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In Vitro Plantlet Regeneration from Shoot Apices of *Lupinus polyphyllus* Lindl.

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ABSTRACT

The present paper reports on the *in vitro* propagation of *Lupinus polyphyllus* Lindl. through multiplication of shoot apices. Shoot apices obtained from *in vitro* germinated seedlings were cultured on MS (Murashige & Skoog) medium supplemented with various phytohormonal regimes. Indirect shoot regeneration was achieved on medium fortified with BAP (3μM) and NAA (1μM). Subsequent subculturing of these explants on half strength basal medium resulted in elongation of microshoots. The rooting of these elongated microshoots was achieved on the MS (x1/2) basal medium and also in presence of various auxins (NAA & IBA). Plantlets thus developed are presently undergoing hardening trials.

Key words: *Lupinus polyphyllus*, Shoot tip culture, Multiple shoots, Plantlets

Abbreviations: - MS (x1/2) – Murashige and Skoog (Half-salt strength); NAA-Napthaleneacetic acid; IBA- Indole-3-butyric acid; IAA- Indole-3-acetic acid; BAP-6 Benzyl-aminopurine; GA- Gibberellic acid

INTRODUCTION

Lupin is a genus of about 200 species of annuals, perennials and shrubs from North and South America and Mediterranean (Anonymous, 1983). As they belong to leguminosae family, the lupin roots, like other legumes, bear nitrogen fixing nodules and some species are used for green manuring (Anonymous, 1983). *Lupinus polyphyllus* is a species of lupins introduced and cultivated in Kashmir. It is a herbaceous perennial plant which grows 75 cm-1.2m high with whorls of long, narrow compound palmate leaves and pea shaped, deep blue, purple, pink, white or yellow flowers (Bremness, 1993). These appear in late spring and early summer and are followed by flattened spherical seeds in long pods. This plant is grown for ornamental purposes for its showy flowers. Seeds of the plant have cosmetic uses and are constituents of skin lotions used to clean oily skin. In addition, this plant has a role in absorbing nuclear radiation.

MATERIAL AND METHODS

In vitro grown shoot apices were used as explants. For this purpose, seeds were collected from plants of *L. polyphyllus* growing in Gulmarg and thoroughly washed using lab wash, surface sterilized with $HgCl_2$ (0.2%) for 15-20 minutes and finally rinsed 3-4 times with autoclaved double distilled water. Medium used for raising the cultures was Murashige and Skoog's medium (1962). The seeds were inoculated on half salt strength of the medium supplemented with 3% sucrose, 0.8% agar as jelling agent and pH adjusted between 5.5 and 5.8. Medium was autoclaved for 20-25 minutes at 121°C temperature and 15 lb pressure. The cultures were maintained at 25± 2°C with 16 - hour photoperiod provided by 3000 lux cool fluorescent tubes.

RESULTS

Effect of different concentrations of BAP, its combination with NAA on shoot tips of *L. polyphyllus* is given in Table1. The shoot tips when cultured on half and full strength MS basal media showed only elongation of explant. Shoot apices were also cultured on MS (x1/2) basal medium supplemented with various concentrations of BAP. On medium containing BAP (1μM and 2μM) there was registered low compact callus induction at the basal end followed by indirect multiple shoot regeneration, but the number of shoots produced was less (3-6 per explant). On increasing BAP concentration to 5μM there was semi compact callus formation at the basal end of the explant followed again by indirect multiple shoot regeneration. The shoot number was slightly higher, i.e., 8-10 shoots per explant (Fig.1). Further increase in BAP concentration (10μM and 15μM) triggered only non-regenerative callus formation. Use of the MS (full salt strength) medium with a combination of BAP (3μM) and NAA (1μM) promoted mild compact callus production at basal end, followed by indirect multiple shoot regeneration. The shoot number was fairly high and reached up to 18-20 shoots per explant (Fig.2). Slightly higher BAP concentration (5μM) in combination with NAA (1μM) decreased the shoot number and only 10-12 shoots formed per explant.

