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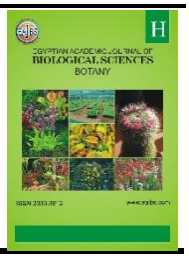

In vitro Propagation and Caulogenesis of *Dracaena draco* Plants

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ABSTRACT

Dracaena draco is a monocot from family Asparagaceae. The dragon tree is a subtropical plant and features many botanic and Mediterranean gardens worldwide. *D. draco* has been categorized as vulnerable in Europe and endangered worldwide in the IUCN Red List of Threatened Species (IUCN, 1998). The aim of this study is to develop an efficient protocol for rapid *in vitro* propagation and caulogensis of *D. draco* Plants via enhanced axillary bud proliferation from single nodal explants cultured on full strength MS medium, 30g/l sucrose, 4g/l gelrite augmented with various concentrations of plant growth regulators as BA, NAA, IBA and their combinations. In general, the present study concluded that the best results for the initiation stage were recorded on MS medium fortified with NAA and BA at 2.00 and 1.00 mg/l, respectively. Meanwhile, supplemented medium with BA and NAA at 4.00 and 1.00 mg/l, respectively, gave rise to the best results for the multiplication stage. Regarding rhizogenesis stage, the best results were recorded when the neoformed shoots of the multiplication stage were divided singly and cultured on MS medium plus IBA and NAA at 1.00 and 0.50 mg/l, respectively which led to the highest mean number of roots formed per propagule. For callus induction, the internodal segments cultured into MS medium supplemented with BA and NAA at 0.50 and 2.00 mg/l, respectively, gave rise to the best results for the percentage of explants formed callus and callus size. Concerning the percentage of direct organogenesis and number of shoots formed per explants best results obtained from BA and NAA at 0.250 mg/l and 0.00 mg/l, respectively.Neoformed plantlets were acclimatized *ex vitro* and *in vivo* vigorously in a mixture of perlite and peat moss at (1:1) or (2: 2) or (2:3) and (3:3) consecutively, in addition to fixed volume (1 portion) of sterile sand; which resulted in the highest mean value of survival percentage/plant (100%) and showed true-to-type plants *ex vitro*.

INTRODUCTION

The genus *Dracaena* comprises about 60–100 species of woody-stemmed plants in tropical regions of Africa and Asia (Bailey 1949, Bos1998). *Dracaena draco* is a monocot from the family Asparagaceae (subfamily Nolinoidae, Chase *et al.* 2009) with secondary growth and a tree-like habit (Halle´ *et al.*1978). The name *Dracaena* is derived from the Greek word ‘drakainia’ meaning a female dragon (Stern, 1992). The dragon tree is a subtropical plant and a relic of an ancient Mio-Pliocene Southern Tethys flora (Marrero *et*

