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RESEARCH ARTICLE

Comparative efficiency of different explants for *in vitro* callus production in *Inula royleana* DC., a threatened medicinal plant growing in Kashmir Himalaya

Samar Amin, Zahoor A Kaloo, Seema Singh
Plant Tissue Culture Laboratory, Department of Botany, University of Kashmir, Hazratbal, Srinagar, J&K, India.

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Abstract

Callus cultures have successfully been used for organogenic differentiation, establishment of cell suspension cultures that in turn help in production of synthetic seeds through somatic embryogenesis and in secondary metabolite production, somaclonal variations etc. So far as the selected plant *Inula royleana* is concerned, it is a threatened and one of the important medicinal plant species of genus *Inula*. It is known to contain sesquiterpene lactones. During the present study callus development has been induced in explants like leaf, petiole, involucral bracts and *in vitro* seedlings on MS medium supplemented with different concentrations of both auxins and cytokinins individually as well as in combination. Best results were obtained when leaf explants were inoculated on medium containing BAP (5mg/l) + IAA (2mg/l), in a time period of 32 days with 100 percent cultures.

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Introduction

Callus cultures have been used for organogenic differentiation in a number of plants like *Vicoa indica* (Thulaseedharan and Vaidyanathan, 1990), *Artemisia absinthium* (Nin et al., 1996), *Artemisia annua* (Kamili et al., 2001), Pluchea lanceolata (Kumar et al., 2003), *Cichorium intybus* (Nandagopal and Kumari, 2006) etc. They have also proved useful in synthetic seed production like in case of *Ipomoea batatas* (Chee et al., 1992) from shoot apex callus, *Arnebia euchroma* (Manjkhola et al., 2005) from leaf explant callus, *Pogonatherum paniceum* (Wang et al., 2007) from seed callus etc. A number of secondary metabolites have been produced in relatively large concentrations from callus and suspension cultures like Tetrahydroanthracene glucosides from *Aloe saponaria* Suspension (Yagi et al., 1983), Alkaloids (Anderson et al., 1987) and Canthinone alkaloids (Anderson et al., 1986) from *Ailanthus altissima* Suspension, Alliin from *Allium sativum* Callus (Malpathak and David, 1986), Acridone and furoquinoline alkaloids and coumarins from *Ruta bracteosa, R. chalepensis* and *R. macrophylla callus* (Baumert et al., 1992), Saponins from callus cultures of *Agave amanuensis* (Andrijany et al., 1999), Altamisine from *Ambrosia tenuifolia* Callus (Goleniowski and Trippi, 1999) etc. Callus cultures have helped in evaluating somaclonal variation in plant species like Wheat with respect to gliadin proteins (*Cooper et al.,* 1986), *Populus alba* × *P. grandidentata* (Son et al., 1993), *Jatropha curcas* (Jose et al., 2012) etc. Keeping in view the importance of callus cultures, present study was carried out to produce callus from *Inula royleana.*

Inula royleana DC. is a perennial medicinal herb native to Western Himalaya (Stojakowska and Malarz, 2004). Sesquiterpene lactones of eudesmane type (Bohlmann et al., 1978; Qurishi et al., 1980), abietane diterpenes (Edwards et al., 1962; Bhat et al., 1975) and diterpene alkaloids (Khaleque et al., 1959; Hegnauer 1964) have been reported from the roots of this plant due to which it acts as insecticidal (Jennings et al., 1986), insect repellent (Ulubelen et al., 2001), antimicrobial (Yang et al., 2001), anti-inflammatory (Dirsch et al., 2000), antiproliferative against different cancer cell lines (Lawrence et al., 2001; Konishi et al., 2002), vasodepressor (Kolak et al., 2001; Ulubelen et al., 2002) and have neuromuscular blocking properties (Manchanda et al., 2000). This plant has also been used traditionally for curing headache (Kala, 2006), dermatitis (Kaul, 1997), throat sores, wounds and inflammation of hooves in cattle (Khuroo et al., 2007), intestinal

problems (Khan and Khatoon, 2008), lowering hypertension (Haq and Alam, 2010) etc. In Kashmir Himalaya it is commonly known as Gugi Phool and is found at an altitude of 2800-3400 m (Khuroo et al., 2007). Illicit trade, uncontrolled grazing and indiscriminate overexploitation for its medicinal use have made this plant threatened (Dar et al., 2002) so there arises a need for conservation of this plant species. In the present study *in vitro* strategies have been adopted for the production of callus that acts as an alternative source for the production of secondary metabolites as well as for its large scale micropropagation.

Inula royleana DC. in natural habitat

Materials and methods

Plant material and sterilisation

For present study four different explants - leaf, petiole, involucral bract and seeds were collected from wild habitat. They were first thoroughly washed under running tap water in order to remove dirt and dust followed by washing with detergent labolene and surfactant tween-20. Detergent was removed by washing the explants with double distilled water. Then they were treated under laminar air flow hood with chemical sterilants like, 2% sodium hypochlorite for 8-10 min in case of leaf, petiole and involucral bract and 0.1% mercuric chloride for 10-15 min in case of seeds. This was followed by washing with autoclaved double distilled water and finally inoculation on sterilised nutrient medium.