The microshoots produced in such processes were isolated and placed on MS (x 1/2) basal medium for elongation. For rooting of microshoots MS (x 1/2) medium and MS (x1/2) basal medium supplemented with auxins (NAA and IBA) were used. Table 2 summarizes the results of rooting trials of microshoots of the plant. On MS (x 1/2) basal medium direct one root was produced per shoot. Direct multiple root regeneration was observed on NAA (5μM) and IBA (2.5μM) when used independently. The number of roots produced on former auxin was less (3-5), but roots were long. However, on IBA (2.5μM) roots were numerous but short in size. Complete plantlets (7-8cm long) were recovered after 8 weeks of culture period (Fig. 3). Then these plantlets were transferred to pots containing peat moss and vermiculite mixture in the ratio of 1:3. Plantlets are presently undergoing such trials (Fig.4).

Fig. 1

Fig. 3

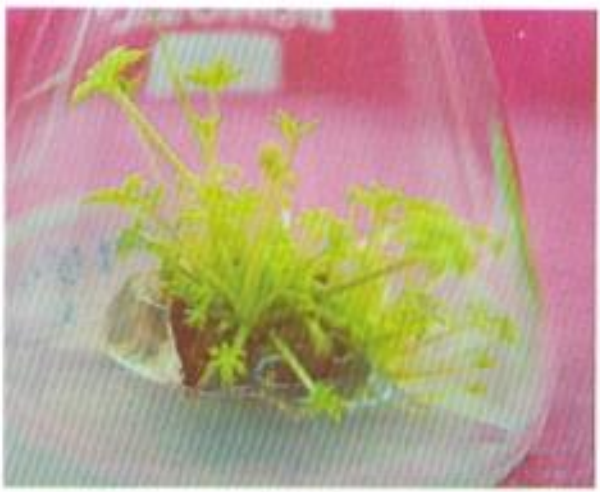

Fig. 2

Fig. 4

Legends
Fig. 1. Multiple shoot formation on MS (x1/2) + BAP (5μM).
Fig. 2. Shoot multiplication on MS (x1/2) + BAP (3μM) + NAA (1μM)
Fig. 3. Complete plantlet formation after 8 weeks.
Fig. 4. Plantlets in peatmoss and vermiculite mixture.

DISCUSSION

The present study showed good potential for plant regeneration. The regenerants were recovered indirectly through callus. The maximum number of multiple shoots (18-20) was recovered on MS medium supplemented with BAP (3μM) and NAA (1μM) followed by BAP (5μM) + NAA (1μM) where 10-12 shoots per explant were observed. Multiple shoots were also produced on different concentrations (1μM, 2μM and 5μM) of BAP which is in accordance with Pniewski *et al.* (2002) who also recorded multiple shoot regeneration on various concentrations of BAP (0.5, 0.1, 0.25 and 0.5 mg/l) from axillary buds of four lupin species. Sator (1985) on the other hand observed multiple shoot formation in *L. polyphyllus* on MS medium supplemented with BAP from embryo and cotyledons while as Upadhyaya *et al.* (1992) have shown *in vitro* shoot formation from hypocotyl explants of *L. texensis* on medium supplemented with kinetin and BAP. Earlier, Korpusenko *et al* (1990) have shown that callus developed from hypocotyl explants of *L. polyphyllus* on MS basal medium with 2,4-D which gave many regenerants after plating on modified MS medium supplemented by BAP (3mg/ l), GA (0.1mg/l) and nicotinic acid (0.15mg/ l). Multiple shoot bud induction in *L. polyphyllus* was achieved from callus cultures using NAA (0.54μM) and BAP (4.4μM) by Sroga (1987) which is in corroboration with present study where maximum shoots were obtained indirectly on MS medium containing BAP (3μM) and NAA (1μM). A range of BAP concentration used in present study reveals that interaction of BAP (3μM) with NAA (1μM) proves to be the optimum concentration for maximum shoot regeneration in shoot apex cultures of *L. polyphyllus*.

