PROC. NAT. ACAD. SCI., INDIA 62 (B) I, 1992

Caulogenesis in cultured needles of *Pinus kesiya* Royle ex Gord

(Key words : *Pinus kesiya*/caulogenesis/plantlets)

Received 14.1.1991; Accepted 1.4.1991

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Abstract

Direct caulogenesis and plantlet regeneration were accomplished in cultured needles of *Pinus kesiya* on MS medium supplemented with cytokinins at varying range of concentrations. Induced adventitious buds were elongated on MS medium containing 1.0 mg/l GA_3 and the shootlets thus obtained were rooted to get complete plantlets. Plantlets were hardened in a growth chamber and the per cent survival was 80.

Introduction

Pinus kesiya is an important conifer of the high altitudes of North East India. Some genotypes have tremendous biomass potential and resin production prospect. Tissue culture has been used for cloning superior genotypes[1]. Induction of adventitious buds on embryonic shoots has been successful in many conifers like *Abies*[2], *Picea*[3, 4], *Pseudotsuga*[5], and *Pinus*[6, 7]. This report describes plantlet regeneration *via* direct adventitious bud induction in the cultured needles of *Pinus kesiya*.

Material and Methods

Seed of commercially favoured genotypes of *Pinus kesiya* Royle ex Gord, obtained from Agro-Forestry Division of ICAR, Shillong, were soaked in water and then put for stratification in the refrigerator for 48 h at 4°C. Seeds after surface sterilization with 6% H_2O_2 (v/v) for 10 min followed by 1% aqueous $HgCl_2$ solution (w/v) for 2 min, were germinated aseptically on moist cotton pad kept in large beakers. Needles from 30 days old seedlings were gently pulled off from the upper region of the shoot and placed horizontally on MS[8] medium containing various growth regulators *viz.* 1-naphthalene acetic acid (NAA), indole–3–acetic acid (IAA), indole–3–butyric acid (IBA), 2, 4–dichlorophenoxyacetic acid (2, 4–D), benzyladenin (BA) and kinetin (KN) at range of concentrations (0.5–5.0 mg/l) for morphogenetic effects. 10 ml of medium and 109 needles were taken per 35×10 mm Laxbor petri dishes. The petri dishes were sealed with Parafilm M (American Can Co) and cultures were maintained at 24 ± 2°C under a photon flux of 50 μm mol m^{-2} s^{-1}. Irradiance was provided by cool white fluorescent tubes at 10h/day.

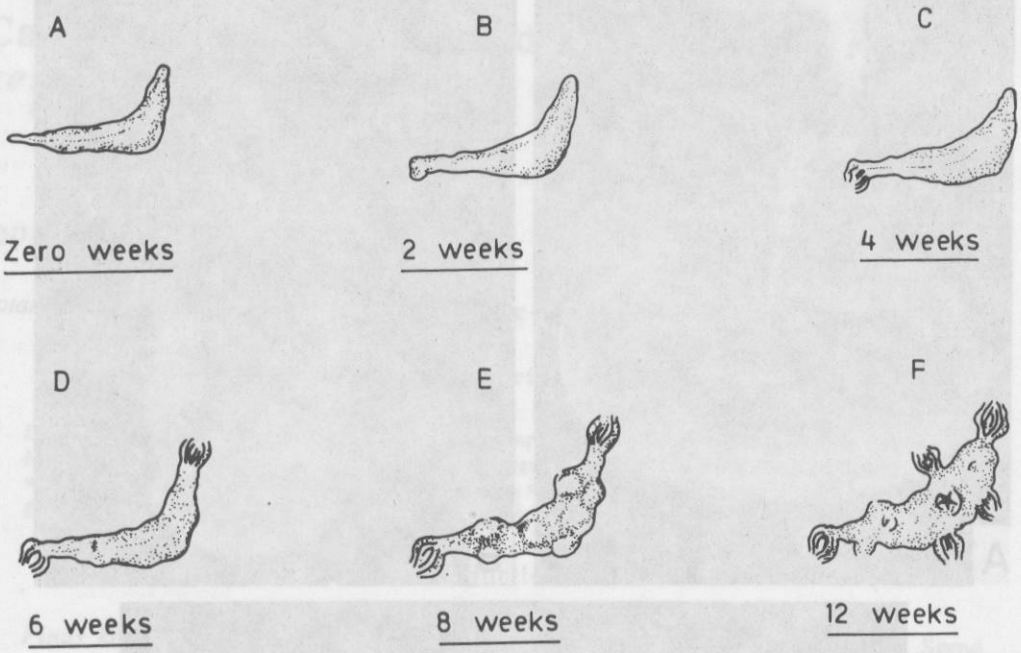
Results and Discussion

Out of various growth regulators used NAA, BA and KN, alone or in combinations, were able to induce morphogenetic effects in the cultured needles (Table 1). Needles cultured on MS medium supplemented with BA and KN at 1–5 mg/l concentration showed appreciable swelling at the apex within 2 weeks of culture (Fig. 1B). A bud primordium began to develop first at the apex later at the base in the form of protuberance within 6 weeks (Figs. 1C, D). The entire needle got coverted into a bunch of adventitious buds under the influence of applied cytokinins, BA and KN at 2 mg/l each or 1.0 mg/1 of each in combination, within 8 weeks (Fig. 1E). A further incubation up to 12 weeks was required for the proper formation and development of the multiple buds on the cultured explants. However, adventitious bud induction in response to KN was better than that of BA in terms of number of shoots produced/

Table 1 – Morphogenetic effects of NAA, BA and KN on the cultured needles of *Pinus kesiya* *.

