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MICROPROPAGATION IN *DELONIX REGIA* THROUGH IMMATURE EMBRYO DERIVED SHOOT TIPS

S.M. RAHMAN, M. HOSSAIN, A.K.M. RAFIUL ISLAM AND O.I. JOARDER

Department of Botany,
University of Rajshahi, Rajshahi, Bangladesh

Abstract

A micropropagation method was developed from immature embryo-derived shoot tips of *Delonix regia* on Murashige and Skoog's medium. Shoot multiplication occurred through proliferation of axillary leaf buds and the best multiplication rates were obtained with 1.0 mg/l BAP and 0.5 mg/l NAA. No response was observed in 2ip supplemented media other than rhizogenesis and callogenesis. *In vitro* formed shoots when ransferred onto half strength of MS salts + 0.5 mg/l IBA & 0.5 mg/l NAA produced profuse roots in all the shoots.

Intorduction

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 (Krikorian, 1982; Murashige, 1974; Vasil & Vasil, 1980). 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. *Delonix regia* is a big leguminous tree and very often planted as an avenue tree on road sides, besides obtaining biomass for fuel and timber.

The enhanced axillary branching method of shoot multiplication may be initially slower than through callusing or through adventitious bud formation but with each passage the number of shoots increases logarithmically and within a year astronomical figures can be obtained. This method is becoming increasingly popular for clonal propagation of crop plants because the cells of the shoot apex are uniformly diploid and are least susceptible to genotypic changes under culture conditions (Bhojwani & Razdan, 1983). Observations on the *in vitro* micropropagation of *Delonix regia* through immature embryo-derived shoot tips are described in this paper.

Materials and Methods

Immature seeds from a 20 year old *Delonix regia* were collected during the first week of May, 1990 and surface sterilized with $HgCl_2$ (0.1% w/v). The embryos (with small part of cotyledon) were excised and seedlings were raised in MS medium (Murashige & Skoog, 1962) with 1.0 mg/l BAP. Shoot tips (0.5 cm in length) were collected from 15 day old seedlings and cultured on MS medium containing different types of cytokinins singly or in combination with auxin or gibberellic acid. The range of concentration of hormones was 0.2-1.0 mg/l. Morphogenetic response of the explants was recorded after 5 weeks of culture. The pH of the media was adjusted to 5.8 before addition of 0.6% agar. The cultures were maintained at $26\pm2^{\circ}C$ with a 16h photoperiod.

Table 1. Response of immature embryo-derived shoot tip explants of *Delonix regia* to various combinations of cytokinins and auxins (20 replicates per treatment).

Hormones mg/l	% of explants produced callus	% of explants produced roots	% of explants produced shoots	Number of shoot/ explant	Nature of response
1 BAP	---	---	55	4.9	HS
1 Kn	---	---	30	1.3	WS
1 2ip	---	---	---	---	---
1 BAP+0.5 NAA	---	---	85	8.7	HS
1 BAF+0.5 IBA	---	---	45	1.5	WS
1 BAP+0.5 IAA	---	---	45	2.6	MS
1 BAP+0.2 GA_3	---	---	25	1.2	WS
1 Kn+0.5 NAA	---	---	40	7.1	HS
1 Kn+0.5 IBA	---	70	---	---	BR
1 Kn+0.5 IAA	---	---	40	2.1	WS
1 Kn+0.2 GA_3	---	---	---	---	---
1 2ip+0.5 NAA	40	---	---	---	FC
1 2ip+0.5 IBA	---	40	---	---	BR
1 2ip+0.5 IAA	10	---	---	---	HC
1 2ip+0.2 GA_3	---	20	---	---	UBR

HS= Healthy shoot, MS= Moderate shoot, UBR= Unbranched root, HC= Hard callus, WS= Weak shoot, BR= Branched root, FC= Friable callus

