



UNIVERSITI PUTRA MALAYSIA

**EFFECTS OF SELECTED PLANT GROWTH REGULATORS ON
REGENERATION OF MANGOSTEEN (GARCINIA MANGOSTANA L.)
USING TISSUE CULTURE TECHNIQUE**

MOHAMMAD HOSSEIN TORABI SIRCHI

FP 2009 6

In The Name of Allah, the Most Gracious and the Most Merciful

Specially Dedicated

To

My heaven dwelling father:

Mohammad Torabi Sirchi



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

**EFFECTS OF SELECTED PLANT GROWTH REGULATORS ON
REGENERATION OF MANGOSTEEN (*GARCINIA MANGOSTANA* L.)
USING TISSUE CULTURE TECHNIQUE**

By

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May 2009

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The *in-vitro* plantlet regeneration system via organogenesis and callus induction was developed from various mangosteen explants (seed, shoot tip, stem and leaf) using various plant growth regulators (PGR). For shoot induction, the mangosteen half seed, leaf and stem explants sufficiently achieved shoot regeneration rates above 60% of total explants cultured (70%, 60% and 60% respectively) on various auxin and cytokinin supplementation into the basal MS medium [4.0 mgL⁻¹ (w/v) BAP with 0.2 mgL⁻¹ (w/v) NAA for seed; 1.0 mgL⁻¹ (w/v) BAP in combination with 0.05 mgL⁻¹ (w/v) KIN for stem; 2.0 mgL⁻¹ (w/v) of BAP for leaf]. Shoot induction from the shoot tip explant was not successful. However,



among the three explants, the leaf explant gave the highest mean number of shoots at 46.5 (seed and stem at 9.3 and 8.9 respectively). Mean shoot height however, was 0.48 cm for leaf, while it was higher for shoots generated from stem and seed explants (0.7 cm and 0.8 cm respectively). The shoots rooted successfully on full strength MS medium without IBA with high percentage of root formation (87%) and number of roots (3.8) (seven months after culture). The plantlets were then successfully potted and acclimatized on medium containing soil + sand+ organic matter+ vermiculite mixture (2:2:1:1) which gave the highest number of leaves (6.5), plant height (3.5cm) and percentage of plant survival (80%). As for callus induction, induction from seed and leaves was 90% on medium with 0.5 mgL⁻¹ (w/v) NAA, while induction from shoot tip and stem was 90% and 83% respectively on medium with 0.2 mgL⁻¹ (w/v) KIN. Our study has successfully developed a plant regeneration system from easily available stem and green juvenile leaf explants, as compared to the seasonally available and limited seed and young leaf explants that are generally reported by other researches. Furthermore, callus induction was successful from various mangosteen explants for future utilization in the development of a plant regeneration protocol from callus, as callus also has the potential of producing improved varieties via various genetic modification techniques today.



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

**KESAN HORMON PENGAWAL ATUR PERTUMBUHAN POKOK
TERPILIH KE ATAS REGENERASI MANGGIS (*Garcinia mangostana* L.)
DENGAN MENGGUNAKAN TEKNIK KULTUR TISU**

Oleh

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April 2009

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Sistem regenerasi anak pokok *in-vitro* melalui organogenesis dan pembentukan kalus telah berjaya dihasilkan dari pelbagai eksplan manggis (biji benih, pucuk, batang dan daun hijau) di bawah pengaruh pelbagai kombinasi hormon pengawal atur pertumbuhan pokok (PGR). Kajian regenerasi pucuk baru dari eksplan biji, daun dan batang telah mencapai kadar regenerasi yang memuaskan iaitu sebanyak 60% dari jumlah eksplan yang dikulturkan (70%, 60% dan 60% masing-masing) dalam medium asas MS yang mengandungi pelbagai kombinasi auksin dan sitokinin [4.0 mgL⁻¹ (w/v) BAP dengan kombinasi 0.2 mgL⁻¹ (w/v) NAA untuk eksplan biji; 1.0 mgL⁻¹ (w/v) BAP dengan kombinasi 0.05 mgL⁻¹ (w/v) KIN



