J. Agric. Sci. Mansoura Univ., 25 (10): 6383 - 6396, 2000.

PRODUCTION OF TROPANE ALKALOIDS IN *Atropa belladonna* L. USING CELL CULTURE TECHNIQUE .

Salwa S. Sakr* and M. M. Dessouky**

*** Department of Horticulture, Faculty of Agriculture, Cairo University, Giza, Egypt.**

**** National Organization for Drug Control and Research (NODCAR), Giza, Egypt.**

ABSTRACT

A promising protocol for enhancement of total alkaloids production from cell cultures of *A. belladonna* L. was established. The effect of MS-medium supplemented with different concentrations from 2,4-D and Kin or NAA and BA on both of growth parameters as well as total alkaloids production were investigated. The effect of various concentrations of phenylalanin and ornithine as a precursors of alkaloids accumulation were studied. The optimum value of cell growth parameters and alkaloids production were obtained from leaf, stem and root cell cultures, respectively. The most effective supplementations to liquid MS-medium (Murashige and Skoog,1962) were 1 mg/l from each of NAA and BA. Phenylalanine at 20 mg/l gave the highest value of cell growth and alkaloids accumulation in the different types of cell cultures. Total alkaloids of different types cultures were identified using HPLC.

INTRODUCTION

Higher plants are very useful, not only as food, but also, as a source of natural drugs or other raw materials. Therefore, it is quite advantageous if these metabolites could be produced by plant cell and tissue culture techniques (Phillipson, 1990).

Many pharmaceutical and other industrial products are based on plant products, much effort has been investigated in the biotechnology production of secondary metabolites by plant cell cultures, Wink (1987).

Atropa belladonna L. Fam., Solanaceae, is one of the most important medicinal plants. Its extract is used as opposes the muscarinic effects of acetylcholine. Containing the alkaloid tropane, which, relaxes the smooth muscles of the stomach, intestines, bladder and gallbladder, and dilates the bronchial tubes. Moreover, it can be useful adjunct to botanical treatment of visceral spasms, spasms, asthma, pertussis, spastic constipation, hypersudation and urinary incontinence.

Alkaloids form a structurally heterogeneous class of secondary compounds that are derived from basically four amino acids namely, ornithine, lysine, phenylalanine (tyrosine) and tryptophane (Mothes and Schütte, 1969; Mothes *et al*., 1985 and Dalton, 1979).

Secondary metabolism in higher plants is strongly influenced by environmental factors. In suspension cultures, plant growth regulators and nutritional factors affect the production of secondary metabolites as well as growth, (Sakuta and Komamine, 1987). Also, types and concentrations of growth regulators affect secondary product formation (Bohm,1980).The presence of2,4-dichlorophenoxy acetic acid (2,4-D), indol acetic acid (IAA) and naphthalenacetic acid (NAA)stimulated scoboletin and scoplin production in tobacco tissue cultures (Okazaki *et al.,* 1982) and also increased the

production of indol alkaloids in cell cultures of *Cinchona ledgeriana* (Harkes *et al,* 1985).

Moreover,Nussbaumer *et al*.,(1998) examined clone of *Datura candida* x *D. aurea* for its growth and hyoscyamine and scopolamine content under various culture conditions .They reported that, half strength Gamborg·s B5 medium supplemented with 5% sucrose and 1 mg/l from each of NAA and BA gave the best value of root culture growth. Meanwhile, full strength of B5 medium supplemented with the same concentrations of NAA and BA gave the best results of hyoscyamine and scopolamine content.

Attempts to induce or increase the production of metabolite formation in cultured tissue by supplying precursors or intermediate compounds have produced encouraging results. The addition of phenylalanine has been reported to increase the accumulation of hydroxycinnamoyl esters in apple cell cultures (Koumba and Macheix,1981), polyphenols in tobacco cells (Sahai and Shuler,1984), and of naphthoquinonesd in *Lithospermum* cell culture (Mizukami *et al*, 1977).

The aim of this investigation was to study the effect of different growth regulators (2,4-D, NAA, Kin and BA) and different precursors (phenylalanine and ornithine)on callus, cell growth and total alkaloids in different cultures for different explants of *A. belladona* L.

