Embryo Culture in *Picea smithiana* (Wall) Boiss

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ABSTRACT

Mature zygotic embryos of *Picea smithiana* (Wall) Boiss were cultured on MS (1962) medium (half salt strength) supplemented with IBA (10μ M), BAP (5μ M) and 3% Sucrose. Green compact and nodular callus was obtained, which resulted in initiation of somatic embryogenesis and shoot bud induction. Subculturing of friable green callus, obtained from radicle and hypocotyl regions of embryo, under the influence of 5μ M NAA and 5μ M BAP resulted in differentiation of shoots. Trials to elongate the shoot buds and the maturation of somatic embryos are in progress.

Keywords: *Picea smithiana*, embryo culture, callus, somatic embryogenesis, shoot buds.

Abbreviations: MS (x½) - Murashige and Skoog (half salt strength); IBA - Indole-3- butyric acid; BAP - 6-benzyl amino purine; NAA - naphthalene acetic acid; AE - Von Arnold and Eriksson.

INTRODUCTION

Picea smithiana (Wall) Boiss. (West Himalayan Spruce) is a very large evergreen tree and belongs to family Pinaceae. It is locally called as "Kachul". It is a tree of considerable economic importance in northwest India on account of its multiple uses in wood based industries and manufacture of paper pulp for news print. Its natural propagation takes place by seeds but it takes two years to produce seeds by sexual reproduction, because of very long life cycle. Vegetative propagation by rooting of cuttings is also not very much promising. However, in order to meet the rising demand for wood products and exploitation due to deforestation, it is very essential to use some new techniques which would prove fruitful as compared to conventional methods. Nowadays the technique of *in-vitro* culture is widely being used in trees as a means of mass propagation in relatively

shorter period and irrespective of season and long life cycle (Jones *et. al.*, 1977; Sommer and Brown, 1979; Mott, 1981; Dunstan, 1988; Gupta *et. al.*, 1991 and Paques and Cremiere, 1996). Published reports on *in vitro* culture of *Picea* sp. reveal that some success has been achieved in plantlet production (Patel and Thorpe, 1986; Lu, *et. al.*, 1991; Tautorus, *et. al.*, 1990; Flinn *et. al.*, 1991; Gupta *et. al;* 1993 and Ruaud, 1993) This communication reports observations on culture of mature zygotic embryos of *Picea smithiana* which has successfully produced somatic embryos and shoot buds in the embryo callus.

MATERIAL AND METHODS

Mature green cones of *Picea smithiana* (Wall) Boiss were collected from Gulmarg and Tangmarg forests of Kashmir in the months of September and October. Seeds separated from these cones were stored at 4^0C for one month. The chilled seeds were thoroughly washed with running tap water. This was followed by their surface sterilization in 0.1% $HgCl_2$ for 15 minutes and then rinsing three times with sterile double distilled water. These seeds were allowed to remain for soaking in sterile double distilled water at 4^0C for 2-4 days in small flasks with their mouths sealed. Mature zygotic embryos were aseptically dissected out at laminar flow and sterilized with 1% NaOCl for 5 –6 minutes followed by 2-3 washes with autoclaved double distilled water. The embryos were then cultured on MS (x ½) medium (1962) supplemented with various growth regulators. The pH of the medium was adjusted between 5.5 –5.6 by using NaOH (0.1N) or HC1 (0.1N) before jelling the medium with 0.8% agar. The medium was finally dispensed into 100 ml flasks plugged and autoclaved for 15-20 mints at 15lb pressure and 121^0C temp. The cultures were maintained at 25 ± 3^0C with 16-18 hour photo period from cool white fluorescent tube lights (3000 lux) at 50-65%R.H.

RESULTS

Different morphogenetic responses observed on *in-vitro* culture of embryos on MS (x ½) medium supplemented with different concentrations and combinations of growth regulators are summarized in Table 1. The embryos of *Picea smithiana* when cultured in presence on IBA (10µm) promoted green friable and non-mucilaginous callus formation at radicle and hypocotyl regions of embryos. Cotyledons elongated upto 1cm and remained green. Subculturing of

Table 1. Morphogenetic responses of cultured zygotic embryos to various concentrations and combinations of growth regulators.

