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Differentiation in explants from mature leguminous trees

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ABSTRACT

Stem and petiole explants, obtained from mature trees, of Albizzia lebbeck, Cassia fistula and C. siamea callused and differentiated shoot-buds and later shoots on B5 medium supplemented with either 0.5 mg/l IAA + 1 mg/l BAP or BM + 2 mg/l NAA + 0.5 mg/l BAP. The stem explants were more responsive than the petiole explants. In A. lebbeck, the IAA substituted medium favoured differentiation from both types of explants. However, in C. fistula, the type of explants rather than the medium composition had an overriding influence on shoot differentiation since those from petiole hardly responded in either medium. It has been possible to obtain plantlets from both A. lebbeck and C. fistula under conditions conducive to rooting. Plantlets of A. lebbeck have also been successfully transferred to the field.

INTRODUCTION

Micropropagation of tree species is being increasingly recognized as a tool with much potential for applications in the field of forestry. It offers a rapid means of afforestation, multiplying woody biomass, and of conserving elite and rare germplasm (Winton 1978, Mott 1981, Bonga and Durzan 1982, Bajaj 1986). However, in this group of plants, young seedling and juvenile tissues are generally manipulated far more easily than the hard, mature tissues. In this context, we now report differentiation in explants obtained from mature trees of three leguminous species, namely Albizzia lebbeck (the 'East Indian Walnut'), Cassia fistula and C. siamea (two ornamental avenue trees).

MATERIALS AND METHODS

Source of Explants

Explants from Albizzia lebbeck, Cassia siamea and C. fistula were obtained from mature trees growing in and around the main university campus. However, for any one plant species, all explants were obtained from a particular adult tree during

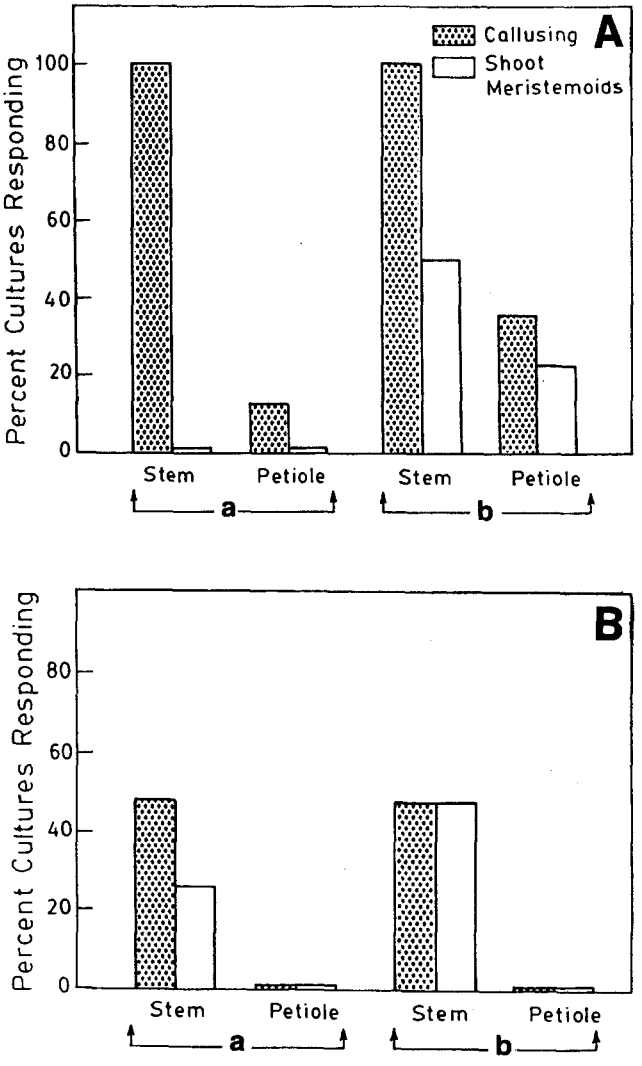

Fig 1. A & B. Induction of callusing and organogenesis from stem and petiole explants of (A) Albizzia lebbeck and (B) Cassia fistula on (a) BM + 2 mg/l NAA + 0.5 mg/l BAP, and (b) BM + 0.5 mg/l IAA + 1 mg/l BAP.

