EFFECTS OF CYTOKININS AND AUXINS ON DIRECT AND INDIRECT REGENERATION OF BANANA (*Musa acuminata* L.) cv. Berangan

By

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

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DEDICATION

This thesis is specially dedicated to my family, friends and colleagues in Universiti Putra Malaysia.

A special dedication to the government of Malaysia for great leadership, which yielded such a powerful and vibrant nation. The uniqueness of Malaysia has set the standard as a peace-loving country where religio-cultural diversity thrives to champion economic successes and political stability in Southeast Asia.

Malaysia's success story was indebted to great leadership and vision of former Prime Minister, Tun Dr Mahathir Mohamad followed by Datuk Seri Abdullah Ahmad Badawi. The government's strategic approaches and visions thus help to unify the nation in such a way to enhance and assured success and prosperity for the citizens of Malaysia as a whole.

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

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September 2004

Chairman : Mihdzar Abdul Kadir, Ph.D

Faculty : Agriculture

The ultimate challenge to banana industry in Malaysia is the constant threat from disease infection such as Fusarium wilt (race 4) and Sigatoka leaf spot. Conventional breeding of banana remains difficult due to high sterility and polyploidy level, therefore biotechnological techniques must be integrated into banana improvement programmes. The present study was divided into two parts and directed to cater for the abovementioned scenario. Therefore, a direct and indirect regeneration protocol is needed for *in-vitro* propagation and genetic manipulation, respectively. To study the effect of cytokinins and auxins on shoot and root proliferation, excised shoot-tip with rhizome and leaf base (1.0 cm^2 base x 1.5 cm) was cultured on modified Murashige and Skoog (1962) nutrient medium. The modified solid medium was supplemented with various concentrations (0.5, 2.0, 4.0, 8.0, 16.0 mg/L) of cytokinins (BAP, Kinetin, Adenine hemisulphate) for shoot proliferation and (0.10, 0.25, 0.50, 1.00, 1.50,

2.00, 2.50 mg/L) auxins (NAA, IBA, IAA) for rooting study. The results demonstrated that shoot and root proliferation were significantly dependent on type and concentration of cytokinins and auxins. The optimum cytokinin concentration for shoot proliferation was 8.0 mg/L BAP with 8.4 shoots per explant in the 5^{th} subculture. Four mg/L BAP is recommended for *in-vitro* shoot proliferation, as 8.0 mg/L BAP had no significant different from the effect on shoot induction. The maximum number of roots, 10.5 and 9.0 per explant was achieved at 2.00 mg/L IBA and 2.00 mg/L IAA, respectively on 30^{th} day of inoculation. Therefore, treatment with either 2.00 mg/L IBA or 2.00 mg/L IAA can be used for *in-vitro* rooting of banana cv. Berangan. Both recommended cytokinin and auxin levels are subjected to *in-vitro* screening and field evaluation to avoid the onset of somaclonal variants. In the second study, rhizome (1.0 x 1.0 x 0.5 cm) was cultured on solid MS media supplemented with various levels (0.5, 2.0, 4.0, 8.0, 16.0 mg/L) of auxins (NAA, PCPA, 2-4,D) to determine their effects on callus induction. PCPA and 2-4,D treatments failed to induce callus. Treatment with 4.0 mg/L NAA produced 5% callus, whereas treatments with 8.0 and 16.0 mg/L both produced 10% callus per replication. Subculturing did not promote callus induction. The follow-up experimental results showed that 12 mg/L NAA induced the maximum percentage of callus (93%) per replication after 2 months culture. However, the results of follow-up experiment must be further evaluated for the type, intensity and regenerability of callus.

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

KESAN SITOKININ DAN AUKSIN TERHADAP PENJANAAN SECARA LANGSUNG DAN TIDAK LANGSUNG UNTUK PISANG (*Musa acuminata* L.) cv. Berangan

Oleh

SIOSI LOLOHEA TUAVAO

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Cabaran utama kepada perusahaan industri pisang di Malaysia adalah serangan penyakit secara berterusan seperti Fusarium wilt (race 4) dan bintik daun Sigatoka. Kaedah pembiakbakaan pisang melalui cara konvensional menghadapi kesukaran disebabkan keadaan steriliti dan paras poliploid yang tinggi, dengan itu kaedah bioteknologi perlu diintergrasikan ke dalam program pembaikan pisang. Kajian ini telah dibahagikan kepada dua bahagian, bagi menghadapi senario yang dinyatakan di atas. Oleh itu protokol penjanaan tanaman secara langsung atau secara tidak langsung diperlukan untuk pembiakan mikro secara *in-vitro* dan untuk tujuan manipulasi genetik. Untuk mengkaji kesan sitokinin dan auksin terhadap penghasilan pucuk dan akar, bahagian pucuk yang telah dipotong yang mengandungi umbisi dan bahagian bawah daun (1.0 cm² bahagian bawah x 1.5 cm) telah dikultur ke dalam media mengandungi nutrien Murashige dan Skoog (1962). Medium modifikasi pepejal berkenaan telah dibekalkan dengan berbagai paras kepekatan sitokinin (0.5,

