APPLICATION OF STMS MARKERS FOR DIVERSITY ANALYSIS IN BABANA

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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.

Selepas DNA diekstrak, pemilihan primer dan pengoptimaan 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 (≥ 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.

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ABBREVATIONS

A_{260}	absorbance at ultraviolet of 260 nm
A_{280}	absorbance at ultraviolet of 280 nm
AFLP	Amplified Fragment Length Polymorphism
$AgNO_3$	silver nitrate
APS	ammonium persulphate
bp	base pair
df	degree of freedom
dH_2O	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
$MgCl_2$	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	**Thermus aquaticus**
TBE	**Tris Borate EDTA**
TEMED	**N, N, N', N' tetramethylethylenediamine**
%	**Percentage**
°C	**Degree Celsius**
µM	**micro Molar**
µL	**microliter**
χ^2	**Chi-square**