

**APPLICATION OF STMS
MARKERS FOR DIVERSITY
ANALYSIS IN BABANA**

MELIKA MIRASKARI

**DISSERTATION SUBMITTED IN FULFILMENT OF THE
REQUIRMENTS FOR PARTIAL DEGREE OF MASTER OF
BIOTECHNOLOGY**

**INSTITUTE OF POSTGRADUATE AND RESEARCH
UNIVERSITY OF MALAYA
KUALA LUMPUR**

NOVEMBER 2003

Perpustakaan Universiti Malaya



A511760400

Abstract

Biodiversity of different banana varieties have been studied by using Sequence Tagged Microsatellite Sites (STMSs) DNA marker.

If short sequence repeats (SSR) loci are cloned and sequenced, primers to the flanking region can be designed to produce a Sequence-Tagged Microsatellite Site (STMS).

STMS markers have special characteristics such as locus specificity, potential to amplify multiple alleles and co-dominant nature. Their transferability makes STMS markers a powerful tool for genetic mapping, diversity analysis and genotyping. These markers were also chosen because they have been successfully used for wheat, barley and rice (Talbert et al., 1994) and also for *Cavendish* banana and more recently for analyzing somaclonal variation in *Mutiara* banana, a variant of *Rastali* banana. (R.Y. Othman pers.comm.).

In this study a variety of banana samples (including wild and cultivars) were analyzed using STMS primer pairs: AGMI 9/93, AGMI 10/103 and AGMI 105/108. After DNA extraction, selection of primers, and optimization of PCR and electrophoresis conditions were carried out. Then the amplified alleles were scored and analyzed.

All three alleles detected for each primer set, conformed to the equilibrium distribution of genotype (Hardy-Weinberg Equilibrium). From the chi-square test results, two of three primer sets produce high levels ($\geq 50\%$) of heterozygosity (AGMI 10/103 & AGMI 9/93) however, the level of heterozygosity for AGMI 105/108 appears to be very low. This shows that this primer may not be useful for examining population diversity.

In brief the result showed that STMS markers could potentially be useful for analyzing diversities in bananas.

Abstrak

Biodiversiti pisang yang berlainan variasi telah dikaji dengan menggunakan penanda DNA Sequence Tagged Microsatellite Sites (STMSs).

Jika lokus "Short Sequence Repeats (SSR)" diklon dan turutannya dibaca, primer-primernya untuk kawasan apitan boleh direka untuk menghasilkan satu Sequence-Tagged Microsatellite Site (STMS). Penanda STMS mempunyai ciri-ciri istimewa seperti lokus spesifik, yang berpotensi untuk mengamplifikasi alel-alel berbilang dan keadaan kodominan. Kebolehannya untuk dipindah membuatkan penanda STMS suatu alat yang penting untuk pemetaan genetik, analisis dan penggenotipan. Penanda-penanda ini juga dipilih kerana telah berjaya digunakan untuk gandum, barli dan beras (Talbert et al., 1994) dan juga pisang Cavendish serta terbaru untuk menganalisis variasi somaklonal dalam pisang Mutiara iaitu varian pisang Rastali (R.Y. Othman pers. comm.).

Dalam kajian ini variasi sampel pisang (termasuk liar dan kultivar) telah dianalisa menggunakan pasangan primer STMS: AGMI 9/93, AGMI 10/103 dan AGMI 105/108.

Selepas DNA diekstrak, pemilihan primer dan pengoptimuman PCR serta keadaan elektroforesis dijalankan. Kemudian, alel yang telah diamplifikasi dianalisa skornya. Ketiga-tiga alel dikesan untuk setiap set primer mengikut pengagihan ekuilibrium genotip (Ekuilibrium Hardy-Weinberg). Daripada keputusan Chi-Square dua dari tiga set primer menghasilkan heterozigositi tahap tinggi ($\geq 50\%$) (AGMI 10/103 & AGMI 9/93). Walaubagaimanapun tahap heterozigositi untuk AGMI 105/108 kelihatan sangat rendah. Ini menunjukkan yang primer ini mungkin berguna untuk mengkaji diversiti populasi. Secara ringkasnya, keputusan menunjukkan bahawa penanda STMS mempunyai potensi digunakan untuk menganalisis diversiti pisang.

ACKNOWLEDGEMENT

I wish to express my appreciation to my supervisor Associate Prof. Dr Rofina Yasmin for her valuable advises, great patience, full support and guidance throughout this thesis.

Many thanks to Dr. Asif Muhammad Javed for his advise, technical assistance and expertise for this study. Also to Ms. Fatimah Kayat and all the members of the Genetic lab and my friends whose names have not be mentioned here.

And last but not least, my special thanks go to my family, if not because of their support, I could never be able to make it.

