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In vitro REGENERATION OF *Atropa belladonna* L. FROM LEAF DISCS

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ABSTRACT

Multiple shoots were regenerated from calli induced from leaf discs of *Atropa belladonna* on Murashige and Skoog (MS) medium supplemented with various combinations and concentrations of naphthalene acetic acid (NAA) and either 6-benzyladenine (BA) or thidiazuron (TDZ). TDZ was more effective than BA. High numbers of adventitious shoots occurred at all levels of TDZ, especially in the presence of NAA. The maximum number of shoots (13.92 shoots/leaf disc) was obtained at 1.5 mg l^{-1} TDZ and 0.5 mg l^{-1} NAA, whereas BA gave the highest number of shoots (9.2 shoots/leaf disc) at 3.5 mg l^{-1} alone. Regenerants were rooted easily (100%) and normally developed roots on auxin-free MS medium. Rooted plantlets were successfully acclimatized (94%) in 1 perlite : 2 sand (w/w). Some of the plants showed morphological and growth characteristics different from those of seed-derived plants.

Key words: *medicinal plants, organogenesis, belladonna.*

1. INTRODUCTION

Belladonna (*Atropa belladonna*) belongs to the family Solanaceae and the genus *Atropa*. The plant is considered to be one of

the most important medicinal plants for its high content of alkaloids . (Phillipson and Handa, 1976; Simola *et al.*, 1988; Bajaj and Simola, 1991). The species is used as a floricultural plant and for the production of some medicinal drugs; mainly atropine (Bajaj and Simola, 1991; Toima *et al.*, 1991).

Regeneration by organogenesis or somatic embryogenesis has been possible in several plant species (James *et al.*, 1988; Leblay *et al.*, 1991; Billings *et al.*, 1988; Beattie and Garrett, 1995; Massimo *et al.*, 1996; Arena and Pastur, 1997). Plant tissue culture and regeneration techniques are useful in the production of somaclonal variants and the development of transgenic plants. There are many studies on regenerating complete plants from callus cultures of excised *A. belladonna* anther/pollen, suspension and hairy root cultures (Knopp *et al.*, 1988; Bajaj, 1988, Bajaj and Simola, 1991). This study was aimed to develop a method for the regeneration of *A. belladonna* from leaf discs *via* adventitious shoot formation, which would be very useful for mass propagation or induction of somaclonal variation.

2. MATERIALS AND METHODS

Seeds, obtained from the Research Station of the Faculty of Agriculture, Menofia University, Egypt, were sown in 10 cm pots filled with 1 peatmoss : 1 sand in the nursery. After the plants reached the height of 15 cm, they were transferred to the lab and kept for 8 months. Fully expanded and healthy leaves were randomly excised and first washed with sterilized distilled water to which a few drops of Dermosept solution (4% Chlorhexidine Gluconate, SPIMACO, Saudi Arabia) were added, followed by dipping in 70% ethanol for few seconds and then immersed for 10 minutes in a 10% Clorox solution (5.25% sodium hypochlorite) containing a few drops of the surfactant Tween 20 and finally rinsed 4 times with sterile distilled water. Leaf discs were prepared using a sterile 8 mm cork borer. The leaf discs, with their lower surfaces on the surface of the medium, were cultured in 100 x 15 mm sterile plastic petri dishes containing 25 ml Murashige and Skoog (MS) (1962) medium supplied with30 g l^{-1} sucrose and 7 g l^{-1} agar (Micro Agar, DUCHEF

Biochemicals, The Netherlands). The culture media were supplemented with various concentrations of BA (0.0, 0.5, 1.0. 1.5, 2.5 or 3.5 mg l^{-1}) or TDZ (0.0, 0.5, 1.0. 1.5, 2.5 or 3.5 mg l^{-1}) singly or in combination with NAA (0.0, 0.5 or 1.0 mg l^{-1}). The pH was adjusted to 5.7 before autoclaving. Six petri dishes were allocated for each treatment and each plate containing three leaf discs. All cultures were incubated in the dark for 3 weeks at 25±2° C and then transferred to a 16 h photoperiod (49-58.6 µmol m^{-2} s^{-1}, cool white fluorescent lamps). To allow shoot elongation, whole clump of shoots, regenerants with leaf disc, were transferred to MS containing no growth regulators. Microshoots were then rooted and acclimatized as described by AL-Wasel (1999). Data were recorded after 8 weeks, and then analyzed using WINKS statistical data analysis program (TexasSoft, Cedar Hill, Texas, USA). Comparisons of treatment means were accomplished by the Tukey test at the 5% level of significance.

