PROPAGATION OF CALLA (*ZANTEDESCHIA* SP.) PLANTS BY TISSUE CULTURE TECHNIQUE

By

NERMEEN EL–SAYED ABDUL–MONEEM ABDUL-QADER
B. Sc. Agric. Sc. (Horticulture), Ain Shams University, 1999
M.Sc. Agric. Sc. (Ornamental Plants), Ain Shams University, 2005

A thesis submitted in partial fulfillment
of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Agricultural Sciences (Ornamental Plants)

Department of Horticulture
Faculty of Agriculture
Ain Shams University

2014

Approval Sheet

Propagation of Calla (*Zantedeschia* sp.) Plants by Tissue Culture Technique

By

NERMEEN EL–SAYED ABDUL–MONEEM ABDUL-QADER
B. Sc. Agric. Sc. (Horticulture), Ain Shams University, 1999
M.Sc. Agric. Sc. (Ornamental Plants), Ain Shams University, 2005

This Thesis for Ph.D. degree has been approved by:

Dr. Emam Mohamed Saber Nofal .....
Prof. Emeritus of Ornamental Plants, Faculty of Agriculture, Khafr El Sheikh University

Dr.Hassan Mohamed Fadel El wakeel ……
Prof. of Pomology Plants, Faculty of Agriculture, Ain Shams University

Dr. Sohair El-Sayed Mohamed Hassan ….....
Prof. Emeritus of Ornamental Plants, Faculty of Agriculture, Ain Shams University

Date of Examination: / / 2014

PROPAGATION OF CALLA (*ZANTEDESCHIA* SP.) PLANTS BY TISSUE CULTURE TECHNIQUE

By

NERMEEN EL–SAYED ABDUL–MONEEM ABDUL-QADER

B. Sc. Agric. Sc. (Horticulture), Ain Shams University, 1999

M.Sc. Agric. Sc. (Ornamental Plants), Ain Shams University, 2005

Under the supervision of:

Dr. Sohair El-Sayed Mohamed Hassan

Prof. Emeritus of Ornamental Plants, Department of Horticulture, Faculty of Agriculture, Ain Shams University (Principal Supervisor)

Dr. Ayman Kamal Ibrahim

Assistant Prof of Ornamental Plants, Department of Horticulture, Faculty of Agriculture, Ain Shams University

Dr. Faisal Mohamed Abdel-Aleam Saadawy

Head of Research Emeritus, Ornamental Plant and Landscape Design Research Department, Horticulture Research Institute, Agricultural Research Center

ACKNOWLEDGEMENT

First and foremost, I feel always indebted to Allah, the best beneficent and merciful God.

This work is dedicated to the souls of my father, my mother and Prof. Dr. Mahmoud Hashim, may Allah be merciful to them.

I would like to express my gratitude to **Prof. Dr. Sohair El-Sayed Mohamed Hassan**, Professor of Ornamentals, Faculty of Agriculture, Ain Shams University for suggesting the current study, her supervision, valuable guidance, kind support, continuous help during the whole work and revision of this manuscript.

Deep thanks to **Dr. Ayman Kamal Ibrahim**, Lecturer of Ornamental Plants, Faculty of Agriculture, Ain Shams University for his supervision, scientific guidance and sincere intentions.

Deep thanks are offered to **Prof.Dr. Faisal Mohamed Abdel-Aleam Saadawy,** Head Researcher Emeritus in the Horticulture Research Institute (HRI), Agriculture Research Center, Giza, for his supervision, scientific remarks, following up during all stages of laboratory work and the great efforts in writing, statistical analysis and financial support through the HRI.

I am deeply indebted to the Director of Tissue Culture Laboratory for all facilities and guidance I found in this lab.

Finally, I would like to thank all **my family** for their supporting me with love and encouragement. The least I can do is to dedicate this work to them.

I can't forget **my colleagues** and **friends** who offered me their help and encouragements. My best wishes for them.

ABSTRACT

Nermeen El-Sayed Abdul-Moneem : Propagation of Calla (*Zantedeschia sp.*) Plants by Tissue Culture Technique. Unpublished Ph.D.theses, Department of Horticulture, Faculty of Agriculture, Ain Shams University, 2014.

This work was carried out in the Tissue Culture Laboratory, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt during the period from 2008 to 2013.

