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IN VITRO PROPAGATION OF CROTON (*CODIAEUM VARIEGATUM*)

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Abstract

Croton (*Codiaeum variegatum*) with its amazing colors and leathery leaves is regarded as a beautiful foliage plant. The plant is native of South East Asia. Generally crotons are multiplied vegetatively by means of cuttings and air layering. This process is slow in response and need to maintain large number of mother plants stock. Micropropagation is an alternative mean of propagation that can meet its high demand in relatively shorter time. An improved and enhanced method was established for the *In vitro* propagation of croton. Murashige and Skoog medium supplemented with 0.5 mg/L of BAP (6-benzyl amino purine) was the best medium for shoot induction). The effect of the selected media was further enhanced by adding adjuvants like malt extract and peptone. The MS basal salt mixtures with BAP (0.5 mg/L) and 25 mg/L of malt extract alongwith 25 mg/L of peptone effectively enhanced the shoot formation. The *In vitro* roots were efficiently induced by using MS media supplemented with 2.0 mg/L of IBA (Indole 3- butyric acid). The sufficiently rooted plants were then transferred to the green house for hardening and 80% sand and 20% farm yard manure as a potting mix was best suited for the acclimatization of plantlets.

Introduction

Codiaeum variegatum, commonly known as Croton and sometimes called Joseph's Coat, belongs to the family Euphorbiaceae, is one of the most popular ornamental plants because of vivid foliage colors and varied leaf shapes. *C. variegatum* is native to Indonesia, Malaysia, Philippines, India, Thailand and Sri Lanka. It is an evergreen shrub, up to 6 m in height but usually maintained at 60-90 cm and grows well in areas having humid climate. More than 200 varieties of croton exist on the globe, available in different leaf sizes, shapes and color patterns. Young leaves are usually green, bronze, yellow or red, later changes to gold, cream, white, scarlet, pink, maroon, purple, black or brown. Sometimes totally different forms of leaves and color variations occur on the same plant. Flowers are small, long, axillary, usually unisexual racemes. Fruits are globular capsules and 3-8 mm in diameter.

In addition to its aesthetic value as an indoor plant, crotons are also well known for its medicinal value. The leaves extracts of crotons are reported to have many medicinal properties including purgative, sedative, antifungal, antiamoebic and anticancerous activities (Deshmukh & Borle, 1975; Kupchan *et al*., 1976). The plant is also well reputed for the production of valuable secondary metabolites of alkaloids, terpenes and flavanoids in nature (Puebla *et al*., 2003; Maciel *et al*., 1998; Martins *et al*., 2002).

Croton can be propagated by various methods such as cuttings, grafting, by seeds and air layering. From shoot tip cuttings, one mother/stock plant can yield only 20 plants per year (our own nursery experience). Due to its slow rate of conventional multiplication, the plant is very high in demand. Micropropagation is a relatively new technology and application of innovative method have served to overcome barriers to

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progress in the multiplication of elite species and further improvements are anticipated. *In vitro* growth and development is considerably influenced by several factors like genotype, the age and size of mother plant and explant, the season, growth conditions, media composition and various other physiological factors. As a mean of securing pathogen free plants, culture of shoot apical meristem is ideal. Other advantages that assure by this method include large number of plant production in shorter time period, irrespective of the season (Mulabagal & Tsay, 2004). Croton was chosen for micropropagation due to its rare success in conventional breeding and very little data is available for its *In vitro* production (Shibata *et al*., 1996; Orlikowska *et al*., 1995, 2000). The present study was aimed to establish an efficient, economical and reliable protocol for *In vitro* propagation of *Codiaeum variegatum* (Croton).

Materials and Methods

Plant material and explant sterilization: The plant material was collected from the nursery of the H.E.J. Research Institute, University of Karachi. The plants were sprayed with the fungicide and insecticide 2-3 week prior to start initiation and over head watering was strictly avoided. Freshly grown shoot tips, with two to three nodes, were selected as an explant source. Shoot tips were washed in running tap water for 10 minutes to remove the dust or sand particles. The tips were surface sterilized by using 0.5% of Sodium hypochlorite for 20 minutes. Few drops of Tween-20 were also added as a surfactant. After 20 minutes the plant material was washed three times with sterile distilled water to remove the traces of bleach with gentle shaking under sterile conditions.

