In Vitro Propagation of Croton (*Codiaeum Variegatum* Gold Dust.) using Nodal Explants from Field Grown Plants

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ABSTRACT: This study was carried out in the tissue culture laboratory , the Faculty of Agriculture, Saba basha, Alexandria University, Egypt during the period from 2012 to 2014 . An efficient and reliable protocol for *in vitro* propagation of croton (*Codiaeum variegatum* Gold Dust.) was optimized. However, nodal explants from field grown of Croton (*Codiaeum variegatum* Gold Dust.) were used during *in vitro* culture study for induction of multiple shoots. Nodal explants were inoculated on various initiation or establishment media with different combinations of NAA and BA and the neoformed shoots were cultured on proliferation (multiplication) media for the development of multiple shoots, and the elongation media to elongate of the neoformed shoot. The subsequent elongated shoots were rooted, and acclimatized *ex vitro,* successfully. The best medium for shoot initiation was WPM medium supplemented with 1.0 mg/l BA. The favourable medium for multiplication was the tested medium augmented with 2.0 mg/l BA and 0.50 mg/l NAA. In addition, the most effective medium for elongation was the used medium enriched with 1.0 mg/l NAA. Furthermore, th *in vitro* shoots showed healthy root development when the tested medium was supplemented with combination of 2.0 mg/l IBA and 0.50 mg/l NAA (rooting stage).The combination of sand: peat moss (4:1) was used as substratum for the hardening of the in vitro plantlets, as a potting mix, was the best suited mix for the acclimatization of plantlets.

Key words: *In vitro* culture, *Codiaeum variegatum*, nodal explants, initiation, multiplication, rhizogenesis, acclimatization

INTRODUCTION

Garden croton grows naturally in southern Asia, Indonesia, and other Eastern Pacific islands where it grows in open forests and scrub. It is an evergreen shrub growing up to 6 m in height but usually maintained at 60 to 90 cm and grows well in areas having humid climate. The family *Euphorbiaceae* comprises nearly 322 genera and 8910 species (Bingtao *et al*., 2008) many of which have their own economic value and hence contribute to the floristic wealth of tropical and subtropical countries of the world. The family comprises a number of endemic and endangered taxa. However the in vitro studies are confined only on a few genera of aesthetic, medicinal, timber yielding, rubber yielding, dye yielding, cottage industries, ornamental and food crops like *Acalypha, Baliospermum, Codiaeum*, *Cleistanthus, Croton,Euphorbia,Emblica, Eryngium, Excoecaria, Givotia, Glochidion, Hevea, Jatropha, Mallotus, Manihot,Phyllanthus, Putranjiva, Ricinus, Sapium and Uapaca* (Rajesh-Kondamudi *et al.,* 2009). In addition to its aesthetic value as an indoor plant, crotons are also well known for its medicinal value. 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). Generally, crotons are multiplied vegetatively by averages of cuttings and air layering. These processes are slow in response and requires large number of mother/stock plants. Despite its slow rate of conventional

multiplication, the plant is very high in demand (Deepa and Shanthi,2013) . Hence, micropropagation is an alternative averages of propagation, to meet its high demand in relatively shorter time. For instance, from shoot tip cuttings, one mother/stock plant can yield only 20 plants per year (Nasib et al.,2008; Mulabagal and Tsay,2004). Micropropagation is a relatively new technology and application of innovative method that have served to overcome barriers to progress in the multiplication of elite species and further improvements are anticipated (Nasib *et al*.,2008 ; Ashish and Sharma, 2011) . *In-vitro* growth and development is considerably influenced by several factors like genotype, the age and size of mother plant and explants, the season, growth conditions, media composition, and various other physiological factors (Ashish and Sharma, 2011). Also, as a averages of securing pathogen free plants, culture of shoot apical meristem is ideal. Other advantages in this method include rapid multiplication of plants within shorter period of time irrespective of the season (Mulabagal and Tsay, 2004). Keeping the above points in mind, chosen croton was for micropropagation due to its rare success in conventional breeding and also due to the meager availability of data for *in-vitro* production (Shibata *et al*., 1996; Orlikowska *et al.*, 2000). An improved and enhanced method was established for the *in-vitro* propagation of croton. Croton is an evergreen shrub with alternate, simple leaves mottled with white, yellow, or red flower. Flowers are small, long, axillary, usually unisexual racemes antiamoebic, and anticancerous activities (Kupchan *et al.,* 1976). The plant may change colour as it matures (Ogunwenmo *et al.,* 2007). Fruits are globular capsules and 3 to 8 mm in diameter.Hence, this species has been selected for the different morphology and color combination of leaves, with contrasting veins. The leaves are alternate, non-serrated but sometimes lobed. The leaf extracts of crotons are reported to have many medicinal properties including purgative, sedative, antifungal, Croton (*Codiaeum variegatum* ,Gold Dust) with its amazing colors and leathery leaves is regarded as a beautiful foliage plant commonly known as croton and sometimes called Joseph's Coat orvariegated croton (Nasib *et al.,* 2008).Propagation of croton by rooting of softwood cuttings has been a good development. Some authors have investigated how different compounds of the substrate can improve root induction (Tillmann *et al* .,1994; Chen *et al*., 2000 ;Dai- Bisheng, 2007). The present study was aimed to establish an efficient and reliable protocol for *in vitro* propagation with focusing on rhizegenesis of this hard –to- root species.

