Name: Ibrahim Mohamed Shams El Din Ali. Degree: Ph.D.

Title of Dissertation: "Biotechnological studies on the insectivorous plants and their effect on the growth of date palm using tissue culture techniques"

Supervisors: Ibrahim A. Ibrahim¹; Hamdy A. Emara¹; Abd El Moneem A. M. El Banna².

1-Plant Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), Menoufiya University. 2 - Central Laboratory of Date Palm Research and Development, Agricultural Research Center, Giza, Egypt.

Department: Plant Biotechnology. **Branch:** Plant Cell, Tissue and Organ Culture.

ABSTRACT

Carnivorous or insectivorous plants belong to several botanical families, the most important of them is Droseraceae, which includes *Drosera* plants. Some economic substances are extracted from Drosera. Tissue culture technique provides the best way to obtain as high and clean quantity as possible of the biomass needed to obtain these substances. This study aimed to propagate the Drosera capensis in vitro and evaluate its content from phytohormones and total amino acids, indoles and phenols. Also studying the effect of Drosera capensis leaf and root extracts as plant growth substances on in vitro growth of Phoenix dactylifera cv. Bartamouda as one of the most important crops. And studying the effect of Drosera capensis residue on larva of red palm weevil. Shoots were visible on leaf explants, apparently forming directly on leaf surfaces without intermediate callus. The best results of shoot number (13.8 shoots per explant) and shoot length (2.93 cm) were obtained at 0.05 mg L⁻¹ BA compared with the control, BA-free media, observed 2.8 shoots per explant and 2.27 cm in length. MS basal medium supplemented with 1.0 mg L^{-1} IBA achieved the best root formation where the root number was 47.3 per plant. Using of $\frac{1}{2}$ MS medium supplemented with NAA at 0.25 mg L⁻¹ and BA at 0.3 mg L⁻¹ gave rise to biggest callus weight. The amounts of phytohormones in *Drosera capensis* plant (mg 100g⁻¹ fresh weight) were as following: Indole acetic acid in leaf was 2.055 mg, while in case of root

was 2.291 mg. Zeatine in leaf was 1.609 mg, while in case of root was 0.418 mg. Other Cytokinins in leaf was 18.791 mg, while in case of root was 1.003 mg. Gibberellic acid in leaf was 70.938 mg, while in case of root was 86.59 mg. Abscissic acid in leaf was 0.500 mg, while in case of root was 0.158 mg. The amount (mg 100g⁻¹ fresh weight) of total amino acids in leaves was 200 mg, while in case of roots were 100 mg. The amount of total indoles in leaves was 17 mg; while in case of roots was 11 mg. The amount of total phenols in leaves was 0.05 mg; while in case of root was 0.02 mg. Concentrations of the extract of Drosera capensis leaves and roots were applied at different ratios in *in vitro* experiments of date palm cv. Bartamouda. The results revealed that Drosera capensis root extract had a significant effect on fresh weight of date palm embryogeneic callus as the best result (4.63g) was observed with using Drosera capensis root extract at 3.0ml L⁻¹ (0.042g residue). Using of Drosera capensis root extract at 0.05ml L⁻¹ (0.0007g residue) gave rise to higher number of mature embryos. The highest significant shoot number (21 shoots) of date palm was obtained with using 1.0ml L^{-1} (0.01g residue) Drosera capensis leaf extract. Also the length of date palm shoots increased significantly by using the same concentration of Drosera capensis leaf extract and reached 3.3cm. In rooting stage, the best result was obtained with the use of Drosera capensis root extract at 1.0ml L^{-1} (0.014g residue). In acclimatization stage, the best result was obtained with the use of Drosera *capensis* root extract at 1.0ml L^{-1} (0.014g residue). Finally, *in vitro* date palm cultivation can be achieved with MS medium supplemented with Drosera capensis extract as a source of phytohormones at different micropropagation stages. The residue of Drosera capensis plants at different concentrations (0.0, 50.0, 100. 500.0 mg per liter) had been given to fully developed larvae of red palm weevil (Rhynchophorus ferrugineus Oliv.) through their feeding diet. Larvae were obtained from the field and

were maintained on the stems of sugarcane prior to mass rearing, artificial diet, which was formulated from sucrose, molasses, potatoes and agar. The residue of *Drosera capensis* had toxicological effects on *R. ferrugineus* larvae. The lethal action of *Drosera capensis* residue had appeared clearly at 500 mg L⁻¹ where the lethal percentage of red palm weevil larva was 65% after ten days.

Key words: *Drosera capensis;* Shoot multiplication; Benzyl adenine; Rooting stage; Indole butyric acid; *Drosera capensis* extract; Phytohormones; *In vitro*; date palm cv. Bartamouda; Embryogeneic callus; Mature embryos; *Rhynchophorus ferrugineus* Oliv.