Rooting of single shoots regenerated from various explants of *L. polyphyllus* has been achieved on medium supplemented with IAA and NAA regimes by Sator (1985) and Sroga (1987). Upadhyaya *et al* (1992) also observed adventitious root formation in tissue culture derived shoots placed on MS media containing various regimes of NAA, IAA and IBA in *L. texensis*. Hence, present studies are in agreement with these studies where also rooting of isolated shoots was achieved with various concentrations of IBA and NAA but are in contrast to Hardy *et al* (1995) who obtained rooting in *L. mutabilis* by using two successive rooting media, one for induction with IBA or NAA followed by another expressive medium which was without auxin. Present studies on rooting behaviour are again contradictory to Korpusenko and Khotyleva (1991) who induced rooting in *L. polyphyllus* with nicotinic acid and GA. Pniewski *et al* (2002) obtained rooting of regenerated shoots of four lupin species on low salt MS medium containing B5 vitamins which is again not in consonance with present results. On medium supplemented with NAA (3.0mg/l) and isopentyl adenine (0.2 mg/l) in *L. albus*, rooting of isolated shoots was achieved by Daza and Chanber (1992) which is nearly close to our results.

The present study thus reveals that protocol developed for *in vitro* plant regeneration of *L. polyphyllus* from shoot apices is viable and after refinement can be utilized for large scale multiplication vis-à-vis conservation of this economically important plant.

Table 1 : Effect of different concentrations of Phytohormones on shoot apices of *Lupinus polyphyllus*.

Growth media & phytohormones	Response*	Percentage response	No. of shoots per explant	Mean ± SD
MS(x1/2) Basal	Elongation of shoot apices	100	-	-
MS Basal	Elongation of shoot apices	100	-	-
MS (x1/2) + BAP (1µM)	Low compact callus induction at the basal end followed by indirect multiple shoot regeneration	70	3 - 6	4.5 ± 1.1
MS (x1/2) + BAP (2µM)	Low compact callus induction at the basal end followed by indirect multiple shoot regeneration	50	4 - 6	5 ± 1.0
MS (x1/2) + BAP (5µM)	Semi compact callus formation at the basal end of the explant followed by indirect multiple shoot regeneration	50	8 - 10	9 ± 0.8
MS (x1/2) + BAP (10µM)	Moderate callus at basal end, elongation of shoot apices	70	-	-
MS (x1/2) + BAP (15µM)	Semi compact callus, induction at the basal end of the explant, elongation of shoot apices	70	-	-
MS + BAP (3iM)+ NAA (1µM)	Mild compact callus formation at the basal end followed by indirect multiple shoot regeneration	80	18 - 20	19 ± 0.8
MS + BAP (5iM)+ NAA (1µM)	Mild compact callus formation followed by indirect multiple shoot regeneration	50	10 - 12	11 ± 0.8

* Data scored after 8 weeks of culture period; 10 replicates per treatment

Table 2: Effect of different auxins on rooting of micro shoots of *L. polyphyllus*

Growth media and phytohormones	Response*	No. of roots	Percentage of response	Mean ± SD
MS (x1/2) basal	Direct root formation	01	80	1 ± 0.0
MS (x1/2) + NAA (2.5 IM)	Low callus formation at basal end of the shoot	-	90	-
MS (x1/2) + NAA (5 IM)	Direct multiple root regeneration	3-5 (long)	100	4 ± 0.8
MS (x1/2) + IBA (2.5 IM)	Direct multiple root regeneration	Numerous (short)	100	-
MS (x1/2) + IBA (5 IM)	No response	-	-	-

* Data scored after 8 weeks of culture period. 10 replicates per treatment

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