

UNIVERSITI PUTRA MALAYSIA

MICROPROPAGATION OF MANGOSTEEN (GARCINIA MANGOSTANA L.) BY DIRECT AND INDIRECT ORGANOGENESIS

SUCI RAHAYU

FP 2007 3



MICROPROPAGATION OF MANGOSTEEN (GARCINIA MANGOSTANA L.) BY DIRECT AND INDIRECT ORGANOGENESIS

SUCI RAHAYU

MASTER OF SCIENCE UNIVERSITI PUTRA MALAYSIA

2007



MICROPROPAGATION OF MANGOSTEEN (GARCINIA MANGOSTANA L.) BY DIRECT AND INDIRECT ORGANOGENESIS

By

SUCI RAHAYU

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

April 2007



DEDICATION

I dedicate this thesis to my father, Gito Soewarno and my mother, Subariah.



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

MICROPOPAGATION OF MANGOSTEEN (Garcinia mangostana L.) BY DIRECT AND INDIRECT ORGANOGENESIS

By

SUCI RAHAYU

April 2007

Chairman : Associate Professor Mihdzar Abdul Kadir, PhD

Faculty : Agriculture

The mangosteen (Garcinia mangostana L.) still remains an under exploited tropical fruit species that has potential to be developed for local consumption and export commodity. The problem for large scale planting of this plant is not having enough planting materials because the conventional vegetative propagation method has low percentage of success. One of the alternative approaches for mass propagation of mangosteen is through tissue culture. The advantages of propagation through tissue culture are mass propagation can be achieved in a short of time period, plantlet can be produced all year round, and require less space of the plant in the laboratory compare with to the field. The result of the study on *in vitro* propagation of mangosteen in terms of production of adventitious shoots through direct organogenesis and indirect organogenesis in term of callus induction was conducted at the laboratory of Faculty of

UPM

Agriculture, Universiti Putra Malaysia. It was observed that among 24 initiation media tested, 16 mg/l BAP gave the highest number of shoots (7.00 shoot/explant), followed by 8 mg/l BAP (6.3 shoots/explant) after eighth weeks of culture. In general, the supplement of AdS in media did not significantly influence the number of shoots produced.

There was negative correlation between the height of shoot and the number of shoot produced. The highest number of shoots was recorded on media with 1 mg/l and 2 mg/l BAP, while the shortest shoot was on media of 16 mg/l and 8 mg/l BAP. Contrary to the height of shoot, there was positive correlation between the weight of shoots and the number of shoots. Treatment with 16 mg/l and 8 mg/l BAP gave the highest weight of shoots produced per seed explant.

During subculture, treatment with BAP 2 mg/l gave the highest number of shoots at every occasion of subculture (39.56 shoots per explant), followed by 1 mg/l BAP (27.71 shoots per explant). Height of shoots on all media of subculture did not significantly differ. Combination of 1 mg/l BAP and 2 mg/l Kinetin was the most suitable media for elongation of the shoot (1.06 cm).

Among the rooting media, ½ MS + 15 mg/l NAA + 0.05% Charcoal + 10 g/l sucrose was most suitable for the emergence of roots with 3.4 roots per shoot). While ½ MS + 1 mg/l IAA + 0.05% Charcoal + 10 mg/l sucrose seemed to be most suitable media for lengthening the roots. The average length of root on this



media was 6.85 cm per shoot. Plantlets from rooting experiments acclimatized on the media of soil + sand + peat (2 : 1: 1) have grown well. 100% of them could survive under misting chamber condition for eight weeks.

In study on organogenesis through callus induction, all media with the exception of MS, BAP alone and 2,4-D alone could produce calli.

It is expected that this findings can contribute to the knowledge of science, particularly on *in vitro* propagation of mangosteen, and can be used as a reference for other students and researchers as well as agricultural industries in developing mass propagation of mangosteen.



