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INVESTIGATION ON THE EFFECTS OF GROWTH HORMONES FOR THE MICROPROPAGATION OF *CEDRUS DEODARA*

Aaradhna Chauhan*, Sanjeev Lal and Vipin Chandra
Department of Botany, R.C.U. Govt. (P.G.) College, Uttarkashi, India
*Email: aradhana.chauhan@gmail.com, Mob. No.-8057409751
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ABSTRACT

Cedar (*Cedrus deodara*) is one of the most important plant species used in timber industry in India. It also has some medicinal properties. In current study, the effect of various concentrations of plant growth regulators (BAP and 2,4-D) and surface sterilant (mercuric chloride) was observed for *in vitro* establishment of *Cedrus deodara*. Nodal explants were inoculated on MS media supplemented with auxin and cytokinin. Present work was carried out at Department of Botany, R.C.U. Govt. (P.G.) College, Uttarkashi, India. Maximum bud response (47%) and number of shoots (3.0) was exhibited by explants inoculated in MS media supplemented with BAP/2,4-D at 1.0 mg/l and 0.2 mg/l concentration. For surface sterilization $HgCl_2$was used at four different concentrations i.e., 0.01, 0.05. 0.1 and 0.5 mg/l. Maximum explants were survived with 0.01% $HgCl_2$.
Keywords **:** *Cedrus deodara, In vitro*, tissue culture, $HgCl_2$, BAP, 2,4-D.

Introduction

Cedrus deodara is commonly known as Cedar or Deodar belongs to family Pinaceae. It is beautiful, evergreen, tall, coniferous tree (Pijut, 2008). Commonly found between the altitude 1,200 and 3,030 m., Himalayan moist temperate forests (Champion and Seth, 1968). It is restricted to montane or high montane zones of western Himalaya (Farjon, 1990). It is an important source of commercial timber in India. Ancient Egyptians used cedar sawdust (cedar raisin) in the process of mummification. (Chaney, 1993; Demetci, 1986; Maheshwari and Biswas, 1970). In spite of this, *Cedrus deodara* has some medicinal properties also like anticancer and antioxidant properties. (Chaudhary *et al*., 2011; Tiwari *et al*., 2001; Saxena *et al*., 2010).

Deforestation, natural calamities, overexploitation and human activities are the main reasons of the depletion of this species in its natural habitat. Propagation through seeds might be irregular and have short supply. Due to high demand in timber industry, there is need for multiplication on large scale. Multiplication through *in vitro* technique is the only answer to fulfil the above objective. Successful rejuvenation from mature trees of a number of woody plants has been achieved (Boulay, 1979; Ahuja, 1986 & 1993; Dunstan *et al*., 1986; Mascarenhas *et al.,* 1987; Gupta and Mascarenhas, 1987) through *in vitro*techniques.

The successful *in vitro* multiplication on mature conifers was earlier reported (Gupta and Durzan, 1985; Gupta *et al*., 1994; Dumas and Monteuuis, 1995; Parasharami *et al*., 2003; Anderson and Levinsh, 2005; Malabadi and Van Staden, 2005; Cortizo*et al*., 2009; Agrawal et al., 1991; De Diego *et al*., 2010). Adventitious shoots were induced 10-20 times more effectively by BAP than by 2-iP in embryonic explants of *Pinus strobes* (Flinn *et al.,* 1996).*In vitro* organ differentiation was achieved by Bhatnagar *et al*., 1983 in *Cedrus deodara* and *Pinus roxburghii*. The *in vitro* regeneration was successfully done using stem segments of *Cedrus deodara* by Tamta and Palni, 2004. They suggested that the more work is required to develop the protocol for *in vitro* propagation of *C. deodara*. More work on successful in vitro regeneration was reported (Piola and Rohr, 1996; Piola *et al.,* 1998 & 1999; Renau-Morata *et al.*, 2005). The objective of our study is to develop the protocol for *in vitro* establishment of *Cedrus deodara*.

