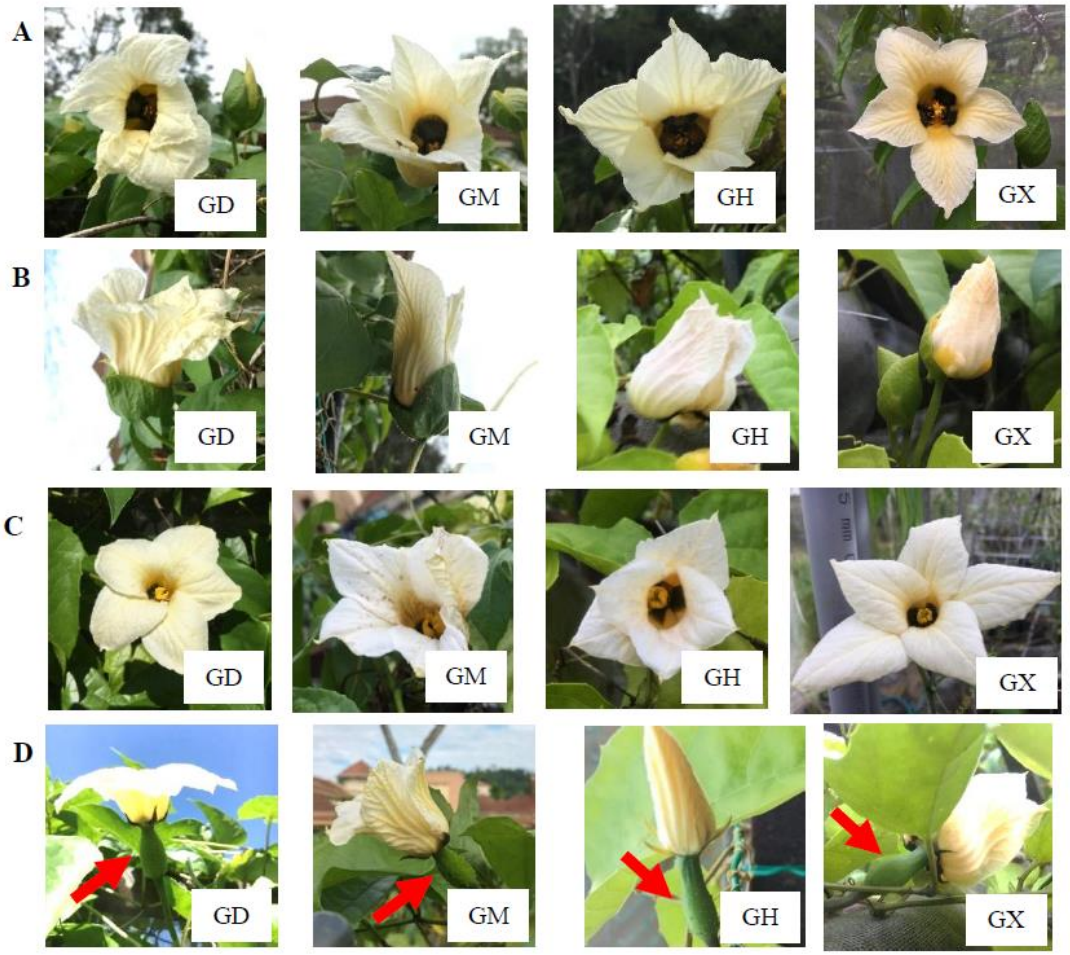

Figure 2. Flower Morphology. (A) Front View of Male Flowers. (B) Side View of Male Flowers. (C) Front View of the Female Flower. (D) Side View of Female Flowers. The Red Arrows Indicate the Presence of the Ovary (Female Reproductive Part).

