PLANTLET REGENERATION FROM EMBRYONAL SHOOT EXPLANTS OF *PINUS ROXBURGHII* SARG.

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Abstract - Embryonal shoot explants from in vitro raised seedlings of Pinus roxburghii were cultured on MS(x1/2) basal medium fortified with various cytokinin concentrations. Explants produced green friable callus at basal ends of explant on MS(x1/2) medium enriched with BAP(2.5µM).However other BAP concentrations(5,10,15µM) favoured multiple shoot regeneration. With BAP(5µM) maximum number of shoots(10±0.5) was recorded. In other trials when BAP was replaced by Kn(2.5 5,10,15 ,20 ,25µM) only calluas induction was observed at the basal ends of explants. The cultures with multiple microshoots were subcultured on basal medium for shoot elongation. Isolated shoots produced rooting response on MS(x1/2) basal medium after 8-10 weeks of culture period. Rooted plantlets were transferred to green house for hardening and field trials.

Keywords- Embryonal shoot,Pinus roxburghii, Explant, multiple shoot, plantlet, MS medium(Murashige and Skoog, 1962).

1. INTRODUCTION

Pinus roxburghii Sarg. (Pinaceae) commonly known as Chir pine is one of the most important conifers in the Himalayan region (Tiwari 1994), which moulds the life of various ethnic and other communities of the region. It is valued for timber, pulp and resin. Oleoresin from Chir pine is the main source of turpentine in India which is chiefly used as a solvent for thinning, paints and varnishes besides having medicinal uses. The need to develop protocols for rapid mass propagation of conifers 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 of such procedure described for several species (Kaul 1990). Present attempt has been made to study the morphogenetic response of embryonal shoots on various cytokinin regimes.

2. MATERIALS AND METHODS

Mature cones of *Pinus roxburghii* were collected from Shankaracharya park (a protected forest, managed by J& K forest Deptt.) Srinagar Kashmir, India. Seeds separated from these cones were srored at 4^oC for one month. The chilled seeds were thoroughly washed with running tap water after cleaning them with detergent (Cedpol 1%) and few drops of tween -20 (surfactant). This was followed by their surface sterilization with $HgCl_2$(0.1%) for 20 minutes and then rinsing three times with double distilled water. Sterilized seeds were inoculated on half strength MS basal medium. The pH of medium was adjusted between 5.5-5.8 by using NaOH (0.1N) or HCl (0.1N) before jelling the medium with 0.8% agar. Seedling formation was observed after 4-6 weeks of culture period. Embryonal shoots were used as explants and cultured on MS(x1/2) medium supplemented with various cytokinin regimes. The cultures were maintained at 25± 3^oC with 16-18 hour photoperiod from cool white fluorescent tube lights at 50-65% relative humidity.

3. RESULTS AND DISCUSSION

Varied morphogenetic responses observed on *in vitro* culture of embryonal shoots of *Pinus roxburghii* on different cytokinin regimes are depicted in Table 1. Explants produced green friable callus at the basal ends on low BAP (2.5µM) enriched medium. However MS(x1/2) medium augmented with other BAP (5,10,15µM) concentrations favoured multiple shoot regeneration. With BAP(5µM) maximum number of shoots (10 ± 0.5) was recorded in 70% culture (Fig.1). Micro shoot number got reduced to 4±0.5 0n BAP (10µM) fortified medium (Fig.2).In another trial only 2+0.8 shoots/ explants were regenerated on BAP (15µM)supplemented medium. Explants cultured on high BAP (20,25µM) did not favoured any shoot regeneration.

Culturing of explants on various Kn (2.5,5, 10,15 20 25 µM) concentrations favour only callus induction at the basal ends without any shoot proliferation.

Table1. Effect of BAP and Kn on Embryonal shoots of *Pinus roxburghii* cultured on MS (× ½) basal medium.

S. No	Cytokinin (µM)	Response*	%age Response	Average No. of shoots / explant X±S.E
1.	BAP(2.5)	Callus at basal end of explants (+)	70	-
2.	BAP (5)	Multiple axillary shoot regeneration	70	10 ± 0.5
3.	BAP (10)	Multiple axillary/ adventitious shoot regeneration	30	4 ± 0.7
4.	BAP (15)	- Do-	50	2±0.8
5.	BAP(20)	No response	-	-
6	BAP (25)	- Do -	-	-
7	Kn (2.5)	Callus at basal end of explants (+)	20	-
8.	Kn (5)	Callus at basal end of explants (++)	20	-
9.	Kn(10)	Callus at basal end of explants (++)	40	-
10.	Kn(15)	Callus at basal end of explants (+)	20	-
11.	Kn(20)	Callus at basal end of explants (+)	20	-

*Ten replicates per treatment, Data scored after 8- weeks of culture period.

+ low friable callus, ++ moderate callus.