MATERIALS AND METHODS

This investigation was carried out in the Laboratory of Plant Tissue Culture, Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University, Giza during1998.The main objective of this investigation was to study the effect of different conditions on total alkaloids production from different explants of *Atropa belladonna* L

Plant material

Atropa belladonna L.seeds were secured from the pharmacognosy Department, Faculty of Pharmacy, Cairo University. Seeds were surface sterilized by immersion in 70% Ethanol for 10 Sec., followed by three washes using sterile distilled water, then immersed in 50% of commercial Clorox solution containing a drop of Twin 20 for 15 min. The seeds were subsequently rinsed several times with sterile water. These seeds were then germinated aseptically on solid MS-medium (Murashige and Skoog,1962). Cultures were solidified by 0.7 % agar added prior autoclaving at 1.2 Kg/cm ^{2} for 15 min. The pH of the medium was adjusted to 5.8 by addition of 0.1 N HCL or 0.1 N KOH. The cultivation was done in 300 ml glass jars containing 50 ml of basal MS-medium, i.e., hormones free. Seeds germination took place within 7-10 days. After one month from germination the different segments i.e., leaf, stem and root were used as a source of callus production.

Callus production

First experiment

In this experiment, the following concentrations of 2,4-dichlorophenoxy acetic acid (2,4-D) and N-(2-furanylmethyl)- purine-6-amino (Kinetin) were added to MS-basal medium as followed:-

1- MS-medium free hormones
2- 0.0 mg/l 2,4-D + 1.0 mg/l Kin.
3- 1.0 mg/l 2,4-D + 0.0 mg/l Kin
4- 1.0 mg/l 2,4-D + 1.0 mg/l Kin
5- 1.0 mg/l 2,4-D + 2.0 mg/l Kin
6- 2.0 mg/l 2,4-D + 1.0 mg/l Kin

Second experiment

The effect of the following concentrations of Naphthalene acetic acid (NAA) and 6-benzylamino purine (BA) was studied at the following concentrations:

1-MS + 0.0 mg/l NAA + 1.0 mg/l BA
2-MS + 1.0 mg/l NAA + 0.0 mg/l BA
3-MS + 1.0 mg/l NAA + 1.0 mg/l BA
4-MS + 1.0 mg/l NAA + 2.0 mg/l BA
5-MS + 2.0 mg/l NAA + 1.0 mg/l BA

Cultures of all treatments were maintained under light condition 16 h/day photoperiod at intensity of 3000 Lux from cool light fluorescent lamps for 30 days. All cultures were incubated at 26 ±1 °C. 5 replicates from each treatment were used.

Determinations of callus growth

Growth patterns of callus production from different types of all cultures were determined after 30 days from incubation as followed:-

1-Fresh weight (gm).
2-Dry weight (gm).
3-Dry matter content(%).

Cell cultures induction

According to Torres (1988), the suspension cultures were induced from friable callus of leaf, stem and root. The obtained cells were maintained in an agitated liquid MS-medium with the following additions :

1-MS-medium + 1 mg/l 2,4-D + 1 mg/l Kin
2-MS-medium + 1mg/l NAA + 1 mg/l BA

Measurements of cell cultures growth.

Growth parameters of cell suspension cultures were measured at intervals during the growth cycle by the following measurements:

1-Cell number : The cell number was calculated according Neumann (1966).
2-Packed cell volume (PCV) was determined according to Patrick, (1984).

The measurements were repeated four times

Third experiment

Phenylalanin and ornithine as a precursors of tropane alkaloids production was added at different concentrations 0.0, 5.0, 10.0, 20.0 and 50.0 mg/l to different types of cell cultures.

Chemical analysis

Tropane alkaloid was determined as total alkaloid by HPLC (High pressure liquid chromatographic) in the obtained types of cultures. The determination of total alkaloids was carried out according the method described by British Pharmacopoeia (1998).

Chromatographic conditions:-

Apparatus:-LDC constametric III solvent delivery system.
LDC spectromanitor III variable wavelength detector.Water WISP 710 B auto injector.
Spectra physics SP 4270 integrator.
Column :-Du Pront Zorbox C8, 4.6 mm i.d.X15 cm, (with Brownlee RP-8 Spheri-5 precolumn, optionol).
Mobile phase:-78 % TEAP Buffer.
18 % Methanol.
4 % Acetonitrile.
Flow rate:- 1.5 ml/min.
Injection volume:- 50 μl.
Run Time :- 5 min.
UV Detector :- 215 mm.
Sensitivity :- 0.02 AUFS.
Chart speed :- 0.5 cm/min.
Dilution :- 1.00000
Temperature :- Ambient
Standard preparation:- As shown in Fig. (1) standard curve of tropan alkaloid had been estimated according Milan *et al*., (1990).

