Journal of Scientific and Innovative Research 2014; 3 (2): 203-206
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2014; 3(2): 203-206

Received: 24-02-2014
Accepted: 26-04-2014

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Rapid in vitro propagation of *Inula royleana* DC. through embryo culture

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Abstract

During present study a rapid in vitro propagation method was developed for *Inula royleana*, a medicinal perennial herb, through embryo culture. Embryo germination and complete plantlet formation was obtained on basal MS medium in a time period of 6 and 11 days respectively with 100% culture response. Indirect shoot regeneration with 25±0.6 mean number of shoots was also obtained in 70% cultures when leaf explants obtained from germinated embryos were inoculated on MS medium containing BAP (5 mg/l) +IAA (2 mg/l).

Keywords: In vitro propagation, Embryo culture, MS medium, Callus, Shoots.

Introduction

Inula royleana DC., a perennial medicinal herb, is native to Western Himalaya and Kashmir.[1] Locally it is known as "Gugi Phool" and found at an altitude of 2800-3400m.[2] This plant is rich in lycoctonine and anthranoyl-lycoctonine alkaloids[3] which were previously named as Royline and Inuline respectively.[4] Moreover, sesquiterpene lactones of eudesmane type[5,6], abietane diterpenes[7,8] and diterpene alkaloids[9,10] are also reported from its roots due to which it acts as insecticidal[11], insect repellent[12], antimicrobial[13], anti-inflammatory[14] and antiproliferative against different cancer cell lines[15,16] and have neuromuscular blocking properties[17]. Moreover, vasodepressor effect of some abietanes is also reported.[18,19] This plant is also used traditionally for curing a number of diseases like headache[20], dermatitis[21], throat sores, wounds and inflammation of hooves in cattle[2], intestinal problems[22], in lowering hypertension[23] and as an anti-allergic and antiseptic[24]. Further, roots are stored for its aroma and protecting garments and in the form of paste, it is applied with leaf on swelling sprains.[24] Roots are also used to control the high blood pressure.[25] Illicit trade, overgrazing and overexploitation[26] have made this plant threatened, so there arises a need for its conservation where plant tissue culture plays an important role.

Materials and Methods

Explant selection and sterilisation

Seeds are the source of embryos which were collected from plants growing in wild habitat. They were thoroughly washed under running tap water in order to remove dirt and dust. This was followed by washing with detergent labolene and surfactant tween-20 that helps the detergent to spread all over the surface of the explants. After washing with double distilled water, seeds were treated with chemical sterilant (0.1% mercuric

chloride) for 10-15 min. This was followed by washing with autoclaved double distilled water. Then embryos were isolated from sterilised seeds by exerting pressure at the base of the seeds and finally inoculated on sterilised nutrient medium. The sterilisation procedure was carried out under laminar air flow hood.

Preparation of Medium and maintenance of culture conditions

Murashige and Skoog's (MS)[27] medium, gelled with 0.8% agar containing 30 g sucrose 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 medium was adjusted to 5.8 before autoclaving at 121ºC and 15 lb. The cultures were incubated at 22±4ºC and exposed to 24h photoperiod supplied by fluorescent tubes.

Results

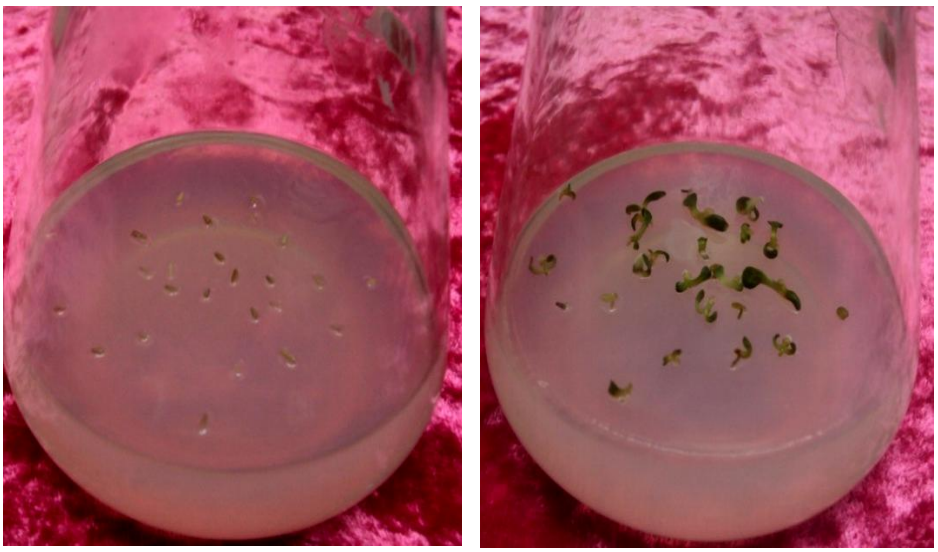
Germination of embryos: When embryos were inoculated (Fig.1a). on MS basal medium, they germinated within 6 days with response percentage of 100% (Fig.1b).