The primary cultures with multiple axillary shoots were subcultured on MS(x1/2) basal medium for shoot elongation. Maximum shoot elongation (4±0.6 cm) was recorded after 8- weeks of culture period (Fig. 3). Micro shoots were separated and subcultured on basal medium for further growth and elongation (Fig.4). Isolated microshoots cultured on basal medium showed rooting in 40% of cultures after 8- 10weeks of culture period (Fig. 5). However no rooting was achieved on NAA (1.5, 2.5, 5, 10,15µM) enriched medium. Shoot elongation and callus development was recorded on these concentrations. Similar response was recorded on IBA (1.5,2.5,5,10 µM) supported medium. Plantlets were deflasked and transferred to green house for hardening and field trials (Fig.6).

Fig.1. Multiple shoot regeneration on MS (x1/2)+BAP (5µM)

Fig.2. Multiple shoot regeneration on MS (x1/2) +BAP (10µM).

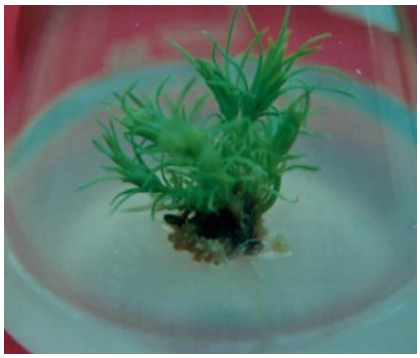

Fig.3. Micro-shoot elongation on Basal medium.

Fig.4. Elongated shoots on Basal medium.

Fig.5. In Vitro rooting of Micro-shoots on Basal medium.

Fig.6. Plantlet in small pot.

Clonal propagation of superior selected trees is desirable in silviculture. However the forest trees are usually difficult to propagate from mature expalnts, therefore most of the studies have been done using juvenile plant material in conifers (Horgen and Aitken- Christie 1981, Baxter et al. 1989, Burns et al.1991, Kalia et al. 2001, Kalia 2002 and Salajova and Salaj 2005). In the present investigation shoot regeneration was also recorded in embryonal shoot explants , excised from 4-6 week old *in vitro* raised seedlings, on BAP fortified medium. The results are very much in conformity with the studies of Kalia et al (2007) in *Pinus roxburghii*. Similar promotory effect of BAP in inducing multiple shoots has been reported in variety of *Pinus species* (Gupta and Durzan 1985, abdullah et al. 1986, Lapp et al. 1996 and Kalia et al. 2001). The concentration of BAP used in the medium significantly influenced multiple shoot regeneration. A maximum of 10±0.5 shoots per explants were induced on media containing 5µM BAP. Lapp et al. (1996) and Kalia et al. (2007) also reported that the concentration and type of cytokinin are important factors affecting the induction of shoots in *Pinus* species. The present study revealed that multiple shoots could be induced from embryonal shoots on medium containing BAP than Kn, BAP was found more effective compared to Kn. Similar resuts were reported in *Pinus roxburghii* by Kalia et al. 2007. Superiority of BAP for shoot proliferation may be attributed to the ability of plant tissues to metabolize BAP more readily then Kn or to the ability of BAP to induce production of natural hormones such as Zeatin within the tissues (Zaerr and Mapes 1982).

Transfer of primary cultures with multiple shoots on MS(x1/2) basal medium favoured shoot elongation. The results are in accordance with the observations of Abdullah et al. 1987 (P.*brutia*), Parasharami et al. 2003 (P. *roxburghii*) and Kalia et al.2007 (P. *roxburghii*).

The potential and efficiency of adventitious rooting is highly variable and challenging task among the conifers and remains one of the key problem in plantlet regeneration *in vitro* (Kalia et al. 2007). Rooting has

been achieved in number of species but the rooting response has been generally very low e.g 4% in *Pinus resinosa* (Noh et al.1988) ,3% in *P. monticola* (Stiff et al. 1989) and 3% in *Abies* hybrids (Gajdosova and Vookova,1994). In present observation rooting of micro shoots was observed on MS(x1/2) basal medium. Similar results were also observed by Toribo and Pardos (1989) in *pinus sylvestris* and Franco and Schwarz(1985) in *Pinus occarpa*. *In vitro* rooting of microshoots was not observed on medium containing various NAA/IBA concentrations , which is quite contrary to the reports of Ellis and Bilderback (1991), Kalia et al.(2001) Kalia et al.(2007) and Arya et al. (2014) who reported *in vitro* rooting of shoots on NAA/IBA supplemented media in different *Pinus* species.

4. CONCLUSION

The present study demonstrates efficient protocol for direct multiple shoot regeneration from embryonal shoots of *Pinus roxburghii*. Such an effort of micropropagation can significantly increase forest productivity with the production of selected genotype. Commercial exploitation of developed protocol is possible as the *in vitro* raised shoots can be employed as propagules for further multiplication of the genotype.

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