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GROWTH AND PRODUCTION OF TROPANE ALKALOIDS BY CELL SUSPENSION CUTURES OF *HYOSCYAMUS ALBUS L.*

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ABSTRACT

Cell suspension of *Hyoscyamus albus* L. were investigated for growth and production of tropane alkaloids in three different media. The effects of sucrose concentration and initial pH were also studied. 2,4- Dichlorophenoxyacetic acid and 1% sucrose enhanced the production of tropane alkaloids. The percent of alkaloids (0.75%) was higher than in the cultivated plant (0.352 %).

INTRODUCTION

Certain genera of the family Solanaceae contain tropane alkaloids as secondary metabolites, and many plants containing them have long been known for their medicinal, hallucinognic and poisonous properties (Evans, 1979).

Hyoscyamus is of special interest for its high content of tropane alkaloids and for many years sexual crosses have been performed between species and varities within this genus (Corduan and Spix, 1978). Tissue cultures of several Solanaceous plants are known to produce alkaloids of pharmaceutical interest (Tabata *et al.*, 1970). The formation of tropane alkaloids in cultured cells of the Solanaceous plants have been widely investigated (Dhoot and Henshaw, 1977; Yamada and Hashimoto 1982). Koul *et al.* in 1983 studied the potentiality of cell suspension cultures of *Hyoscyamus muticus* for the production of tropane alkaloids under various cultural conditions. These attempts have not met with much success (Kurz and Constabel, 1985). *Hyoscymus albus* L. has been mentioned in the litereature (Planchen *et al.*, 1946) as a substitute for *H. niger* L. which is used medically as an oficial drug. Organgi (1987) reported that the content of tropane alkaloids in a cultivated *H. albus* is about 0.352 % which fulfils the international pharmacopoeial requirements for the exploitation of the plant as a source of tropane alkaloids. The culture cells of *H. muticus* L. generally contain much less scopolamine and hyoscyamine than in the intact plants (Oksman - Caldenty *et al.*, 1986).

Hashimoto *et al.* in 1986 studied the production of tropane alkaloids in callus and root cultures of seven *Hyoscyamus* species including *H. albus.*

The present work aims at studying the effect of some factors on the growth and content of tropane alkaloids by cell suspension cultures of *H. albus* L.

EXPERIMENTAL

1 - Source of Material

a) Seeds

Seeds of *Hyoscyamus albus* L. were kindely supplied by prof. Dr. R. A. Organgi, Research Unit of Plant Ecology, Biol. Dept., Fac. of App. Sci., Umm Al- Qura Univ., Makkah, Saudi Arabia.

b) Explant Tissue

Seeds were surface sterilized in 10 % (v/v) Clorox (commercial bleach containing 5.25 % sodium hypochlorite) for 25 min., washed five times with sterile distilled water and placed on a medium containing MS mineral salts and vitamins (Murashige and Skoog, 1962) supplemented with 3 % (w/v) sucrose and 0.7 % (w/v) agar (BDH). The seeds were incubated under a 16 hr. photoperiod of 500 Lux (daylight fluorescent tube) at 25 ± 2°C. After seven days, 3-4 mm hypocotyl segments were aseptically excised and transferred to 9 cm sterile Petri dishes containing 10 ml UM medium, each (Ushima and Murashige, 1974) supplemented with 3 % (w/v) sucrose, 0.7 % (w/v) agar (BDH) and 2 mg/L of 2,4- Dichlorophenoxyacetic acid (2,4-D), 0.25 mg/L of Kinetin (K) and 2 gm/L casein hydrolyzate at pH 5.6. Callus cultures were grown in the dark as creamy and friable after 8-10 weeks at 25±2°C. Cell suspension cultures were established from callus and maintained in liquid UM medium on a rotary shaker (125 r.p.m.) at 25°C with a continuous illumination of 500 Lux. These cultures were used as a stock for obtaining suspension cultures.

2 - Effect of Media

The basal medium consists of : MS salt (Murashige and Skoog, 1962) and 3 % (w/v) sucrose.

The MS medium [A] : 2 mg/L of ∝ -naphthalene acetic acid (NAA) was added to the basal medium.

The MS medium plus vitamins [B] : MS medium [A] plus 0.5 mg/L nicotinic acid, 0.1 mg/L thiamine HC1 and 0.5 mg/L pyridoxine HCL.

The UM medium [C] (Ushima and Murashige, 1974) : The basal medium was supplemented with 5 mg/L nicotinic acid, 10mg/L thiamine HC1, 10 mg/L pyridoxine

HC1 and 2 mg/L casein hydrolyzate in addition to 2 mg/L 2,4 -D and 0.25 mg/L kinetin.

