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BRIEF COMMUNICATION

Adventitious root formation in young shoots of *Cedrus deodara*

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Abstract

The effect of auxins [indole-3-butyric acid (IBA) and α-naphthaleneacetic acid (NAA)], phenolics (phloroglucinol and coumarin), a combination of auxins and phenolics, and a systemic fungicide (*Bavistin*, containing 50 % carbendazim) on adventitious root formation in stem cuttings (current season's growth) of *Cedrus deodara* L. during winter and monsoon (rainy) seasons has been examined. Significant stimulation of rooting due to treatments was observed in cuttings planted in winter in the following order: 0.25 mM IBA (87.5 % rooting) > 5 mM coumarin (70.8 %) > IBA (0.5 mM) + coumarin (5 mM) (50.0 %). In cuttings planted in monsoon only 0.05 % *Bavistin* was found to be effective in inducing rooting (83.3 %). Other treatments were ineffective and in some treatments drying of cuttings was noticed.

Additional key words: auxins, carbendazim, cedar, clonal propagation, stem cuttings, phenolics, rooting.

The advantages of clonal (vegetative) propagation in forest trees have long been realized (Schreiner 1939, Libby 1974). In general propagation of gymnosperms is difficult because of low rooting efficiency. There is a need for the development of rapid and simple methods of clonal multiplication for large scale afforestation programmes. Interfascicular shoots and seedling cuttings of *Pinus* spp. (Mergen and Simpson 1964, Whitehill and Schwabe 1975, Smith and Thorpe 1975) and current years stem cuttings of *Taxus baccata* (Nandi *et al.* 1996) have been reported to form adventitious roots following chemical treatments, possibly due to juvenile state. The present investigation was carried out to examine the effect of various chemicals on rooting in *Cedrus deodara,* using young and juvenile shoots which could provide an inexhaustible supply of propagules. *C. deodara* is a conspicuous evergreen gymnosperm and a highly valued tree.

Terminal cuttings were taken from growing shoots of well established plants of *Cedrus deodara* L. (age 15 years, 4 m height) from Almora town (29°37'N, 79°40'E, 1600 m a.s.l.) in the last week of January and July 1994. Cuttings were brought to the laboratory in perforated polythene bags and used within 4 h after collection. The auxins, indole-3-butyric acid (IBA) and α-naphthaleneacetic acid (NAA), were obtained from *Himedia Laboratory Pvt. Ltd.*, Mumbai, India. The phenols, phloroglucinol and coumarin were from *Sigma Chemical Co.*, St. Louis, USA, and *Bavistin* (containing 50 % carbendazim, m/m) was from *BASF India Ltd.*, Mumbai, India.

The final cuttings (current season's growth; 10 - 15 cm in length and without lateral shoots) were clipped and the needles were removed from the basal 3 - 4 cm; the basal portion was dipped in various solutions for 24 h at 20 °C. The solutions included IBA (0.25 and 1.0 mM), NAA (0.25 and 1.0 mM), phloroglucinol (5 and 15 mM), coumarin (5 and 15 mM), IBA (0.5 mM) + phloroglucinol (5 mM), IBA (0.5 mM) + coumarin (5 mM) and *Bavistin* (0.05 % a.i.). All chemicals were dissolved in 1.5 % (v/v) aqueous ethanol; control cuttings were dipped in aqueous ethanol (1.5 %, v/v). Each treatment consisted of 24 cuttings.

Following chemical treatment, cuttings were planted in polythene sleeves (16 cm height × 8 cm diameter) containing sieved rooting medium (equal parts of sand,

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Abbreviations: IBA - indole-3-butyric acid; NAA - α-naphthaleneacetic acid.

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soil and farm yard manure) and these were then placed under a polythene tunnel (8.5 m length, 2.5 m width and 1.8 m height in the middle). The mean air temperatures during winter (January and February) and monsoon (July, August and September) seasons were 8 - 10 °C and 21 - 24 °C, and relative humidity 70-74 % and 80 - 85 %, respectively. Generally 80 % reduction in photosynthetically active radiation was recorded inside the polytunnel. Based on random observations, root initiation was first noticed after about 14 weeks in majority of cuttings and, therefore, the final data were recorded at 15 weeks after planting. All observations and statistical analyses were carried out as previously described (Nandi *et al.* 1996).

