**Effects of types and concentrations of auxins on rooting of stem-cuttings of croton (*Codiaeum variegatum* L.)**

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**ABSTRACT**

The development of an efficient system of vegetative propagation is essential for establishment of commercial and clonal production of ornamental plants. The aims of this paper were the test effects of auxins and the development of stem-cutting rooting induction system of croton (*Codiaeum variegatum*), an species with mix-colored leaves used for ornamental purposes around the world. Apical stem-cuttings from adult shrubs of croton were collected at spring season and were treated for 24-h with different types and concentrations of auxins and planted in substrate consisted of sand. After 60-d there was observed that 100% of rooting of stem-cuttings were obtained only in the treatments with 400 mg L-1 IBA and IBA+NAA (200 mg L-1 each). Similarly, best number of roots (40.5) and fresh weight (1.06 g) per stem-cutting was obtained in the IBA+NAA treatment. The survival of plants of rooted-stem-cuttings of croton transplanted to soil:sand 1:1 (*v/v*) substrate varying from 80 to 100% according to the auxin treatment.

**Key-words**: *Codiaeum variegatum*, ornamental plant, vegetative propagation, Plant Growth Regulators

**Running titles**: Stem cutting propagation of *Codiaeum variegatum*

**INTRODUCTION**

Floriculture represents and provide not only economical, but also social importance in the world, mainly because the generation and high necessity of human-labor and because the better conditions of labor than conventional agriculture (Benschop et al. 2010).

Floriculture has more than ten different sectors of production of ornamental and flower plants, as pot-flowers, cut-flowers, foliages, bulbs market, plants for landscape garden, among others. Ornamental horticulture or floriculture is composed by several types of botanical families, as Orchidaceae, Liliaceae, Araceae, Arecaceae, Zingiberaceae and others from Monocots group and Asteraceae, Rosaceae, Euphorbiaceae and other from Dicots group.

Euphorbiaceae family includes around of 7,500 species included in around 300 genera (IES 2012), many with economic importance as medicinal plants (Catapan et al. 2002), oil and biodiesel production (Comar et al. 2004, Ibáñez-Torres 2004), weed plant in agriculture of different part of the world (Ibáñez-Torres 2004, Vargas et al. 1999), and ornamentals application as *Euphorbia pulcherrima*, commonly called poinsettia, used main as pot culture (Candido et al. 2008), the crotons (*Codiaeum variegatum*) and other species as Euphorbia punicea (van Veldhuisen 2006).

*Codiaeum variegatum* is a shrub species from Southeast Asia and Pacific Islands and has more than 200 varieties classified by the main differences in its leaves, commonly variegated and combined the green, bronze, yellow, red, rose, black, brown and other intermediary colors (Nasib et al. 2008), justifying its use as ornamental in gardens. Medicinal, toxic and antimicrobial properties also were found in leaves extracts of this species (Naidu 1988).

Propagation of croton can be realized using cuttings, grafting, and by seeds. From apical stem cuttings around 20 plants can be obtained per year from each donor plant (Nasib et al. 2008) and normally, the commercial production were maintained by stocks of donor plants.

Micropropagation techniques also were established for croton, but not efficient protocol for multiplication and rapid growth was obtained (Nasib et al. 2008). Rooting of stem cuttings are the main type and fast propagation of croton in commercial production (Papafotiou et al. 2001).

Pop et al. (2011) characterized the adventitious rooting induction as a complex process and key step in the vegetative propagation of economically important crops, playing an important role in the successful production of elite clones, and several types of factors can be influence the rooting of stem cuttings as genotype, season of the year, physiological conditions of mother plant, type of substrate, plant growth regulators, and environmental conditions of growth.

Ornamental horticultural importance of croton species, associated with the few information about its propagation result in our hypothesis that improving rooting of stem-cuts only is possible with treatments of auxins. The aim of this work was to test different types and concentrations of auxins in the rooting of apical stem cuttings of croton.