Each experiment was carried out twice at one week intervals for four weeks. The cited values are means of duplicate analysis.

3 - Sucrose Experiments

These were carried out using UM medium [C]. Concentrations of 1% , 3% and 5% sucrose were added. Each experiment was carried out twice at one week intervals for four weeks. The cited values are means of duplicate analysis.

4 - pH Experiments

The initial pH of the medium was adjusted in the range from 2.5 to 6.0 either with HC1 (0.1N) or KOH (10%) prior to autoclaving. Suspension cultures were allowed to grow for three weeks and then harvested. Other cultural conditions were kept the same as in the prementioned UM medium [C].

5 - Determination of Alkaloids

The harvested cell cultures; grown in 100 ml. Medium; were dried at 110°C for 15 min. and then at 60°C. until having constant weight. Each sample was finally powdered and extracted with 30 ml. of mixture ether, ethanol (4:1). 2 ml. Ammonia solution were added and the mixture was left for one hour. Then, it was filtered and the filtrate was extracted with mixture of HCI (0.5N), ethanol (3:1) until complete extraction of alkaloids. The acidic solution was rendered alkaline using dilute ammonia solution and finally extracted with chloroform. The chloroformic extract was evaporated to dryness *in vacuo.* The residue was dissolved in 10 ml of 0.02 N H_2SO_4 and titrated against 0.02 N NaOH using methyl red as indicator. Each ml. of 0.02 N H_2SO_4 is equivalent to 0.005787 gm. alkaloid calculated as hyoscyamine (Koul *et al.,* 1983).

RESULTS AND DISCUSSION

The suspension cells grew as yellowish and friable cultures with mainly single cells. Formation of alkaloids in stock suspension cultures started at the end of the first week.

1 - Effect of Media

Cells grew best in UM medium [C], fairly well in MS plus vitamins medium [B] and poorly in the MS medium [A] (Fig. 1). Maximum growth was reached at the end of the third week.

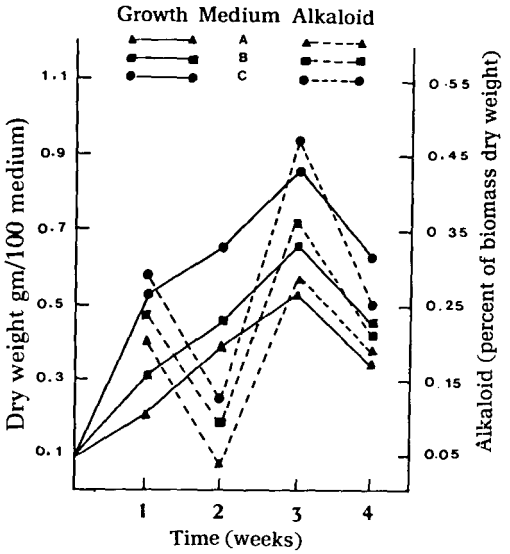

Fig. 1 : Effect of Media on growth and Alkaloid content in *Hyoscyamus Albus.*

Suspension cultures grew as fine yellowish friable cells in the UM medium [C]. In case of the MS medium [A] as well as the MS plus vitamins medium [B], the suspension cultures were yellowish white. After the end of the third week, the colour of the cultures turned yellowish brown in case of UM medium only. The formation of total alkaloids started in the second week and reached its maximum at the end of the third week in each of the tested media (Fig. 1). It is obvious that selection and medium-composition are essential factors in obtaining good growth and tropane alkaloids production (Yoshihiro, *et al.,* 1989). Many reports suggested that, in undifferentiated cells, an inverse relationship between primary metabolism, such as growth, and production of secondary metabolites exists (Böhm, 1977 and Lindsey and Yeoman, 1983). In general, attempts to modify the basal medium rusulted in determintal influences on alkaloid production, especially when NAA in medium [A] or [B] was substituted with 2,4-D in medium [C]. This conclusion is similar to previous observation of Carew and Krueger (1977).

In our *Hyoscyamus albus* cell suspension cultures, we could not detect any tropane alkaloids in any of the studied media used for growing the cells. Hashimoto and Yamada (1983) reported that in spite of adding ten to twenty percent of hyoscyamine to their medium, neither hyoscyamine nor scopalamine was detected in that medium. The UM medium [C] was selected for further study because it proved to be the best medium for the growth and alkaloidal formation of harvested cells. An alkaloid percent of 0.47 which was obtained from cell suspension in case of UM medium [C] (Fig. 1); at the end of the third week, is higher than that reported by Organgi (1987) (0.352%) for the cultivated *H. albus* plant.

