

In Vitro multiplication of *Pinus roxburghii* Sarg

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Pinus roxburghii Sarg commonly known as chirpine is an important plant of Indian subcontinent. It contributes significantly to the local and industrial economy of the country being an important source of timber and resin of commercial value. It also protects the watersheds, which sustain and regulate the water supply for millions who inhabit the Himalayan river basin. The need to develop procedures for rapid mass propagation of forest trees has been recognized for many years and is a prerequisite for employing tissue culture technique for *in vitro* conservation. *In vitro* micropropagation of conifers via adventitious shoot bud induction is one such procedure described for several species (Kaul, 1990). In an attempt to optimize adventitious bud differentiation, effects of mode of application of phytohormones to the mature zygotic embryos of *Pinus roxburghii* was studied.

The mature seeds of chirpine were surface sterilized with 0.1% HgCl₂ and 3.0% H₂O₂ (w/v) for 20 and 10 min., respectively. Each treatment was followed by 3 or 4 wash with sterilized distilled water. The megagametophytes were dissected out and further sterilized for 5 min. with 0.1% HgCl₂ prior to embryo excision. The excised zygotic embryos were cultured on MS (Murashige and Skoog, 1962) medium supplemented with 3% sucrose, gelled with 0.8% agar and pH 5.8. The embryos were exposed to BAP in two ways:

1. Continuous exposure to lower BAP (5-25 μ M) concentrations supplemented in the culture medium for 5 weeks.

2. Exposure to high BAP (125 or 250 μ M) concentrations for 1, 2 or 4 h followed by culturing on MS basal medium for 5 weeks.

For elongation, the explants with induced adventitious buds were transferred to 1/2 MS basal medium and for rooting 2.5 μ M NAA was incorporated in 1/2 MS medium. The cultures were incubated under 16 h photoperiod at 25 \pm 2°C.

Preliminary investigations were carried out using BAP and kinetin individually for adventitious bud differentiation. BAP was found better compared to kinetin in terms of per cent response as well as average number of buds/explant. Pulido *et al.*, (1990) and Sen *et al.*, (1994) have also emphasized the efficiency of BAP over other cytokinins in inducing adventitious buds on embryos of other pine species.

The concentration of BAP in the semi-solid medium during continuous exposure also influenced the response (Table 1). Increase in BAP concentration produced clustered and stunted buds compared to the buds scattered on whole explant surface when exposed to lower BAP (5-10 μ M) or given a pulse treatment (Fig. 1A). It was also recorded that the embryos provided with pulse treatment of BAP developed buds in 3-4 weeks compared to 4-5 weeks when continuous exposure was provided with BAP. Zel *et al.*, (1988) also reported that soaking explants in higher BAP was the most effective treatment for bud induction, which agrees with the results of Bornman (1985), where short-time treatment with high hormone concentration was found most effective.

The mode of application of BAP had profound effect on the number of adventitious buds produced as well as the placement of buds on explant surface. The embryo explants that were provided a pulse treatment with 250 μ M BAP for 2 h produced 38.7 buds/explant compared to 26.4 buds on 10 μ M BAP supplemented medium. It was observed that pulse treatment for 4 h with both 125 μ M and 250 μ M BAP was detrimental compared to 2 h (Table 2). Increase in explant mortality was observed with increasing exposure to pulse treatment of BAP (4 h).

Table 1: Effects of BAP on induction of adventitious buds on mature zygotic embryos of *Pinus roxburghii* (Culture age: 5 weeks)

BAP (μ M)	Responsive explants (%)	Average number of buds	Average length of buds (mm)
0	0.0	0.0	0.00
5	40.0	9.2 \pm 6.1	1.3 \pm 0.4
10	66.7	26.4 \pm 16.3	1.8 \pm 0.8
15	60.0	16.2 \pm 12.9	1.7 \pm 0.5
20	56.7	16.2 \pm 7.9	1.4 \pm 0.5
25	43.3	13.7 \pm 5.4	1.2 \pm 0.4

\pm Values represent the standard deviation.

Table 2: Effects of pulse treatment of BAP on induction of adventitious buds on mature zygotic embryos (Culture age: 5 weeks)

BAP (μ M)	Duration of pulse treatment (h)	Responsive explants (%)	Average length of buds	Average number of buds (mm)
125	1	43.3	12.1 \pm 9.2	1.3 \pm 0.5
	2	46.7	12.6 \pm 5.0	1.4 \pm 0.5
	4	30.0	12.3 \pm 10.2	1.0 \pm 0.3
250	1	60.0	14.7 \pm 11.2	1.3 \pm 0.4
	2	66.7	38.7 \pm 18.3	1.8 \pm 0.6
	4	16.7	11.4 \pm 3.3	1.5 \pm 0.5

\pm Values represent the standard deviation

Elongation of buds was carried out on 1/2 MS medium (Fig. 1B). The concentration of cytokinin and its exposure period during the induction phase had a bearing on the per cent buds elongating as well as the average bud length. Higher the BAP concentration used in the medium, longer was the time required for bud elongation. It was also observed that the shoot buds clustered together have a lower frequency and rate of bud elongation compared to the buds scattered on whole explant surface. Chesick *et al.*, (1991) also reported similar results. Kaul (1990) reported that the buds induced on higher cytokinin concentrations were difficult to elongate. The buds induced in response to pulse treatment had a better rate of elongation compared to those provided continuous exposure. Amerson and Mott (1982) recorded similar observations in *Pinus sylvestris*. The buds were serially subcultured 3-4 times on basal medium to obtain shoots 1.0-1.5 cm long for rooting.

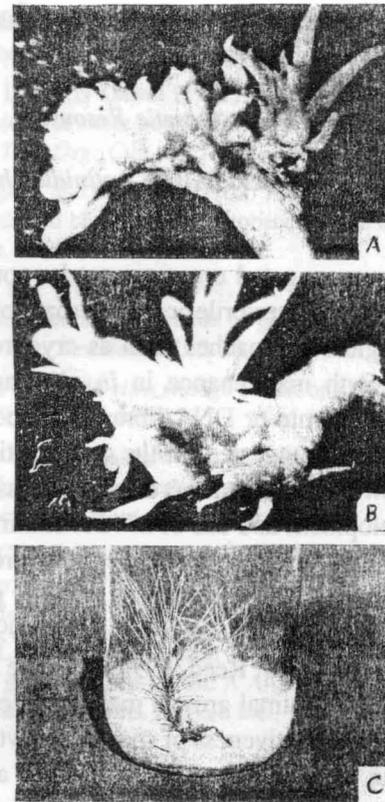
Induction of adventitious rooting in *in vitro* raised shoots of conifers is a key step in micropropagation, determining the pace of commercial application of tissue culture in clonal forestry. Rooting was induced in 70% shoots when exposed to 2.5 μ M NAA-supplemented medium for 1 month followed by transfer to liquid basal medium for elongation of the induced root primordia (Fig. 1C). The plantlets were transferred to soil after hardening and acclimatization.

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Fig. 1. Multiplication of *Pinus roxburghii* through adventitious bud differentiation

- A: Adventitious buds scattered on whole cotyledon surface, cultured on basal medium after giving pulse treatment with 250 μ M BAP for 2 h (x 7)
- B: Elongation of induced adventitious buds after first subculture to 1/2 MS basal medium (x 7)
- C: Rooted plantlet in liquid 1/2 MS basal medium (with cotton support)

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