Shoot multiplication: Shoot multiplication of croton was evaluated by varying different parameters like type of basal media, BAP concentration and addition of adjuvants which include peptone and malt to the media. In the first series of experiments, the simple MS (Murashige & Skoog, 1962) and WPM (Lloyd & McCown, 1980) were used to identify the optimum basal media for *In vitro* multiplication. Different concentrations of BAP (6-Benzyl amino purine) were also added to both the basal media to study its effect on shoot multiplication (Table 1). In the second series of experiments, two different adjuvants (malt extract and peptone) were added to the resultant formulation of the first series of experiments (Table 2) and their effects on shoot multiplication were evaluated.

All the media formulations for shoot multiplication had 2.5% sugar and 2.5 gm/L phytagel was used as a solidifying agent. Each media formulation was inoculated by 15 explants. Data were recorded after every week for 6 weeks and values are the means of 5 replications (Table 1 and Table 2).

Root formation: The *In vitro* rooting in croton was studied using MS medium with different concentrations of IAA and IBA (Table 3). All the media formulations for root induction had 2.5% sugar and 2.5 gm/L phytagel as a solidifying agent. Fifteen explants were inoculated to each formulation. Data were recorded after every week uptill six weeks and values are the means of five replications (Table 1 and Table 2).

Acclimatization: The rooted plants were then transferred to the green house for hardening. The potting mix used in this study comprised of 80% sand and 20% farm yard manure. The transferred plants were monitored after every week for at least 6 weeks.

Table 1. Different Media formulations of croton and plant response.

Codes	Basal media	BAP (mg/l)	Mean no. of leaves	Mean no. of buds	Mean length of shoot (cm)
W1		0.00	1.05 b	1.26 c	1.50 d
W2		0.10	1.32 cd	1.26 c	1.73 c
W3		0.20	1.46 bc	1.30 bc	1.82 bc
W4		0.30	1.42 bd	1.33 bc	1.94 bc
W5		0.40	1.50 c	1.38 bd	2.27 bd
W6	WPM	0.50	1.52 abcd	1.41 cd	2.35 cd
W7		0.60	1.58 acd	1.45 bcd	2.39 cd
W8		0.70	1.77 bd	1.55 cd	2.30 bd
W9		0.80	2.00 bd	1.79 ad	2.37 cd
W10		0.90	2.50 ab	2.28 ab	2.35 bcd
W11		1.00	3.52 cbd	2.34 a	2.34 bcd
M1		0.00	1.38 cb	1.41 cd	1.64 d
M2		0.10	1.43 cd	1.55 cd	1.74 c
M3		0.20	1.48 bd	1.57 cd	1.86 bc
M4		0.30	2.02 abc	1.82 ad	2.16 bcd
M5		0.40	2.82 bd	2.45 abc	2.29 bc
M6	MS	0.50	3.94 a	2.60 a	2.55 a
M7		0.60	3.80 acb	2.57 a	2.54 a
M8		0.70	3.83 a	2.32 ab	2.53 a
M9		0.80	3.81 cd	2.20 bc	2.53 a
M10		0.90	3.83 a	2.14 c	2.43 cb
M11		1.00	3.80 ad	2.31 a	2.49 cd

Note: Each value in the Table is the average of five replicates. Values sharing the same letter in each column are not significantly different from each other by DMRT (α=0.05).

Table 2. Effect of peptone and malt extract on axillary shoots formation of croton.

Codes	Adjuvants (mg/l)		Mean no. of leaves	Mean no. of buds	Mean length of shoot (cm)
	Peptone	Malt extract			
A1	0.00	0.00	3.84 c	2.37 e	2.55 b
A2	25.00	0.00	4.12 d	9.96 bc	3.26 bc
A3	50.00	0.00	4.26 cd	10.00 cd	4.03 cd
A4	75.00	0.00	4.33 bd	10.06 cd	4.12 d
A5	0.00	25.00	4.32 bd	9.52 bc	3.66 bc
A6	0.00	50.00	5.04 cb	9.82 bc	4.54 cd
A7	0.00	75.00	5.06 cb	10.14 cd	4.55 d
A8	25.00	25.00	6.44 a	11.59 a	5.64 a
A9	50.00	50.00	6.34 a	11.32 b	5.36 ad
A10	75.00	75.00	6.36 a	11.36 b	5.48 a

Note: Each value in the Table is the average of five replicates. Values sharing the same letter in each column are not significantly different from each other by DMRT (α=0.05).

