UNIVERSITI PUTRA MALAYSIA

OPTIMIZATION OF PARAMETERS INVOLVED IN THE TRANSFORMATION OF OIL PALM USING THE BIOLISTIC METHOD

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FSMB 1998 7

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By

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Thesis Submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Food Science and Biotechnology, Universiti Putra Malaysia.

January 1998

“IN THE NAME OF ALLAH, MOST GRACIOUS, MOST MERCIFUL”

Dedicated To :

My Parents : Hj. Ghulam Kadir and Hjh. Aishan Bibi

My Wife : Nor Muriani

My Sons : Haziq, Iman and Najib

My Special Brother : Salim

My Brothers and Teachers

"By command of my Lord : Of knowledge it is only a little that is communicated (thought) to you (O men!)"

(Surah Al-Isra' : 85)

"It is He Who produces gardens, with trellises and without, and date-palms, and crops of diverse flavour, and the olive and the pomegranate, similar (in kind) and different (in variety)"

(Surah Al-An'am : 141)

He Who created seven heavens in harmony. You can see no fault in the Beneficent One's Creation; then look again: can you see any fault? Then look again and yet again; your sight will come back to you weakened and worn out"

(Surah Al-Mulk : 3-4)

ACKNOWLEDGEMENTS

All praise be to the Almighty ALLAH, the Merciful and the Compassionate. Due to His willingness, the completion of this study was made possible.

I would like to express my deep appreciation and gratitude to the chairman of my supervisory committee, Dr. K. Harikrishna, for his help, guidance and constant support in making the completion of this thesis a success. A special gratitude to Dr. Paul Christou, my external supervisor, for becoming my "transformation teacher" and giving invaluable guidance and encouragement throughout my studies. The help rendered by my co-supervisors, Dr. Suhaimi Napis and Dr. Cheah Suan Choo is greatly appreciated. Thanks also due to Dr. Norihan Saleh and Dr. M.K.U. Chowdhury, my former supervisors, for their guidance and help at the early stage of my study. A special thank you to Dr. Douglas A. Chamberlain for some essential editorial help.

A heartiest acknowledgment to Mrs. Fatimah Tahir for her invaluable assistance during my study. I am grateful to all staff members of the Plant Science and Biotechnology Unit, PORIM, especially to Feshah, Shamsul, Rosli, Zaiton, Mohd Shah, Jeya and Wai, to Drs. Rod Casey, Mark, Sudhakar, Yolande and Messrs Ajay, Rahat, Salem, Janet and Nicky at the Department of Applied Genetics and Virology, John Innes Centre and Mrs Hamidah and Zaiton of MARDI for their help and support.

My utmost thanks to Datuk Dr. Yusof Basiron (Director-General of PORIM); Professor Dr. Jalani Sukaimi (Deputy Director-General), Dr. Ariffin Darus (Director of Biology) and Dr. N. Rajanaidu (Head, Plant Science and Biotechnology Unit) for their support and encouragement in this study.

My invaluable appreciation to PORIM, JPA and ADB for the financial support to undertake this study.

Last but not least, my heartiest appreciation to my parent, my wife, my sons, my mother-in-law and my brothers for giving me the strength during the course of my study.

May ALLAH bless us always

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LIST OF ABBREVIATIONS

The following abbreviations were used in the text :

ACP	Acyl carrier protein
Act1	Rice actin 1 gene's promoter
Adh1	Maize alcohol dehydrogenase 1 gene's promoter
Anova	Analysis of variance
ATP	Adenosine triphosphate
bar	Gene coding for phosphinothricin acetyltransferase
BSA	Bovine serum albumin
$CaCl_2$	Calcium chloride
35S CaMV	Cauliflower mosaic virus 35S gene's promoter
CsCl	Cesium Chloride
2,4-D	2,4-dichlorophenoxyacetic acid
dNTP	Deoxynicotinamide triphosphate
EDTA	Ethylenediaminetetra acetic acid
Emu	A recombinant truncated maize alcohol dehydrogenase 1 gene promoter with enhancers elements from Adh1 gene and *Agrobacterium*
EtBr	Ethidium Bromide
GUS	β-Glucuronidase
hpt	Gene coding for hygromycin phosphotransferase
HCl	Hydrochloric acid
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
KAc	Potassium acetate
Kb	Kilobase
KCl	Potassium chloride
Kda	Kilodalton