3. RESULTS

Callus formation was visibly observed after two weeks of incubation in all media having growth regulators. Leaf discs placed on a medium containing no growth regulators did not form callus and the explants dessicated. The interactions between cytokinins and auxin were significant. TDZ stimulated callus and adventitious shoot formation better than BA. TDZ alone resulted in lower shoot formation which increased significantly in the presence of NAA (Table 1). TDZ at 1.5 mg l^{-1}, or in combination with NAA, was found to be the best concentration for adventitious shoot induction (Fig.1). The maximum number of regenerated shoots (13.92 shoots/leaf disc) was obtained with 1.5 mg l^{-1} TDZ and 0.5 mg NAA. The higher levels of TDZ decreased the number of shoot formation. BA alone, especially at the highest level, resulted in higher shoot formation (9.2 shoots/leaf disc) (Table 2). Most of the regenerated shoots originated from the cut surface. The addition of NAA, in the presence of TDZ or BA, improved shoot regeneration rate and promoted the formation of numerous fibrous roots, especially in media lacking cytokinins (Fig 2). BA had less effect on hairy roots formation than TDZ which

visibly inhibited their formation. Regenerated shoots rooted readily (100%) in basal MS salt medium (Fig. 3). All rooted plantlets survived when transferred to a soil mixture (1 perlite : 2 sand),. Some of these plants had different leaf shape from those of seed-propagated plants (Fig. 4). These plants are under investigation to assure that their phenotype is stable. All plants will be also evaluated for their alkaloid contents in order to obtain a variant that yields high amount of alkaloids.

Table (1):Effects of TDZ and NAA on shoot regeneration from leaf discs of *A. belladonna In vitro*.

TDZ (mg l^{-1})	NAA (mg l^{-1})	Discs with shoots (%)	Mean no. of adventitious shoots/leaf disc
0.0	0.0	0.0	0.0f[1]
0.5		72.2	2.61 ef
1.0		66.7	1.94 ef
1.5		100.0	5.39 de
2.5		94.4	3.72 def
3.5		88.9	2.39 ef
0.0	0.3	0.0	0.0 f
0.5		83.3	5.89 cde
1.0		100.0	12.33 ab
1.5		100.0	12.93 a
2.5		100.0	12.56 a
3.5		100.0	9.94 abc
0.0	0.5	16.7	0.17 f
0.5		100.0	12.17 ab
1.0		100.0	13.42 a
1.0		100.0	13.92 a
2.5		100.0	11.33 ab
3.5		100.0	7.5 bcd

[1] Means, in the same column, followed by different letters are significantly different by Tukey test at the 5% level.