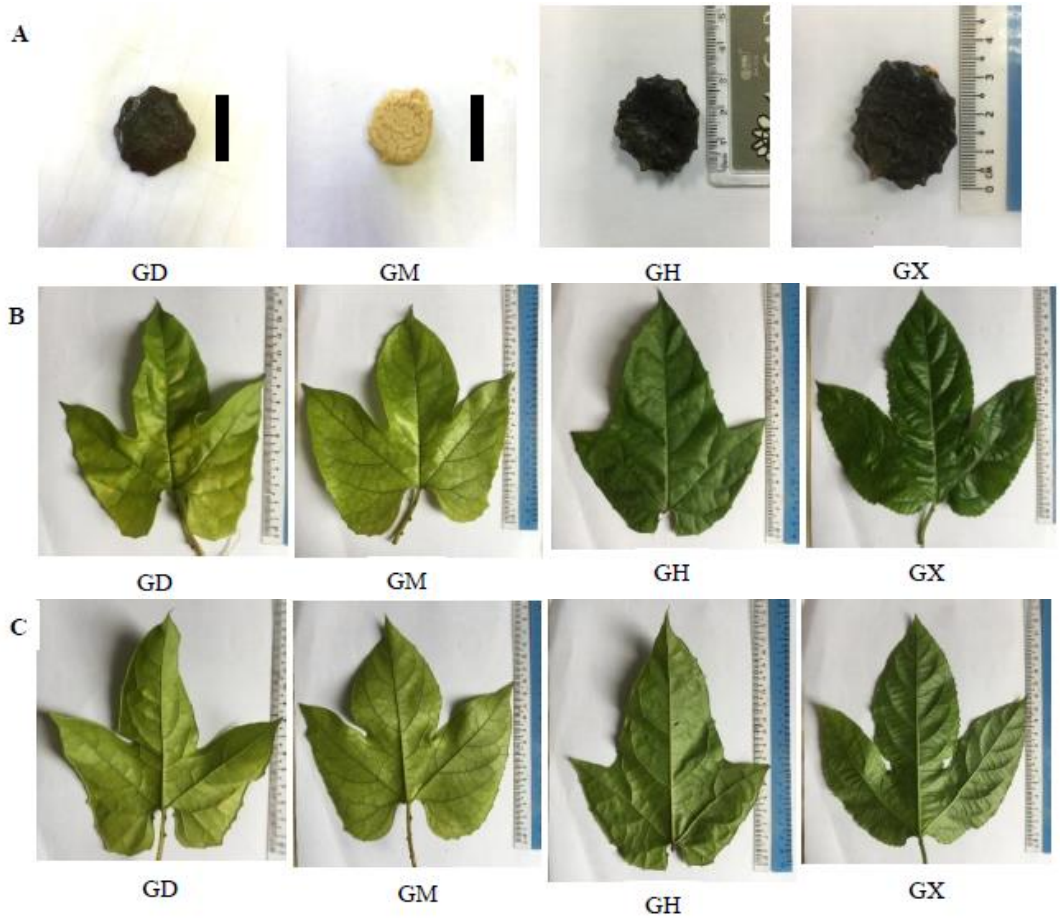

Figure 3. Leaves and Seeds Extracted from Aril. (A) Seeds Extracted from Aril. (B) Adaxial Part of Leaves. (C) Abaxial Part of Leaves. Scale Bars: 2cm.

unripe fruit was yellowish-orange, globose-oval in shape with dense spike density for GM accessions. At the same time, the fruit characteristics for GH accession were orange in color with a tapered shape and sparse spike density. In comparison, GX has ripe orange fruit with no spike density and a globose oval shape. In previous literature, the spine density of the fruits varies, ranging from dense spines to hard and widely spaced [23] and no spines [9] (Table 2). It is uncertain if these distinctions are genetic or environmental in origin, however, they may be impacted by abiotic variables such as collecting locations or soil nutrients. Figure 4 illustrates the fruit morphology of gac.

In another study [9], the fruit shape ranged from globose to globose oval in Southern Vietnam, Northern Vietnam (globose, globose oval, oval and tapering), Central Vietnam (globose oval and oval) and Thailand (globose, globose oval and oval). Globose and oval-shaped fruits were identified in both Vietnam and Thailand accessions, whereas tapered fruits were only observed in accessions from the northern part of the country. The fruits have a variety of spiky surfaces, ranging from densely to lightly covered with spines. The presence of sparsely spiky surfaces was only detected in 1 accession from Northern Vietnam. It was discovered that Vietnam accessions had the greatest variety of fruit shapes, surface texture and seeds, with globose to tapered shapes strongly associated with sparsely spiky surfaces. The fruits were harvested after they reached maturity and turned from orangey-red to dark red in color [24]. The duration for the fruit to mature took 4 to 10 weeks.

The size of the ripe gac fruit can be varied. However, from the observations, different accessions required different duration for the fruit to ripen as the sizes were different. Bigger fruits took longer to mature, especially those collected from GH (Hulu Langat). Fruits from GD (Damansara) appeared smaller than GH, even though they had reached the mature ripeness stage. At this rate, GD