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

PEMBIAKAN MIKRO MANGGIS (Garcinia mangostana L.) MELALUI ORGANOGENESIS SECARA LANGSUNG DAN TAK LANGSUNG

Oleh

SUCI RAHAYU

April 2007

Pengerusi: Profesor Madya Mihdzar Abdul Kadir, PhD

Fakulti

: Pertanian

Manggis (Garcinia mangostana) merupakan antara spesies buah tropika yang

belum diterokai, meskipun ianya mempunyai potensi untuk dikembangkan sama

ada untuk pemakanan tempatan mahupun untuk komoditi eksport. Permasalahan

yang berlaku adalah tidak cukupnya anak pokok untuk penanaman skala besar.

Pembiakan vegetatif secara biasa mempunyai peratus kejayaan yang rendah.

Salah satu pilihan untuk pembiakan secara besar-besaran ialah dengan kultur

tisu. Keuntungan pengembang biakan dengan kultur tisu adalah pembiakan

secara besar-besaran dapat diperolehi dalam waktu yang lebih pendek, produksi

anak pokok dapat diperolehi sepanjang tahun dan jimat ruang penyimpanan

dalam makmal berbanding penyimpanan anak pokok di lapang. Hasil kajian dari

penyelidikan kultur tisu dengan tajuk Pembiakan Tampang Manggis Melalui

Organogenesis Secara Langsung dan Tidak langsung (Induksi Kalus) adalah

vi

sebagai berikut: Diantara 24 media inisasi yang dikaji, 16 mg/l BAP memberikan jumlah tunas yang paling banyak, iaitu 7.00 tunas tiap eksplan, diikuti oleh 8 mg/l BAP yang menghasilkan 6.3 tunas selepas lapan minggu penanaman di media. Secara amnya, pencampuran AdS ke dalam media tidak mempunyai kesan yang nyata terhadap jumlah tunas yang dihasilkan.

Terdapat hubung kait secara negatif antara tinggi tunas dan jumlah tunas yang dihasilkan. Tunas paling tinggi diperolehi pada media 1 mg/l and 2 mg/l BAP, sementara tunas paling pendek pada media 16 mg/l and 8 mg/l BAP. Kebalikan dari tinggi tunas, terdapat hubungan yang positif antara berat tunas dan jumlah tunas, dengan demikian media 16 mg/l and 8 mg/l BAP memberikan berat tunas yang tertinggi.

Selama subkultur, 2 mg/l BAP selalunya memberikan jumlah tunas yang paling banyak pada setiap subkultur (39.56 tunas per eksplan), diikuti oleh 1 mg/l BAP (27.71 tunas per eksplan). Secara am, tinggi tunas pada semua media subkultur tidak ada perbezaan yang bererti. Gabungan antara 1 mg/l BAP dan 2 mg/l Kinetin adalah merupakan media yang paling pantas untuk pemanjangan tunas (1.06 cm).

Media ½ MS + 15 mg/l NAA + 0.05% 'Charcoal' + 10 g/l 'sucrose' merupakan antara media yang paling sesuai untuk pemunculan akar, di mana jumlah akar yang tumbuh pada media ini mencapai 3.4 per tunas. Sedangkan media ½ MS +



1 mg/l IAA + 0.05% 'Charcoal' + 10 g/l 'sucrose' didapati paling sesuai untuk pemanjangan akar. Purata panjang akar pada media ini adalah 6.85 cm per tunas. Eksplan-eksplan dari pada kajian perakaran yang diaklimatisasi pada media tanah dicampur pasir dan gambut dengan nisbah 2 : 1: 1 dapat tumbuh dengan baik. 100% plantlet hidup pada kebuk kalbus (misting chamber) selama 8 minggu.

Dalam kajian organogenesis melalui induksi kalus, semua media kecuali MS, BAP sahaja dan 2,4-D sahaja dapat menghasilkan kalus.