**MATERIAL AND METHODS**

Experiment was conducted in Pompeia city (Latitude 22°06’31” and Longitude 50°10’18”), Brazil, and the stem cuttings were obtained from adult five donor plants shrubs with around 3 m of height growth in field environmental conditions in last week of September and was repeated in the second week of October, period that corresponding to the spring season in this locality, with an temperature average around 21 – 23°C and 50 – 100 mm of pluviometry (CIIAGRO 2012).

The treatments consisted of two types of auxins, indole-3-butyric acid (IBA) and naphtaleneacetic acid (NAA), both at 200 or 400 mg L-1 used isolate, and one treatment that combine IBA and ANA at 200 mg L-1 each. Tap water was used as control.

The liquid solutions of auxins used as treatments of stem cuttings of croton were prepared solving IBA and NAA powder (Sigma-Aldrich, USA) in 10 ml of 92.8 °GL of alcohol, and completed with tap water for one liter.

Only apical stem cuttings were used in the experiment. Stem cuttings with 10 -12 cm with four expanded leaves were collected from de donor plants and 50% of its basal part were immediately soaked in water solution with different types and concentrations of auxins for 24 hours at 25 ± 1 °C and then, the stem cuts were planted in plastic trays (200 ml per cell) using sand as substrate. The stem cuttings were cultivated in greenhouse under 20 to 35 °C of temperature, around 12.000 lux of luminosity in 11.5 to 12-h of natural photoperiod, 50 – 95% of air humidity obtained in small tunnels protected with transparent plastic tray film to conserve the air humidity and to prevent the dehydration of the cuts. Irrigation was made using micro-sprinklers and around 2 mm of water were used per day. Fertilization was not used.

Each four repetition per treatment was consisted of ten stem cuts and total of 40 stem cuts were used per treatment conducted in full randomized blocks. The experiment was repeated twice.

Percentage of rooting, number and length of roots per stem cut, as well as, fresh weight of roots were evaluated at 60 days after the planting of stem cuttings. Data were submitted to ANOVA and the means were compared using Duncan’s Multiple Range test at 5% of probability.

Evaluation of the percentage of survival transferred plants from stem cuttings propagation was observed after 90 days of planting in commercial plastic pot culture using soil and sand 1:1 (*v/v*) as substrate.

**RESULTS AND DISCUSSION**

The highest percentage of rooting, 100% of stem cuttings that rooting, were obtained in IBA at 400 mg L-1, NAA at 200 and 400 mg L-1 and in the treatment that combine IBA + NAA at 200 mg L-1 each. The stem cuttings from control and the treatment with 200 mg L-1 IBA resulted in 83.3% of stem cuttings with roots (Table 1).

Few papers were dedicated to study the propagation of *Codiaeum variegatum*, including the factors that influence the rooting of stem cuttings. Tillmann et al. (1994) tested different substrates for rooting of apical stem cuttings of croton and observed that highest percentage of rooting were obtained with vermiculite (95%) and phenolic foam (95%) without the use of PGRs and in the period among march and june (correspondent to Autumn season), the best period for stem cut propagation of croton according these same authors. In these same conditions, the use of sand showed lower percentage of stem cuttings with roots (68%) and shoots (85%) and dry weight of roots (0.17 g) than other substrates yet described (Tillmann et al. 1994). However, the actual experiment conducted in August and September (spring season) and using only sand as substrate, resulted in an high percentage of stem cuttings with induction of adventitious roots, both without (83.3%) or with auxins (83.3 – 100%) treatments. Almeida et al. (2008) also obtained better percentage of rooting induction in apical stem cuttings of *Ixora coccinea* ‘Compacta’ using sand (68%) than commercial substrate Plantmax® (48%). The use of sand for rooting of stem cuttings shows advantages such as low cost, high availability, good water drainage and high porosity, and is mainly used in herbaceous and semi-woody cuttings (Fachinello et al. 1994, Kämpf 2000).

