

CHLOROPLAST DNA DIVERSIFICATION OF MALAYSIAN PINEAPPLE

NORFADILAH BINTI HAMDAN

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To my Parent,

Sisters,

and Friends,

Thanks for your pray, attention and spiritual support...

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ABSTRACT

There are several genes normally used for the phylogenetic study such as Mitochondria DNA (mtDNA), Chloroplast DNA (cpDNA) and Nuclear DNA (nDNA). Among these three genomes, cpDNA is commonly being used in phylogenetic study because it is easy to amplify via PCR, evolves at a conservation rate and it has appropriate length and base substitution rate for inferring phylogeny at higher levels. In this study, phylogenetic tree of eight Malaysian pineapple (*Ananas comosus*) cultivars were constructed using sequences of large subunit of the ribulose-bisphosphate carboxylase (*rbcL*) gene. A *rbcL* gene was isolated from genomic DNA, amplified and sequenced. Phylogenetic analysis was carried out using maximum parsimony method. Results revealed that *rbcL* gene of *Ananas comosus* is about 1250 bps. Based on the tree, eight Malaysian pineapple cultivars were classified into two groups. The first group consisted of Yankee and Gandul cultivars while second group consisted of Moris, Moris Bentanggur, Moris Gajah, N36, Josaphine and Sarawak. Bootstrap value in some branches were low this is reflected by the small number of informative characters (981 were conserved and 85 were potentially informative) to build the tree. Formation of several group or subclades is due to its similar genetic pattern, thus support this system classification. The study suggested that *rbcL* gene could be used to determine the phylogenetic relationship distinguish the pineapple cultivars.

ABSTRAK

Terdapat beberapa jenis gen yang biasanya digunakan untuk kajian filogenetik seperti DNA Mitokondria (mtDNA), DNA Kloroplas (cpDNA) dan DNA Nuklear (nDNA). Antara ketiga-tiga genom ini, cpDNA banyak digunakan dalam kajian filogenetik kerana ianya mudah diampifikasi melalui PCR, dan ia mempunyai saiz dan kadar pengantian *base* yang sesuai untuk membuat kesimpulan filogeni pada peringkat taksonomi yang tinggi. Dalam kajian ini, rajah pokok filogenetik dari lapan kultivar nanas (*Ananas comosus*) Malaysia telah dibina menggunakan gen *ribulose-bisphosphate carboxylase* (*rbcL*). Gen *rbcL* telah diekstrak dari genomic DNA nanas. Kemudian, ia diampifikasi dan diklon sebelum di hantar untuk penjujukan. Jujukan DNA ini seterusnya melalui proses analisis filogenetik dan ianya dijalankan menggunakan kaedah *Maximum Parsimony*. Hasil analisa menunjukkan bahawa saiz gen *rbcL* bagi *Ananas comosus* adalah ± 1250 bp. Berdasarkan filogeni ini, lapan kultivar nanas Malaysia boleh dikelaskan kepada dua kumpulan. Kumpulan pertama terdiri daripada kultivar Yan Kee dan Gandul manakala kumpulan kedua terdiri daripada kultivar Moris, Bentanggur Moris, Moris Gajah, N36, Josaphine dan Sarawak. Nilai *Bootstrap* di beberapa kumpulan adalah rendah dan ini disebabkan oleh bilangan karakter yang konservatif (981 konservatif dan 85 adalah karakter berpotensi) untuk membina pokok. Pembentukan kumpulan atau beberapa subkumpulan adalah disebabkan oleh persamaan ciri genetik, seterusnya menyokong sistem klasifikasi ini. Kajian ini membuktikan bahawa jujukan dari gen *rbcL* boleh digunakan untuk menentukan hubungan filogeni bagi pembezaan antara kultivar nanas.

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LIST OF ABBREVIATIONS/ SYMBOLS

±	-	plus minus
%	-	percentage
° C	-	degree Celcius
µL	-	microliter
bp	-	basepair
BS	-	bootstrap support
CI	-	consistency index
cm	-	centimetre
cpDNA	-	chloroplast DNA
g	-	gram
h	-	hour
IPTG	-	isopropyl β- D- 1- thiogalactopyranoside
ITS	-	internal transcribed spacer
kb	-	kilobase
kg	-	kilogram
LAIX	-	LB, ampicillin, IPTG, X- gal
LB	-	Luria- Bertani Broth
m	-	meter
min	-	minute
mL	-	mililiter
mM	-	micromolar
MP	-	Maximum Parsimony
MPIB	-	Malaysian Pineapple Industry Board

mtDNA	-	mitochondria DNA
nDNA	-	nuclear DNA
nm	-	nanometer
PCR	-	Polymerase chain reaction
<i>rbcL</i>	-	large subunit of ribulose- bisphosphate carboxylase gene
RI	-	retention index
rpm	-	revolutions per minute
sec	-	second
SOC	-	Super Optimal Broth with Catabolite repression
T_m	-	annealing temperature
U	-	unit
X- gal	-	5- bromo-4- chloro- indolyl- β - D- galactopyranoside

CHAPTER 1

INTRODUCTION

1.1 Study background

Chloroplasts are organelles which present abundantly in the leaf cells which play important role in metabolic activity known as photosynthesis process. The other important roles plays by the chloroplast are producing the starch, give the colour pigments to the flowers, certain amino acids, lipids and vitamins. Chloroplasts have their own genome whereby it possess a full complement of transcriptional and translation machinery in order to express their genetic information.