Table 3. Effect of IBA and IAA on root formation in croton.

Code	IBA (mg/l)	IAA (mg/l)	Number of roots	Length of roots (cm)	Root emergence (days)
CR1	0.50	0.00	1.20 b	1.40 c	20
CR2	1.00	0.00	1.60 bc	1.60 d	19
CR3	1.50	0.00	1.80 cd	1.80 cd	16
CR4	2.00	0.00	2.80 a	2.80 a	12
CR5	2.50	0.00	2.60 a	2.60 ab	11
CR6	0.00	0.50	1.20 b	1.20 b	19
CR7	0.00	1.00	1.80 d	1.60 d	17
CR8	0.00	1.50	2.40 cd	1.60 d	16
CR9	0.00	2.00	2.60 ad*	2.20 ac*	11
CR10	0.00	2.50	2.60 ad*	2.20 ac*	10

Note: Each value in the Table is the average of five replicates. Values sharing the same letter in each column are not significantly different from each other by DMRT (α=0.05).
Values with a * sign means Roots with Callus

Statistical Analysis: A completely randomized design was used for all the experiments. Each experiment was replicated five times with three samples in each replicate. Duncan's Multiple Range Test (DMRT) was used to separate means.

Results and Discussion

An efficient and reliable system for the *In vitro* propagation of *Codiaeum variegatum* has been optimized. These set of experiments have produced valuable results which are discussed in the following sections:

Shoot multiplication: Two basal salt mixtures (high and low), Woody Plant Media (Lloyd & McCown, 1980) and MS Media (Murashige & Skoog, 1962), were used initially to study the effect of basal media on shoot multiplication. In the light of our results, the MS medium proved better than WPM media since it showed highest number of leaves and buds alongwith the maximum length of shoot, both with or without BAP (Table 1). The Woody Plant Media was basically designed to overcome the chloride ion susceptibility of the woody plants (George, 1993). Since our results showed better on MS media, we can predict that unlike most of the woody plants like croton is not susceptible to chloride ion. Thus the MS media, which is a high salt media as compared to WPM, is better for the enhanced multiplication of axillary shoots.

In the present study different concentrations of BAP (6-Benzylamino purine), were used to evaluate its effect on the axillary shoot formation. The plant growth varied as the concentration of the BAP changed. There is an increment in axillary shoot formation until the BAP concentration reached to 0.5 mg/L Table 1. The concentrations above 0.5 mg/L did not show any significant increment in the axillary shoot induction. This might be due to the fact that the requisite concentration of each type of growth regulator differs greatly according to the plant being cultured (George, 1993). Our results clearly suggest that maximum shoot induction can be achieved by the use of 0.5 mg/L of BAP (Fig. 1B).

Two different types of adjuvants (Peptone and Malt Extract), in different concentrations, were tested to evaluate their effect on axillary shoot induction and multiplication, both in single and in combination (Table 2). Peptone and malt extract generally consist of low molecular weight proteins, amino acids, vitamins and plant

growth substances, which may promote plant growth but they are relatively undefined supplement (George, 1993). In the present study, the higher levels of both these adjuvants didn't ensure the maximum promotion of axillary shoots formation (Table 2). The basal media use for this study was MS with 0.5 mg/L of BAP. The peptone and malt extract both were able to enhanced the axillary shoot formation greatly, in combination of 25 mg/L each, as compared to their individual effect. Thus our results clearly suggest that the use of peptone and malt extract markedly enhanced the axillary shoots formation in croton (Fig. 1B).

Rooting: To evaluate the *In vitro* root formation in croton, different concentrations of IBA and IAA were used for the induction of roots. As indicated in Table 3, IBA in the concentration of 2 mg/L was best suited for the induction of roots because maximum number and length of roots were achieved on that formulation and even the higher concentration of IBA (2.5 mg/L) failed to produce significantly different response. The media supplemented with IAA also showed root induction in relatively shorter time but the only problem was, apart from root induction, IAA also induced callus even in a very low concentrations (Table 3 and Fig. 1C).

