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Plant regeneration from shoot apical meristems of *Melia azedarach* L. (*Meliaceae*)

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Abstract

A protocol was developed for plant regeneration of *Melia azedarach* L. by *in vitro* culture of apical meristem (0.5 mm in length). The influence of six clones was investigated. The culture procedure comprised two sequential steps: 1) Induction of shoots by *in vitro* culture of axillary buds from adult trees (10-15 years old) by culture on Murashige and Skoog (1962) medium (MS) supplemented with 0.5 mg·dm^{-3} BAP (6-benzylaminopurine), 0.1 mg·dm^{-3} IBA (indolebutyric acid), and 0.1 mg·dm^{-3} GA_3 (gibberellic acid). The Multiplication of the regenerated shoots was achieved in MS + 0.5 mg·dm^{-3} BAP + 0.1 mg·dm^{-3} GA_3. 2) *In vitro* culture of the apical meristems from the regenerated shoots in MS medium (0.7 %) supplemented with various combinations of BAP and IBA.

Maximum shoot proliferation was obtained on MS medium supplemented with 0.5 mg·dm^{-3} BAP and 0.1 mg·dm^{-3} IBA.

Regenerated shoots were rooted on MS + 3.5 mg·dm^{-3} IBA (4 days) followed by subculture on MS lacking growth regulators (30 days). Complete plants were transferred to soil.

Introduction

Meristem culture techniques proved to be a very useful tool for accomplishing three objectives: 1) production of disease-free plants 2) micropropagation of selected plants, and 3) preservation of germoplasm (Styer and Chin 1983).

The "Paradise tree" (*Melia azedarach* L.), a member of the *Meliaceae* family, was introduced into Argentina from Southern Asia. It is a fast-growing tree and its wood is prized for its technological traits (Mangieri *et al.* 1977, Cozzo 1994).

Although plant regeneration from both axillary bud culture and nodal explants of *Melia azedarach* was done (Domecq 1988, Ahmad *et al.* 1990, Thakur *et al.* 1998), there is no report on successful *in vitro* plant regeneration from meristems to obtain disease free plants.

The present investigation was undertaken to induce plant regeneration from *in vitro* cultured meristems of six selected clones of *Melia azedarach*.

Materials and Methods

All the plant material came from DANZER FORESTACIÓN S.A. (Posadas, Misiones, Argentina). Six clones (3, E, H, J2, Lp, and 20) of *Melia azedarach* L. were used in this study. The culture procedure comprised two sequential steps:

1). Induction of shoots by *in vitro* culture of axillary buds from adult trees (10-15 years old) according to the procedure reported by Domecq (1988) consisting of: **a)** Disinfection of axillary buds by immersion in 70 % ethanol (3min), followed by NaOCl (2 % active Cl) with 0.1 % TRITON-X-100®, during 20 min and finally washed with abundant sterile distilled water. **b)** Initiation of culture in Murashige and Skoog (1962) medium (MS) supplemented with 0.5 $mg{\cdot}dm^{-3}$ BAP (6-benzylaminopurine), 0.1 $mg{\cdot}dm^{-3}$ IBA (indolebutyric acid), and 0.1 $mg{\cdot}dm^{-3}$ GA_3 (gibberellic acid), and **c)** Multiplication of the regenerated shoots in MS + 0.5 $mg{\cdot}dm^{-3}$ BAP + 0.1 $mg{\cdot}dm^{-3}$ GA_3.
2). *In vitro* culture of the apical meristems from the regenerated shoots. Meristems (0.5 mm in length, consisting of the domo and a pair of leaf primordia) were aseptically isolated and cultured in small glass test tubes (11 ml) containing 3 ml of nutrient medium. Media were prepared using the MS salts and vitamins, 3 % sucrose and 0,7 % Sigma agar (A-1296). BAP and IBA at several concentrations were used. The pH was adjusted to 5.8 using a few drops of either 0.1 N HCl or 0.1 N KOH prior to the addition of agar. Tubes were covered with aluminium foil and autoclaved at 1.46 $Kg{\cdot}cm^{-2}$ for 20 min.