Results and Discussion

The excised embryos produced normal plants on the germination medium (MS + 1.0 mg/l BAP). After 5 weeks of culture these *in vitro* raised seedlings had 3-4 leaves and were 6-8 cm in height. Shoot tips isolated from these plants produced multiple shoots. The explants showed their first response by unfurling of leaves and within 2 weeks of culture shoot multiplication was found to start. Media containing BAP alone or with NAA showed better result for shoot proliferation (Table 1, Fig.1a). Kinetin was also effective but at lower frequencey (Fig.1b). Number of shoots produced per explant varied greatly (1-15) among the cultures. Maximum number of shoots (8.7) was recorded in 1.0 mg/l BAP + 0.5 mg/l NAA. However, media containing Kn with NAA showed more or less similar results with regard to multiple shoot formation. Addition of IAA or IBA to BAP or Kn containing medium did not improve the degree of shoot proliferation. Moreover shoot growth was retarded and leaves turned yellowish. In media containing BAP + NAA and Kn + NAA a good amount of callus was found to develop at the base of the explants. In the same hormonal combinations some of the proliferating cultures produced roots at their bases. No response was observed in 2ip supplemented media other than rhizogenesis or callogenesis.

It is evident from the observations that BAP is the suitable cytokinin for multiple shoot formation from seedling shoot tips in *Delonix ragia*. The superiority of BAP over other cytokinins for multiple shoot proliferation in trees has also been reported (Lundergan & Janik, 1980; Vieitez & Vietez, 1980; Hu & Wang, 1983). Consequently, for shoot

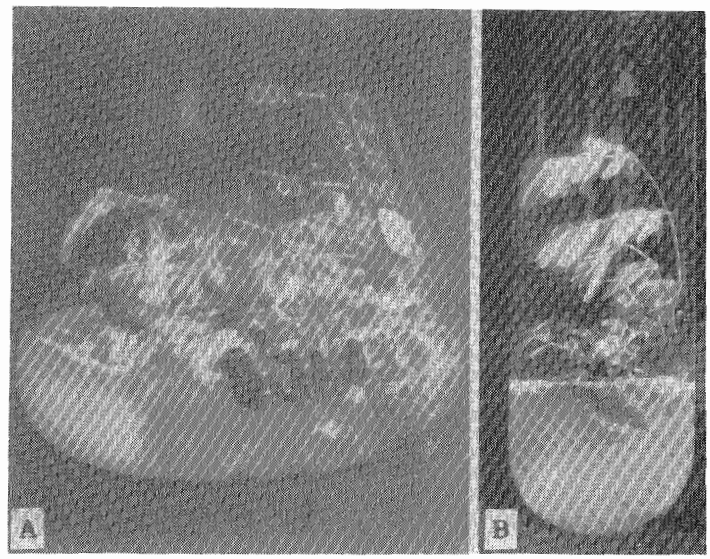

Fig.1. Micropropagation in *Delonix regia*. A. Multiple shoot proliferation from shoot tip explants on MS + 1.0 gm/l BAP + 0.5 mg/l NAA. B. Multiple shoot proliferation from shoot tip explants on MS + 1.0 mg/l Kn + 0.5 mg/lAA.

multiplication presence of an auxin in the medium is helpful but not obligatory. Hundred percent rooting occurred in the *in vitro* formed shoots when transferred onto half strength MS salts + 0.5 mg/l IBA + 0.5 mg/l NAA. IBA (0.5 mg/l) alone also induced roots but media containing both IBA and NAA improved root and shoot growth. Similar results have been reported by Ellyard (1981), Datta (1984) and William *et al*., (1984) wherein an equimolar concentration of IBA + NAA gave improved rooting than either of the auxins alone. It has been observed that the proliferated shoots from cultured shoot tip explants could be used as explants in the next subculture and in this way stock culture could be maintained indefinitely at an interval of 4-5 weeks. The results illustrate the potentiality of immature embryo-derived shoot tips to produce high frequency shoot proliferation in *Delonix regia* and afterwards plantlets as well.

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