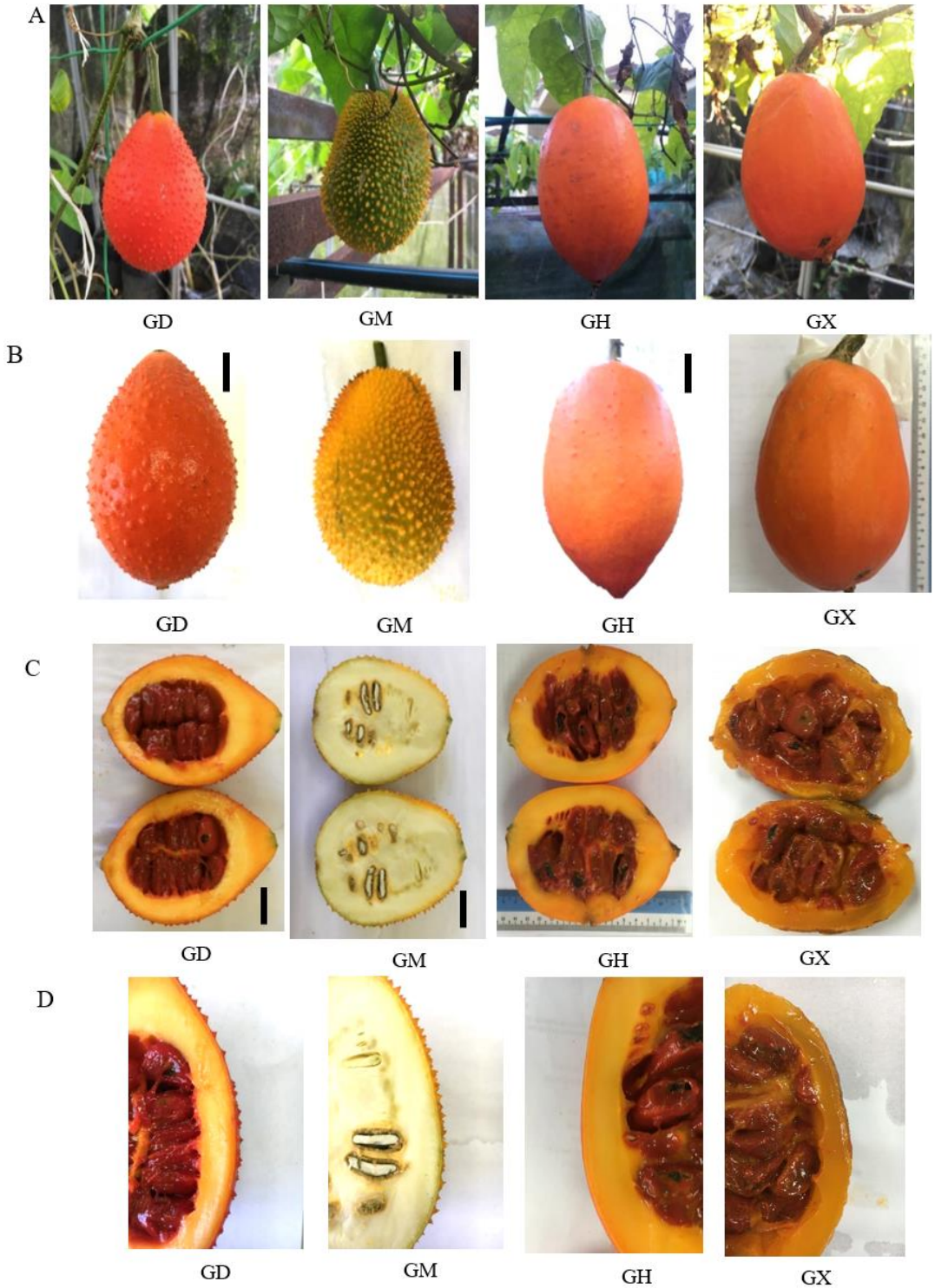

Figure 4. Fruit Morphology. (A) Gac Fruit at the Field. (B) Front View of Gac Fruit. (C) Cross-Section of Gac Fruit. (D) Close-up of the Cross-Section. Abaxial Part of Leaves. Scale Bars: 2 cm.

only took less than 5 weeks for the fruit to be harvested. In this instance, the essential stage is important as the gac fruit needs to be harvested before fully ripe to avoid wilting [25].

The reproductive components, the gac fruit seeds, can be classified into mature and immature seeds, whereas the mature seeds for GD and GX were dark brown in color and GH seeds were black. The immature gac fruit seeds were creamy-

yellow in color. Previous literature stated that the seed varied in shape and colors from brown to light-black, brown to blackish, black and dark black (Table 2; Figure 3) [9, 24].

may be due to different site collections of the seeds in Vietnam and Thailand.

Quantitative data analysis of M. cochinchinensis

The morphological evaluations of gac fruit quantitative data indicated significant differences among the 10 morphological characters studied. Quantifying quantitative differences in morphological characteristics such as fruit weight, fruit width, fruit length, seed weight, seed length, leaf length and leaf width is possible. Table 3 shows the means comparison of quantitative morphological characteristics of 4 gac accessions at $p \leq 0.05$. Results from this analysis were in accordance, which showed high genetic diversity in the gac fruit studied [22]. In the study, fruit-morphological characters revealed highly significant differences among 16 gac accessions, with a high confidence interval at $P \leq 0.001$.

The leaf of the gac fruit was selected randomly. For the width of the leaf, GD recorded the smallest with 14.63 cm, while the biggest was GM with 18.17 cm, which showed highly significant differences between these two accessions. GH and GX respectively recorded 15.93 cm and 17.20 cm. The lowest length recorded for GD was 14.93 cm, while the longest was GX with 18.6 cm. GH and GX had the leaf length of 18.50 cm and 18.43 cm, respectively (Table 3). The leaf length parameter showed that there was a high significant difference especially in accessions GD compared to the other 3 accessions (GM, GH and GX). Comparable leaf width results were observed. On average, leaf size is about 10 to 16 cm [21], and it was reported that the length of the leaf is 8 to 18 cm long [8]. All of the gac fruit accessions in this study had three-lobed leaves. However, gac fruit leaves have been observed in three-lobed and five-lobed [9]. This

From the observation, the number of flower petals for both male and female flowers was 5 petals. The result was in line with the previous study reported [22]. Based on Table 3, GX accession recorded the highest fruit weight with 384.73 g, followed by GH (334.70 g), GM (285.57 g) and GD (193.72 g). This result showed that GD and GH showed a highly significant difference between each other. The highest fruit width was recorded in GH, with 8.67 cm. The second highest width was GX with 8.57 cm, then GM with 8.13 cm while GD had the lowest fruit width with 7.93 cm. GH attained the maximum fruit length with 12.13 cm, followed by GX (11.68 cm), GM (11.17 cm) and GD (9.42 cm). In a study, fruit weight ranged from 0.6 to 2.7 kg per fruit and fruit length ranged from 13.7 to 21.3 cm [24]. This showed that the gac fruits had considerable variability in the fruit morphological parameters studied.

The random collection of gac fruit seeds from 4 accessions showed that the average weight per 10 seeds for GD (22.9 g), GH (34.9 g), the least was GM (14.3 g) and the highest was GX (39.4 g) which showed that accessions GX and GM showed high significant different (Table 3). Seed length of the gac fruit showed that GD had 2.98 cm, GM had the lowest length of 1.43 cm, GH had 3.49 cm and lastly, the highest was 3.94 cm for GX. This showed that accessions GM and GX had high significant differences from each other (Table 3). However, in a study, 10-seed weight of 16 gac accessions ranged from 21.9 to 44.4 g [26]. Moreover, the total number of seeds per fruit was 21 (GD, GH and GX) and 20 for GM. The research in Thailand, the highest number of seeds per fruit recorded was 40 seeds compared to South

Table 3. Means comparison of quantitative morphological characteristics of 4 gac accessions

