In vitro differentiation of plantlets from tissue cultures of *Albizzia lebbeck* L.

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(Revision received 6 December, 1982)

Key words: *Albizzia lebbeck*, shoot differentiation, root differentiation, plantlet formation

Abstract. Attempts at inducing differentiation in various explants of *Albizzia lebbeck* resulted in the production of abundant shoot buds from the hypocotyl, root, cotyledon and leaflet explants, both directly and indirectly (i.e. without and with the intervention of callus formation). Rooting was achieved on transfer of the shoots to BM + 2 mg/l IAA after some growth. The plants could be successfully transferred to soil, providing a method for mass propagation of this important leguminous tree species.

Introduction

In recent years, culture of plant cells and tissues has gained considerable importance as a promising tool in improving and multiplying economically important crop plants [7, 9, 12]. The legumes are of considerable value, but so far work on them has been restricted to the herbaceous seed and forage crops; the woody species which comprise some well-known ornamental, medicinal and timber crops of the subtropics have generally been neglected.

In the present communication we report the *in vitro* differentiation of plantlets through organogenesis in *Albizzia lebbeck*, an important leguminous timber tree popularly known as the "East Indian Walnut". Differentiation of plantlets via somatic embryogenesis has been reported earlier by us [3].

Material and methods

Seeds of *Albizzia lebbeck* were obtained from Professor R.S. Mehrotra at the University of Kurukshetra as well as from the Old Delhi Ridge. After soaking in tap water for 8–10 h, they were surface-sterilized by chlorine-water, and, after washing with sterile water, grown *in vitro* under a light intensity of 750 lux on B_5 basal medium (BM [2]). The source of iron was supplied by 5×10^{-5} M $FeSO_4$ and 5×10^{-5} M Na_2EDTA and the medium was gelled with 0.9% Difco Bacto-agar. The pH was adjusted to 5.8 before autoclaving. Different concentrations – approximately in the range of 10^{-6} to 2×10^{-5} M – of indole-3-acetic acid (IAA), α-naphthaleneacetic acid (NAA),

Plant Cell Tissue Organ Culture 2: 49–53 (1983).

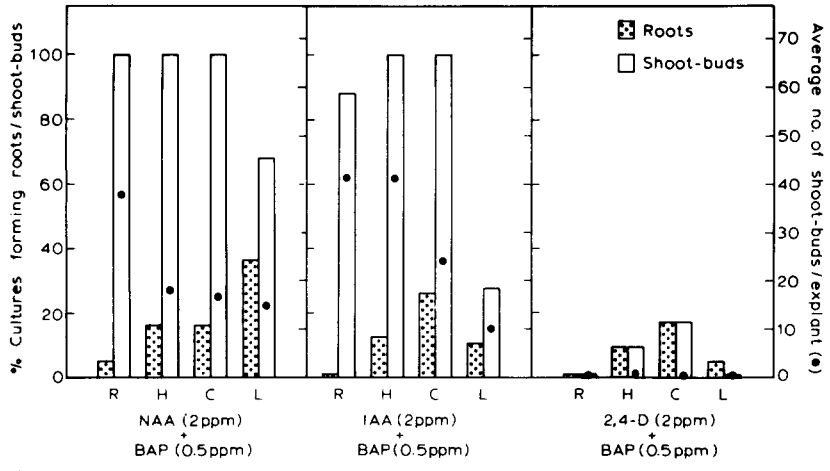

Figure 1. Effect of various auxins (NAA, IAA and 2,4-D) together with BAP on differentiation in tissue cultures of *Albizzia lebbeck*. The histograms represent the mean values of percentage cultures forming shoots and roots in two replicate experiments with a minimum of 12 cultures raised per experiment. The average number of shoot buds formed per explant was scored after 45 days of culture and is indicated by black dots.

2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BAP) were added to BM in various combinations. Seedlings, 10–15 days old, were used in all experiments. A minimum of 12 cultures with 1 or 2 explants per culture were evaluated in each treatment. The explant size was ca. 1 cm from various regions of the *in vitro* grown seedling i.e. roots, hypocotyls and leaves (segments of the rachis each bearing 4 leaflets). Cotyledons, which were also ca. 1 cm long, were split longitudinally into two halves before inoculation. Culture were routinely maintained for a period of 8 weeks, though the data presented were scored after 6 weeks.

Results

Of the various explants cultured, only the hypocotyl explants showed differentiation of somatic embryos and plantlets on BM [3]. The failure of the other explants (roots, cotyledons and leaflets) to differentiate on BM, thus, led to further trials i.e. experimentation with media containing both a cytokinin and an auxin. Callusing as well as differentiation were observed. Callusing occurred in practically all explants cultured in the various media (only the root explants showed lower response). However, formation of shoot buds required more exacting conditions; the maximal response was when the exogenous auxin level was approximately 10^{-5} M (2 mg/l) and that of the cytokinin 5×10^{-6} M (0.5 mg/l). NAA and IAA were more effective than 2,4-D. Shoot buds from the various explants originated via two modes of differentiation, i.e. directly, from the explant without any callus formation, and, indirectly, with the intervention of a phase of callus formation. The results with each type of explant are given in Figure 1. Since the data were