TABLE OF CONTENTS

ABSTRACT	iii
ABSTRAK	iv
ACKNOWLEDGEMENT	v
TABLE OF CONTENTS	vi
LIST OF FIGURES	ix
LIST OF TABLES	x
ABBREVIATION	xi
1.0 Introduction	1
1.1 General introductions	1
1.2 Importance and production constrain of bananas	3
1.2.1 Socioeconomic importance of bananas	5
1.3 Molecular markers in banana breeding	8
1.3.1 Advantages of microsatellites as genetic markers in brief	10
1.3.2 Application of microsatellites in different areas	11
1.4 Objective of study	12
2.0 Literature Review	13
2.1 Origin and distribution of banana	13
2.2 Taxonomic classification of <i>Musa</i>	14
2.2.1 <i>Ensete</i>	15
2.2.2 <i>Musa</i>	16
2.3 <i>Musa</i> species in Malaysia	18

2.4 Numerical taxonomy	21
2.5 Morphological studies in <i>Musa</i>	22
2.6 Biochemical markers	24
2.7 Molecular methods for detecting genetic diversity	26
2.7.1 Molecular markers	27
2.7.1.1 Non-PCR based markers	27
2.7.1.1.1 Restriction Fragment Length	
Polymorphism	27
2.7.1.2 PCR-based Markers	28
2.7.1.2.1 Random Amplified Polymorphic DNA	29
2.7.1.2.2 Amplified Fragment Length	
Polymorphism	30
2.7.1.2.3 Sequenced Tagged Microsatellite Sites	30
3.0 Materials and Methods	33
3.1 Banana samples	33
3.2 DNA extraction	35
3.2.1 Determination of DNA Quality and Concentration	37
3.2.2 Dilution	37
3.3 Polymerase Chain Reaction (PCR)	38
3.3.1 Preparation of PCR	38
3.3.2 PCR Technique	39
3.3.3 Optimization	40
3.4 Polyacrylamide Gel Electrophoresis (PAGE)	41

3.4.1 Preparation of Gel	41
3.4.2 Preparation samples and Running of PAGE	43
3.4.3 Silver Staining of Denaturing Polyacrylamide Gels	43
3.5 Data Analysis	44
3.5.1 Chi-Square Test (χ^2)	44
3.5.2 Polyacrylamide gel analysis	44
3.5.3 Combination of Data	44
4.0 Results	45
4.1 DNA analyzing	45
4.2 Polymerase Chain Reaction	46
4.2.1 Optimization	46
4.3 Polyacrylamide Gel Electrophoresis	47
4.3.1 Optimization	47
4.3.2 Result of PAGE Screening	51
4.3.3 Data Analysis	58
5.0 Discussion	64
5.1 STMS General Discussion	64
5.2 Discussion Based On Results	66
5.2.1 Extraction of DNA	66
5.2.2 Optimization of PCR and Gel Electrophoresis	66
5.2.3 STMS Analysis	68
6.0 Conclusion	71
7.0 References	72

LIST OF FIGURES

- Figure 4.1:** A comparison between the results of a 6% gel (a) and a 7% gel (b) with the same samples and primer set AGMI 10/103. **Page 49**
- Figure 4.2:** A comparison between the results of a 6% gel (a) and a 7% gel (b) with the same samples and primer set AGMI 9/93. **Page 50**
- Figure 4.3:** STMS analysis using primer set AGMI 9/93. **Page 52**
- Figure 4.4:** STMS analysis using primer set AGMI 10/103. **Page 53**
- Figure 4.5:** STMS analysis using primer set AGMI 105/108. **Page 54**

LIST OF TABLES

Table 1.1: Cultivated area, production and yield of bananas and plantains.	Page 3
Table 1.2: Fruit hectareage in Peninsular Malaysia in 1997.	Page 4
Table 2.1: Classification of the genus <i>Musa</i> .	Page 18
Table 3.1: The accession, ploidy level and genome constitution of 18 varieties of banana.	Page 34
Table 3.2: Sequence and annealing temperature of studied <i>Musa</i> STMS primers.	Page 39
Table 4.1: DNA concentration and OD readings from spectrophotometer.	Page 45
Table 4.2: The recommended Temperature and our results after optimization are compared.	Page 47
Table 4.3: The percentage used for each primer set.	Page 48
Table 4.4: The result of primer AGMI 10/103.	Page 55
Table 4.5: The result of primer AGMI 9/93.	Page 56
Table 4.6: The result of primer AGMI 105/108.	Page 57
Table 5.1: A comparison of the main feature of different DNA markers techniques.	Page 65

ABBREVIATIONS

A₂₆₀	absorbance at ultraviolet of 260 nm
A₂₈₀	absorbance at ultraviolet of 280 nm
AFLP	Amplified Fragment Length Polymorphism
AgNO₃	silver nitrate
APS	ammonium persulphate
bp	base pair
df	degree of freedom
dH₂O	distilled water
dNTP	deoxyribonucleoside triphosphate
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetra-acetic acid
et al	et alii; (and other people)
FAO	Food and Agriculture Organization
g	gram
M	Molar
mg	milli gram
mM	milli Molar
MgCl₂	magnesium chloride
NaOH	sodium hydroxide
ng	nano gram
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphism

RFLP	Restriction Fragment Length Polymorphism
STMS	Sequence Tagged Microsatellite Sites
Taq	<i>Thermus aquaticus</i>
TBE	Tris Borate EDTA
TEMED	N, N, N', N' tetramethylethylenediamine
%	Percentage
°C	Degree Celsius
μM	micro Molar
μL	microliter
χ²	Chi-square