



UNIVERSITI PUTRA MALAYSIA

SOMATIC EMBRYOGENESIS IN MUSA SPP.

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By

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of Requirement for the Degree of Doctor of Philosophy**

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Dedicated to
Departed soul of my grand-father
Hj. M. Mohi uddin Khan



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of requirement for the degree of Doctor of Philosophy

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Embryogenesis competent material (scalp) was initiated from shoot tip of *Musa* spp. cultivar Mas (AA), Berangan (AAA), Intan (AAA), Raja (AAB) and Tanduk (AAB). Somatic embryogenesis were investigated from four explant sources viz., scalps, male flower primordia, *in vitro* corm slices and immature ovules of *Musa acuminata* cv. Mas. Scalp formation was optimal on Murashige and Skoog (MS) medium with modified vitamins supplemented with 100 μ M BAP and 1.0 μ M IAA. Among the cultivars investigated, cv. Mas was the most responsive for scalp formation whereby 40% of the shoot tips formed scalps by the 7th month of culture. Cultivar Mas was also the most responsive for meristematic globule formation from scalps attaining 100% meristematic globule formation by week 7 of culture of scalps in Z medium. Cells with embryogenic potential were released from the meristematic globules of cv. Mas after 10 to 12 months of culture of the meristematic globules in Z medium. The embryogenic cell suspension was transferred to liquid S medium and formed globular embryos after 3 to 4 months in culture. Matured globular embryos upon transfer to liquid S regeneration medium supplemented with 0, 1.0, 5.0, 10, 20, 40 and 80 μ M BAP germinated to form roots but without shoots. Typical bi-polar



structure with prominent shoot and root poles was detected through a longitudinal section of a germinating somatic embryo.

In male flower primordia, $60 \pm 7.07\%$ of the cultured explants initiated callus after 3 months on MS medium supplemented with $5.7 \mu\text{M}$ IAA, $18.0 \mu\text{M}$ 2,4-D, $5.4 \mu\text{M}$ NAA and $4.0 \mu\text{M}$ biotin. Improved callus growth was observed on a reduced 2,4-D concentration of $4.5 \mu\text{M}$ with $5.7 \mu\text{M}$ IAA, $5.4 \mu\text{M}$ NAA and $4.0 \mu\text{M}$ biotin after 4 months of culture. Somatic embryo formation was observed in culture after 1 month on MS medium supplemented with $4.7 \mu\text{M}$ ABA. On transfer of the somatic embryo into germination medium containing MS/SH salts supplemented with $1.0 \mu\text{M}$ NAA, $0.5 \mu\text{M}$ kinetin, $0.2 \mu\text{M}$ zeatin, $2.0 \mu\text{M}$ BAP, $4.0 \mu\text{M}$ biotin, 100 mg/l glutamine, 100 mg/l malt extract and 45 g/l sucrose only plumule development occurred while root formation was not observed even after 1 month of culture.

Embryogenic callus formation from *in vitro* corm slices was observed in two media type, which were MS medium with modified vitamins supplemented with $0.5 \mu\text{M}$ 2,4-D and MS medium with modified vitamins supplemented with $5.0 \mu\text{M}$ 2,4-D, $1.0 \mu\text{M}$ proline, 100 mg/l casein hydrolysate and 40 mg/l cystein-HCl. Seventy percent of the cultured explants formed embryogenic callus at week 18 of culture. Embryogenic callus from the first medium formed root-like structures upon transfer to regeneration medium containing MS salts supplemented with $5.0 \mu\text{M}$, $10 \mu\text{M}$, $20 \mu\text{M}$ and $30 \mu\text{M}$ BAP. Embryogenic callus from the second medium also formed root-like structures on transfer to regeneration medium containing liquid

½ strength MS salts supplemented with 5.0 µM, 10 µM, 20 µM, 40 µM, 60 µM and 80 µM BAP.

Immature ovule explants responded to form vitreous callus instead of embryogenic callus in all the treatments tested. Among the five cultivars and four explant sources investigated, scalps and male flower-primordia of cultivar Mas could be considered promising for the induction of somatic embryogenesis.