MATERIALS AND METHODS

Plant material and explants sterilization

The plant material was collected from shrubs grown in garden of Ornamental and landscape, of the Research Department of EL-Montazh, Alexandria, Egypt. 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 explants' source in August. The collected material was brought to the plant tissue culture laboratory of the Plant Production Department of the Faculty of Agriculture ,Saba Basha, Alexandria University during 2012-2014 seasons and washed, thoroughly, with running tap water for 30 minutes to remove the dust or sand particles. The shoot tips were cut to nodal segments (single node) as an

explants source (Bhattatacharya *et al*., 1990).The excised explants were dipped in 70% ethanol for 60 sec. After treatment with ethanol the explants were rinsed with double distilled water twice, so as to lower the toxic effect of ethanol. The nodal segment's surfaces were sterilized using 20% of sodium hypochlorite for 20 minutes and 1.5 mg/l mercuric chloride for 5 min. Few drops of Tween-20 , also, were added as a surfactant to sterilized water with sterile gentle shaking under sterile conditions . After 20 minutes the plant material was washed three times with sterilized water and became ready for culture.

Microprogation stages

1-Intiation stage

The explants were cultured on solidified woody plant medium coined as WPM (Lloyd and McCown, 1980) which contained different concentrations of the cytokinin benzyl adenine (BA) at four concentrations:0.0(nil), 0.5,1.0 and 1.5 mg/l,in combinations with the auxin Naphthalene acetic acid (NAA) at four concentrations: 0.0(nil) ,1.0, 2.0 and 3.0 mg/l.Three explants were cultured in each jar which containing 30ml of medium and were placed, vertically. Each treatment was replicated three times and each has 3 explants (i.e.9 explants /treatment).The jars were capped with aluminum foil closures. The cultured jars were incubated in growth chamber at $25\pm1^{\circ}$ C temperature under 16 hr daily light and 8hr darkness illumination by a florescent light intensity of 2880 Lux (40μ mol $m^{-2}S^{-1}$ PPF).

2-Multiplication stage

The neoformed propagule of the initiation stage was sectioned into single leaflet node. The excised nodal cutting explants of the different positions were cultured onto the multiplication medium which was woody plant medium (WPM) supplemented with BA at four concentrations: 0.0(nil), 1.0, 2.0 and 3.0 mg/l, in combinations with NAA at four concentrations:0.0(nil) ,0.25, 0.50 and 0.75 mg/l.

3-Rooting (rhizogenesis) stage

The obtained shoots of croton from the multiplication stages were, individually separated and cultured on a rooting medium for rhizogenesis to achieve this stage ,two types of auxins were used as Indole butyric acid (IBA) at four concentrations: 0.00(nil),1.00, 2.00 and 3.00 mg/l , in combinations with NAA at five concentrations:0.00 ,0.25 ,0.50 ,0.75 and1.00 mg/l. Generally, the data were recorded per propagule at initiation, multiplication and rooting stages after 35 days in culture. The tested characters were as follows:

-Average shoot length(cm)/propagule.
-Average number of shoots formed/ propagule.
-Average number of leaflets formed/ propagule.
-Average number of nodes formed/ propagule.
-Average number of roots formed/ propagule.
-Average root length(cm)/ propagule.(at rooting stage)
-Average rooting time / propagule. (at rooting stage)

4-Acclimatization stage

The neoformed plantlets (rooted shoots) were then transferred to the greenhouse for hardening. The potting mix used in this study comprised of sand and peat moss (4:1) .The transferred plants were monitored weekly for at least 6 weeks.