Statistical analysis:

All experiments were designed in a completely randomized design and obtained data were statistically analyzed using standers error (SE) according to the method described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

1. Effect of 2,4-D and Kin on callus production.

Data in Table (1) show the effect of 2,4-D and Kin on callus production from different explants; leaf, stem and root of *Atropa belladonna* L. MS-medium free hormones gave no effect on callus initiation from different explants. Meanwhile, the supplementation of MS-medium with low concentration from 2,4-D or Kin alone gave a low effect on callus initiation. But, the optimum value of callus production was obtained from using mixture

FIG1

of 2,4-D and Kin. The highest value of callus production (9.45; 7.53 and 5.26 gm) at the same sequence were recorded for leaf, stem and root explants, respectively. The addition of (1mg/l) from each of 2,4-D and Kin to MS-medium. Fresh weight (gm) of different calli cultures were recorded after 8 weeks (at the end of the first subculture) (Fig.2). Meanwhile, the dry weight of different cultured explants was 0.88, 0.59 and 0.35 (gm/jar) for leaf, stem and root callus cultures, respectively. The dry matter content (%) was calculated. The descending order was 9.35 %, 7.80 % and 6.75 % for leaf, stem and root callus cultures of *A. belladonna* L., respectively.

2.Effect of NAA and BA on callus production.

The effect of supplementation of MS-medium with the previously mentioned concentrations from NAA as auxin alone or in a combination with BA as cytokinin on callus production from different explants of *A. belladonna* L. are tabulated in Table (2) and Fig.(3). The conditioned of MS-medium with low concentration of NAA or BA alone gave some little effect on callus production. On the other hand, the optimum value of callus production was obtained from addition of both of NAA and BA to MS-medium.

As mentioned above, leaf explants resulted in the maximum value of callus fresh weight (11.85 gm/jar followed by 8.65 then 6.35 gm/ jar) for stem and root calli cultures, respectively using 1 mg/l from both of NAA and BA resulted in the highest values of callus production as compared with other supplementations to MS-medium. Dry weight; as growth dynamic was 1.12, 0.79 and 0.51 for leaf, stem and root callus cultures, respectively. The calculated dry matter content was 9.55, 9.33 and 8.23 for leaf, stem and root callus cultures of *A. belladonna* L., respectively.

The above results clear that, the supplementation of MS-medium with NAA as auxin and BA as cytokinin was more suitable for callus production from different explants of *A. belladonna* than using 2,4-D and Kin. It was found that, addition of 1 mg/l from each of NAA and BA to MS-medium gave the highest values as compared with other concentrations.

In this connection our results were in close with Gamborg and Shyluk (1981), concluded that, callus initiation and production was depending on the presence of auxin and cytokinin, which stimulates cell division and cell elongation, respectively. Moreover, Nussbaumer *et al*., (1998) reported that supplementation of B5 medium with 1 mg/l of each of NAA and BA gave the best results of growth value for *Datura candida X D. aurea.*

Cell cultures induction

Cell cultures were obtained from friable calli cultures of different explants; i.e. leaf, stem and root of *A. belladonna*. The calli cultures were sieved to single cells, and recultured on liquid MS-medium supplemented with 1 mg/l from each of 2,4-D and Kin or NAA and BA.

In case of subculturing on MS-medium containing 1 mg/l from each of 2,4-D and Kin. the maximum values for cell number ($x10^5$) were counted during 21 days from cultivation, in the presence of 1 mg/l from each of 2,4-D and Kin in MS-medium. The obtained maximum number was 5.856, 5.233 , 5.125 $x10^5$ cells / 1 ml for leaf, stem and root explants, respectively.

Fig.(2):Callus production from leaf, stem and root explants of *belladonna*, after 8 weeks from cultivation on MS- medium supplemented with 1 mg/l 2,4-D + 1 mg/l Kin cultured under 16/8 hr light condition. in.

Fig.(3):Callus production from leaf, stem and root explants of *belladonna*, after 8 weeks from cultivation on MS- medium supplemented with 1 mg/l NAA + 1 mg/l BA cultured under 16/8 hr light condition.

1.