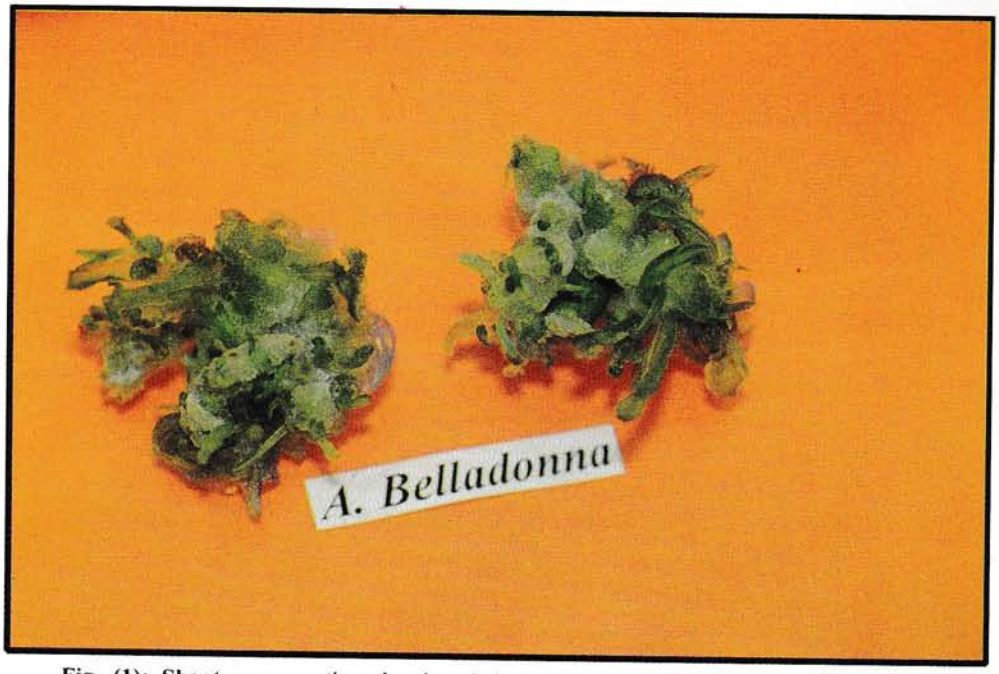

Fig. (1): Shoot regeneration developed from callus induced from leaf discs of *A. belladonna* cultured on MS medium supplemented with 1.5 mg l^{-1} TDZ and 0.5 mg l^{-1} NAA.

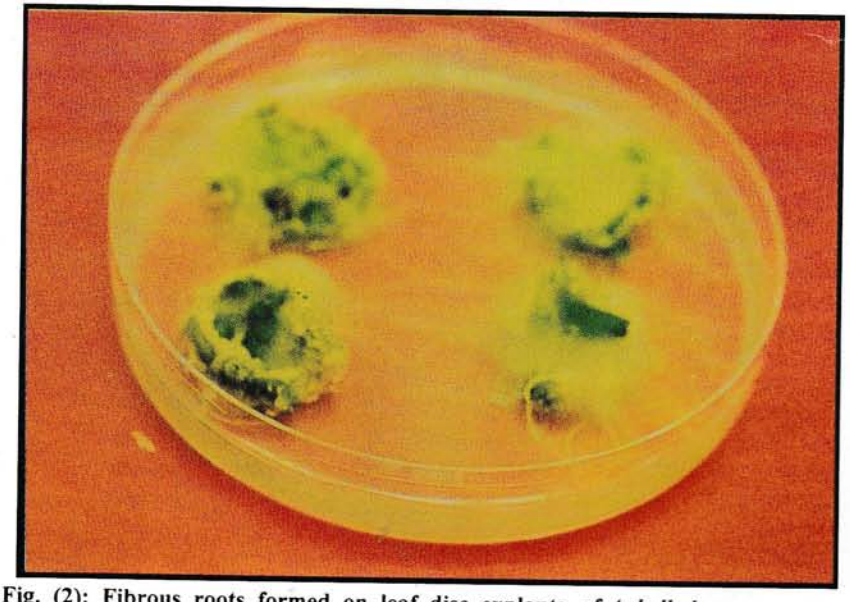

Fig. (2): Fibrous roots formed on leaf disc explants of *A. belladonna* cultured on MS medium containing NAA.

Table (2): Effects of BA and NAA on shoot regeneration from leaf discs of *A. belladonna In vitro*.

BA (mg l^{-1})	NAA (mg l^{-1})	Discs with Shoot (%)	Mean no. of adventitious shoots/leaf disc
0.0	0.0	0.0	0.0g[1]
0.5		38.9	1.22 fg
1.0		83.3	2.61 defg
1.5		33.3	1.8 efg
2.5		88.9	6.17 abcde
3.5		93.3	9.2 a
0.0	0.3	20.0	0.33 fg
0.5		93.3	3.13 cdefg
1.0		100	5.0 abcdef
1.5		93.3	6.27 abcde
2.5		88.9	4.33 bcdefg
3.5		100	8.61 ab
0.0	0.5	13.3	0.17 g
0.5		55.6	2.06 efg
1.0		72.2	3.89 cdefg
1.5		94.4	6.56 abcd
2.5		94.4	7.06 abc
3.5		94.4	6.78 abcd

[1] Means, in the same column, followed by different letters are significantly different by Tukey test at the 5% level.

4. DISCUSSION

The results of the present study indicated that *A. belladonna* can be regenerated easily by culturing leaf segments *in vitro*. Both cytokinins and auxin affected the ability of the leaf discs to develop adventitious shoots. However, cytokinins were found to be the limiting factor for obtaining the maximal adventitious shoot formation. TDZ was more effective than BA in inducing multiple adventitious shoots. This would be attributed to the inhibitory effect of TDZ on fibrous root formation; therefore, explants exploited their regenerability potential to form adventitious shoots only, whereas BA did not suppress fibrous roots formation and the potential of the

Fig. (3): Rooted plantlets of *A. belladonna* on auxin-free MS medium.

