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The in vitro Proliferation of Forest Trees

1. Dalbergia sissoo Roxb. ex Dc

By B. SUWAL, A. KARKI[1]) and S. B. RAJBHANDARY[2])

Tissue culture Laboratory, Royal Botanical Garden,
Godawary, Kathmandu, Nepal

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Abstract

Multiple shoots were induced on cotyledonary node culture of *Dalbergia sissoo* in the presence of benzylaminopurine (BAP) at 1 mg/l and naphtaleneacetic acid (NAA) at 0.1 mg/l. These shoots continued to proliferate at a sustained rate of 10—15 microshoots over two years of 8 weekly subcultures in the basic medium, with the supplement of BAP at 0.25 mg/l. Such microshoots rooted readily in non-sterile sand beds with subsequent successful field establishment.

Key words: Dalbergia sissoo, cotyledonary culture, multiple shoots non-sterile rooting, field establishment.

Zusammenfassung

Bei Kotyledonen-Nodien-Kulturen von *Dalbergia sissoo* wurden durch die Anwendung von Benzylaminopurin (BAP) (1 mg/l) und Napthalenessigsäure (NAA) (0,1 mg/l) multiple Sprosse induziert. Diese Sprosse pflanzten sich weiterhin mit einer ununterbrochenen Rate von 10—15 Sprossen über 2 Jahre fort, wobei die Subkulturen 8 Wochen dauern, bei Verwendung des Basismediums unter Zugabe von 0,25 mg/l BAP. Solche Mikrosprosse wurden in nicht sterilem Sand leicht bewurzelt und erfolgreich in Feldversuche ausgebracht.

Introduction

Dalbergia sissoo is a multipurpose native species which is extensively used in afforestation in the Nepal Terai for production of fodder, fuel and timber. Since there is considerable phenotypic variation between trees in provenance trials of *D. sissoo* raised from seed, genetic improvement will depend on the selection of individual trees with superior characteristics and propagation of these by clonal means. However, cloning of superior material through the use of conventional vegetative cuttings resulted in uneven growth, as only tip-cuttings gave rise to straight-growing plants, whereas branch cuttings produced trees which retained their characteristics as branches (plagiotrophic growth) (K. WHITE, personal communication). Moreover only 25 percent of the cuttings were successfully rooted (SINGH, 1982). An alternative to vegetative propagation by cuttings is to use the tissue culture method of multiplying clones using meristem or shoot-tips or buds as the explants.

To develop a process for cloning elite candidate trees of *Dalbergia sissoo* it was thought convenient first to devise a method of producing tissue culture plants using explants from seedlings grown in culture. Once a successful proliferation medium has been established it can then easily be tested for cloning superior individuals through meristem or shoot tip or bud culture.

We report here the shoot proliferation, followed by non-sterile rooting of these shoots, and subsequently field estab-

Present address:

[1]) Department of Botany, University of Nottingham, School of Biological Sciences, University Park, Nottingham, U. K.

[2]) Department of Medicinal Plants, Thapathali, Kathmandu, Nepal

lishment of rooted plants, by culturing *D. sissoo* cotyledons excised from *in vitro* grown seedlings.

Materials and Methods

Seeds of *D. sissoo* were obtained from the Afforestation Division, Hattisar, Kathmandu. The seeds were kept in running water for one hour, followed by a brief dip in water containing teepol, at the rate of two drops of teepol in 100 ml of water. The seeds were then washed five times in distilled water. Finally they were sterilized in 0.1 percent $HgCl_2$ solution for 20 minutes, and subsequently washed five times in sterilized distilled water. The seeds were transferred to culture flasks (100 ml conical flasks containing 40 ml solidified medium), when the seedlings germinated within four days. Cotyledons excised from the seedlings were cultured on MURASHIGE and SKOOG medium (1962) (MS), with the addition of benzylaminopurine (BAP) at 1.0 mg/l and naphtaleneacetic acid (NAA) at 0.1 mg/l, and 1000 mg/l caseinhydrolysate. The medium was solidified with 0.6 percent bacteriological agar with pH adjusted to 5.8 before sterilization. It was sterilized by autoclaving at 15 lb/sq. in for 15 minutes. The cultures were incubated at 25 ± 4^0 C under a 16 h photoperiod, with light provided by fluorescent tubes at ca 3000 lux.