Statistical analysis

A completely randomized design was used for all the experiments (Gomez and Gomez, 1984).Recorded data were analyzed, statistically, using analysis of variance technique (ANOVA) and averages were compared by Duncan's multiple range test (Steel *et al*.,1997) and significance was determined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Achievement of optimal and reliable system for micropropagation of croton (*Codiaeum variegatum* Gold Dust) was urgent and in focus. Therefore, a set of experiments was conducted, and the obtained results were presented and discussed in the following section as follows:

1-Initiation stage

Data presented in Table (1) and Fig. (1) exhibit that both applied growth regulators' levels and their combinations exerted highly significant effects on the initiation stage's characters of croton ,where single nodal explants were grown *in vitro* for 35 days . Respecting the main effect of BA, the highest average value of shoot length (2.78 and 2.75 cm) was recorded at the absence of BA or its existence in the culture medium at 0.50mg/l. While, supporting the culture medium with NAA at 2.00 mg/l, gave rise to the highest average value (3.65 cm). Regarding the average number of shoots that formed per propagule, the obtained results of BA manifested that BA at 1.0 mg/l gave the highest number of shoots (1.51) which expressed, significantly, the highest average value comparing to the other treatments .In this respect, BA levels consider as in favour of stimulation cell division , morphogenesis (shoot initiation/bud formation) in tissue culture, and break of apical dominance and release growth of lateral buds (Raven,1992;Salisbury and Ross,1992;Davies,1995) and their combinations exerted highly significant effects on the initiation stage's characters of croton ,where singles nodal explants were grown *in vitro* for 35 days).

Table (1). Effect of different levels of BA and NAA (mg/l) and their combinations on the initiation stage of croton cultured *in vitro* for 35 days.

Characters	NAA levels (mg/l)	BA levels (mg/l) 0.00	0.50	1.00	1.50	Average NAA	Significance BA	NAA	BA X NAA
(a)Average shoot length (cm)/ propagule:									
	0.00	1.26	2.57	2.61	2.26	2.18	**	**	**
	1.00	4.37	3.47	3.23	3.53	3.65			
	2.00	3.44	2.78	2.45	2.43	2.77			
	3.00	2.05	2.19	1.78	1.98	2.00			
Average (AB)		2.78	2.75	2.52	2.55				
L.S.D.(0.05)							0.11	0.11	0.22
(b)Average number of shoots formed/propagule:									
	0.00	0.33	1.11	1.33	1.16	0.98	**	**	**
	1.00	0.60	1.88	2.33	1.49	1.58			
	2.00	1.11	0.76	0.88	1.33	1.02			
	3.00	0.55	0.66	1.72	0.83	0.94			
Average(BA)		0.65	1.16	1.51	1.20				
L.S.D.(0.05)							0.27	0.27	0.55
(c)Average number of leaflets formed/propagule:									
	0.00	2.55	4.61	5.66	6.00	4.70		**	**
	1.00	6.50	5.88	5.78	5.27	5.86			
	2.00	6.27	6.27	5.11	5.66	5.83			
	3.00	5.72	5.61	4.55	4.33	5.05			
Average(BA)		5.26	5.59	5.27	5.31				
L.S.D(0.05)							0.32	0.32	0.65
(d)Average number of nodes formed/propagule:									
	0.00	1.44	2.78	2.72	2.83	2.44		**	**
	1.00	5.50	4.44	4.39	5.50	4.95			
	2.00	5.05	4.27	3.99	3.77	4.27			
	3.00	3.50	3.55	4.16	4.16	3.84			
Average (BA)		3.87	3.76	3.81	4.06				
L.S.D.(0.05)							0.24	0.24	0.48
(e)Average number of roots formed/propaglue:									
	0.00	0.22	2.33	0.00	0.00	0.63		**	**
	1.00	3.72	2.99	1.66	0.36	2.18			
	2.00	5.11	3.55	3.33	3.77	3.94			
	3.00	2.33	1.33	0.66	0.00	1.08			
Average (BA)		2.84	2.55	1.41	1.03				
L.S.D.(0.05)							0.50	0.50	1.00

L.S.D.(0.05)=Least significant difference teast at 0.05 level of probability

*,**:Significant or highly significant.

