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INDUCTION OF ADVENTITIOUS BUDS AND PLANTLET REGENERATION IN *PINUS SYLVESTRIS* L.

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THE application of tissue culture in forestry is still in its infancy, and it provides an alternative means for cloning trees. Furthermore, it can be used to genetically improve trees over a much shorter time than with traditional technology. Direct induction of adventitious buds on the primary explants without formation of callus, will reduce the risk of chromosomal variation due to cultural conditions. This approach may be applied for clonal propagation in forest trees. By using tissue culture, adventitious buds have successfully been initiated in several pine species[1,5].

In this study, our objective was to induce adventitious buds from embryos, develop shoots and regenerate plantlets.

Seeds of *Pinus sylvestris* L. were stored at 5°C for 2 months. They were sterilized in 10% sodium hypochlorite solution for 15 min and washed in sterile water 4 times. Seeds were placed on a wet filter paper in a petri dish for 3–4 days in the dark. Whole mature embryos were isolated under a binocular microscope and planted horizontally on an agar medium in petri dishes.

Several media were tested for the induction of adventitious buds — Schenk and Hildebrandt[6] (SH) and Gupta and Durzan[7] (DCR). The pH of all the media was adjusted to 5.7 with 1N NaOH or 1N HCl and then autoclaved for 15 min.

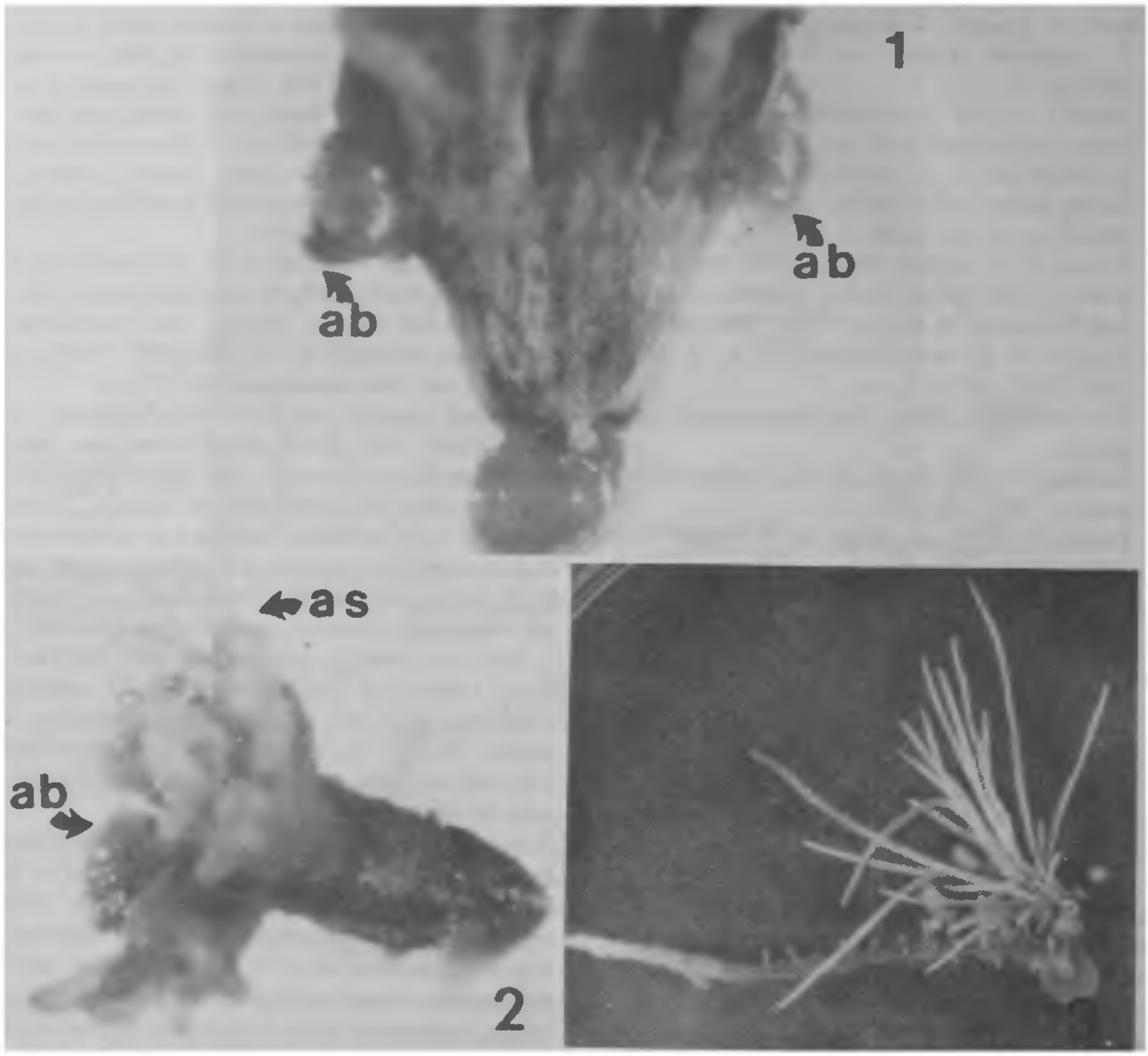
Isolated mature embryos were cultured in 100 × 20 mm petri dishes (10 embryos per dish) containing 20 ml of medium. The plates were sealed with parafilm and incubated at 25 ± 1°C, under 2,000 lux light intensity. Induction of adventitious buds directly from embryos was achieved on SH and DCR media supplemented with BAP (0–5 mg/l). All observations were recorded after 4 weeks.