Growth regulators (mg/l)			Number of shoots/explant ±SE	Remarks
NAA	BA	KN		
0.5	0	0	—	swollen
1.0	0	0	—	swollen, elongated
2.0	0	0	—	swollen, elongated
5.0	0	0	—	swollen, pale
0	0.5	0	—	swollen, green
0	1.0	0	4 ± 1.3	blunt growth
0	2.0	0	8 ± 1.8	green, healthy, stout
0	5.0	0	5 ± 2.1	pale, poor
0	0	0.5	1 ± 0.8	mostly at the apex
0	0	1.0	5 ± 1.7	normal, tiny
0	0	2.0	15 ± 1.8	green, healthy
0	0	5.0	6 ± 2.1	green, stunt
0.5	0.5	0	—	callus
1.0	0.5	0	—	callus
1.0	1.0	0	—	callus++
1.0	2.0	0	—	callus
0.5	0	0.5	—	callus
1.0	0	0.5	—	callus
1.0	0	1.0	—	callus
1.0	0	2.0	—	callus
0	0.5	0.5	6 ± 1.4	healthy, green
0	1.0	1.0	10 ± 1.8	healthy, green
0	2.0	2.0	4 ± 1.1	tiny, pale

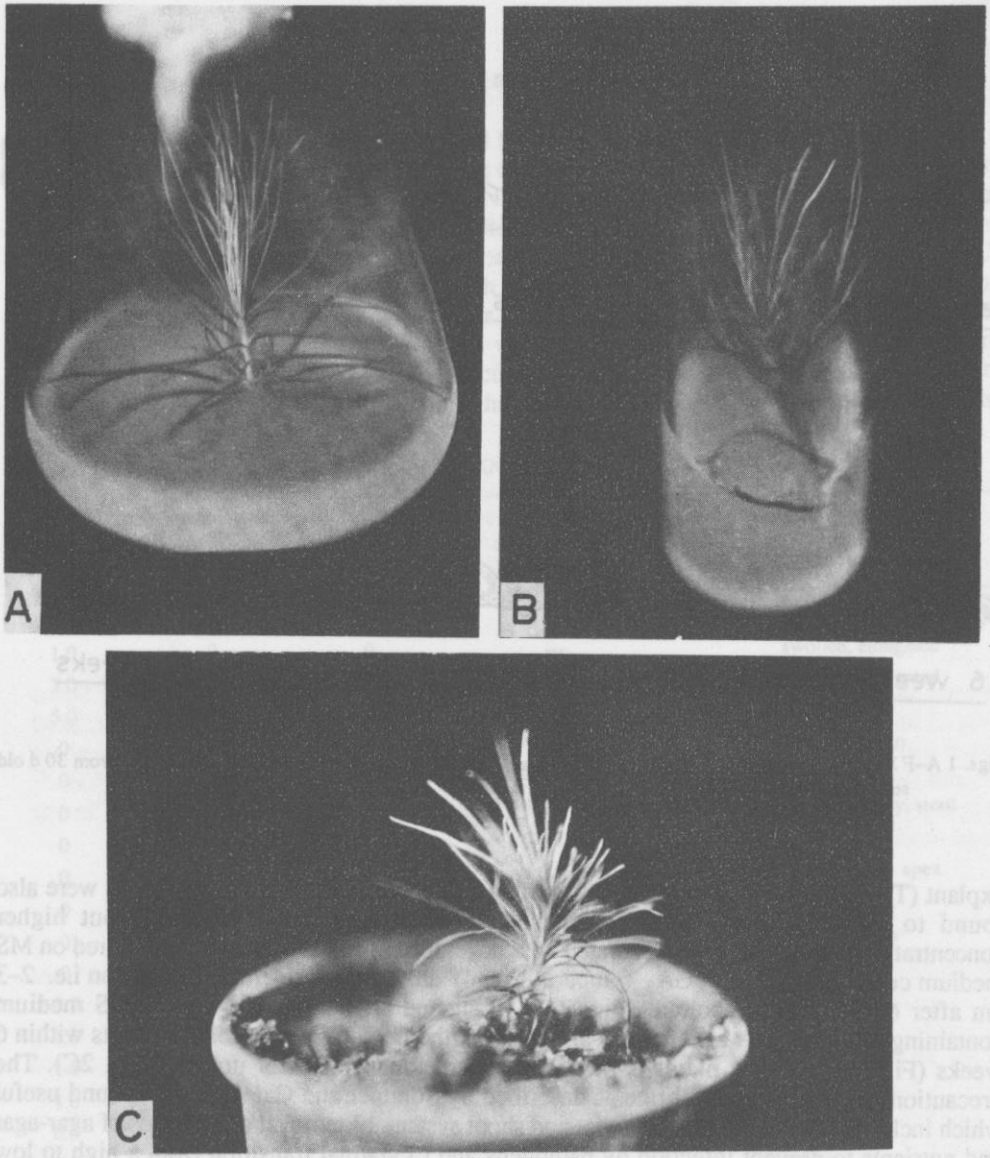
*Data scored after 12 weeks, 10 replicates per treatment, experiment repeated twice; — no response; ++ appreciable.



