



**UNIVERSITI PUTRA MALAYSIA**

**IN VITRO CULTURE OF MUSKMELON -  
Cucumis melo var. BIRDIE**

**ZULAINI ISMAIL**

**FSMB 1989 1**

IN VITRO CULTURE OF MUSKMELON -  
Cucumis melo var. BIRDIE

by

ZULAINI ISMAIL

Thesis submitted in Partial Fulfilment of the  
Requirements for the Degree of Master of Science  
in The Faculty of Food Science and Biotechnology,  
Universiti Pertanian Malaysia

May 1989



**DEDICATED**

**TO**

**My Husband, Md Akhir**  
who has been a constant source of  
inspiration for me throughout this study

**AND**

**My Mother, Senah**  
for her patience and understanding



## ACKNOWLEDGEMENTS

It pleases me to have the opportunity of expressing my sincere gratitude to Universiti Pertanian Malaysia and in particular the Faculty of Food Science and Biotechnology for supporting this research as part of a full-time post graduate programme.

I am extremely grateful and greatly indebted to my supervisor Associate Professor Dr. Zaliha Christine Alang, Biotechnology Department, Faculty Food Science and Biotechnology, for her dedicated efforts, stimulating discussions, active participation and constant encouragement throughout the planning and execution of this research.

I take this opportunity also to thank to Associate Professor Khalip Raffar, Deputy Dean, Faculty of Agriculture and Associate Professor Dr. Lim Eng Siong, Department of Agronomy and Horticulture, Faculty of Agriculture, Universiti Pertanian Malaysia for their expert advice in some parts of this study.

I am grateful to Assistant Professor Dr. S. Hisajima, Institute of Applied Biochemistry, University of Tsukuba, Japan, for useful discussions on progress of this study.



My sincere appreciation is extended to Encik Abdul Ghani Hashim, Faculty of Science and Environmental studies for his professional photographic work; Mr. Ho Oi Kuan, Faculty of Veterinary Medicine and Animal Science, for technical assistance in the Scanning Electron Microscopy work; Mr. How Peng Guan, Agronomy Department for technical assistance in the preparation of the histological slides; staff of the Nursery Unit, Faculty of Forestry and staff of the Hydroponic Unit, Faculty of Agriculture, for their help in the planting out study.

To all of the Central Tissue Culture Research Laboratory staff and all of my friends, I express my sincere gratitude for their assistance and help throughout this study.

Lastly, I wish to thank to ALLAH for allowing me to finish this study.



## TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENTS	iii
LIST OF TABLES	ix
LIST OF PLATES	x
LIST OF ABBREVIATIONS	xiv
ABSTRACT	xiv
ABSTRAK	xvii
CHAPTER	
1 INTRODUCTION	1
Cucurbits in Malaysia	3
LITERATURE REVIEW	6
<u>In vitro</u> Culture of <u>Cucumis melo</u>	5
Somatic Embryogenesis and Organogenesis from Callus	6
Cell Suspension and Protoplast culture	9
Bud Formation and Plant Regeneration	12
The Aim of the Investigation	14
2 MATERIALS AND METHODS	16
General	16
Supply and Storage of Seeds	16
Glassware and Cleaning	16

Chemicals	18
Preparation of Stock Solutions and Basal Medium	19
Incubation of Cultures	20
Excision and Surface - Sterilization of Cotyledons, Embryos and Separated Cotyledonary Halves	20
Surface - Sterilization of Seeds	23
Preparation and Sterilization of Testaless Seeds	23
Induction of Buds	24
Effect of Cytokinins and GA <sub>3</sub> on Excised Cotyledons	24
Effect of Cytokinins and GA <sub>3</sub> on Excised Embryos	25
Effect of Cytokinins, GA <sub>3</sub> and NAA on Testaless Seeds	25
Determination of Medium for Continued Production of Buds and Elongation of Shoots	27
Rooting Study	28
Determination of Suitable Shoot Size for Rooting	28
Effect of Light and Support Medium on Root Development From Single Shoot in the Presence of IBA	28
Effect of Strength of Basal Medium, Activated Charcoal and Auxin on Root Development	29