Traits	Accessions				p-value
	GD	GM	GH	GX	
Leaf width (cm)	14.63 ± 0.85^{b}	18.17 ± 1.04^{a}	15.93 ± 0.51^{ab}	17.20 ± 0.98^{a}	0.00549
Leaf length (cm)	$14.93 + 0.15^{b}$	18.6 ± 0.66^{a}	18.5 ± 1.25^{a}	18.43 ± 1.01^{a}	0.002067
Fruit length (cm)	9.42 ± 0.07^{c}	11.17 ± 0.2^{b}	12.13 ± 0.15^{a}	11.68 ± 0.08^{b}	5.34e-08
Fruit weight (g)	193.72 ± 0.33^{c}	285.57 ± 0.51^{b}	334.70 ± 0.44^{a}	384.73 ± 0.21^{a}	2e-16
Fruit width (cm)	7.93 ± 0.15^{b}	8.13 ± 0.15^{b}	8.67 ± 0.21^{a}	8.57 ± 0.06^{a}	0.00104
Mature seed weight (g)	2.29 ± 0.02^{a}	1.43 ± 0.03^{c}	3.49 ± 0.10^{a}	3.94 ± 0.01^{b}	2.67e-15
Seed length (cm)	2.98 ± 0.19^{a}	2.20 ± 0.26^{b}	3.10 ± 0.10^{a}	3.93 ± 0.03^{c}	1.40e-05

Table 4. ISSR data for molecular characterization 4 gac accessions

Primer	NPB	NMB	NTB	PIC	PP	MI
ISSR1	11	7	18	0.39	61.11	23.74
ISSR2	9	4	13	0.44	69.23	30.47
MISSR1	13	0	13	0.50	100.00	49.50
MISSR4	9	1	10	0.46	90.00	41.65
MISSR8_1	9	2	11	0.46	81.82	37.87
MISSR8_2	5	2	7	0.48	71.43	34.07
IS20	6	2	8	0.38	75.00	28.20
IS21	7	0	7	0.50	100.00	50.00
IS22	6	0	6	0.49	100.00	48.61
IS25	9	0	9	0.50	100.00	49.85
IS30	13	1	14	0.50	92.86	46.37
IS34/1	7	1	8	0.47	87.50	41.01
IS34/2	11	0	11	0.48	100.00	48.35
IS42/1	11	1	12	0.50	91.67	45.52
IS42/2	11	2	13	0.49	84.62	41.31
IS44/1	11	2	13	0.50	84.62	42.06
IS44/2	2	1	3	0.49	66.67	32.41
IS45/1	8	1	9	0.50	88.89	44.31
IS45/2	18	0	18	0.50	100.00	50.00
IS50	5	5	10	0.42	50.00	21.00
IS52/1	15	3	18	0.50	83.33	41.63
IS52/2	6	3	9	0.46	66.67	30.76
IS54	7	3	10	0.47	70.00	32.81
IS56	6	2	8	0.49	75.00	36.91
IS57	9	4	13	0.45	69.23	31.34
IS58/1	9	2	11	0.49	81.82	40.15
IS58/2	12	0	12	0.50	100.00	49.65
IS78	12	1	13	0.50	92.31	46.15
IS83	9	3	12	0.49	75.00	36.46
IS85	14	3	17	0.48	82.35	39.43
Total	280	56	336	14.25	2491.11	1191.58
Average/Primer	9.33^{EMR}	1.87	11.20^{MR}	0.48	83.04	39.72

Note. NPB: Number of polymorphic bands, NMB: number of monomorphic bands, NTB: number of total bands scored, PIC: polymorphic information content, PP: percentage of polymorphism, MI: marker index, MR: multiple ratios, EMR: effective multiple ratio.

Vietnam, which was 10 seeds. The seed weight ranged from 0.88 to 4.64 mg and the length ranged from 18.03 to 34.84 mm [9]. The length of the seed varied from 18.03 to 34.84 mm. The number of seeds per fruit may range from 7 to 54. The country and location where the seeds were collected impact the seeds' morphological variabilities.

Molecular studies

DNA-based molecular markers are preferable compared to morphological markers as they can give a better estimate of genetic diversity and are not influenced by the environment [27]. The ISSR method was adopted in this study. Apart from its capacity to assess genetic diversity amongst closely related species, ISSR offers

Table 5. Jaccard's similarity among the 4 gac fruit accessions collected.

Accessions	GD	GM	GH	GX
GD				
GM	0.67			
GH	0.51	0.53		
GX	0.33	0.27	0.32	
Mean	0.50	0.40	0.32	0.41[a]

DNA extraction and quantification

The total genomic DNA of all 4 gac accessions were successfully extracted from the young leaves through a modified CTAB extraction method. The average concentration of genomic DNA ranged from 135.5 (GH) to 196.8 (GD) ng/µL. DNA quantification was conducted through spectrometry adjusted at 260/280 nm and agarose gel electrophoresis to confirm the purity of DNA. GD has the highest concentration of nucleic acid (196.8 ng) from all 4 samples, with a purity of 1.34. However, sample GX has the second-lowest nucleic acid concentration (171.9 ng) even though the lowest purity recorded was the lowest with 0.88. If the ratio is significantly lower (1.6), it may suggest the presence of proteins, phenol or other contaminants with a strong absorption band at or near 280 nm.

ISSR analysis

Thirty ISSR primers were used to identify the variability of 4 gac fruit accessions in this study. From all the 30 primers used, all of them showed variable banding patterns. All the banding patterns of 30 ISSR primers were used to identify the DNA variability in gac fruit.

Marker informativeness

Overall, there were a total of 336 bands produced using 30 ISSR primers in 4 gac fruit accessions (Table 4). The total number of band scores ranged from 3 (IS44/2) to 18 (ISSR1, IS45/2 and IS52/1) with an average of 11.20 bands per primer. The size of amplified products ranged from 200 bp to 10037 bp.

From the 336 amplified bands, 280 were polymorphic bands and 56 were monomorphic bands. The number of polymorphic bands varied from 2 (IS44/2) to 18 (IS45/2). The average number of polymorphic bands was 9.33 per primer. The relative polymorphism was lowest for primer IS50 (50%) and primer IS21, IS22, IS25, IS34/2 IS45/2 and IS58/2 displayed the highest percentage of polymorphism (100%) with an average of 83.04%. This is corroborated by a study that used 2 ISSR primers i.e., $(GACA)^4$ to amplify all 20 loci that were polymorphic between 550 and 2500 bp and $(GTG)^5$ to amplify all 42 gac samples that were polymorphic between 350 and 3000 bp [9].

