

PHYTOHORMONES SCREENING OF *Drosera capensis* AS INSECTIVOROUS PLANTS AND ITS EXTRACT EFFECT ON *in-vitro* GROWTH AND DEVELOPMENT OF DATE PALM (*Phoenix dactylifera* cv. Bartamouda)

Ibrahim A .Ibrahim**; *Hamdy A. Emara**; *Abdel-Moneam M. El-Banna and *Ibrahim M. Shams El-Din*****

* Plant Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), Minufiya University.

** Central Laboratory of Date Palm Research and Development, Agricultural Research Center, Giza, Egypt.

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 evaluate the Drosera capensis content from phytohormones. Also studying the effect of Drosera capensis leaf and root extracts as plant growth substances on in-vitro growth of one of the most important crops that is Phoenix dactylifera cv. Bartamouda.

The amounts of phytohormones in this plant (mg 100g⁻¹ fresh weight) were as following: Indole acetic acid in leaf was 2.055, while in case of root was 2.291. Zeatine in leaf was 1.609, while in case of root was 0.418. Other cytokinins in leaf was 18.791, while in case of root was 1.003. Gibberellic acid in leaf was 70.938, while in case of root was 86.59. Abscissic acid in leaf was 0.500, while in case of root was 0.158. 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 embryogenic callus as the best result (4.63 g) was observed with using Drosera capensis root extract at 3.0 ml L⁻¹. Using of Drosera capensis root extract at 0.05 ml L⁻¹ gave rise to higher number of mature embryos. The highest significant shoot number (21 shoots) of date palm was obtained with using 1.0 ml L⁻¹ 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.

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.

Key words: *Drosera capensis* extract, Phytohormones, *In-vitro*, date palm cv. Bartamouda, *Embryogenic calli*, Mature embryos, Shoot formation.

INTRODUCTION

The *in-vitro* growth and development of a plant is determined by a number of complex factors: The genetic make-up of the plant; Physical growth factors (Light -Temperature - CO₂ - pH); Nutrients: water, macro and micro elements and sugars and some organic substances (plant growth regulators, vitamins, amino acids). Media used in tissue culture are composed of a high number of ingredients such as macro and micro-nutrient salts, plant-growth regulators, amino acids, vitamins, sugar, gelling agents and activated charcoal (Pireik, 1987). Most of these compounds are expensive especially plant growth regulators, so some workers tried to use natural-organic products as a substitute to these ingredients. Hassan *et al.* (2008) reported that Pollen extracts of date palm were used in tissue culture medium for banana as growth substances in comparison with growth regulators. Most of treated plants showed highly growth characteristics such as shoot number, shoot length, root number, root length, fresh weight and dry weight compared to either the cytokinin (Benzyl Adenine) and/or the auxin (Indole Butyric Acid and Naphthalene Acetic Acid). El-Assar *et al.* (2004) studied the effects of natural extract Date Palm meristematic tissue on length, diameter, and color of "Sewi" apical tip growing tissues. They found that the extract of date palm meristematic tissue produced significant increasing of growing tissues. The carnivorous-medicinal plant *Drosera capensis* (Cape Sundew) contains many of important substances that have a huge economic importance because their useful effect in agriculture and drug manufacture to cure many diseases (Roberto (1984); Bunney (1992); Marczak *et al.* (2005); Jindaprasert *et al.* (2008)).

Date palm (*Phoenix dactylifera* L.), a monocotyledonous and dioecious species belonging to the Arecaceae family, is widely cultivated in arid regions of the Middle East and North Africa (Al-Khayri, 2001) and it needs especial media through its micropropagation. This study aimed to evaluate the *Drosera capensis* content from phyto-hormones and using its

plant extract, as a source of plant growth regulators containing MS medium, on *in-vitro* growth and development of date palm (*Phoenix dactylifera* cv Bartamouda).