In-vitro produced explants of *Zantedeschia rehmannii* cv. Hot Cherry" were obtained from the Tissue Culture Laboratory of Zohriya Garden, where they were subcultured on MS medium supplemented with BA at 4 ppm. However, explants remained unresponsive, lost their green color and became pale. To solve this problem, the effect of N, Fe and Mg levels in MS Medium was investigated. Further experiments on multiplication, rooting and tuberization were carried out. Results of all these experiments could be briefed in the following.

Raising the level of NH_4NO_3 in MS medium to 175% of the original one resulted in the highest values of total number of shoots and total number of leaves and reasonable chlorophyll content.

It is much better to use MS medium with the normal levels of both Fe and Mg contents.

Using BA at 4 ppm in the multiplication stage proved to be the better choice as it in resulted the best number of shoots, total fresh weight of shoots and total number of leaves. Cytokinin-free media failed to produce any number of shoots at all.

Using IBA at 5 ppm in the rooting stage guaranteed the highest values in regard to number of roots, root length, fresh weight of roots and percentage of rooting, in addition to the least number of days to rooting.

Adjusting incubation temperature to 20 °C resulted in the highest number of mini-tubers when sucrose level was 60 g/l. On the other hand, raising incubation temperature to 25 °C gave rise to the heaviest fresh

weight of mini-tubers at 60 g/l sucrose and to the highest mini-tuber diameter at 70 g/l sucrose.

The highest values of number of shoots and total number of leaves were a result of using sucrose at 30 g/l and BA at 4 ppm, while the greatest number of mini-tubers and percentage of tuber formation was obtained when sucrose at 90 g/l and BA at 4 ppm were applied.

The highest records of shoot length, fresh weight of shoot, shoot diameter, number of leaves, number of mini-tubers, mean fresh weight of mini-tuber, mini-tuber diameter and chlorophyll content were obtained when 3 g/l of KNO_3 were added to MS medium. The quickest tuber formation took place when KNO_3 at 4 g/l was added.

Key Words: BA, FeSO4.7H2O , IBA, incubation temperature, KNO_3, MgSO4.7H2O, mini-tuber, MS medium, NH_4NO_3, tissue culture and *Zantedeschia*.

ABBREVIATIONS

BA, BAP:	Benzyl adenine
DKW:	Driver Kuniyuki Walnut
IAA:	Indol-3-acetic acid
IBA:	Indol-3-butyric acid
Ki, Kin, KT:	Kinetin
L2:	Phillips and Collins medium
Meq:	Milliequivalent
MS:	Murashige and Skoog medium
MSB:	Mitis salivarius bacitracin medium
NAA:	Naphthalene acetic acid
N.B.:	Nota bene
TDZ:	Thidiazuron
WH:	White's medium
WPM :	Woody plant medium

CONTENTS

		Page
	LIST OF TABLES…….	v
	LIST OF FIGURES……..	viii
	LIST OF PLATES…….	xvi
	LIST OF ABBREVIATIONS……	xvii
1.	**INTRODUCTION ……...…**	1
2.	**REVIEW OF LITERATURE ……..**	6
3.	**MATERIALS AND METHODS ……**	15
4.	**RESULTS AND DISCUSSION ……..…**	19
	Experiment 1. Effect of level of NH_4NO_3 in MS medium	19
	1– Effect of NH_4NO_3 level in MS medium on number of shoots ...…….……..…..…	19
	2– Effect of NH_4NO_3 level in MS medium on shoot length	20
	3– Effect of NH_4NO_3 level in MS medium on total fresh weight of shoots ……	21
	4- Effect of NH_4NO_3 level in MS medium on total number of leaves ……..	21
	5- Effect of NH_4NO_3 level in MS medium on chlorophyll content (SPAD units) ……..……..	22
	Experiment 2. Effect of $FeSO_4.7H_2O$ levels in MS medium on growth ……	26
	1- Effect of $FeSO_4.7H_2O$ level in MS medium on number of shoots ..…….	26
	2- Effect of $FeSO_4.7H_2O$ level in MS medium on shoot length …….	26
	3-Effect of $FeSO_4.7H_2O$ level in MS medium on total fresh weight of shoots …….	26
	4- Effect of $FeSO_4.7H_2O$ level in MS medium on total number of leaves ……..	27
	Experiment 3. Effect of $MgSO_4.7H_2O$ level in MS medium on growth ……	30
	1- Effect of $MgSO_4.7H_2O$ level in MS medium on propagules weight ……..……	30
	Experiment 4. Effect of cytokinin type, concentration and	