Cuttings planted in winter formed adventitious roots quite well (50 % and above rooting success compared with no rooting in control cuttings) following treatment with some chemicals (Fig. 1, Table 1). Among the two auxins, the lower concentration of IBA (0.25 mM) induced callusing (58.3 % compared to 8.3 % in control) and profuse rooting (87.5 %) with mean root number of 4.3 ± 0.7 and mean root length of 32.4 ± 2.5 mm; NAA at

Fig. 1. Representative rooting response of *Cedrus deodara* cuttings treated with various chemicals (a - untreated control, b - 0.25 mM NAA (callusing), c - 5 mM coumarin, d - *Bavistin* (0.05 %), e - 0.25 mM IBA). The photograph was taken 15 weeks following treatment. *Bar* represents 2 cm.

Table 1. The effect of various chemicals on rooting of *Cedrus deodara* cuttings. Cuttings with roots ≥ 1 mm were considered to have rooted and included for calculating % rooting; the data were recorded 15 weeks after treatment and planting. Root initials > 2 mm were considered for calculating mean number of roots and mean root length. Means ± SE, n = 24. A *dash* indicates that the cutting had dried at the time of data recording. Higher concentrations of IBA, NAA (1 mM each), coumarin and phloroglucinol (15 mM each) when applied alone failed to induce any response and hence not included in this table.

Treatment	January				July			
	callusing [%]	rooting [%]	root number [cutting^{-1}]	root length [mm]	callusing [%]	rooting [%]	root number [cutting^{-1}]	root length [mm]
Control	8.3	0	0	0	0	0	0	0
Bavistin (0.05 %)	29.2	4.2	0.5 ± 0.4	4.0 ± 3.5	70.8	83.3	3.9 ± 0.4	13.2 ± 0.9
IBA (0.25 mM)	58.3	87.5	4.2 ± 0.7	32.4 ± 2.5	12.5	0	0	0
NAA (0.25 mM)	50.0	8.3	2.8 ± 1.6	9.4 ± 5.5	8.3	0	0	0
Coumarin (5 mM)	54.2	70.8	3.6 ± 0.3	42.7 ± 5.8	12.5	0	0	0
Phloroglucinol (5 mM)	45.8	4.2	0.5 ± 0.4	0.8 ± 0.7	4.2	0	0	0
IBA + coumarin (0.5 + 5 mM)	37.5	50.0	3.4 ± 0.1	47.2 ± 2.6	16.7	0	0	0
IBA + phloroglucinol (0.5 + 5 mM)	16.7	0	0	0	20.8	-	-	-
$LSD_{0.05}$	2.1	1.1	2.2	11.1	0.9	0.7	0.4	1.0

the same concentration was, however, much less effective in rooting (8.3 %) although good callusing (50 %) occurred. At higher concentration (1.0 mM), both the auxins caused tissue mortality within 4 weeks of planting. Amongst the two phenolics tested, the lower concentration of coumarin (5 mM) stimulated callusing (54.2 %) as well as profuse rooting (70.8 %, mean root number 3.6 ± 0.3 and mean root length 42.7 ± 5.8 mm). However, coumarin and phloroglucinol (5 and 15 mM) were not effective at all. The combined treatment of IBA (0.5 mM) + coumarin (5 mM) also stimulated rooting (50 %) with good number of roots (3.4 ± 0.1) and maximum root length (47.2 ± 2.6 mm); treatment with IBA (0.5 mM) + phloroglucinol (5 mM) had no influence on rooting although some callusing (16.7 %) was observed. Treatment of cuttings with *Bavistin* (0.05 %) resulted in some callusing (29.2 %) with very low rooting (4.2 %). None of the chemicals used for treatment of cuttings collected and planted in monsoon were effective in stimulating rooting except *Bavistin* (0.05 %), which induced callusing (70.8 %) and profuse rooting (83.3 % compared with no rooting in control cuttings; Table 1).

The stimulation of adventitious root formation in stem cuttings with auxins and commercial rooting mixtures is well known (Blazich 1988). As observed in *C. deodara,* stimulatory as well as seasonal effect of auxins, phenolics and a systemic fungicide, *Bavistin* on root formation in stem cuttings of *Taxus baccata* have been reported (Nandi *et al.* 1996, 1997). In *T. baccata* very good rooting response was obtained during the monsoon season. In *C. deodara*, *Bavistin* treatment was effective only during the monsoon while treatment with other chemicals, including auxins, were ineffective during this period. In winter, however, IBA and/or coumarin treatment were better in *C. deodara* compared to *T. baccata.* The seasonal effect on rooting of cuttings in conifers has been reported to vary (refs. in Whitehill and Schwabe 1975). The reported variation could be attributed to species difference and/or sensitivity of tissues (Trewavas and Cleland 1983). Besides other factors, both internal as well as the stock plant environment do exert an influence on root formation (Davis *et al.* 1988).