MATERIALS AND METHODS

This study was conducted at the Central Laboratory of Development of Date Palm Research, Agricultural Research Center, Giza, Egypt and Genetic Engineering and Biotechnology Research Institute (GEBRI), Minufiya University, Sadat city during the period from 2006 to 2010.

In-vitro Cultures of *Drosera capensis* plants were established on half strength of MS (Murashige and Skoog, 1962) basal medium according to the method described by Shams El-Din (2002). *In-vitro* shoots were multiplied for six subcultures and transferred to rooting stage. Plantlets produced from rooting stage were subjected for extraction of samples then separated with HPLC. *Drosera capensis* extraction from leaves and roots were used as a source of plant growth regulators to *in-vitro* propagation of date palm cv. Bartamouda.

Acidic plant hormones, indole acetic acid (IAA), gibberellic acid (GA₃) and abscisic acid (ABA) and cytokinins were extracted from *Drosera capensis* plants (leaves and roots) and determined according to the method described by Shindy and Smith (1975) using high performance liquid chromatography (HPLC) at Arid Land Agricultural Research Unit, Faculty of Agriculture, Ain Shams University.

Micropropagation of date palm using *Drosera capensis* extract:

Forty grams fresh weight of *Drosera capensis* leaves or sixty grams of roots (*in-vitro*) were extracted with 100ml of ethyl alcohol 95% at room temperature for three days. The extract was filtered and the solvent removed at low temperature (35- 40 °C) and reduced pressure on a rotary evaporator. The residue was re-dissolved in two ml ethanol 95% and completed to 10ml by sterilized water. This final solution was subjected to use in experiments of date palm. Whereas the both extract of leaves and roots were filter sterilized and added to sterilized culture medium of date palm (Tisserat, 1981; Ibrahim and Hegazy, 2001 and Hegazy *et al.* 2009).

The effect of *Drosera capensis* root extract on production of embryogenic callus:

Callus formation of date palm cv. Bartamouda was obtained from date palm offshoots (from Aswan governorate) according to the method described by Ibrahim *et al.* (2008) and Hegazy *et al.* (2009).

In this experiment the Embryogenic calli resulted from previous *in-vitro* cultures were divided into pieces of approximately 0.5 g and cultured on $\frac{3}{4}$ MS medium supplemented with 200 mgL⁻¹ KH₂PO₄.2H₂O, 170mg L⁻¹ NaH₂PO₄.2H₂O, 200mg L⁻¹ glutamine, 100mg L⁻¹ myo-inositol, 1.0g L⁻¹ activated charcoal, 40g L⁻¹ sucrose, 5g L⁻¹ Bacteriological agar and different amounts of *Drosera capensis* root extract was added at 0, 0.75, 1.5, 3.0 or 6.0 ml L⁻¹, without plant growth regulators. While the control contained 2,4-D at 0.5 mg L⁻¹ and Kinetin at 1.0 mg L⁻¹ and Medium pH was adjusted to 5.8 (Gadalla, 2007). Each treatment included three replicates (12 small jars (200ml) contained 35 ml of nutrient MS medium). Each jar contained about (0.5 g) embryogenic callus. All cultures were incubated in total darkness, 25±2°C. The fresh weight of embryogenic calli were recorded after four, eight and 12 weeks from onset of the culture.

The effect of *Drosera capensis* root extract on Maturation stage:

Embryogenic calli resulted from previous stage were divided into pieces of approximately (1cm²) and cultured on $\frac{3}{4}$ MS medium supplemented with *Drosera capensis* root extract at 0, 0.05, 0.1, 0.2 or 0.4 ml L⁻¹, while the control medium which was contained NAA at 0.1 mg L⁻¹. Each treatment included three replicates (12 small jars). Each jar contained about (1cm²) embryogenic callus. All cultures were incubated in 500-1000 Lux at 16 hrs, 25±2°C for 12 weeks (4 weeks interval). The number of mature embryos recorded after four, eight and 12 weeks from onset of the culture.