Under these conditions 2—5 shoots developed from the nodal region of the cotyledon after seven days. These shoots were used in a number of experiments using different concentrations of BAP (0.01 to 10 mg/l) and kinetin (2—4 mg/l), either alone or in combination with NAA (0.01—0.1 mg/l). The cytokinin concentration that supported a high rate of shoot proliferation was selected for further subculture.

The sterile rooting step was skipped, and the multiplied shoots were separated out treated in 0.01 percent indoleacetic acid for 5 minutes and transplanted, through the year 1985, to sand beds in a greenhouse. To facilitate rooting relative humidity was maintained at 70—80 percent.

Rooted plants, 10—20 cm high, were transplanted into the soil in the greenhouse, followed by planting out in the field.

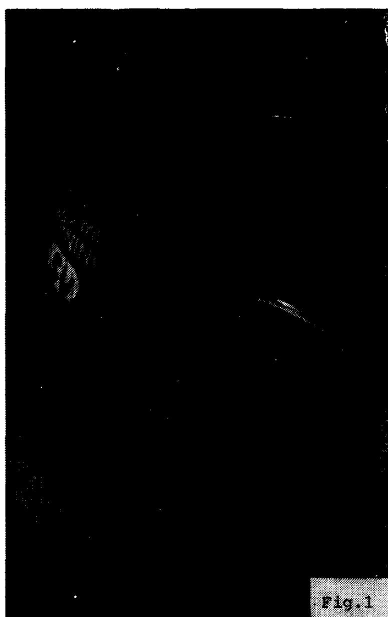

Fig. 1. — Development of shoots from cotyledonery node on MS supplemented with 1 mg/l BAP + 0.1 mg/l NAA, and 1000 mg/l caseinhydrolysate.

Fig. 2. — Multiple shoot formation on MS supplemented with 0.25 mg/l BAP and 1000 mg/l caseinhydrolysate. Eight weeks in culture.

Results

When the multiple shoots developed on cotyledonary node *(Fig. 1)* were transferred to the media supplemented with both BAP at 10—0.01 mg/l and NAA at 0.1 mg/l, callus formation increased with the increase in BAP concentration. At the higher concentrations of BAP (2—10 mg/l), with 0.1 mg/l NAA, brown callus tissues were formed on the parts of the explant in contact with the medium and no development of shoots occurred. BAP at or below 1.0 mg/l with NAA at 0.1 mg/l induced the formation of callus as well as shoots and roots. With 0.01 mg/l NAA, BAP at 1.0 mg/l or below this level also produced callus, shoots, and roots.

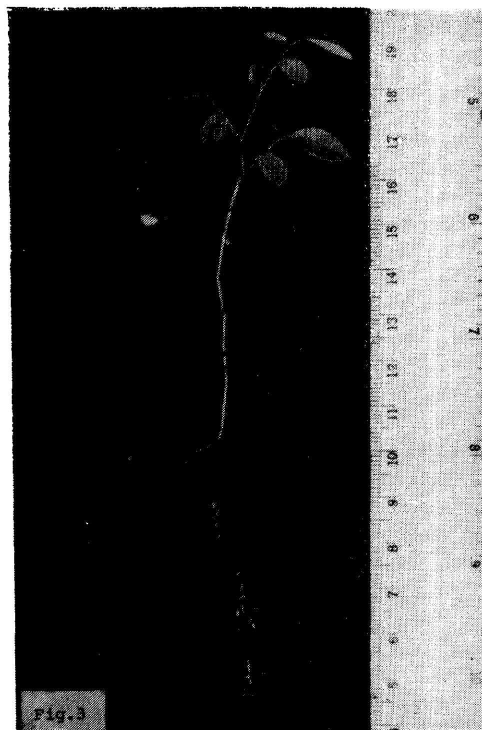

Fig. 3. — Rooted plantlets after 4 weeks in the sandbeds.

