UNIVERSITI PUTRA MALAYSIA

SCREENING OF POLYMORPHIC RANDOM AMPLIFIED POLYMORPHISM DNAs (RAPDs) MARKERS FOR THE AMPLIFICATION OF PHYSALIS MINIMA DNA

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By

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2010

APPROVAL SHEET

This project entitled "**Screening of Polymorphic Random Amplified Polymorphism DNAs (RAPDs) Markers for the Amplification of *Physalis Minima* DNA"** has been prepared and submitted by Stefan Nyua anak Joshua to the Faculty of Agriculture, University Putra Malaysia in fulfillment of his requirement of PRT 4999 (Project) for the award of degree of Bachelor of Agricultural Science.

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DECLARATION

I hereby declare that the thesis is based on my original except for quotations and citations which have been duly acknowledged. I also declared that it has not been previously or currently submitted for any other degree at Universiti Putra Malaysia or any other institutions.

STEFAN NYUA ANAK JOSHUA

……..

Date:……

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LIST OF ABBREVIATIONS

%	percentage
ºC	degree Celsius
µ	micro
bp	base pair
CtDNA	chloroplast DNA
dH_2O	distilled deionised water
dNTPs	deoxynucleotide-5-triphosphate
DNA	deoxyribonucleic acid
Et Br	ethidium bromide
g	gram
h	hour
ISSR	Inter-Simple Sequence Repeats
kb	kilobase
L	litre
m	mili
min	minute
mtDNA	mitochondrion DNA
M	molar
Mg^{2+}	magnesium ion
$MgCl_2$	magnesium chloride

n	nano
NaCl	sodium chloride
Naoh	Sodium hydroxide
PCR	polymerase chain reaction
rDNA	ribosomal DNA
rpm	revolution per minute
RNA	ribonucleic acid
Rnase	ribonucleas
RFLP	Restricted Fragment Length Polymorphism
RAPD	Random Amplified Polymorphic DNA
s	second
SSR	Simple Sequence Repeats
Taq	Thermus aquatics
UV	Ultraviolet
vol	volume

ABSTRACT

Cape gooseberry or *Physalis minima* is from the Solanaceae family and originates from Central South America. In European countries the fruit which resembles cherry tomatoes are widely used as vegetables. It is considered as weeds in Malaysia despite the medicinal value and high vitamin C content. This plant has the potential as cultivated crop. Prior to cultivation, the fundamental aspects are required and one of it is the genetic profile. Molecular tools are widely used in genetic studies and Random Amplified Polymorphism DNA (RAPDs) is one of the early PCR based marker. It is favourable because it is a dominant marker, easy to use and randomly amplified genomic DNA. A study was carried out to determine the genetic variation of *P. minima* from two populations with the objectives of (1) to screened 10 OPA and 20 OPB RAPD primers for amplification of *P. minima* DNA and (2) to obtain polymorphic RAPD primers for *P. minima.*. DNA extraction was carried out using GeneAll® Plant DNA extraction kit and quantification was done using spectrophotometer. Polymerase chain reaction (PCR) was carried out in a total volume of 25 µL consisting of 30 ng of genomic DNA, 10× PCR Mastermix, 10× PCR Buffer, 0.2 µM of the primers and distilled water. Amplifications were carried out in a thermalcycler programmed, with an initial step at 94°C for 3 minutes, followed by 40 cycles of 60 seconds at 94°C; 60 seconds at 43.6°C, and 120 seconds at 72°C, followed by a cycle of final extension step of 10 minutes at 72°C. From this study a total of 11 OPA primers and 20 OPB primers were obtained that showed positive amplification on *P. minima* DNA band.

