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Comparative Effect of Different Nutrient Media on Regeneration Potential of Petiole Explants of Inula Royleana DC., A Multipurpose Plant Species of Kashmir Himalaya

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ABSTRACT

During present study, effect of different nutrient media viz., MS, Nitsch, B_5 and White media, was observed on petiole explants Inula royleana; in order to establish an efficient regeneration protocol for conservation of this one of the medicinally reputed plant species of Kashmir Himalaya. Among these nutrient media, callus induction and shoot regeneration were obtained only on MS and Nitsch media while as White medium proved effective in regenerating roots. MS proved more effective than Nitsch medium in inducing both maximum amount of callus as well as maximum mean number of shoots (9.5 with 4.7 cm mean shoot length) but number of days taken were more in comparison to Nitsch medium. When MS was supplemented with BAP (0.8 mg/l), maximum amount of callus was produced within 35 days in 40% cultures. However, for regenerating maximum shoots from this callus MS required BAP in combination with IAA at concentrations 5 mg/l and 2 mg/l respectively. Regenerated shoots produced roots on White basal medium and complete plantlets were then acclimatized with 70% survival rate by using vermicompost and soil in 3:1 ratio.

Keywords: Comparative effect of media, Petiole, Regeneration potential

INTRODUCTION

I. royleana belongs to family Asteraceae, the dicotyledonous taxon, which is the second largest family of flowering plants with about 11,000 genera and about 23,000 species [1], constituting one-tenth of all the flowering taxa [2]. The genus *Inula* L., placed under subfamily Asteroideae, tribe *Inuleae,* was first reported by Linnaeus [3], in which he identified and described thirteen species. Later workers, however, broadened the generic circumscription of the genus and included number of heterogenic taxa under the broad concept of *Inula* L. *I. royleana*, a perennial medicinal herb, is native to Western Himalaya [4] growing at an altitude of 2567-3425 m.a.s.l.. The plant is rich in lycoctonine and anthranoyl-lycoctonine alkaloids [5], previously named as royline and inuline respectively [6]. Moreover, sesquiterpene lactones of eudesmane type [7,8], abietane diterpenes [9,10] and diterpene alkaloids [11,12] have also been reported from the roots of this plant. During present study, the comparative effect of different nutrient media was observed so as to establish successful regeneration protocol for its rapid *in vitro* propagation. To our knowledge, this is the first study regarding the comparative efficiency of various nutrient media for development of micropropagation protocols from petiole explants of *I. royleana.*

MATERIALS AND METHODS

I. royleana collected from Khillanmarg (Gulmarg, 3098 m.a.s.l.) and transplanted in Kashmir University Botanical Garden (KUBG, 1585 m.a.s.l.) was used as the source of petiole explants. The explants were first surface sterilised by using detergent Labolene and surfactant Tween-20 and then chemically sterilised in Laminar Air Flow Hood by using

2% sodium hypochlorite for 8 min. Before inoculation, the explants were washed with double distilled water so as to make them free from the toxic effects of hypochlorite solution.

For callus production and shoot regeneration, the explants were inoculated on nutrient media *viz*., MS and B_5 [13,14] containing 30 g sucrose and Nitsch medium [15] containing 20 g Sucrose, gelled with 0.8% Agar adjuvanted with different growth hormones individually as well as in different combinations. The pH of the media was adjusted to 5.8 prior to autoclaving at 121^{0}C for 15 min. Cultures were then incubated in culture room with controlled condition (Temperature: 22±4^{0}C, 18 h photoperiod). This was followed by periodic observation of cultures.

For initiating roots in regenerated shoots, White basal medium [16] with 20g Sucrose and 0.8% Agar was used. The medium was autoclaved at 12^{0}C for 20 min. Well-developed plantlets were transferred to pots containing vermicompost and garden soil in 3:1 ratio. After testing them against humidity, the pots were kept in green house and subsequently transferred to field conditions where the plants survived successfully. The experiments were repeated thrice and effects of different treatments were quantified. Data was analysed through DMRT at 5% level.