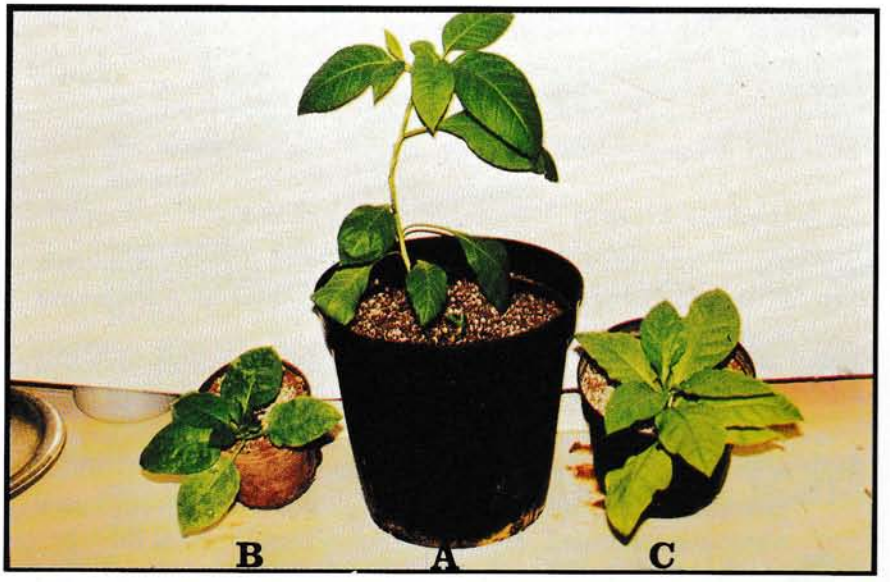

Fig . (4): Morphological differences in leaf shape between the mother plant and the *in vitro*-derived plants: The middle plant= mother plant; The left plant= tissue cultured-derived plant having similar leaf shape as mother plant; The right plant= tissue cultured-derived plant having different leaf (heart shape and rough leaves) from those of the mother plant.

explants was devoted for both adventitious shoot and root formation. The optimal combination for belladonna adventitious shoot formation was 1.5 mg l^{-1} TDZ and 0.5 mg l^{-1} NAA. TDZ is a substituted phenylurea with cytokinin like activity (Thomas and Katterman, 1986). This has been recently exploited to stimulate shoot regeneration in a number of plant species (Beattie and Garrett, 1995; Faure *et al.*, 1998). Nugent *et al.*, (1991) reported that TDZ was more effective than BA or Kinetin for inducing shoot regeneration from carnation petals.

Some regenerants showed different morphological characters. Off-type plants have been also reported among regenerated plants from stem and root explants of *A. belladonna in vitro* (Toth, *et al.*, 1991). This reliable regeneration system would have promising applications for improving belladonna through somaclonal variation, genetic transformation or mass propagation.

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5. REFERENCES

AL-Wasel A.S. (1999). *In vitro* multiplication of *Atropa belladonna* L. using nodal segments. Alexandria Journal of Agricultural Research. 44 (3): 263-274.

Arena E. M. and Pastur G.J.M. (1997). Adventitious shoot induction from leaf explants of *Ribes magellanicum* cultured *in vitro*. Scientia Horticulturae. 72: 73-79.

Bajaj Y.P.S. (1988). Regeneration of plants from frozen (-196°C) protoplasts of *Atropa belladonna* L., *Datura innoxia* Mill., and *Nicotiana tabacum* L. Indian Journal of Experimental Biology. 26 (4): 289-292.

Bajaj Y.P.S. and Simola L.K. (1991). *Atropa belladonna* L.: *In vitro* culture, regeneration of plants, cryopreservation, and the production of tropane alkaloids. In: Bajaj Y.P.S. (Eds.), Medicinal and Aromatic Plants III, Biotechnology in Agriculture and Forestry. Springer- Verlag, Berlin Heidelberg, New York, vol. 15, pp 1-23.

Beattie L.D. and Garrett R.G. (1995). Adventitious shoot production from immature embryos of white clover. Plant Cell Tissue and Organ Culture. 42: 67-72.

