MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *Momordica cochinchinensis* SPRENG DIFFERENT ACCESSIONS CULTIVATED UNDER TROPICAL CONDITION

BY

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ABSTRACT

Momordica cochinchinesis 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 is a new and less known plant. This study aimed to characterize gac using morphological analysis involving both vegetative and reproductive parts and to characterize the genetic diversity in gac by using Inter-simpler sequence repeat (ISSR) analysis. Four different gac accessions were collected from different areas and were cultivated under tropical conditions. The gac accessions showed differences in morphological characters. Generally, the gac fruits were reddish-orange in color, the leaf was dark green on adaxial part and light green on the abaxial part, 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 shape 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 0.67 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 consist of only GX. Results from both morphological and molecular analysis showed genetic diversities in all four gac studied.

خلاصة البحث

المومورديكا كوكينكينينسيس أو فاكهة الجاك هي فاكهة خارقة معروفة في فيتنام. الجاك فاكهة برتقالية بيضاوية الشكل ولها ملمس شائك ناعم. تعتبر فاكهة الجاك في ماليزيا من الثمار الجديدة وقليلة الشهرة. هدفت هذه الدراسة إلى توصيف الجاك باستخدام التحليل المورفولوجي الذي شمل كلا من الأجزاء النباتية والتناسلية وتوصيف التنوع الوراثي في الجاك باستخدام تحليل التكررات المترادفة القصيرة. تم جمع أربعة مدخلات مختلفة من الجاك من مناطق مختلفة وزرعت في ظروف استوائية. أظهرت مدخلات الجاك اختلافات في الصفات المورفولوجية، حيث كانت ثمار الجاك بشكل عام ذات لون برتقالي محمر، وكانت الأوراق خضراء داكنة على الجزء المتجه للمحور وخضراء فاتحة على الجزء البعيد عن المحور، وكانت الزهرة الأنثوية صفراء فاتحة والزهرة الذكرية كانت بيضاء. تراوح وزن الثمرة من 193.72 جم إلى 384.90 جم بمختلف الأشكال وكثافة الأشواك. استخرج الحمض النووي بطريقة CTAB، وأظهرت جميع المشرعات الثلاثين مستويات عالية من تعدد الأشكال (83٪) وكان محتوى معلومات تعدد الأشكال بمتوسط 0.48. تراوح معامل المسافة الجينية لني (Nei) بين 0.32 و 0.67 بمتوسط قيمة قدرها 0.41. بناء على تحليل UPGMA قام مخطط الرسم الشجري بتجميع مدخلات الجاك الأربعة في مجموعتين رئيسيتين. تألفت المجموعة الأولى من المدخلات GD و GM و GH بينما تتألف المجموعة II من GX فقط، وأظهر التحليل المورفولوجي والجزيئي تنوعا وراثيا في جميع ثمار الجاك الأربعة المدروسة

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Biosciences).

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I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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Allah says in the Quran,

“If you are grateful, I will surely increase you (in favour)”

(Surah Al-Baqarah 14:7)

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LIST OF SYMBOLS

$	Dollar
%	Per cent
°C	Degree Celsius
µL	Microliter
$A_{260/280}$	Absorbance ratio
AUD	Australian dollar
bp	Base pair
cm	Centimeter
g	Gram
K	Potassium
kb	Kilobase
kPa	Kilo Pascal
L	Liter
M	Molar
Mg	Milligram
mg/L	Milligram per liter
mg/mL	Milligram per milliliter
min	Minute
mL	Milliliter
mm	Millimeter
mM	Millimolar
N	Nitrogen
ng	Nanogram
ng/µL	Milligram per microliter
P	Phosphorus
sec	Second
V	Volt
v/v	Volume by volume
w/v	Weight by volume
w/w	Weight by weight
β	Beta
µL	Microliter

LIST OF ABBREVIATIONS

AFLP	amplified fragment length polymorphism	NaCl	sodium chloride
ANOVA	analysis of variance	PCR	polymerase chain reaction
CRD	completely randomized design	PIC	polymorphic information content
CTAB	cethyl trimethyl ammonium bromide	PVP	polyvinylpyrrolidone
dH2O	distilled water	RAPD	random amplified polymorphic DNA
DNA	deoxyribonucleic acid	RFLP	restriction fragment length polymorphism
EDTA	ethylenediaminetetraacetic acid	SE	standard error
EtOH	ethanol	SSR	simple sequence repeats
HSD	Honestly Significant Different	TAE	tris-acetate-EDTA
IIUM	International Islamic University Malaysia	TE	tris-EDTA
ISSR	inter-simpler sequence repeat	Tris-HCl	tris aminomethane hydrochloride
MCF	Michigan Cancer Foundation	NaCl	Sodium chloride
HCl	Hydrochloric acid		

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Momordica cochinchinensis often referred to as gac, is a popular 'superfruit' in Vietnam (Phan-Thi & Waché, 2014). Other names for *Momordica cochinchinensis* are baby jackfruit, sweet gourd and Cochinchin gourd. The family of Cucurbitaceae is called Momordica (Jia et al., 2017) while *cochinchinensis* named from a northern part of Vietnam called Cochinchina (Aamir and Jittanit, 2017). *Momordica cochinchinensis* has been widespread across Southeast Asia, Malaysia and India (Kha et al., 2013).