S No.	Medium	Response*	Degree of Callus formation	% age of response
1.	MS (x ½) + IBA (10μM)	Elongation of cotyledons, green friable callus formation at hypocotyl and radicle regions.	++	80
2.	MS (x ½)+NAA (10μM)	Elongation of cotyledons, poor growth, no callusing.	-	80
3.	MS (x ½) + BAP (5μM)	Friable green callus formation	+++	30
4.	MS (x ½)+ BAP (10μM)	No elongation, no callusing	-	0
5.	MS (x ½) + IBA (10μM) + BAP (5μM)	Green nonmucilaginous compact nodular callus, somatic embryogenesis (4-6 embryos/culture), shoot bud induction (4-6 shoot buds/culture)	++	60 20 20
6.	MS (x ½)+NAA (10μM) BAP (10μM)	Green, compact, non-mucilaginous, nodular callus.	+	90

* Data recorded at the end of 6 weeks; 10 replicates/treatment
\- No callus growth, + low, ++ moderate, +++ high

this callus after 4-weeks under the combined effect of NAA (5μm) and BAP(5μm) resulted in differentiation of shoots from callus in 15% of cultures (Table 2, Fig. 1). Culture of embryos on NAA (10μm) comparatively showed poor growth without any callus formation. When the embryos were grown on medium fortified with BAP(5μm), friable green callus was produced (Fig.2). Sub-culturing of this callus after 4 weeks under the combined influence of NAA (10μm), BAP(5μm) and 0.5 mg/l glutamine resulted in profuse growth of callus. There was change of callus colour from green to light brown in some regions. No organogenesis was observed (Table 2). However, if BAP conc. was increased to 10μm in primary culture no callusing was observed (Fig.3). Combined effect of IBA(10μm) and BAP(5μm) resulted in compact, nodular and green callus

formation which at the end of 4th week of culturing showed induction of somatic embryos and shoot buds in the callus (Fig.4). When a combination of NAA(10μm) and BAP(10μm) was used it resulted only in the formation of green nodular callus. Subculturing of this nodular callus on NAA(10μm), BAP(5μm) and 0.5mg/l glutamine resulted in shoot bud induction (Table 2, Fig.5). Attempts will be continued to subculture the shoot buds and somatic embryos produced in different trials for their elongation and maturation respectively. This is an encouraging result which will ultimately lead to the development of multiple shoots and plants.

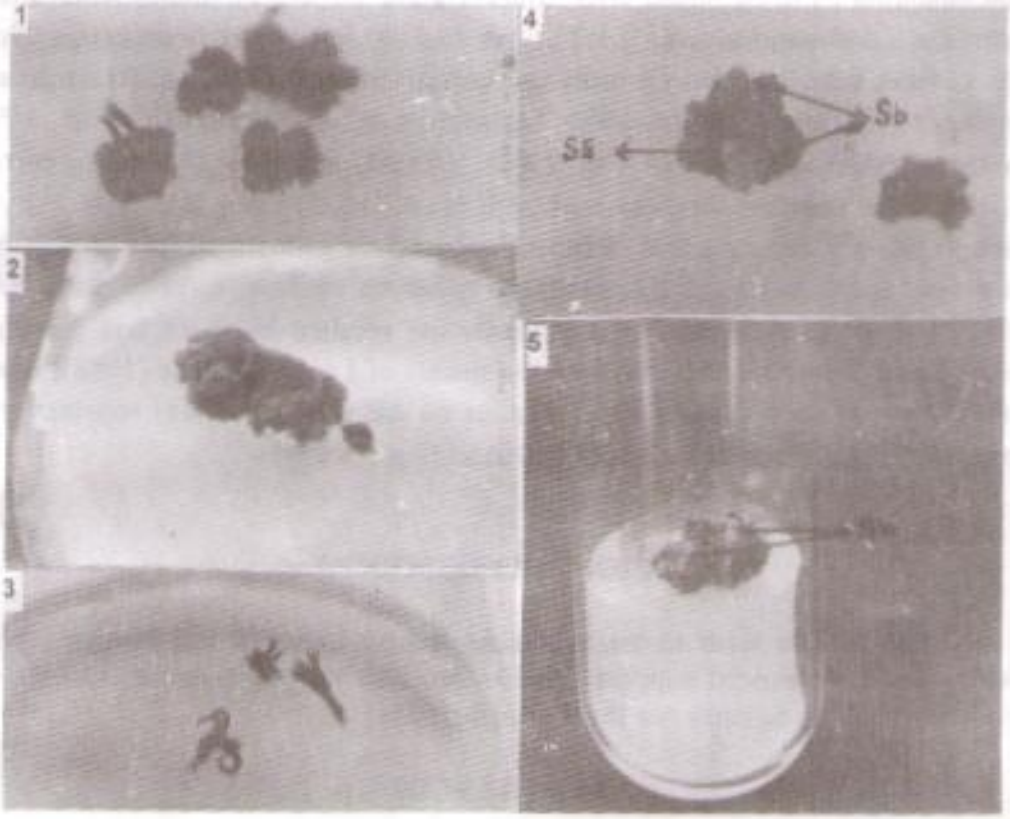
Table 2. Subculturing response of embryo callus to different growth regulators.

