Research Paper

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Studies on the optimization conditions of root and callus initiation in Atropa belladonna in in-vitro conditions

Biotechnology

KEYWORDS : Atropa belladonna, Atropine, hyoscine, scopolamine, hyoscyamine, Callus

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ABSTRACT

Atropa belladonna L. is used in traditional treatments for an assortment of conditions including headache, menstrual symptoms, pectic ulcer, histaminic reactions, inflammation and motion sickness. It is used to overcome bronchial spasms, whooping cough. It is used for Parkinson's disease, antidote for snakebite, gastric agent. The plant species is the source of alkaloid atropine ($C_{17}H_{23}NO_3$, d1-hyoscyamine) which has proven to be a cornerstone in the study of autonomic pharmacology. Root and callus cultures have been established from explants of Atropa belladonna. Explants selected for the present study included leaf midrib and petiole region. MS media constituent with hormone NAA was used in the present investigation. Under 25±3°C explants with NAA showed root initiation. Under same conditions explants with KI differentiated into callus and further formation of roots. But roots obtained from explants (with MS+NAA) directly transferred to MS+KI+2% sucrose media showed callus formation under 25±3°C. Initial media without excess sucrose did not give rise to callus or root formation. Use of MS+BA also did not show any initiation of callus at 25°C but other workers point out a lower temperature of 20-24°C for development of callus.

INTRODUCTION:

The term micropropagation means a technological process consisting of several steps in order to produce numerous propagula from a chosen mother plant under in vitro conditions. Large scale technologies have been developed following long experimental procedures worldwide. We all are aware that medicinal plants are most important source of life saving drug for majority of world's population. But growing population and exploitation of natural resources cannot compete the demand of these medicinal plants. Plant tissue culture would be the best answer. Among the list of plant species Atropa belladonna is known for its own importance.

From the ancient time Atropa belladonna is a well known medicinal plant. It is a perennial herb belongs to family Solanaceae. Name of the plant was published by Linnaeus in Species Plantarum in 1753. The common name were Deadly nightshade, Dwale, Death cherries etc,. Plant grows in saline soil and in shade with optimum temperature 24˚c. it is 5 feet perennial herb which consist of purple color stem, dark green leaves, creepy root, dark shaped purple flower with black blue berries. Whole plant is toxic because by the production of toxic substance called tropane alkaloids. The plant species is native to Europe (Austria, Ukraine, and Albania), Northern Africa (Algeria, Morocco) and Western Asia (Iran, Turkey). It is cultivated in Europe, Northern and Southern America and in few parts of India and Pakistan. It belongs to Solanaceae family, Subfamily Solanoideae and tribe Hyoscyameae. A. belladonna is a perennial herb with purplish colored stem, thick roots, darkish color leaves with short petiole, purplish colored bell shaped flower and shining black color fruit berry with disperse seeds. The plant grows well in shady moist environment in cold zones with saline in soil.

The active agents of belladonna are atropine, hyoscine and hyoscyamine. Used in traditional treatments for conditions including headache, menstrual symptoms, inflammation, rheumatism, neuralgia and much noted for eye disorders. It is a powerfull muscle relaxant and relieves spasm. It is best Homeopathic drug for Irritable bowel syndrome. It combats cardiac palpitation. Belladonna is very toxic plant and should avoid intake of leaves or berry as it may leads to death.

Because of pharmaceutical interest in this plant, a considerable amount of work has done on morphology, in vitro culture and alkaloid production. Embryogenesis in protoplast from cell suspension was reported (G. Gosch, Y. P. S. Bajaj, 1975). Kanamycine resistance gene was expressed in belladonna (Natalia cucu, Gabriela Negoianu, 2001). Germinational study on belladonna was carried out using seeds at different conditions (Elena Genova, Gergana komitska, 1997). Variation in alkaloid production reported in belladonna with Agrobacterium rhizogenes (Toshio Aoki, Hideki Matsumoto, 1997). Metabolic steps are artificially altered by genetic transformation of Atropa belladonna (M. Jaziri, K. Yoshimatsu, 1999). Many experiments were conducted to increase the productivity of plants and its culture.

MATERIALS & METHODS:

The pure breed plants were obtained from Jammu Kashmir Horticultural Department, Srinagar. Maintenance and reproduction of plant is maintained in green house and later transferred to M S media for further results.

Media: MS media, MS major salt, MS minor salt, Iron EDTA, MS vitamins, Sucrose 3%, Agar-6.5-8.0%. Various concentration of the three hormone solutions were prepared and added to the best media culture. Napthaleneacetic acid (NAA), Kinetin (KIN) were added at different concentration to culture media which was retained by factorials design. Two concentrations of NAA and Kinetin concentration were applied in the present study listed below in detail. (Table1). Leaf parts, lamellae (5mm length), Midrib (5-7mm length) and petiole (5mm length) are used as explants. Each piece planted on a solid agar medium in five replicates (jars) with frequency of 2 explants pre jar for callus induction.

Table 1:- MS media supplemented with different growth regulators at different concentrations used in the present research from leaf explants of Atropa belladonna.

