SOMATIC EMBRYOGENESIS OF *CARICA PAPAYA CV*. EKSOTIKA

By

BEVERLIEN CHRISTINE

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Agricultural Science

November 2005

Dedicated TO:

My Parents (Daiman Lamat and Maina Godoun)

My Sisters (Jeniffer and Gwendoline)

My Brothers (Sherwin and Reno)

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Agriculture Science

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Faculty : Agriculture

This study was carried out with the main objective of establishing somatic embryo production in *Carica papaya* cv. Eksotika that can be used for further genetic improvement of the crop. The study included induction of somatic embryogenesis from immature zygotic embryos and anatomical study of the embryo development, establishment of plant regeneration from the somatic embryo, and establishment of cell suspension culture from embryogenic callus.

The specific objective of the study on induction of somatic embryogenesis from zygotic embryos was to study the effect of growth regulators on the induction of somatic embryos with or without monthly subculture. The combination of 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BAP) at various concentrations were assessed. The experiment was conducted in a Randomized Completely Block Design.

In the experiment without subculture, MS medium supplemented with 5 mg/L 2,4-D induced the highest percentage (100 %) of somatic embryo formation on the second and third month of culure. At higher 2,4-D concentrations (6-8 mg/L) the percentage of callus formation increased. Monthly subculturing delayed the maturation of somatic embryos and increased callus formation. The percentage of somatic embryo formation was highest (97.5 %) on MS medium supplemented with 5, 6 and 8 mg/L 2,4-D by the third subculture.

The specific objectives of the study on plant regeneration from somatic embryos were to determine suitable plant growth regulators, as well as medium formulation on regeneration of somatic embryos. The combination of BAP and NAA at various concentrations were assessed. Different medium formulations evaluated were MS medium (Murashige and Skoog, 1962), LS medium (Linsmair & Skoog, 1965) and B5 medium (Gamborg, 1968) either at full strength or half strength in

macronutrients. All experiments were conducted and arranged in a Completely Randomised Design (CRD).

MS medium without plant growth regulator induced the highest percentage (59.3 %) of regeneration from the somatic embryos. It was observed that the higher the concentration of BAP and NAA either alone or in combination, the lower the percentage of regeneration and the higher the percentage of callus formation. In the experiment on effect of different medium formulations on regeneration, MS full strength medium produced the highest percentage of regeneration of the somatic embryos.

The third study was the establishment of cell suspension culture from embryogenic callus of papaya cv. Eksotika. Four weeks after the transfer of embryogenic callus into liquid medium, single cells were released and after eight weeks pro-embryogenic masses (PEMs) were formed. The highest mean number of cells per ml (56.9) was obtained on liquid MS medium containing 2 mg/L 2,4-D. Maturation of somatic embryos was achieved on transferring the globular or heart-shaped somatic embryos to liquid MS medium without growth regulator. Germination of somatic embryos occurred following transfer of cotyledonary embryos from liquid MS medium onto solid hormone-free MS medium

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Pertanian

EMBRYOGENESIS SOMA BAGI *CARICA PAPAYA* KULTIVAR EKSOTIKA

Oleh

BEVERLIEN CHRISTINE

November 2005

Pengerusi : Profesor Madya Maheran Abdul Aziz, PhD

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Penyelidikan ini dilaksanakan dengan objektif utama untuk mewujudkan produksi embrio soma betik kultivar Eksotika, yang boleh digunakan untuk pembaikan genetik tanaman tersebut. Kajian ini terdiri daripada induksi embrio soma daripada embrio zigotik yang belum matang dan kajian anatomi ke atas pembentukan embrio soma tersebut; mewujudkan regenerasi pokok daripada embrio soma, dan mewujudkan kultur ampaian sel daripada kalus yang embriogenik.

Objektif khas daripada kajian induksi embrio soma daripada embrio zigotik yang belum matang adalah untuk mengkaji kesan pengawalatur tumbesaran terhadap induksi embrio soma sama ada dengan subkultur setiap bulan atau tanpa subkultur. Kombinasi asid diklorofenoksi asetik (2,4-D) dan 6-benzilaminopurina (BAP) pada beberapa kepekatan telah ditaksirkan. Kajian ini dijalankan dengan menggunakan Rekabentuk Rawak Berblok Penuh (RCBD).

Dalam eksperimen tanpa subkultur, medium MS yang mengandungi 5 mg/L 2,4-D menghasilkan peratus pembentukan embrio soma tertinggi (100 %) pada bulan kedua dan ketiga. Pada kepekatan 2,4-D yang lebih tinggi (6-8 mg/L) peratus pembentukan kalus bertambah. Subkultur setiap bulan melambatkan kematangan embrio soma dan meningkatkan pembentukan kalus. Peratus pembentukan embrio soma tertinggi (97.5 %) adalah pada medium MS yang mengandungi 5, 6 dan 8 mg/L 2,4-D pada subkultur ketiga.