their interaction on multiplication …… 33
1- Effect of cytokinin type, concentration and their interaction on number of shoots …… 33
2- Effect of cytokinin type, concentration and their interaction on shoot length ……. 41
3- Effect of cytokinin type, concentration and their interaction on total fresh weight of shoots ……. 46
4- Effect of cytokinin type, concentration and their interaction on total number of leaves …….. 50
Experiment 5: Effect of auxin type, concentration and their interaction on rooting …… 55
1- Effect of auxin type, concentration and their interaction on number of roots ……. 55
2- Effect of auxin type, concentration and their interaction on root length ……. 59
3- Effect of auxin type, concentration and their interaction on fresh weight of roots …… 63
4- Effect of auxin type, concentration and their interaction on root diameter …… 66
5- Effect of auxin type, concentration and their interaction on number of days to root formation 69
6- Effect of auxin type, concentration and their interaction on percentage of rooting…… 71
Experiment 6: Effect of incubation temperature, sucrose concentration and their interaction on mini-tuber formation …….. 77
1.- Effect of incubation temperature, sucrose concentration and their interaction on number of mini-tubers …… 77
2- Effect of incubation temperature, sucrose concentration and their interaction on fresh weight of mini-tubers … 82
3. Effect of incubation temperature, sucrose concentration and their interaction on mini-tuber diameter ….. 87
Experiment 7: 1. Effect of sucrose and BA concentrations on tuber formation ……. 92
1- Effect of sucrose and BA concentrations and their 92

interaction on number of shoots ……
2- Effect of sucrose and BA concentrations and their interaction on shoot length …… 95
3- Effect of sucrose and BA concentrations and their interaction on total fresh weight of shoots …… 99
4- Effect of sucrose and BA concentrations and their interaction on shoot diameter …….. 102
5- Effect of sucrose and BA concentrations and their interaction on total number of leaves …… 105
6- Effect of sucrose and BA concentrations and their interaction on number of mini-tubers …… 108
7-Effect of sucrose and BA concentrations and their interaction on total fresh weight of mini-tubers 113
8- Effect of sucrose and BA concentrations and their interaction on mini-tuber diameter …… 117
9. Effect of sucrose and BA concentrations and their interaction on percentage of tuber formation 120
Experiment 8: Effect of KNO_3 level in MS medium on growth ……..…….. 125
1- Effect of KNO_3 level in MS medium on shoot length .. 126
2- Effect of KNO_3 level in MS medium on total fresh weight of shoot ……. 126
3- Effect of KNO_3 level in MS medium on shoot diameter 126
4- Effect of KNO_3 level in MS medium on total number of leaves ……. 127
5- Effect of KNO_3 level in MS medium on number of mini-tubers …… 127
6- Effect of KNO_3 level in MS medium on mean fresh weight of mini-tuber …… 128
7- Effect of KNO_3 level in MS medium on mini-tuber diameter ……. 129
8- Effect of KNO_3 level in MS medium on chlorophyll content (SPAD units) …….. 130
9- Effect of KNO_3 level in MS medium on number of days 130

to mini-tuber formation ……
5. **SUMMARY and CONCLUSION** …… 137
6. **REFERENCES** …….. 142
7. **ARABIC SUMMARY** …….. 139

LIST OF FIGURES

		page
Fig. (1)	Effect of NH_4NO_3 level in MS medium on number of shoots of *Zantedeschia rehmannii* cv. Hot Cherry …….	22
Fig. (2)	Effect of NH_4NO_3 level in MS medium on shoot length (cm) of *Zantedeschia rehmannii* cv. Hot Cherry ……	23
Fig. (3)	Effect of NH_4NO_3 level in MS medium on total fresh weight of shoots (g) of *Zantedeschia rehmannii* cv. Hot Cherry ……	23
Fig. (4)	Effect of NH_4NO_3 level in MS medium on number of leaves of *Zantedeschia rehmannii* cv. Hot Cherry	24
Fig. (5)	Effect of NH_4NO_3 level in MS medium on chlorophyll content (SPAD units) of *Zantedeschia rehmannii* cv. Hot Cherry	24
Fig. (6)	Effect of $FeSO_4.7H_2O$ level in MS medium on number of shoots of *Zantedeschia rehmannii* cv. Hot Cherry	28
Fig. (7)	Effect of $FeSO_4.7H_2O$ level in MS medium on shoot length (cm) of *Zantedeschia rehmannii* cv. Hot Cherry ……..	28
Fig. (8)	Effect of $FeSO_4.7H_2O$ level in MS medium on fresh weight of shoots (g) of *Zantedeschia rehmannii* cv. Hot Cherry ……	29
Fig. (9)	Effect of $FeSO_4.7H_2O$ level in MS medium on number of leaves of *Zantedeschia rehmannii* cv. Hot Cherry ……	29
Fig. (10)	Effect of $MgSO_4.7H_2O$ level in MS medium on fresh weight of propagules (g) of *Zantedeschia rehmannii* cv. Hot Cherry	31