Gac fruit is an orange-red, ovoid-shaped fruit with a soft spiky texture. Gac is a climber plant that can climb up to 20 meters. It has tuberous roots and brown or grey-black seeds. Kubola and Siriamornpun (2011) stated that the gac fruit tastes like papaya. Traditionally, Vietnamese people use gac 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 (Tinrat et al., 2014).

Moreover, gac fruit has been studied to have high carotenoids. In Tinrat et al. (2014), carotenoids are the phytochemical properties containing nutrition values that give various health benefits. Gac fruit has β-carotene and lycopene and these 2 phytochemical properties are antioxidant substances. The highest antioxidant activity of gac fruit can be seen during the immature stage of the fruit (Kubola & Siriamornpun, 2011). Apart from that, it also has potential as antimicrobial properties. As gac fruit is rich in carotenoids, the concentration of lycopene is at least 5 times that of other well-known fruits analyzed, including tomato, papaya, guava and watermelon. Above all, gac aril has the greatest known quantity of β-carotene of any fruit or vegetable. It is, for example, 8 times the amount found in carrots, which are known to be high in β-carotene (Kha et al., 2013).

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 to prevent severe diseases. The lycopene concentration has a cholesterol-reducing effect and can lower the risk of death from chronic diseases such as cancers, cerebral diseases, heart disease and stroke. According to Petchsak and Sripanidkulchai (2015), gac aril's extract can generate the death of cells which occurs in human MCF-7 breast cancer cells.

1.2 STATEMENT OF THE PROBLEM

Gac is a new and less-known plant in Malaysia. Malaysians are still not familiar with gac. This is because gac fruit is originally from Vietnam. It is not well-introduced and common in Malaysia like any other fruits available. The research study of gac is still lacking in Malaysia (Rozana, 2019). Information on gac, particularly in classifying its morphology and genetic variability, is still inadequate (Wimalasiri, 2015). The limitation of available information on genetic variation hinders efforts to develop gac varieties. Information on genetic diversity and population relationships is essential for plant breeding programmes, since it facilitates the selection of suitable genetic material.

Presently, the lack of genetic information on gac populations in Vietnam restricts access to advantageous features present in adapted gac varieties in Vietnam. Using morphological and molecular markers, crop species' genetic diversity may be measured. In the Momordica genus, variations in fruit form are common; however, it is uncertain how much diversity may be seen in *M. cochinchinesis* depending on its growing environment and the plant developmental (Dey et al., 2006; Toan et al., 2018). The need for uniformity in morphology in order to improve the capacity to compare and communicate morphological data. Examining the reasons behind why morphological data may only be represented by morphological descriptions and not by character matrices, as well as the reasons behind why morphological terminology must be devoid of homology assumptions. Images merely support and verify data; they're not data. Comparing physical features with DNA sequence data reveals conceptual flaws in the former due to their high average individuality. Delimitating

morphological, datum, and evidentiary units each has their own problems (Vogt et al., 2010). In this research, fruit-morphological and ISSR markers were utilised to analyse the genetic diversity of gac accessions collected around Malaysia to select divergent genotypes for breeding and create efficient conservation measures.

In the context of the breeding development of gac, the biggest challenge is the lack of information on gac cultivation in Malaysia. Gac being a dioecious plant requires adequate amount of pollen from the male flower to the female flower is crucial for cross-pollination to produce fruit. Gac requires hand pollination or natural pollinator to increase its fruit set. Without pollination can become the main factor contributing to low gac fruit yield. Of equal importance, the sex of gac cannot be determined from seed and can only be defined upon flowering. The sex determination of gac can be determined between the male and female flowers. The fact is that sex determination is primarily physiological. The flower information can be studied on the shape of the flower and the presence of the female reproductive organs. Growing the male vines is essential not for fruit but for making pollens for the female vines. A limited number of female vines can cause the male to outgrew and this may imply to carry out a lot in both of land and water to cultivate the unneeded male vines. Moreover, growing gac is also long before they start producing fruit. At an early stage, it is difficult to determine the sex of the plant. The data for both morphology and molecular is essential in context for breeding development of gac. In order to overcome these issues, clear and thorough morphological and molecular characterization of gac is required.