untuk eksplan batang; 2 mgL⁻¹ (w/v) BAP untuk eksplan daun]. induksi pucuk dari eksplan pucuk manggis tidak berjaya. Namun begitu, di antara tiga eksplan yang berjaya menghasilkan regenerasi tersebut, regenerasi dari eksplan daun menghasilkan min bilangan pucuk yang tertinggi iaitu 46.5 pucuk (eksplan biji dan batang masing-masing hasilkan sebanyak 9.3 dan 8.9 bilangan pucuk). Min tinggi pucuk yang terbentuk dari eksplan daun mencapai 0.48 cm, manakala min tinggi pucuk dari eksplan batang dan biji benih adalah lebih tinggi (0.7 cm and 0.8 cm masing-masing). Seterusnya, pucuk-pucuk *in-vitro* tersebut berjaya melengkapkan pembentukan akar dalam medium MS pada kekuatan penuh tanpa pengaruh hormon IBA, dan mencatatkan peratus pembentukan akar (87%) serta bilangan akar (3.8) yang tinggi (selepas tujuh bulan pengkulturan). Berikutan pembentukan anak benih (lengkap dengan pucuk dan akar), anak-anak benih berjaya dipasukan dan diaklimatasi dalam medium yang mengandungi campuran tanah + pasir + bahan organik + vermiculite (pada kadar 2: 2: 1: 1), dengan menghasilkan pembentukan min bilangan daun (6.5), min tinggi pokok (3.5cm) dan peratus kehidupan pokok (80%). Kajian pembentukan kalus pula mencatatkan peratus pembentukan sebanyak 90% dari eksplan biji dan daun dalam medium yang mengandungi 0.5 mgL⁻¹ (w/v) NAA, manakala eksplan pucuk dan batang mencatatkan 90% dan 83% masing-masing dalam medium yang mengandungi 0.2 mgL⁻¹ (w/v) KIN. Kesimpulannya, kajian ini telah berjaya menghasilkan protokol regenerasi pokok dengan menggunakan pelbagai organ



pokok manggis yang sedia ada dan mudah didapati seperti batang dan daun hijau, juvena, berbanding dengan penggunaan biji benih bermusim dan daun merah muda yang terhad atau bermusim, sepertimana yang diamalkan oleh kebanyakan penyelidik. Tambahan pula, kejayaan pembentukan kalus dari pelbagai eksplan manggis ini merupakan langkah pertama penjanaan kajian regenerasi pokok dari kalus, kerana protokol ini berpotensi diterapkan dalam penghasilan baka-baka manggis yang lebih berkualiti melalui pelbagai teknik modifikasi genetik.

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I certify that a Thesis Examination Committee has met on 26 May 2009 to conduct the final examination of Mohammad Hossein Torabi Sirchi on his thesis entitled "Effects of Selected Plant Growth Regulators on Regeneration of Mangosteen (*Garcinia mangostana* L.) Using Tissue Culture Technique" 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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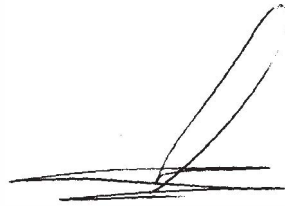


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DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or any other institutions.



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TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGMENT	viii
APPROVAL	ix
DECLARATION	xi
LIST OF TABLES	xvi
LIST OF FIGURES	xvii
LIST OF PLATES	xx
LIST OF ABBREVIATIONS	xxi
 CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	4
2.1 Botany and production of mangosteen	4
2.1.1 Sexual system	7
2.1.2 Seed	7
2.1.3 Variation	8
2.2 Propagation, Cultivation and Harvesting	9
2.2.1 <i>In-vitro</i> system	10
2.3 Organogenesis	11
2.3.1 Types of organogenesis	12
2.4 Micropropagation Stages	15
2.4.1 Establishment of cultures	16
2.4.2 Shoot multiplication	17
2.4.3 Rooting	18
2.4.4 Acclimatization to <i>ex-vitro</i> conditions	20