Fig. (11.a)	Effect of cytokinin type on number of shoots of *Zantedeschia rehmannii* cv. Hot Cherry …..	34
Fig. (11.b)	Effect of cytokinin concentration on number of shoots of *Zantedeschia rehmannii* cv. Hot Cherry ……..	35
Fig. (11.c)	Effect of interaction between cytokinin type and concentration on number of shoots of *Zantedeschia rehmannii* cv. Hot Cherry …….	35
Fig. (12.a)	Effect of cytokinin type on shoot length (cm) of *Zantedeschia rehmannii* cv. Hot Cherry …..	43
Fig. (12.b)	Effect of cytokinin concentration on shoot length (cm) of *Zantedeschia rehmannii* cv. Hot Cherry …	44
Fig. (12.c)	Effect of interaction between cytokinin type and concentration on shoot length (cm) of *Zantedeschia rehmannii* cv. Hot Cherry ……	44
Fig. (13.a)	Effect of cytokinin type on fresh weight of shoots (g) of *Zantedeschia rehmannii* cv. Hot Cherry	48
Fig. (13.b)	Effect of cytokinin concentration on fresh weight of shoots (g) of *Zantedeschia rehmannii* cv. Hot Cherry ……..	49
Fig. (13.c)	Effect of interaction between cytokinin type and concentration on total fresh weight of shoots (g) of *Zantedeschia rehmannii* cv. Hot Cherry	49
Fig. (14.a)	Effect of cytokinin type on total number of leaves of *Zantedeschia rehmannii* cv. Hot Cherry …	51
Fig. (14.b)	Effect of cytokinin concentration on number of leaves of *Zantedeschia rehmannii* cv. Hot Cherry ……..	52
Fig. (14.c)	Effect of interaction between cytokinin type and concentration on total number of leaves of *Zantedeschia rehmannii* cv. Hot Cherry ……	52
Fig. (15.a)	Effect of auxin type on number of roots of	56

Zantedeschia rehmannii cv. Hot Cherry

Fig. (15.b) Effect of auxin concentration on number of roots of *Zantedeschia rehmannii* cv. Hot Cherry 57

Fig. (15.c) Effect of interaction between auxin type and concentration on number of roots of *Zantedeschia rehmannii* cv. Hot Cherry 57

Fig. (16.a) Effect of auxin type on root length (cm) of *Zantedeschia rehmannii* cv. Hot Cherry 61

Fig. (16.b) Effect of auxin concentration on root length (cm) of *Zantedeschia rehmannii* cv. Hot Cherry … 61

Fig. (16.c) Effect of interaction between auxin type and concentration on root length (cm) of *Zantedeschia rehmannii* cv. Hot Cherry ……. 62

Fig. (17.a) Effect of auxin type on fresh weight of roots (g) of *Zantedeschia rehmannii* cv. Hot Cherry 64

Fig. (17.b) Effect of auxin concentration on fresh weight of roots (g) of *Zantedeschia rehmannii* cv. Hot Cherry ……. 65

Fig. (17.c) Effect of interaction between auxin type and concentration on fresh weight of roots (g) of *Zantedeschia rehmannii* cv. Hot Cherry ……. 65

Fig. (18.a) Effect of auxin type on root diameter (mm) of *Zantedeschia rehmannii* cv. Hot Cherry 67

Fig. (18.b) Effect of auxin concentration on root diameter (mm) of *Zantedeschia rehmannii* cv. Hot Cherry 68

Fig. (18.c) Effect of interaction between auxin type and concentration on root diameter (mm) of *Zantedeschia rehmannii* cv. Hot Cherry ……. 68

Fig. (19.a) Effect of auxin type on number of days to root formation of *Zantedeschia rehmannii* cv. Hot Cherry ……. 70

Fig. (19.b) Effect of auxin concentration on number of days to 70