The effect of *Drosera capensis* leaf extract on Shoot formation stage:

The mature embryos resulted from previous stage were germinated on $\frac{3}{4}$ MS medium free from plant growth regulators and were cultured (cluster contained from three to four embryos per jar) for four weeks and then cultures were transferred to $\frac{3}{4}$ MS medium supplemented with 30g L⁻¹ sucrose, 5 g L⁻¹ Bacteriological agar, 0.5 g L⁻¹ activated charcoal and 0.5 mg L⁻¹ BA plus 0.1 mg L⁻¹NAA as a control and five concentrations of *Drosera capensis* leaf extract at 0.0, 0.25, 0.5, 1.0, and 2.0ml L⁻¹ in three replicates each replicate contains four jars. All cultures were incubated in 3000 Lux at 16 hrs, 25±2°C. These cultures were transferred into the same fresh medium every four weeks. After four, eight and 12 weeks shoot number and shoot length (cm) were recorded. The best date palm shoots (6-8 cm length) were transferred to date palm rooting medium for 4.5 months through three recultures and subsequently to adaptation stage in greenhouse (Gadalla, 2007). Data obtained were subjected to the analysis of variances of randomized complete design as recommended by Sendecor and Cochran (1980).

RESULTS AND DISCUSSION

Qualitative and quantitative analysis of phytohormones by high performance liquid chromatography (HPLC):

Data presented in Table (1) reveal that the amounts of phytohormones ($\text{mg } 100\text{g}^{-1}$ fresh weight) in *Drosera capensis* leaf and root extract are differing. The amount of Indole acetic acid in leaf was 2.055, but in case of root was 2.291. The amount of zeatine in leaf was 1.609, but in case of root was 0.418. The amount of other cytokinines in leaf was 18.791, but in case of root was 1.003.

Table 1. The amount of phytohormones in leaf and root of *in-vitro* *Drosera capensis* ($\text{mg } 100\text{g}^{-1}$ fresh weight)

Type of Hormone	Leaf	Root
Indole acetic acid	2.055	2.291
Zeatine	1.609	0.418
Substances-like Cytokinines	18.791	1.003
Gibberellic acid	70.938	86.59
Abscissic acid	0.500	0.158

The amount of gibberellic acid in leaf was 70.938, but in case of root were 86.59. The amount of abscissic acid in leaf was 0.500, but in case of root was 0.158. These results coincided with many researchers such as Straus (1960); Steinhart *et al.* (1961); Roberto (1984) and Puchooa and Ramburn (2004).

Effect of *Drosera capensis* root extract concentration on fresh weight (g) of embryogenic callus of date palm cv. Bartamouda:

Data in Table (2) show that *Drosera capensis* root extract concentration had a significant effect on fresh weight of embryogenic callus of date palm cv. Bartamouda. In case of using 2,4-D at 0.5 mg L^{-1} with 1 mg L^{-1} Kinetin, treatment No. (1), as a control to compare with *Drosera capensis* root extract concentrations at 0.0, 0.75, 1.5, 3.0 and 6.0 ml L^{-1} , it was found that the effect of control somewhat equal to the effect of *Drosera capensis* root extract at 6.0 ml L^{-1} , whereas there is no a significant different between them. Where, the fresh weight of embryogenic callus at treatment No. (1) reached to (1.57), (2.90) and (4.13) g after four, eight and 12 weeks, respectively. While in case of *Drosera capensis* root extract at 6.0

Table 2. Effect of *Drosera capensis* root extract concentration on fresh weight (g) of embryogenic callus of date palm cv. Bartamouda.