With regared to the number of leaflets formed, the obtained data disclosure that BA at 0.50 mg/l, brought about the highest average value (5.59). On the other hand, augmenting the WPM with NAA at either 1.00 or 2.00 mg/l led to the highest average values (5.86 or 5.83, orderly) comparing to the other treatments. Meanwhile, the interaction between nil level(0.00 mg/l) and 1.00 mg/l BA and NAA, respectively, gave the highest average value (6.50 leaflets/explants). As for the average number of nodes formed /propagule, the obtained results showed that BA at 1.50 mg/l, led to the highest average value (4.06). On the other side, supplemented the tested medium with NAA at1.00 mg/l, gave the highest average value (4.95) comparing to the other treatments.

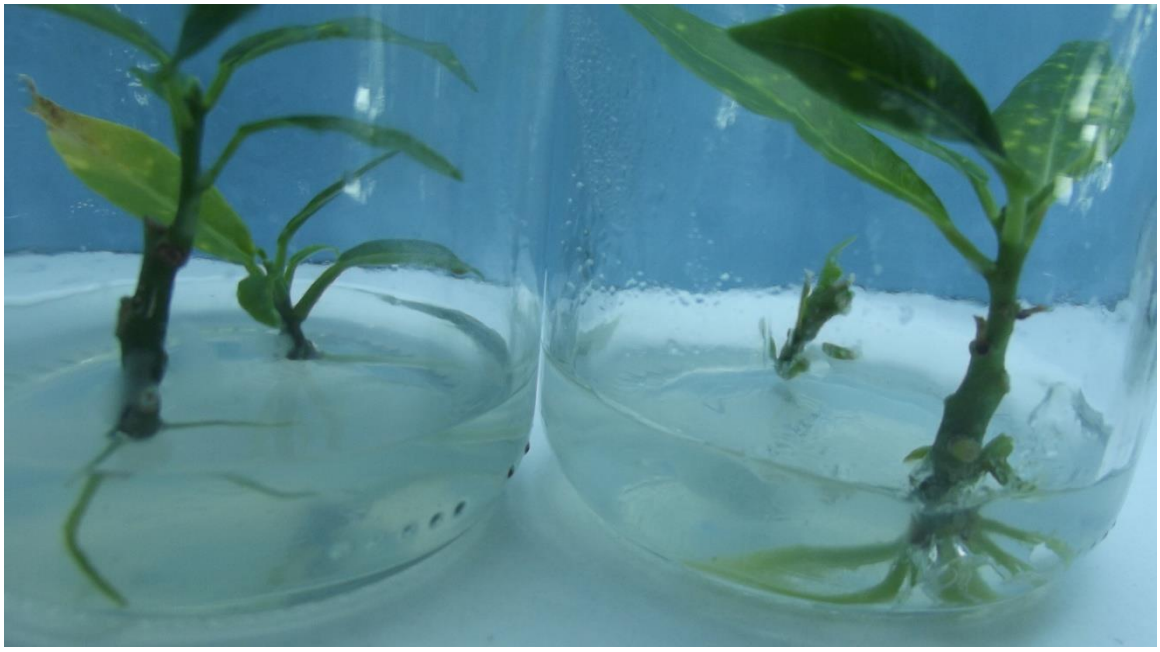

Figure (1): Initiation stage of croton nodal explants cultured on WPM +1mg/l NAA only.

On the other hand, the main effect of NAA, the obtaind data declared that NAA at 1.0 mg/l, resulted in the highest average values of the most studies characters except rooting trait. As regarded to the average number of roots/ propagule, the obtained results expressed that BA at either nil (0.00 mg/l) or 0.50 mg/l, led to the highest average values (2.84 or 2.55, consecutively).While BA at 2.00 mg/l , at 2.00 mg /l, gave the highest average value (3.94).The interaction between BA at 0.00 and NAA at 2.00 mg /l, resulted in formation the highest rooting mass (5.11). Decreseasing the average values of the studied characters (length of shoot and number of roots) were concomitant with increasing BA. WPM This finding could be due to accumulation supra-optimal level of cytokinin within tissues which exerts adverse effects on growth performance (Murashige,1974;Tomas,1987;George *et al*.,2008).Hence , medium without BA resulted in the highest average value of shoot length was taken place. This finding could be attributed to the mode of action of auxin NAA within cultured tissues which is capable of controlling various distinctive processes such as cell growth and elongation (George and herrington,1984;Georrge *et* al.,2008).On the other hand, extreme the lowest