al., 1998) endemic to a few Atlantic Ocean archipelagos and North Africa (Cabrera Pe´rez 1999). As an iconic plant and an herbal symbol of the Canary Islands, it features manybotanic and Mediterranean gardens worldwide. The plant can attain a massive size and reach very old age, the plant is characterized by a single or multiple trunks growing up to 12 m tall, with a dense umbrella-shaped or mushroom-like shape canopy of thick leaves (Symon 2000, Chen *et al.,* 1998).This shape comes from the growth pattern called dracoid habitus (Beyhl 1996) that requires each branch to grow only until it flowers and then to re-branch from its terminal buds. This is an adaptation of the tree to its Macaronesian habitat (Beyhl 1995). It grows slowly, requiring about ten years to reach 1 m tall (Monteiro *et al.,* 1999, von Humboldt 1850, Gebauer 2009). Young trees have only a single stem; branching occurs when the tree flowers, when two side shoots at the base of the flower panicle continue the growth as a fork in the stem. Some specimens are believed to be up to 650 years old; the oldest is growing at Icod de Los Vinos in Northwest Tenerife (Krawczyszyn and Krawczyszyn 2016, Lengálová 2020). The massive trunk seems to arise from the contribution of aerial roots that, growing from the low branches, join it increasing its waist (Krawczyszyn and Krawczyszyn 2014). *Dracaena draco* possesses several medicinal properties and is used as an herbal remedy in traditional medicine (Mimaki *et al.,* 1999). Voyagers to the Canary Islands in the 15th century obtained Dragon's blood as dried garnet-colored drops from *Dracaena draco* a native to the Canary Islands and Morocco. The Canarian dragon tree *D. draco* was first described in 1402 (Boutier and Le Verrier, 1872). The resin is exuded from the wounded trunk or a branch of the plant (Deepika *et al.,* 2008).The resin obtained from the trunk is used in traditional medicine [International Union for Conservation of Nature (IUCN), Bañares *et al.*1998]. There are two tissues responsible for the red resin secretion that differ in terms of location and morphology, these are the groups of ground parenchyma cells of both primary and secondary origin and the cells of the cortex containing polyphenolic-like inclusions(Joanna and Mirela 2015). On the basis of the assessment of the World Conservation Monitoring Center (1998) using a now-outdated set of criteria (Version 2020-3), *Dracaena draco* has been categorized as vulnerable in the IUCN Red List of Threatened Species (IUCN, 1998) on the Canary Islands due to overexploitation of the trees for Dragon's Blood in the middle ages (Lucas and Synge, 1978, Bañares *et al.* 1998). Three compounds (Bexarotene, Taspine and 2-hydroxy-1-naphthaldehyde isonicotinoyl hydrazone) from the resin of *D. draco* with anti-inflammatory, and pro-proliferative as well as antimicrobial activities isolated (Ticona *et al.,* 2020). Most *Dracaena* species are propagated vegetatively by stem cuttings, but some species such as *D. draco* and *D. ombet* are mainly propagated by seeds (Vinterhalter and Vinterhalter, 1997). Micropropagation offers many advantages because it potentially can facilitate large-scale production of valuable clones and allow plant reintroduction in its natural ecosystem (Miller and Murashige 1976, Blanco *et al.,* 2004). In vitro propagation methods have been developed for a few ornamental Dracaena sp., including *D. surculosa* (Miller and Murashige, 1976; Liu *et al.,* 2010), *D. deremensis* (Badawy *et al.,* 2005; Blanco *et al.,* 2004; Debergh, 1976), *D. fragrans* (Debergh, 1975, 1976; Debergh and Maene, 1981; Lu, 2003; Vinterhalter, 1989; Vinterhalter and Vinterhalter, 1997; Jazib *et al.,* 2019), *D. sanderiana* (Aslam *et al.,* 2013; Beura *et al.,* 2007), and *D. marginata* (Chua *et al.,* 1981; El-Sawy *et al.,* 2000), *D.ombit* (Dewir *et al.,* 2019). However, propagation of ornamental *Dracaena* sp. still relies on imported cuttings as a commercial practice (Dewir *et al.,* 2019). Plant cell, tissue and organ culture could be an alternative approach for economic production of Dragon's blood plants and the secondary metabolites they produce and to speed up the propagation rate and to reduce the need for mother plants (George and Sherrington, 1993, Deepika *et al.,* 2008). Therefore, the aim of the present study was to develop an efficient protocol for *in vitro*

propagation, caulogensis and acclimatization of *Dracaena draco* plants under *in vitro* and *ex vitro* culture conditions.

MATERIALS AND METHODS

The experiments regarding the effect of different concentrations of certain growth regulators and their combinations on *in vitro* propagation and caulogensis of *Dracaena draco*plantlets using nodal segments as explants were conducted in the Plant Tissue Culture Laboratory of The Faculty of Agriculture Saba Basha, Alexandria University, during the period of 2017 and 2019.

Plant Materials:

1. **Seeds preparation:** Fully ripe fruits were picked up from a healthy *Dracaena draco* old tree (Fig. 1a) cultivated 1860 in Antoniades Botanical Garden (lat.31.210610 N, long. 29.953420 E), Horticultural Res. Inst., Agric. Res. Center, Alexandria, Egypt. The collected material was brought to the laboratory to process, the seeds were manually removed from the fruit and washed after that to be ready for sterilization and tissue culture manipulation.

