UNIVERSITI PUTRA MALAYSIA

IN VITRO GROWTH RESPONSE AND ACCLIMATIZATION PERFORMANCE OF CHITOSAN TREATED HERMAPHRODITE PAPAYA (Carica papaya L. cv. Eksotika)

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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 Science

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IN VITRO GROWTH RESPONSE AND ACCLIMATIZATION PERFORMANCE OF CHITOSAN TREATED HERMAPHRODITE PAPAYA (_Carica papaya_ L. cv. Eksotika)

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In vitro propagation of papaya has been reported to be hindered by slow explant initiation and proliferation as well as the production of abnormal shoots and roots which resulted in low plantlets survival rate during transplantation. Chitosan supplementation to culture media and through foliar application has been reported to give positive effects on *in vitro* growth and *ex vitro* acclimatization of several crop species.

The present study aims at establishing a complete and reliable method of propagation of hermaphrodite papaya (*Carica papaya* L. cv. Eksotika) through the determination of best sterilization procedure, type of explant, medium and plant growth regulators (PGRs) requirements, the application of chitosan to improve *in vitro* shoot and root growth and plantlets performance during *ex vitro* acclimatization. In attempts to minimize the possible variations, a single mother plant was used as source of planting material throughout the study. This is the first report on the application of chitosan on Eksotika papaya.

Explants taken from greenhouse mother plant and treated with 20% NaOCl solution for 20 minutes experienced low percentage of explant contamination (35.20%) with comparatively high rate of explant viability (74.67%). Shoot tip was identified as the suitable planting material with higher percentage of explant viability (73.33%).

Full-MS salt was superior to half-MS in shoot growth and development. The combination of 1.0 mg L^{-1} BAP and 0.1 mg L^{-1} NAA was selected as the basal plant growth regulators for experiment with chitosan application based on its maximum performance on shoot proliferation. The treatment induced shoot after 13 days of culture, resulted in highest rate of proliferation (128.9 shoots per explant) and an average shoot height of 1.96 cm.

Chitosan supplementation had significantly enhanced growth and development of papaya shoot tip explants. Chitosan at 20 mg L^{-1} induced earliest shoot initiation which occurred after seven days of culture. This was six days earlier than control treatment. Treatment with 15 mg L^{-1} chitosan resulted in maximum rate of shoot proliferation (220 shoots per explant). This was approximately two-fold higher than control. Longest shoots (4.18 cm) were obtained on medium supplemented with 5 mg L^{-1} chitosan.

Chitosan application enhanced *in vitro* rooting and acclimatization of papaya. Half-MS was superior to full-MS in papaya rooting procedure. Earliest root induction was observed on half-MS with 1.0 mg L^{-1} IBA and 15 mg L^{-1} chitosan after nine days of culture. Half-MS with 1.0 mg L^{-1} IBA and 5 mg L^{-1} chitosan recorded highest mean number of roots per explant (8.00). Longest roots (7 cm) were produced on half-MS supplemented with 1.0 mg L^{-1} IBA and 5 mg L^{-1} chitosan. Foliar application of chitosan at 30 mg L^{-1} resulted in highest survival percentage (93.33%) and maximum mean difference of shoot growth (11.33 cm) of the resultant plantlets during *ex vitro* acclimatization.

In conclusion, the present study showed that the addition of chitosan had profound effects on *in vitro* growth performance and *ex vitro* acclimatization of papaya explants. It offers the potential use of chitosan to overcome the shortcomings in *in vitro* culture of papaya.

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

PENGARUH DAN KESAN KITOSAN TERHADAP PERTUMBUHAN *IN VITRO* DAN AKLIMATISASI BETIK HERMAFRODIT (*Carica papaya* L. cv. Eksotika)

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Penyelidik menemui beberapa masalah dalam kultur tisu betik. Percambahan dan pembiakan pucuk yang perlahan, penghasilan pucuk dan akar yang tidak normal yang akibatnya merendahkan peratusan hidup plantlet semasa transplantasi telah dilaporkan. Pembekalan kitosan ke dalam medium kultur dan penggunaan secara foliar telah dilaporkan memberi kesan yang positif dalam pertumbuhan *in vitro* eksplan dan aklimatisasi bagi beberapa spesies tanaman.

Kajian ini bertujuan untuk mewujudkan kaedah propagasi yang lengkap dan berkesan bagi betik hermafrodit (*Carica papaya* L. cv. Eksotika) melalui penentuan kaedah pensterilan dan jenis eksplan yang bersesuaian, komposisi medium dan hormon yang diperlukan dan penggunaan kitosan untuk merangsangkan pertumbuhan eksplan semasa pengkulturan *in vitro* dan aklimatisasi plantlet. Eksplan yang digunakan sepanjang kajian diperolehi daripada pokok induk yang sama untuk mengurangkan peluang berlakunya variasi. Ini merupakan laporan yang pertama mengenai pengunaan kitosan ke atas betik Eksotika.