Anatomical study of the shoot tip of banana cv. Mas (AA) indicated a conical-shaped structure consisting of several layers of leaf primordia covering the meristem apex. Shoot-bud proliferation which were of axillary origin and induced due to the inclusion of high cytokinin especially BAP in the medium were seen at the leaf bases of the shoot tips. Anatomical investigation of the meristematic globules indicated single cells originating from the starch riched cells in the peripheral layer of the meristematic globules.

Transformation study showed 9 cm target distance along with helium pressure of 1100 and 1350 psi to be the efficient variables for the transformation of scalps of cv. Mas whereby 40% of the scalps were transformed. A target distance of 6 cm along with helium pressure of 900 psi was optimal for the transformation of embryogenic cell suspension whereby 70% of the bombarded samples were transformed.

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EMBRIOGENESIS SOMA BAGI *MUSA* SPP.

Oleh

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‘Scalp’ iaitu struktur yang berkeupayaan menjadi embryogenik telah dijanakan dari mercu pucuk spesies *Musa* kultivar Mas (AA), Berangan (AAA), Intan (AAA), Raja (AAB) dan Tanduk (AAB). Embryogenesis soma daripada 4 sumber eksplan telah dikaji iaitu ‘scalp’ primodia bunga jantan, potongan umbisi daripada kultur *in vitro* dan ovul yang belum matang dalam *Musa* spp. kultivar Mas (AA). Pembentukan ‘scalp’ adalah optima pada media Murashige dan Skoog (MS) yang mengandungi vitamin terubah suai, 100 μ M BAP dan 1.0 μ M IAA. Di antara kultivar yang dikaji, kultivar Mas menunjukkan respon yang terbaik dari segi pembentukan ‘scalp’ dengan 40% mercu pucuk membentuk ‘scalp’ pada bulan ke 7 pengkulturan di dalam medium Z. Kultivar Mas juga didapati menunjukkan respon yang terbaik terhadap pembentukan dari ‘scalp’ dengan penghasilan 100% globul meristematik pada minggu ke 7 ‘scalp’ dikultur di dalam medium Z. Sel dengan potensi embriogenik telah diperolehi daripada globul meristematik kultivar Mas selepas 10 ke 12 bulan globul meristematik dikultur di dalam medium Z. Sel ampai embriogenik telah dipindahkan ke medium S dan membentuk embrio

globular selepas 3 ke 4 bulan dikultur. Embrio globular yang matang selepas dipindahkan ke media regenerasi yang mengandungi cecair S dengan 0, 1.0, 5.0, 10, 20, 40 dan 80 μM BAP bercambah membentuk akar tetapi tanpa pucuk. Struktur bi-polar yang tipikal dengan pucuk dan akar yang menonjol keluar telah dikenalpasti melalui keratan memanjang embrio somatik yang sedang cambah.

Bagi primodia bunga jantan, $60 \pm 7.07\%$ daripada eksplan yang dikultur mula membentuk kalus selepas 3 bulan di dalam media MS yang dibekalkan dengan 5.7 μM IAA, 18.0 μM 2,4-D, 5.4 μM NAA dan 4.0 μM biotin. Pertumbuhan kalus yang lebih baik diperolehi dengan pengurangan kepekatan 2,4-D kepada 4.5 μM serta mengandungi 5.7 μM IAA, 5.4 μM NAA dan 4.0 μM biotin selepas 4 bulan dikultur. Pembentukan embriogenesis soma telah diperolehi selepas 1 bulan pengkulturan pada medium MS mengandungi 4.7 μM ABA. Apabila embrio soma tersebut dipindahkan ke dalam media percambahan yang mengandungi garam MS/SH yang dibekalkan dengan 1.0 μM NAA, 0.5 μM Kinetin, 0.2 μM Zeatin, 2.0 μM BAP, 4.0 μM biotin, 100mg/l glutamine, 100 mg/l ekstrak malt dan 45 g/l sucrose, hanya perkembangan pucuk berlaku sementara pembentukan akar tidak diperolehi selepas 1 bulan dikultur.