Treatments		Fresh weight (g), after		
No. of Treatment	Concentration	4 weeks	8 weeks	12 weeks
1	0.5 mg L ⁻¹ 2,4-D + 1mg L ⁻¹ Kinetin	1.57	2.90	4.13
2	0.0 ml L ⁻¹ <i>Drosera capensis</i> root extract	0.77	0.93	1.10
3	0.75 ml L ⁻¹ <i>Drosera capensis</i> root	0.83	1.13	2.03
4	1.5 ml L ⁻¹ <i>Drosera capensis</i> root extract	1.23	2.47	3.57
5	3.0 ml L ⁻¹ <i>Drosera capensis</i> root extract	1.6	3.20	4.63
6	6.0 ml L ⁻¹ <i>Drosera capensis</i> root extract	1.47	2.83	4.10
L S D at 0.05		0.13	0.15	0.21

mg L⁻¹ were 1.47, 2.83 and 4.10 g after four, eight and 12 weeks, respectively. But the best result was obtained with the use of *Drosera capensis* root extract at 3,0 mg L⁻¹ where this character reached its utmost, 1.6, 3.2 and 4.63 g after four, eight and 12 weeks, respectively. In-general the effect of *Drosera capensis* root extract had a stimulatory effect on fresh weight of embryogenic callus at lowest concentrations, but at highest concentrations it had inhibitor effect. Whereas fresh weight of embryogenic callus increased gradually and significantly, except treatments No. 2 and 3 after four weeks, as *Drosera capensis* root extract concentration increased from 0.00 to 0.75, 1,5 and 3,0 ml L⁻¹ where this character increased from 0.77, 0.93 and 1.10 g after four, eight and 12 weeks, respectively to 0.83, 1.13 and 2.03 g after four, eight and 12 weeks, respectively, (1.23), (2.47) and (3.57) g after four, eight and 12 weeks, respectively and 1.6, 3.2 and 4.63 g after four, eight and 12 weeks, respectively.

Raising *Drosera capensis* root extract concentration to 6.0 ml L⁻¹ affected fresh weight of embryogenic callus inversely, as it declined to 1.47, 2.83 and (4.10) g after four, eight and 12 weeks, respectively. In this concern, it has been reported that in many plant species adding of plant extracts juice of coconut, tomato, potato, onion, banana, orange, apple, pineapple, and yeast to the culture medium enhanced the growth of tissues. Hassan *et al.* (2008) reported that pollen extracts of date palm were used in tissue culture medium for banana as growth substances in comparison with growth regulators. Most of treated plants showed highly growth characteristics such as fresh weight compared to either the cytokinin (benzyl adenine) and/or the auxin (indole butyric acid and naphthalene acetic acid).

Effect of *Drosera capensis* root extract concentration on number of mature embryos of Date Palm cv. Bartamouda in production of embryos :

Data in Table (3) show that *Drosera capensis* root extract concentration had a significant effect on number of mature embryos of Date Palm cv. Bartamouda in production of embryos stage after 12 weeks. Whereas in case of using *Drosera capensis* root extract at 0.05 ml L⁻¹ the number of mature embryos reached to the highest record 12.7 compared with media free *Drosera capensis* root extract 6.3, and also the medium which containing NAA at 0.1 mg L⁻¹ where the number of mature embryos was 10.3. The effects of using plant extracts in *in-vitro* culture have been investigated by a number of workers. One of the earliest report is that of Overbeek *et al.* (1941), who succeeded in growing immature *Datura* embryos in culture by including the liquid endosperm of *Cocos nucifera* (coconut milk) in their culture medium.

Table 3. Effect of *Drosera capensis* root extract concentration on number of mature embryos of Date Palm cv. Bartamouda in production of embryos stage

No. of treatment	Treatments Concentration	No. of mature embryos,		
		4 weeks	8 weeks	12 weeks
1	0.1 mg L ⁻¹ NAA	7	9	10.3
2	0.0 mg L ⁻¹ <i>Drosera capensis</i> root	4.3	5.3	6.3
3	0.05 ml L ⁻¹ <i>Drosera capensis</i> root	9	11	12.7
4	0.1 ml L ⁻¹ <i>Drosera capensis</i> root	8.3	10	12.0
5	0.2 ml L ⁻¹ <i>Drosera capensis</i> root	6.3	8.3	9.8
6	0.4 ml L ⁻¹ <i>Drosera capensis</i> root	5	6.3	7.5
L S D at 0.05		2.1	1.1	1.0

