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Comparison studies on callus induction of fresh and reused explants of *Atropa belladonna*

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ABSTRACT

Atropa belladonna belongs to the family Solanaceae. It is the most poisonous plant of western Hemisphere. All parts of the plant contain tropane alkaloids. The active agents of belladonna are atropine, hyoscine and hyoscyamine. Belladonna has been used in traditional treatments for centuries for an assortment of conditions including headache, pectic ulcer, histaminic reactions, motion sickness etc. Micropropagation of ancient medicinal plant is necessary to produce many such secondary products. Micropropagation of Atropa belladonna at higher temperature had showed differentiated results. 25±3 °C incubation for 16h light/8h dark showed many altering results with different hormonal concentration. Fresh explants and reused explants (intact and healthy explants transferred from hormone free media to new media), when transferred to MS media with NAA and Kinetin resulted in altered callus formation. Growth rate was varied, recorded and callus dry weight calculated for comparison study between fresh and reused explants.

Keywords: *Atropa belladonna,* Atropine, Hyoscine, Scopolamine, Callus, Organogenesis

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INTRODUCTION

Atropa belladonna is a perennial herb and belongs to the family *Solanaceae*, included under the threat category Rare in the Red Data Book of the People's Republic of Bulgaria and as Vulnerable in the Red list of Bulgarian vascular plants [1]. The name Atropa belladonna was published by Linnaeus in Species Plantarum in 1753. The common names of species include belladonna, deadly nightshade, dwale, beautiful death. A member of the family Solanaceae, it is closely related to plants such as tomato and potatoes, as well as other toxic plants, such as *Datura*, *Hyoscyamus*, and *Nicotiana* [2]. Belladonna has been introduced and naturalised in a few areas, and is found as a weedy species in north to southern Scandinavia and in some areas of Canada and the United States (Scott 1991). The highly toxic alkaloids atropine, hyoscyamine and bellodonnine are present throughout the plant [3]. Atropa belladonna has been used in traditional treatments for centuries for an assortment of conditions including headache, menstrual symptoms, pectic ulcer disease, inflammation and motion sickness. Atropine can also be used to lower blood pressure and lessen the effects of hypertension [4]. Hyoscine is also used in anti-vertigo drugs and other drugs that aid in the prevention of motion sickness [5].

In the history of belladonna plant, the yearly production rate during that time in the above mentioned areas was to be about 60-100 tons of dry leaves & 150-200 tons of dry roots annually. Till today, belladonna plant is very important in the scientific and medical communities because of the chemicals it contains. India imports 300-350 tones of belladonna every year and only few tones of belladonna grown in Delhi Research Laboratory, Shimla.

Micropropagation methods may help to meet the growing demand of belladonna. Plant Tissue Culture idea to develop transgenic plants may minus the import of lot such medicinal plants into India. Even stress free Atropa belladonna may lead to commercial cultivation of this plant.

MATERIALS AND METHODS

Atropa belladonna plant: The plant material was obtained from Kashmir Horticulture department, Srinagar, Kashmir.

Media and supplements: Media used is simple M S Media with two different hormonal supplements, NAA and Kinetin.

Explants: Two different explants were used for the present study. One is freshly excised explants from mother plant. Another is explants which were already incubated in hormone free media, does not showed any result and same explants transferred into new hormonal supplemented media. Hence called Reused explants.

Incubation is carried out at 25±3°C for 16h light/8h dark at sterile conditions.

Reused explants are those explants which were collected from the mother plant, surface sterilized and transferred into hormone free media. This hormone free media recorded no change and explants were intact after a month of incubation. Same explants were transferred on other media with different hormone supplement provided the explants are healthy and intact. In the present experiment the reused explants were healthy, green and hence were not again conducted to surface sterilization.

The Reused explants and fresh explants (after surface sterilization) were transferred into fresh M S media containing NAA and Kinetin. Fresh explants with NAA showed root initiation, but reused explants showed callus initiation. When Kinetin is used in the above mentioned initiation period, fresh explants differentiate into callus and then root initiation occurs usually (Indirect Organogenesis). Reused explants also showed callus initiation but failed to develop roots. Both wet calluses after 60 days were dried at 60˚C for 48h and dry weight calculated. Dry calluses were stored for phytochemical analysis. Comparison between Atropa belladonna fresh and reused explants are recorded in Table 1.

RESULT AND DISCUSSION

This work was aimed at reusing explants which did not show results in the previous exposure. The main intension of using old intact explants was not to waste the material as it is rarely available (extinct plant) and also for differential results.

The above experimental analysis showed that the explants reused were slow in growth and also the yield of callus was less compared to fresh explants. With NAA no root differentiation was observed in reused explants, but callus developed. No indirect organogenesis was recorded in reused explants but fresh explants gave same result as expected. The reused explant's physiological conditions may be the reason for change. Shoot tip and auxiliary meristem of the plant Atropa belladonna was raised on BA supplemented MS media and subsequently maintained [6]. Best rooting was noted in 0.5mg/l IBA. 0.1 mg/l IBA in combination with 0.0025mg/l BAP promoted root induction [7]. Highest rooting was also reported in IBA in tomato family [8]. Hormone free media did not have any effect on explants. Atropa baetica was successfully supplemented with BAP and NAA at higher temperature reported shoot [9]. MS with IBA and BAP recorded multiple shoot in Atropa acuminata [10].

Fig. 1 Callus growth in both NAA & KI and their weight respectively

Table 1 Comparison between Atropa belladonna fresh and reused explants

Hormone	Hormonal concentration (mg/l)	Explants type	Result	Callus wet weight (mg)	Callus dry weight (mg)
NAA	0.2	Fresh Lamellae	Root initiation	No callus formation	
		Reused lamellae	Callus initiation	1.09	0.28
	0.3	Fresh Lamellae	Root initiation	No callus formation	
		Reused lamellae	Callus initiation	1.36	0.41
KI	0.2	Fresh Lamellae	Callus differentiated into root	1.32	0.44
		Reused lamellae	Callus formation. No root observed	1.06	0.19
	0.3	Fresh Lamellae	Callus differentiated into root	1.49	0.52
		Reused lamellae	Callus formation. No root observed	1.22	0.26

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