3	SHOOT REGENERATION OF <i>GARCINIA MANGOSTANA</i> L.	22
3.1	Introduction	22
3.2	Material and Methods	23
3.2.1	Effect of low concentrations of BAP and NAA on shoot regeneration and callus formation from mangosteen half seed explants	23
3.2.2	Effect of high concentrations of BAP and NAA on shoot regeneration and callus formation from half seed explants	28
3.2.3	Effect of BAP and KIN concentration on shoot height and callus formation from shoot tip explants	30
3.2.4	Effect of BAP and KIN concentrations on shoot regeneration and callus formation from mangosteen stem explant	33
3.2.5	Effect of BAP and NAA concentrations on shoot induction and callus formation from mangosteen leaf explant	36
3.3	Results and Discussions	39
3.3.1	Effect of low concentrations of BAP in combination with NAA on shoot regeneration from mangosteen half seed explant	39
3.3.2	Effect of high concentrations of BAP in combination with NAA on shoot regeneration from mangosteen half seed explant	47
3.3.3	Effect of BAP and KIN concentrations on shoot height and callus formation from shoot tip explant	55
3.3.4	Effect of BAP and KIN concentrations on shoot induction and callus formation from stem explant	61
3.3.5	Effect of BAP and NAA concentrations on shoot induction and callus formation from leaf explants	69
3.4	Summary	76
3.4.1	Effect of BAP and NAA concentrations on shoot induction and callus formation from mangosteen seed explant	76
3.4.2	Effect of BAP and KIN concentrations on shoot height and callus formation from shoot tip explant	77
3.4.3	Effect of BAP and KIN concentrations on shoot induction and callus formation from stem explants	77
3.4.4	Effect of BAP and NAA concentrations on shoot induction and callus formation from leaf explant	78

4	THE EFFECT OF DIFFERENT IBA CONCENTRATIONS AND MS SALT STRENGTHS ON <i>IN VITRO</i> GROWN MANGOSTEEN PLANTLETS	79
4.1	Introduction	79
4.2	The effect of different IBA concentrations and MS salt strengths on <i>in vitro</i> rooting of mangosteen shoots	80
4.2.1	Materials and Methods	80
4.2.2	Explant material	80
4.2.3	Culture medium and condition	80
4.2.4	Treatment	80
4.2.5	Parameters taken	81
4.2.6	Experimental design and statistical analysis	82
4.3	Result and Discussion	82
4.3.1	Effect of different IBA concentrations and MS Salt strengths on rooting	82
4.4	Summary	89
4.4.1	Effect of different IBA concentrations and MS salt strengths on rooting	89
5	THE EFFECT OF DIFFERENT POTTING MEDIA ON ACCLIMATIZATION TO <i>EX-VITRO</i> CONDITIONS	90
5.1	Introduction	90
5.2	Materials and Methods	91
5.2.1	Explant material	91
5.2.2	Culture Medium and Condition	91
5.2.3	Treatment	92
5.2.4	Parameters taken	92
5.2.5	Experimental design and statistical analysis	92
5.3	Result and Discussion	93
5.3.1	Effect of different potting medium on acclimatization of mangosteen plantlets	93
5.4	Summary	99
5.4.1	Effect of different potting mixture on acclimatization	99
6	SUMMARY, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDIES	100
	REFERENCES	102
	APPENDICES	109



BIODATA OF STUDENT
LIST OF PUBLICATIONS

1453
1464



LIST OF TABLES

Table		Page
1	The combination of BAP and NAA (low concentrations) for shoot induction and callus formation from mangosteen half seed explant.	26
2	The combination of BAP and NAA (high concentrations) for shoot induction and callus formation from mangosteen half seed explant.	29
3	The combination of BAP and KIN concentrations for induction of shoots on shoot-tip of mangosteen.	32
4	The combination of BAP and KIN concentrations for induction of shoots on mangosteen stem explant.	35
5	The combination of BAP and NAA concentrations for shoot regeneration on leaf explants.	38
6	The combination of IBA and MS salt strengths used on mangosteen root induction.	81
7	Mean number of root formation on different MS salt strength and IBA concentrations after seven months of culture	85
8	Percentage of root formation (%) on different MS salt strength and IBA concentrations after seven months of culture	86