2.0, 4.0, 8.0, 16.0 mg/L) yang terdiri daripada BAP, Kinetin, Adenine hemilsulphate, untuk penghasilan pucuk dan (0.10, 0.25, 0.50, 1.00, 1.50, 2.00, 2.50 mg/L) auksin (NAA, IBA, IAA) untuk kajian pengakaran. Keputusan kajian menunjukkan penghasilan pucuk dan akar adalah bergantung dengan signifikannya kepada jenis dan kepekatan auksin dan sitokinin. Paras optimum sitokinin untuk penghasilan pucuk adalah 8.0 mg/L BAP dengan penghasilan 8.4 pucuk bagi setiap eksplan pada subkultur ke lima. Rawatan 4.0 mg/L adalah dicadangkan untuk penghasilan pucuk secara *in-vitro*, memandangkan ia tidak menunjukkan perbezaan yang bererti jika dibandingkan dengan paras 8.0 mg/L BAP. Bilangan akar tertinggi, 10.5 dan 9 bagi setiap eksplan telah diperolehi bagi 2.0 mg/L IBA dan 2.0 mg/L IAA secara berturut setelah 30 hari dikultur. Oleh itu, rawatan 2.0 mg/L IBA atau IAA boleh digunakan untuk pengakaran pisang cv. Berangan secara *in-vitro.* Kedua-dua paras sitokinin dan auksin yang dicadangkan adalah tertakluk kepada saringan *in-vitro* dan penilaian ladang bagi mengenalpasti variasi somaklonal. Dalam kajian kedua, umbisi (1.0 x 1.0 x 0.5 cm) telah dikultur ke dalam media MS pepejal yang dibekalkan dengan berbagai paras (0.5, 2.0, 4.0, 8.0, 16.0 mg/L) auksin (NAA, PCPA dan 2,4-D) untuk mengenalpasti kesannya ke atas penghasilan kalus. Rawatan PCPA dan 2,4-D gagal untuk menghasilkan kalus. Subkultur tidak menggalakkan penghasilan kalus. Kajian lanjutan seterusnya menunjukkan NAA (12 mg/L) memberi perbezaan bererti dalam penghasilan jumlah kalus terbanyak (93%) bagi setiap eksplan dalam masa dua bulan dikultur. Keputusan kajian memerlukan pengajian seterusnya untuk mengenalpasti jenis, intensiti dan keupayaan penjanaan kalus.

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A special gratitude to Universiti Putra Malaysia (UPM) for granting admission to further my study in Malaysia. The study environment and laboratory facilities were second to none. I was blessed to pursue my study in UPM at the Department of Agricultural Technology.

I certify that an Examination Committee met on 3rd September 2004 to conduct the final examination of Siosi Lolohea Tuavao on his Master of Agricultural Science thesis entitled "Effects of Cytokinins and Auxins on Direct and Indirect Regeneration of Banana (*Musa acuminata* L.) cv. Berangan" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 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 fulfilment of the requirement for the degree of Master of Agricultural Science. The members of Supervisory Committee are as follows:

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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.

SIOSI LOLOHEA TUAVAO

Date: 26 OCT 2004

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ACIAR	Australian Centre for International Agricultural Research
ANOVA	analysis of variance
2,4-D	2,4-dichlorophenoxyacetic acid
2,4,5-T	2,4-5 trichlorophenoxyacetic acid
2-iP	2-isopentyl adenine

BAP	6-benzylaminopurine
RCBD	Randomized Complete Block Design
cm	centimetre
cv.	cultivar
DNMRT	Duncan's New Multiple Range Test
DNA	Deoxyribonucleic acid
DGT	direct gene transfer
et al.	*et alia*
FHIA	Fundacion Hondurena de Investigacion Agricola
g	gramme
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
INIBAP	International Network for the Improvement of Banana and Plantain
IITA	International Institute of Tropical Agriculture
Kinetin	6-furfurylaminopurine
mg/L	milligramme per litre
mL	millilitre
MS	Murashige and Skoog (1962)
M	molar
mM	millimolar
NAA	Naphthalene acetic acid
pH	- log (H^+)
PCPA	p-Chlorophenoxy acetic acid
PGR	plant growth regulator

PGPR	plant growth promoting rhizobacteria
RM	ringgit
SAS	statistical analysis system
spp.	species
%	percentage
µM	micromolar
µm	micrometer
UPM	Universiti Putra Malaysia
USD	US dollar
var.	variety