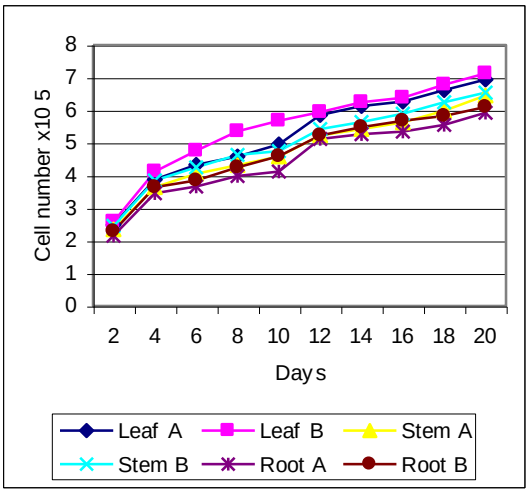

Fig.(4):Cell number x10^5 of different types cultures of leaf, stem and root explants of *A. belladonna* L cultured on MS-medium supplemented with 1 mg/l 2,4-D +1 mg/l Kin or 1 mg/l NAA +1 mg/l BA.
Where:- A: MS+1 mg/l 2,4-D +1 mg/l Kin
B: MS+1 mg/l NAA +1 mg/l BA

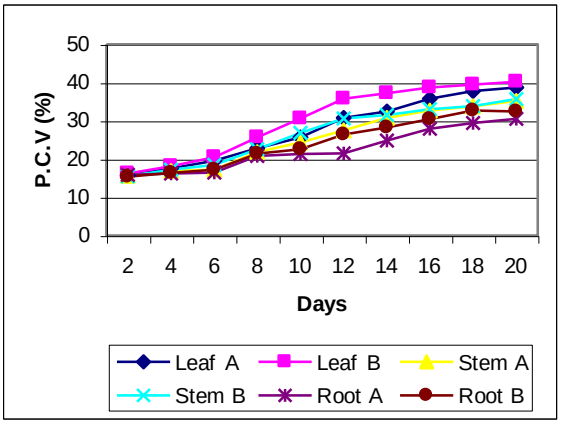

Fig.(5):Packed cell volume (%) of different types cultures of leaf, stem and root explants of *A. belladonna* L cultured on MS-medium supplemented with 1 mg/l from each of 2,4-D and Kin or NAA and BA Where:-A: MS+1 mg/l 2,4-D +1 mg/l Kin B: MS+1 mg/l NAA +1 mg/l BA

Table (1): Effect of MS-medium supplemented with 2,4-D and Kin (mg/l) on fresh weight (gm), dry weight (gm) and dry matter content (%) of callus cultures of leaf, stem and root explants of *A. belladonna* L. cultured under light condition. ± SE (Standard error). N=5 replicates .

Growth regulators (mg/l)		Explants		
2,4-D	Kin	Leaf	Stem	Root
		Fresh weight		
0	0	---	---	---
0	1	3.12±0.25	2.65±0.45	2.14±0.54
1	0	1.25±0.12	1.00±0.14	0.82±0.015
1	1	9.45±0.65	7.58±0.75	5.26±0.87
1	2	8.23±0.85	5.42±0.66	4.76±0.42
2	1	6.54±0.25	3.93±0.33	3.25±0.66
Dry weight				
0	0	---	---	---
0	1	0.21±0.0036	0.08±0.0015	0.06±0.0025
1	0	0.11±0.0033	0.006±0.0002	0.004±0.0001
1	1	0.88±0.0025	0.59±0.0024	0.35±0.0032
1	2	0.72±0.0043	0.43±0.0034	0.35±0.0045
2	1	0.49±0.0035	0.25±0.0009	0.24±0.0065
Dry matter content %				
0	0	---	---	---
0	1	6.48±0.46	5.25±0.23	4.84±0.58
1	0	5.21±0.58	4.17±0.58	3.15±0.47
1	1	9.35±1.15	7.80±0.69	6.75±0.73
1	2	8.56±0.98	6.35±0.33	5.19±0.84
2	1	7.54±0.59	6.18±0.46	5.09±0.44

However, it was 5.942, 5.422 and 5.225 X10^5 cells / 1 ml. In case of subculturing on MS-medium supplemented with 1 mg/l from each of NAA and BA for leaf, stem and root calli cultures, respectively (Fig4). The maximum value of cell number for different types of cell cultures, was recorded at the 2 th day of cultivation.

