24

IN-VITRO PROPAGATION OF *PINUS KESIYA* ROYLE EX. GORD.

A. KUMAR AND PRAMOD TANDON
Plant Biotechnology Laboratory, Department of Botany,
North-Eastern Hill University, Shillong 793 014

SUMMARY

Regeneration of plantlets in *Pinus kesiya* has been achieved through axillary bud induction on seedling apices. Murashige and Skoog medium with higher BAP concentrations (20–30 μM) was favourable for both bud induction and for increasing their number. After 30 days of culture, axillary buds were separated and transferred to the medium free of growth regulators for subsequent elongation. Highest rooting (about 64%) was observed when elongated shoots were placed on 0·6% agar supplemented with 50 μM of NAA for 24 hr and then transferred to 1/2 MS nutrient salts medium with 1% sucrose and 0·9% agar.

KEY WORDS

Pinus kesiya, seedling apices, bud induction, rooted shoots, regeneration

ABBREVIATIONS

BAP, 6–benzylaminopurine; 2,4–D, 2, 4–dichlorophenoxyacetic acid; IAA, indole-3-acetic acid; IBA, indole–3–butyric acid; KN, kinetin; MS, Murashige and Skoog; NAA, α–naphthaleneacetic acid; ZN, zeatin

INTRODUCTION

The commercial importance of conifers for their wood and wood products has drawn considerable attention of plant biotechnologists to evolve means for rapid clonal propagation of selected genotypes. In contrast to the angiospermic trees, conifers have received little attention as far as their tissue culture is concerned. Cell and tissue culture has been used for cloning superior genotypes (Ho, 1989) and there have been reports of adventitious buds on embryonic shoots in conifers like *Abies* (Bonga, 1981), *Picea* (Arnold and Eriksson, 1979; Jansson and Bomman, 1983), *Pseudotsuga* (Dunstan *et al.*, 1986) and *Pinus* (David *et al.*, 1979).

Pinus kesiya is important among conifers of higher altitudes of North-East India as some genotypes have tremendous biomass potential and oleo-resin prospect. Here we report regeneration of plantlets of *Pinus kesiya* through bud induction on seedling apices.

MATERIALS AND METHODS

Seeds of superior genotypes of *Pinus kesiya* Royle ex. Gord. (obtained from Agro-forestry Division of ICAR, Shillong), after soaking in water were stratified in refrigerator for 48 hr at 4°C. Seeds were surface sterilized with 6% H_2O_2 (v/v) for 10 min followed by 1% aqueous $HgCl_2$ solution (w/v) for 2 min and germinated aseptically on moist cotton pads kept in large beakers. Roots, hypocotyl part and cotyledons were removed from 20 day old seedlings to get the primary explants, i.e., seedling apices. These were aseptically cultured on Murashige and Skoog (1962) nutrient medium supplemented with various auxins *viz.*, NAA, IAA, IBA, 2,4–D and cytokinins *viz.*, BAP, KN and ZN at a range of concentrations (1–20 μM) for morphogenesis. The cultures were maintained at 24 ± 2°C under an illumination of 3,000 lux by cool white flourescent tubes for 10hr a day.

RESULTS AND DISCUSSION

Among the various growth regulators used, only cytokinins (BAP, KN and ZN) were able to induce caulogenetic response in the cultured seedling apices. Axillary bud induction as influenced by the cytokinins was assessed by examining each seedling apex under stereo microscope and visually counting the number of buds. It was observed that BAP at all concentrations was better than KN and ZN (Table 1). However, the influence of BAP did

Table 1. Effect of various cytokinins on shoot bud induction in seedling apices of *Pinus kesiya*.

Concentration	Mean shoot buds/seedling apice		
µM	BAP	ZN	KN
1·0	1·8	1·0	1·0
5·0	2·6	1·6	2·1
10·0	5·2	1·8	2·4
20·0	6·4	1·8	3·2

*Data scored after 6 weeks, 10 replicates per treatment; repeated twice.