concentration of NAA used, affected well the initiation stage of croton *in vitro.*This might be owing to the reason that higher concentration of NAA, which is usually ineffective against shoot proliferation (Vijaya *et al*.,1991;Waseem *et al*.,2011).Furthermore, it is known the role and mode of action of auxin for their abilities to enhance root formation, as stated by research workers(Chen *et al.,*1985; Liu *et al*.,1994;George *et al*.,2008;Waseem *et al*.,2011). Silva *et al.,* (2013) reported that the longest shoots on croton production being produced on medium supplemented with 1.0 mg/l NAA, and the combination of NAA and IBA at a ratio of 1:1mg/l of BA and NAA ,respectively led to the production of both number of leaves and longest shoots after a 60 days of subculture period , successfully, especially when cultures were initiated from explants taken from sprouted shoots of croton (*Codiaeum vareiegatum* , L.). The establishment and multiplication stages were possible when 1mg/l BA or 1mg/l IAA and 3 mg/l 2ip were added to the selected cultivar, respectively (Radice, 2010). Chitra and Madhusoodanan (2005)who studied the influence of auxins in direct *in vitro* morphogenesis of *Euphorbiaceae.* Rout *et al.* (2006) reviewed critically on present scenario and future prospects of tissue culture. However Indole butyric acid (IBA) induced the rooting very effectively and various studied characted plants established in the soil. (Ahn *et al*., 2007).

2-Multiplication stage

Results presented in (Table 2) and Fig. (2) revealed that both applied growth regulators and their combinations affected highly singinficantly the studied characters of multiplication stage .However, regarding the average shoot length (cm) / propagule, BA was in adverse relationship in the given trait; whereas BA level increased, the studied trait decreased ,therefor the nil level (0.00 mg/l) gave the highest average value (2.17).On the contrary, NAA levels were in proportional relationship, where, as the levels increased the highest average values increased, especially at 0.50 or 0.75 mg/l, which gave the highest average values (2.42 or 2.49, consecutively).Meanwhile, the interaction between BA at nil level and NAA at 0.50 mg/l ,recorded the highest average value (2.76).As for the average number of shoots formed / propagule, the main effect of BA showed that augmenting the culture medium with BA at 2.00 mg/l, led to the highest average value (3.06).On the other hand, providing the culture medium with either 0.50 or 0.75 mg/l of NAA, resulted in the highest average values (2.73 or 2.65,each in turn)

Meanwhile, the combination of BA and NAA at 2.00 and 0.50 mg/l, respectively led to the highest average value (3.89).With reference to the average number of leaflets formed/ propagule, fortifying the tested medium with either 1.00 or 2.00 mg/l of BA, resulted in the highest average values of the given trait (7.51 or 7.59,each in turn), comparing to the other treatments. On the other hand ,as NAA added to the culture media increased the gained values increased , especially at either 0.50 or 0.75mg/l, led to the highest average values (7.87 or 7.88, consecutively) of the given trait. But the interaction between BA and NAA at 2.00 and 0.50mg/l , in series resulted in the highest average value (7.22) of the tested trait .

Table (2). Effect of different levels of BA and NAA (mg/l) and their combinations on the multiplication stage of croton cultured *in vitro* for 35 days.

Characters	NAA levels (mg/l)	BA levels (mg/l) 0.00	1.00	2.00	3.00	Average NAA	Significance BA	NAA	BA X NAA
(a)Average shoot length(cm/propaglue:									
	0.00	1.01	1.67	1.39	1.01	1.27	**	**	**
	0.25	2.33	2.16	1.69	2.13	2.08			
	0.50	2.76	2.32	2.61	2.00	2.42			
	0.75	2.57	2.11	2.61	2.66	2.49			
Average(BA)		2.17	2.07	2.07	1.95				
L.S.D.(0.05)							0.09	0.09	0.18
(b)Average number of shoots formed/ propagule:									
	0.00	0.33	1.66	2.61	1.33	1.48	**	**	**
	0.25	0.72	2.27	2.60	1.05	1.66			
	0.50	1.44	3.22	3.89	2.38	2.73			
	0.75	1.72	3.11	3.16	2.61	2.65			
Average(BA)		1.05	2.56	3.06	1.84				
L.S.D.(0.05)							0.30	0.30	0.61
(c)Average number of leaflets formed/ propaglue:									
	0.00	3.44	5.55	6.00	5.88	5.22	**	**	**
	0.25	6.55	6.99	6.39	8.00	6.98			
	0.50	7.89	8.27	8.66	6.66	7.87			
	0.75	7.11	9.22	9.33	5.89	7.88			
Average(BA)		6.25	7.51	7.59	6.61				
L.S.D.(0.05)							0.40	0.40	0.80
(d)Average number of nodes formed/ propaglue:									
	0.00	2.44	5.89	5.16	2.00	3.87	**	**	**
	0.25	3.89	5.11	6.27	6.05	5.33			
	0.50	4.16	4.50	7.22	7.22	5.77			
	0.75	5.44	5.33	6.33	7.05	6.04			
Average(BA)		3.98	5.20	6.24	5.58				
L.S.D.(0.05)							0.49	0.49	0.99
(e)Average number of roots formed/ propaglue:									
	0.00	0.29	0.94	0.33	0.00	0.38	**	**	**
	0.25	3.66	3.50	0.72	1.39	2.31			
	0.50	5.00	1.78	3.44	2.00	3.05			
	0.75	6.28	3.55	3.38	4.11	4.33			
Average(BA)		3.80	2.44	1.97	1.87				
L.S.D.(0.05)							0.55	0.55	1.11