Effect of *Drosera capensis* leaf extract concentration on shoot formation of Date Palm cv. Bartamouda:

Data presented in Tables (4 and 5) show the effect of *Drosera capensis* leaf extract concentration on shoot formation estimated as shoot number and shoot length (cm) of Date Palm cv. Bartamouda: It's noticed that when the *Drosera capensis* leaf extract concentration increased from 0.0 to 0.25, 0.5 and 1.0 ml L⁻¹ the number of shoots increased significantly from 5.7, 7, and 7.7 to 7.3, 9.7 and 10.3, 9.7, 11.7 and 13.3 and 15.3, 17.3 and 21 after 4, 8 and 12 weeks respectively. But when *Drosera capensis* leaf extract increased to 2.0 ml L⁻¹ the number of shoots decreased significantly to 13.3,

Table 4. Effect of *Drosera capensis* leaf extract concentration on Shoot Number of Date Palm cv. Bartamouda in shoot formation stage

No. of Treatment	Treatments Concentration	Shoot Number, after		
		4 weeks	8 weeks	12 weeks
1	0.1 mg L ⁻¹ NAA and 0.5 mg L ⁻¹ BA	13.3		16.3
2	0.0 ml L ⁻¹ <i>Drosera capensis</i> leaf	5.7	7.0	7.7
3	0.25 ml L ⁻¹ <i>Drosera capensis</i> leaf	7.3	9.7	10.3
4	0.5 ml L ⁻¹ <i>Drosera capensis</i> leaf	9.7	11.7	13.3
5	1.0 ml L ⁻¹ <i>Drosera capensis</i> leaf	15.3	17.3	21
6	2.0 ml L ⁻¹ <i>Drosera capensis</i> leaf	13.3	15	17
L S D at 0.05		1.1	0.9	0.7

15 and 17 after 4, 8 and 12 weeks respectively. While in case of medium containing NAA at 0.1 mg L⁻¹ plus BA at 0.5 mg L⁻¹ the shoots number were 13.3, 14.7 and 16.3 after 4, 8 and 12 weeks respectively. These results are in agreement with that of Henderson *et al.*, 1952 and Archibald, 1954 they reported that coconut milk was shown to stimulate cell division in cultured tissues and its use as a supplement was adopted in many laboratories. Al-khateeb (2008) studied the effect of date syrup concentration on *in-vitro* multiplication of date palm cv. Rhanezi. He found that lower concentration of date palm syrup 5% has successfully induced buds and shoots formation.

Effect of *Drosera capensis* leaf extract concentration on shoot length of date palm cv. Bartamouda:

Data in Table (5) show that, shoot length of date palm was affected significantly by *Drosera capensis* leaf extract.

The highest records of this character was 2.77cm., 3.07 and 3.3cm after 4, 8 and 12 weeks respectively at 1.0 ml L⁻¹ *Drosera capensis* leaf extract followed by 2.5, 2.7 and 2.9 after 4, 8 and 12 weeks respectively at 2.0 ml L⁻¹ followed by 2.27, 2.47 and 2.8 after 4, 8 and 12 weeks respectively at 0.5 ml L⁻¹, also these records were at using combination of NAA at 0.1 mg L⁻¹ and 0.5 mg L⁻¹ BA. While using *Drosera capensis* leaf extract at 0.25 ml L⁻¹ the shoot length was 2.1, 2.2 and 2.4 after 4, 8 and 12 weeks respectively. Also using medium free of *Drosera capensis* leaf extract showed shoots of 2.07, 2.17 and 2.4 in length after 4, 8 and 12 weeks respectively. It was noticed that no significant differences between the use of *Drosera capensis* leaf extract at 0.0 and 0.25 ml L⁻¹.