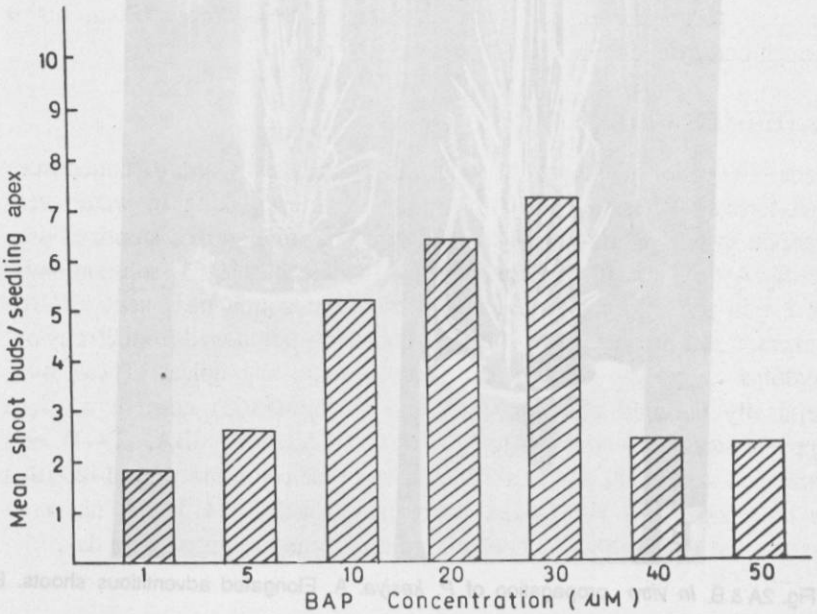

Fig. 1. Effect of BAP on shoot bud induction in cultured seeding apices of *Pinus kesiya*.

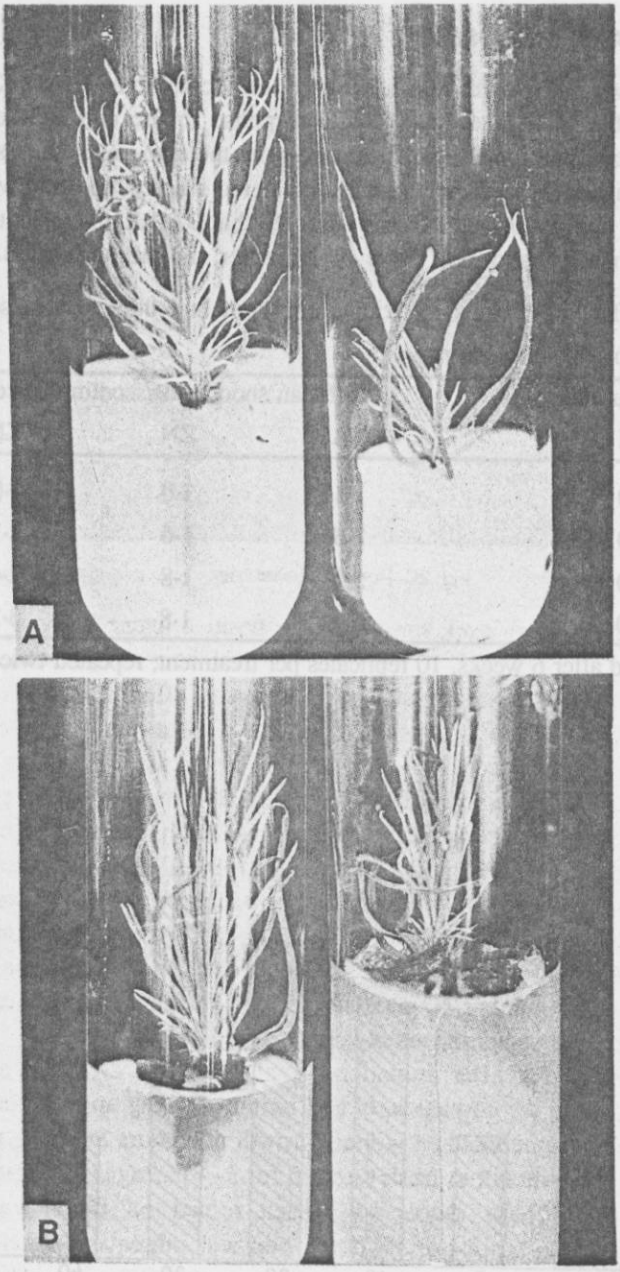

Fig. 2A & B. *In vitro* propagation of *P. kesiya*. A. Elongated adventitious shoots. B. Rooted shoots.