The tubes with one meristem were covered with Resinite AF-50® film (Casco S. A. C. Company, Buenos Aires) and incubated during 30 days in a growth room at 27±2 °C under a 14/10 h daylight cycle with an irradiance of 4.5 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ provided by cool white fluorescent lamps.

Each treatment consisted of 6 meristems and each experiment was repeated 3 times. Means are given with the standard error (± SE).

Root differentiation on the shoots was induced by subculture the regenerated shoots on MS supplemented with 3.5 $mg{\cdot}dm^{-3}$ IBA for 4 days and subsequently in MS without growth regulators for 30 days (Domecq 1988).

Results and Discussion

Approximately 50-75 % of the axillary buds from all the clones tested started to grow shortly after incubation. After 1 month a single shoot of 1.5-3.0 cm was obtained. By subculturing this shoot on the multiplication medium, after 20 days of culture, multiple shoots (on average 5-8 shoots) were induced. When apical meristems from these shoots were excised and subsequently cultured in nutrient medium, most of them showed the earliest sign of growth within 1 week of culture: the most of them formed callus at the cut end of the explant and just swelled and turned green. By the end of the 2nd week the first leaves appeared (Fig. 1a). After 3-4 weeks of culture *Melia azedarach* meristems differentiated into either single or multiple shoots (Fig. 1b). Root regeneration and subsequent plantlet formation occur occasionally in media containing the highest concentration of IBA (0.5 $mg{\cdot}dm^{-3}$) tested. Unlike other woody plant species (Bonga 1987, Debergh and Read 1991), culture initiation of the meristems is not the critical step. It was possible to establish all the clones. Contamination with microorganisms (bacteria and/or fungi) as well as browning of the explants was relatively low (less than 5 % of the explants).

The growth responses of meristems of six clones of *Melia azedarach* cultured *in vitro* under the influence of a wide range of both BAP and IBA are presented in Fig. 2. An analysis of these results permits to draw the following conclusions:

1. Although individual clones differed in their ability to organogenesis, it was possible to induce shoot growth in all of them. By using an appropiate combination of growth regulators in the nutrient medium (one of the best combination is 0.5 $mg{\cdot}dm^{-3}$ BAP and 0.1 $mg{\cdot}dm^{-3}$ IBA) 80-100 % of the meristems produced shoots (Fig. 2).

Table. Rooting of shoots derived from meristem culture of six clones of *Melia azedarach*.

Clon	Rooted shoot (%)
20	95.5
3	68.4
E	94.3
H	100.0
J2	93.5
Lp	100.0

Fig. 1. Plant regeneration by *in vitro* culture of apical meristems of *Melia azedarach* clon Lp. Bars represent 1 cm.
a) After two weeks culture.
b) Multiple shoots after 4 weeks culture in MS + 0.5 mg·dm^{-3} BAP + 0.1 mg·dm^{-3} IBA.
c) Leaves abscission in MS + 0.1 mg·dm^{-3} BAP + 0.1 mg·dm^{-3} IBA.
d) Rooted shoot.