Pembentukan kalus embriogenik daripada potongan umbisi kultur *in vitro* telah diperolehi di dalam 2 media iaitu media MS dengan vitamin yang dimodifikasi yang dibekalkan dengan 0.5 μM 2,4-D dan media MS yang dibekalkan dengan 5.0 μM 2,4-D, 1.0 μM prolin, 100 mg/l kasein hidrolisat dan 40 mg/l sistein-HCl. Tujuh puluh peratus daripada eksplan yang dikultur membentuk kalus embriogenik

selepas 18 minggu dikultur. Kalus embriogenik daripada media pertama membentuk struktur seperti akar apabila dialihkan ke media regenerasi yang mengandungi garam MS yang dibekalkan dengan 5.0 μM , 10 μM , 20 μM dan 30 μM BAP sementara kalus embriogenik daripada media kedua membentuk struktur seperti pucuk atau akar apabila dialihkan ke media regenerasi yang mengandungi $\frac{1}{2}$ garam MS cecair yang dibekalkan dengan 5.0 μM , 10 μM , 20 μM , 40 μM , 60 μM dan 80 μM BAP.

Eksplan daripada ovul yang belum matang membentuk kalus yang bersifat 'vitreous' dan tidak membentuk kalus embriogenik di dalam semua rawatan yang diuji. Di antara lima kultivar dan 4 sumber eksplan yang dikaji, eksplan 'scalp' dan primodia bunga jantan daripada kultivar Mas boleh di anggap berpotensi untuk membentuk embriogenesis soma.

Kajian anatomi ke atas mercu pucuk pisang kultivar Mas (AA) menunjukkan struktur berbentuk kon yang terdiri daripada beberapa lapisan primodia daun yang menutupi meristem apeks. Tunas baru yang berproliferasi yang berasal daripada tunas aksil dan teransang akibat penambahan sitokinin yang tinggi khususnya BAP ke dalam media telah dilihat pada pangkal daun mercu pucuk tersebut. Penyelidikan anatomi ke atas globul meristematik, menunjukkan sel tunggal muncul daripada sel yang kaya dengan kanji di dalam lapisan persisian globuls meristematik tersebut.

Kajian transformasi menunjukkan jarak sasaran 9 cm dengan tekanan helium pada 1100 dan 1350 psi merupakan pemboleh ubah yang efisien untuk transformasi 'scalp' kultivar Mas dengan 40% daripada 'scalp' tersebut mengalami transformasi.

Jarak sasaran 6 cm dengan tekanan 900 psi adalah optima bagi transformasi sel ampaiian embriogenik dengan 70% daripadanya berjaya ditransformasikan.

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TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	x
APPROVAL SHEETS	xii
DECLARATION FORM	xiv
LIST OF TABLES	xix
LIST OF FIGURES	xxi
LIST OF PLATES	xxiii
LIST OF ABBREVIATIONS/NOTATIONS	xxviii
CHAPTER	
1. GENERAL INTRODUCTION	1.1
1.1 Background	1.1
1.2 Objectives of the Study	1.5
2. LITERATURE REVIEW	2.1
2.1 Tissue Culture of Banana and Plantain	2.1
2.1.1 Growth Regulator Requirement in Tissue Culture of Banana and Plantain	2.1
2.1.2 Organogenesis and Scalp Formation	2.2
2.1.3 Somatic Embryogenesis	2.5
2.2 Histological Studies	2.15
2.3 Plant Genetic Transformation	2.17
3. ESTABLISHMENT OF EMBRYOGENIC CELL SUSPENSION AND PLANT REGENERATION FROM SCALPS DERIVED FROM SHOOT TIPS OF <i>MUSA</i> SPP.	3.1
3.1 Preface	3.1
3.2 Materials and Methods	3.2
3.2.1 Plant Materials.....	3.2
3.2.2 Preparation of Explants.....	3.2
3.2.3 Preparation of Stock Solutions	3.4
3.2.4 Preparation of Culture Media.....	3.4
3.2.5 Incubation of Cultures	3.4
3.2.6 Experimental Design and Data Analysis	3.5