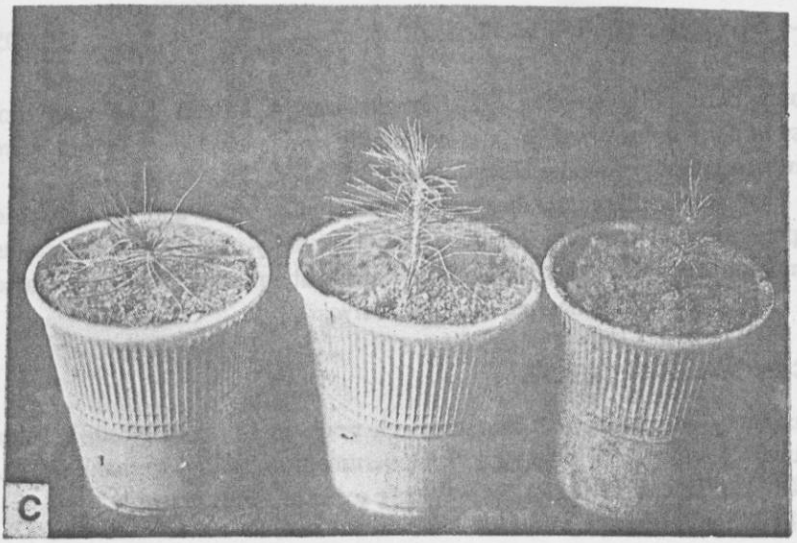

Fig. 2C. Potted regenerants.

not reach its optimum level, since it gave a positive linear response with the concentrations tested in contrast to the typical hormonal response curve which normally shows a constant or decreasing effect after its optimum peak. Therefore, a broader range of BAP was used to find out the optimum level for shoot production. The maximum effect on shoot induction through axillary buds was observed within a concentration range of 20–30 µM (Fig. 1). Combination of auxins and cytokinins invariably resulted in callusing. In our experiment, BAP appeared to be the most effective cytokinin in initiating shoot primordia, whereas in case of loblolly pine ZN was found more suitable (Mehra–Palta *et al.*, 1978). The shoot production in the present study was terminated after several weeks by discarding the primary explant. A large number of plantlets may be obtained through continued transfer of seedling apices to shoot initiation medium.

Eight weeks after initiation, small buds/shoots were separated individually from the cotyledonary axil on the seedling apices. These were cultured on the same medium without growth regulators for elongation and transferred every month to fresh medium for 3–4 passages until grown to 1 cm (Fig. 2A). These shoots were then rooted on 0·6% water agar supplemented with 50 µM of NAA for 24hr and subsequent transfer to 1/2 MS nutrient salt medium with 1% sucrose and 0·9% agar. About 64%

rooting was observed (Fig. 2B). Regenerants with well developed root system were transferred to pots containing soil : sand (1 : 1) and were hardened under glasshouse conditions (Fig. 2C).

Tissue culture provides means for cloning trees and their genetic improvement. Direct bud induction on the primary explant without callus formation will reduce the risk of chromosomal aberration and holds promise for cloning superior genotypes of *Pinus kesiya*.

ACKNOWLEDGEMENTS

This study was supported by a research grant to PT from North-Eastern Council, Ministry of Home Affairs, Government of India. We thank Dr. D. K. Dhyani, Scientist (ICAR) for providing seeds of *Pinus kesiya*.

REFERENCES

Arnold, S. V. and T. Eriksson : Induction of adventitious buds on buds of Norway Spruce *(Picea abies)* grown *In vitro*. Physiol. Plant. 45, 29–34 (1979).

Bonga, J. M. : Organogenesis *in vitro* of tissues from mature conifers. I*n Vitro* 17, 511–518 (1981).

David. A., M. Faye and K. Isemukali : Culture *in vitro* et micropropagation du Pin maritime *(Pinus pinaster* Sol). In : Micropropagation d' Arbres Forestiers. Annales AFOCEL, No. 12·6/79, 33–40. AFOCEL, Nangis, France (1979).

Dunstan, D. I., G. H. Mohammed and T. A. Thorpe : Shoot production and elongation on explants from vegetative buds excised from 17–20 year old *Pseudotsuga menziesii*. N. Z. J. For Sci. 16, 269–282 (1986).

Ho, R. H. : Adventitious root and shoot regeneration in cultured explants of black spruce. Plant Sci. 62, 129–135 (1989).

Jansson, E. and C. H. Bornman : Morphogenesis in dormant embryonic shoots of *Picea abies* : influence of the crown and cold treatment. Physiol. Plant. 59, 1–8 (1983).

Mehra–Palta, A., H. Smeltzer and R. L. Mott : Hormonal control of induced organogenesis : Experiments with excised plant parts of loblolly pine. Tappi 61, 37-40 (1978).

Murashige T. and F. Skoog : A revised medium for rapid growth and bioassays with tobacco cultures. Physiol. Plant. 15, 473–497 (1962).