RESULTS AND DISCUSSION

MS induced callusing from petiole explants when supplemented with BAP both individually as well as in different combinations with IAA. Among different concentrations of BAP, callus was produced when BAP was added in a concentration range of 0.5-6 mg/l, maximum amount produced on medium containing 0.8 mg/l BAP in 40% cultures within 35 days. The callus obtained was nodular and green in color (Figure 1a). Among different combinations of BAP and IAA, callus was obtained on three combinations viz., BAP(3 mg/l)+IAA(1 mg/l); BAP(2 mg/l)+IAA(3 mg/l) and BAP(5 mg/l)+IAA(2 mg/l) but maximum amount was obtained on MS adjuvanted with BAP(5 mg/l)+IAA(2 mg/l) in 50% cultures within 35 days. The callus obtained was nodular and creamish in color (Figure 1b). Similar results were obtained by Ramrao et al. [17] from petiole explants of *Jatropha curcas* wherein they obtained maximum callus proliferation on MS containing cytokinin and auxin in combination but instead of IAA, they used 2,4-D.

Shoot regeneration was obtained without sub-culturing the callus on medium containing BAP (1 to 4 mg/l) individually and the three combinations of BAP and IAA on which callus was obtained (Table 1). When MS was containing BAP individually, maximum mean number of shoots (7±1.08) with 4.24 cm mean shoot length was produced when BAP was added at a concentration of 3 mg/l (Figure 1c). Among the combinations, maximum mean number of shoots (9.5±1.71) with 4.7 cm mean shoot length was regenerated in 80% cultures within 37 days on medium containing BAP(5 mg/l)+IAA(2 mg/l) (Figure 1d). Our results are in accordance with that of Choffe et al. [18] who obtained maximum shoot regeneration in *Echinacea purpurea* from petiole callus on MS containing BAP and IAA in combination. Ramrao et al. [17] obtained shoot regeneration from petiole derived callus of *Jatropha curcas* on MS medium containing different hormones in combination but instead of IAA they used IBA and in addition to cytokinin and auxin they also adjuvanted the medium with GA_3.

Table 1: Effect of MS medium containing cytokinins individually as well as in different combinations with auxins on shoot regeneration from petiole callus

Medium	BAP (mg/l)	IAA (mg/l)	Mean number of shoots regenerated	Mean shoot length (cm)	Mean number of days taken for shoot regeneration	Percent culture response
MS	1	-	3^{d}	4.1^{c}	39^{a}	75
MS	2	-	4.25^{cd}	3.22^{d}	37^{b}	100
MS	3	-	7^{bcd}	4.24^{bc}	35^{c}	100
MS	4	-	6^{bcd}	4.5^{ab}	35^{c}	75
MS	3	1	4.6^{cd}	4.05^{c}	38^{ab}	75
MS	2	3	8.3^{bc}	2.3^{e}	37^{b}	75
MS	5	2	9.5^{a}	4.7^{a}	37^{b}	80

Ten replicates per treatment. The significance was tested by DMRT. The values with the same alphabet are non-significant with respect to each other. Different alphabets represent significance at p=0.05

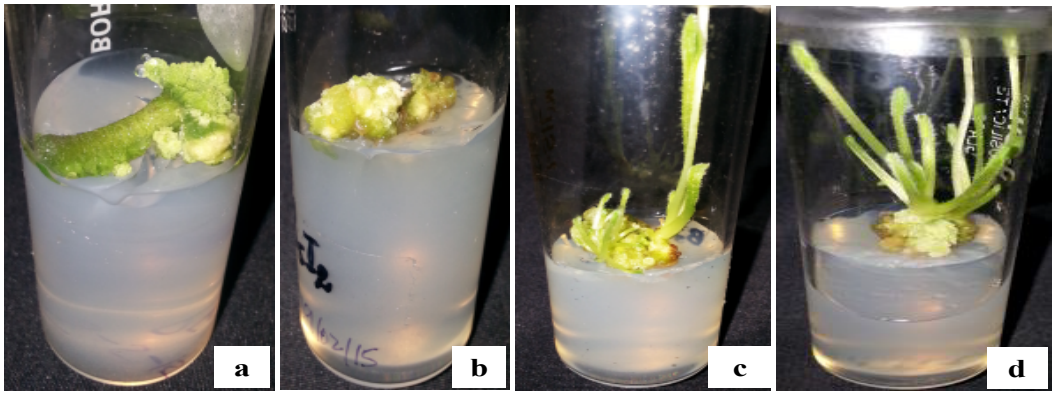

Figure 1: a) Callus induction from petiole explant of *I. royleana* on MS containing 0.8 mg/l BAP; b) Callus induction on MS containing BAP(5 mg/l)+IAA(2 mg/l); c) Shoot regeneration from petiole callus on MS containing 3 mg/l BAP; d) Shoot regeneration on MS containing BAP(5 mg/l)+IAA(2 mg/l)