Billings S.G., Chin C.K. and Jelenkovic G. (1988). Regeneration of blueberry plantlets from leaf segments. HortScience. 23 (4): 763-766.

Faure O., Diemer F., Moja S. and Jullien F. (1998). Mannitol and thidiazuron imroved *In vitro* shoot regeneration from spearmint and peppermint leaf disks. Plant Cell, Tissue and Organ Culture. 52: 209-212.

James D.J., Passey A. and Rugini E. (1988). Factors affecting high frequency plant regeneration from apple leaf tissues cultured *in vitro*. J. Plant Physiol. 132: 148-154.

Knopp E., Strauss A. and Wehrli W. (1988). Root induction on several Solanaceae species by *Agrobacterium rhizogenes* and the determination of root tropane alkaloid content. Plant Cell Reports. 7 (7): 590-593.

Leblay C., Chevreau E. and Raboin L.M. (1991). Adventitious shoot regeneration from *in vitro* leaves of several pear cultivars (*Pyrus communis* L.). Plant Cell Tissue and Organ Culture. 25: 99-105.

Massimo H.M.S., Shattuck V.I. and Strommer J. (1996). Rapid plant regeneration of pea using thidiazuron. Plant Cell Tissue and Organ Culture. 45: 165-168.

Murashige T. and Skoog F. (1962). Revised medium for rapid growth and bioassays with tabacco tissue culture. Physiol. Plant. 15: 473-497.

Nugent G., Wardley-Richardson T. and Lu C.(1991). Plant regeneration from stem and petal of carnation (*Dianthus caryophyllus* L.). Plant Cell Report. 10: 477-480.

Phillipson J. D. and Handa S. S. (1976). Hyoscyamine N-oxide in

Atropa belladonna. Phytochemistry. 15: 605-608.

Simola L.K., Nieminen S., Huhtikangas A., Ylinen M., Naaranlahti T. and Lounasmaa, M. (1988). Tropane alkaloids from *Atropa belladonna*, Part II. Interaction of origin, age, and environment in alkaloid production of callus cultures. J. Nat. Prod. 51: 234-242.

Thomas J. C. and Katterman F. R. (1986). Cytokinin activity induced by thidiazuron. Plant Physiol. 81: 681-683.

Toima N.M., Boselah N.A.E., Youssef A.K. and Amine I.S. (1991). Seed germination of *Atropa belladonna* Linn. Bull. Fac. of Agri., Univ. of Cairo. 42 (7): 31-18.

Toth E. T., Onisie T., Amariei D. and Lazurca D. (1991). Variability in tissue culture regenerated plants of *Atropa belladonna*. Acta Horticulturae. 289: 269-270.

تشجيع تكوين النموات الخضرية العرضية لنبات البلادونا (*Atropa belladonna* L.) من حلقات ورقية

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ملخص

تم تشجيع تكوين النموات الخضرية من الكلس الذي تكون على الحلقات الورقية لنبات البلادونا على بيئة موراشيجي وسكوج المضاف إليها توليفات وتراكيز مختلفة من هرمون نفثالين حمض الخليك والبنزيل أدنين أو الثايديزرون. وقد بينت النتائج أن هرمون الثايديزرون كان أكثر فعالية من هرمون البنزيل أدنين، حيث أدت جميع التراكيز المستعمله منه إلى تشجيع أعلى المعدلات للنموات الخضرية العرضية خاصة في وجود هرمون النفثالين حمض الخليك. وقد حدث أعلى معدل للنموات الخضرية العرضية (13.92 نمو خضري عرضي/ حلقة ورقية) في تركيز 1.5 ملجم-1 ثيايديزرون و 0.5 ملجم-1 نفثالين حمض الخليك، بينما كان أعلي معدل تضاعف للنموات الخضرية العرضية (9.2 نمو خضري عرضي/ حلقة ورقية) عند إضافة 3.5 ملجم-1 بنزيل أدنين منفرداً.

تم تشجيع تكوين الجذور العرضية الطبيعية الشكل علـــى هـــذه النمـــوات الخضرية العرضية بنسبة 100% على بيئة موراشيجي وســـكوج الخاليـــة مـــن الأوكسينات. وتم أقلمة هذه النباتات بنجاح (94%) في خلطة 1 بيرليت : 1 رمل. وقد لوحظ أن بعض هذه النباتات تختلف في شكلها الظاهري عن النبات الأم.

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