Micropropagation of *Codiaeum variegatum* (L) Blume cv "Norma"

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Abstract

Codiaeum variegatum (L) Blume cv "Norma" (croton), was successfully micropropagated when culture were initiated with explants taken from newly sprouted shoots. The establishment and multiplication steps were possible when 1 mgl^{-1} IAA and 3 mgl^{-1} 2iP were added to MS medium, with an average bud propagation ratio of between 2 and 3 every 6 weeks starting after three subcultures on the same medium. Root induction was observed on basal medium plus 1 mgl^{-1} IBA. Plantlets in the greenhouse had variegated leaves that were true-to-type.

Key words: *Codiaeum variegatum*, micropropagation, nodal segments, cytokinin.

Introduction.

Codiaeum variegatum (L) Blume cv "Norma" (croton) is a hardy ornamental plant (*Euphorbiaceae*) with large red, yellow and green colored leaves, which render this plant ideal for home flower pots and a very economically important species. At present, a large proportion of the plants sold are propagated by seed or cuttings. Micropropagation would provide an alternative method to replace mother plant stocks with microcuttings maintained *in vitro*. The problems of rejuvenation, dormancy or delayed flowering sometimes observed when hardy ornamental plants are produced *in vitro* (1) do not apply to *Codiaeum* spp., whose appear lies in its foliage.

A protocol developed for micropropagation of *C variegatum* cv “Corazon de oro” (2) was found not to work for this variety, cv. “Norma”. The aim of this work was to establish a practical method for rapid and large-scale multiplication of *C. variegatum* cv “Norma”.

Material and Methods.

Vegetative nodal segments of *C. variegatum* (L) Blume cv “Norma”, were obtained from greenhouse mother plants that had been fertilized and pruned to encourage new shoots. The plants were sprayed with a mix of Benlate and Agrimicine to minimize disease and contamination problems. Explants of 20 mm long were disinfected with 70 % alcohol for a few minutes, 25 % commercial bleach (2 % chlorine) for 30 min, and 0.1 % mercuric chloride for 5 min. They were then rinsed thoroughly with sterile distilled water. Nodal segments were cut at the basal ends and transferred onto modified Murashige & Skoog medium (5) supplemented with 1 mgl^{-1} indole acetic acid (IAA) + 1, 3 or 5 mgl^{-1} of N6 benzyladenine (BA) or 6 $\gamma\gamma$ isopentenyladenine (2iP). All media were solidified with 8 g/L of agar and autoclaved at 0,1 Mpa for 15 min. The pH was previously adjusted to 5,7±0,1. Shoots with 2-3 leaves and 50 mm high were rooted on MS + 1 mgl^{-1} indole butyric acid (IBA). Each treatment consisted of five explants inoculated in glass flasks with 50 ml of media, replicated 10 times. Data were analyzed by χ^2.

Explants were subcultured every 42 days and were maintained at 25 ± 2 °C under a 16 h light photoperiod provided by cool white fluorescent light (57 $\mu E.m.^{-2}$ $s.^{-1}$) TLT 110 W/54 RS Philips day-light tubes.

Plantlets with roots were transferred to pots containing a mixture of 1:2:1 soil: crushed pine leaves: peat and they were maintained in a greenhouse for 2 weeks under mist (up to 85

% RH), wherefore humidity conditions were gradually reduced to normal by 30 days from transfer.

Results and Discussion

The aseptic establishment of croton was very difficult. Strongly pruned shoots provided useful explants, whereas nodal and shoot tips from the primary axis were not used successfully in culture establishment, as also observed in *Cordyline spp*. (4).

Seventy % of the pre-existing buds in the explants grew normally, when 1 mgl^{-1} IAA and 3 mgl^{-1} 2iP was added to MS medium, after 20 days of the culture initiation. Furthermore, 22 % were contaminated and 8 % died (Table 1). Fewer explants sprout when 2iP concentration was reduced to 1 mg/L in the medium, whereas abnormal shoots were observed when it was increased to 5 mg/L (Table 1).

After 50 days of culture, nodal segments initiated on MS with 3 mgl^{-1} 2iP produced propagules suitable to start the multiplication stage. In contrast, when BA was used, callus growth occurred after 10 days of culture, arresting the growth of buds. This response was not expected as BA was reported effective in micropropagation of another croton cultivar (2). Similar variation in media specificity were observed in other hardy ornamental plants (3).

Multiplication of plant material started after three subcultures on MS with 3 mgl^{-1} 2iP, where the average bud propagation ratio was between 2.5 fold every 6 weeks. Explants were subcultured every 42 days without any deviation in phenotype even after 24 months of continuous subculturing.

The cytokinin present in the medium had no effect on root initiation when explants were up to 4 cm high and carried 2 expanded leaves. Then, some of the biggest shoots showed

sprouted buds and roots simultaneously on the same medium. However, the addition of 1 mgl^{-1} IBA to MS medium consistently stimulated a synchronous root induction in all shoots. Plantlets produced with the protocol described here were successfully acclimatized in greenhouse conditions without the need for any special care, as *in vitro* leaves were not particularly prone to desiccation.

Conclusion

The micropropagation of *C. variegatum* cv "Norma" was facilitated when culture was initiated with explants taken from newly sprouted shoots. The same medium used to initiate *in vitro* culture was suitable for propagule multiplication. Starting from a single nodal segment, it was possible to produce 730 plantlets after 1 year when a combination of 1 mgl^{-1} IAA and 3 mgl^{-1} 2iP was added to MS medium. The plantlets in the greenhouse showed variegated leaves with phenotype characteristics as in the mother plants.

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Table 1: Effect of concentration and cytokinin selected on *in vitro* sprouted shoots of *C. variegatum* cv Norma, grown for 20 days on MS medium supplemented with 1 mgl^{-1} IAA. Values are expressed by percentage.

Cytokinin conc mg/L		Explants contam.	Explants Not grown	Explants with callus	Sprouted shoots
BA	1	20 a	3 c	77 a	0 c
	3	18 a	4 c	89 a	1.2 c
	5	21.5 a	1.4 c	85 a	2,2 c
2iP	1	21 a	26 a	0 c	47 b
	3	22 a	8 bc	0 c	70 a
	5	18.6 a	11 b	10 b	60.4 ab

Values followed by the same letter in each column, are not significantly different at $P<0.05$ level by χ^2.