Table 5. Effect of *Drosera capensis* leaf extract concentration on Shoot Length (cm) of Date Palm cv. Bartamouda in shoot formation stage:

Treatments		Shoot Length (cm), after		
No. of treatment	Concentration	4 weeks	8 weeks	12 weeks
1	0.1mg L ⁻¹ NAA and 0.5 mg L ⁻¹ BA	2.27	2.47	2.8
2	0.0 ml L ⁻¹ <i>Drosera capensis</i> leaf	2.07	2.17	2.4
3	0.25 ml L ⁻¹ <i>Drosera capensis</i> leaf	2.1	2.2	2.4
4	0.5 ml L ⁻¹ <i>Drosera capensis</i> leaf	2.27	2.47	2.8
5	1.0 ml L ⁻¹ <i>Drosera capensis</i> leaf	2.77	3.07	3.3
6	2.0 ml L ⁻¹ <i>Drosera capensis</i> leaf	2.5	2.7	2.9
L.S.D at 0.05		0.09	0.1	0.1



Starting stage
 $\frac{3}{4}$ MS + 100 mg L⁻¹ 2, 4-D + 3 mg L⁻¹ 2-ip



Swelling
 $\frac{3}{4}$ MS + 10 mg L⁻¹ 2, 4-D + 3 mg L⁻¹ 2-ip



Embryogenic callus
 $\frac{3}{4}$ MS + 3.0 ml L⁻¹ *Drosera capensis* root extract



Somatic embryos
 $\frac{3}{4}$ MS + 0.05 ml L⁻¹ *Drosera capensis* root extract



Shooting
 $\frac{3}{4}$ MS + 1.0 ml L⁻¹ *Drosera capensis* leaf extract



Elongation
 1.0 ml L⁻¹ *Drosera capensis* leaf extract



Rooting
 $\frac{3}{4}$ MS + 1.0 ml L⁻¹ *Drosera capensis* root extract



Pre-acclimatization
 1.0 ml L⁻¹ *Drosera capensis* root extract



Acclimatization
 1.0 ml L⁻¹ *Drosera capensis* root extract

***In-vitro* propagation of date palm using *Drosera capensis* extract.**

In this Concern, Hassan *et al.* (2008) reported that Pollen extracts of date palm were used in tissue culture medium for banana as growth substances in comparison with growth regulators. Most of treated plants showed highly growth characteristics such as shoot number, shoot length compared to either the cytokinin (benzyl adenine) and/or the auxin (indole butyric acid and naphthalene acetic acid). In conclusion, natural extract such as pollen extract of date palm provided to be an excellent economic resources as growth substances.

The results of shoot proliferation and elongation response to different concentrations of BA as control, water pollen extract or ethanol pollen extract revealed that pollen extracts succeeded as plant growth substances instead of cytokinins at shoot multiplication stage of banana tissue culture.

Conclusively, results of this study indicated that. Micropropagation of date palm can be achieved using MS medium supplemented with extract of *Drosera capenses* as a source of phytohormones at the different micropropagation stages. Extract of *Drosera capensis* showed a significant effect on fresh weight of embryogenic callus, and the same extract at 0.05 mg/l observed the highest number of mature embryos. Moreover, the extract of leaves at 1mg/l gave the highest shoot number and length.

REFERENCES

- Al-khateeb, A. A. (2008).** Enhancing the growth of Date palm (*Phoenix dactylifera*) *in-vitro* tissue by adding date syrup to the culture medium. *Scientific Journal of King Faisal University (Basic and Applied Sciences)*.**9** (1): 71-84.
- Al-Khayri, J. M. (2001).** Optimization of biotin and thiamine requirements for somatic embryogenesis of date palm (*Phoenix dactylifera* L.). *IN VITRO CELL. DEV. BIOL. PLANT* , **37**:453-456.
- Archibald, J. F. (1954).** Culture *in-vitro* of cambial tissues of cacao. *Nature*, 173: 351-353.
- Bunney, S. (1992).** *The Illustrated Encyclopedia Of Herbs Their Medicinal And Culinary Uses*. Chancellor press, Michelin House, London PP.:138.
- El-Assar, A. M.; El-Messeih, W. M. and El-Shenawi, M. R. (2004).** Applying of some natural extracts and growth regulators to culture media and their effects on "Sewi" cv. Date palm tissues grown *in-vitro*. *Assiut Journal of Agricultural Sciences*, **35**(4): 155-168.