CONTENTS

No.	Subject	Page
1	INTRODUCTION	1
2	REVIEW OF LITERATURE	6
2-1	Identification of carnivorous plants.	6
2-2	Effect of BA concentration on shoot multiplication of cape sundew	17
	(Drosera capensis).	
2-3	Effect of IBA concentration on rooting of cape sundew.	21
2-4	Effect of NAA and BA concentration on callus formation.	23
2-5	Identification of phytohormones.	25
2-6	Importance of Drosera spp. (specifying their substances in an attempt	26
	to use them as active gradient).	
2-7	Using natural substances in plant tissue culture.	30
2-8	Micropropagation of date palm.	35
2-9	Using plant extracts as insecticides.	47
3	MATERIAL AND METHODS	
4	RESULTS AND DISCUSSION	72
4-1	Propagate the Drosera capensis plant in vitro and evaluates their	72
	contents from phytohormones, total amino acids, indoles, and phenols.	
4-1-1	Effect of BA concentration on multiplication of Drosera capensis.	72
4-1-1-1	Effect of BA on shoot number of Drosera capensis.	72
4-1-1-2	Effect of benzyl adenine on shoot length(cm) of Drosera capensis.	74
4-1-2	Effect of IBA concentration on root formation of Drosera capensis.	77
4-1-2-1	Effect of IBA on root number of Drosera capensis.	77
4-1-2-2	Effect of IBA on root length of Drosera capensis.	80
4-1-2-3	Effect of IBA on shoot length of Drosera capensis.	81

No.	Subject	Page
4-1-3	Effect of NAA and BA concentration on callus fresh weight	83
	of <i>Drosera capensi<u>s</u>.</i>	
4-1-3-1	Effect of NAA and BA concentration on callus weight of Drosera	83
	capensis leaves after four weeks.	
4-1-3-2	Effect of NAA and BA concentration on callus weight of Drosera	86
	capensis leaves after six weeks.	
4-1-3-3	Effect of NAA and BA concentration on callus weight of Drosera	89
	capensis leaves after eight weeks.	
4-1-4	Evaluation of Drosera capensis extract.	94
4-1-4-1	The qualitative and quantitative analysis of phytohormones by high	94
	performance liquid chromatography.	
4-1-4-2	Determination of total amino acids, indoles, and phenol compounds in	100
	leaf and root of Drosera capensis by using a spectrophotometer.	
4-2	Micropropagation of date palm using Drosera capensis extract.	101
4-2-1	Effect of Drosera capensis root extract concentration on fresh weight	101
	(g) of embryogenic callus of date palm cv. Bartamouda.	
4-2-2	Effect of Drosera capensis root extract concentration on number	105
	of mature embryos of date palm cv. Bartamouda in production	
	of embryos stage.	
4-2-3	Effect of Drosera capensis leaf extract concentration on shoot	108
	formation stage.	
4-2-3-1	Effect of <i>Drosera capensis</i> leaf extract concentration on shoot number	108
	of date palm cv. Bartamouda in shoot formation stage.	
4-2-3-2	Effect of <i>Drosera capensis</i> leaf extract concentration on shoot length	111
	of date palm cv. Bartamouda.	

No.	Subject	Page
4-2-4	Effect of <i>Drosera capensis</i> root extract concentration on rooting stage.	112
4-2-4-1	Effect of Drosera capensis root extract concentration on root number	112
	of date palm cv. Bartamouda.	
4-2-4-2	Effect of Drosera capensis root extract concentration on root length of	115
	date palm cv. Bartamouda.	
4-2-5	Effect of Drosera capensis root extract concentration on survival	117
	percentage of date palm cv. Bartamouda after acclimatization stage.	
4-3	Effect of Drosera capensis residue concentration on lethal percentage	120
	of RPW in vitro as an evaluation of their using in bio-resistance.	
5	SUMMARY	125
6	CONCLUSION	131
7	LITERATURE CITED	133
8	ARABIC SUMMARY	160

LIST OF ABBREVIATIONS

2,4-D	2, 4-Dichlorophenoxyacetic acid
2iP	6-(y-y-Dimethylallylamino)purine
AC	Activated charcoal
ВА	Benzyladenine (6-Benzylaminopurine)
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
Kin	Kinetin(6-furfuryl aminopyrine)
mg L ⁻¹	Milligram per liter
MS medium	Murashige and Skoog medium (1962)
NAA	α -Naphthalene acetic acid
NOA	naphthoxyacetic acid
PGRs	Plant growth requlators
RT	Room temperature
TDZ	Thidiazuron (1-phenyl-3- [1,2,3-thiadiazol-5-yl] urea)
Zea	Zeatin, 6-[4-hydroxy-methyl-but-ethylamino]purine
μΜ	Micro mole