The differences observed in the percentage of rooting between actual and the experiment conducted by Tillmann et al. (1994) with rooting of apical stem cuttings of croton in sand could be explained by two main endogenous factors, the genotype used and the season of the year that the different authors used for collect the stem cuttings.

Both the type and concentration of the auxins has a strong effect on adventitious rooting induction of stem segments, but the stimule for adventitious rooting induction by auxins are not well understood at molecular level (Ludwig-Müller et al. 2005, Pop et al. 2011). However, adventitious rooting induction were dependent of the genotype used, and this genotype-dependence can be correlated with its differences in auxin metabolism (Epstein and Ludwig-Müller 1993; Han et al. 2009), as observed in different cultivars of *Prunus avium*, where was observed that the difficult-to-root cultivar conjugated IBA more rapidly than easy-to-root cultivar (Epstein et al. 1993). The season of the year is another factor that influences the rooting of stem cuttings, as observed in *Erythrina falcata* (Neves et al. 2006), *Actinidia deliciosa* (Ono et al. 1998), and other species.

The high percentage of rooting of croton even in the control treated with water (83.3%) can be explained by the presence of auxins in the latex of some Euphorbiaceae species, as showed in *Euphorbia abyssinica* (0.06 µg of indole acetic acid / g of latex) (Negussie et al. 2009). The endogenous levels of IAA in *Euphorbia esula* varying according the season of the year (Nissen and Foley 1987a) and phonological stage of plants (Nissen and Foley 1987b), resulting in a endogenous factor that control the adventitious root induction success in this species and other correlated, as crotons.

The number of roots were increased significantly when the apical stem cuttings of croton were treated with auxins IBA at 400 mg L-1 and the treatment that combine IBA + NAA (200 mg L-1 each) resulted in the best numbers of roots per stem cuttings (44 and 40.5, respectively), showing an improvement of more than 200% in these treatments compared with the control (Table 1).

Lengths of roots were not influenced by the treatments of stem cuttings with the auxins IBA and NAA in any concentrations of these PGRs (Table 1). However, the treatment that combined IBA + NAA at 200 mg L-1, in each, result in an improvement of 136% of fresh weight of roots when compared with the control treatment (Table 1), improving the quality of adventitious rooting obtained.

Fascella and Zizzo (2009) observed higher rooting capacity (78.8%), and higher number of roots per stem-cutting (13.4) in the treatment with NAA powder at 4,000 mg L-1 compared with the control treatment without auxin in *Euphorbia* x *lomi* hybrids. For successful induction of adventitious rooting of stem cuttings of poinsettia (*E. pulcherrima*) is recommended the use of IBA powder or in solution isolate at 1,500 to 2,000 mg L-1 or in a combination between IBA at 1,000 mg L-1 and NAA at 500 mg L-1 (Lopes 2009). The present paper with croton species showed the best results for propagation of stem cuttings with the same PGRs (IBA alone or combined with NAA) but at lower concentrations, 400 mg L-1 in total than that described by *Euphorbia* genus.

The highest percentage of survival plants (100%) in conditions of pot culture was obtained in IBA 400 mg L-1 and IBA + ANA 200 mg L-1 each (Table 1), showing a relationship not only with the best percentage of rooting, but also with quality of rooting for the success of cutting propagation.

The obtaining of an efficient system, with high percentage of rooting and survival of plants in the field, combined with the reduction of time and costs are a continuous challenge in vegetative propagation, main in shrubs and woody species with economical purposes. The association of works with several factors that result in an increase of rooting with reduction of the time to obtain roots from stem-cuttings, as the testing of types and concentration of auxins used in this experiment is a solution for increasing the ex vitro vegetative propagation system in croton and other species from Euphorbiaceae. In fact, the actual paper is the first that studied the influence of auxins IBA and NAA on rooting of stem-cutting of croton (*Codiaeum variegatum*), an important plant for horticulture ornamental purposes.

