VEGETATIVE PROPAGATION OF SCOTS PINE (PINUS SYLVESTRIS L.) THROUGH TISSUE CULTURE

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Abstract.--A pulse treatment with a high-concentration NAA solution (125 mg/L) not only enhanced rooting up to 33% but also increased the number (up to 10 roots/propagule) and size of roots (2mm in diameter) in cultured Scots pine adventitious shoots. This induced multiple roots system should increase the vigor of regenerated plantlets, and hence, shorten the adaptation period while being transferred to soil.

Additional keywords: Adventitious buds, adventitious roots, pulse treatment, seedling, embryonic cotyledons.

Tissue culture methods have been evaluated for Scots pine by several research groups. Tranvan (8) studied the formation, localization of adventitious buds on seedlings, and the initiation of cotyledon adventitious buds. Bornman and Jansson (2) attempted to increase the rooting percentage of four types of explants by applying the growth-active compound, coumarin, alone or in combination with auxin. Shen and Arnold (7) completed the culture sequences to regenerate plantlets from cultured embryonic tissue with an overall regeneration rate of 10% over a 10-month period.

This experiment was directed to improve the survival rate at elongating stage of adventitious buds and then to promote the rooting percentage of those elongated adventitious shoots to provide mass, clonal propagules for improvement of a Christmas tree program.

METHODS

Scots pine seeds of Central Massif were chosen from germination tests as experimental material from among twelve varieties purchased from F.W. Schumacher Co.

Two types of explants were established from seeds by embryo culture. Seeds were pretreated with 1% H_2O_2 for 1 week to facilitate the removal of seed coats and to stimulate germination (6). After the removal of seed coats, seeds were surface-disinfected with 1/6-strength Clorox solution for 15 min and then rinsed with autoclaved, distilled water. Embryos were aseptically separated from endosperms and planted on culture media. Seedlings were produced by growing the embryos on 1/3-strength M.S. minimal organic medium (5), and cotyledonous adventitious buds were initiated on 1/3-strength M.S."B" medium with supplements of 1.0 mg/L kinetin and 30 g/L sucrose. The whorl of cotyledon with a stub of subtended hypocotyl was excised from 2- to 4-week-old seedlings and planted on the M.S."B"

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medium described above (Fig. 1A).

Four adventitious shoots, in addition to the apical shoot, were formed on the basal area of the cotyledon whorl (Fig. 1B). Those shoot bundles were separated from the mother explant at 2- to 3-month intervals and subcultured on M.S."B" medium for continuous multiplication. Cotyledonous adventitious buds were transferred to 1/6-strength M.S."B" medium to encourage the elongation of adventitious buds into shoots. Elongated shoots with a visible stem region were suitable to be rooted (4). Each of two chemical (adenine sulfate at 66 mg/L concentration and coumarin at 1.46 mg/L concentration) was incorporated into the basal medium (1/6-strength M.S."B" medium) separately to test for effect on rooting. Finally, a pulse treatment with a high concentration NAA solution (125 mg/L) was applied for 24 h before transferring the shoots to medium free of growth regulator (1, 3).

RESULTS AND DISCUSSION

Adventitious shoots were produced in two ways: 1) The cotyledon whorl excised from 2- to 4-week-old seedling readily produced four adventitious shoots on the basal area when it was planted on 1/3-strength M.S."B" medium with supplements of 1.0 mg/L kinetin and 30 g/L sucrose; 2) The adventitious buds initiated on embryonic cotyledons were transferred to 1/6-strength M.S."B" medium to encourage the elongation of buds into shoots. Those adventitious shoots developed on the basal area of the cotyledon whorl can be excised from mother explant at 2- to 3-month intervals and cultured on the same medium for continuous multiplication, or they are ready to be rooted.

The adventitious buds initiated on embryonic cotyledons were numerous, but the elongation of those adventitious buds was sporadic. Efforts to stimulate the growth of adventitious buds have been without much success so far. Increase in boron concentration to minimize the phenolic compound synthesis in order to prevent the stunting of the adventitious buds might be tried in further experiments.

Adenine sulfate and coumarin incorporation in culture medium did not show any significant effect over the control. But, those pretreated adventitious shoots responded quickly to pulse treatment with high-concentration NAA solution. In 3 months, ten adventitious shoots (33%) produced root primordium with four of them having actual root protrusion and root growth. The NAA solution pulse treatment not only increased the number of rooted propagules, but also increased the number and size of root formed in the individual propagule (Fig. 2A, 2B). A single root is typical in cultured pine tissue (6), but the above 8-month culture sequences resulted in an increase in number (up to 10 roots/propagule) and size (2 mm in diameter) of rooting which should increase the vigor of the regenerated plantlets, and hence, shorten the adaptation period when being transferred to soil. One regenerated plantlet from previous culture without the pulse treatment has been transferred to vermiculite for one year (Fig. 1D). A single rooting of 7 cm long was observed when being transferred to soil recently (Fig. 1C).

