

**STUDIES ON THE PROPAGATION OF SOME
ORNAMENTAL PLANTS BY
TISSUE CULTURE**

BY

FAISAL MOHAMED ABDEL ALEAM SAADAWY
B. Sc. Agric. Sci. (Horticulture), Ain Shams Univ., 1965
M. Sc. Agric. Sci. (Floriculture), Al-Azhar Univ., 1975

**Thesis submitted in partial fulfillment
of
the requirements for the degree of
DOCTOR OF PHILOSOPHY
in
AGRICULTURAL SCIENCES
(ORNAMENTAL HORTICULTURE)**

**Department of Horticulture
Faculty of Agriculture
Ain Shams University**

2000

APPROVAL SHEET

FAISAL MOHAMED ABDEL ALEAM SAADAWY

M. Sc. Agric. Sci. (Floriculture), Al-Azhar Univ., 1975

Prof. Dr. Abou-Dahab M. Abou-Dahab

Prof. Dr. Mahmoud R. Shedeed

Prof. Dr. Mahmoud E. Hashim

Date of examination / / 2000

**STUDIES ON THE PROPAGATION OF SOME
ORNAMENTAL PLANTS BY
TISSUE CULTURE**

BY

FAISAL MOHAMED ABDEL ALEAM SAADAWY

B. Sc. Agric. Sci. (Horticulture), Ain Shams Univ., 1965

M. Sc. Agric. Sci. (Floriculture), Al-Azhar Univ., 1975

Under the supervision of :

Prof. Dr. Mahmoud E. Hashim

Prof. of Horticulture, Faculty of Agriculture
Ain Shams University

Prof. Dr. Mohamed K. A. Nada

Ex-Director of the Dept. of Ornamental Plants
Horticulture Research Institute
Agriculture Research Center, Ministry of Agriculture

Dr. Mohamed I. Ragab

Assoc. Prof. of Horticulture, Faculty of Agriculture
Ain Shams University

Abstract

Faisal Mohamed Abdel Aleam Saadawy, Studies on the Propagation of Some Ornamental Plants by Tissue culture. Unpublished Doctor of Philosophy Dissertation, Horticulture Dept., Fac. of Agric., Ain Shams Univ., 2000

Orchid flowers are the most fascinating and beautiful of all flowers. They are of highest value as cut-flowers and can be grown in garden beds, pots, hanging baskets, and indoor containers. Cultivation of orchid plants, both for plant sale as well as cut-flower production, has become a very profitable occupation. In spite of their commercial value, orchids have not yet gained the attention and popularity they deserve in Egypt.

Two orchid species, *Laelia anceps* and *Cymbidium devonianum* were used in this study. Results of this study could be abstracted in the following :

- 1 - For the multiplication of *Laelia anceps* and *Cymbidium devonianum* orchids by tissue culture technique it is better to use MS medium supplemented with 6-benzyl adenine (BA) at 1 ppm in order to encourage explant multiplication to get a lot of shoots.
- 2 - Rooting of the induced shoots could be achieved by growing these shoots on MS medium supplemented with naphthalene acetic acid (NAA) at 10 ppm.
- 3 - In order to reduce the high expenses of the tissue culture technique for economic purposes, MS Medium could be substituted by some natural, cheap and easily available products such as broad bean, wheat corn or rice flour. Chemical and hormonal analyses of these substances unveiled their richness of almost all elements represented in

the standard MS medium, in addition to their content of a naturally balanced phytohormones. Corn starch could be the source of sugars needed for the explants to grow, and it can be used to some extent instead of the expensive agar.

- 4 - To avoid great losses caused by contamination. 8-hydroxyquinoline sulphate (8-HQS) at 150 ppm could be used incorporated in MS medium. This procedure eliminate contamination and abolish the need to autoclave media. Besides its effectiveness in controlling contamination, it proved to have some beneficial effects on the multiplication and rooting of the plant material. The biochemical finger print showed that the dissimilarity coefficient between the 8-HQS-treated plantlets and the control was very small, indicating that this substances did not induce somaclonal variations.
- 5 - A lot of differences were found between the two orchids used, *Laelia anceps* and *Cymbidium devonianum*. The former was more responsive and easily to propagate than the later.