L.S.D.(0.05)=Least significant difference test at 0.05 level of probability
*,**:Significant or highly significant.

Figure (2): Multiplication stage neoformed croton shoot of initiation stage culture on WPM+ 2.0mg/lBA+0.50 mg/l NAA.

Respecting the average number of roots formed / propagule , the obtained results, divulged that the absence of BA from the culture medium, brought the highest average value of the tested trait (3.80),then as the BA levels increased the given results decreased . For NAA levels, the highest average value of the given trait (4.33) was recorded due to augmenting the culture medium with NAA at 0.75 mg/l., comparing to the other treatments. In general , these results could be brought about to the mode's action of cytokinins on stimulation both cell division and promotion growth of axillary shoots in plant tissues culture as, also, found by Tomas(1987),Triginano and Gray(2000) and George *et al*. (2008). Nasib *et al*. (2008) grew the shoot tip explants of *Codiaeum variegatum* on MS + BAP (0.5mg/l) + peptone (25mg/l). Sana (2012) reported that enhanced shoots and buds proliferation formation can be achieved by using the MS media with 2 mg/l of both KIN and BA. The higher concentration of these hormones (5mg/l each) resulted in shoot formation of various cultivars of *Codieaum variegatum* (L.). Martin *et al*. (2005) reported the influence of auxins in direct *in vitro* morphogenesis of mesophyll cells of *Euphorbia nivulia*, where the KIN reduced the rate of morphogenesis, whereas BAP induced somatic embryogenesis. The combination of BA with NAA and IAA had positive effect on morphogenesis. The shoot tips of *Glochidion multiloculaire* produced multiple shoots when cultured on MS + BA (1.0 mg/l) and IAA (1.0 mg/l). Callus was derived from the leaf and stem explants on a medium containing 2,4-D (0.5-2.0

mg/l),and produced shoot buds when transferred to MS+ BA (1.0-2.0 mg/l) + CM (Coconut milk, 10% v/v)(Yamuna *et al*., 1995). Bhot *et al.* (2010) concluded that nodal explants from field grown plants of *Codiaeum variegatum* (L.) Blume when inoculated on B5 medium fortitified with NAA (0.1mg/l), BAP (2.0 mg/l) and phloroglucinol (100 mg/l), showed the highest number of shoot bud development and proliferation in var. Undulatum while with spermidine (0.1mg/l), BAP(2.0 mg/l) and phloroglucinol (100mg/l) showed shoot bud development and proliferation in var. *Norwood Beauty* and *Punctat aureum.* With reference to the rooting mass per propagule, this finding might be taken place due to the well-known role of auxins in inducing of root formation (Tomas, 1987 and George *et al*.,2008).

3-Rooting (rhizogenesis) stage

Results in Table (3) and Fig. (3) showed that the applied both auxin levels and their combination exerted, significant effects on the studied characters of croton, except, the average number of node formed /propagule, whereas, However the interaction between IBA at 2.00 mg/l and NAA at 0.50mg/l. gave the highest nmber of root and root length per propagule (11.78, 3.96),respectively and also gave the high level of shoot length (4.02) which may equal at the same average the highest shoot length(4.08) was produced when medium supplemented with NAA at1.00 mg/l and IBA at nil(0.0).The data showed significant effect on the number of shoots characters when applied both growth regulators,using NAA at 0.75 gave the highest average effect (1.27).While using IBA at 2.00 mg/l gave high average effect comber to anther concentration of IBA (0.94) .On other hand ,the interaction between IBA and NAA gave the highest average number of shoots per propagule (1.72).But the lowest average of rooting time produced with IBA at 3.00 mg/l and NAA at1.00 mg/l (10.27).As for the average number of leaflets formed/ propagule ,the main effect of IBA and NAA showed that augmenting the culture medium with IBA at 2.00 mg/l and NAA at 0.25 gave the highest average value(8.89).