Objektif khas daripada kajian regenerasi pokok daripada embrio soma adalah untuk menentukan pengawalatur tumbesaran serta formulasi medium yang sesuai untuk regenerasi embrio soma tersebut. Kombinasi NAA dan BAP pada beberapa kepekatan telah dicuba. Pelbagai formulasi medium yang dikaji adalah medium MS (Murashige dan Skoog, 1962), medium LS (Linsmair dan Skoog, 1965) dan medium B5 (Gamborg, 1968) sama ada nutrien makronya berkepekatan penuh atau separuh. Kesemua kajian ini dijalankan dengan menggunakan Rekabentuk Rawak Lengkap (CRD).

Medium MS tanpa pengawalatur tumbesaran menghasilkan peratus regenerasi embrio soma yang tertinggi (59.3 %). Pemerhatian menunjukkan lebih tinggi kepekatan NAA dengan atau tanpa BAP lebih rendah peratus regenerasi dan lebih tinggi peratus pembentukan kalus. Di dalam eksperimen ke atas kesan pelbagai formulasi medium terhadap regenerasi juga menunjukkan medium MS pada kepekatan penuh memberikan peratus regenerasi embrio soma yang tertinggi.

Kajian ketiga adalah mewujudkan kultur ampaian sel daripada kalus embriogenik. Empat minggu selepas kalus embriogenik dipindahkan ke dalam media cecair, sel-sel baru terbentuk dan selepas lapan minggu 'pro-embryogenic masses' (PEM) terbentuk. Min bilangan sel per ml (56.9) tertinggi diperoleh pada medium MS cecair yang mengandungi 2 mg/L 2,4-D. Kematangan embrio soma dicapai dengan memindahkan embrio berbentuk globul atau hati ke dalam medium cecair MS

tanpa pengawalatur tumbesaran. Percambahan embrio soma berlaku berikutan pemindahan embrio soma berkotiledon dari media cecair MS ke medium pepejal MS tanpa pengawalatur tumbesaran.

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I certify that an Examination Committee has met on 22nd November 2005 to conduct the final examination of Beverlien Christine on her Master of Agriculture Science entitled "Somatic Embryogenesis of *Carica papaya* cv. Eksotika" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian

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DECLARATION

I hereby certify that this thesis is based on my original work except quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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BEVERLIEN CHRISTINE

Date :

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2,4-D	**2,4-dichlorophenoxyacetic acid**
AC	**activated charcoal**
ACC	**1-aminocyclopropane-1-carboxylic**
ANOVA	**analysis of variance**
BAP	**6-benzylaminopurine**
BA	**6-benzyladenine**
B5	**Gamborg (1968) basal medium**
CH	**casein hydrolysate**
CRD	**Completely randomised design**
cv.	**cultivar**
DNMRT	**Duncan new multiple range test**
EC	**embryogenic cell**
e.g.	**exempli gratia (for example)**
et al.	***et alia***
etc.	**et cetera**
FAA	**formaldehyde acetic acid**
FDA	**fluorescein diacetate**
Fe-EDTA	**iron ethylene diamine tetraacetic acid**
GA_3	**gibberellic acid**
g/L	**gram per liter**
IAA	**indole acetic acid**
IBA	**indole butyric acid**
i.e	**id est (that is)**
IEDCs	**induced embryogenic determined cells**
LS	**Linsmaier and Skoog (1965) basal medium**
M	**molar**
MARDI	**Malaysian Agriculture Research and Development Institute**
mg/L	**milligram per liter**
MS	**Murashige and Skoog (1962) basal medium**

NAA	**naphthalene acetic acid**
PBA	**6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine**
PCR	**polymerase chain reaction**
PEDCs	**pre-embryogenic determined cells**
PEMs	**pro-embryogenic masses**
PGR	**plant growth regulator**
pH	**$^{-}$ log $[H^{+}]$**
PRV	**papaya ringspot virus**
Ri	**root-induction**
RNA	**ribonucleic acid**
Rpm	**rotation per minute**
SAS	**statistical analysis system**
SEM	**scanning electron microscopy**
sp.	**species**
Ti	**tumor-induction**
v/v	**volume to volume**
w/v	**weight per volume**
%	**percent**
α	**level of significance**
UKM	**Universiti Kebangsaan Malaysia**
μ**g**	**microgram**
μ**m**	**micrometer**
μ**M**	**micromolar**
UV	**ultra violet light**