1.3 JUSTIFICATIONS

Gac is nutritious and therapeutic which was once common throughout Asia, is now only found in a few places in Vietnam, Thailand, and India. Gac is genetically and morphologically varied, but there is not much knowledge on how diverse it is. The limited selection of germplasm and cultivars for high aril production, nutrition, or anticancer action results from the lack of information on the morphological and genetic variety of this species.

Based on the morphological data from previous researches by Toan et al. (2018) and Wimalasiri (2015), there are differences in morphological characterization. The difference in leaves, fruits, flowers and seeds showed that there are variations in gac. The phytochemical contents in gac aril are secondary metabolites created as plant defence mechanisms against environmental stress situations like drought and severe temperatures. Gac has the most carotenoids of any known fruit or vegetable. Lycopene and β-carotene are the two most abundant carotenoids in the fruit, with the aril holding more than 7 times more lycopene than β-carotene (Gul et al., 2015). Lycopene levels in the aril were more than 20 times greater than in field-grown cherry tomatoes. Similarly, the β-carotene content of gac aril was more than 15 times that of carrots. These two syntheses of carotenoid are an essential factor to consider in next research on molecular breeding for gac cultivars for increased of carotenoid production. Not only is gac fruit high in β-carotene and lycopene, but it also contains α-tocopherol (vitamin E), phenolic compounds, and flavonoids. is made up of a variety of fatty acids in their individual compositions. For instance, it was discovered that fresh gac aril has 10.2 percent mg of oil, which is on par with the amount of oil found in other oil-rich fruits such as avocados (Chuyen et al., 2015). Due to the success of the products that are now on the market and the biological activities that this fruit has, it has become more popular in recent decades as a potential use in the functional food industry.

Globally, research involving gac has increased tremendously over the years. Specifically, in Australia, emphasize is given on genetic, gac processing and crop production. For example, in Australia gac production could be profitable in both field and greenhouse systems, according to estimation methods of potential gross margins based on costs of about $5-6 per kg and a fresh fruit sale price of $8 per kg (Park et al., 2013b). Gac genetics research is currently underway in Australia and the results will aid in the development of new varieties with desirable agronomic characteristics.

The surge of interest in gac is catalyzed by the many potentials that gac can offer which are yet to be discovered. In line with its renowned designation as the fruit of heaven, there is a dire need to identify the genetic diversity of gac in order to open avenues for further research. Thus, this study is initiated in aim to identify the variability of gac using both morphological and molecular analysis. This is feasible as

the limitation of morphological characterization can be complemented with data generated through molecular analysis. Molecular evidence will continue to enhance the knowledge of phylogenetic relations for ever-increasing molecular datasets.

Based on the data of morphological and molecular study of other researchers, this research on morphological and molecular characterization of gac will help to overcome the limitations of other studies. The data and information that have been generated from this research will able to add more information not only for morphology and molecular, but also for other future research on cultivation. Future cultivator and modern agriculture will gain beneficiaries.

1.4 RESEARCH OBJECTIVES

The study aimed to achieve the following objectives:

1. To characterize gac using the morphological characterization of both vegetative and reproductive parts.
2. To characterize the genetic variation in gac using Inter-simple sequence repeat (ISSR) analysis.

1.5 RESEARCH QUESTIONS

1. Are there any morphological differences among the 4 gac studied?
2. Is there any genetic diversity in 4 gac accessions using ISSR?

1.6 RESEARCH HYPOTHESIS

The hypotheses are as follows:

1. There will be variations in morphological characteristics of different accessions of gac studied.
2. ISSR can be utilized to detect genetic variabilities among the gac.

1.7 SIGNIFICANCE OF THE STUDY

The output of this research is data on the gac genetic diversity on both morphology and molecular. Furthermore, evaluation involving relationship between the examined variables were also conducted. Correlation of two different analysis using molecular and morphological study can help to confirm the exact diversification of gac. Since evaluation involving only morphological analysis is insufficient in differentiating the gac diversity, integration study alone is not enough to differentiate the gac. In brief, evaluation involving both morphological and molecular study are effective if used together.

The fact that the samples were taken from various areas throughout Malaysia revealed a greater degree of genetic variety, which indicated the existence of a diversified gene pool which this is something that would be helpful for the crop's development. The results of ISSR studies showed that the samples from various collecting locations in Malaysia were separated from one another, suggesting the existence of a variety of gac genotypes throughout Malaysia.

ISSR that are used in this research will be crucial for producing commercially desirable traits which include better seeds, fruit yield and nutrients. These markers will be important in breeding programs of gac to select plants with desirable traits in its early stages of growth. Gac from these areas should be selected for future breeding programs for commercial propagation.