	Anatomical Study	29
	Scanning Electron Microscopy	29
	Light Microscopy	30
<b>3</b>	<b>RESULT</b>	
	Induction of Buds	32
	Effect of Cytokinins and GA <sub>3</sub> on Excised Cotyledons and separated Cotyledonary Halves	32
	Effect of Cytokinins and GA <sub>3</sub> on Excised Embryos	38
	Effect of Cytokinins, GA <sub>3</sub> and NAA on Testaless Seeds	40
	Determination of Medium for Continued Production and Elongation of Shoots	47
	Rooting Study	52
	Determination of Suitable Shoots Size for Rooting	52
	Effect of Light and Support Medium on Root development from Single Shoots in the Presence of IBA	54
	Effect of Strength of Basal Medium, Activated Charcoal and Auxin on Root Development	54
	Preliminary Investigation of Bud Formation	60
<b>4</b>	<b>DISCUSSION</b>	<b>69</b>
<b>5</b>	<b>SUMMARY</b>	<b>79</b>



<b>BIBLIOGRAPHY</b>	<b>81</b>
<b>APPENDICES</b>	
<b>A</b> Media Formulation after Murashige and Skoog (1962)	<b>85</b>
<b>B</b> SPSS - X ANOVA and DNMRT Computer Print Out	<b>87</b>
<b>C</b> Preliminary Study on Planting Out	<b>95</b>
<b>BIOGRAPHICAL SKETCH</b>	<b>103</b>



## LIST OF TABLES

TABLE		PAGE
1	The Botanical and Common Names of Some Cultivated Species of the Cucurbitaceae	2
2	Effect of Cytokinins and GA <sub>3</sub> (5.0 μM) on the Production of Buds from Excised Cotyledons	33
3	Effect of Cytokinins on Shoot Production from Testaless Seeds	41
4	Effect of Cytokinins and GA <sub>3</sub> (5.0 μM) on Shoot Production from Testaless Seeds	44
5	Effect of Cytokinins and NAA on the Production of Buds from Testaless Seeds	46
6	The Number of Shoots Produced in Media for Continued Production and Elongation	49
7	Mean Percentage of Rooted Shoots in Medium Containing IBA (3.0 μM)	53
8	Mean Percentage of Rooted Shoots in the Light and in the Dark on Liquid or Agar Medium Containing IBA (3.0 μM)	55
9	Mean Percentage of Shoots Which Developed Roots on Full or Half Strength MS Medium, With or Without Charcoal, in the Presence of NAA or IBA	61



10	ANOVA and DNMRT for Cotyledon Culture on Media Containing BAP (Cytokinin) with or without GA <sub>3</sub>	87
11	DNMRT for Testaless Seeds Culture on Media Containing Cytokinin	88
12	ANOVA and DNMRT for Testaless Seeds Culture on Media Containing Cytokinin with or without GA <sub>3</sub>	89
13	ANOVA and DNMRT for Testaless Seeds Culture on Media Containing BAP and NAA	90
14	DNMRT for Cotyledonary Nodes Culture on Media Containing BAP and Basal Medium	91
15	DNMRT for Shoot Sizes on Agar Media Containing IBA (3.0 uM)	91
16	ANOVA and DNMRT for Individual Shoots Culture in the Dark for One Week Followed by Two Weeks in the Light, on a Filter-paper Bridge in Liquid Media Containing IBA (3.0 uM)	92
17	ANOVA and DNMRT for Individual Shoot Culture in the Light for Two Weeks on a Filter-paper Bridge in Liquid Media Containing NAA (2.0 uM)	93



## LIST OF PLATES

PLATE		PAGE
1	Seeds of Muskmelon ( <u>Cucumis melo</u> ) used throughout the Study	17
2	Explants of <u>C. melo</u> used in the Study a. entire seeds b. testaless seeds c. excised cotyledons d. excised embryo e. micropylar halves of excised cotyledons f. chalazal halves of excised cotyledons	21
3	Bud Development from Excised Cotyledons After Four Weeks in Culture on Medium Containing BAP (2.0 $\mu$ M)	34
4	Excised Cotyledons After Four Weeks in Culture on Medium Containing BAP (2.0 $\mu$ M)	35
5	Excised Cotyledons After Four Weeks in Culture on Medium Containing BAP (2.0 $\mu$ M) and GA <sub>3</sub> (5.0 $\mu$ M)	37
6	Excised Cotyledons After Four Weeks in Culture on Basal Medium without Hormones	37
7	Excised Embryo After Four Weeks in Culture on Medium Containing BAP (10.0 $\mu$ M)	39
8	Excised Embryo After Four Weeks in Culture on Medium Containing BAP (10.0 $\mu$ M) and GA <sub>3</sub> (5.0 $\mu$ M)	39
9	Testaless seeds After Three Weeks in Culture on Medium Containing BAP (3.0 $\mu$ M)	42