The precise mechanism of stimulation of rooting by phenolics is not very clear; it is likely that these compounds, at least partly protect auxins from destruction by the enzyme IAA oxidase (Donoho *et al.* 1962). Phenolics when applied alone (James and Thurbon 1979) or in combination with auxins (Kling and Meyer 1983) are known to enhance root formation. As observed in this study, coumarin alone also induced root formation in hypocotyl cuttings of *Impatiens balsamina* (Dhawan and Nanda 1982); it also acted synergistically with IBA in the present investigation. Similarly, phloroglucinol alone stimulated *in vitro* root formation in apple root stocks (James and Thurbon 1979) and also in conjunction with IBA in apple and other species (refs. in Nandi *et al.* 1996). Stimulation of rooting in *C. deodara* by *Bavistin* may be related to the auxin-like property of carbendazim as observed earlier in other species (Thurston *et al.* 1979, Nandi *et al.* 1996).

The high success of adventitious root formation in current season's shoots of *C. deodara* and *T. baccata* indicates that juvenile material can be a promising source of explants for large scale clonal (vegetative) multiplication of gymnosperms required for use in afforestation programmes. Since many plant species including conifers exhibit plagiotropic growth habit, care should be taken while selecting material for preparing cuttings. The use of small sized cuttings (10 - 15 cm) needed should further reduce handling cost, time and nursery space.

References

Blazich, F.A.: Chemicals and formulations used to promote adventitious rooting. - In: Davis, T.D., Haissig, B.E., Sankhla, N. (ed.): Adventitious Root Formation in Cuttings. Pp. 132-149. Dioscorides Press, Portland 1988.

Davis, T.D., Haissig, B.E., Sankhla, N. (ed.): Adventitious Root Formation in Cuttings. - Dioscorides Press, Portland 1988.

Dhawan, R.S., Nanda, K.K.: Stimulation of root formation in *Impatiens balsamina* L. cuttings by coumarin and the associated biochemical changes. - Biol. Plant. **24**: 177-182, 1982.

Donoho, C.W., Mitchell, A.E., Sell, H.N.: Enzymatic destruction of ^{14}C labeled indoleacetic acid and naphthalene acetic acid by developing apple and peach seeds. - Proc. amer. Soc. hort. Sci. **80**: 43-49, 1962.

James, D.J., Thurbon, I.J.: Rapid *in vitro* rooting of the apple rootstock M9. - J. hort. Sci. **54**: 309-311, 1979.

Kling, G.J., Meyer, M.M., Jr.: Effect of phenolic compounds and indoleacetic acid on adventitious root initiation in cuttings of *Phaseolus aureus, Acer saccharinum, Acer grisetum.* - HortScience **18**: 352-354, 1983.

Libby, W.J.: The use of vegetative propagules in forest genetics and tree improvement. - New Zeal. J. Forest Sci. **4**: 440-447, 1974.

Mergen, F., Simpson, B.: Asexual propagation of pines by rooting needle fascicles. - Silvae Genet. **13**: 133-139, 1964.

Nandi, S.K., Palni, L.M.S., Rikhari, H.C.: Chemical induction of adventitious root formation in *Taxus baccata* cuttings. - Plant Growth Regul. **19**: 117-122, 1996.

Nandi, S.K., Rikhari, H.C., Nadeem, M., Palni, L.M.S.: Clonal propagation of *Taxus baccata* L. - a Himalayan asset under threat. - Physiol. mol. Biol. Plants **3**: 15-24, 1997.

Schreiner, E.J.: The possibilities of the clone in forestry. - J. Forest. **37**: 61-62, 1939.

Smith, D.R., Thorpe, T.A.: Root initiation in cuttings of *Pinus radiata* seedlings. II. Growth regulator interactions. - J. exp. Bot. **26**: 193-202, 1975.

Thurston, K.C., Spencer, S.J., Arditti, J.: Phytotoxicity of fungicides and bactericides in orchid culture media. - Amer. J. Bot. **66**: 825-835, 1979.

Trewavas, A.J., Cleland, R.E.: Is plant development regulated by changes in the concentration of growth substance or by changes in the sensitivity to growth substance? - Trends Biochem. Sci. **8**: 354-357, 1983.

Whitehill, S.J., Schwabe, W.W.: Vegetative propagation of *Pinus sylvestris*. - Physiol. Plant. **35**: 66-71, 1975.