Material and Methods

Collection of Plant Material

Fresh stem nodal segments were collected from the trees which are already planted in the Botanical Garden, at college campus during the month of September.

Explant preparation

The explants were prepared by trimming to 1.5-2.0 cm in size (Fig.-1). They were washed thoroughly in running tap water to remove dust particles followed by washing with detergent and distilled water.

Surface sterilization

Explants were taken to the laminar air flow for further surface sterilization. For surface sterilization, mercuric chloride was used at different concentration followed by quick dip with 70% ethyl alcohol and three times washing with autoclaved double distilled water.

Culture establishment

Surface sterilized explants were inoculated in MS media supplemented with BAP and 2,4-D at different concentration. The cultures were kept in incubation room at 25±2°C under photoperiod of 16 hrs light and 8 hrs dark. Data on all parameters was recorded after 4 weeks.

Results and Discussion

Bud response was observed after 20-25 days of inoculation (Fig.-2). Maximum bud response (47%) was obtained with BAP at 1.0 mg/l and 2,4-D 0.2 mg/l followed by 41% showed by BAP 1.0 mg/l+2,4-D 0.5 mg/l (Table-1). While minimum bud response (25%) was exhibited by micro shoots inoculated in MS media supplemented with BAP 1.5 mg/l+ 2,4-D 0.5 mg/l (Table-1). Similar to our work, Tamta and Palni (2004) also studied the effect of different concentration of cytokinin and auxin on *in vitro* shoot development. Maximum bud response was obtained in higher concentration of BAP. MS media supplemented with 1.0 mg/l BAP gave the best bud response which corroborates with our results.

In current investigation, maximum number of shoots (3) were augmented with BAP (1.0 mg/l) and 2,4 D (0.2 mg/l) followed by BAP (1.5 mg/l) + 2,4 D (1.0 mg/l), in which 2.8 number ofshoots was reported (Fig.-1). Shoot length was recorded maximum (2.69 cm) with MS medium supplemented with BAP 0.5+ 2,4 D 1.0 mg/l. Minimum number of shoots (1.3) and shoot length (1.44 cm.) was recorded with 0.5 mg/l BAP+0.5 mg/l 2,4 D (Fig.-1). Present study shows that the *in vitro* establishment is affected by the different concentrations of plant growth regulators and sterilant. Higher concentrations (0.1 mg/l) of mercuric chloride proved toxic for bud response. Minimum survival (17.5%) was recorded in explants which are surface sterilized with 0.5 mg/l $HgCl_2$. Bud response (34.1%) was highest with 0.05 mg/l concentration, while survival was observed maximum with 0.01 mg/l mercuric chloride (Table-2).

In present work, we observed that the concentration of growth hormones and sterilant plays an important role for*in vitro* establishment similar to the observations of Ahmad *et al.* (2013). They also observed that mercuric chloride at higher concentration proved toxic for *in vitro* survival. The effect of plant growth regulators, explant size, incubation temperature and cytokinin was observed by Renau- Morata *et al*. (2005). They demonstrated that in MS media supplemented with0.1 mM BA, highest bud sprouting was recorded in *C. libani*. Although, in *C. atlantica*, BA has negative effect on bud sprouting.

The types of explants and culture media also found significant in culture establishment in the work of Nazemi and Salehi (2018). In this work, the effect of genotypes and growth regulators was observed. BAP had important role to increase the rate of survival and bud response. BAP at very high concentration may suppress the shoot regeneration and has toxic effect for *in vitro* establishment (Sarmast *et al*., 2012). In our study, maximum rate of establishment was achieved with 1.0 mg/l BAP.

Conclusion

The protocol was developed for *in vitro* multiplication of *Cedrus deodara*. Very less work was earlier reported for *in-vitro* establishment of this species. Therefore, more work was needed for tissue culture of *Cedrus deodara*. Our study reveals that the concentration of cytokinin and auxin affects the rate of *in-vitro* establishment.We can achieve maximum rate of multiplication by manipulating the concentration of growth hormones.