CHAPTER TWO

LITERATURE REVIEW

2.1 ORIGIN, HISTORY, VERNACULAR NAMES AND GEOGRAPHICAL DISTRIBUTION

Momordica cochinchinensis, or gac, is called "fruit from heaven" by the Vietnamese because of its skyward vines and as a testament to its nutritional benefits (Phan-Thi and Waché, 2019). Gac is still undergoing domestication (Bharathi and John, 2013). It was speculated to have developed independently from *M. dioica* in South Asia around 19 million years ago, most likely in the Cochinchina area of Vietnam (Mondal et al., 2006). Moreover, gac is primitive from South China through Southeast Asia to North-Eastern Australia, Vietnam, Thailand and India (Lim, 2012). However, Phan-Thi and Waché (2019) recorded that there are still lacked information regarding the fruit's natural origins, stating just the area where it was picked or purchased.

The vernacular name of gac is depended on the tribe and region of the country (Table 2.1) which is due to different dialect even though within the same region. The common name for *Momordica cochinchinensis* is Chinese Bitter Cucumber, Chinese Cucumber, Cochinchin Gourd, gac, Giant Spine Gourd, Spiny Bitter Cucumber, Spiny Bitter Gourd and Sweet Gourd. Gac is known as Karkotaka, in Ayurvedic which is also the name of an ancient northern Indian whose ancestors are of Austroasiatic heritage (Phan-Thi and Waché, 2019).

Table 2.1 The common names of gac based on different countries.
Source: Lim (2012)

Country	Vernacular name
Bangladesh	Kakrol
Chinese	Da Ye Mu Bie Zi, Mu Bie, Mù-Biē-Guǒ, Mu Bie Zi, Teng Tong, Tu Mu Bie
Czech	Tykvice Končičinská
French	Margose À Piquants, Muricie
India	Bhat Kerala (Assamese), Golkara, Kakrol (Bengali), Bhat-Karela, Gangerua, Gulkakra, Kakur, Kakrol, Kantola, Kathaamla (Hindu), Karkataka, Kaadu Kaakara (Kannada), Karol, Karot (Manipuri), Gulkara, Kshudramalakasanda (Malayalam), Kakana, Kartalen (Marathi), Gangeruka, Jalakaravalli, Karka, Karkaphala, Karkata, Karkataka, Katamala, Krindana, Kshudradhatri, Kshudramalakasandna, Mrigalendaka, Mrigavitsadrisha, Todana (Sanskrit), Palupakal, Adavi-Kakara (Tamil), Adavikakara, Adavikaakara, Varivalli (Telugu)
Indonesia	Pupia, Torobuk, Toropu
Italian	Cetriolino Spinoso
Japanese	Mokube Tsushi, Nanban Kikarasuuri
Khmer	Makkao
Laos	Khaawz
Malaysia	Teruah
Nepal	Jhuse Karelaa
Philippines	Tabog-Ok, Tabog-Uak (Bikol), Tambua-Uang (Bontok), Malakaban, Taboo (Cebu Bisaya), Parum-Parung (Ibanag), Tambaching (Igorot), Libas, Parug-Parug, Parog-Parog-Ti-Noang, Parog-Parog-Ti-Tau, Sugod-Sugod (Iloko), Tabala (Manabo), Tambalosan (Panay Bisaya), Tabola (Subanum), Balbas-Bakiro, Buyok-Buyok, Patolang-Uak (Tagalog)
Spanish	Cundeamor, Pepino Amargo Espinoso, Pepinillo Del Diablo

Table 2.1 Continued

Country	Vernacular name
Sri Lanka	Tumba Karavila (Sinhalese)
Thailand	Bai-Khai-Du, Fakkhao, Phak-Khao, Khika-Khrua Yawd-Fak-Kao
Vietnam	Dia Ta Pieu (Dao), Ga'c, Day Ga'c, Moc Miet Tu, Mac Khau (Tay), Ma Khau (Thai)

2.2 CLASSIFICATIONS OF *Momordica cochinchinensis*

Momordica cochinchinensis taxonomical classification is shown below:

Kingdom Plantae
Subkingdom Viridiplantae
Infrakingdom Streptophyta
Superdivision Embryophyta
Division Tracheophyta
Subdivision Spermatophytina
Class Magnoliopsida
Superorder Rosanae
Order Cucurbitales
Family Cucurbitaceae
Genus *Momordica*
Species *Momordica cochinchinensis*

2.2.1 Family Cucurbitaceae

Cucurbitaceae is derived from the Latin word "Corbis," which means "bottle" or "basket." Traditionally, mature cucurbits have been used as containers or musical equipment. Cucurbit cultivation for fresh consumption began more than 3000 years ago in Western Asia (Lebeda et al., 2007). Cucurbit's fruits and seeds have many variations, which are practicable to identify genera. Cucurbitaceae crops that are locally known include the *Cucurbita maxima* (squash or pumpkin), *Cucurbita. pepo*