Table (2): Effect of MS-medium supplemented with NAA and BA (mg/l) on fresh weight (gm), dry weight (gm) and dry matter content (%) of callus cultures of leaf, stem and root explants of *A. belladonna* L. cultured under light condition. ± SE (Standard error). N=5 replicates

Growth regulators (mg/l)		Explants		
NAA	BA	Leaf	Stem	Root
		Fresh weight		
0	0	---	---	---
0	1	4.58±0.44	3.45±0.35	2.25±0.25
1	0	2.52±0.24	2.12±0.15	1.56±0. 12
1	1	11.85±1.95	8.65±1.24	6.35±0.85
1	2	10.25±1.14	6.85±0.86	5.45±0.56
2	1	8.56±1.33	4.25±0.25	3.17±0.33
Dry weight				
0	0	---	---	---
0	1	0.35±0.003	0.29±0.005	0.18±0.002
1	0	0.19±0.005	0.12±0.003	0.08±0.004
1	1	1.12±0.25	0.79±0.0015	0.51±0.005
1	2	0.85±0.012	0.54±0.0017	0.43±0.001
2	1	0.78±0.004	0.35±0.0015	0.29±0.0012
Dry matter content %				
0	0	---	---	---
0	1	6.5±0.86	6.2±0.45	5.8±0.47
1	0	5.8±0.43	5.4±0.32	4.9±0.45
1	1	9.6±0.25	9.3±0.52	8.2±0.33
1	2	8.5±0.97	7.9±0.35	8.1±0.25
2	1	7.8±0.43	7.2±0.18	6.5±0.18

The previously mentioned results reveals that, using cytokinins may enhance cell division, while auxins stimulates cell enlargement (Torres,1989). This effect was clear when they were used in combination in MS-medium. The obtained results are in agreement with both of Skoog and Schmitz, (1972) they reported that, cytokinins are generally added to a culture medium aiming to promote cell division in calli cultures of plant tissue and stimulating the rate of protein synthesis in tobacco cell cultures.

Effect of different precursors on cell growth and total alkaloids accumulation:

The effect of different precursors phenylalanin and ornithine at different concentrations 0.0 ,5.0 ,10.0, 20.0 and 50.0 mg/l on cell growth as well as alkaloid production from different types of cell cultures was investigated. Data tabulated in Table (3) revealed that, leaf explants of *A. belladonna* L. resulted in the maximum value for cell growth and total alkaloids accumulation (which was calculated as scopolamine (μg/100 mg dry weight) using HPLC). On the other hand, low value of cell growth as well as alkaloids production was obtained from root cell cultures. MS-medium supplemented with 1 mg/ l NAA + 1 mg/l BA resulted in the highest value as compared with the addition of 1 mg/l 2,4-D + 1 mg/l Kin. Phenylalanin showed highest value of tropane alkaloids and cell growth parameters than ornithene at the same concentration. Using phenylalanin at 20 mg/l was the most effective concentration for tropane alkaloids than others. The highest value of total tropane alkaloids were 30.5, 26.7 and 23.7 for leaf (Fig.6), stem and roots , respectively.

The obtained results are in agreement with Tiburcio *et al*., (1985) since they indicated that total alkaloids content in tobacco cell cultures can be increased to 3.7 % on a dry weight basis by the addition of organic acids to the medium.

The addition of phenylalanine has been reported to increase the accumulation of hydroxycinnamoyl esters in apple cell cultures (Koumba and Macheix,1981). As well as formation of tropan alkaloids in cell cultures of *Datura spp* caused mainly by strong repression in the biosynthesis of tropic acid, the acidic moiety of hyosyamine and scopolamine (Lindsey and Yeoman,1983). Koumba and Macheix,(1981) reported that, supplying precursors or intermediate compounds have produced encouraging results. In this respect, Sahai and Shuler (1984) found that, polyphenols in were increased by the addition of precursors to tobacco and of naphthoquinones to *Lithospermum* cell culture.

It may be concluded that; cultivation of leaf explants of *A*. *belladonna* L. in liquid MS-medium containing 1mg/l of each of NAA + BA in the presence of 20 mg/l phenylalanine was the most favourable condition for stimulating total tropane alkaloids production.

REFERENCES

Bohm, H. (1980). The formation of secondary metabolites in plant tissue and cell cultures. Int. Rev.Cytol., Suppl.,11B: 183-208.

British Pharmacopoeia. B.P .(1998). vol. I. 709 –711; The Stationery Office Dept.,BP. KE 5833 Norwich, NR 3-1BR.