Rawatan pensterilan dengan 20% cecair NaOCl selama 20 minit ke atas eksplan yang diperolehi daripada rumah hijau mencapai peratusan kontaminasi yang rendah (35.20%) bersama dengan peratusan kehidupan eksplan yang tinggi (74.67%). Hujung pucuk merupakan jenis eksplan yang sesuai untuk digunakan dengan peratusan kehidupan yang lebih tinggi (73.33%).

Medium MS sepenuh memberikan prestasi yang lebih unggul berbanding dengan medium MS separuh dalam pertumbuhan pucuk. Kombinasi 1.0 mg L^{-1} BAP dan 0.1 mg L^{-1} NAA dipilih sebagai hormon basal dalam eksperimen yang melibatkan penggunaan kitosan disebabkan prestasi yang unggul dalam kadar pertumbuhan pucuk. Rawatan ini merangsangkan induksi pucuk selepas pengkulturan selama 13 hari, mencapai purata bilangan pucuk per eksplan yang optimum (128.9 pucuk per eksplan) dan purata ketinggian pucuk pada 1.96 cm.

Penggunaan kitosan meningkatkan pertumbuhan dan perkembangan eksplan betik secara ketara. Kepekatan kitosan pada 20 mg L^{-1} merangsangkan induksi pucuk selepas pengkulturan selama tujuh hari. Ini adalah enam hari lebih awal berbanding dengan rawatan kawalan. Rawatan dengan 15 mg L^{-1} kitosan memberikan kadar pembiakan pucuk yang maksimum (220 pucuk per eksplan). Ini adalah kira-kira dua kali ganda lebih tinggi daripada rawatan kawalan. Pucuk yang terpanjang (4.18 cm) diperolehi dalam rawatan yang mengandungi 5 mg L^{-1} kitosan.

Pengakaran *in vitro* betik dan aklimatisasi plantlet telah dirangsangkan melalui penggunaan kitosan. MS separuh adalah lebih unggul daripada MS sepenuh dalam pertumbuhan akar. Pengeluaran akar yang terawal ditemui dalam MS separuh yang dibekalkan dengan 1.0 mg L^{-1} IBA dan 15 mg L^{-1} kitosan selepas pengkulturan selama sembilan hari. Medium MS separuh dengan 1.0 mg L^{-1} IBA dan 5 mg L^{-1} kitosan mencatatkan purata bilangan akar per eksplan yang tertinggi (8.00). Akar yang terpanjang (7 cm) dihasilkan dalam medium MS separuh dengan 1.0 mg L^{-1} IBA dan 5 mg L^{-1} kitosan. Penggunaan kitosan secara foliar dalam 30 mg L^{-1} menghasilkan peratusan kehidupan plantlet yang tertinggi (93.33%) dan purata perbezaan pertumbuhan pucuk yang maksimum (11.33 cm) semasa aklimatisasi.

Kesimpulannya, kajian ini menunjukkan bahawa penambahan kitosan memberikan kesan yang positif terhadap pertumbuhan *in vitro* dan aklimatisasi eksplan betik. Ini memberi potensi penggunaan kitosan untuk menyelesaikan kelemahan yang dihadapi dalam kultur *in vitro* betik.

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I certify that a Thesis Examination Committee has met on 7th November 2014 to conduct the final examination of Lee Sin Yee on her thesis entitled "*In Vitro* Growth Response and Acclimatization Performance of Chitosan Treated Hermaphrodite Papaya (*Carica papaya* L. cv. Eksotika)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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

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This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
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LIST OF ABBREVIATIONS

2,4-D	2,4-dichlorophenoxy acetic acid
2iP	6-γ-γ-dimethylaminopurine
ANOVA	analysis of variance
BA	6-benzyladenine
BAP	6-benzylaminopurine
C_2H_4	ethylene
CaOCl	calcium hypochlorite
cv	cultivar
DNMRT	duncan new multiple range test
et al.	*et alia*
EtOH	ethanol
GlcN	β-1,4-linked glucosamine
H_2O_2	hydrogen peroxide
HCl	hydrogen chloride
$HgCl_2$	mercuric chloride
IAA	1H-indole-3-acetic acid
IBA	1H-indole-3-butyric acid
kinetin	N-2-furanylmethyl-1H-purine-6-amine
MAFC	Malaysia Agrifood Corporation Berhad
MARDI	Malaysian Agricultural Research and Development Institute
MS	Murashige and Skoog