KOH	Potassium hydroxide
KV	Kilovolt
LB	Luria Bertani broth
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulfate
MS	Murashige and Skoog
MU	Methyl umbelliferone
MUG	4-methyl umbelliferyl β-D-glucuronide
NAA	α-Naphthaleneacetic acid
NaCl	Sodium chloride
NaOH	Sodium hydroxide
$NaPO_4$	Sodium phosphate
NH_4Ac	Ammonium acetate
nptII	Gene coding for neomycin phosphotransferase
PCR	Polymerase chain reaction
PMSF	Phenylmethylsulfonylfluoride
PVP	Polyvinyl Pyrrolidone
SDS	Sodium dodecyl sulfate
T0	First generation of transgenic plants
T1	Progeny of T0 plant
T2	Progeny of T1 plant
Tris	Tris [hydroxymethyl] aminomethane
Triton X-100	T-octylphenoxypoly-etoxyethanol
Tween 20	Polyoxyethylene sorbiton monolaurate
Ubi1	Maize ubiquitin 1 gene's promoter
X-gluc	5-bromo-4-chloro-3-indoyl-glucuronide
YLMP	Young leaves from mature palm
YLSP	Young leaves from seedling palm

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the Degree of Doctor of Philosophy.

OPTIMIZATION OF TRANSFORMATION TECHNIQUES TO OBTAIN TRANSGENIC OIL PALM

By

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JANUARY 1998

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Physical and biological parameters affecting DNA delivery into oil palm embryogenic calli using the biolistic device have been optimized. The physical parameters tested were : helium pressure, distance from rupture disc to the macrocarrier, distance from macrocarrier to the stopping plate, distance from stopping plate to the target tissue, vacuum pressure, number of bombardments, particle types and sizes, and the effect of calcium chloride and spermidine on microcarrier-DNA binding. The optimized biological parameters were: explant types with gold microcarrier, explant types with tungsten, duration of callus culture in fresh medium prior to bombardment, duration between bombardment and GUS staining, genotype, immature embryo preculture duration, DNA concentration, osmoticum type and concentration and osmoticum treatment duration before and after bombardment. Independent experiments were carried out to study the effects of each parameter and its

variables on transient expression. Two days after bombardment, the tissues were stained with GUS assay buffer for 16-20 hours at 37^0C and the blue spots counted under a binocular microscope. All the variables used in these experiments were found to be significantly different except for vacuum pressure, bombardment number and genotype.

The efficiency of GUS gene expression was measured in embryogenic calli and young leaves of mature and seedling palms using five constructs carrying different promoters : Emu; Ubi1; Act1, 35S and Adh1 were evaluated to identify the most suitable promoter for use in oil palm. The GUS gene expression from the different promoters was assayed histochemically and fluorometrically from a total of 200 plates of target tissues in eight independent experiments. Significant effects on transient GUS gene expression were demonstrated by each of the different promoters tested.

The effectiveness of kanamycin; geneticin (G-418); neomycin, hygromycin and basta as selection agents to inhibit growth of oil palm embryogenic calli was evaluated. Embryogenic calli were separately exposed to all these selection agents at different concentrations ranging from 1 to 2000 mg/l for a period of one month. This was done in two replicates and repeated twice to ensure reproducibility of the selection system. Of the five compounds tested, hygromycin and basta were found to be most suitable as selection agents for oil palm as they can stop the growth of embryogenic calli at lower concentrations.