The data collected from ISSR profiles using 30 primers were used to evaluate the efficacy of markers in genetic diversity studies using 2 parameters i.e., polymorphic information content (PIC) and marker index (MI) (Table 4).

The PIC values varied from 0.39 to 0.50 with an average of 0.48. This indicates that level of gene variation, when PIC>0.5, the locus was of high diversity among the gac fruit accessions. Most primers showed PIC values between 0.46 and 0.50. The high PIC values may be due to the abundance of diverse gac fruit accessions and highly informative markers. This high level of polymorphism demonstrates the markers' usefulness in the genetic analysis of gac fruit.

The MI for each primer is reflective of the general usefulness of the marker system used [28]. MI is derived from the product of PIC and EMR which reflects the positive correlation between these two. Primers IS21 and IS45/2 gave the highest MI (50.00) and primer IS50 showed the lowest MI (21.00). The average MI recorded was 39.72. EMR readings are often positively correlated with the degree of polymorphism and are proportional to polymorphic loci. Primer IS45/2 gave the highest EMR value (18) and the average EMR was 9.33 per primer (Table 4). Marker indices are used extensively as tools to resolve the discriminatory power of numerous markers evaluation of wild *Salvia* [29], *Gladiolus hybridus* Hort. [30], *Perovskia abrotanoides* [31] and Bambara groundnut [32]. From these, ISSR markers used in this study revealed high polymorphism in gac fruit, suggesting the numerous high variation microsatellite loci in the gac fruit genome.

The percentage of polymorphism across all the samples was 83.04% (Table 4). This finding demonstrated that the 30 ISSR primers used were capable of showing a high degree of variations in gac fruit accessions. This is further supported by a previous study on the use of ISSR markers to show the genetic diversity of gac fruit in Vietnam and Thailand which showed a high degree of poly-

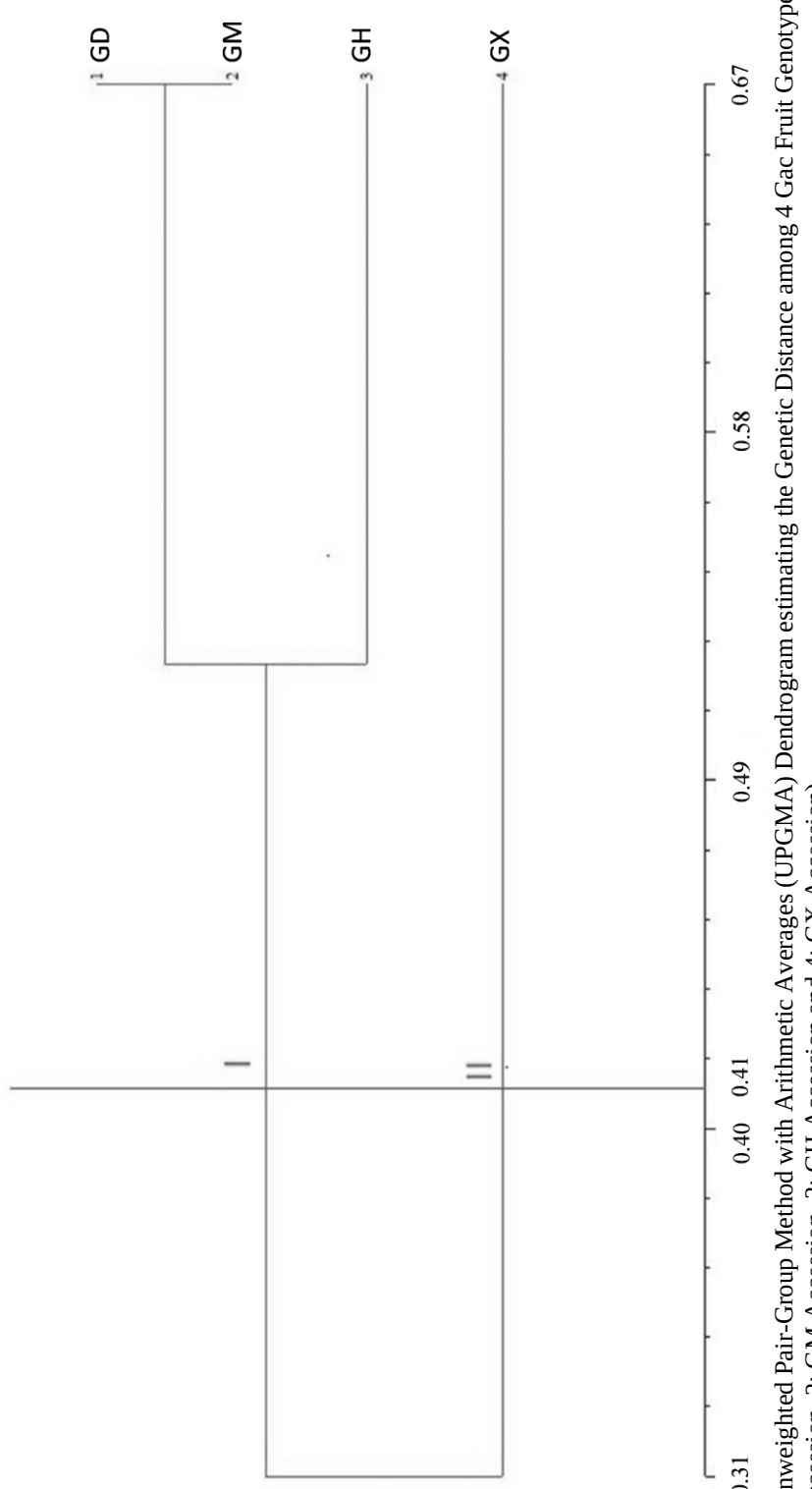

Figure 5. Unweighted Pair-Group Method with Arithmetic Averages (UPGMA) Dendrogram estimating the Genetic Distance among 4 Gac Fruit Genotypes (1: GD Accession, 2: GM Accession, 3: GH Accession and 4: GX Accession)