- Gadalla, E. (2007).** High frequency somatic embryo production and maturation into plantlets in date palm (*Phoenix dactylifera* L.) Through suspension culture. *Egyptian Journal of Agriculture Research*, **85** (1): 349- 365.
- Hassan, H. M. M.; Ahmed, O.K.; El-Shemy, H.A. and Afify, A.S. (2008).** Palm pollen extracts as plant growth substances for Banana tissue culture. *World Journal of Agricultural Sciences*, **4** (4): 514-520.
- Hegazy, A. E.; Nasr M. I.; Ibrahim I. A. and El-Bastawissy H. H. (2009).** Micropropagation of date palm cv. Malakaby through embryogenesis: 2- Effect of adenine hemisulfate, glutamine and glutathione. *Journal of Agri. Sci., Mansoura Univ.*, **34** (3): 1545-1560.
- Henderson, J.H.M.; Durrell, M.E. and Bonner, J. (1952).** The cultures of normal sunflower callus. *Am. J. Bot.*, 39: 467-472.
- Ibrahim, I. A.; Gabr, M.F.; Nasr, M.I. and Fadl, R.A. (2008).** Effect of cobalt chloride on somatic embryogenesis of Egyptian dry date palm cultivars. 1st International Conference on Environmental Studies and Research, Environmental Studies and Research Institute (ESRI), *Minufiya University- Sadat Branch*, April 2008.
- Ibrahim, I.A. and Hegazy, A.E. (2001).** *In-vitro* cultivation of date palm. 3- Date palm abnormalities during micropropagation via embryogenesis. *Mid-Atlantic Plant Molecular Biology Society, Eighteenth Annual Meeting*, 2, Beltsville –MD, USA, August 2-3, 2001 p 39.
- Jindaprasert, A.; Springob, K.; Schmidt, J.; De-Eknamkul, W. and Kutchan T. M. (2008).** Pyrone polyketides synthesized by a type III polyketide synthase from *Drosophyllum lusitanicum*. *Photochemistry*, **69** (18): 3043-3053.
- Marczak, L.; Kawiak, A.; Lojkowska E. and Stobiecki M. (2005).** Secondary metabolites in *in-vitro* cultured plants of the genus *Drosera*. *Phytochem Anal.*, **16**(3):143-149.
- Murashige, T. and Skoog, F. (1962).** A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, 15: 473- 497.
- Overbeek, J.V.; Conklin, M.E. and Blakeslee, A.F. (1941).** Factors in coconut milk essential for growth and development of very young *Datura* embryos. *Science*, **94**: 350-352.
- Pierik, R.L.M. (1987).** Preparation and composition of nutrient media. In: *In Vitro Culture of Higher Plants. Martinus Nijhoff, Dordrecht*, 45-82p.

- Puchooa, D. and Ramburn, R.(2004).** A study on the use of carrot juice in the tissue culture of *Daucus carota*. *African Journal of Biotechnology*, **3 (4)**: 248-252.
- Roberto, C. (1984).** Encyclopedia of Medicinal Plants, The Macdonald, Maxwell House, London, Number 117.
- Snedecor, G.W. and Cochran, W. G. (1980).** *Statistical Methods*. 6th ed., Iowa state University Press, Iowa, USA.
- Shams El-Din, I. M. (2002).** Evaluation of some species of insectivorous plants and their propagation through tissue culture. Department of Agricultural Science. Institute of Environmental Studies and Research. Ain Shams University.
- Shindy, W.W. and Smith, O. (1975).** Identification of plant hormones from cotton ovules. *Plant Physiol.*, **55**: 550-554
- Steinhart, C.E.; Standifer, L.G. and Skoog, F. (1961).** Nutrient requirements for *in vitro* growth of spruce tissue. *Am. J. Bot.*, **48**: 465-472.
- Straus, J. (1960).** Maize endosperm tissue grown *in vitro* : Development of a synthetic medium. *Am. J. Bot.* , **47**: 641-646.
- Tisserat, B. (1981).** Date palm tissue culture. *Advances-in-Agricultural-Technology,-Western-Series,-United States Department of Agriculture*, (17): 50.