Key words: Orchids, *Laelia*, *Cymbidium*, BA, kinetin, IBA, NAA, media, natural complexes, 8-HQS, Physan 20. vilamen, cortex, stele, pith.

Acknowledgment

The writer wishes to express his sincere gratitude and appreciation to Professor Dr. Mahmoud El-Sayed Hashim, Professor of Floriculture, Department of Horticulture, Faculty of Agriculture, Ain Shams University for his outstanding supervision, valuable guidance and constructive suggestions given throughout the course of this study.

The writer is greatly indebted to Professor Dr. Mohamed Kamal Nada, Ex-Director of the Department of Floriculture, Horticulture Research Institute for his continuous support, sincere encouragement and valuable assistance.

Gratitude is also extended to Dr. Mohamed Imam Ragab Associate-Professor of Floriculture, Department of Horticulture, Faculty of Agriculture, Ain Shams University for his kind help, fruitful advices and particularly for permitting this work to be carried out in the Tissue Culture Laboratory, Strawberry Improvement Center.

Thanks and gratitude should also be given to Professor Dr. Alfred Ibrahim Messeha, Director of the Department of Floriculture, Horticulture Research Institute, Associate-Professor Azza El-Malt, Department of Floriculture, Horticulture Research Institute, and to the great physician Professor Dr. Kamal L. Samy for their kind and generous help in providing the plant material used in this work.

Gratitude and appreciation are due to Dr. Dale Sokkary, Director of Rio Grand Botanic Garden, Albuquerque , New Mexico , USA for his scientific help, cordial support and sincere advices.

I feel very obliged to the staff of the Tissue Culture Laboratory for Improvement of Strawberry and Unconventional Crops for their kind help and guidance throughout the course of this study.

Finally, I would like to express my thanks and appreciation to my family for their kind understanding, sincere sympathy and unlimited patience throughout the course of this work.

Contents

1. Introduction	1
2 . Review of Literature	5
2.1. Experiment 1 : Effect of Cytokinins on Multiplication	5
Use of Cytokinins for Multiplication of Different Explants	5
.....	
Protocorm-like Bodies Formation by Cytokinins.	6
Effect of Cytokinins on Shoot and Root Fresh Weight	9
2.2. Experiment 2 : Effect of Auxins on Rooting	10
Effect of Auxins on Roots	10
Effect of Auxins on Shoot Growth	13
2.3. Experiment 3 : Effect of Media	13
The use of Complex Organic mixtures	14
Effect of Sucrose Concentration	15
The Use of Agar in the Tissue Culture Media	16
Starch as a substitute for Agar and Sucrose	17
Solid Media vs. Liquid Media	17
Strength of the Medium	18
2.4. Experiment 4 : Effect of Biocides	20
Phyosan-20	21
8-hydroxyquinoline sulphate (8-HQS)	21
Disadvantages of Using Antibiotics	22
Other Chemicals as Anticontaminants	23
3. Materials and Methods	25
3.1. Source of Explants	25
3 .2. Preparation of Explants	26
3.3. Experiment 1 - Effect of Cytokinins on Multiplication	27
.....	
3.4. Experiment 2 - Effect of Auxins on Rooting	28
3.5. Experiment 3 - Effect of Media	29

3.6. Experiment 4 - Effect of Biocides	30
3.7. Comparison Between Two Orchids	31
4. Results and discussion	35
4.1. Experiment 1 - Effect of Cytokinins on Multiplication	35
4.2. Experiment 2 - Effect of Auxins on Rooting	66
4.3. Experiment 3 - Effect of Media	105
4.4. Experiment 4 - Effect of Biocides	143
4.5. Comparison Between <i>Laelia</i> and <i>Cymbidium</i>	155
5. Summary	162
6. References	169
7. Appendix	198
8. Arabic Summary	213