This results coud be explaind on the bases that auxin induced number of responses which involved cell division, cell enlargement, protein and nucleic acids synthesis which are concomitants of auxin-induced growth and changes in wall plasticity of plant cell and increase the apical dominance as there are essential and rapid processes involved in growth and elongation (Wilkins,1989).Our results were further confirmed by the previous findings of Komalavalli and Rao (2000); Sarker and Shaheen(2001); Munshi *et al.*(2004);Awal *et al*.(2005);Rajani and Patil(2009); Waseem *et al.*(2011) who suggested IBA as the best auxin for root induction and development. Nasib *et al*. (2008) grew the shoot *Codiaeum variegatum* which enhanced the rooting was induced on MS+ IBA (2.0mg/l) medium, and they were acclimatized with 95% survival rate. Chitra and Madhusoodanan (2005) studied the influence of auxins in direct *in vitro* morphogenesis of Euphorbiaceae. Rout *et al.* (2006) reviewed critically on present scenario and future prospects of tissue culture of some *Euphorbiaceae* members. While Indole butyric acid (IBA) induced the rooting very effectively .

Table(3).Effect of different levels of IBA and NAA (mg/l) and their combinations on the rooting stage of croton cultured *in vitro* for 35 days.

Characters	NAA levels (mg/l)	BA levels (mg/l) 0.00	1.00	2.00	3.00	Average NAA	IBA	Significance NAA	IBA	IBA X NAA
(a)Average shoot length(cm)/ propagule:										
	0.00	1.01	2.06	2.48	2.24	1.94		**	**	**
	0.25	2.94	3.34	2.47	2.23	2.74				
	0.50	3.04	3.12	4.02	3.89	3.52				
	0.75	3.56	3.31	2.71	2.22	2.95				
	1.00	4.08	3.49	2.97	2.86	3.30				
Average(IBA)		2.92	3.06	2.93	2.65					
L.S.D.(0.05)								0.19	0.21	0.42
(b)Average number of shoots formed/propagule:										
	0.00	0.00	0.00	0.83	0.00	0.20		**	**	**
	0.25	1.16	0.66	0.55	1.33	0.93				
	0.50	1.00	0.88	1.61	1.33	1.20				
	0.75	1.11	1.00	1.72	1.27	1.27				
	1.00	0.00	0.00	0.00	0.00	0.00				
(IBA)		0.65	0.50	0.94	0.78					
L.S.D.(0.05)								0.16	0.17	0.35
(c)Average number of leaflets formed/propagule :										
	0.00	2.38	5.78	5.66	6.00	4.95		ns	**	**
	0.25	7.22	5.55	8.89	4.89	6.46				
	0.50	6.89	7.00	6.00	6.11	6.50				
	0.75	8.44	7.11	5.72	5.94	7.05				
	1.00	7.33	4.83	5.89	5.00	5.76				
Average(IBA)		6.45	6.05	6.43	5.78					
L.S.D.(0.05)								0.39	0.43	0.87
(d)Average number of nodes formed/propogule:										
	0.00	2.00	3.39	3.27	2.72	2.84		**	**	**
	0.25	3.55	3.11	3.11	4.66	3.61				
	0.50	3.11	4.49	3.78	3.33	3.68				
	0.75	4.22	3.33	3.77	3.94	3.81				
	1.00	4.22	3.88	3.89	3.00	3.75				
Average(IBA)		3.42	3.64	3.56	3.53					
L.S.D.(0.05)								0.34	0.34	0.68
(e)Average number of roots formed/propaglue:										
	0.00	0.44	4.55	4.94	5.44	3.84		**	**	**
	0.25	5.77	6.72	7.38	9.44	7.33				
	0.50	7.38	7.33	11.78	7.50	8.50				
	0.75	7.33	7.33	5.77	7.11	6.88				
	1.00	5.16	6.55	7.22	6.44	6.34				
Average(IBA)		5.22	6.49	7.42	7.18					
L.S.D.(0.05)								0.42	0.47	0.94

To be Continued..

