THE DEVELOPMENT OF PLANT REGENERATION SYSTEM FROM CALLUS OF PINEAPPLE (*Ananas comosus* L.)

By

ANGELA EE DE SILVA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science.

February 2005

To all

whose chief end

is to

glorify God

and to

enjoy Him forever.

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

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Chairman: Mihdzar Abdul Kadir, PhD

Faculty: Agriculture

Malaysia's production of canned pineapples has been decreasing since 1992. Two important factors that have been a hindrance to the progress of this industry are competition from other producers and the increasing demand for fresh pineapples. Current varieties need to undergo qualitative improvements. Genetic modification, breeding and selection are some crop improvement techniques that are not successful at the moment in developing varieties that can replace current world varieties. Somaclonal variation is another technique for obtaining desirable variants, which have been achieved in crops such as sugarcane, wheat and sorghum. Highly stable variants that can be transmitted to progenies, and a more controlled change of their characteristics than those of induced mutations were achieved. However, this technique requires a plant regeneration system from callus cells. These cells have a tendency to mutate, and more cells are mutated under prolonged culture and rapid proliferation, and so generate more variants for selection. Therefore, the objectives of this project are to induce calli, proliferate old calli and regenerate shoot from calli.

For calli induction, meristemic globular bodies (MGB) of Moris and Josapine, were cultured in various levels of auxin naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic (2,4-

D). The highest percentage of MGB forming calli was observed in treatment NAA 14 and 10 mg/L for Moris and Josapine respectively, at the end of 16 weeks.

For calli proliferation, 18 month-old calli were cultured in various levels of NAA, 2,4-D, b-naphtoxyacetic acid (BNOA) and p-chlorophenoxyacetic acid (4-CPA) auxins for 12 weeks, and then in various levels of casein hydrolysate (CH) and coconut water (CW) in the presence of NAA 6 mg/L for 12 weeks. Among the various levels of the four auxins, NAA 6 mg/L proliferated healthy and high mean calli fresh weight. However, NAA 6mg/l supplemented with CW and CH also gave healthy and generally higher mean calli fresh weight than NAA 6mg/L alone. NAA 6 mg/L alone was considered the most economical treatment for calli proliferation, while NAA 6mg/L supplemented with 15%v/v CW and 300mg/L CH gave significantly highest mean calli fresh weight and was considered the best treatment for rapid calli proliferation.

For shoot regeneration, 18 month-old calli were cultured in various levels of NAA, 2,4-D, BNOA and 4-CPA auxins for 12 weeks, and then in combinations of various levels of auxins (BNOA and 2,4-D) and cytokinins (Benzylaminopurine [BAP], Kinetin and Adenine) for 12 weeks. Among the various levels of the four auxins, 2,4-D at 1mg/L regenerated high number of shoots, and was considered the best treatment for high shoot regeneration from calli that were considered as high competency calli. However, regeneration response from these calli were gradually decreasing, such that treatment BNOA 6mg/L combined with BAP 1mg/L (with subculture) (that statistically gave highest number of regenerated shoots) and an extended culture period of 12 weeks (without subculture), generated mean number of shoots was considered as not satisfactory. Subsequently, the 18 month old-calli (now 27 months) were cultured in various combination levels of CH and CW for 12 weeks, but these failed to

show any regenerated shoots from calli that were now considered low competency calli. However, calli obtained from treatment NAA 6 mg/L + 300mg/l CH + 15%v/v CW (rapid calli proliferation treatment, and now 27 months old), preserved calli competency and regenerated highest mean number of shoots in treatment 10%v/v CW and 200mg/l CH, and was considered the best treatment for regeneration of shoots from low competency calli.

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

PEMBENTUKKAN KALUS DAN REGENERASI PUCUK DARI KALUS UNTUK NANAS (*Ananas comosus* L.)

Oleh

ANGELA EE DE SILVA

February 2005

Pengerusi: Mihdzar Abdul Kadir, PhD

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Sejak tahun 1992, prestasi pengeluaran nanas tin Malaysia semakin merosot. Dua faktor penting dan cabaran utama perkembangan industri ini adalah persaingan dari pengeluar nanas dunia dan permintaan terhadap nanas segar yang semakin meningkat. Baka yang sedia ada perlu dipertingkatkan nilai kualitatifnya. Setakat ini, modifikasi genetik, pembiakbakaan dan pemilihan adalah teknik pemajuan tanaman yang kurang berjaya dalam menghasilkan baka yang lebih baik atau setanding dengan baka dunia. Variasi somaklonal, adalah satu lagi teknik yang berupaya menghasilkan baka baru yang lebih baik, dan teknik ini telah berjaya diaplikasikan pada beberapa tanaman seperti tebu, gandum dan sorghum. Teknik ini membolehkan modifikasi genetik yang lebih terkawal berbanding dengan teknik modifikasi genetik secara langsung, serta menghasilkan baka yang stabil dan boleh mewariskan kualiti barunya kepada generasi seterusnya. Namun begitu, teknik ini memerlukan sistem regenerasi pokok melalui sel kalus. Sistem ini merangkumi tiga bahagian iaitu; pembentukkan kalus, pertumbuhan kalus, dan regenerasi pucuk dari kalus. Kalus adalah fasa sel yang cenderung mengalami mutasi, dan lebih banyak kalus mengalami mutasi apabila dikultur untuk jangkamasa yang panjang dan dipercepatkan pertumbuhannya, dan seterusnya menghasilkan

variasi untuk pemilihan baka yang lebih baik. Maka, objektif projek ini adalah, pembentukkan kalus, pertumbuhan kalus dan regenerasi pucuk dari kalus.

