BIOLISTIC TRANSFORMATION OF OIL PALM USING THE PHOSPHOMANNOSE ISOMERASE (*pmi*) GENE AS THE POSITIVE SELECTABLE MARKER

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ABSTRACT

The selectable marker system based on the *Escherichia coli* phosphomannose isomerase (*pmi*) gene was adapted for genetic transformation of oil palm. This system makes use of the *pmi* gene that encodes phosphomannose isomerase, which converts mannose-6-phosphate to fructose-6-phosphate and uses mannose as the selection agent. This is to anticipate the future requirement of using non-antibiotic resistance genes for the commercialization of transgenic oil palm. The use of antibiotic or herbicide-based selection systems has caused much public concern due to inadequate knowledge of the agents' impact on the environment and on human health. In this study, four transformation vectors, namely pMI3, pMI11, pMI3G and pMI11G were constructed for transforming oil palm. The *pmi* gene is driven by CaMV*35S* promoter in pMI3 and pMI3G, and *Ubi1* promoter in pMI11 and pMI11G. *gusA* gene was also included in the pMI3G and pMI11G constructs. The gene constructs were transferred into oil palm embryogenic calli via biolistic-mediated transformation. Bombarded calli were selected on medium supplemented with mannose as a carbon source. Results from kill curve experiment indicated that oil palm embryogenic callus have little or no PMI activity and cannot utilize mannose as a carbon source. However, when calli were transformed with a *pmi* gene, the PMI activity was greatly increased and they could utilize mannose efficiently as carbon source. For early identification of transgenic events, histochemical staining with 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide (X-Gluc) was used. Transgenic plants were confirmed by PCR and the transgene expression was detected using RT-PCR analysis. Transient expression results demonstrate that the *Ubi1* promoter is more efficient than the CaMV*35S* promoter in oil palm embryogenic calli. The insertion verification was confirmed with 98% homology observed to its corresponding *pmi* gene from *E. coli* (Genbank accession no: M15380) via PCR direct sequencing. In conclusion, the results of this study indicated that mannose selection system can be used for oil palm transformation. Potentially this will make transgenic oil palm acceptable in the future.

ABSTRAK

Sistem penanda pemilihan berdasarkan gen phosphomanose isomerase (*pmi*) *Escherichia coli* telah diadaptasi untuk tranformasi genetik sawit. Sistem ini menggunakan gen *pmi* yang mengekodkan phosphomanose isomerase, yang menukarkan manos-6-fosfat kepada fruktos-6-fosfat dan menggunakan manos sebagai ejen pemilihan. Ini adalah sebagai langkah persediaan bagi mengunakan gen tidak rintang antibitiotik dan racun herba di dalam pengkomersialan sawit transgenik. Penggunaan antibiotik atau racun herba sebagai agen pemilihan telah banyak meningkatkan keresahan awam kerana kurangnya pengetahuan ke atas kesan jangka panjang ejen-ejen ini terhadap kesihatan manusia dan alam sekitar. Di dalam kajian ini, empat vektor tranformasi, iaitu pMI3, pMI11, pMI3G dan pMI11G telah dihasilkan untuk tranformasi sawit. Gen *pmi* dipacu oleh promoter CaMV*35S* di dalam pMI3 dan pMI3G, dan promoter *Ubi1* di dalam pMI11 dan pMI11G. Gen *gusA* juga turut dimasukkan ke dalam konstruk pMI3G dan pMI11G. Konstruk gen yang telah dibina dipindahkan ke kalus embriogenik sawit dengan cara tranformasi menggunakan biolistik. Kalus yang ditembak telah dipilih di atas media yang mengandungi manos sebagai sumber karbon. Keputusan dari ujikaji lengkuk pembunuhan menandakan bahawa kalus embriogenik sawit mempunyai sedikit atau tiada langsung aktiviti PMI dan tidak boleh menggunakan manos sebagai sumber karbon. Walaubagaimanapun, apabila kalus ditransformasi dengan gen *pmi*, aktiviti PMI dapat digandakan dan dapat menggunakan manos sebaik mungkin sebagai sumber karbon. Untuk pengesanan awal sebarang acara transgenik, pengasaian histokimia menggunakan 5-bromo-4-kloro-3-indolyl-*ß*-D-glucuronide (X-Gluc) telah digunakan. Pokok transgenik disahkan dengan analisis PCR dan pengekspressan transgen telah dikesan melalui analisis RT-PCR. Manakala pengekspressan transien yang dilakukan ke atas kalus embriogenik sawit menunjukkan bahawa transformasi menggunakan promoter *Ubi1* adalah lebih efisyen jika dibandingkan dengan menggunakan promoter CaMV*35S*. Pembuktian penyelitan telah disahkan dengan 98% homologi kepada gen *pmi* dari *E. coli* (Genbank accession no: M15380) melalui penjujukan secara langsung PCR. Secara kesuluruhannya, keputusan kajian ini menunjukkan bahawa sistem pemilihan manos boleh digunakan untuk transformasi sawit. Ini adalah untuk memastikan penghasilan sawit transgenik yang lebih diterima oleh pengguna di masa akan datang.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