LIST OF FIGURES

Figure	Page
1. Effect of BAP in combination with NAA (low concentration) on percentage of shoot formation per half seed explant after 12 weeks of culture.	44
2. Effect of different combinations of BAP and NAA (low concentration) on mean shoot height after 12 weeks of culture	45
3. Effect of different combinations of BAP and NAA (low concentrations) on percentage of callus formation from half seed explant after 12 weeks of culture.	46
4. Effect of BAP in combination with NAA (high concentrations) on percentage of shoot per half seeds after 12 weeks of culture.	51
5. Effect of BAP in combination with NAA (high concentrations) on mean number of shoots produced per half seed explant after 12 weeks of culture.	52
6. Effect of BAP in combination with NAA (high concentrations) on mean shoot height (cm) of half seeds attained after 12 weeks of culture.	53
7. Effect of BAP in combination with NAA (high concentrations) on percentage of half seeds that responded to callus formation after 12 weeks of culture.	54
8. Effect of BAP in combination with KIN on mean shoot height (cm) after 12 weeks of culture of mangosteen shoot tip explants.	59
9. Effect of BAP in combination with KIN on percentage of shoot tip explant that responded to form callus (%) after 12 weeks of culture.	60
10. Effect of BAP in combination with KIN on percentage of stem explants producing shoots after 12 weeks of culture.	65



11. Effect of BAP in combination with KIN on mean number of shoots produced per stem explant after 12 weeks of culture.	66
12. . Effect of BAP in combination with KIN on mean shoot height (mm) attained after 12 weeks of stem culture.	67
13. Effect of BAP in combination with KIN explant that responded to form callus (%) after 12 weeks of stem culture.	68
14. Effect of BAP either alone or in combination with NAA on mean number of shoots per leaf explant after 12 weeks of culture.	72
15. Effect of BAP either alone or in combination with NAA on percentage of leaf explants producing shoots after 12 weeks of culture	73
16. Effect of BAP either alone or in combination with NAA on mean shoot height (cm) attained after 12 weeks of culture on mangosteen leaf explant.	74
17. Effect of BAP either alone or in combination with NAA on percentage of leaf explant that respond to form callus	75
18. Percentage of root formation (%) on different IBA concentrations after 7 months of culture	83
19. Percentage of root formation (%) with different MS salt strength after 7 months of culture.	84
20. Mean number of root formation on different IBA concentrations after 7 months of culture.	84
21. Mean number of root formation on different MS salt strength after 7 months of culture.	85
22. Effect of potting mixture on number of leaves developed per plant and plant height (cm) after 12 weeks of acclimatization	96



23. Percentage of plants survival after 12 weeks of acclimatization

97



LIST OF PLATES

Plate		Page
1	Effect of BAP in combination with NAA (low concentration) on mangosteen half seed explant.	43
2	Effect of BAP either alone or in combination with NAA (high concentrations) on mangosteen half seed explant.	50
3	Effect of BAP and KIN either alone or in combination on mangosteen shoot tip explant	58
4	Effect of BAP either alone or in combination with KIN on mangosteen stems explant.	64
5	Effect of BAP either alone or in combination with NAA on leaf explant.	71
6	Effect of IBA on rooting of mangosteen shoots (A) Effect of full MS salt strength medium without IBA on root formation	87
7	Effect of different potting medium on <i>ex vitro</i> establishment and acclimatization of mangosteen plantlets	95



LIST OF ABBREVIATION/NOTATIONS

ANOVA	Analysis of Variance
BAP	6-Benzylaminopurine
cm	centimeter
°	Centigrade Degrees
2, 4-D	2, 4-Dichlorophenoxyacetic acid
DNMRT	Duncan's New Multiple Range Test
g	Gram
H	Hour (s)
½	half
¼	Quarter
IAA	Indol-3-Acetic acid
IBA	Indole-3-Butyric acid
KIN	Kinetin, (6-furfurylaminopurine)