Dalam kajian pembentukkan kalus, MGB baka Moris dan Josapine telah dikultur dalam pelbagai kepekatan auksin NAA dan 2,4-D selama 12 minggu. Peratus pembentukkan kalus yang tertinggi dari MGB telah dikenalpasti dalam rawatan NAA 14 dan 10 mg/L untuk baka Moris dan Josapine masing-masing, pada akhir minggu ke-16.

Dalam kajian pertumbuhan kalus, kalus berumur 18 bulan telah dikultur dalam pelbagai kepekatan auksin NAA, 2,4-D, BNOA dan 4-CPA selama 12 minggu, dan kemudian dikultur dalam kombinasi pelbagai kepekatan CH dan CW berserta NAA 6 mg/L untuk 12 minggu. Rawatan NAA 6mg/L telah menghasilkan pertumbuhan kalus yang sihat dan purata berat basah kalus yang tinggi berbanding di antara rawatan keempat auksin tersebut. Tetapi rawatan NAA 6mg/L berserta CW dan CH, telah menghasilkan purata berat basah kalus yang lebih tinggi. Rawatan dengan hanya NAA 6mg/L telah diterima sebagai rawatan yang berekonomi untuk penghasilan kalus, manakala rawatan NAA 6 mg/L + 15%v/v CW + 300mg/L CH telah menghasilkan purata berat basah kalus yang tertinggi dan menunjukkan perbezaan yang bererti.

Dalam kajian regenerasi pucuk dari kalus, kalus berumur 18 bulan telah dikultur dalam pelbagai kepekatan auksin NAA, 2,4-D, BNOA dan 4-CPA selama 12 minggu, dan kemudian dikultur dalam gabungan pelbagai kepekatan auksin (BNOA dan 2,4-D) dan sitokinin (BAP, Kinetin dan Adenine) selama 12 minggu. Di antara rawatan keempat-empat auksin tersebut, rawatan 2,4-D 1mg/L telah menghasilkan bilangan regenerasi pucuk yang tertinggi dari kalus, maka rawatan ini diterima sebagai rawatan yang terbaik untuk regenerasi bilangan pucuk yang tinggi dari kalus, dan kalus ini secara relatif dianggap sebagai kalus cergas.

Tetapi bilangan regenerasi pucuk dari kalus semakin berkurang dengan bertambahnya jangkamasa pengkulturannya, sehinggakan gabungan rawatan BNOA 6mg/L dengan BAP 1mg/L (walaupun bilangan regenerasi pucuknya adalah tertinggi mengikut statistik, melalui amalan subkultur), serta tambahan pengkulturan selama 12 minggu lagi (amalan tanpa subkultur), telah menghasilkan bilangan pucuk yang tidak memuaskan. Seterusnya, kalus 18 bulan tersebut (sekarang genap 27 bulan), dikultur dalam gabungan pelbagai kepekatan CH dan CW selama 12 minggu, tetapi kalus ini telah langsung hilang kecergasannya untuk menghasilkan pucuk, dan kalus ini dianggap sebagai kalus kurang cergas. Tetapi, kalus yang diperolehi dari rawatan NAA 6mg/L + 15%v/v CW + 300mg/L CH (rawatan yang telah diterima sebagai terbaik untuk penghasilan kalus yang cepat, sekarang turut genap 27 bulan), telah berjaya memelihara kecergasan kalus dan menghasilkan bilangan pucuk yang tertinggi dalam rawatan 10%v/v CW + 200mg/L CH.

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Last but not least, her gratitude to her trusted academic mentor and friend, who was determined that she finish what she started.

I certify that an Examination Committee met on 14th February 2005 to conduct the final examination of Angela Ee De Silva on her Master of Science thesis entitled "The Development of Plant Regeneration System from Callus of Pineapple (*Ananas comosus* L.)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for 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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ANGELA EE DE SILVA

Date:

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2,4-D	2,4-dichlorophenoxyacetic acid
4-CPA	p-chlorophenoxyacetic acid
ADE	Adenine
BAP	6-benzylaminopurine
BNOA	b-naphtoxyacetic acid
CH	casein hydrolysate
CW	coconut water
MS	Murashige and Skoog basic culture media
NAA	naphthaleneacetic acid
MGB	Meristemic globular bodies