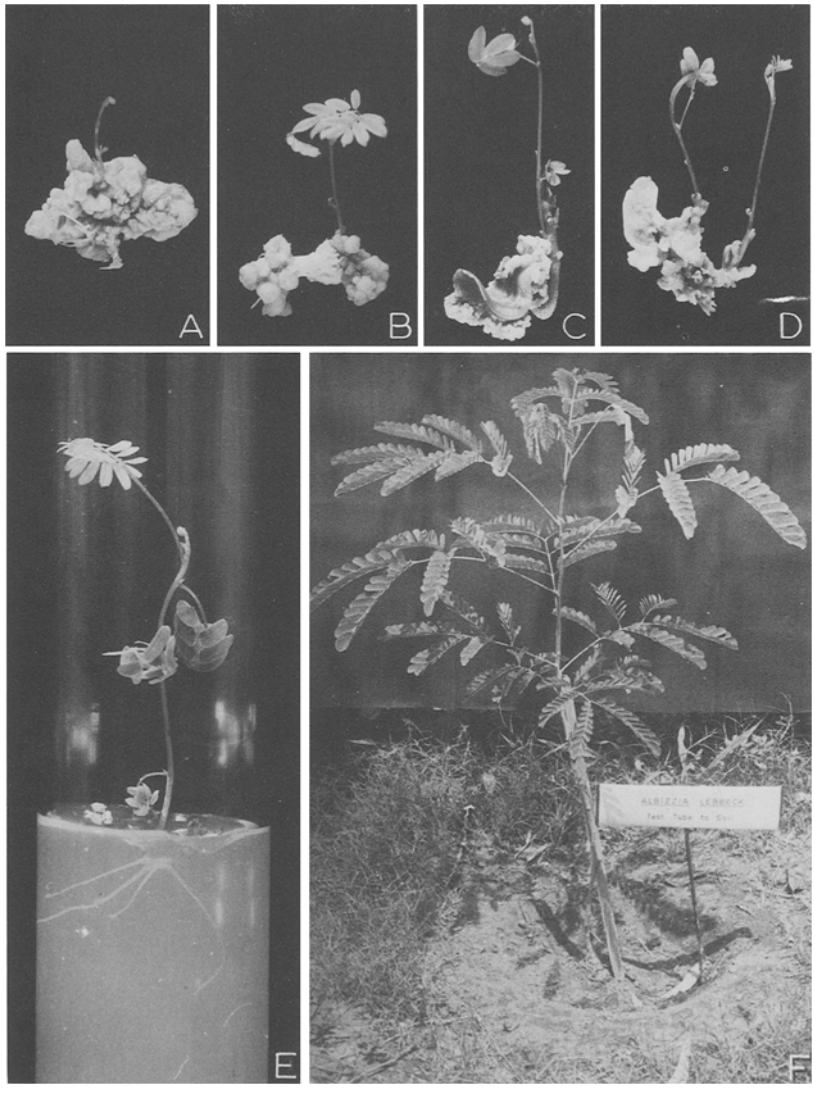

Figure 2. A–F. Differentiation of plantlets in tissue cultures of *Albizzia lebbeck* via organogenesis and their successful transfer to soil.
A–D: Development of shoot buds from root, hypocotyl, cotyledon and leaflet explants respectively, on BM + IAA (2 ppm) + BAP (0.5 ppm).
E: Differentiation of roots from shoot implanted on BM after 15 days of culture.
F: A one-year-old plantlet, produced through tissue culture, after transfer to field conditions.

taken after 6 weeks (a period which is long enough for organogenesis to occur in calli), they represent the sum total of roots and shoot-buds arising both directly as well as indirectly.

Transfer of well-developed shoots from the various explants (Figure 2A–D)

to BM or BM + 2 mg/l IAA resulted in rooting in 45–50% of the cultures after two weeks (Figure 2E). With time, the percentage rises to 80. After 2 months, the plantlets with well developed roots were transferred to pots containing garden soil and subsequently to the field (Figure 2F).

In addition, keeping in mind that the species under investigation is an important tree species of India, culture of apical meristem (obtained from seedlings grown *in vitro*) was also tried with the viewpoint of developing a clonal propagation programme. Such meristems (about 1 cm from the tip), on aseptic transfer to BM or BM + 2 mg/l IAA, readily produced vigorous plantlets with roots in 85% of the cultures.

Discussion

Despite the widespread occurrence of somatic embryogenesis as well as differentiation of shoot-buds [6, 7, 9, 12], comparatively little success has been gained in inducing them in the more important crop plants such as cereals and legumes [1, 5, 10]. From the viewpoint of the tree improvement programme, tissue culture studies on legumes are of much potential value, and it is in this connection that our results on *A. lebbeck* are of special interest. Earlier, three leguminous tree species (*Acacia koa, Prosopis cineraria* and *Dalbergia sissoo*) have been studied, but only organogenesis has been reported [11, 4, 8]. Further, because of the fact that in our studies, organogenesis and embryogenesis have been obtained in the same plant, it is possible that *A. lebbeck* could serve also as a useful system for fundamental studies on the control of the two basic modes of differentiation in plant tissue cultures.

Acknowledgements

Our grateful thanks are due to Dr. A. Rashid for helpful suggestions. Financial support by the Department of Science and Technology, in the form of a research scheme to SCM, and by the University Grants Commission, in the form of a Research Fellowship to PKG, are gratefully acknowledged.

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