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— SHORT COMMUNICATION —

Micropropagation of *Emilia zeylanica* C.B. Clarke, by using explants of inflorescence rachis

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Tissue culture techniques are useful for *ex situ* conservation of rare, endemic or threatened plant species. This report describes a protocol for micropropagation of *Emilia zeylanica* (Compositae), an endangered plant species, using segments of inflorescence rachis, as explants. Only 15% of the explants were contaminated by using this material to start the *in vitro* culture. The highest shoot proliferation was obtained on Murashige and Skoog (1962) medium supplemented with 0.05 mg l^{-1} 6-benzylaminopurine. Shoot length gradually decreased by addition of increasing concentrations of 6-benzylaminopurine. Maximum number of roots was obtained in Murashige and Skoog (1962) medium supplemented with 0.1 mg l^{-1} indole-3-butyric acid. High survival percentage, over 75%, was obtained when the plantlets were transferred to greenhouse conditions. *Emilia zeylanica* can be successfully micropropagated with a single inflorescence rachis and without a significant damage to the mother plant.

Key words: endangered plant, micropropagation, multiple shoots, plantlets, peduncle.

Abbreviations: MS: Murashige and Skoog (1962) medium; BAP: 6-benzylaminopurine; Kin: kinetin; IAA: indole-3-acetic acid; IBA: indole-3-butyric acid.

INTRODUCTION

There is an increasing concern throughout the world about the uncontrolled exploitation and depletion of the earth's natural resources, especially of those affecting plant biodiversity of tropical forests. The extinction of a species is related to the degree of threat by biotic and abiotic factors. Therefore, the need for conservation is exceptionally high and of paramount importance to preserve heritage for posterity of a plant. The habitats where endangered species live are under strong anthropogenic and natural pressures and proper management of plant diversity through local measures of protection is required (Cuenca & Amo-Marco, 2000).

Emilia zeylanica (Compositae) is a tropical annual herb. Owing to biotic factors, destruction of habitat, forest fragmentation and prolonged drought, there is a fast depletion of its natural population. Regeneration and reestablishment of the plant through *in vitro* culture is one of the most effective biological techniques to maintain its diversity.

Nodal segments have been proved successful as a material for micropropagation of field grown plants, such as *Centaurea spachii* (Cuenca & Amo-Marco, 2000) *Centaurea paui* (Cuenca *et al.,* 1999) and *Delphinium malabaricum* (Agarwal *et al.,* 1991). The objective of this work was to develop an efficient protocol for rapid micropropagation of *Emilia zeylanica* by using segments of inflorescence rachis, as explants.

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MATERIALS AND METHODS

Plant material

Emilia zeylanica C.B. Clarke was collected from the Shola forests at the Palni hills of Tamil Nadu, India. The inflorescence rachises, which were excised from healthy plants, were washed with running tap water for 10 min and were pretreated with 1% (v/v) detergent (3 drops of Tween 80), followed by 70% (v/v) ethanol. The explants were then surface sterilized with 0.1% (w/v) mercuric chloride for 3 min, rinsed 3-5 times in sterile double distilled water and finally cut into 10-15 mm segments.

Culture medium

The explants were transferred to MS medium (Murashige & Skoog, 1962) containing 3% (w/v) sucrose, 0.8% agar and various concentrations of BAP, IAA, and Kin. The pH of all media was adjusted to 5.7 before adding agar. The media were sterilized in an autoclave at 121 °C, for 20 min. The explants were transferred to 25 × 150 mm glass culture tubes containing 15 ml of culture medium and plugged with non absorbent cotton. The cultures were maintained in a growth room at 25 ± 1 °C during the light and dark periods. The photoperiod was 16 h and the light was supplied by fluorescent lamps (3000 lux). The cultures were maintained by a regular subculture at 4 week intervals on a fresh medium with the same hormone composition.

Effects of plant growth hormones on shoot multiplication

The sterilized explants were cultured on a MS medium supplemented with BAP, Kin, and IAA, each at five concentrations (0.05, 0.10, 0.50, 1.0, and 1.5 mg l^{-1}). A control treatment without cytokinins was also included.

Rooting and acclimatization of plantlets

The rooted plantlets were removed from the culture tubes, washed thoroughly with tap water to remove traces of agar, treated with 0.3% bavistin and planted in small pots filled with a mixture of garden soil, sand and farmyard manure (2:1:1). Plants were maintained in a growth chamber. They were acclimatized to a reduced relative humidity by gradual opening of the plastic cover, and after 3 weeks they were completely uncovered and hardened to greenhouse conditions.