CONCLUSIONS

Before the survival rate at the elongation stage of cotyledonous adventitious buds can be increased or the embryogenesis method of Scots pine callus can be developed, the most practical way for vegetative propagation of Scots pine is through induction of adventitious shoots on the cotyledon whorl and rooting of those adventitious shoots with NAA solution pulse treatment.

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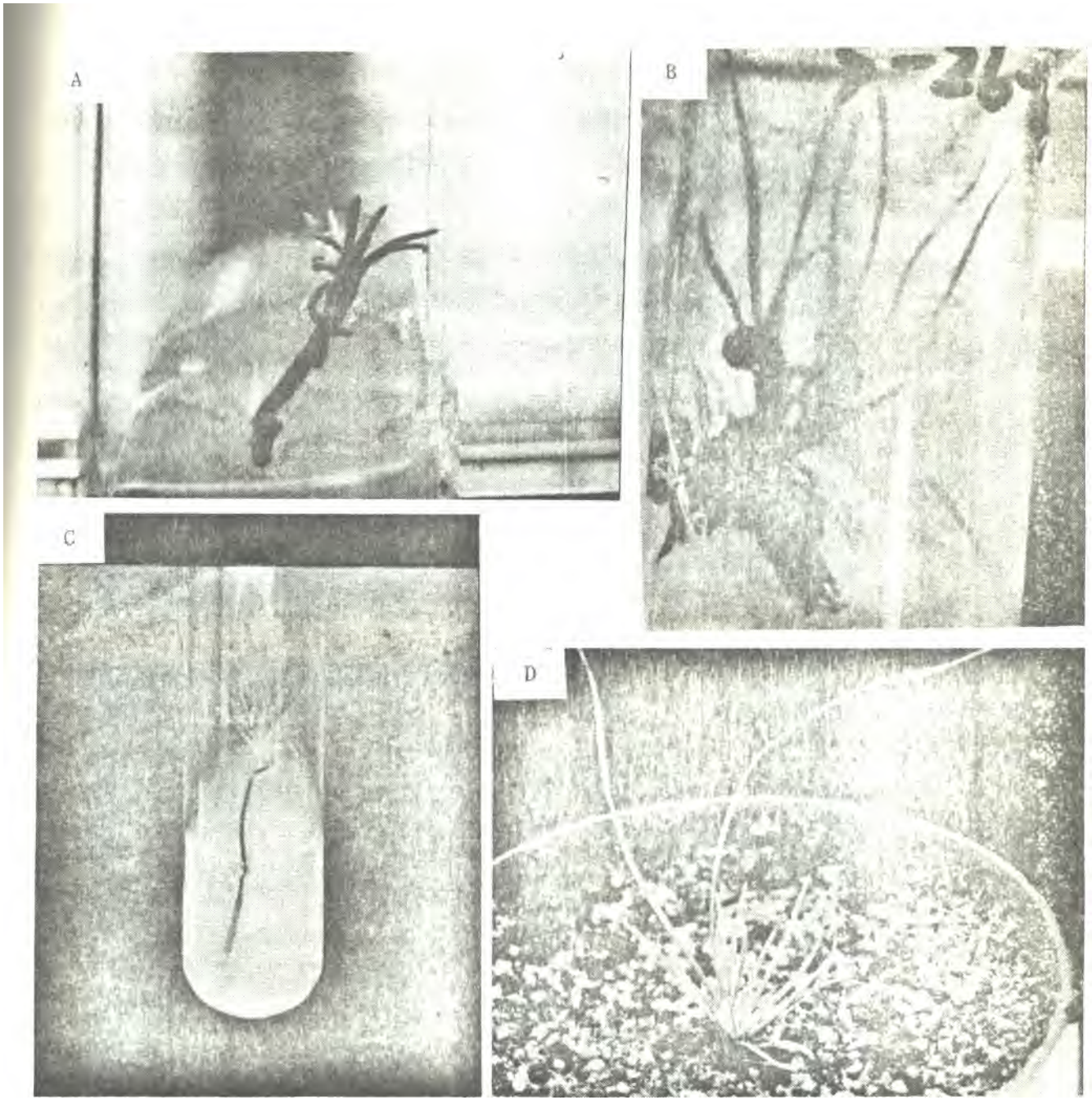

Fig. 1 (A) 4-week-old seedling from which apical slices were excised. Apical slices included the base of the cotyledonary whorl subtended by a stub of 2mm hypocotyl.