2 - Effect of Sucrose Concentration

The effect of various concentrations of sucrose is shown in Fig. 2. A concentration of five percent sucrose was more suitable for the growth than the other concentrations (1%, 3%). Maximum growth was obtained at the end of the fourth week in case of 3% and 5% sucrose while it was at the end of the second week in case of 1% sucrose.

Suspension cultures with 1% sucrose grew as brownish friable suspensions after the end of the second week. With 3% and 5% sucrose the cultures were yellowish brown.

1% Sucrose yielded the highest alkaloidal content (0.75%) followed by 3% sucrose (0.46%) and the lowest alkaloidal content (0.126%) was obtained with 5% sucrose. The maximum alkaloidal formation was observed at the end of the third week (Fig.2) in the three studied sucrose concentrations. This conclusion; of using 1% sucrose; coincided with that reported for *H. muticus* by Koul *et al.* (1983). The high yield of tropane alkaloids (0.75%) is the first record by using cell suspension cultures as a source of tropane alkaloids.

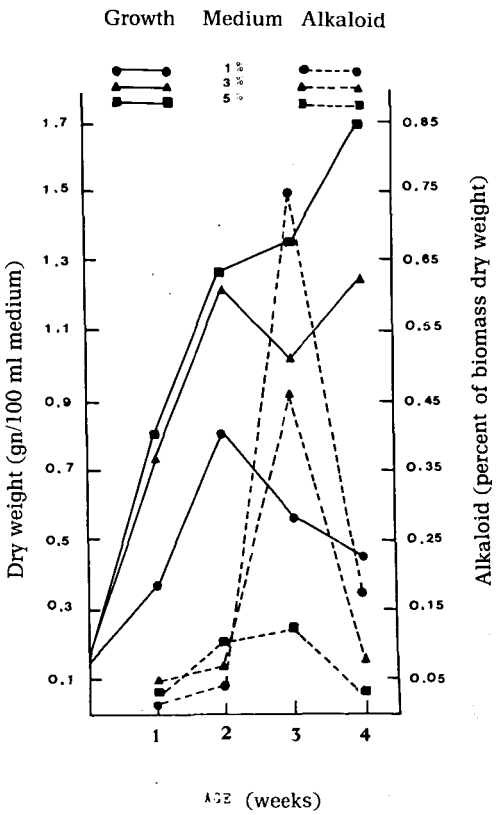

Fig. 2 : Effect of sucrose concentration on gowth and Alkaloid content in *Hyoscyamus Albus*.

3 - Effect of initial pH

The effect of initial pH was studied at the end of the third week as the maximum formation of alkaloids was conducted at that stage (Fig. 1).

Table 1 depicts the effect of different pH ranges on the growth as well as the percent of alkaloidal formation at the end of the third week. The cells were able to aclimatize

Table. 1

Effect of initial pH on growth and alkaloid formation in suspension cultures of *H. albus.*

Initial pH	Dry WT. gm/100ml Medium	Alkaloid %
2.5	0.361	0.381
3.0	0.310	0.402
3.5	1.193	0.465
4.0	1.399	0.345
4.5	1.589	0.132
5.0	2.052	0.084
5.5	1.823	0.036
6.0	1.796	0.012

⋆ Mean of duplicate analysis, calculated on the basis of dry weight.

themselves in UM medium [C] at the studied pH range (2.5-6). The dry cell yield reaches its maximum at pH 5. The pH did not affect the nature of the cell suspension. Maximum alkaloid formation (0.465%) was obtained at pH 3.5 while the minimum formation (0.012%) was conducted at pH 6.0. At the end of the experiment (three weeks), the final pH remained in range of 5.5 to 5.9 whatever was the initial pH. The data obtained suggest that the pH influenced the growth as well as the formation of tropane alkaloids (Martin and Rose, 1976 and Koul *et al.*, 1983). On absolute bases maximum alkaloid formation was at the end of the third week. Similar reports, where metabolite synthesis was independent of cell mass and morphogensis have been made for *Catharanthus roseus* (Carew and Krueger, 1977) and for *H. muticus* (Grewal *et al.* 1979). Further investigation will be carried out concerning the combination of the reached best conditions in order to have higher content of tropane alkaloids and for having a qualitative and quantitative picture of the formed alkaloids.

REFERENCES

Böhm, H., 1977. Secondary metabolism in cell cultures of higher plants and problems of differentiation. In : Kleinzeller, A., G.F. Springer and H.G. Wittman (eds). Molecular Biology, Biochemistry and Biophysics, 23: 118-119. Springer-Verlag; Berlin.