Diharapkan, hasil daripada kajian ini dapat mempertingkatkan ilmu pengetahuan sains, khasnya pembiakan dengan kultur tisu, dan dapat diguna pakai sebagai rujukan sama ada oleh pelajar, penyelidik, mahupun pihak industri pertanian dalam penghasilan manggis secara besar-besaran.



ACKNOWLEDGEMENTS

First, and most importantly, I am obligated to express gratitude to Allah, Most Merciful, for the blessing me granted onto me to execute and complete the study. I am very indebted to those who have helped me in preparing and conducting the study. I wish to express my deepest gratitude.

I would like to thank the Chairman of the Supervisory Committee, Associate Professor Mihdzar Abdul Kadir, PhD for his guidance and advice in the preparation of project proposal, implementation of research, presentation of seminars and completion of the thesis. I appreciate his patience and sincere approach to motivate, help, advise and guide me to finish my study at the Universiti Putra Malaysia. Thanks also are extended to my Committee members, Associate Professor Maheran Abdul Aziz, PhD in particular for her advice, comments, guidance, critical discussion and encouragement in the completion of this thesis and to Mr. Azmi Abdul Rashid who supported me by his encouragement and by the stimulating discussions held with me. They have given me the experience and knowledge, resulting in significant and positive impact on my work.

I am also deeply grateful to the Head of the Agency for Agricultural Research and Development, Department of Agriculture, Indonesia for awarding the scholarship that enables me to pursue a Master program at Universiti Putra



Malaysia, and to the Director of the Indonesia Centre Agricultural Agency for Biotechnology and Genetic Resources Development, Bogor, Indonesia for granting the study leave.

My appreciation goes to all staff of the Tissue Culture Laboratory, Department of Agriculture Technology, Universiti Putra Malaysia: Encik Rozaidi, Encik Ahmad Nazri, Cik Rostina Rolon, Cik Suharni, and Cik Ramisah for their help and assistance while conducting the study. I also wish to extend my gratefulness to Encik Rustam for his kind help to get the fresh-mangosteen fruits as study materials at the Agriculture Training Center in Kalumpang, Selangor - Darul Ehsan, Malaysia. I am indebted to Encik Zainuri, head the Department of Agriculture in Serdang, Selangor-Malaysia.for his help to get information on mangosteen in Malaysia. I am also indebted to Encik Mohammad Yusof Hashim, head of Fruits Division, Department of Agriculture in Kuala Lumpur, Malaysia.for the interest and kindness extended to me by asking Encik Anwar and Encik Amir Hamzah, staff of the Agricultural Department in Rembau, Negeri Sembilan - Darul Khusus and Agriculture Training Center in Kalumpang, Selangor - Darul Ehsan, Malaysia respectively, to provide me with freshmangosteen fruits as study material from their orchards. Without their assistance I would have difficulties conducting the experiments.



My special thanks are addressed to my housemates, Rozliza Ismail, Wan Rozidayani Wan Mohammad, Uswati Mohammad Ghazali, Norhazlina Nodin and Siti Khairani Khalid for sharing the joy and sorrow during my stay in Malaysia.

A special note of thanks also to Ibu Dr. Ir. Ika Mariska for her valuable encouragement and attention. Last but not least, I would like to express my deepest thanks to my father, Gito Soewarno and mother, Subariah for their support, encouragement and endless prayers. To my brothers, Teguh Setiono and Kukuh Setiyadi and my Sister, Sari Hening Andajani, thanks for your prayers and moral support. May God bless us all.