Dalton,D.R.(1979)..“The alkaloids. The fundamental Chemistry” Dekker, New York.

Gamborg, O. L. and Shyluk, J.P. (1981). Nutrition, media and characteristics of plant cell and tissue cultures. *In*” Plant Tissue culture: Methods and Application in Agriculture”. By. T.A. Thorp (ed.). 21-44, Academic Press, New York.

Harkes, P., Krijbolder, L., Libbenga, K., Wijnsma, R., Nsengiyaremge, T. and Verpoorte, R.(1985). Influence of various media constituents on the growth of *Cinchona ledgeriana* tissue cultures and the production of alkaloids and anthraquinones. Plant Cell, Tissue Organ Cult., 4: 199 - 214.

Koumba, D. and Macheix, J. (1981). Biosynthesis of hydroxycinnamic derivatives in apple fruit cell suspension culture. Physiol. Veg., 20: 137-142.

Lindsey, K. and Yeoman, M. M. (1983). Novel experimental system for studying the production of secondary metabolites by plant tissue cultures. *In* “Plant Biotechnology, S.E.B. Seminar Series, 18, Eds. H.Smith and S.M.Mantell. Cambrige Univ. Press.

Milan P., Jan, F and Vlastimil T.(1990). Chromatographic Science Series, Vol.,53: Chromatographic analysis of alkaloids, 224-229

Mizukami, H.,Konoshima, M.,and Tabata, M.(1977). Effect of nutritional factors on shikonin derivative formation in *lithospermum* callus cultures. Phytochemistry 16: 1183-1186.

Mothes, K. and Schütte, H.R. (1969). “Biosynthesis der alkaloids” VEB Dtsch. Verlag Wiss., Berlin.

Mothes, K. and Schütte, H.R.and Luckner, M.(1985). “ Biochemistry of alkaloids”. Verlag Chemie, Weinheim.

Murashige, T. and Skoog, F., (1962). A revised medium for rapid growth of bioassays with tobacco tissue cultures. Physiol. Plant, 15: 473.

Neumann, K. H. (1966). Wurzelbildung und Nucleinsaure- gehalt bei Phloem Gewbekulturen der Karottenwurzl auf synthetischen Nahrmedium verschiedener Hormonkombination. Les Phytohormones et l'Oranogenese 38: 95-102.

Nussbaumer, P., Kapetanidis, T. and Christen, P.(1998). Hairy roots of *Datura candida* X *D. aurea* : Effect of culture medium composition on growth and alkaloid biosynthesis. Plant Cell Reports,17 (5): 405-409.

Okazaki, M., Hino, F., Nagasawa, K., and Miura, Y.(1982). Effect of nutritional factors on formation of scopoletin and scopolin in tobacco tissue cultures. Agric.Biol.Chem.46: 601-607.

Patrick, J., (1984). Induction and maintenance of cell suspension cultures. pp 130-136. In" Cell Culture and Somatic Cell Genetics of Plants" Vol 1(Indra vasil ed.). Academic Press, Inc., New York.

Phillipson, J, D.(1990).Plants as source of valuable products: In. Secondary Products from Plant Tissue Culture. (eds) Charwood, B.V. and Rhodes, M.J.C. 1-23 Press in Oxford, New York, Toronto.

Sakuta, M. and Komamine, A.(1987). Cell growth and accumulation of secondary metabolites.*In*: "Cell Culture and Somatic Cell Genetics of Plants", Vol.,4 (Cell Culture in Phytochemistry) Vasil, I (ed.) pp.97-114. Academic press, New York, London and Tokyo.

Sahai, O., and Shuler, M.(1984). Environmental parameters influencing phenolic production by batch cultures of *Nicotiana tabacum*. Biotechnol. Bioeng.26: 111-120.

Snedecor, G.M. and W.G. Cohchran (1967). Statistical Methods 5 th Ed. Iowa State Collage, Press, Ames Iowa, U.S.A.

Skoog, F. and Y.R., Schmitz (1972). IX. Cytokinins. In: Plant Physiology A. Treatise, vol. 6 B, Steward, F.C., (ed.). Academic Press, new York.

Tiburcio, A. F., Ingersoll, R and Galston, A.W.(1985). Modified alkaloid pattern in developing tobacco callus. Plant Science, 38, 207.