2.**Seeds sterilization**: The seeds were washed thoroughly in the water, using liquid soap for 30 min., and then the excised explants were placed under running tap water for 90 minutes then dipped in 70% ethanol for 15 sec. After pretreatment with ethanol, the seeds were washed with double distilled water twice to lower the toxic effect of ethanol. The seeds were surface sterilized with a concentration of mercuric chloride (HgCl2) at 0.1% (v/v) with a few drops of wetting agent "Tween-20" (surfactant agent) for fifteen minutes. A similar procedure was repeated, but the seeds were immersed in the concentration of sodium hypocholrite solution (NaOCl) at 30%. After the surface sterilization of seeds with mercuric chloride and sodium, hypocholrite solution was decanted and the seeds were washed with sterile double distilled water four times, so as to lower the toxic effects of HgCl2 and NaOCl and became ready for culturing. Repetitive subcultures of the shoot tips and nodes of *Dracaena draco* seedlings on to hormone-free MS medium for four months produced sufficient stock of shoots for further experiments.

***In vitro* Experimental Stages**:

1. Initiation stage, during this stage explants, were cultured on solidified Murashige and Skoog medium (1962) solidified with gelrite (4g/l). The pH of the tested media was adjusted to 5.7 before adding gelrite, and then sterilized autoclaving at 121°C for 20 min., then explants were cultured into the given MS medium which contained different concentrations of cytokinin (BA at four concentrations: 0.00 (nil), 0.25, 0.50, and 1.00 mg/l, in combinations with auxin (NAA) at five concentrations 0.00 (nil), 0.5, 1.00, 2.00, and 4.00 mg/l.

2. Multiplication stage, in this stage the neoformed propagules of the initiation stage were sectioned into single leaflets nodes (*ca.* 1 cm) cultured randomly, on multiplication media which supplemented with BA at five concentrations: 0.00 (nil), 1.00, 2.00 2.00, and 8.00 mg/l, in combinations with NAA at four concentrations: 0.0 (nil), 0.50, 1.00 and 2.00 mg/l.

3. Rhizogenesis, the obtained shoots from the multiplication stage were, individually, cultured on a rooting medium, contained two types of auxins were tested, Indole-3-Bytric acid (IBA) at four concentrations: 0.00 (nil), 0.50, 1.00 and 2.00 mg/l, in combinations with (NAA) at four concentrations: 0.0 (nil), 0. 25, 0.50 and 1.00 mg/l.

4 **Callus Formation Experiment:**

The obtained shoots from the initial explants were used for the induction of callus. The leaves were excised from shoots and cut into (0.5 cm^2) segments, more or less. The explants were cultured in to MS medium supplemented with different concentrations of BA at four concentrations; 0.00 (nil), 0.125, 0.250 and 0.500 mg/l, in combinations with NAA

at four concentrations; 0.0 (nil), 0.5, 1.0 and 2.0 mg/l. The percentage of callus formation, size of callus, percentage of direct organogenesis, number of shoots and number of roots per propagule were recorded after 35 days in culture. Callus size was determined, macroscopically, and for statistically convenience, the minus or plus symbols were converted to a numerical code as follows: (-), 0; (+), 1; (++), 2; (+++), 3; (++++), 4. These symbols refer to no callus, low, moderate, high, and intensive callus formed per propagule, respectively.

Generally, each treatment was represented by 10 jars and three explants per jar (175 ml) containing 20 ml medium. The culture jars and the tested media were solidified and autoclaved as mentioned earlier. The explants were cultured on the sterilized media, vertically, and incubated in a growth chamber at 25 ± 1°C temperature under 16 hr daily light and 8 hr darkness illumination by a florescent light intensity of 2880 Lux (40µmol m-2s-1 PPF). After 6 weeks of culture, the data were collected.

Acclimatization of neoformed plantlets, the plantlets produced from the rooting stage was washed gently out of solidified medium under running tap water, followed by immersing them into fungicide for 25 sec. They were, then, transplanted *ex vitro* in small plastic pots (6 cm) plastic pots contained a combination of an autoclaved mixture of the perlite (0, 1, 2 and 3 volume) and peat moss at (0, 1, 2, 3 volume) each; and one constant volume of washed and autoclaved sand. Then, they were arranged in a factorial experiment and finally placed in transparent plastic bags (*ex vitro*), to maintain high relative humidity at (RH) 85% and 27±1°C, for hardening-off. However, the tested pots with different media were rearranged, randomly, weekly within the same plot to avoid the experimental error. Ten days later, the plastic bags were perforated for gaseous exchange, then transferred into the plastic house (*in vivo*) and continued for further hardening. After four weeks, the plastic bags were removed and the acclimatized plantlets were watered, as needed, and fertilized weekly with mineral fertilization (20:20:20) at 0.5g/l.