No of Media	MS Media components
1	MS (25.0g/l)+Sucrose(30g/l)+Agar(6.5g/l). {Hormone free}
2	MS(25.0g/l) +Sucrose(30g/l)+Agar(6.5g/l)+NAA(0.2mg/l)
3	MS(25.0g/l)+Sucrose(30g/l)+Agar(6.5g/l)+NAA(0.3mg/l)
4	MS(25.0g/l)+Sucrose(30g/l)+Agar(6.5g/l)+KI(0.2mg/l)
5	MS(25.0g/l)+Sucrose(30g/l)+Agar(6.5g/l)+KI(0.4mg/l)

Treatment of Material: Atropa belladonna grows well in incubation conditions set at 26±3˚c with 16hour light/8hour dark photoperiod.

Dry matter content (%): The fresh weight (mg) of different calli was dried at 60˚c for 48 hours in hot air oven. Dry matter content was estimated by below equation

$$\text{Callus dry weight} = \frac{\text{Callus dry weight}}{\text{Callus fresh weight}} \times 100$$

Dry callus of different hormone concentration is stored for fur-

ther analysis of phytochemical at 16˚c in airtight containers.

RESULT AND DISCUSSION

Atropa belladonna fresh explants were cut and separated as lamellae part, midrib and petiole. Explants are surface sterilized and transferred into MS media supplemented with definite hormonal concentration. Conditions of lab were maintained as noted above. Explants showed results exactly after 30-35 days of inoculation. 5-6 week after inoculation explants showed morphological changes. Roots appeared on inoculation site, where NAA was growth hormone. Root growth rate was observed in interval of time and changes in growth rate were recorded. (Table2) The same roots differentiated into callus with exposure for long time in same conditional media. Kinetin showed successful callus induction. Callus growth was also promising even at higher temperature. 2 months old callus from NAA and KI used for dry weight calculation, and tabulated. (Table3). There was no further changes in morphology of any of the explants above, even on prolong exposure to same media.

Atropa belladonna showed its best root growth with less concentration of NAA, where other hormones like IAA, BA and 2, 4 D did not showed any promising result. Callus rose from leaves of belladonna supplemented with KI showed highest growth and healthy callus. MS media with 3% sucrose supplemented with 1.25mg/l of BAP provided shoots from explants; 0.25 mg/l NAA induced roots in Atropa baetica (Rafael et al., 1997). MS+BAP+IAA most suitable for in vitro propagation was reported (Dimitrova D et al., 1996). NAA too showed best root initiation and hence micropropagation. Report proved use of NAA for both root and shoot initiation with Schenk & Hildebrandt media (H. C. Chaturvedi, 2004). Even MS media also showed same report as previously mentioned media. root induction in hormone free media at 25˚c under 16h day light was successfully recorded (K. Yoshimatsu, 1997). But at 27˚c there were no changes in explants with hormone free media. Same intact explants were transformed to hormone media. Induction of adventitious shoot or embryogenesis were completely absent in above mentioned media under mentioned environmental conditions. IBA 1mg/l proved good root inducer in Atropa acuminate and IAA reported for shoot elongation of same species of Atropa (Ashok Ahuja et al, 2002).

Table 2:- MS media supplemented with NAA hormone at different concentration showed altering results with different explants tabulated.

Each culture jar: Having 2 explants on incubation day. No of replicates: 3

Hormone	Hormone concentration (mg/l)	Explants (Leaf parts)	Length of root in 30days (cm)	Length of root in 45days (cm)
Hormone Free	Nil	Lamellae	No Result	
		Midrib	No Result	
		Petiole	No Result	
NAA	0.2	Lamellae	0.5 ± 0.1	1.7 ± 0.2
		Midrib	0.4 ± 0.1	0.9 ± 0.1
		Petiole	No Result	
NAA	0.3	Lamellae	0.7 ± 0.1	2.1 ± 0.2
		Midrib	0.4 ± 0.1	0.9 ± 0.1
		Petiole	0.3 ± 0.1	0.8 ± 0.1

Data from Table 2 conclude that NAA with 0.3 mg/l showed best root initiation compared to higher concentration. MS+0.3mg/l lamellae showed maximum root growth. Other concentration also showed promising results.

Table 3: Interactions between Atropa belladonna and different hormones concentrations of callus fresh weight and callus dry weight.

Hormone	Concentration(mg/l)	Callus fresh weight (mg)	Callus dry weight (mg)
NAA	0.2	1320.0	120.0
NAA	0.3	1290.0	90.0
Kinetin	0.2	1350.0	110.0
Kinetin	0.4	1460.0	160.0

By observation of callus dry weight, we can conclude that callus from KI 0.4mg/l reported maximum callus growth.

Figure 1: Root initiation in NAA supplemented media.

Figure 2: Callus induction in MS media supplemented with NAA & KI.

CONCLUSION

Hormone free media proved unsuccessful for root initiation or for callus induction but the explants were found to be intact. NAA at lower concentration proved best for root induction even at higher temperature. KI successfully induced best callus compared to NAA and had higher growth rate. From the above data it was noted that Atropa belladonna showed good result at higher temperature of 25±3°C. This indicates that the natural cold conditions under which the plant grows can be modified under controlled conditions to grow at higher temperatures, which in turn results in the growth of this plant in different regions unsuited presently.

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