3.2.7	Effect of BAP on Shoot-bud Proliferation in Banana Cultivar Mas (AA), Berangan (AAA), Intan (AAA), Raja (AAB) and Tanduk (AAB).....	3.5
3.2.8	Scalp Formation from Shoot Tip Explants of Banana Cultivar Mas (AA), Berangan (AAA), Intan (AAA), Raja (AAB) and Tanduk (AAB) on MS Salts with Modified Vitamins Supplemented with 100 μ M BAP and 1.0 μ M IAA	3.5
3.2.9	Formation of Meristematic Globules from Scalps of Banana Cultivar Mas (AA), Berangan (AAA), Intan (AAA) and Raja (AAB).....	3.6
3.2.10	Establishment of Embryogenic Cell Suspension from Scalp derived Meristematic Globules of <i>Musa acuminata</i> cv. Mas (AA) in Z medium.....	3.6
3.2.11	Formation of Somatic Embryos from Embryogenic Cell Suspension and Attempts for Plant Regeneration in <i>M. acuminata</i> cv. Mas (AA)	3.7
3.2.12	Response of Meristematic Globules Obtained from Scalps of <i>M. acuminata</i> cv. Mas (AA) in/on Different Regeneration Media.....	3.9
3.3	Results	3.11
3.3.1	Effect of BAP on Shoot-bud Proliferation in Banana Cultivar Mas (AA) Berangan (AAA), Intan (AAA), Raja (AAB) and Tanduk (AAB).	3.11
3.3.2	Scalp Formation from Shoot Tip Explants of Banana Cultivar Mas (AA), Berangan (AAA), Intan (AAA), Raja (AAB) and Tanduk (AAB) on MS Salts with Modified Vitamins Supplemented with 100 μ M BAP and 1.0 μ M IAA.....	3.22
3.3.3	Formation of Meristematic Globules from Scalps of Banana Cultivar Mas (AA), Berangan (AAA), Intan (AAA) and Raja (AAB) in Z Medium	3.26
3.3.4	Establishment of Embryogenic Cell Suspension from Scalp-derived Meristematic Globules of <i>M. acuminata</i> cv. Mas (AA) in Z Medium.....	3.30
3.3.5	Formation of Somatic Embryos from Embryogenic Cell Suspension and Attempts for Plant Regeneration in <i>M. acuminata</i> cv. Mas (AA).....	3.35
3.3.6	Response of Meristematic Globules Obtained from Scalps of <i>M. acuminata</i> cv. Mas (AA) in/on Different Regeneration Media...	3.43
3.4	Discussion	3.46
4.	INDUCTION OF SOMATIC EMBRYOGENESIS AND PLANT REGENERATION IN MALE FLOWER-PRIMORDIA, <i>IN VITRO</i> CORM SLICES AND IMMATURE OVULE EXPLANTS OF <i>Musa acuminata</i> cv. MAS (AA)	4.1
4.1	Preface.....	4.1
4.2	Materials and Methods	4.1
4.2.1	Induction of Embryogenic Callus and Attempts for Regeneration in Male Flower-primordia Explants of <i>M. acuminata</i> cv. Mas (AA)....	4.2