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the first flush of 'spring growth'. The explants (from stems and petioles) were first treated with a dilute detergent solution and then washed thoroughly by leaving them under running tap water for about a hour. The explants were then cut into ca. 4 cm long segments and treated with freshly prepared chlorine water for 30-45 minutes. Subsequently they were washed three times with sterile distilled water and prior to inoculation, the exposed ends were trimmed of leaving ca. 1 cm long pieces.

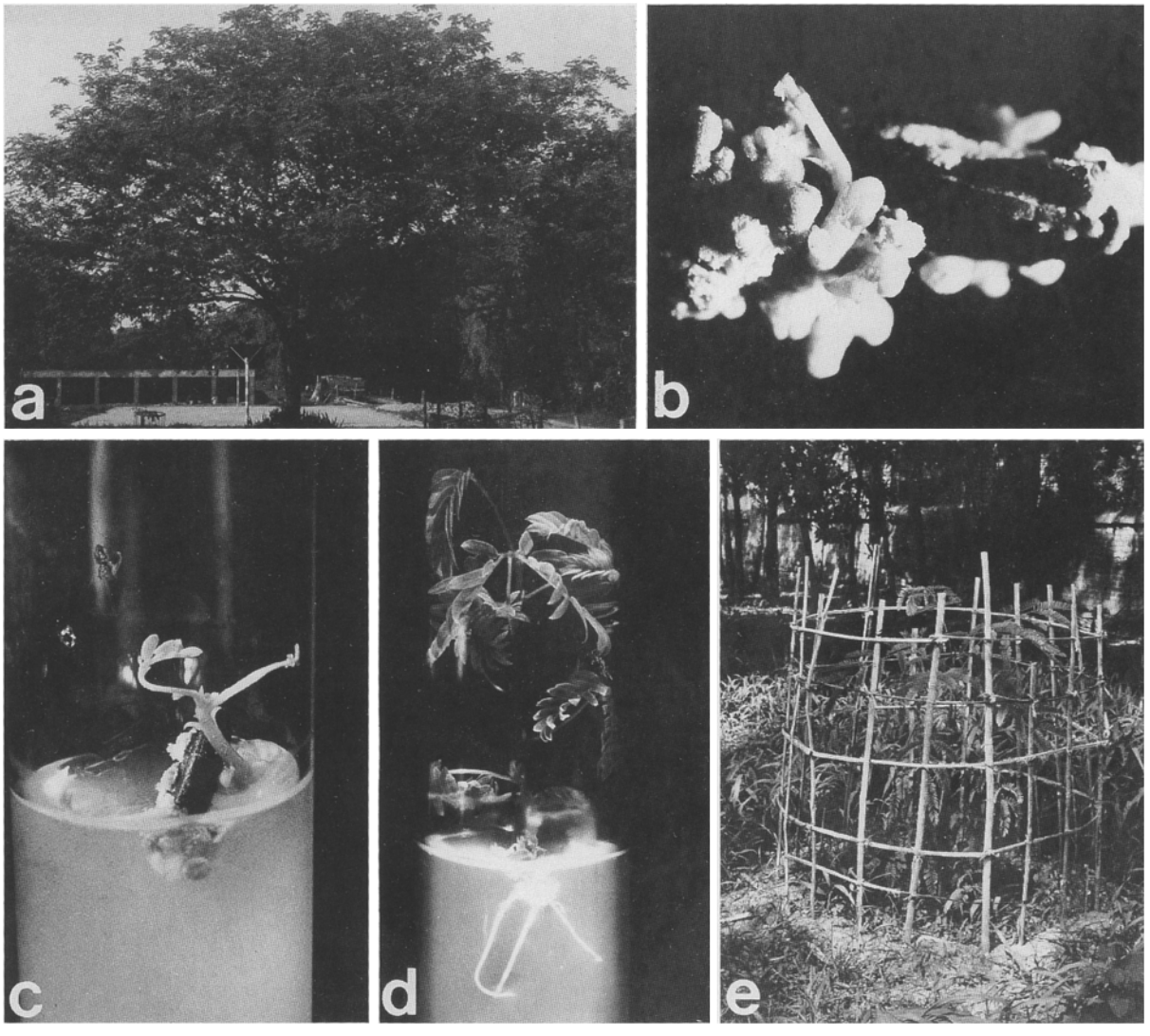
Culture Medium and Cultural Conditions

For induction of morphogenesis and plant regeneration, the B5 medium of Gamborg et al. (1968) was employed. However, iron was supplied through a combination of $FeSO_4$ and Na_2EDTA rather than FeEDTA. Sucrose at 2% concentration was used as the carbohydrate source. The medium was solidified with 0.8% Difco Bacto-agar and the pH of the medium was adjusted to 5.8 by NaOH or HCl before autoclaving for 15 min at 1.08 Kg/cm^2. All cultures were maintained under a daily photoperiod of 16 hrs light and 8 hrs darkness. The temperature was maintained at $27\pm1^{o}C$ during light hours and $25\pm1^{o}C$ during darkness. Light was provided by a bank of mixed cool-white daylight fluorescent tubes (Philips TL 65-90 W/54 TLF 40 W/54) and incandescent lamps (Philips Argenta, 100 W). The light intensity varied from 750 to 1000 lux.

Transfer of Plantlets to Field Conditions

Plantlets with well-developed root-system were maintained in the original culture tube for about two months for 'hardening'. When the medium had dried considerably, plantlets were removed and washed thoroughly under running tap water to free any adhering agar. These were subsequently transplanted in small plastic pots, and later to larger pots for 4-5 months prior to transplantation in the field.

Fig 2. a-e. Plantlets from mature tree explants in Albizzia lebbeck. a: The parent tree; b & c: Differentiation of shoots from stem (b) and petiole (c) explants on BM + 0.5 mg/1 IAA + 1 mg/1 BAP, 9.5 and 2x, respectively; d: a month-old plantlet from a stem explant on BM, 1.76x; e: 6-month old plantlet transferred to field.