Medium and culture conditions

Murashige and Skoog's (MS, 1962) medium, gelled with 8% agar was supplemented with different concentrations of auxins and cytokinins both individually and in combination. Auxins like 2,4- D; IAA; NAA; IBA and cytokinins like BAP and Kn were used in concentration range of 0.1-5 mg/l. The pH of the media was adjusted to 5.8 before autoclaving at 121 °C and 15 lb. The cultures were incubated at 22±4 ^{0}C and exposed to 24h photoperiod supplied by fluorescent tubes.

Results

Callus production from leaf explants

Callus was produced when leaf explants were inoculated on MS medium supplemented with BAP(1mg/l) (fig.1); BAP (5mg/l) + IAA (2mg/l) (fig.2); 2,4-D (0.2mg/l) (fig.3), BAP (2mg/l) + IAA (3mg/l) (fig.4), BAP (3mg/l) + IAA (1mg/l) (Fig.5) and Kn (2mg/l) + IAA (1mg/l) (Fig.6) in time period of 30, 32, 34, 39, 43 and 56 days respectively. But amount of callus and percent response were maximum in case of BAP (5mg/l) + IAA (3mg/l).

Callus production from petiole explants

Petiole explants produced callus when they were inoculated on MS medium supplemented with BAP (5mg/l) + IAA (3mg/l) (Fig.7); BAP (5mg/l) + IAA (2mg/l) (Fig.8), IAA (1mg/l) (Fig.9), BAP (1mg/l) (Fig.10), BAP (3mg/l) + IAA (1mg/l) (Fig.11) and Kn (0.5mg/l) (Fig.12) in time period of 35, 38, 39, 49, 55 and 62 days respectively. But amount of callus and percent response were maximum in case of BAP (5mg/l) + IAA (3mg/l).

Callus production from involucral bract explants

Involucral bracts produced callus when inoculated on MS medium containing BAP (2mg/l) + NAA (1mg/l) (fig.13), Kn (2mg/l) + IAA (1mg/l) (fig.14) and BAP (2mg/l) + 2,4-D (1mg/l) (fig.15) in a time period of 48, 52 and 60 days respectively.

Callus production from *in vitro* seedling explants

Seeds were first inoculated on basal medium. They germinated within 20 days (fig.16). The *in vitro* seedlings were then inoculated on MS containing hormones individually or in different combinations but medium containing BAP (3mg/l) + NAA (5mg/l) (fig.17) and BAP (2mg/l) + IAA (3mg/l) (fig.18) proved effective in producing callus in a time period of 58 and 63 days respectively.

Fig. 1

Fig. 2

Fig. 3

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Fig. 17

Fig. 18

Table No. 1: Effect of different hormones on callus production from leaf explant

MS medium	BAP	Kn	2,4-D	IAA	Mean No. of days for callus production	% Response
+	-	-	-	-	-	-
+	1mg/l	-	-	-	30	80
+	5mg/l	-	-	2mg/l	32	100
+	-	-	0.2mg/l	-	34	50
+	2mg/l	-	-	3mg/l	39	40
+	3mg/l	-	-	1mg/l	43	40
+	-	2mg/l	-	1mg/l	56	30

(30 replicates per treatment)

Table No. 2: Effect of different hormones on callus production from petiole explant

MS medium	BAP	IAA	Kn	Mean No. of day for callus production	% Response
+	-	-	-	-	-
+	5mg/l	2mg/l	-	35	70
+	5mg/l	3mg/l	-	38	90
+	-	1mg/l	-	39	60
+	1mg/l	-	-	49	60
+	3mg/l	1mg/l	-	55	50
+	-	-	0.5mg/l	62	40

(30 replicates per treatment)

Table No. 3: Effect of different hormones on callus production from involucral bract explant

MS medium	BAP	Kn	2,4-D	IAA	NAA	Mean No. of days for callus production	% Response
+	-	-	-	-	-	-	-
+	2mg/l	-	-	-	1mg/l	48	60
+	-	2mg/l	-	1mg/l	-	52	40
+	2mg/l	-	1mg/l	-	-	60	40

(30 replicates per treatment)

Table No. 4: Effect of different hormones on callus production from *in vitro* seedling explant

MS medium	BAP	IAA	NAA	Mean No. of days for callus production	% Response
+	-	-	-	-	-
+	3mg/l	-	5mg/l	55	60
+	2mg/l	3mg/l	-	63	40

(30 replicates per treatment)

Discussion

During present study, different hormones both auxins and cytokinins either individually or in different combinations were tried to produce callus from *I. royleana*. Best results with 100% response were obtained from leaf explant on medium containing BAP 5mg/l + IAA 2mg/l. The callus produced was hard, green and nodular. This is in contrast with the result of callus induction studies of *Onobrychis sativa* where leaf explants produced maximum callus on MS supplemented with 2.5 mg/L BAP and 0.5 mg/L NAA (Mohajer et al., 2012). Wani et al., 2010 produced callus in case of *Tridax procumbens* leaf explants by using BAP 0.5mg/l in combination with 2,4-D 0.5mg/l. In *Rauwolfia serpentine* profuse callus induction was obtained on MS medium containing NAA 2.0 mg/l + BAP 0.5mg/l but from nodal explants (Salma et al., 2008).

Conclusion

A procedure was developed for callus production in *Inula royleana* from four different explants viz., leaf, petiole, involucral bract and *in vitro* seedling explants. MS medium was supplemented with different growth regulators. Among all the explants, leaf explants proved to be most responsive as they produced maximum amount of callus in less number of days when inoculated on medium containing BAP in combination with IAA. The Callus produced can be used for inducing shoots and roots and also as an alternative source for secondary metabolite production.

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