In this work there was possible to obtain an efficient protocol (100% of rooting and plant survival) for stem-cutting (10-12 cm in length) propagation of croton (*Codiaeum variegatum*) only when was used a liquid solution of IBA 400 mg L-1 or a combination of two auxins, IBA + NAA at 200 mg L-1 each.

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**REFERENCES**

Almeida E.F.A., da Luz P.B., Lessa M.A., Paiva P.D.O., Albuquerque C.J.B., de Oliveira M.V.C. (2008) Different substrates and environments for mini-ixora (*Ixora coccinea* Compacta ) rooting. Ciência Agrotécnica, 32: 1449-1453.

Benschop M., Kamenetsky R., Le Nard M., Okubo H., De Hertogh A. (2010) The global flower bulb industry: production, utilization, research. Horticultural Reviews, 36: 1-115.

Candido V., Miccolis V., Castronuovo D. and Manera C. (2008) Poinsettia (*Euphorbia pulcherrima*) cultivation in biodegradable pots: mechanical and agronomical behaviour of pots and plants traits. Acta Horticulturae (ISHS), 801: 1563-1570.

Catapan E., Luís M., da Silva B., Moreno F.N., Viana A.M. (2002). Micropropagation, callus and root culture of *Phyllanthus urinaria* (Euphorbiaceae). Plant Cell, Tissue and Organ Culture, 70: 301-309.

CIIAGRO (Centro Integrado de Informações Agrometereológicas) (2012) Garça e Marília. Disposable in <http://www.ciiagro.sp.gov.br/MonitoramentoCafe/MAPAPrincial/Marilia.htm>, Accessed on 07 of September of 2012.

Comar V., Tilley D., Felix E., Turdera M., Neto M.C. (2004). Comparative emergy evaluation of castorbean (Ricinus communis) production systems in Brazil and the U.S. *In*:Ortega, E. & Ulgiati, S. (Eds.): Proceedings of IV Biennial International Workshop “Advances in Energy Studies”, Unicamp, Campinas, Brazil: 227-237.

Epstein E., Ludwig-Müller J. (1993). Indole-3-butyric acid in plants: occurrence, synthesis, metabolism and transport. Physiologia Plantarum, 88: 382-389.

Epstein E., Zilkah S., Faingersh G., Rotebaum A. (1993). Transport and metabolism of indole-3-butyric acid in sterile easy-to-root and difficult-to-root cuttings of sweet cherry (*Prunus avium* L.). Acta Horticulturae, 329: 292–295.

Fachinello J.C., Hoffmann A., Nachtigal J.C., Kersten E., Fortes G.R.L. (1994). Propagação de plantas frutíferas de clima temperado. Editora e Gráfica UFPEL, Pelotas, 179p.

Fascella G., Zizzo G. (2009) Efficient propagation technique for Euphorbia x lomi thai hybrids. HortScience, 44: 495-498.

Han H., Zhang S., Sun X. (2009). A review on the molecular mechanism of plants rooting modulated by auxin. African Journal of Biotechnology, 8: 348-353.

Ibáñez-Torres A. (2004). Rooting experiments with *Euphorbia lagascae* cuttings. Anales de Biología, 26: 101-104.

IES (International Euphorbia Society) (2012). About Euphorbiaceae. Disposable in <http://www.euphorbia-international.org/euphorbias/about_euph.htm>, Accessed on 07 of September of 2012.

Kämpf, A.N. (2000). Seleção de materiais para uso como substrato. *In*: Kämpf A.N., Fermino M.H. (Eds.). Substratos para plantas:a base da produção vegetal em recipientes. Gênesis: 139-145.

Lopez R.G. (2009) Successful poinsettia propagation. OFA Bulletin, 915: 3-5.

Ludwig-Müller J., Vertocnik A., Town C.D. (2005) Analysis of indole-3-butyric acid-induced adventitious root formation on Arabidopsis stem segments. Jornal of Experimental Botany, 56: 2095-2105.