MSO	MS medium without plant growth regulators
mM	millimolar
NAA	1-napthalene acetic-acid
NaOCl	sodium hypochlorite
NaOH	sodium hydroxide
PGR	plant growth regulator
pH	$- \log [H^+]$
PLBs	protocorm-like-bodies
RAPD	random amplified polymorphic DNA
RCBD	randomized complete block design
SAS	statistical analysis system
TDZ	thidiazuron
UPM	Universiti Putra Malaysia
UV	ultraviolet
v/v	volume per volume
zeatin	6-4-hydroxy-3-methyl-trans-2-butenylaminopurine
$\mu mol\ m^{-2}\ s^{-1}$	micromole per meter square per second
α	level of significance

CHAPTER 1

INTRODUCTION

1.1 Background of Papaya

Papaya (*Carica papaya* L.) belongs to the family *Caricaceae*, said to be native to tropical America and is widely distributed throughout tropical and sub-tropical regions of the world (Krishna *et al*., 2008). Papaya is an important fruit crop grown for fresh fruit and other processed products. Global export volume of fruits has achieved tremendous increase with Mexico, Brazil, Belize, Malaysia and India as major exporters (FAOSTAT, 2012).

One of the most popular cultivars in Malaysia is Eksotika papaya, released by the Malaysian Agricultural Research and Development Institute (MARDI) in 1987 from a backcross breeding of Hawaiian Sunrise Solo and Subang 6 (Chan, 1987). The cultivar is well-known for its sweetness and firm fruit texture and size with convenience in packaging as well as storage attributes.

1.2 Conventional Propagation and *In Vitro* Culture of Papaya

Conventionally, papaya is propagated from seeds, as well as by grafting or use of cuttings. Seed germination often results in high degree of genotypic and phenotypic variations due to cross-pollination nature of plant (Agnithori *et al*., 2004). Seed germination often involved sowing of multiple seeds per planting point, which later, thinned out to a single hermaphrodite plant once the sex of each plant is identified at flowering. A hermaphrodite plant is preferable for its commercially superior fruit quality and uniformity (Hsu *et al*., 2012). Thus, the practice of seed propagation has been considered commercially as uneconomical in terms of time consumption, labour and planting materials requirements. Propagation by grafting or use of cuttings is considered impractical due to severe apical dominance which restricts shoot proliferation (Yeh and Fitch, 2009).

Tissue culture, or *in vitro* culture, offers one pathway to propagate true-to-type, homogenous papaya plants in large scale. However, several researchers have reported several problems in the procedure. Slow initiation and proliferation due to apical dominance nature of papaya (Panjaitan *et al*., 2007) and the production of abnormal shoots and roots in prolonged culture of explants have been widely published. This has resulted in low survival rate during transplantation (Yu *et al*., 2000; Agnithori *et al*., 2004).

1.3 Chitosan Application in Agriculture

In attempts to overcome problems encountered in tissue culture propagation of several crop plants, culture media formulations have been manipulated and optimized. Chitosan supplementation to culture media has been reported to give profound effects on *in vitro* growth and development of several crop species including oil palm (Kanchanapoom *et al*., 2010), orchid (Pornpienpakdee *et al*., 2010; Sopalun *et al*., 2010) and potato (Asghari-Zakaria *et al*., 2009). Chitosan, a natural and biodegradable polysaccharide polymer obtained from exoskeletons of crustaceans, has been widely used in agriculture as plant growth enhancer to improve plant growth and quality as well as improvement in resistance to various pests and diseases (Boonlertnirun *et al*., 2008). Kanchanapoom *et al*. (2010) observed that chitosan supplementation into culture medium triggered earlier organogenesis of oil palm (*Elaeis guineensis* Jacq. var. *tenera*) callus. Limpanavech *et al*. (2008) reported that the addition of 10 mg L^{-1} chitosan into culture medium increased the proliferation rate of protocorm-like-bodies (PLBs) of *Dendrobium* orchid. Pornpienpakdee *et al*. (2010) observed that resulted plantlets of *Dendrobium* 'Eiskul' treated with chitosan achieved 100% survival rate of during transplantation.

1.4 Justification and Objectives

In vitro culture of papaya is hindered by several problems whereas chitosan was proven to improve *in vitro* growth performance and acclimatization of several crops. Therefore, the present study investigates *in vitro* growth performance of *Carica papaya* L. cv. Eksotika plantlets cultured on media supplemented with varying amounts of chitosan and during *ex vitro* acclimatization through foliar application of chitosan. Although there are several publications on *in vitro* culture of papaya, there has been no report on the application of chitosan in the procedure. In justification, the study offers the potential use of chitosan in improving growth performance of papaya plantlets while in culture and during acclimatization condition.

The main objectives include:

1. to formulate an effective explant sterilization protocol and identify the suitable type of explant for *in vitro* propagation of papaya;
2. to determine the appropriate medium and plant growth regulators (PGRs) requirements, and their interactions for optimum shoots growth and development;
3. to observe *in vitro* growth performance of shoots and roots treated with chitosan;
4. to observe growth performance of resultant plantlets while in *ex vitro* acclimatization through foliar application of chitosan.

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