Bombarded embryogenic calli were exposed to 40 or 80mg/l of selective agents after 1 or 3 weeks. It was found that there were no significant differences in the number of resistant embryogenic calli produced per plate when selected at different concentrations and time. The presence of transgenes in the resistant embryogenic calli was confirmed by PCR and Southern analysis. Transgenic embryogenic calli were later regenerated into whole plants and their transgenic status verified by PCR and Southern analysis. Problems faced during the study and their solutions are also discussed.

As oil palm has a long breeding cycle, inheritance of transgenes cannot be demonstrated within the period of this study. Therefore, rice, a model crop for monocot transformation, was also used for transformation experiments. Calli derived from immature embryos were bombarded and were selected on hygromycin. Resistant calli isolated were regenerated into whole plants. Two transgenic lines were obtained. T1 and T2 from one of the clones were also produced and analysed. Integration and inheritance of the transgenes were followed by phenotypic and genotypic analysis.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi syarat untuk memperolehi Ijazah Doktor Falsafah.

PENGOPTIMUMAN TEKNIK-TEKNIK TRANSFORMASI UNTUK MEMPEROLEHI KELAPA SAWIT TRANSGENIK

Oleh

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Parameter-parameter biologi dan fizikal yang mempengaruhi penghantaran DNA ke dalam kalus embriogenik kelapa sawit menggunakan alat biolistik telah berjaya dioptimakan. Parameter-parameter fizikal yang telah diuji adalah : tekanan helium, jarak diantara cakera pecah ke pembawa makro, jarak diantara pembawa makro ke piring penghenti, jarak diantara piring penghenti ke tisu sasaran, tekanan hampagas, bilangan tembakan, saiz dan jenis pembawa mikro serta kesan kalsium klorida dan spermidin terhadap pengabungan DNA dan pembawa mikro. Parameter-parameter biologi yang telah diuji pula adalah : jenis eksplan menggunakan pembawa mikro emas, jenis eksplan menggunakan pembawa mikro tungsten, jangkamasa pengsubkulturan ke media segar sebelum tembakan, jangkamasa antara tembakan dan pewarnaan GUS, genotip, jangkamasa pra-pengkulturan embrio tidak matang

matang, kepekatan DNA, jenis dan kepekatan bahan osmotik dan jangkamasa tindakan osmotik sebelum dan selepas tembakan. Ujikaji berasingan telah dijalankan untuk mengkaji kesan setiap parameter dan pembolehubah ke atas ungkapan sementara. Dua hari selepas tembakan, tisu diwarnakan menggunakan penimbal asei GUS selama 16-20 jam pada suhu 37^0C dan bintik-bintik biru yang dihasilkan telah dikira dibawah mikroskop binokular. Setiap pembolehubah yang digunakan menunjukkan perbezaan bererti kecuali, tekanan hampagas, bilangan tembakan dan genotip.

Keupayaan ungkapan gen GUS oleh lima plasmid yang membawa promoter-promoter berbeza : Emu; Ubi1; Act1, 35S dan Adh1 telah dinilai keatas kalus embriogenik dan daun-daun muda dari pokok semaian dan pokok matang. Ini adalah untuk memilih promoter-promoter yang sesuai untuk kelapa sawit. Ungkapan gen GUS oleh promoter-promoter berbeza telah diasei menggunakan kaedah histokimia dan florometrik ke atas 200 piring tisu sasaran dan di dalam 8 ujikaji berasingan. Promoter-promoter tersebut telah menunjukkan kesan yang bererti terhadap ungkapan sementara gen GUS.

Kecekapan lima agen pemilihan : kanamisin; genetisin G-418; neomisin, higromisin dan basta untuk merencat pertumbuhan kalus embriogenik kelapa sawit telah dinilai. Kalus embriogenik telah didedahkan secara berasingan kepada kepekatan berbeza (1-2000mg/l) ejen pemilihan untuk tempoh satu bulan. Ujikaji ini telah dijalankan secara replikasi dan diulang sebanyak dua kali untuk memastikan kebolehulangan hasil. Dari kelima-lima ejen pemilihan