morphism (>90%) [9]. Polymorphism in ISSR is highly variable in a few different species studied before where *Rhodiola chrysanthemifolia* recorded a polymorphism level of 89.7% [31], 92% in mulberry [13], 91.03% in *Vigna unguiculata* spp. *Sesquipedalis* [33], 57.5% in *Viagna mungo* [34] and 73.6% in *Tribulus terrestris* [35]. Generally, ISSR markers are useful and efficient for identifying hybrids in controlled crosses from the different genetic backgrounds [36], but also can be used extensively for fingerprinting, phylogenetic analysis, population structure analysis, varietal/line identification, genetic mapping and marker-assisted selection [37].

Phylogenetic analysis

The phylogenetic analysis result is critical for elucidating biological diversity and genetic classifications among 4 gac fruit accessions to aid in the development of future plant breeding systems.

The genetic relationship between 4 gac fruit accessions was illustrated using Jaccard's similarity coefficient (Table 4) in a dendrogram (phylogenetic tree). Sequential agglomerative hierarchical nested clustering (SAHN) analysis and the unweighted pair group method of arithmetic averages were used to create the phylogenetic tree (UPGMA) (Figure 5). NTSYSpc software was used to calculate Jaccard's pairwise similarity coefficient using 30 primers. The mean similarity coefficient was 0.41, with values ranging from 0.31 (GH and GX) to 0.67 (GD and GM) (Table 5). The wide range of genetic similarities between gac fruit accessions reveals the significant heterogeneity among them. In a study, the genetic similarities of 42 gac fruit accessions using ISSR data and found that T1 toT3 accessions were the most genetically similar (100% similar to each other) [22].

Phylogenetic tree analysis showed the grouping of all gac fruit accessions into 2 main groups at the similarity coefficient of 0.31 (Figure 5). Cluster I was the biggest group which consisted of 3 accessions (GD, GM and GH). This proved that individual segments are most similar to each other with a similarity coefficient of 0.50. Data suggested that GD and GM were the closest to each other (0.67). While GH was closer to GD (0.51) and GM (0.53) in Group I.

Group II consisted of only accession GX. It is obvious that GX was excluded from the rest of the accessions and grouped in a separate branch (clade) at a similarity coefficient of 0.31 as the most distant of all in Group II. This showed that GX had substantially different characteristics from the distribution in the remaining accessions. This showed that there were high genetic variabilities among the gac fruit accessions. GX was observed to be divided into Group II, implying that human-induced purposeful and unintentional seed, fruit and plant interchange enhances species dispersion and, as a long-term effect, may alter the genetic structure of the species. GX also was collected on the East Coast of Malaysia (Pahang) while the other 3 accessions were collected on the West Coast of Malaysia (Selangor and Melaka). This shows that differences identified at the genetic level might be due to geographical variations between the collection's locality. Farmers collected gac fruits and seeds from the wild and exchanged them with other farmers, relatives and acquaintances. Due to human and environmental interactions, this random exchange of seeds contributes to increasing genetic diversity and gene flow [38, 39].

GD and GM, which had the highest similarity coefficient values of 0.67, indicated that these accessions evolved in close proximity and may have been influenced by similar evolutionary factors. Group I accession (GD, GM and GH) had the greatest intra-group distances. This indicates that these 3 accessions share a nearly identical genetic makeup with only a few minor differences due to the evolutionary channel. Indeed, several accessions have developed with close associations, possibly as a result of exchanges between similar localities as the gac fruits were collected in areas around Malaysia.

Gac fruit is a dioecious plant that reproductive procedures often result in a population with a high degree of genetic heterogeneity due to gene material exchanged via outcrossing breeding systems [26, 40]. Corresponding with this, dioecious *Momordica* species exhibit a more significant proportion of genetic diversity than monoecious species [10], probably owing to their pollination strategy, which includes wind and insects [10, 41]. However, this strategy's reproductive success may be compromised by self-incompatibility [42], geographical constraints and pollinators' short flight distance. The human factor has previously been proven to be responsible for the absence of association between genetic and geographical distance in some circumstances [43].

Conclusion

In conclusion, gac fruit accessions collected from different locations in Malaysia showed high genetic diversity in both morphological and molecular. Due to many morphological similarities observed in the four gac fruit accessions studied, the use of ISSR markers had enabled us to differentiate between these four genotypes genetically. ISSR markers have been proven to be a practical approach to identifying gac fruit's genetic diversity, soon initiating a large-scale Malaysian gac fruit breeding program.

Acknowledgment

This project was supported by IIUM Research Acculturation Grant Scheme (IRAGS18-046-0047). Special thanks to all colleagues for their support.