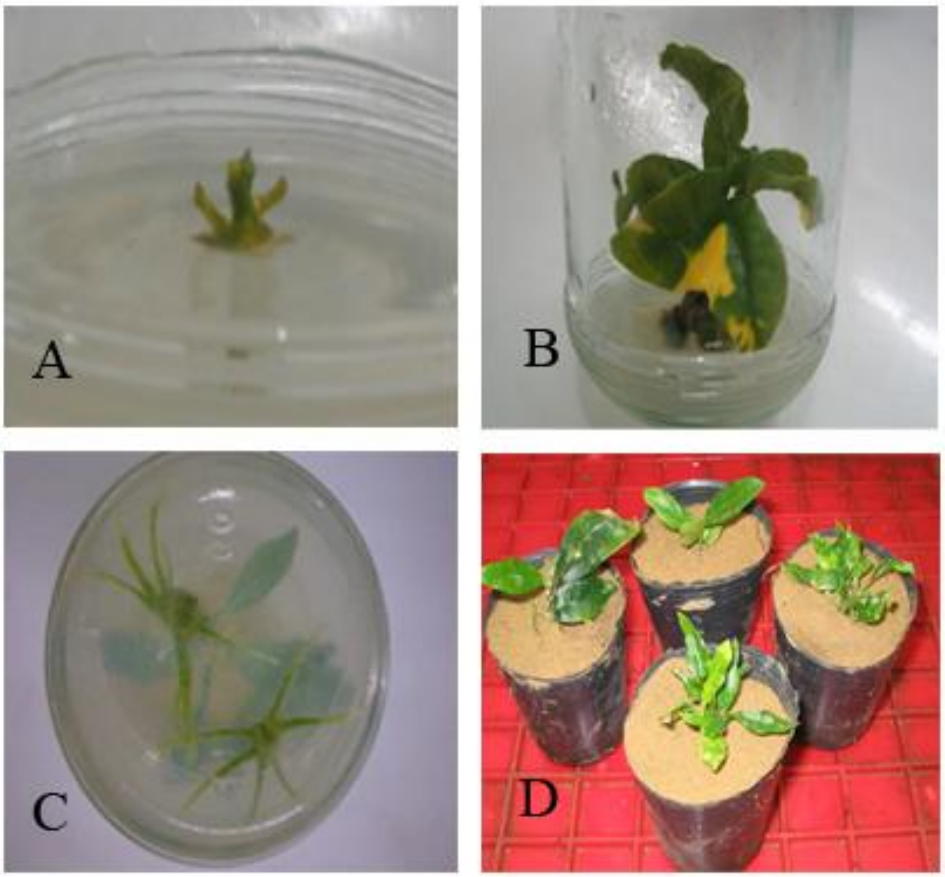

Fig. 1. A Initiation of explant bearing two nodes. B Development of leaves, buds and shoot after 6 weeks. C *In vitro* roots development after 6 weeks. D Acclimatization of the tissue cultured plantlets of croton.

Acclimatization: The *In vitro* grown plantlets with at least two to three roots were transferred to the green house for the acclimatization. The potting mix (20% Farm Yard Manure and 80% Sand, v/v), routinely used in the nursery of our institute, was found suitable for the hardening of the plants. The survival rate of the *In vitro* grown plants was 95% and so there was no need for any experimental study for acclimatization (Fig.1D).

Conclusion: The protocol here in described is very much efficient for the *In vitro* multiplication of *Codiaeum variegatum*. In the light of our results, it can be suggested that enhanced shoots and buds formation can be achieved by using the MS medium with 0.5 mg/L of BAP. The high concentrations of peptone and malt extract didn't exponentially increase the axillary shoots formation. The optimum concentrations of peptone and malt extract was found to be 25 mg/L each. The *In vitro* roots were successfully induced by 2.0 mg/L of IBA. The rooted plants were then effectively acclimatized with the potting mix of 80% sand and 20% farm yard manure (v/v).

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