I certify that an Examination Committee has met on 25 April 2007 to conduct the final examination of Suci Rahayu on her Master of Science entitled "Micropropagation of Mangosteen (*Garcinia mangostana* L.) by Direct and Indirect Organogenesis" in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and Universiti Putra Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Associate Professor Halimi Mohd Saud, PhD

Faculty of Agriculture Universiti Putra Malaysia (Chairperson)

Associate Professor Saleh Kadzimin, PhD

Faculty of Agriculture Universiti Putra Malaysia (Internal Examiner)

Associate Professor Datin Siti Nor Akmar Abdullah, PhD

Faculty of Agriculture Universiti Putra Malaysia (Internal Examiner)

Ahmad Tarmidzi Hashim, PhD

Malaysian Oil Palm Board (External Examiner)

HASANAH MOHD. GHAZALI, PhD

Professor/Deputy Dean School of Graduate Studies University Putra Malaysia

Date: 3 AUGUST 2007



This thesis submitted to 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 are as follows:

Mihdzar Abdul Kadir, PhD

Associate Professor Faculty of Agriculture Universiti Putra Malaysia (Chairman)

Maheran Abdul Aziz, PhD

Associate Professor Faculty of Agriculture Universiti Putra Malaysia (Member)

Azmi Abdul Rashid, M.Phil

Lecturer Faculty of Agriculture Universiti Putra Malaysia (Member)

AINI IDERIS, Ph.D

Professor/Dean School of Graduate Studies Universiti Putra Malaysia

Date: 9 AUGUST 2007



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.

SUCI RAHAYU

Date: 25 JUNE 2007



TABLE OF CONTENTS

				Page
DF	DICAT	ION		ii
	STRAC			iii
	STRAK			vi
		LEDGEN	MENTS	ix
	PROVA			xii
	CLARA			xiv
		ABLES		XVII
		IGURES		XX
		PLATES		xxii
		PPENDI	CES	xxiii
			ATIONS/NOTATIONS	xxix
	71 01 1			АМА
СН	APTE	R		
1	GEN	ERAL IN	TRODUCTION	1
	1.1	Backgro	ound	1
	1.2	Objecti	ves of the Study	3
2	LITE	RATURE	EREVIEWS	4
	2.1	Organo	genesis	4
		2.1.1	Direct Organogenesis	5
		2.1.2	Indirect Organogenesis	8
	2.2	Microp	ropagation Status of Mangosteen	14
		2.2.1	Direct Organogenesis in Mangosteen culture	14
		2.2.2	Indirect Organogenesis in Mangosteen Culture	19
		2.2.3	Acclimatization of Mangosteen Plantlet	20
3	DIRE	CT ORG	ANOGENESIS	22
	3.1	3.1 Introduction		22
	3.2	Study o	n Shoot Proliferation	23
		3.2.1	Materials and Methods	23
		3.2.2	Results	31
		3.2.3	Discussions	79
	3.3	Study o	n Shoot Elongation	91
		3.3.1	Materials and Methods	91
		3.3.2	Results	94



		3.3.3	Discussions	102
	3.4	Study	of Rooting	103
		3.3.1	Materials and Methods	104
		3.3.2	Results	107
		3.3.3	Discussions	114
	3.5	Study	on Acclimatization	118
		3.5.1	Materials and Methods	118
		3.5.2	Results	119
		3.5.3	Discussions	121
4	INDI	RECT O	ORGANOGENESIS	123
	5.1	Introdu	uction	123
	5.2	Materi	ials and Methods	123
	5.3	Results	S	130
	5.4	Discus	ssions	134
5	CON	CLUSIO	ONS	137
RE	FEREN	ICES		140
AP	APPENDICES SIODATA OF THE AUTHOR			154
BIC				186



LIST OF TABLES

l'able		Page
1	The number of shoots produced per half seed explant of mangosteen on various proliferation media at first week of culture	33
2	The number of shoots produced per half seed explant of mangosteen on various proliferation media at second week of culture	34
3	The number of shoots produced per half seed explant of mangosteen on various proliferation media at third week of culture	35
4	The number of shoots produced per half seed explant of mangosteen on various proliferation media at fourth week of culture	36
5	Mean number of shoots produced per half seed explant of mangosteen on various proliferation media at fifth, sixth, seventh and eighth week of culture	38
6	The average shoot height produced from half seed explant of mangosteen on various proliferation media of culture at first week of culture	42
7	The average shoot height produced from half seed explant of mangosteen on various proliferation media of culture at second week of culture	43
8	The average shoot height produced from half seed explant of mangosteen on various proliferation media of culture at third week of culture	44
9	The average shoot height produced from half seed explant of mangosteen on various proliferation media of culture at fourth week of culture	45
10	The average shoot height produced from half seed explant of mangosteen on various proliferation media at fifth week of culture	46