Table 1 : Effect of hormones on shoot response

Hormone concentration (mg/l)	Bud Response (%)	Days of Bud response
BAP-0.5	38.7±2.29	40 Days
BAP-1.0	27±2.70	35 Days
BAP-1.5	26.3±2.77	30 Days
BAP-2.0	31±2.33	30 Days
BAP-0.5/2,4 D-0.2	34.5±1.89	25 Days
BAP-0.5/2,4 D-0.5	36±2.08	28 Days
BAP-0.5/2,4 D-1.0	32.5±2.26	35 Days
BAP-1.0/2,4 D-0.2	47±2.49	22 Days
BAP-1.0/2,4 D-0.5	41±4.93	25 Days
BAP-1.0/2,4 D-1.0	40.5±5.24	28 Days
BAP-1.5/2,4 D-0.2	36.3±4.94	30 Days
BAP-1.5/2,4 D-0.5	25±5.42	35 Days
BAP-1.5/2,4 D-1.0	37.3±3.06	25 Days
BAP-2.0/2,4 D-0.2	25.5±2.16	38 Days
BAP-2.0/2,4 D-0.5	32.5±3.09	27 Days
BAP-2.0/2,4 D-1.0	33±2.00	35 Days

Table 2: Effect of Mercuric chloride on establishment

$HgCl_2$ Concentration (mg/l)	Bud Response %	Survival %
0.01	32±3.59	31.5±2.9
0.05	34.1±2	28±3.43
0.1	28±3.34	24.5±2.52
0.5	27.5±2.38	17.5±3.09