TABLE 1. Effects of BAP, KIN, and IAA concentrations on the percentage of reactive explants, the number of shoots and the maximum shoot length per explant after five weeks of culture on MS medium. The means and standard errors (± SE) are presented for each column. Means sharing at least one letter are no significantly different at the $p \leq 0.05$ level (Duncan's multiple range test)

Hormone	Concentration mg l^{-1}	Percentage of responsive explants	No of shoots per explant	Maximum shoot length (mm)
Control	–	70	1.6 ± 0.3 ^{lm}	0.9 ± 1.0 ^{hi}
BAP	0.05	93.3	2.8 ± 0.4 ^{jk}	38.3 ± 2.9 ^{b}
	0.10	97	3.4 ± 0.6 ^{h}	28.7 ± 3.4 ^{cd}
	0.50	100	11.7 ± 0.8 ^{a}	40.9 ± 5.4 ^{a}
	1.00	90	6.7 ± 1.1 ^{e}	26.1 ± 2.8 ^{de}
	1.50	87	4.9 ± 1.1 ^{fg}	25.1 ± 3.3 ^{e}
Kin	0.05	97	9.4 ± 1.0 ^{c}	26.9 ± 3.8 ^{d}
	0.10	90	7.0 ± 1.2 ^{de}	21.9 ± 3.2 ^{f}
	0.50	86	3.3 ± 1.1 ^{hi}	19.5 ± 2.8 ^{fg}
	1.00	83	3.0 ± 1.0 ^{ij}	9.2 ± 1.8 ^{h}
	1.50	79	1.8 ± 0.3 ^{l}	8.0 ± 1.8 ^{i}
IAA	0.05	93	7.3 ± 1.3 ^{d}	36.4 ± 4.6 ^{bc}
	0.10	97	11.3 ± 1.3 ^{ab}	3.4 ± 5.2 ^{ij}
	0.50	93	5.1 ± 0.7 ^{f}	29.3 ± 5.7 ^{c}
	1.00	83	2.9 ± 0.7 ^{ijk}	12.1 ± 2.4 ^{gh}
	1.50	80	3.3 ± 0.8 ^{hi}	4.0 ± 2.0 ^{gl}

RESULTS AND DISCUSSION

Inflorescence rachis segments of *E. zeylanica* were selected as explants for *in vitro* establishment. Peduncles have been shown to be the best material for in *vitro* establishment of endangered plants, such as *Centaurea spachii* (Cuenca & Amo-Marco, 2000) and *Gentiana lutea* L. ssp. *aurantiaca* (Feijoo & Iglesias, 1998). Explants exhibited only 15% of contamination, lower than that of buds and leaves.