10	Cotyledonary Node From Testaless seed After Three Weeks in Culture on Medium Containing BAP (3.0 $\mu$ M)	48
11	Cotyledonary Node After Four days in Subculture on Medium Containing BAP (0.5 $\mu$ M)	50
12	Cotyledonary Node After Seven days in Subculture on Medium Containing BAP (0.5 $\mu$ M)	50
13	Clumps of Buds From Cotyledonary Node Prior to Subculture on Basal Medium	51
14	Shoot Development From Buds at Cotyledonary Node After Two Weeks in Subculture on Basal Medium	51
15	Individual Shoot Cultured in the Light for Three Weeks on Filter-paper Bridges in Liquid Medium Containing IBA (3.0 $\mu$ M)	56
16	Individual Shoot Cultured in the Light for Three Weeks on Agar Medium Containing IBA (3.0 $\mu$ M)	57
17	Individual Shoot Cultured in the Dark for One Week Followed by Two Weeks in the Light, on a Filter-paper Bridge in Liquid Medium Containing IBA (3.0 $\mu$ M)	58
18	Individual Shoot Cultured in the Dark for One Week Followed by Two Weeks in the Light on Agar Medium Containing IBA (3.0 $\mu$ M)	59
19	Individual Shoot Cultured in the Light for Two Weeks on Filter-paper Bridge in Liquid Medium Containing NAA (2.0 $\mu$ M)	62
20	Scanning Electron Micrograph of Cotyledonary Node from Testaless Seed Cultured on MS Medium Containing BAP (3.0 $\mu$ M) After Two Weeks in Culture (C - cotyledons; B -bud; MS - main shoot)	64



21	Close-up of Bud Formation at Cotyledonary Node on Bud Induction Medium After Two Weeks (B - bud; C - cotyledon; MS -main shoots)	65
22	Longitudinal Section Through Axillary Buds at Cotyledonary Node of Testaless seed Cultured on Bud Induction Medium (MS + 3.0 $\mu$ M BAP) after Two Weeks (AB - axillary bud)	66
23	Scanning Electron Micrograph of Cotyledonary Node From Testaless Seed Cultured on Control Medium for Three Weeks (C - cotyledon; MS - main shoot)	67
24	Longitudinal Section Through Main Shoot from Testaless Seed Cultured on Bud Induction Medium for Three Weeks (B - bud; MS - main shoot)	68
25	<u>In vitro</u> -propagated Muskmelon Plantlet, One Week After Transplanting to vermiculite	98
26	<u>In vitro</u> -propagated Muskmelon Plant, One Week After Transplanting to Sand	98
27	<u>In vitro</u> -propagated Plantlet, One Week After Transplanting to Vermiculite : Sand (1 : 1)	99
28	<u>In vitro</u> -propagated plantlet, One Week After Transplanting to Hydroponic Nutrient/Pebbles	99
29	<u>In vitro</u> -propagated Muskmelon Plants, Two Months After Transplanting to the Hydroponic Glasshouse	100
30	<u>In vitro</u> -propagated Muskmelon Plants Showing Vigorous Root Development.	101
31	<u>In vitro</u> -propagated Muskmelon Plants Showing Male (M) and Female (F) Flowers	101



32      In vitro-propagated Muskmelon      102  
Plant, Two to Three Months After  
Transfer to the Hydroponic  
Glasshouse, Showing Normal-shaped  
Fruit.



## ABSTRACT

Abstract of thesis submitted to the Senate of Universiti Pertanian Malaysia in partial fulfilment of the requirements for the degree of Master of Science.

### IN VITRO CULTURE OF MUSKMELON

- CUCUMIS MELO VAR. BIRDIE

By

ZULAINI ISMAIL

May, 1989

Supervisor : Dr. Zaliha Christine Alang

Faculty : Food Science and Biotechnology

Several explants from F1 hybrid seeds and aseptically-germinated seedlings of muskmelon Cucumis melo var. Birdie, were tested for their capacity for plant regeneration through direct organogenesis in vitro.

Excised cotyledons and embryos from imbibed, ungerminated seeds were cultured on Murashige and Skoog (MS) medium containing 1.0 - 5.0  $\mu$ M and 1.0 - 50.0  $\mu$ M





benzylaminopurine (BAP), kinetin, or isopentenyl adenine (2iP) respectively, in the presence or absence of 5.0  $\mu\text{M}$  gibberellic acid ( $\text{GA}_3$ ). Embryo explants produced callus on all media tested, and only 39% of cotyledon explants produced buds on medium containing 2.0 - 3.0  $\mu\text{M}$  BAP.