After 4–5 weeks, adventitious buds (1–4 mm) were transferred individually to SH medium containing BAP (0–2 mg/l) for the production of shoots. Shoots (5–10 mm in height) were then removed and cultured on SH medium supplemented with 0.1 mg/l NAA for root induction.

Induction of adventitious buds: Two different media — SH and DCR — were tested for the induction of adventitious buds directly from embryos and development of shoots. Embryos began germination after 72–96 h. The basal end of the embryos turned reddish after 4–5 days. Adventitious buds were formed from the cotyledons of

Table 1 *Effect of medium and BAP on induction of adventitious buds in mature embryos of Pinus sylvestris after 4 weeks*

Medium	Conc. of BAP (mg/l)	No. of mature embryos cultured	No. of mature embryos which formed buds	Per cent
SH	0	42	0	0
	1	40	16	40
	2	40	12	30
	3	40	18	45
	5	38	8	21
DCR	0	40	0	0
	1	40	10	25
	2	40	8	20
	3	40	13	33
	5	40	4	10



Figures 1–3. 1. Induction of adventitious buds (ab) in *P. sylvestris* mature embryos; **2.** Development of *P. sylvestris* adventitious buds (ab) into adventitious shoots (as), and **3.** Root induction in a regenerated *P. sylvestris* shoot.

whole embryos after 4–5 weeks on DCR and SH media containing BAP (1–5 mg/l) (figure 1). Similar results have been obtained with isolated whole embryos of *Pinus brutia*[3]. The highest induction of adventitious buds was observed in 45% of the cultured embryos on SH medium supplemented with 3 mg/l BAP (table 1) as compared to 33% on DCR medium containing the same amount of BAP. SH medium contains a high concentration of potassium and the beneficial effects of this element on organogenesis are well-recognized[8]. The level of calcium and its ratio to other macro-elements is also important for organogenesis[9].

Table 2 *Shoot induction from cultured adventitious buds of Pinus sylvestris after 4 weeks on SH medium supplemented with BAP*

Conc. of BAP (mg/l)	No. of adventitious buds which formed shoots	Per cent
0	28	70
0.1	22	55
0.5	16	40
1.0	11	28
2.0	7	18

The number of adventitious buds cultured in all cases was 40.

The browning of adventitious buds was observed inhibiting adventitious shoot formation. To prevent this, frequent subculturing on a fresh medium was necessary every 5–6 days.

Shoot and root regeneration: Adventitious buds were individually isolated once and cultured on SH medium containing BAP (0–2 mg/l) for shoot development. Shoot elongation was observed in 70% of the buds on SH medium with no hormonal supplement (table 2). Shoot elongation started from adventitious buds after 2 weeks (figure 2).

For the induction of roots, well-developed shoots were cultured on SH medium containing 0.1 mg/l NAA. Root initiation was observed after 2 weeks (figure 3). Fifty per cent of the shoots developed roots. Ishii[10] induced rooting in about 70% of shoots developed from adventitious buds of *Chamaecyparis obtusa*.

Conclusions: 1) SH medium is better than DCR medium for the induction of adventitious buds. 2) Shoot elongation from adventitious buds is highest on SH medium without growth hormones. 3) Root induction in the regenerated shoots is achieved on SH medium. 4) Direct adventitious bud induction, without formation of callus, may be applicable for clonal propagation of forest trees. However, more work is needed to study different types of variation among the regenerated plantlets.

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INFLUENCE OF CARBON, NITROGEN AND PHOSPHORUS NUTRITION ON GROWTH OF *DIOSCOREA DELTOIDEA* CALLUS AND PRODUCTION OF DIOSGENIN AND STEROLS

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THE influence of nutritional factors on the growth of plant cells and secondary product formation has been a subject of intense study[1,2]. It is now realized that a separate media need to be devised for the growth of plant cells and metabolite production. The importance of carbon-to-phosphate ratio, in the nutrient medium, on secondary metabolite formation in microbes is well known[3,4]. However, only limited attention has been paid towards this aspect in plant cell cultures[2].

In this communication we report the influence of carbon (C), nitrogen (N) and phosphorus (P) nutrition on the growth and diosgenin production in callus tissues of *D. deltoidea*.

Seeds of *D. deltoidea* collected from the Kashmir valley were aseptically germinated. The callus was initiated from hypocotyls of 10-day-old seedlings on modified MS medium[5] supplemented with 1 mg/l 2,4-D and 3% sucrose and maintained on the same medium by regular subcultures of 45 days interval. Cultures were incubated at 25 ± 2°C in continuous light (3000 Lux).

The growth of callus was measured in terms of dry weight, after drying the tissue in hot air oven at 60°C to constant weight.

The harvested tissue was dried and powdered. One gram dry powder was soxhlet-extracted with petroleum ether (30–50°) for 24 h. The extract was evaporated and examined for free sterols[6]. The residual cells were hydrolysed by refluxing with 10% HCl for 2 h, cooled and filtered. The residue was washed first with water and then with 0.1 N NaOH and finally with water to neutrality. The residue was then dried at 60°C, powdered and soxhlet-extracted with $CHCl_3$ for 48 h. The $CHCl_3$ extract was evaporated and the diosgenin and bound sterols were quantified by comparing OD values with the standard curve constructed by preparative TLC method[7].

It is clear from figures 1A,B that diosgenin synthesis in *D. deltoidea* callus is restricted to the stationary phase of growth. Hence, the growth and