Experimental design and statistical analysis, all the experiments carried out during this study were designed as factorial experiments layout in completely randomized design (Gomez and Gomez, 1984). Recorded data were analyzed statistically using the analysis of variance technique (ANOVA) and means were compared by L.S.D tests (Steel *et al*., 1997) and significance was determined at $p \leq 0.05$.

Fig. 1:*In vitro* seed germination of *Dracaena draco* on MS medium (a) Old *D. draco* tree in Antoniades Botanical Garden, (b) Seed preparation and sterilization, (c and d) Germinated seeds after 2 and 4 weeks.

RESULTS AND DISCUSSION

1. Initiation Stage:

Data presented in Table (1) and Fig (2) exhibit that both applied growth regulators (BA and NAA) and their combinations exerted significant effects on the initiation stage characters of *Dracaena draco* single node explants grown *in vitro*. The highest mean values were always recorded at 1.00 mg/l BA in the culture medium for numbers of the shoot (2.33), shoot length (12.72 cm) and the number of leaflets (14.07) formed per propagule. However, the highest mean values for the number of nodes (15.78) and the number of roots (0.84) formed per propagule were always recorded at 0.50 mg/l BA in the culture medium, but the lowest ones were noticed at either the absence of BA or at 0.25 mg/l for the number of shoots, shoot length, the number of leaflets, nodes number and roots number formed per propagule. Regarding the main effect of NAA tested levels on the above-mentioned traits commonly, the highest mean values were always recorded at 2.00 mg/l of NAA for numbers of the shoot (2.94), shoot length (13.64 cm), number of leaflets (14.72) number of nodes (16.36) and number of roots (1.81) formed per propagule in the culture medium, and the lowest ones were noticed at its absence from the cultured medium (0.00 mg/l) or at (0.250 mg/l). Concerning the interaction between levels of both factors under the study, BA at 1.00 or 0.50 and NAA at 2.00 mg/l, led to the highest mean values of the studied characters, in general. The lower concentrations of NAA in combination with BA play a crucial role in there generation of plants 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 *et al.,* 2008). additionally, stated 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. It is known that the average number of leaves per shoot and number of nodes is one of the important growth factors and is an indirectly proportional relationship to the length of the shoot, and if the shoot length increases, the number of leaves and number of nodes increases as well (Waseem *et al*., 2009a).

This was in agreement with the results obtained by Atta- Alla *et al.* (1996) and El-Sawy *et al.* (2000) on *Dracaena marginata* cv. Tricolor, who demonstrated that the highest shoot proliferation was obtained on MS medium containing BA at 4.0 mg/l and NAA at 0.05 mg/l. Beaura *et al.* (2007) showed that the presence of BAP at 2 mg/l with NAA at 0.5 mg/l produced significantly longer shoots and more leaves in *Dracaena*. Khan *et al.* (2004) reported profuse adventitious shooting of Cordyline in media containing a combination of kinetin at 4.0 mg/l and NAA at 0.5 mg/l.

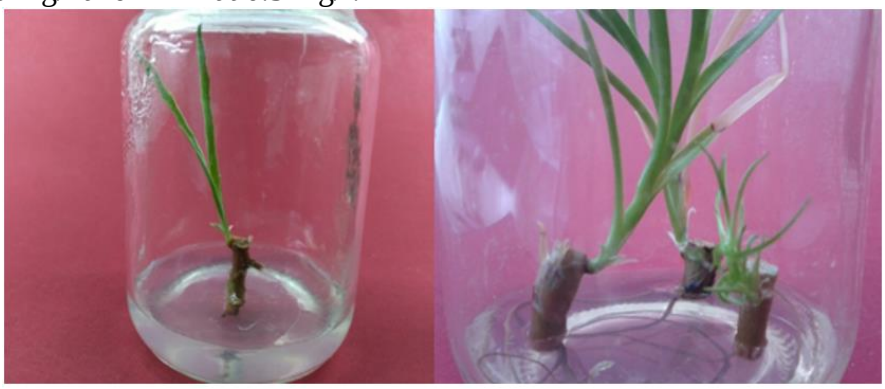

Fig. 2: Initiation stage of *Dracaena draco* single nodal grown *in vitro* on MS medium augmentedwith BA at 1.00 mg/l and NAA at 2.00 mg/l over 40 days

Table 1: Effect of different levels of NAA and BA (mg/l) and their combinations on the initiation stage of *Dracaena draco* cultured *in vitro* for 40 days.