Callus was also obtained on Nitsch basal medium and medium supplemented with BAP 0.8, 1, 2, 3 mg/l and BAP(3 mg/l)+IAA(1 mg/l). Maximum amount of callus was obtained on BAP 2mg/l supplemented medium in 40% cultures and 27 days (Figure 2a). Number of days taken for callus production was less as compared to that obtained of MS medium but amount of callus was more in latter case. For shoot regeneration only one concentration of BAP *viz*., 3 mg/l was effective. The mean number of regenerated shoots was 4.3±0.67 with mean shoot length 2.9 cm and the number of days taken was 33 (Figure 2b). Here again the number of days taken for shoot regeneration is less than that of MS medium but number of shoots is much lesser. This is the first report of callus production and shoot regeneration from petiole explant by using Nitsch medium in case of any medicinal plant.

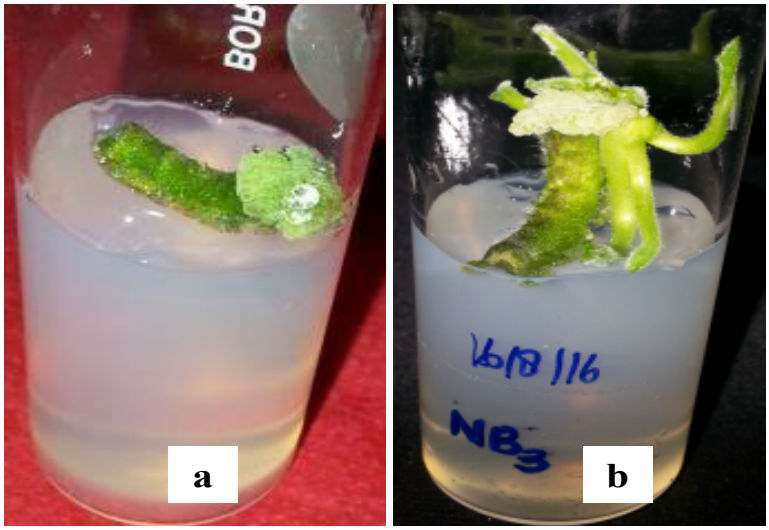

Figure 2: a) Callus production on Nitsch containing 2 mg/l BAP; b) Shoot regeneration on Nitsch containing 3 mg/l BAP

White basal medium proved effective in regenerating 3.5±0.22 mean number of roots with mean root length of 3.10±0.4 cm within 34 days with 90% culture response (Figure 3a, 3b).

Three month old *in vitro* rooted plantlets were washed free of agar and transferred to pots containing vermicompost and soil in 3:1 ratio. After hardening them against humidity they were shifted to green house where they grew successfully under controlled environmental conditions (27±1°C temp., 16 h/8 h photoperiod and relative humidity RH 80–90%). Plants were watered on alternate days with tap water. After three months, hardened plants were first transplanted in earthen pots containing soil:sand mixture (3:1) and then after two months to field conditions with a survival rate of about 100%. After transplantation, the plants were regularly irrigated using tap water (Figures 4a-4c) and are growing successfully in the field.

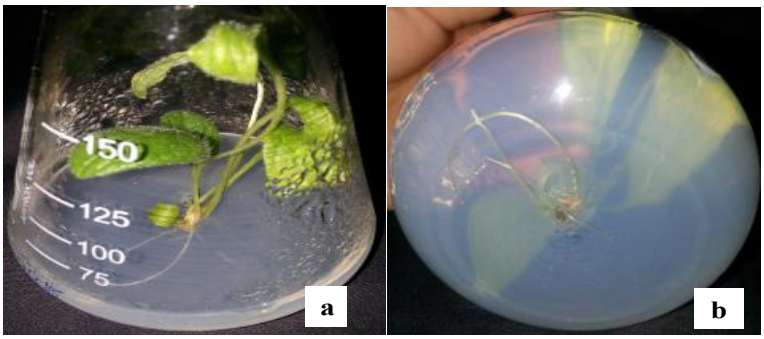

Figure 3: a) Root regeneration on White medium; b) Root regeneration on White medium

Figure 4: a-c Acclimatization procedure from culture room to field conditions

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