4.2.2	Induction of Embryogenic Callus and Attempts for Regeneration from <i>In Vitro</i> Corm Slice Explants of <i>M. acuminata</i> cv. Mas (AA).....	4.4
4.2.3	Investigation of Embryogenic Potential of Immature Ovule Explants of <i>M. acuminata</i> cv. Mas (AA).....	4.6
4.3	Results.....	4.8
4.3.1	Induction of Embryogenic Callus and Attempts for Regeneration from Male Flower-primordia Explants of <i>M. acuminata</i> cv. Mas (AA).....	4.8
4.3.2	Induction of Embryogenic Callus and Attempts for Regeneration of <i>In Vitro</i> Corm Slice Explants of <i>M. acuminata</i> cv. Mas (AA).....	4.13
4.3.2.1	Attempts for Regeneration of Embryogenic Callus Obtained from <i>In Vitro</i> Corm Slice Explants of <i>M. acuminata</i> cv. Mas (AA) on MS Media Supplemented with Different Concentrations of BAP ...	4.19
4.3.3	Investigation of Embryogenic Potential of Immature Ovule Explants of <i>M. acuminata</i> cv. Mas (AA)	4.22
4.4	Discussion	4.26
5.	ANATOMICAL AND MICRO MORPHOLOGICAL STUDTES ON	
	<i>IN VITRO</i> TISSUES OF <i>Musa acuminata</i> cv. MAS (AA)	5.1
5.1	Preface	5.1
5.2	Materials and Methods	5.1
5.2.1	Light Microscopy	5.1
5.2.1.1	Fixation	5.3
5.2.1.2	Dehydration	5.3
5.2.1.3	Infiltration	5.3
5.2.1.4	Embedding	5.3
5.2.1.5	Microtoming	5.4
5.2.1.6	Mounting	5.4
5.2.1.7	Staining	5.4
5.2.1.8	Processing of Germinated Somatic Embryo	5.5
5.2.2	Scanning Electron Microscopy (SEM)	5.5
5.3	Results	5.6
5.3.1	Light Microscopy	5.6
5.3.1.1	Shoot Tip	5.6
5.3.1.2	Proliferated Shoot-buds	5.8
5.3.1.3	Formation and Released of Meristematic Globules in Scalp Tissue..	5.8
5.3.1.4	Meristematic Globules	5.8
5.3.1.5	Embryo with Bi-polar Structure	5.10
5.3.2	Scanning Electron Microscopy	5.12
5.4	Discussion	5.16

6. PRELIMINARY STUDY ON GENETIC TRANSFORMATION OF <i>Musa acuminata</i> cv. MAS USING PARTICLE GUN	6.1
6.1 Preface.....	6.1
6.2 Materials and Methods.....	6.4
6.2.1 Plant Materials and Media Composition.....	6.4
6.2.2 Plasmid DNA	6.4
6.2.3 Gold Particles	6.6
6.2.4 Particle Bombardment	6.6
6.3 Results	6.7
6.4 Discussion	6.11
7 GENERAL DISCUSSION AND CONCLUSION	7.1
REFERENCES	R.1
APPENDICES	A.1
VITA	V.1



LIST OF TABLES

Table	Page
3.1 Response of scalps (%) to meristematic globules formation in Z medium with duration of culture in different banana cultivar of Mas (AA), Berangan (AAA), Intan (AAA) and Raja (AAB).....	3.28
3.2 Number of meristematic globules released/scalp in different banana cultivar of Mas (AA), Berangan (AAA), Intan (AAA) and Raja (AAB) during 7-10 weeks of scalp culture in Z medium	3.28
3.3 Effect of sub-culture of scalps of <i>Musa acuminata</i> cv. Mas in Z medium.....	3.31
3.4 Response of somatic embryos obtained from embryogenic cell suspension of <i>M. acuminata</i> cv. Mas (AA) in liquid S regeneration medium with different concentrations of BAP	3.39
3.5 Response of globular somatic embryos obtained from embryogenic cell suspension of <i>M. acuminata</i> cv. Mas (AA) on semi-solid S regeneration medium with different concentrations of BAP	3.41
3.6 Response of globular somatic embryos obtained from embryogenic cell suspension of <i>M. acuminata</i> cv. Mas (AA) on semi-solid MS regeneration medium with different concentrations of BAP	3.41
3.7 Response of globular somatic embryo of <i>M. acuminata</i> cv. Mas (AA) derived from embryogenic cell suspension on different semi-solid regeneration media	3.42
3.8 Response of meristematic globules of <i>M. acuminata</i> cv. Mas (AA) in/on different regeneration media	3.44
4.1 Stages in somatic embryogenesis of floral-primordia explants of <i>M. acuminata</i> cv. Mas	4.10
4.2 Response of <i>in vitro</i> corm slice explants of <i>M. acuminata</i> cv. Mas on different media composition and duration of culture.....	4.14