11	explant of mangosteen on various proliferation media at sixth, seventh, and eighth week of culture	4/
12	The weight of shoots produced per half seed explant of mangosteen on various proliferation media after eight weeks of culture	53
13	The number of shoots produced per half seed explant of mangosteen on various media at fourth week of subculture	58
14	The number of shoots produced per half seed explant of mangosteen on various media at eighth week of subculture	59
15	The number of shoots produced per half seed explant of mangosteen on various media at twelfth week of subculture	60
16	The number of shoots produced per half seed explant of mangosteen on various media at sixteenth week of subculture	61
17	The number of shoots produced per half seed explant of mangosteen on various media at twentieth week of subculture	62
18	The average height of shoot produced from half seed explant of mangosteen on various media at fourth week of subculture	67
19	The average height of shoot produced from half seed explant of mangosteen on various media at eighth week of subculture	68
20	The average height of shoot produced from half seed explant of mangosteen on various media at twelfth week of subculture	69
21	The average height of shoot produced from half seed explant of mangosteen on various media at sixteenth week of subculture	70



22	The average height of shoot produced from half seed explant of mangosteen on various media at twentieth week of subculture	71
23	The weight of shoots produced per half seed explant of mangosteen on various media at fourth week of subculture	75
24	The weight of shoots produced per half seed explant of mangosteen on various media at eighth week of subculture	76
25	The weight of shoots produced per half seed explant of mangosteen on various media at twelfth week of subculture	77
26	The weight of shoots produced per half seed explant of mangosteen on various media at sixteenth week of subculture	78
27	The weight of shoots produced per half seed explant of mangosteen on various media at twentieth week of subculture	79
28	The average length of shoot on different types of elongation media	95
29	The average weight of shoot on different types of elongation media	100
30	Mean root number on mangosteen shoot cultured on various rooting media	108
31	Mean root length on mangosteen shoot cultured on various rooting media	112
32	The percentage of plantlet surviving under misting chamber for eight weeks	120
33	Mean percentage of callus formation on media of BAP combined with concentration levels of 2,4-D	131
34	Mean percentage of callus formation on media of BAP combined with Glutamine	133



LIST OF FIGURES

Figure		Page
1	Weekly mean number of shoots produced per half seed explant of mangosteen on media containing BAP and Kinetin either with or without AdS	32
2	Weekly mean height of shoots produced per half seed explant of mangosteen on media containing BAP and Kinetin either with or without AdS	40
3	The effect of BAP and Kinetin on the mangosteen shoot height at sixth, seventh and eighth week of culture	47
4	The mean height of shoot on media containing BAP regardless of the AdS levels at sixth to eighth week of culture	48
5	The mean height of shoot on media containing Kinetin regardless of the AdS levels at sixth to eighth week of culture	49
6	Relationship between the height of shoot and the number of shoots produced per half seed explant of mangosteen at sixth, seventh and eighth week; $F = 27.19$; $df = 1$, 16; $P < 0.01$)	50
7	Relationship between the initial fresh weight and the final fresh weight of mangosteen half seed explant; $F = 2.05$; $df = 1, 94$; $P > 0.05$	52
8	Regression between the number of shoots produced per explant and the weight; $F = 69.63$; $df = 1, 22$; $P < 0.01$	54
9	The number of shoots produced per explant, as affected by the interaction between AdS and medium of BAP and Kinetin at first to fifth four weekly of subculture. Means separation at each week by LSD at 5% level	55
10	The rate of increase in number of shoots produced from medium of 1 mg/l BAP, 2 mg/l BAP, and 4 mg/l BAP at first to fifth four weekly of subculture.	57