Figs. 1 A–F Schematic outline of induction and development of adventitious buds on a cultured needle from 30 d old seedling of *Pinus kesiya*

explant (Table 1). The combinations of BA and KN at concentration 1.0 mg/l each were also found to be satisfactory for caulogenesis in the present study (Table 1) but higher concentrations were inhibitory. Separated well developed adventitious buds subcultured on MS medium containing 1.0 mg/l GA_3 (gibberillic acid) attained considerable elongation i.e. 2–3 cm after 6 weeks of subculture (Fig. 2A). Elongated shoots were rooted in MS medium containing 1.0 mg/l IBA as reported[7] and 60% of the treated shoots produced roots within 6 weeks (Fig. 2B). Young plantlets of 3–5 cm were found ideal for potting (Fig. 2C). The precautions for better survival rate as described by Sommer and Caladas[9] were found useful which included, a) balance between root and shoot system, b) removal of all traces of agar-agar and nutrients to prevent infection by pathogens and c) gradual transition from a high to low humidity.

In plant tissue culture it is believed that *in vitro* shoot proliferation is controlled by the balance of auxins and cytokinins. However, present study on *Pinus* indicates that cytokinin only is required to induce caulogenesis which is in agreement with earlier reports on conifers[10,11]. Nevertheless, the fact that auxin can be omitted in the present study does not exclude the role of auxin-cytokinin interaction in controlling morphogenesis as there might be involvement of endogenous auxins at the organ forming loci. The suitability of KN over BA



Figs 2A–C. Elongation of excised adventitious buds and their subsequent rooting.

Fig. 2A Elongation of excised adventitious buds on MS medium containing 1.0 mg/l GA.

Fig. 2B. Rooted shoots of *Pinus kesiya* in MS medium containing 1.0 mg/l IBA.

Fig. 2C. Potted healthy rooted shoots of *Pinus kesiya*.

in this case suggests very specific requirement of cytokinin for the present system. The type of cytokinin and their specific requirement has been emphasised in other *in vitro* studies related to conifers[11, 12].

Hardening of the potted plants was accomplished within 15 days in Heraeus Votsch growth chamber and rate of survival was recorded to be 80% under glass house conditions. Following the sequence of bud induction, elongation and rooting of shootlets thus obtained, ca. 36 plantlets/seedlings could be produced within 6 months. Micropropagation of selected genotypes would be useful in producing mass planting stock for reforestation.

Acknowledgment

Financial assistance from North Eastern Council, Ministry of Home Affairs, Govt. of India, is gratefully acknowledged.

References

1. Ho, R.H. (1989) *Pl. Sc.* **62** : 129.
2. Bonga, J.M. (1981) *In Vitro* **13** : 41.
3. Arnold, S.V. & Erikson, T. (1979) *Physiol. Plant* **45** : 29.
4. Jonsson, E. & Bomman, C.H. (1983) *Physiol. Plant* **59** : 1.
5. Dunstan, D.I., Mohammed, G.H. & Thorpe, T.A. (1986) *N.Z.J. For. Sc.* **16** : 269.
6. David, A.H., David, M.F. & Isemukali, K. (1979) *AFOCEL Etudes et Recherches* **12** : 33.
7. Kumar, A. & Tandon, P. (1989) *Proc. XIII Plant Tissue Culture Conference,* Oct. 18–20, 1989, Shillong, India.
8. Murashige, T. & Skoog, F. (1962) *Physiol. Plant* **15** : 473.
9. Sommer, H.E. & Caladas, L.S. (1981) in *Plant Tissue Culture : Methods and Application in Agriculture,* ed. Thorpe, T.A., Academic Press, New York, p. 349.
10. Rumary, C. & Thorpe, T.A. (1984) *Can. J. For. Res.* **14** : 10.
11. Patel, K.R., Kim, H. & Thorpe, T.A. (1986) *For. Ecol. Manag.* **15** : 147.
12. Abdullah, A.A., Yeoman, M.M. & Grace, J. (1987) *Tree Physiol.* **3** : 123.