Carew, D.P. and R.J. Krueger, 1977. *Catharanthus roseus* Tissue culture : The effects of medium modifications on growth and alkaloid production, Lloydia, 40: 326-336.

Corduan, G. and C. Spix, 1978. Production of natural compounds by cell culture methods, A.W. Alfermann and E. Reinhard (eds.), 295, Munchen, GSF, BPT-Report 1.

Dhoot, G.K. and G.G. Henshaw, 1977. Organization and alkaloid production in tissue cultures of *Hyoscyamus niger,* Ann. Bot., 41: 943-949.

Evans, W.C., 1979. The Biology and Taxonomy of the Solanaceae, Linnean Society Symposium Series, 7:241-254.

Grewal, S., S. Koul, A. Ahuja, and C.K. Atal, 1979. Hormonal control of growth, organogensis and alkaloid production in *in vitro* cultures of *Hyoscyamus muticus* Linn, Ind. J. Exp. Biol., 17: 558-561.

Hashimoto, T. and Y. Yamada, 1983. Scoplamine production in suspension cultures and redifferentiated roots of *Hyoscyamus niger,* Planta Med., 47: 195-199.

Hashimoto, T., Y. Yukimune and Y. Yamada, 1986. Tropane alkaloid production in *Hyoscyamus* root cultures, J. Plant Physiol., 124: 61-75.

Koul, S., A. Ahuja and S. Grewal, 1983. Growth and production in suspension cultures of *Hyoscyamus muticus* as influenced by various cultural parameters. Planta Med., 47: 11-16.

Kurz, W.G.W. and F. Constabel, 1985. Aspects affecting biosynthesis and biotranstormation of secondry metabolites in plant cell cultures, the CRC Critical Reviews in Biotechnology, 2: 105-118.

Lindsey, K. and M.M. Yeoman, 1983. The relationship between growth rate, differentiation and alkaloid accumulation in cell cultures, J. Exp. Bot., 34: 1055-1065.

Martin, S.M. andD. Rose, 1976. Growth of plant cell *(Ipomoea)* suspension cultures at controlled pH levels, Can. J. Bot., 54: 1264-1270.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures, Physiolgia PL., 15: 473-497.

Oksman-Caldenty, K.M., A. Strauss and R. Hiltunen, 1986. Optimization of some parameters in cultivating scopolamine-producing cells of *Hyoscyamus muticus,* 34th Annual Congress on Medicinal Plant Research (G.A.), Hamburg, 22-27 Sep., 25.

Organgi, R.A. 1987. Habitat and alkaloidal content of *Hyoscyamus albus* L. grown in Saudia Arabia, Bull. Fac. of App, Sci. and Eng., Res. Unit of plant Ecology. 25-34.

Planchen, L., P. Bretin and P. Manceaux, 1946. Précis de matiére medicale. 5 émeed. Paris, 1750.

Tabata, M., H. Yamamoto and N. Hiraoka, 1970. Alkaloid production in the tissue cultures of some solanaceous plants. In les cultures de tissus de plantes. Collegues Internationaux. C.N.R.S. No 193, Paris, 177-186.

Ushima, H. and T. Murashige, 1974. Evaluation of parameters in isolation of viable protoplasts from cultured *Tobacco* cells, Plant Physiol., 54: 936-944.

Yamada, Y. and T. Hashimoto, 1982. Production of tropane alkaloids in cultured cells of *Hyoscyamus niger.,* Plant Cell Rep., 1: 101-103.

Yoshihiro, M., H. Ohkawa and Y. Yamada, 1989. Production of tropane alkaloids by hairy root cultures of *Duboisia leichhardtii* transformed by *Agrobacterium rhizogenes,* Plant Science, 59: 191-201.

نمو وانتاج قلويدات التروبان بواسطة مزارع المعلق الخلوي لنبات السكران الأبيض

أحمد محمد كنسارة و مدحت سيف النصر

أجـرى فحص لنمو وانتاج قلويدات التروبان لمزارع المعلق الخلوي في ثلاث بيئات مختلفـة لنبـات السكران الأبيض ، وقد درس أيضاً تأثير تركيز السكروز ورقم الأس الهيـدروجيني في بدايـة التجـارب كمـا تم التوصل إلى زيادة انتاج قلويدات التروبان بإستخدام هرمون ٢ ، ٤ ـ ثنائي كلوروفينوكسي حامض الخليك و١٪ سكروز ، حيث بلغت نسبة تكوين قلويدات التروبان (٧٥,٠٪) وهي أعلى منها في النبات المنزرع (٣٥٢,٠٪) .