During the shoot multiplication phase, the per-

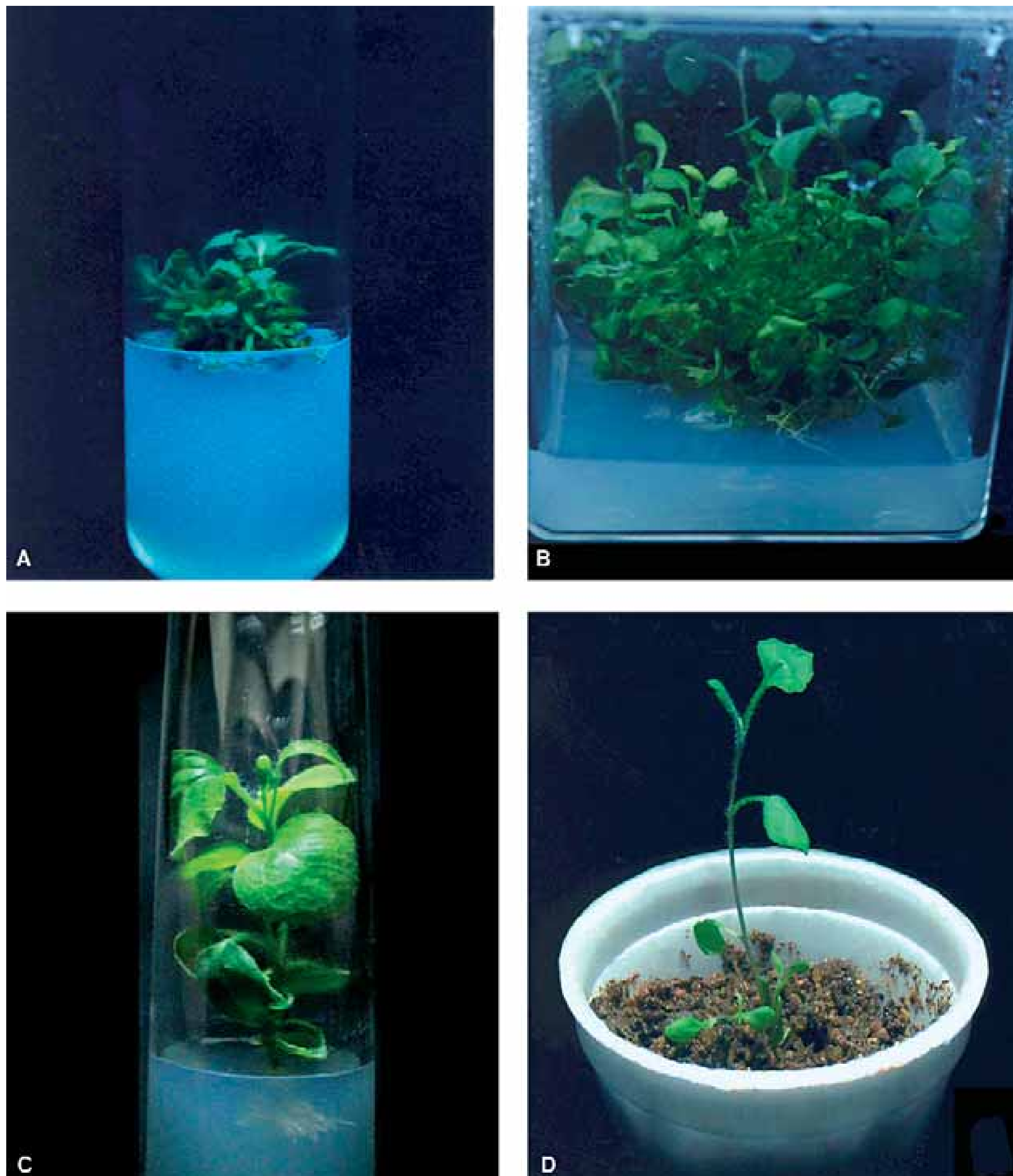

FIG. 1. *In vitro* plant regeneration of *Emilia zeylanica* C.B. Clarke. (A) Response of inflorescence rachis explants on shoot initiation; (B) High frequency of multiple shoots from a single explant (0.50 mg l^{-1}); (C) Rooting of *in vitro* shoots in MS medium with 3.0 mg l^{-1} IBA; (D) Hardened plantlet.

TABLE 2. Effects of auxin on *in vitro* rooting of shoots cultured on MS medium. Means and standard errors (± SE) are presented for each column. Means sharing at least one letter are no significantly different at the $p \leq 0.05$ level (Duncan's multiple range test)

Auxin	**Concentration mg l^{-1}**	**Percentage of rooting**	**No of roots per explant**	**Root length per explant (mm)**
IAA	0.05	70	3.4 ± 0.6^{f}	3.7 ± 0.5^{f}
	0.10	80	4.9 ± 0.7^{e}	6.0 ± 0.9^{de}
	0.50	80	6.1 ± 0.9^{c}	8.5 ± 1.0^{c}
	1.00	90	5.3 ± 1.2^{de}	5.2 ± 0.8^{e}
IBA	0.05	90	11.1 ± 0.6^{ab}	10.2 ± 0.7^{b}
	0.10	100	12 ± 1.1^{a}	12.1 ± 0.7^{a}
	0.50	90	7.8 ± 1.0^{b}	8.2 ± 0.8^{cd}
	1.00	80	5.5 ± 1.0^{cd}	6.5 ± 0.6^{d}

centage of reactive explants (83.3-100%) was consistently high in all media studied. In the MS medium without any hormone, an average of 1.6 ± 1.1 shoots per explant was recorded after five weeks of culture (Table 1). It is significant to note that multiple shoots were induced from inflorescence rachis explants without an intervening callus phase on MS medium containing different concentrations of BAP and Kin.

Multiple shoots were induced from inflorescence rachis explants after four weeks of culture on MS medium supplemented with different concentrations of BAP at 0.50 mg l^{-1} (11.7 ± 2.6) (Fig. 1) and Kin at 0.05 mg l^{-1} (9.4 ± 3.4) (Table 1). The induction of shoots by using BAP has been well-documented in *Piper* spp. (Bhat *et al.,* 1995), *Ocimum* spp. (Pattnaik & Chand, 1996), *Withania somnifera* (Manickam *et al*., 2000), and *Phyllanthus caroliniersis* (Catapan *et al*., 2000). Shoot elongation, decreasing slightly with increasing BAP concentration, has also been observed in other micropropagation protocols (Ault, 1994; Iriondo *et al*., 1995). With a further increase in cytokinin concentration there is an enhancement in callusing. However, in the medium containing higher concentrations of IAA, shoots showed stunted growth with a lesser number of shoots (2.9 ± 2.3) produced.

Rooting and acclimatization

The *in vitro* multiple shoots were sub-cultured to develop whole plants for root induction in media supplemented with different concentrations of IAA and IBA. When the rooting media were supplemented with IBA concentration 0.1 mg l^{-1} the number and length of roots greatly increased (Fig. 1) and then decreased at 2.0 mg l^{-1} (Table 2). IBA was more resistant than IAA to degradation in the tissue culture media, both during autoclaving and at room temperature (Nissen & Sutter, 1990). As with *Wedelia chinensis* (Kameri *et al*., 2005), this concentration of IBA seems to play a stimulatory role in the process of root formation in *E. zeylanica* shoots. In the IBA medium, the majority of roots developed three weeks earlier than in the IAA. IBA concentration was beneficial also for both root system development and for shoot quality. The medium supplemented with IAA (0.05 - 1.0 mg l^{-1}) had poor rooting, with an intervening callus. For acclimatization, *in vitro* plantlets were transferred to pots containing a mixture of soil, vermiculate and sand (2:1:1) and healthy roots appeared after two weeks. The survival rate of the clones was about 95%. The plants were hardened for 10-15 days before being transferred to the greenhouse. Based on these observations we propose an efficient protocol to micropropagate the rare species *E. zeylanica* from nodal segments of inflorescence rachis.

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