Shoot and root regeneration: Germinated embryos regenerated shoots and roots again on basal medium (Fig.1c).

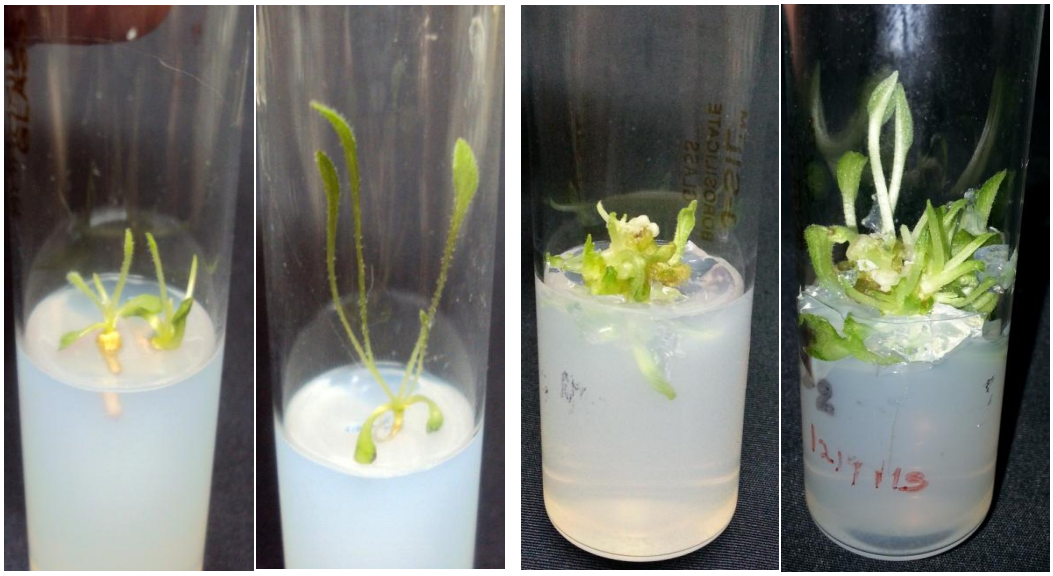
Callus production and subsequent shoot regeneration: Callus was also obtained when leaf explants obtained from germinated embryos were inoculated on MS medium supplemented with BAP (5 mg/l) +IAA (2 mg/l) in 70% cultures in a time period of 39 days. Later, 25±0.6 mean number of shoots were regenerated from this callus on the same medium without sub-culturing in a time period of 65 days (Fig.1d).

Discussion

The present work has been carried out to develop rapid micropropagation protocols for *Inula royleana* DC. using embryos as explants. Work on embryo culture of *I. royleana* confirmed 100% germination of the excised embryos within 6 days on simple MS basal medium. The germinated embryos did not require any exogenous hormonal support for the direct shoot and root organogenesis. They developed shoots and roots in 100% cultures within 11 days on basal MS medium. These observations highlight the regeneration potential of the embryo without the associated tissues of the seed. There are also many reports on the rapid germination of embryos upon the removal of seed coat.[28,29] Khodaparast and Hosyni[30] have also reported that the removal of seed coat causes an increase in germination rate upto 40% in case of Salvia leriifolia. The present study on embryo culture is in agreement with that of Mohan *et al*.[31] who also obtained embryo germination in *Jatropha curcas* L. and shoot regeneration from these embryos on basal MS medium but they achieved rooting on MS medium supplemented with IBA (0.5 mg/l). Modarres *et al*.[32], however, observed that the best treatment for rapid growth of embryo of Salvia leriifolia was full strength MS basal medium and half strength MS medium containing 1 mg/l NAA and BAP in combination.



(a) **(b)**



(c) **(d)**

Figure legends: (a) Inoculation of embryos on basal MS medium **(b)** Germination of embryos on basal MS medium **(c)** Direct shoot and root organogenesis on MS basal medium **(d)** Callus production and subsequent shoot regeneration

Conclusion

A protocol was developed in order to see the hormonal control of shoot organogenesis and subsequent complete plantlet formation in *Inula royleana* through embryo culture. For embryo culture, the best medium for embryo germination and subsequent shoot and root organogenesis was MS basal medium. The embryos germinated within 6 days and the complete shoot and root regeneration was obtained in next 11 days. In addition to direct organogenesis, indirect shoot regeneration was also obtained in 70% cultures when leaf portions of germinated embryos were inoculated on MS medium containing BAP in combination with IAA.

Acknowledgement

Authors acknowledge the great help received from the scholars whose articles cited and included in references of the manuscript.

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