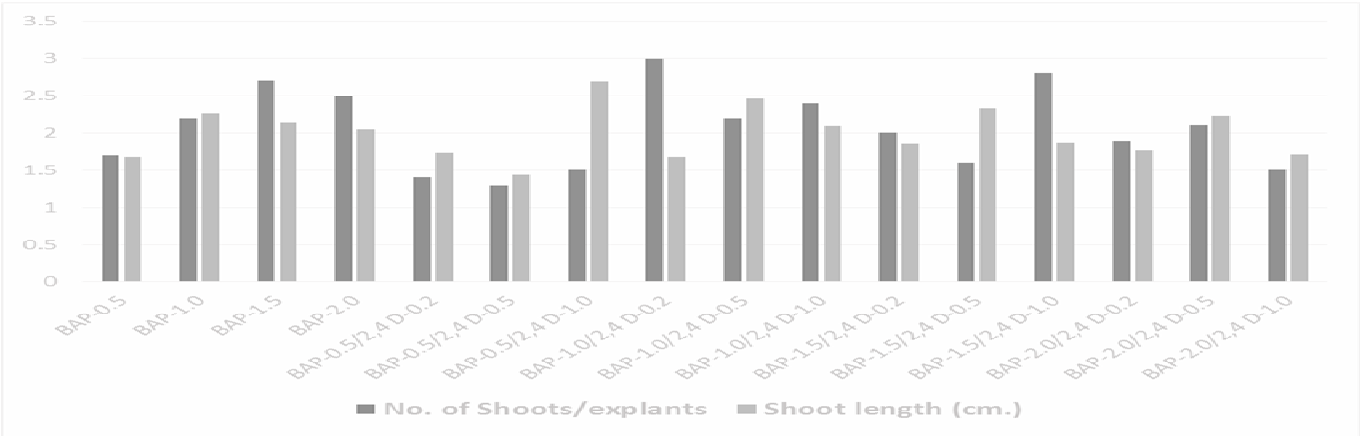

Fig. 1: Effect of plant growth regulators on shoot proliferation

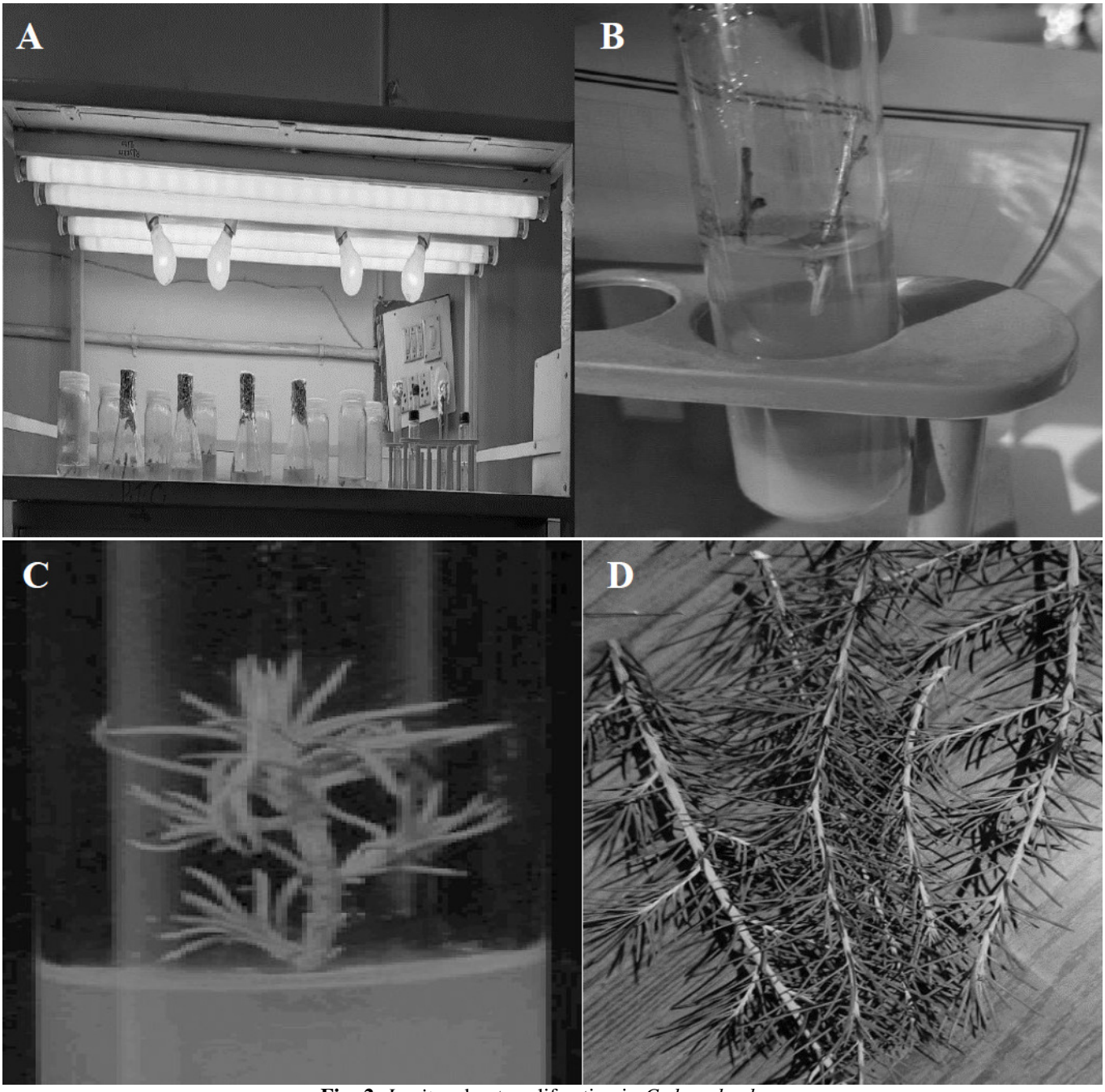

Fig.-2: *In vitro* shoot proliferation in *Cedrus deodara*

Conflict of interest- The authors declare that they do not have any conflict of interest.

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