Unimbibed, testaless seeds were germinated aseptically on MS medium in the presence of 1.0 - 50.0  $\mu\text{M}$  BAP, kinetin or 2iP. On medium containing 3.0  $\mu\text{M}$  BAP, 68% of cultures produced small buds in the region of the cotyledonary node within three weeks. The addition of  $\text{GA}_3$  (5.0  $\mu\text{M}$ ) or naphthalene acetic acid (NAA) (0.1 - 1.5  $\mu\text{M}$ ) did not increase the percentage of cultures which produced buds.

Cotyledonary nodes were subcultured to media containing the same (3.0  $\mu\text{M}$ ) or lower concentrations of BAP for two weeks and then to MS basal medium for another two weeks to allow further bud development and shoot elongation. The highest number of shoots per cotyledonary node (9.7) was obtained when the first subculture medium contained 0.5  $\mu\text{M}$  BAP.

Individual shoots less than 1.0 cm, 1.0 - 2.5 cm and more than 2.5 cm in length, were tested for rooting



ability in the presence of 3.0  $\mu$ M IBA. The highest percentage rooting (49%) occurred in shoots more than 1.0cm long. Further studies on root induction showed that the highest percentage of rooting (85%), occurred when shoots 1.0 - 2.5 cm long were cultured in the light, on filter-paper bridges in liquid, half-strength MS medium containing 2.0  $\mu$ M NAA, without charcoal.

Complete plantlets were successfully transplanted to the glasshouse and grown in hydroponic culture. The plants flowered and produced normal fruit.



## ABSTRAK

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia bagi memenuhi sebahagian daripada syarat-syarat untuk memperolehi Ijazah Master Sains.

### IN VITRO CULTURE OF MUSKMELON

#### - CUCUMIS MELO VAR. BIRDIE

Oleh

ZULAINI ISMAIL

Mei, 1989

Penyelia : Dr. Zaliha Christine Alang  
Fakulti : Sains Makanan dan Bioteknologi

Beberapa jenis eksplan dari biji benih F1 dan anak benih aseptik muskmelon Cucumis melo var. Birdie digunakan untuk memastikan keupayaan kehidupan semula tumbuhan melalui organogenesis terus secara in vitro.

Kotiledon dan embrio dari biji benih yang dipedap air, dikultur ke atas medium Murashige dan Skoog (MS) mengandungi 1.0 - 5.0  $\mu\text{M}$  and 1.0 - 50.0  $\mu\text{M}$  benzilaminopurin (BAP), kinetin atau isopentenil adenin



(2iP) masing-masing, dengan kehadiran atau tanpa 5.0  $\mu\text{M}$  asid giberelik ( $\text{GA}_3$ ). Embrio menghasilkan kalus di atas semua medium yang digunakan dan hanya 39% dari kotiledon menghasilkan tunas di atas medium yang mengandung 2.0 dan 3.0  $\mu\text{M}$  BAP.

Biji benih tanpa testa yang tidak dipedap, dicambahkan secara aseptik di atas medium MS dengan kehadiran 1.0 - 50.0  $\mu\text{M}$  BAP, kinetin atau 2iP. Pada medium yang mengandung 3.0  $\mu\text{M}$  BAP, 68% kultur menghasilkan tunas-tunas kecil dikawasan nod kotiledon dalam masa tiga minggu. Penambahan  $\text{GA}_3$  (5.0  $\mu\text{M}$ ) atau asid asetik naftalin (NAA, 0.1 - 1.5  $\mu\text{M}$ ) tidak meningkatkan peratus kultur yang menghasilkan tunas.

Nod kotiledon disubkulturkan ke medium yang mengandung kepekatan BAP yang sama (3.0  $\mu\text{M}$ ) atau kurang, selama dua minggu dan kemudiannya ke medium asas MS selama dua minggu lagi untuk penerusan pembentukan tunas dan pemanjangan pucuk. Bilangan pucuk per nod kotiledon yang paling tinggi (9.7) diperolehi apabila medium subkultur pertamanya mengandung 0.5  $\mu\text{M}$  BAP.

Keratan pucuk-pucuk kurang dari 1.0 cm, 1.0 - 2.5 cm dan lebih dari 2.5 cm panjang, telah diuji untuk pengakaran dengan kehadiran 3.0  $\mu\text{M}$  (IBA). Peratus



pengakaran paling tinggi (49%) diperoleh pada pucuk yang panjangnya melebihi 1.0 cm. Kajian lanjut ke atas induksi pengakaran menunjukkan peratus yang paling tinggi (85%) diperoleh apabila pucuk 1.0 - 2.5 cm dikulturkan di dalam cahaya, di atas jambatan kertas turas di dalam medium cecair, setengah kepekatan medium MS mengandungi 2.0 uM NAA, tanpa charcoal.