LIST OF ABBREVIATIONS

^{o}C	degree Celcius
%	Percentage
µg	Microgram
µl	Microliter
• M	Micromolar
18S	18 subunit of ribosome
ACP	Acyl carrier protein
Adh1	Maize alcohol dehydrogenase 1 gene's promoter
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BAP	Benzylaminopurine
Bar	phosphinothricine acetyltransferase gene
BLAST	Basic local alignment search tool
bp	Base pair
$CaCl_2$	Calcium chloride
CaMV*35S*	Cauliflower Mosaic Virus *35S*
cDNA	Complementary DNA
CIP	Calf Intestinal Phosphatase
cm	Centi meter
CTAB	hexadecyl trimethyl ammonium bromide
DNA	Deoxyribonucleic acid
DNAse	Deoxyribonuclease (RNAse free)
EC	Embryogenic callus
EDTA	Ethylenediaminetetra acetic acid
EtBr	Ethidium bromide
GFP	Green fluorescent protein
GUS	• -glucuronidase
g	Gram
h	hour
hpt or *hph*	Hygromycin phosphotransferase gene
KAc	Potassium acetate
kb	Kilo base pair

l	Liter
LB	Luria-Bertani Broth
LiCl	Lithium chloride
LUC	luciferase
M	Molar
Man	Mannose
mg	Milligram
$MgCl_2$	Magnesium chloride
min	Minute
$MnCl_2$	Manganese chloride
MS	Murashige and Skoog (1962)
MSO	Hormone-free Murashige and Skoog media (1962)
ml	Milliliter
mM	Millimolar
mm	Millimeter
MUG	4-methyl umbelliferyl β-D-glucuronidase
MOPS	3-(N-morpholino) propane sulfonic acid
NAA	1-Naphthaleneacetic acid
NaOH	Sodium hydroxide
ng	Nanogram
nm	Nanometer
npt II	neomycin phosphotransferase II gene
Nos	gene coding for nopaline syntase
OD	Optical density
PCR	Polymerase chain reaction
PHB	polyhydroxybutyrate
PMI/*pmi*	Phosphomannose isomerase
Psi	Pound per square inch
$RbCl_2$	Rubidium chloride
RNA	Ribonucleic acid
RT-PCR	Reverse transcription PCR
rpm	Revolution per minute
sec	Second
T-DNA	transferred DNA

Ti-plasmid	tumor-inducing plasmid
U	Unit
Ubi1	Maize ubiquitin 1 gene's promoter
UV	Ultraviolet
vir	virulence
w/v	Weight per volume
X-gluc	5-bromo-3-indolyl-glucuronide
Xyl	Xylose