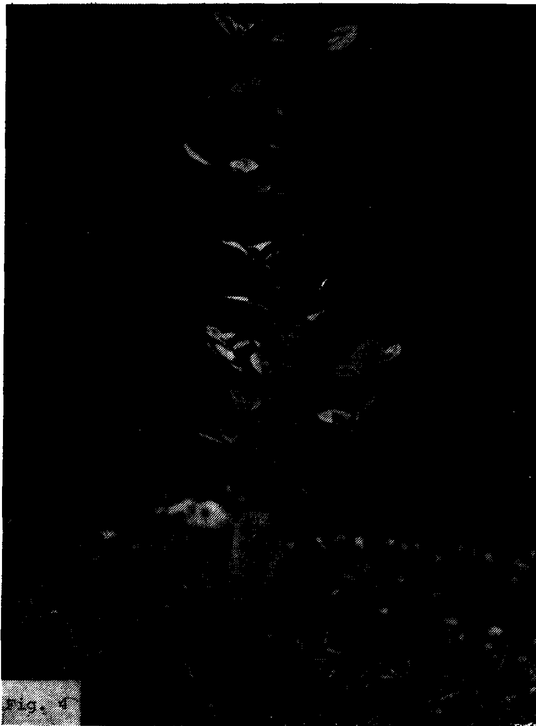

Fig. 4. — Field established plant. Growth of plant in 12 weeks after the transfer.

BAP at 1.0, 0.5, 0.25, and 0.12 mg/l induced the formation and development of shoots. The number of elongating shoots was always higher on the medium containing 0.25 mg/l BAP concentrations. The multiplication rate was 10—15 shoots (0.5—6 cm high) per explant after four weeks of culture in presence of 0.25 mg/l BAP. The shoots have been subcultured for two year without any loss of multiplication potential (*Fig. 2*).

Kinetin at 2—4 mg/l alone or in combination with NAA at 0.1 or 0.01 mg/l gave rise to only callus tissues.

On the transfer of *in vitro* produced shoots to sand beds over 85 percent developed into rooted plantlets within 10 days, under day and night temperatures of 34° C/15° C under 70—80 percent humidity. The rooted plantlets grew to a height of 10—15 cm in four weeks (*Fig. 3*). Such plantlets were transferred to soil in pots and subsequently planted out in the field. All the plants grew straight, without any tendency to horizontal growth (*Fig. 4*).

Discussion

Cotyledons have been reported to be the most regenerative part of the plant (MURASHIGE, 1974). Of 25 legume species that have been regenerated *in vitro* in five, *Ceratonia siliqua, Indigofera enneaphylla, Psophocarpus tetragonolobus, Stylosanthes hamata* and *Trifolium repens* cotyledons were used as explants (FLICK *et al.*, 1983). CHANG *et al.* (1980) reported multiple shoot bud formation of soybean from cotyledonary node culture. USHA MEHTA and MOHAN RAM (1980) observed fasciated shoot bud development at the cotyledonary node culture of *Cajanus cajan*. Our results corroborate the findings of the above workers.

In vitro micropropagation of *Dalbergia sissoo* through axillary bud proliferation was easily accomplished using excised cotyledons as explants. Therefore it is reasonable to assume that the procedure developed in the present work has possibilities in the cloning of elite genotypes of *D. sissoo* from meristem or shoot tip or bud cultures.

The fact that it was possible to omit the sterile rooting stage (KARKI and RAJBHANDRY, 1984), and the successful rooting of over 85 percent of the shoot cuttings in non-sterile sand beds, suggests the commercial applicability of using this method in large scale plantations of *D. sissoo*. MUKHOPADHYA and MOHAN RAM (1981) reported the formation of 2 or 3 plantlets in 30—45 days from root culture of *D. sissoo*. Their method involved the use of aseptic cultures in both the shoot induction and rooting stages. This would, therefore, limit the possibility of using this method in the large scale production of *D. sissoo* plants.

Since no loss of multiplication potential in *D. sissoo* shoot culture was observed in over two years of 8 weekly subcultures, production of more than a million *D. sissoo* plants from a single plant in a year is apparently feasible by use of the present method. Our observations are consistent with the suggestion by BONGA (1977), that the solution to the problem of producing large number of trees of improved qualities on shortened rotations could possibly be obtained through the use of micropropagation techniques.

However, despite the great advantages offered by tissue culture propagation methods, to date only a few species in the genera *Populus, Eucalyptus, Betula, Liquidambar, Acacia, Ulmus, Castanea, Tectona, Santalum*, and *Alnus*, have been micropropagated (PERINET and LALONDE, 1983).

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