List of Tables

Experiment 1

1	Effect of Type and Concentration of Cytokinin on Number of Roots ..	36
2	Effect of Type and Concentration of Cytokinin on Root Length	36
3	Effect of Type and Concentration of Cytokinin on Average Number of Roots	42
4	Effect of Type and Concentration of Cytokinin on Weight of Propagules	42
5	Effect of Type and Concentration of Cytokinin on Number of Propagules	48
6	Effect of Type and Concentration of Cytokinin on Length of Propagule	48
7	Effect of Type and Concentration of Cytokinin on Number of Rooted Plantlets	57
8	Effect of Type and Concentration of Cytokinin on Percentage of Rooted Plantlets	57
9	Effect of Type and Concentration of Cytokinin on Chlorophyll Content	62

Experiment 2

10	Effect of Type and Concentration of Auxin on Root Weight	67
11	Effect of Type and Concentration of Auxin on Number of Roots	67
12	Effect of Type and Concentration of Auxin on Root Length	73
13	Effect of Type and Concentration of Auxin on Plantlet Weight	73
14	Effect of Type and Concentration of Auxin on Shoot Weight	79
15	Effect of Type and Concentration of Auxin on Shoot Length	79
16	Effect of Type and Concentration of Auxin on Number of Leaves	85
17	Effect of Type and Concentration of Auxin on Leaf Width	85
18	Effect of Type and Concentration of Auxin on Chlorophyll Content	90

....		
19	Effect of Type and Concentration of Auxin on Root Diameter	90
20	Effect of Type and Concentration of Auxin on Vilamen Thickness	96
....		
21	Effect of Type and Concentration of Auxin on Cortex Thickness	96
22	Effect of Type and Concentration of Auxin on Stele Diameter	100
.....		
23	Effect of Type and Concentration of Auxin on Pith Diameter	100
	Experiment 3	
24	Effect of Media and Cytokinin Application on Number of Roots	106
.....		
25	Effect of Media and Cytokinin Application on Root Length	111
26	Effect of Media and Cytokinin Application on Ave. Number of Roots	114
27	Effect of Media and Cytokinin Application on Weight of Propagules	118
..		
28	Effect of Media and Cytokinin Application on Number of Propagules	124
29	Effect of Media and Cytokinin Application on Length of Propagule	130
...		
30	Effect of Media and Cytokinin Application on Number of Rooted Plantlets	134
31	Effect of Media and Cytokinin Application on Percentage of Rooted Plantlets	137
32	Effect of Media and Cytokinin Application on Chlorophyl Content	140
....		
	Experiment 4	
33	Effect of Biocides	144
34	Effect of Biocides.....	144
35	Effect of Biocides	151
36	Comparison between Laelia and Cymbidium Orchids	156
37	Studies on some Anatomical Characters of Roots of Laelia and Cymbidium Orchids	156

List of Figures and Plates

Experiment 1

1	Effect of Type and Concentration of Cytokinin on Number of Roots	37
..		
	Effect of Type and Concentration of Cytokinin on Root Length	38
3	Effect of Type and Concentration of Cytokinin on Average Number of Roots	43
4	Effect of Type and Concentration of Cytokinin on Weight of Propagules	44
5	Effect of Type and Concentration of Cytokinin on Number of Propagules	49
6	Effect of Type and Concentration of Cytokinin on Length of Propagule	50
7	Effect of Type and Concentration of Cytokinin on Number of Rooted Plantlets	58
8	Effect of Type and Concentration of Cytokinin on Percentage of Rooted Plantlets	59
9	Effect of Type and Concentration of Cytokinin on Chlorophyll Content	63

Experiment 2

10	Effect of Type and Concentration of Auxin on Root Weight	68
11	Effect of Type and Concentration of Auxin on Number of Roots	69
12	Effect of Type and Concentration of Auxin on Root Length	74
13	Effect of Type and Concentration of Auxin on Plantlet Weight	75
14	Effect of Type and Concentration of Auxin on Shoot Weight	80
15	Effect of Type and Concentration of Auxin on Shoot Length	81
16	Effect of Type and Concentration of Auxin on Number of Leaves	86
17	Effect of Type and Concentration of Auxin on Leaf Width	87
18	Effect of Type and Concentration of Auxin on Chlorophyll Content	91