Primary medium	Subculturing medium	Response*	Percentage response
MS (x ½)+IBA (10μM)	MS (x ½)+NAA (5μM)+ BAP (5μM)	Shoot differentiation	15
MS (x ½)+BAP (5μM)	MS (x ½)+NAA (10μM) + 0.5 mg/l glutamine + BAP (5μM)	Profuse, friable callus	85
MS (x ½)+NAA (10μM) + BAP (10μM)	MS (x ½)+NAA (10μM) + BAP (5μM) + 0.5 mg/l glutamine	Shoot bud induction, 4-5 shoot buds/ culture	65

* Data recorded at the end of 4-weeks; 10 replicates / treatment.

DISCUSSION

The present investigations carried on embryo culture of *Picea smithiana* (Wall) Boiss under different phytohormonal regimes resulted in various morphogenetic responses. Use of 10μM IBA resulted in callus initiation in radicle and hypocotyl regions but the use of auxin alone in any *Picea* sp. has not been reported yet. Subculturing of this embryonal callus, in present studies, on NAA(5μm) and BAP (5μM) resulted in shoot differentiation which shows conformity with that of Lu and Thorpe(1988) where shoots have been reported in *Picea engelmanni* by subculturing of semi compact embryo callus on Arnold and Eriksson (AE) medium (Arnold and Eriksson, 1981) augmented with NAA (10μM) and BAP (10μM). When BAP 10μM was used in the medium only



Figs.1-5 : Morphogenetic response of embryos of *Picea smithiana* to various concentrations and combination of phytohormones.

1- **Shoot differentiation from embryo callus on MS (x ½)+NAA (5μM)+ BAP (5μM);**
2- **Formation of green friable callus on MS (x ½)+ BAP (5μM);**
3- **Embryo germination on MS (x ½)+ BAP (10μM);**
4- **Induction of shoot buds (sb) and Somatic embryos (SE) on MS (x ½)+BA (10μM)+ BAP (5μM);**
5- **Induction of shoot buds (sb) on MS (x ½)+NAA (10μM)+ BAP (5μM)+ 0.5 mg/l glutamine.**

germination of embryo was recorded without any callusing or bud induction which is contrary to the results obtained by Harry and Thorpe (1991) where buds have been observed by culturing of embryo on AE medium (1981) supplemented with BAP (10μM) but is in agreement with Eliss *et. al.* (1991) who have reported bud inhibition by using higher BAP concentration. In present studies when auxin-cytokinin combinations were used it resulted in somatic embryogenesis. Such results have been reported by many workers in different *Picea* sp. (Hakman and Fowke, 1987; Lu and Thorpe, 1988; Tautorus *et. al.*, 1990 and Afele *et. al.*, 1992) by using different auxins NAA or 2,4-D (10μM) and BAP (5μM). In current studies when medium supplemented with NAA (10μM) and BAP (10μM) was used in primary culture green, compact and nodular callus with low growth was recorded. Subculturing of this compact callus on medium fortified with NAA (10μM), BAP (5μM) and 0.5 mg/l glutamine resulted in shoot bud induction which again shows resemblance with the results of Lu and Thorpe (1988), where subculturing of semicompact embryo callus on AE medium (1981) supplemented with BAP (25μM) resulted in shoot bud induction.

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