ABSTRAK

Cape gooseberry ataupun *Physalis minima* dari famili Solanaceae berasal dari kawasan tengah Amerika Selatan. Di negara Eropah di mana buahnya seperti tomato ceri digunakan dengan meluas sebagai sayur-sayuran. Di Malaysia, ianya dianggap sebagai rumpai di sebalik nilainya dalam bidang perubatan dan kandungan vitamin C yang tinggi. Tumbuhan ini mempunyai potensi sebagai tanaman makanan. Sebelum penanaman, aspek-aspek asas diperlukan dan salah satunya ialah profil genetik. Peralatan molecular digunakan secara meluas dalam kajian genetik dan Random Amplified Polymorphism DNA (RAPDs) merupakan salah satu penanda berasaskan PCR yang awal. RAPDs menguntungkan kerana ianya penanda dominan, senang digunakan dan mengamplikasi DNA genom secara rawak. Satu kajian dilakukan untuk menentukan variasi genetik pokok *P. minima* daripada dua populasi dengan objektif (1) untuk menapis 10 OPA dan 20 OPB primer RAPD untuk amplifikasi DNA *P. minima*. (2) untuk mendapatkan primer RAPD yang polimorfik terhadap *P. minima*. Ekstraksi DNA dilakukan dengan menggunakan GeneAll® Plant DNA kit ekstraksi dan kuantifikasi dilakukan dengan menggunakan spektrofotometer. Polymerase Chain Reaction (PCR) dijalankan dalam jumlah keseluruhan 25 µL terdiri daripada 30 ng DNA genom , 10 × PCR Mastermix, 10 × PCR Buffer, 0.2 µM dari primer dan air suling. Amplikasi dilakukan dalam thermalcycler yang telah diprogramkan, dengan langkah awal pada suhu 94ºC selama 3 minit, diikuti dengan 40 kitaran 60 saat pada suhu 94ºC; 60 saat pada 43.6ºC, dan 120 saat pada suhu 72ºC, diikuti dengan langkah terakhir pada 72ºC selama 10 minit. Dari kajian ini sebanyak 11 primer OPA dan 20 primer OPB yang diperolehi menunjukkan amplikasi yang positif terhadap DNA *P. minima*.

CHAPTER 1

INTRODUCTION

Physalis is a genus of plants classified into the nightshade family (Solanaceae). It is native to warm temperate and subtropical regions throughout the world. The genus is characterized by the small orange fruit similar in size, shape and structure to a small tomato, but partly or fully enclosed in a large papery husk derived from the calyx. Many *Physalis* species are called ground cherries, but it is commonly known as cape gooseberry. *Physalis* comprises of about 90 species, all but one being native to tropical and temperate America. The species are variable and taxonomically confusing, and no comprehensive study of the genus exists.

In European countries, *P. minima* also known as sunberry is cultivated widely, and has been produced as hybrids. This fruit is edible and can be eaten fresh, and it also can be use in salad or as decorative in culinary. Malaysia imports this fruit with Colombia as it main exporters and is being sold at hypermarket at a high price.

In Malaysia, local ecotypes are considered as weeds. *P. minima* is also known as native gooseberry or 'Pokok Letup-Letup' and is a pantrophical annual herb having a very delicate stem and leaves. Its height is around 20 to 50 cm. *P. minima* is commonly found on the bunds of the fields, wastelands, around the houses, on roadsides, etc., where the soil is porous and rich in organic matter. These plants grow in most soil types and do

very well in poor soils and in pots. They requires lots of water throughout the growing year, except towards fruit-ripening period. Propagation is by seed. The typical *Physalis* fruit is similar to a firm tomato (in texture) and have a mild, refreshing acidity taste. *Physalis* fruit have around 53 kcal for 100 grams, and are rich in cryptoxanthin. This plant is highly resistant to insect pests and diseases. Although the price of this fruit is quite high, it have high contain of vitamin C and anti-cancer activity. So, the cultivating of this crop is lucrative. However, it is not known as cultivated crops. Exploitation of local varieties is still unknown. Prior to cultivation it is essential to establish their germplasm for future selection of ecotypes with desirable traits. It is also important to study the fundamental aspect of their geographical, distributions, genetic variations, genetic relationships, and genetic distance among ecotypes. Although advanced molecular markers such as microsatellites, Inter Simple Sequences Repeats (ISSR) and Single Nucleotide Polymorphism (SNPs) are highly accurate and co-dominant, they are however costly. For preliminary profiling, dominant markers particularly Random Amplified Polymorphism DNA (RAPDs) are normally employed as they are cheap, less laborious and primers are universal and do not need prior development for a specific genus.

A study was carried out with the objectives of:

1. To screened 10 OPA and 20 OPB RAPD primers for amplification of *P. minima* DNA.
2. To obtain polymorphic RAPD primers for *P. minima.*

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