(B) Adventitious buds induced from apical slices after 4-week culture

(C) Plantlets regenerated in vitro after one month in the rooting medium.

(D) Plantlets potted in vermiculite.

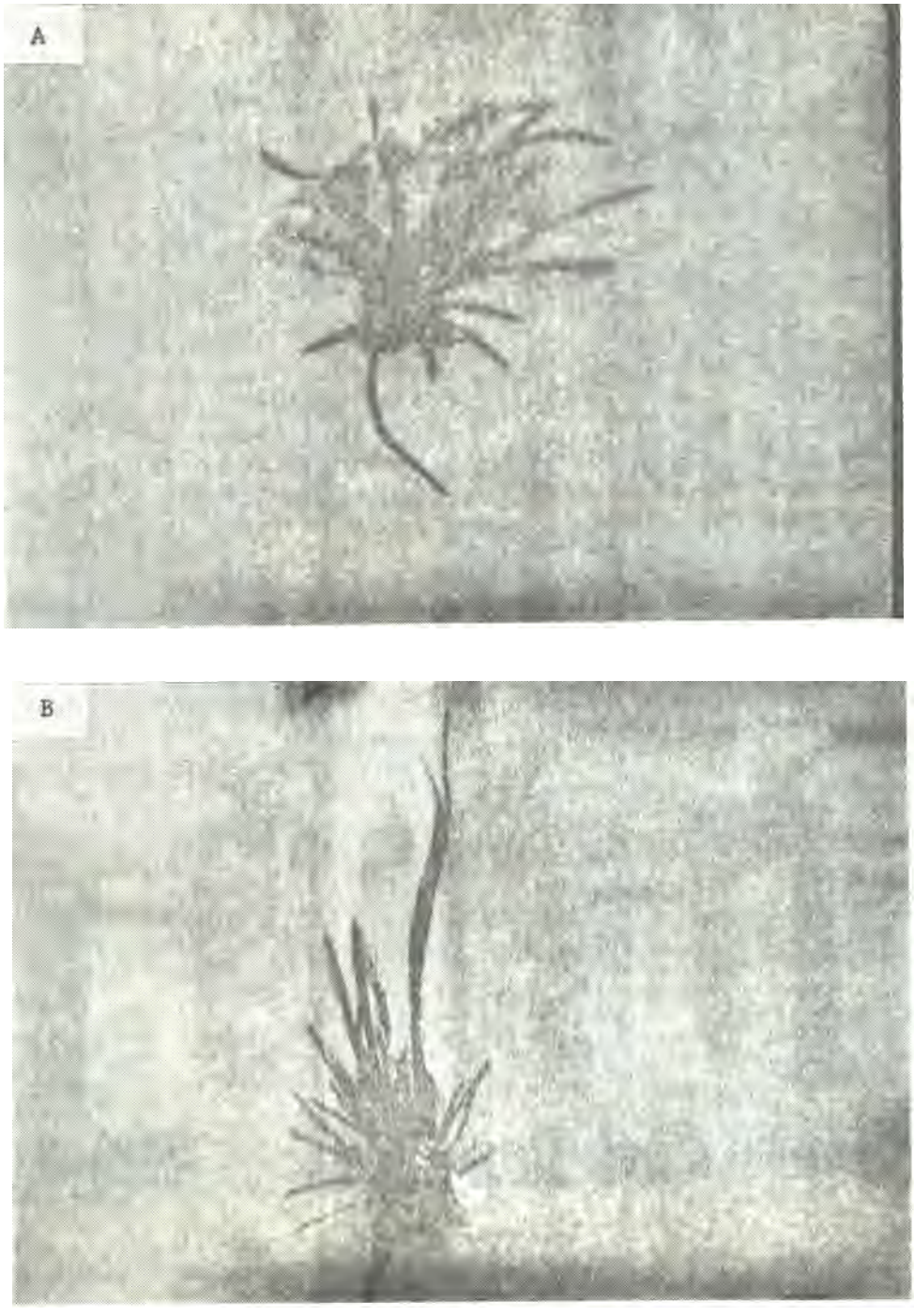

Fig. 2 (A) 10 roots per plantlet

(B) 2 mm in diameter root