Torres, K., (1988). Isolation and culture of protoplasts from carrot cell suspension cultures. 187-200. In" Tissue Culture Techniques for Horticulture Crops". (Torres, K. ed.). Academic Press, New York.

Torres, K., (1989). Tissue culture techniques for horticultural crops. Van Nostrand Reinhold, New York, Chapt., 2: 26-51.

Wink, M.(1987). Sites of lupine alkaloid biosynthesis. Z. Naturforsch., C: Biosci, 42: 23-35.

إنتاج الأتروبين من نبات البلادونا باستخدام تكنيك مزارع الخلايا

سلوى سالم صقر ، محمود مصطفى دسوقى*

قسم نباتات الزينة ، كلية الزراعة جامعة القاهرة ، جيزة مصر.

*** الهيئة القومية لبحوث الرقابة الدوائية ، جيزة ، مصر**

تم تثبيت طريقة واعدة لإنتاج القلويدات الكلية من مزارع خلايا نبات البلادونا . تم دراسة تأثير التركيزات المختلفة من منظمات النمو :

داى كلوروفينوكسى حمض الخليك مع الكينتين وكذلك تأثير نفثالين حمض الخليك مع البنزيل أدنين على كل من مظاهر النمو المختلفة وإنتاج القلويدات . هذا بالإضافة لدراسة تأثير التركيزات المختلفة من البادئات (الفنيل ألينين والأورنثين) على نمو المزارع وكذلك معدل تراكم القلويدات الكلية . وسجلت مزارع خلايا الأجزاء النباتية (الأوراق – السوق – الجذور) أعلى القيم فى قياسات النمو المختلفة وكذلك إنتاج القلويدات على الترتيب . أعطت بيئة موراشيج وسكوج المحتوية على ١ ملليجرام/لتر من كل من نفثالين حمض الخليك و البنزيل أدنين أفضل النتائج . إضافة الفنيل ألينين بتركيز ٢٠ ملليجرام/لتر سجلت أعلى معدل لمظاهر نمو الخلايا وكذلك تراكم القلويدات الكلية فى المزارع المختلفة. وقد امكن التعرف على القلويدات الكلية باستخام جهاز HPLC.

Table (3): Effect of different precursors from phenylalanine and ornithine added to MS-medium supplemented with 1mg/l from 2,4-D + Kin or NAA + BA (1 mg/l) in growth pattern and alkaloids production (μg/100mg dry weight) of different types of cultures ofrom *A. belladonna* L.

Precursors concentrations (mg/l)	*A. belladonna explants*											
	Leaf				Stem				Root			
	Phenylalanine		Ornithine		Phenylalanine		Ornithine		Phenylalanine		Ornithine	
	Cell growth	Total alkaloids	Cell growth	Total alkaloids	Cell growth	Total alkaloids	Cell growth	Total alkaloids	Cell growth	Total alkaloids	Cell growth	Total alkaloids
	MS-medium + 1 mg/l 2,4-D + 1 mg/l Kin											
0.0	---	24.3	---	24.3	---	23.8	---	23.8	---	22.7	---	22.7
5.0	+	25.3	+	25.1	+	24.2	---	23.9	---	23.2	---	22.4
10.0	++	28.5	+	25.4	+	25.0	+	24.1	---	23.4	---	22.8
20.0	++	30.5	+	28.4	+	26.7	+	24.3	+	23.7	+	23.0
50.0	---	24.4	---	24.4	---	24.0	---	23.2	---	23.0	---	22.5
	2-MS-medium + 1 mg/l NAA + 1 mg/l NAA											
0.0	---	32.5	---	32.5	---	30.4	---	30.4	---	27.5	---	27.5
5.0	+	33.5	+	32.7	+	31.0	---	31.4	---	29.6	---	28.5
10.0	++	35.4	+	33.1	+	32.7	+	33.2	+	30.8	+	30.4
20.0	+++	36.4	++	34.4	++	35.7	+	33.8	++	34.6	+	32.2
50.0	---	34.2	---	33.0	---	30.6	---	31.0	---	28.3	---	27.9

--- Control + Low increase ++ Medium increase +++ High increase

J. Agric. Sci. Mansoura Univ., 25 (10), October, 2000.

Fig. (6): HPLC of leaf cell suspension culture of *A. belladonna* L. cultured on MS-medium Supplemented with 1mg/l NAA + 1mg/l BA and modified with 20 mg/l with phenylalanine