References

1. Phan-Thi H, Waché Y (2014) Isomerization and increase in the antioxidant properties of lycopene from Momordica cochinchinensis (Gac) by moderate heat treatment with UV–Vis spectra as a marker. Food Chemistry 156: 58-63. doi: 10.1016/j.foodchem.2014.01.040.
2. Jia S, Shen M, Zhang F, Xie J (2017) Recent advances in Momordica charantia: functional components and biological activities. International Journal of Molecular Sciences 18(12): 2555. doi: 10.3390/ijms18122555.
3. Aamir M, Jittanit W (2017) Ohmic heating treatment for Gac aril oil extraction: Effects on extraction efficiency, physical properties and some bioactive compounds. Innovative Food Science & Emerging Technologies 41: 224-234. doi: 10.1016/j.ifset.2017.03.013.
4. Kha TC, Nguyen MH, Roach PD, Parks SE, Stathopoulos C (2013) Gac fruit: nutrient and phytochemical composition and options for processing. Food Reviews International 29(1): 92-106. doi: 10.1080/87559129.2012.692141.
5. Kubola J, Siriamornpun S (2011) Phytochemicals and antioxidant activity of different fruit fractions (peel, pulp, aril and seed) of Thai Gac (Momordica cochinchinensis Spreng). Food Chemistry 127(3): 1138-1145. doi: 10.1016/j.foodchem.2011.01.115.
6. Kubola J, Siriamornpun S (2011) Phytochemicals and antioxidant activity of different fruit fractions (peel, pulp, aril and seed) of Thai Gac (Momordica cochinchinensis Spreng). Food Chemistry 127(3): 1138-1145. doi: 10.1016/j.foodchem.2011.01.115.
7. Petchsak P, Sripanidkulchai B, (2015) Momordica cochinchinensis aril extract induced apoptosis in human MCF-7 breast cancer cells. Asian Pacific Journal of Cancer Prevention 16(13): 5507-13. doi: 10.7314/apjcp.2015.16.13.5507.
8. Tropical Fruit Farm (n.d.) Gac fruit. https://tropicalfruitfarm.com.my/fruits/f-j,g/gac-fruit/. Accessed date: January 2022.
9. Wimalasiri D (2015) Genetic diversity, nutritional and biological activity of *Momordica cochinchinensis* (Cucurbitaceae). (Doctoral dissertation, RMIT University, Australia). https://researchrepository.rmit.edu.au/esploro/outputs/doctoral/Genetic-diversity-nutritional-and-biological-activity-of-Momordica-cochinchinensis-Cucurbitaceae/9921863787101341. Retrieved date: August 2021.
10. Bharathi LK, Parida SK, Munshi AD, Behera TK, Raman KV, Mohapatra T (2012) Molecular diversity and phenetic relationship of Momordica spp. of Indian occurrence. Genetic Resources and Crop Evolution 59(5): 937-948. doi: 10.1007/s10722-011-9735-0.
11. Costa R, Pereira G, Garrido I, Tavares-de-Sousa MM, Espinosa F (2016) Comparison of RAPD, ISSR, and AFLP molecular markers to reveal and classify orchard grass (Dactylis glomerata L.) germplasm variations. PloS One 11(4), e0152972. doi: 10.1371/journal.pone.0152972.
12. Abdulqader, A., Ali, F., Ismail, A., & Esa, N. M. (2019) Antioxidant compounds and capacities of Gac (*Momordica cochinchinensis* Spreng) fruits. *Asian Pacific Journal of Tropical Biomedicine*, *9*(4), 158. doi: 10.4103/2221-1691.256729.
13. Aboul-Maaty NAF, Oraby HAS (2019) Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. Bulletin of the National Research Centre 43(1): 25. doi: 10.1186/s42269-019-0066-1.
14. Rohlf FJ (2000) NTSYS-pc: numerical taxonomy and multivariate analysis system, Version 2.1. Exeter software. Setauket, New York.
15. Nei M, (1972) Genetic distance between populations. The American Naturalist 106 (949): 283–292. http://www.jstor.org/stable/2459777. Accessed date: November 2021.
16. Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular breeding 2(3): 225-238.doi: 10.1007/BF00564200.
17. Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML, Ziegle J (1997) An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPs and pedigree. Theoretical and Applied Genetics 95(1-2): 163-173. doi: 10.1007/s001220050544.
18. Lübberstedt T, Melchinger AE, Dußle C, Vuylsteke M, Kuiper M (2000) Relationships among early European maize inbreds: IV. genetic diversity revealed with AFLP markers and comparison with RFLP, RAPD, and pedigree data. Crop Science 40(3): 783-791.doi: 10.2135/cropsci2000.403783x.
19. Gaikwad AB, Behera TK, Singh AK, Chandel D, Karihaloo JL, Staub JE (2008) Amplified fragment length polymorphism analysis provides strategies for improvement of bitter gourd (Momordica charantia L.). American Society of Horticultural Science,43(1): 127-133. doi: 10.21273/HORTSCI.43.1.127.
20. Nakano M (2020). *Red seal landscape horticulturist identify plants and plant requirements*. Victoria, B.C.: BCcampus. https://opentextbc.ca/plantidentification/. Accessed date: December 2022.
21. Bharathi LK, John KJ (2013) Momordica Genus in Asia-An Overview. 1st Edition. New Delhi: Springer.
22. Wimalasiri D, Piva T, Urban S, Huynh T (2016) Morphological and genetic diversity of *Momordica*