Table (3) Continued..

(f)Average root length(cm)/propagule:								
0.00	0.23	1.20	1.43	1.80	1.16	**	**	**
0.25	1.43	2.28	1.63	1.50	1.71			
0.50	1.61	3.10	3.96	3.33	3.00			
0.75	2.33	2.56	2.70	2.60	2.55			
1.00	2.83	2.33	2.26	2.13	2.39			
Average(IB)	1.68	2.29	2.40	2.27				
L.S.D.(0.05)						0.19	0.21	0.43
(g)Average rooting time/ propagule:								
0.00	35.00	31.83	32.16	28.22	31.80	**	**	**
0.25	34.00	34.11	20.99	19.33	27.11			
0.50	18.22	20.50	16.78	15.27	17.69			
0.75	31.38	21.54	18.89	24.22	24.01			
1.00	17.27	19.33	11.94	10.27	14.70			
Average(IBA)	27.17	25.64	20.15	19.46				
L.S.D.(0.05)						0.89	0.99	1.99

L.S.D.(0.05)=Least significant difference test at 0.05 level of probability *,**:Significant or highly significant.

Figure (3):Rhizogenesis of neoformed croton shoot of multiplication stage grown on WPM+2.0 mg/l

4-The fourth stage (acclimatization)

Acclimatization of *in vitro* grown plants is an important step in micropropagation (Smart, 2008; Rout *et al*.,2006).The *In vitro* grown plantlets with at least two to three roots were transferred to the greenhouse for the acclimatization *ex vitro*. The potting mix (sand and peat moss,4:1), 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 90% as shown in Fig. (4).

Figure (4):Acclimatized croton tissue culture plants derived plants *ex vitro*.

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الملخص العربي

الاكثار المعملى الدقيق لنبات الكروتن باستخدام اجزاء العقل الساقية للنباتات النامية حقليا

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اجريت هزه الدراسه في معمل زراعة الانسجه - قسم الانتاج النباتى– كلية الزراعه – سابا باشا- جامعة لاسكندريه خلال السنوات ما بين 2012- 2014 لتطوير أو إيجاد بروتوكول فعال للأكثار المعملى الدقيق لنباتات الكروتن"جولد دست" . ولقد تم أستخدام عقل ساقية من نباتات الكروتن النامية بحدائق قسم بحوث الزينه بقصر المنتزه (النباتات الأم) خلال دراسة معملية لأستحثاث اكثار (تضاعف) المجاميع الخضرية . تم زراعة الأجزاء النباتية العقدية على بيئات مغذية للتدشين او البدء باستخدام توليفات مختلفة من الأوكسين (NAA) والسيتوكينين (BA)، و تمت زراعة المجاميع الخضرية المتكونة خلال مرحلة البدء او التدشين علي بيئات مختلفة للتضاعف او الأكثار للحصول على اعداد كبيرة (متضاعفه) من تلك المجاميع الخضرية ، ثم أستطالتها و كذلك تجذيرها ، هذا بالأضافة الى اقلمة تلك النبيتات خارج المعمل ، بنجاح . كانت افضل بيئة لتدشين او بدء المجاميع الخضرية تحت الظروف المعملية هى بيئة أكثار النباتات الخشبية (WPM) المزودة بالسيتوكنين BA بتركيز 1

ملليجرام/ لتر . و كانت بيئة التضاعف او الاكثار هى نفس البيئة المزودة 2 ملليجرام/لتر من السيتوكنين بالأضافة الى 5,. مللجرام/ لتر من الاوكسين NAA . و كانت أفضل بيئة للأستطالة هى نفس البيئة المزودة بالأوكسين NAA عند تركيز 1مللجرام/لتر . و الأكثر من ذلك، عند تعريض تلك المجاميع الخضرية النامية معمليا لتراكيز مختلفة من الأوكسين NAA و كذلك IBA اظهرت مجاميع جذرية قوية و سليمة ، خاصة عند تزويد البيئة بالأوكسين IBA عند تركيز 2 مللجرام/ لتر ، والأوكسين NAA عند تركيز 5,. مللجرام/لتر (مرحلة التجذير) كما ان الخلطة من الرمل : البيتموس (1:4) لأقلمة تلك النباتات ناتج زراعة الأنسجة كانت الأفضل في هذا الصدد .