On the other hand, Pineapple (*Ananas comosus*) is the third most important tropical fruit crop in the world after bananas and mangoes (Botella and Smith 2008; Carlier *et al.*, 2007). It also has become one of the leading commercial fruit crops of the tropics over the past of 100 years. Other than other than Thailand, Philippines, Indonesia, Hawaii, Ivory Coast, Kenya, Brazil, Taiwan, Australia, India and South Africa countries, it was reported that Malaysia has become one of the world producer of pineapple fruit. Development of pineapple industry in Malaysia is monitored by Malaysian Pineapple Industry Board (MPIB). MPIB was established since the year of 1957. The role of MPIB is to manage and develop Malaysian Pineapple Industry.

Pineapple is a type of tropical plant which is believed to originate from East Area of South America. It was introduced in Malaya in the 16th century by the Portuguese. During a year of 1921, pineapple started to be planted in Singapore, Johor and Selangor as cash crop. Now, pineapple plantation is continued to be expended in peat soil area especially in Johor (Malaysian Pineapple Industry Board, 2012). There are many cultivars and 'Smooth Cayenne' is the most commonly grown worldwide. In Malaysia, there are about three common cultivars widely planted in Malaysian which are Spanish (also known as Maspine, Josapine and Hybrid pineapple), Smooth Cayenne (Sarawak pineapple) and Queen (Morris pineapple) (Malaysian Pineapple Board, 2012).

Recently, molecular systematics in plants has progressed rapidly with DNA amplification or known as polymerase chain reaction, PCR is mediated by the direct sequencing methods (Schulte *et al.*, 2008; Sheng-Guo *et al.*, 2008; Osaloo and Kawano, 1999). Molecular approach has offered effective method in addressing many phylogenetic questions which cannot be solved using morphology characters. Previous studies (Chase *et al.*, 1993; Spreitzer *et al.*, 2002) claimed that large subunit of the ribulose- biphosphate carboxylase (*rbcL*) gene is suitable for inference phylogenetic relationship at higher taxonomic levels. The *rbcL* gene is usually up to 1250 bp in size and the used of this gene in phylogenetic analysis has been reviewed in many studies (Chase *et al.*, 1993; Clegg, 1993; Spreitzer *et al.*, 2002). This is due to its advantages where it is easy to amplify using PCR, have appropriate length and base substitution rate for inferring phylogeny at higher taxonomy levels and it evolves at a conservation rate which make it suitable to study plant phylogeny (Clegg, 1993).

Pineapple is well known among user and it is highly consumed due to its pleasant taste and medical values. Though it is has become popular, very little is known about the molecular genetics of pineapple. It is due to limitation on available data of *Ananas* genetic diversity and most of it is based on morphology character only. They apparently arose due to spontaneous mutation, followed by natural selection and cross hybrid with unknown ancestor (Ruas *et al.*, 1996). Hence, in this study, phylogenetic analysis of Malaysian pineapple or *Ananas comosus* was

conducted by using sequence data from the chloroplast gene which is known as large subunit of the ribulose- biphosphate carboxylase (*rbcL*). The information about genetic variability at the molecular level is said to be useful to identify and characterize the unique germplasm that compliments the existing cultivars.

1.2 Problem statement

Pineapple is the third most important tropical fruit in the world after banana and mangoes (Botella and Smith, 2008). However, there is limitation on available data at molecular level for Malaysian pineapple. Previously, most of the classification of pineapple cultivars is based on morphology character. The drawback of classification based on morphological characteristics is inconsistency that arose due to disagreements among morphologist who applied different classification for interpretation of the characteristics.

Hence, in this study, phylogenetic analysis of Malaysian pineapple or *Ananas comosus* was conducted using sequence data from the chloroplast gene, *rbcL* to reconstruct a more detailed phylogenetic frame work of Malaysian pineapple and investigate the evolutionary relationships among these cultivars. The information about genetic variability at the molecular level is said to be useful to identify and characterize the Malaysian pineapple cultivars.

1.3 Objectives of the study

The objectives of the study are:

1. To amplify *rbcL* gene from eight Malaysian pineapple cultivars.
2. To clone the *rbcL* into pGEM-T Easy Vector System (Promega) and sequence the *rbcL* gene from eight Malaysian pineapple cultivars.
3. To construct a phylogenetic tree from isolated sequence of *rbcL*.

1.4 Scope of study

The scope of the study covered the construction of phylogenetic tree from eight commercial Malaysian pineapple cultivars based on *rbcL* gene. In order to achieve this, genomic DNA was isolated from leaf samples of eight commercial Malaysian pineapple cultivars; Moris, Moris Gajah, Moris Bentanggur, Yan Kee, Sarawak, Gandul and N36. PCR was carried out using *rbcL* primers and purified PCR product was subjected to cloning procedure comprising ligation of *rbcL* gene into the pGEM- T Easy Vector System (Promega) followed by transformation into NEB-5 α competent cell (New England BioLabs). Positive transformants were sent for sequencing and sequences obtained were aligned using ClustalX software. Phylogenetic tree were constructed using MEGA 5 software and analysis were carried out using Maximum Parsimony method.

1.5 Significance of the study

Chloroplast DNA (cpDNA) has been used widely to infer plant systematic at different taxonomy levels (Clegg, 1993; Gielly and Taberlet, 1994). In this study, gene of the large subunit of ribulose –bisphosphate carboxylase (*rbcL*) was used as an alternative approach for morphology identification and to study the evolutionary status and relationship among the eight cultivars of Malaysian pineapple. The important of this study was to provide additional information on the relationship pattern among the cultivars which in future can be used as source of knowledge and information for successful interbreeding on creating new cultivars.

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