Planlet telah dipindahkan ke rumah kaca dan dibesarkan dalam kultura hidroponik. Pokok tersebut telah berbunga dan menghasilkan buah yang normal.

## CHAPTER 1

### INTRODUCTION

The family Cucurbitaceae consists of about 90 genera and 750 species, almost equally divided between the New and Old World (Whitaker and Davis, 1962). The cultivated species of this family (Table 1) are not nearly as significant in man's economy as the cereals or the legumes, but they are important crops in the tropics, subtropics, and milder portions of the temperate zones of both hemispheres.

In 1980, commercial planting of cultivated cucurbits occupied 1.35 million ha. in more than 70 countries; of these 39.1% were pumpkins, squash, and gourds, and 60.9% cucumbers and gherkins.

Cultivated cucurbits have many diseases in common, which cause reduction in yield and quality. In some instances, a single organism is the causal agent, or different host species. Virus diseases that attack cucurbits are also numerous and widespread, and evoke tremendous crop losses. They are extremely difficult to



control; though various control measures have been attempted, none has yet proved really successful. Detailed information can be found in the treatise by Whitaker and Davis (1962).

**Table 1**

**The Botanical and Common Names of Some Cultivated Species of the Cucurbitaceae.**

Latin name	Common name
<u>Citrullus vulgaris</u> Schrad	Watermelon
<u>Cucumis sativus</u> L.	Cucumber
<u>Cucumis anguria</u> L.	West India gherkin
<u>Cucumis melo</u> L.	Muskmelon
<u>Luffa cylindrica</u> Roem.	Dish-rag gourd
<u>Lagenaria siceraria</u> (Mol.) Standl.	White-flowered gourd
<u>Cucubita pepo</u> L.	Winter squash, summer squash, pumpkin, marrow
<u>Cucurbita mixta</u> Pang	Winter squash, pumpkin
<u>Cucurbita moschata</u> Poir.	Winter squash, pumpkin
<u>Cucurbita maxima</u> Duch	Winter squash, marrow turban squash, pumpkin
<u>Cucurbita ficifolia</u> Bouche	Malabar gourd, fig-leaf gourd
<u>Sechium edule</u> SW.	Chayote

Source

Jelaska S., (1986)

The main objectives of breeding programmes are thus not only to increase yield and quality, but also to select cultivars resistant to pests and diseases. This is especially important in the absence of effective chemical control.

### Cucurbits in Malaysia

Many types of Cucurbits can be grown in Malaysia, including watermelon, cucumber and muskmelon.

Muskmelon is a crop well suited for production especially in hydroponic culture provided that adequate pest and disease control measures are taken. However, there is no local production of muskmelon seeds, and imported hybrid seeds are expensive - costing between 10 and 30 cents each, depending on the cultivar and seed source. The seed cost makes up between 5 - 10% of the cost of production under hydroponic culture and much more under conventional cropping methods.

A breeding program is currently in progress at U.P.M. for the production of local hybrid seeds in order to reduce the cost of seeds and to select varieties well suited to local tropical conditions. Each melon fruit is capable of producing about 300 seeds and each plant is allowed to produce two fruit. On the basis of the present average cost of seed, hybrid seeds produced from one plant would fetch a retail value of M\$100. The present hydroponic unit at UPM is planted with over 1,500 muskmelon plants per glasshouse.





In the production of hybrids, breeding lines remain the exclusive property of seed companies, and a fresh supply of hybrid seed has to be purchased from the supplier for each planting. It would thus be prudent to develop local breeding lines from the muskmelon germplasm available in Malaysia. A collection of about 50 source populations is currently being used to develop local breeding lines. Unfortunately, conventional breeding procedures for eventual combination into hybrids takes many generations. It is essential that the parental lines be genetically pure in order that they may be sexually maintained for the production of hybrids of consistent performance. If the parental lines can be maintained asexually, the breeding programme could be shortened tremendously. Besides enabling the testing of locally-produced heterozygous material, it would also allow the selected parents to be maintained indefinitely without loss of vigour.

Conventional vegetative propagation of muskmelon is slow and usually limited to the number of shoots available from a single parent plant, whereas micropropagation is capable of producing a large number of plantlets from a single plant.