Characters	BA Levels (mg/l)	NAA levels (mg/l)					Mean (BA)	Significance		
		0.0	0.5	1.0	2.0	4.0		NAA	BA	NAA X BA
(a) Mean number of shoots formed/propagule:										
	0.0	0.44	0.89	1.00	1.67	1.00	1.00	**	**	**
	0.25	0.89	1.56	1.78	2.00	1.33	1.51			
	0.50	1.11	1.33	2.89	3.44	1.78	2.11			
	1.00	1.11	1.22	3.22	4.67	1.44	2.33			
Mean (NAA)		0.89	1.25	2.22	2.94	1.39				
L.S.D. (0.05)								0.47	0.42	0.94
(b) Mean shoot length (cm)/propagule:										
	0.0	3.17	6.07	8.38	9.23	7.89	6.95	**	**	**
	0.25	8.29	10.47	12.67	13.46	9.12	10.80			
	0.50	9.49	12.09	14.24	15.39	10.00	12.24			
	1.00	9.77	12.54	14.56	16.50	10.24	12.72			
Mean (NAA)		7.68	10.29	12.46	13.64	9.31				
L.S.D. (0.05)								0.97	0.86	1.94
(c) Mean number of leaflets formed/propagule:								**	**	*
	0.0	4.11	7.33	9.33	11.89	10.11	8.56			
	0.25	6.22	10.44	14.44	15.11	9.78	11.20			
	0.50	12.67	14.22	15.67	16.33	11.44	14.07			
	1.00	11.56	12.89	13.56	15.56	10.89	12.89			
Mean (NAA)		8.64	11.22	13.25	14.72	10.56				
L.S.D. (0.05)								1.35	1.21	2.71
(d) Mean number of nodes formed/propagule:								**	**	**
	0.0	4.67	8.33	10.33	13.78	12.00	9.82			
	0.25	7.78	12.00	15.89	16.67	11.44	12.76			
	0.50	14.67	16.11	17.22	18.00	12.89	15.78			
	1.00	13.11	15.00	15.11	17.00	12.22	14.49			
Mean (NAA)		10.06	12.86	14.64	16.36	12.14				
L.S.D. (0.05)								1.49	1.34	2.98
(e) Mean number of roots formed/propagule:								**	**	**
	0.0	0.00	0.11	0.33	1.22	0.56	0.44			
	0.25	0.11	0.22	0.67	1.78	0.56	0.67			
	0.50	0.11	0.22	0.78	2.44	0.67	0.84			
	1.00	0.11	0.44	0.67	1.78	0.22	0.64			
Mean (NAA)		0.08	0.25	0.61	1.81	0.50				
L.S.D. (0.05)								0.21	0.19	0.44

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

2. Multiplication Stage:

Results in Table (2) and Fig (3) describe the effect of various levels of both growth regulators (BA and NAA) and their combinations on the studied characters of *Dracaena draco*. BA levels had a highly significant effect on this trait. The highest mean value was recorded at 4.00 mg/l BA for the number of theshoot (12.42), the number of leaflets (11.44) and nodes (13.11) formed per propagule. However, the highest mean values for the shoot length (5.57 cm) and the number of roots (1.83) formed per propagule were always recorded at 1.00 mg/l BA in the culture medium but, the least response was observed with the absence

of BA. On the other hand, NAA had a highly significant effect on the given traits at 1.00 mg/l. Meanwhile, the interaction between both growth regulators exerted a highly significant effect. The interaction between both BA and NAA at 4.00 and 1.00 mg/l, respectively, resulted in the highest mean value of the number of the shoot (31.56), the number of leaflets (13.67) and the number of nodes (15.33) formed per propagule. With respect the highest mean value of shoot length (7.34 cm) and the number of roots (3.00) formed per propagule was recorded at 1.00 mg/l BA combined with NAA at 1.00 mg/l; both growth regulators had a highly significant effect on the given trait.

It could be inferred from the above results that BA at 1.0 mg/l, was the optimal concentration for better performance of this particular hormone. Whereas, any above or lower deviation from this concentration of that growth regulator, the propagules showed poor performance. These results could be brought about to the mode of action of cytokinins on stimulation both cell division and promotion growth of axillary shoots in plant tissue culture as, also, found by George *et al*. (2008).