دراسة الهرمونات النباتية للدروسييرا كابنسييس كنبات آكل للحشرات وتأثير المستخلص النباتي علي نمو نباتات نخيل البلح صنف برتمودا في مزارع الأنسجة النباتية

إبراهيم عبد المقصود إبراهيم^١ - حمدي أحمد عمارة^١ - عبد المنعم محمد البنا^٢ -
إبراهيم محمد شمس الدين^٢

١ - قسم التكنولوجيا الحيوية النباتية، معهد الهندسة الوراثية والتكنولوجيا الحيوية،
جامعة المنوفية فرع مدينة السادات - مصر.

٢ - معمل بحوث النخيل ، مركز البحوث الزراعية بالجيزة- مصر.

تنتمي النباتات آكلة الحشرات لعدة عائلات نباتية أهمها العائلة الدروسييرية التي ينتمي إليها
نبات الدروسييرا . ويستخرج من نبات الدروسييرا عدة مواد فعالة ذات أهمية اقتصادية كبيرة،
وتعتبر تقنية زراعة الأنسجة النباتية أفضل وسيلة للحصول على المادة النباتية المطلوبة
للحصول على المواد الفعالة التي بها .

تهدف هذه الدراسة إلى عمل دراسات فيتوكيميائية علي نبات دروسييرا كابنسييس لمعرفة
محتواه من الهرمونات النباتية. كما تهدف الدراسة إلى دراسة تأثير مستخلص نبات الدروسييرا
كابنسييس علي نمو نخيل البلح صنف برتمودا داخل المعمل.

أظهر تحليل جهاز الكروماتوجرافيا السائلة عالية التشخيص محتوى كلا من الأوراق
والجذور من الهرمونات النباتية مقدره بالمليجرام/١٠٠ جرام مادة طازجة كالتالي: كان كمية
أندول حامض الخليك في الأوراق (٢.٠٥٥) بينما في حالة الجذور (٢.٢٩١). و كان كمية
الزياتيين في الأوراق (١.٦٠٩) بينما في حالة الجذور (٠.٤١٨). و كانت كمية أشباه
السيتوكينينات في الأوراق (١٨.٧٩١) بينما في حالة الجذور (١.٠٠٣). و كانت كمية حامض
الجبريليك في الأوراق (٧٠.٩٣٨) بينما في حالة الجذور (٨٦.٥٩). أما كمية حامض الأبسيسك
فكانت في حالة الأوراق (٠.٥٠٠) بينما في حالة الجذور (٠.١٥٨).

كان لمستخلص الأوراق والجذور تأثيرا معنويا علي نمو نخيل البلح داخل المعمل، ففي مرحلة
تضاعف الكالس الجنيني كان لمستخلص الجذور تأثير معنوي علي الوزن الطازج للكالس
الجنيني وكانت أحسن النتائج (٤.٦٣ جرام) عند استخدام هذا المستخلص بتركيز
٣.٠ مليلتر/لتر.

استخدام مستخلص الجذور بتركيز ٠.٥ مليلتر/لتر أعطى أعلى عدد من الأجنة الناضجة.
استخدام مستخلص الأوراق بتركيز ١.٠ مليلتر/لتر أعطى أكبر عدد من الأفرع
(٢١ فرع)، وأيضا أعلى ارتفاع للأفرع (٣.٣سم) وبالتالي أمكن إنتاج نباتات نخيل البلح في
مزارع الأنسجة النباتية باستخدام مستخلص نباتات الدروسييرا آكلة الحشرات كمصدر
للهرمونات النباتية.