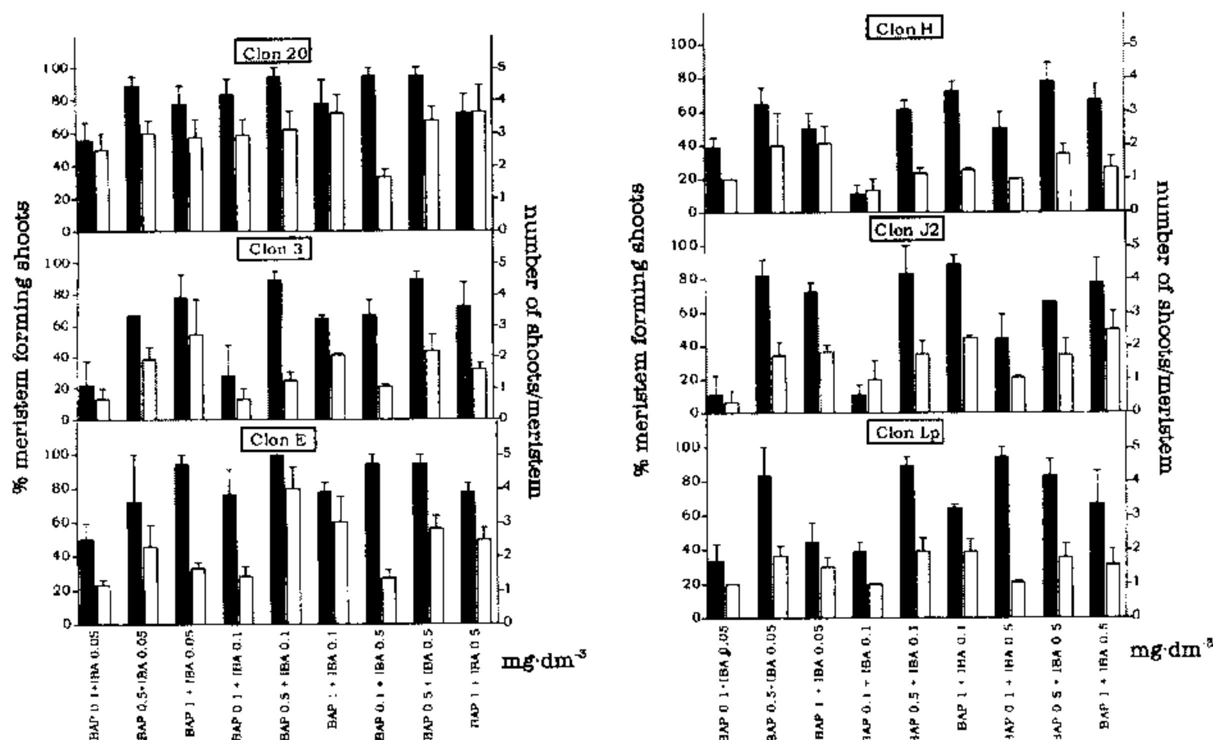

Fig. 2. Plant regeneration by *in vitro* culture of six clones of *Melia azedarach*. Effect of BAP and IBA on the percentage of meristem forming shoot (■) and on the number of shoots/meristem (□).

2. Although all the clones tested produced multiple shoots (Fig. 2), clon E regenerated the greatest mean number of shoots per explant (4). These results are in agreement with those obtained in tissue culture of other woody plant species where differences among clones were described (Kunze 1994, Scaltsoyiannes *et al.* 1997, Coleman and Ernest 1989). However, the number of shoot/meristem depended also on the concentrations of BAP and IBA.

3. Although all combinations of growth regulators permitted shoot formation, this response was greatly affected by the combination of growth regulators in the nutrient medium employed. In previous experiments (data not shown) it was established that for shoot production by meristem culture of *Melia azedarach* the addition to the MS medium of both a cytokinin and an auxin was necessary. In this study, independently of the clones, the best results were obtained in the presence of 0.5 mg·dm^{-3} BAP combined with 0.1 mg·dm^{-3} IBA. This combination BAP-IBA was also successfully employed for micropropagate old trees of *Melia azedarach* (Domecq 1988). It is interesting to note that in media with the lowest BAP concentration (0.1 mg·dm^{-3}) most of the regenerated shoot showed a pronounced abscission of leaves (Fig. 1c).

Table summarises the results obtained when shoots regenerated by culture of meristem on MS + 0.5 mg·dm^{-3} BAP + 0.1 mg·dm^{-3} IBA were subcultured in the rooting medium (MS + 3.5 mg·dm^{-3} IBA, 4 days) and subsequently in MS lacking growth regulators. All the clones exhibited a high percentage of rooting (Fig. 1d). The rooted plants were acclimatized and transferred, in a greenhouse, to soil with a 80 % survival rate where they grew normally and attained a height of 7-10 cm within 25-30 days. All of them looked similar to the mother plants.

In conclusion, in the present investigation, meristem culture was found to be a quick, effective and reproducible procedure for establishing *in vitro* culture of *Melia azedarach* in order to use either for micropropagation or for preservation of germplasm.

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