LIST OF TABLES

Table		Page
4.3	Callus weight of <i>in vitro</i> corm slice explants of <i>M. acuminata</i> cv. Mas on different media composition and duration of culture	4.15
4.4	Response of embryogenic callus from corm slice explants of <i>M. acuminata</i> cv. Mas on regeneration media	4.21
4.5	Response of immature ovule explant of <i>M. acuminata</i> cv. Mas on different media composition....	4.24
6.1	GFP gene expression in transformed scalps and embryogenic cell suspension of <i>M. acuminata</i> cv. Mas (AA) 48 hours after bombardment	6.9

LIST OF FIGURES

Figure		Page
1.1	A flowchart on the improvement programme of <i>Musa</i> spp. through biotechnological approach.....	1.7
3.1	Shoot-bud proliferation from shoot tips of <i>M. acuminata</i> cv. Mas on MS medium with modified vitamins supplemented with 10, 50 and 100 μ M BAP and 1.0 μ M IAA with sub-culture	3.12
3.2	Height of shoot-buds attained on MS medium with modified vitamins supplemented with 10, 50 and 100 μ M BAP and 1.0 μ M IAA with sub-culture in <i>M. acuminata</i> cv. Mas.....	3.12
3.3	Shoot-bud proliferation from shoot tips of <i>M. acuminata</i> cv. Berangan on MS medium with modified vitamins supplemented with 10, 50 and 100 μ M BAP and 1.0 μ M IAA with sub-culture..	3.15
3.4	Height of shoot-buds attained on MS medium with modified vitamins supplemented with 10, 50 and 100 μ M BAP and 1.0 μ M IAA with sub-culture in <i>M. acuminata</i> cv. Berangan..	3.15
3.5	Shoot-bud proliferation from shoot tips of <i>M. acuminata</i> cv. Intan on MS medium with modified vitamins supplemented with 10, 50 and 100 μ M BAP and 1.0 μ M IAA with sub-culture.....	3.17
3.6	Height of shoot-buds attained on MS medium with modified vitamins supplemented with 10, 50 and 100 μ M BAP and 1.0 μ M IAA with sub-culture in <i>M. acuminata</i> cv. Intan. ...	3.17
3.7	Shoot-bud proliferation from shoot tips of <i>Musa</i> sp. cv. Raja on MS medium with modified vitamins supplemented with 10, 50 and 100 μ M BAP and 1.0 μ M IAA with sub-culture..	3.19
3.8	Height of shoot-buds attained on MS medium with modified vitamins supplemented with 10, 50 and 100 μ M BAP and 1.0 μ M IAA with sub-culture in <i>Musa</i> sp. cv. Raja.....	3.19
3.9	Shoot-bud proliferation from shoot tips of <i>Musa</i> sp. cv. Tanduk on MS medium with modified vitamins supplemented with 10, 50 and 100 μ M BAP and 1.0 μ M IAA with sub-culture.....	3.21

3.10	Height of shoot-buds attained on MS medium with modified vitamins supplemented with 10, 50 and 100 μM BAP and 1.0 μM IAA with sub-culture in <i>Musa</i> sp. cv. Tanduk.....	3.21
3.11	Scalp formation from shoot tips of banana cultivar Mas (AA), Berangan (AAA), Intan (AAA), Raja (AAB) and Tanduk (AAB) on MS medium with modified vitamins supplemented with 100 μM BAP and 1.0 μM IAA with sub-culture.....	3.24
4.1	Increment in callus weight with duration of culture of male flower-primordia explants of <i>M. acuminata</i> cv. Mas.....	4.12
6.1	Schematic diagram of plasmid vector pGEM with the inserted GFP gene.....	6.3