cochinchinensis (Cucurbitaceae) in Vietnam and Thailand. Genetic Resources and Crop Evolution 63(1): 19-33. doi: 10.1007/s10722-015-0232-8.
23. Vuong LT (2000) Underutilized β-carotene–rich crops of Vietnam. Food and Nutrition Bulletin, 21(2), 173-181. doi: 10.1177/156482650002100211.
24. Toan PD, Van Biet H, Hue VTT, Dang Sang H, Tri BM, Tuyen BC (2018) Analysis of genetic diversity of gac ['*Momordica cochinchinensis*'(Lour.) Spreng] in Southern Vietnam using fruit-morphological and microsatellite markers. Australian Journal of Crop Science 12(12): 1890. doi: 10.21475/ajcs.18.12.12.p1170.
25. Tran XT, Parks SE, Roach PD, Nguyen MH (2020) Improved propagation methods for gac (*Momordica cochinchinensis* Spreng.). Experimental Agriculture 56(1): 132-141. doi:https://doi.org/10.1017/S001447971900022X.
26. Loveless MD, Hamrick JL (1984) Ecological determinants of genetic structure in plant populations. Annual Review of Ecology and Systematics 15(1): 65-95. doi: 10.1146/annurev.es.15.110184.000433.
27. Xia T, Chen S, Chen S, Zhang D, Zhang D, Gao Q, Ge X (2007) ISSR analysis of genetic diversity of the Qinghai-Tibet Plateau endemic *Rhodiola chrysanthemifolia* (Crassulaceae). Biochemical systematics and Ecology 35(4): 209-214. doi: 10.1016/j.bse.2006.09.
28. Gallucci MARCELLO, Perugini M (2007) The Marker Index: A new method of selection of marker variables in factor analysis. TPM-Testing, Psychometrics, Methodology in Applied Psychology 14(1): 3-25.
29. Etminan A, Pour-Aboughadareh A, Noori A, Ahmadi-Rad A, Shooshtari L, Mahdavian Z, Yousefiazar-Khanian M (2018) Genetic relationships and diversity among wild Salvia accessions revealed by ISSR and SCoT markers. Biotechnology & Biotechnological Equipment 32(3): 610-617. doi: 10.1080/13102818.2018.1447397.
30. Chaudhary V, Kumar M, Sharma S, Kumar N, Kumar V, Yadav HK, Sirohi U (2018) Assessment of genetic diversity and population structure in gladiolus (Gladiolus hybridus Hort.) by ISSR markers. Physiology and Molecular Biology of Plants 24(3): 493-501. doi: 10.1007/s12298-018-0519-2.
31. Pourhosseini SH, Hadian J, Sonboli A, Nejad Ebrahimi S, Mirjalili MH (2018) Genetic and chemical diversity in Perovskia abrotanoides Kar. (Lamiaceae) populations based on ISSRs markers and essential oils profile. Chemistry & Biodiversity 15(3): e1700508. doi: 10.1002/cbdv.201700508.
32. Khan MMH, Rafii MY, Ramlee SI, Jusoh M, Al Mamun M, Halidu J (2021) DNA fingerprinting, fixation-index (Fst) and admixture mapping of selected Bambara groundnut (Vigna subterranea [L.] Verdc.) accessions using ISSR markers system. Scientific Reports 11(1): 1-23. doi: 10.1038/s41598-021-93867-5.
33. Tantasawat P, Trongchuen J, Prajongjai T, Seehalak W, Jittayasothorn Y (2010) Variety identification and comparative analysis of genetic diversity in yardlong bean (*Vigna unguiculata* spp. sesquipedalis) using morphological characters, SSR and ISSR analysis. Scientia Horticulturae 124(2): 204-216. doi: 10.1016/j.scienta.2009.12.033.
34. Souframanien, J. & Gopalakrishna, T. (2004). A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. Theoretical and Applied Genetics 109(8): 1687-1693. doi: 10.1007/s00122-004-1797-3.
35. Sarwat M, Das S, Srivastava PS (2008) Analysis of genetic diversity through AFLP, SAMPL, ISSR and RAPD markers in Tribulus terrestris, a medicinal herb. Plant Cell Reports 27(3): 519-528. doi: 10.1007/s00299-007-0478-5.
36. Khajudparn P, Prajongjai T, Poolsawat O, Tantasawat PA (2012) Application of ISSR markers for verification of F_1 hybrids in mungbean (Vigna radiata). Genetics and Molecular Research 11(3): 3329-3338. doi: 10.4238/2012.September.17.3.
37. Awasthi AK, Nagaraja GM, Naik GV, Kanginakudru S, Thangavelu K, Nagaraju J (2004) Genetic diversity and relationships in mulberry (genus Morus) as revealed by RAPD and ISSR marker assays. BMC Genetics 5(1): 1-9. doi: 10.1186/1471-2156-5-1.
38. Du X, Sun Y, Li X, Zhou J, Li X (2011) Genetic divergence among inbred lines in Cucurbita moschata from China. Scientia Horticulturae 127(3): 207-213. doi: 10.1016/j.scienta.2010.10.018.
39. Vargas-Ponce, O., Zizumbo-Villarreal, D., Martínez-Castillo, J., Coello-Coello, J. & Colunga-García Marín, P. (2009). Diversity and structure of landraces of gave grown for spirits under traditional agriculture: a comparison with wild populations of *A. angustifolia* (Agavaceae) and commercial plantations of *A. tequilana*. American Journal of Botany 96(2): 448-457. doi: 10.3732/ajb.0800176.
40. Hamrick JL, Godt MJW, Sherman-Broyles SL (1992) Factors influencing levels of genetic diversity in woody plant species. In population genetics of forest trees. Forestry Sciences. Dordrecht, Springer.
41. Schaefer H, Renner SS (2008) A phylogeny of the oil bee tribe Ctenoplectrini (Hymenoptera: Anthophila) based on mitochondrial and nuclear data: evidence for Early Eocene divergence and repeated out-of-Africa dispersal. Molecular Phylogenetics and Evolution 47(2): 799-811. doi: 10.1016/j.ympev.2008.01.030.
42. Torres E, Iriondo JM, Pérez C (2002) Vulnerability and determinants of reproductive success in the narrow endemic *Antirrhinum microphyllum* (Scrophulariaceae). American Journal of Botany 89(7): 1171-1179. http://www.jstor.org/stable/4122203. Accessed date: December 2021.
43. Stankiewicz M, Gadamski G, Gawronski SW (2001) Genetic variation and phylogenetic relationships of triazine-resistant and triazine-susceptible biotypes of *Solanum nigrum*–analysis using RAPD markers. Weed Research 41(4): 287-300. doi: 10.1046/j.1365-3180.2001.00238.x.

This page is intentionally left blank.