19	Effect of Type and Concentration of Auxin on Root Diameter	92
20	Effect of Type and Concentration of Auxin on Vilamen Thickness	97
21	Effect of Type and Concentration of Auxin on Cortex Thickness	98
22	Effect of Type and Concentration of Auxin on Stele Diameter	101
23	Effect of Type and Concentration of Auxin on Pith Diameter	102
	Experiment 3	
24	Effect of Media and Cytokinin Application on Number of Roots	107
25	Effect of Media and Cytokinin Application on Root Length	112
26	Effect of Media and Cytokinin Application on Ave. Number of Roots	115
27	Effect of Media and Cytokinin Application on Weight of Propagules ..	119
28	Effect of Media and Cytokinin Application on Number of Propagules	125
29	Effect of Media and Cytokinin Application on Length of Propagule ...	131
30	Effect of Media and Cytokinin Application on Number of Rooted Plantlets	134
31	Effect of Media and Cytokinin Application on Percentage of Rooted Plantlets	138
32	Effect of Media and Cytokinin Application on Chlorophyll Content	141
	Experiment 4	
33	Effect of Biocides	145
34	Effect of Biocides.....	146
35	Effect of Biocides	152
36	Comparison between Laelia and Cymbidium Orchids	157
37	Comparison between Laelia and Cymbidium Orchids	158
38	Comparison between Laelia and Cymbidium Orchids	159
	Plate 1 - Peaks of the hormonal analysis - Acidic fraction	203
	Plate 2 - Peaks of the hormonal analysis - basic fraction	204

Plate 3 - Effect of cytokinins on Laelia and Cymbidium orchids	205
Plate 4 - Effect of auxins on Laelia and Cymbidium orchids	206
Plate 5 - Effect of media on Laelia orchid	207
Plate 6 - Effect of media on Cymbidium orchid	208
Plate 7 - Effect of biocides on Laelia and Cymbidium orchids	209
Plate 8 - Comparison between Laelia and Cymbidium orchids	210
Plate 9 - Effect of auxins on anatomical features of roots	211
Plate 10 - Effect of auxins on anatomical features of roots	212
Plate 11 - Effect of auxins on anatomical features of roots	213
Plate 12 - Diagram of the root cross section of Laelia and Cymbidium	214

1 . Introduction

Orchids are the most fascinating and beautiful of all flowers. They exhibit a wide range of diversity in form, size, color, and texture of flowers. Growing orchid is a fascinating hobby throughout the world. There is joy in watching new growths and roots, waiting for an offshoot to become a mature plant and seeing the new hybrids in flower. Orchids are excellent items for garden and can be grown in beds, pots, hanging baskets, split hollows of bamboo pieces and many other kinds of containers. They are the most beautiful items for indoor decoration. Orchids, having flowers of wonderful beauty and very good keeping qualities, are of highest value as cut-flowers. Some orchid flowers last for 1-3 month if remain attached to the plant, and as cut-flowers they remain fresh for 1-6 weeks.

Cultivation of orchids, both for plant sale as well as cut-flower production, has become a very profitable occupation, millions of dollars worth, to many south east Asian countries. Development of new hybrids and commercial production of cut-flowers in orchids have expanded tremendously in Europe, USA, South America, Thailand, Singapore, Malaysia, Japan and Sri Lanka. In spite of their commercial value, orchids have not yet gained the attention and popularity they deserve in Egypt. They are almost confined to a number of amateurs interested in growing these lovely plants in their small private greenhouses by the conventional propagation methods.

Orchids belong to the monocotyledonous family “Orchidaceae”, the second largest family in the plant kingdom. They are perennial herbs of varying habits. In tropical regions they are found frequently on trees and shrubs and sometimes