LIST OF PLATES

Plate	Page
3.1 A) Sword suckers of <i>Musa acuminata</i> cv. Mas collected from Field 2, UPM B) Trimmed sucker blocks prepared from sword suckers, ready for surface sterilization.....	3.3
3.2 Excised shoot tip obtained from sterilized sucker-blocks placed on culture medium.....	3.3
3.3 Meristematic globules released from scalps of <i>M. acuminata</i> cv. Mas in Z medium at sub-culture 10.....	3.8
3.4 Sieving of cell suspension with 100 µm nylon sieve	3.8
3.5 Meristematic globules obtained from scalps of <i>M. acuminata</i> cv. Mas in Z medium at sub-culture 10	3.10
3.6 Shoot-bud proliferation from shoot tip explants of <i>M. acuminata</i> cv. Mas on different levels of BAP observed at sub-culture 4 A) elongated plantlet on 10 µM BAP B) stunted shoots on 50 µM BAP and C) multiple stunted buds on 100 µM BAP.....	3.13
3.7 Various types of scalps obtained from shoot tips of different banana cultivars on MS medium with modified vitamins supplemented with 100 µM of BAP and 1.0 µM IAA at sub-culture 7: A) cv. Mas (AA), B) cv. Berangan (AAA), C) cv. Intan (AAA), D) cv. Raja (AAB) and E) cv. Tanduk (AAB).....	3.25
3.8 Meristematic globules formed on a scalp surface in Z medium at sub-culture 10.....	3.27
3.9 A) Non-uniform, elongated and vacuolated cells at sub-culture 4 and B) Uniform and less vacuolated cells at sub-culture 9 in Z medium in <i>M. acuminata</i> cv. Mas.....	3.32
3.10 A) Sieved cell suspension of <i>M. acuminata</i> cv. Mas obtained at sub-culture 9 in Z medium B) Sieved cells stained with fluorescein diacetate (FDA).	3.32
3.11 Uniform cells with embryogenic characteristics obtained from meristematic globules of <i>M. acuminata</i> cv. Mas in Z medium at A) sub-culture 10 and B) sub-culture 12	3.34

3.12	Somatic embryo formation from the embryogenic cell suspension of <i>M. acuminata</i> cv. Mas in liquid S medium A) Embryogenic cells released from embryogenic cell aggregates underwent mitotic division after 2 months of culture B) Proembryogenic mass formed from the embryogenic cells after 3 months of culture C) Globular embryos formed from the proembryogenic mass after 4 months of culture.	3.36
3.13	Development of globular somatic embryos from the embryogenic cell suspension of <i>M. acuminata</i> cv. Mas in liquid S medium A) after 1 month of globular embryo formation B) after 2 months of globular embryo formation.....	3.37
3.14	Globular somatic embryos of <i>M. acuminata</i> cv. Mas formed roots in liquid S regeneration medium supplemented with 10 μ M BAP after 1 month of culture.....	3.40
3.15	Globular somatic embryos of <i>M. acuminata</i> cv. Mas formed callus and recurrent embryos on semi-solid S regeneration medium supplemented with 10 μ M BAP after 3 months of culture.....	3.40
3.16	Meristematic globules of <i>M. acuminata</i> cv. Mas forming root-like fuzzy structures in liquid S regeneration medium at week 10 of culture	3.45
3.17	Meristematic globules of <i>M. acuminata</i> cv. Mas turned brown to black and died in liquid S regeneration medium supplemented with 5.0 μ M BAP at week 8 of culture.....	3.45
4.1	Male flower bud of <i>M. acuminata</i> cv. Mas with female bunch exposed and bending upwards.	4.3
4.2	Sterilised male flower bud (yellow arrow) and excised flower clusters (white arrow) of <i>M. acuminata</i> cv. Mas.	4.3
4.3	<i>In vitro</i> shoot-buds with thick basal corms obtained at sub-culture 4 of shoot tip of <i>M. acuminata</i> cv. Mas on MS medium with modified vitamins supplemented with 100 μ M BAP and 1.0 μ M IAA.....	4.5
4.4	Corm slices (Size: 1.0 x 0.3 cm ²) of <i>M. acuminata</i> cv. Mas excised from the base of <i>in vitro</i> shoot-buds.....	4.5
4.5	Immature fruits of <i>M. acuminata</i> cv. Mas collected from Field 2, UPM.....	4.7
4.6	A longitudinal section of an immature fruit showing globular whitish immature ovules attached to the placenta in a chain.	4.7