MS	Murashige and Skoog
NAA	α -Naphthalene Acetic Acid
%	Percentage
PGR	Plant Growth Regulator
pH	$-\log(H^+)$
L	liter
M	Molar
μ M	micromolar, 10^{-3} mM
mg	milligram
ml	milliliter



CHAPTER 1

INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) with high promising economic value is one of the tropical fruit trees belonging to the family of *Guttiferae*, order of *Malpighiales*, and class of *Magnoliophyta* (Almeyda and Martin, 1976; Felipe and Dela, 2001; Verheij and Coronel, 1991). The sweet and creamy fruits are popularly consumed fresh, and used as a source for candy, nut, preserve production and topping for ice-cream and sherbets. The leaves, rind and bark of the tree are used as herbal remedies for thrush, dysentery, diarrhea, cystitis, gonorrhoea, eczema and as wound astringents. Root decoctions are also used for regulation of the female menstruation cycle. Fruit rinds contain tannins used for tanning leather and as natural black dye. The wood is used to manufacture spear handles, rice pounders, wooden shelter and cabinets (Felipe and Dela, 2001; Suci *et al.*, 2007). In 1997, Malaysia exported 1,802 tonnes of fruits (mostly to Singapore and Hong Kong) valued at RM 1.7 million which continued to increase to about 2,447 tonnes and valued at RM4.7 million by the year 2006 (FAMA, 2007; Suci *et al.*, 2007). However, there is a need for greater production potential as mangosteen trees are very slow in growth, taking 10-12 years before fruiting

(Felipe and Dela, 2001; Normah *et al.*, 1995). Furthermore, the fruits are produced seasonally and each fruit normally contains two seeds which are recalcitrant, meaning they are sensitive to desiccation (Normah *et al.*, 1995; Schmidt, 2000). Recalcitrant seeds have low vitality and short viability (Normah *et al.*, 1995; Suci *et al.*, 2007). Generally, the planting materials which were used by farmers were from seedlings or conventional vegetative propagation which are slow growing and non homogenous (Felipe and Dela, 2001; Normah *et al.*, 1995; Te-Chato and Aengyong, 1988). Plant regeneration through tissue culture is an alternative approach for mass propagation of mangosteen planting materials (Felipe and Dela, 2001; Normah *et al.*, 1995; Te-Chato and Aengyong, 1988; Te-Chato and Lim, 1999). Tissue culture as a new technology has been used for propagating various horticultural and forest species successfully (Pierik, 1987). Large number of planting materials can be produced by this technique within a short period of time (Pierik, 1987; Razdan, 2005). Woody plants are slow growing and with poor root induction (Almeyda and Martin, 1976; Meera *et al.*, 2006). Rooting is still the main problem that has not been satisfactorily overcome in mangosteen (Normah *et al.*, 1995; Te-Chato and Aengyong, 1988; Te-Chato *et al.*, 1992). Some researchers have reported on the low success of regeneration and rooting in mangosteen (Felipe and Dela, 2001; Normah *et al.*, 1995; Te-Chato and Aengyong, 1988; Te-Chato *et al.*, 1992). Due to this, more studies on the regeneration and rooting of mangosteen need to be conducted (Normah *et al.*,

1995). In Malaysia, several papers have reported on regeneration of mangosteen using seeds (Normah *et al.*, 1992, Rashid *et al.*, 1995). This study was carried out with the following objectives: (1) to determine the most suitable concentration and combination of plant growth regulators for *in vitro* shoot regeneration and callus production from seed segment, (2) to determine the most suitable concentration and combination of plant growth regulators for *in vitro* shoot regeneration and callus production from shoot tip and stem, (3) to determine the most suitable concentration and combination of plant growth regulators for *in vitro* shoot regeneration and callus production from leaf segments, (4) to determine the most suitable medium salt strength and IBA concentrations for rooting of *in vitro* shoots, and finally (5) to determine the most suitable potting medium for plantlet performance during acclimatization.