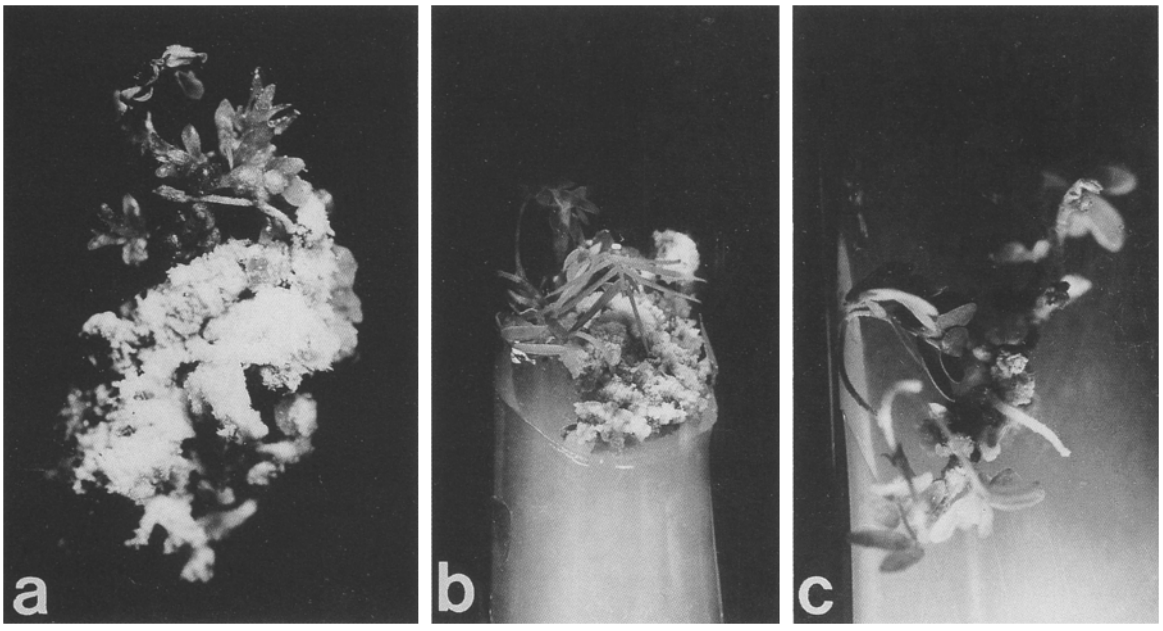


Fig. 3. a-c. Differentiation from mature tree explants of Cassia fistula. a & c: Shoot-buds (a) and shoots (b) from stem explants on BM + 0.5 mg/1 IAA + 1 mg/1 BAP; 3.6 and 2.12x, respectively, c: plantlet on BM + 0.5 mg/1 IAA, 3.4x.

RESULTS AND DISCUSSION

In continuation of our earlier work on embryogenesis, organogenesis and androgenesis of Albizzia lebbeck (Gharyal and Maheshwari 1981, 1983a, b, respectively), and our initial success with androgenesis in Cassia siamea (Gharyal et al. 1983), we now report organogenesis and plantlet formation from explants obtained from adult trees of both these genera.

In all three species, the explants were obtained during the first flush of 'spring growth', when the tissue is in an active phase of growth. The stem and the petiole explants were cultured on B5 basal medium supplemented with either 2 mg/1 NAA + 0.5 mg/1 BAP (medium a) or 0.5 mg/1 IAA + 1 mg/1 BAP (medium b). In A. lebbeck, on medium (a), 100 and 14% of the stem and petiole explants, respectively, callused (Fig. 2b), but rarely showed any signs of differentiation (Fig. 1Aa). On the other hand, on medium (b), 100% of the stem and 36% of petiole explants callused followed by differentiation of numerous shoot-buds over a two-month period (Figs 1Ab and 2c). The percentage of differentiation was 50% from callus masses obtained from stem and 36% from the petioles, and clearly higher than in medium a.

As described above for Albizzia, callus cultures of two species of Cassia, namely, C. fistula and C. siamea were similarly raised from stem and petiole explants of young sprouting shoots. In C. fistula, of the two types of explants inoculated, the stem explants were more responsive (Fig 1B). On both the media, about 50% stem explants callused within the first month of culture. The petiole explants produced a more friable and translucent callus than the stem explants. Clearly, stem explants were better for work, but since these browned and necrosed quite often, they had to be subcultured after a few weeks either on the same medium or on one supplemented with PVP/PVPP. Upon such frequent and serial subculture, green meristemoids (Fig 3a), were observed from 25% of the total stem explants on NAA and from as many as 55% explants on IAA supplemented medium (Fig 1B). On BM + 0.5 mg/1 IAA + 1 mg/1 BAP, the meristemoids developed into well-differentiated shoots (Fig 3b). However, the same response from the petiole explants was sporadic and low (Fig 1B).

In C. siamea too, on BM + 0.5 mg/1 IAA + 1 mg/1 BAP, frequent callusing was observed from both types of explants. But as in C. fistula, the calli turned brown after a month of growth and posed much difficulty in its maintenance during successive subcultures even on media supplemented with PVP/PVPP. Although browning was observed also on BM + 0.5 mg/1 IAA + 1 mg/1 BAP, the petiole and stem explants both developed green meristemoids. However, differentiation of shoots was observed only from the stem explants (data not presented).

Upon transfer of the shoots -- developed in vitro from the callus masses mentioned above -- to either BM or BM + 0.1 mg/1 IAA, differentiation of roots was also observed (see Figs 2d and 3c). Plantlets thus observed were normal in all respects and were even successfully transferred to the field (Fig 2e).

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