Naidu G.P. (1988) Antifungal activity in *Codiaeum variegatum* leaf extract. Current Science, 57: 502-504

Nasib A., Ali K., Khan S. (2008) *In vitro* propagation of croton (*Codiaeum variegatum*). Pakistan Journal of Botany, 40: 99-104.

Negussie A., Aerts R., Gebrehiwot K., Prinsen E., Muys B. (2009) *Euphorbia abyssinica* latex promotes rooting of *Boswellia* cuttings. New Forests, 37: 35-42.

Neves T.S., Carpanezzi A.A., Zuffellato-Ribas K.C., Marenco R.A. (2006) Enraizamento de corticeira-da-serra em função do tipo de estaca e variações sazonais. Pesquisa Agropecuária Brasileira, 41: 1699 – 1705.

Nissen, S.J., Foley M.E. (1987a) Dormancy and correlative inhibition in root buds of *Euphorbia esula*. Weed Sci 35: 155-159.

Nissen, S.J., Foley M.E. (1987b) Euphorbia escula L. root and root bud indole-3-acetic acid levels at three phonological stages. Plant Physiology, 84: 287-290.

Ono E.O., Rodrigues, J.D., de Pinho S.Z. (1998) Efeito de auxinas e boro no enraizamento de estacas de kiwi retiradas em diferentes épocas. Pesquisa Agropecuária Brasileira, 33: 213-219.

Papafotiou M., Chronopoulos J.G., Kargas M., Voreakou N., Leodaritis O., Lagogiani S. Gazi. (2001). Cotton gin trash compost and rice hulls as growing medium components for ornamentals. Journal of Horticultural Science and Biotechnology, 76: 431–435.

Pop T.I., Pamfil D., Bellini C. (2011) Auxini control in the formation of adventitious rooting. Notulae Botanicae, 39: 307-316.

Tillmann M.A.A., Cavariani C., Piana Z., Minami K. (1994) Comparison among several media for the rooting of cróton (*Codiaeum variegatum* L.) cuttings. Scientia Agricola, 51: 17-20.

van Veldhuisen R. (2006) Some notes on Euphorbia punicea Swartz and relate species. Euphorbia World, 1. Disposable in <http://www.euphorbia-international.org/journal/pdf_files/EW1-3-sample.pdf>, Accessed in 07 of September of 2012.

Vargas L., Borém A., da Silva A.A. (1999) Crossing technique in *Euphorbia heterophylla* L. Bragantia, 58: 23-27.

Table 1 – Influence of types and concentrations of auxins in the rooting of stem cuts of croton (*Codiaeum variegatum*) [Influência de diferentes tipos e concentrações de auxinas no enraizamento de estacas caulinares de croton (*Codiaeum variegatum*)]. Pompéia, 2012.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Auxin (s)** | **Concentration** | **Rooting** |  | **Roots** | | |  | **Plant Survival** |
|  | **(mg L-1)** | **Percentage** |  | **Number** | **Length (cm)** | **FW (g)** |  | **Percentage \*\*** |
| Control | 0 | 83.3 |  | 13.2 b | 1.91 a | 0.45 b |  | 80 |
| IBA | 200 | 83.3 |  | 29.2 ab | 1.34 a | 0.49 b |  | 80 |
|  | 400 | 100 |  | 44.0 a | 1.71 a | 0.67 ab |  | 100 |
| NAA | 200 | 100 |  | 22.5 ab | 1.74 a | 0.70 ab |  | 90 |
|  | 400 | 100 |  | 20.7 ab | 1.63 a | 0.78 ab |  | 90 |
| IBA + NAA | 200 + 200 | 100 |  | 40.5 a | 2.07 a | 1.06 a |  | 100 |
| F |  |  |  | 2.85\* | 0.63 ns | 2.17\* |  |  |
| CV (%) |  |  |  | 31.3 | 42.8 | 11.8 |  |  |