APPLICATION OF STMS MARKERS FOR DIVERSITY ANALYSIS IN BABANA

MELIKA MIRASKARI

DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR PARTIAL DEGREE OF MASTER OF BIOTECHNOLOGY

INSTITUTE OF POSTGRADUATE AND RESEARCH UNIVERSITY OF MALAYA KUALA LUMPUR

NOVEMBER 2003
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 (≥ 50%) of heterozygocity (AGMI 10/103 & AGMI 9/93) however, the level of heterozygocity 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-primer 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 Covendish 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.

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 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
  2.1 Origin and distribution of banana 13
  2.2 Taxonomic classification of *Musa* 14
    2.2.1 *Ensete* 15
    2.2.2 *Musa* 16
  2.3 *Musa* species in Malaysia 18
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4 Numerical taxonomy</td>
<td>21</td>
</tr>
<tr>
<td>2.5 Morphological studies in <em>Musa</em></td>
<td>22</td>
</tr>
<tr>
<td>2.6 Biochemical markers</td>
<td>24</td>
</tr>
<tr>
<td>2.7 Molecular methods for detecting genetic diversity</td>
<td>26</td>
</tr>
<tr>
<td>2.7.1 Molecular markers</td>
<td>27</td>
</tr>
<tr>
<td>2.7.1.1 Non-PCR based markers</td>
<td>27</td>
</tr>
<tr>
<td>2.7.1.1.1 Restriction Fragment Length Polymorphism</td>
<td>27</td>
</tr>
<tr>
<td>2.7.1.2 PCR-based Markers</td>
<td>28</td>
</tr>
<tr>
<td>2.7.1.2.1 Random Amplified Polymorphic DNA</td>
<td>29</td>
</tr>
<tr>
<td>2.7.1.2.2 Amplified Fragment Length Polymorphism</td>
<td>30</td>
</tr>
<tr>
<td>2.7.1.2.3 Sequenced Tagged Microsatellite Sites</td>
<td>30</td>
</tr>
<tr>
<td>3.0 Materials and Methods</td>
<td>33</td>
</tr>
<tr>
<td>3.1 Banana samples</td>
<td>33</td>
</tr>
<tr>
<td>3.2 DNA extraction</td>
<td>35</td>
</tr>
<tr>
<td>3.2.1 Determination of DNA Quality and Concentration</td>
<td>37</td>
</tr>
<tr>
<td>3.2.2 Dilution</td>
<td>37</td>
</tr>
<tr>
<td>3.3 Polymerase Chain Reaction (PCR)</td>
<td>38</td>
</tr>
<tr>
<td>3.3.1 Preparation of PCR</td>
<td>38</td>
</tr>
<tr>
<td>3.3.2 PCR Technique</td>
<td>39</td>
</tr>
<tr>
<td>3.3.3 Optimization</td>
<td>40</td>
</tr>
<tr>
<td>3.4 Polyacrylamide Gel Electrophoresis (PAGE)</td>
<td>41</td>
</tr>
</tbody>
</table>
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 Musa. 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 Musa 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
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>RFLP</td>
<td>Restriction Fragment Length Polymorphism</td>
</tr>
<tr>
<td>STMS</td>
<td>Sequence Tagged Microsatellite Sites</td>
</tr>
<tr>
<td>Taq</td>
<td>Thermus aquaticus</td>
</tr>
<tr>
<td>TBE</td>
<td>Tris Borate EDTA</td>
</tr>
<tr>
<td>TEMED</td>
<td>N, N, N', N' tetramethylethylenediamine</td>
</tr>
<tr>
<td>%</td>
<td>Percentage</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>μM</td>
<td>micro Molar</td>
</tr>
<tr>
<td>μL</td>
<td>microliter</td>
</tr>
<tr>
<td>( \chi^2 )</td>
<td>Chi-square</td>
</tr>
</tbody>
</table>