11	Four weekly mean total height of shoots produced per half seed explant of mangosteen on media containing BAP and Kinetin either with or without AdS	65
12	The height of shoot on media enriched with AdS and without AdS at four weekly subculture	65
13	The rate of increase in height of shoots produced on medium of 0-16 mg/l Kinetin at first to fifth four-weekly of subculture	72
14	The growth of shoot explants on several media of elongation	97
15	Relationship between the shoot height and the shoot weight at the final subculture (twenty four-week) of mangosteen shoot explant ($F = 3.46$; $df = 1, 7$; $P > 0.05$)	101
16	The percentage of secondary root growing on the primary root of shoot explant on several rooting media after 28 weeks of subculture	109
17	The relationship between concentration of 2,4-D and callus formation; $F = 3.42$; $df = 1, 3$; $P < 0.05$	132



LIST OF PLATES

Plate		Page
1	The mangosteen. (A) Trees growing in the field, (B) Whole and half fruit, (C) Peeled seed before sterilization, and (D) Seed after sterilization	25
2	Mangosteen shoots formed on media supplemented with 8 mg/l BAP (A) and 16 mg/l BAP (B) at eighth week of culture	39
3	The shoot height attained on medium with 1 mg/l Kinetin (A) and 2 mg/l BAP (B) at eighth week of culture	49
4	Mangosteen shoots formed on media with 1 mg/l BAP (A) and 2 mg/l BAP (B) at twentieth week of subculture	63
5	Mangosteen shoots generated on media with 1 mg/l BA (A), 4 mg/l BA + 100 mg/l AdS (B), 4 mg/l Kinetin (C), and 4 mg/l Kinetin + 100 mg/l AdS (D) at twentieth week of subculture	73
6	Shoot of mangosteen on elongation media with (A) 1 mg BAP + 2 mg/l Kinetin, (B) 0.5 mg/l BAP + 2 mg/l Kinetin, (C) 0.5 mg/l BAP + 4 mg/l Kinetin, and (D) MS without plant growth regulator at twenty fourth week of subculture	98
7.	The secondary root growing on the primary root of shoot explant on media with 10 mg/l IBA (A) and 5 mg/l NAA (B)	110
8.	Root developed from shoot explants cultured on various rooting media. (A). IBA 1 mg/l, (B). IAA 1 mg/l, (C). NAA 0.5 mg/l, (D). ½ MS 0, (E). NAA 10 mg/l, (F). NAA 15 mg/l	113
9.	The eight weeks old plantlets growing in misting chamber	120
10.	Callus formation on media with 5 mg/l 2,4-D + 1 mg/l BAP (A) and 10 mg/l 2,4-D + 2 mg/l BAP (B) on eight week of culture	133



LIST OF APPENDICES

Appendix Table		Page
1	Analysis of variance for the number of shoots produced per half seed explant of mangosteen on various proliferation media at first week of culture	155
2	Analysis of variance for the number of shoots produced per half seed explant of mangosteen on various proliferation media at second week of culture	155
3	Analysis of variance for the number of shoots produced per half seed explant of mangosteen on various proliferation media at third week of culture	156
4	Analysis of variance for the number of shoots produced per half seed explant of mangosteen on various proliferation media at fourth week of culture	156
5	Analysis of variance for the number of shoots produced per half seed explant of mangosteen on various proliferation media at fifth week of culture	157
6	Analysis of variance for the number of shoots produced per half seed explant of mangosteen on various proliferation media at sixth week of culture	157
7	Analysis of variance for the number of shoots produced per half seed explant of mangosteen on various proliferation media at seventh week of culture	158
8	Analysis of variance for the number of shoots produced per half seed explant of mangosteen on various proliferation media at eighth week of culture	158
9	Analysis of variance for the average height of shoot produced from half seed explant of mangosteen on various proliferation media at first week of culture	159