The above-mentioned results, generally, indicated that increasing the mean values of the studied characters was concomitant with increasing BA levels. BA has been the most efficient cytokinin for multiple shoot induction. Structural stability and quick metabolization of BA by plant tissue in comparison to other synthetic cytokinins are the reasons for the supremacy of BA for shoot induction. BA also causes the production of natural hormones such as zeatin within the plant tissue causing enhanced shoot production (Ahmad *et al.,* 2013; Malik *et al.,*2005).

Likewise, this might be due to the mode of action of auxin (NAA) at the above-mentioned level within cultured tissues may enhance, control various distinctive processes such as cell growth and elongation (George and Sherrington, 1984). Wilkins (1989), additionally, stated 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(Waseem *et al*., 2009).

These results are in agreement with those obtained by Paek *et al.,* (1985) on *Cordyline terminalis*. They reported that using 1.0 mg/l IAA + 3.0 mg\l Kin was the most effective treatment for increasing multiplication activity. Also, Atta- Alla *et al.,* (1996) and El- Sawy *et al.,* (2000) on *Dracaena marginata* cv. Tricolor, demonstrated that the highest value of shoot proliferation was produced on MS containing BA at 4.0 mg/l and NAA at 0.05 mg\l. Tian- Lang *et al.,*(1999) found that using 3-3.5 mg/l BA + 0.02 mg/l NAA was the most effective treatment for increasing multiplication on *Dracaena sanderiana* cv. Virescens. (Singh *et al.,* 2001; Aziz *et al.,* 1996), reported a successful shoot proliferation using BAP in combination with auxin on Dracaena species, including *D. deremensis*, *D. fragrans* 'Massangeana'. the highest shoot multiplication (83.33%) with a maximum number of shoot per unit callus (5.62±1.24) and maximum shoot length (3.27±0.82 cm) was observed when the nodal calli of *Dracaena fragrans* cv. Victoria was transferred in culture medium in combination with 4.5 mg/l BAP and 0.5 mg/l NAA(Jazib *et al.,* 2019).

Table 2: Effect of different levels of BA and NAA (mg/l) and their combinations on the multiplications stage of *Dracaena draco* cultured *in vitro* for 60 days.

Characters	NAA Levels (mg/l)	BA levels (mg/l) 0.0	1.0	2.0	4.0	8.0	Mean (NAA)	Significance BA	NAA	BA X NAA
(a) Mean number of shoots formed/propagule:										
	0.0	0.22	1.00	1.22	2.89	1.67	1.40	**	**	**
	0.50	0.33	3.22	4.56	4.56	2.22	2.98			
	1.00	1.44	4.33	13.00	31.56	9.22	11.91			
	2.00	1.44	5.78	9.44	10.67	6.11	6.69			
Mean (BA)		0.86	3.58	7.06	12.42	4.81				
L.S.D. (0.05)								3.76	3.31	7.33
(b) Mean shoot length (cm)/propagule:										
	0.0	0.50	2.61	2.89	2.98	1.87	2.17	**	**	**
	0.50	1.22	5.84	6.22	3.80	2.40	3.90			
	1.00	5.44	6.47	4.69	3.80	2.46	4.57			
	2.00	4.42	7.34	2.76	2.66	2.27	3.89			
Mean (BA)		2.90	5.57	4.14	3.31	2.25				
L.S.D. (0.05)								1.01	0.91	2.03
(c) Mean number of leaflets formed/propagule:								**	**	*
	0.0	1.56	4.44	5.44	8.33	6.22	5.20			
	0.50	6.33	6.89	7.22	10.33	7.11	7.58			
	1.00	10.00	10.22	10.33	13.44	7.22	10.24			
	2.00	10.33	12.67	12.22	13.67	7.89	11.36			
Mean (BA)		7.06	8.56	8.81	11.44	7.11				
L.S.D. (0.05)								1.15	1.03	2.30
(d) Mean number of nodes formed/propagule:								**	**	**
	0.0	1.78	5.44	7.22	10.00	7.22	6.33			
	0.50	7.33	7.89	9.11	11.78	8.11	8.84			
	1.00	11.00	11.22	11.78	15.33	8.22	11.51			
	2.00	11.33	13.67	13.78	15.33	8.89	12.60			
Mean (BA)		7.86	9.56	10.47	13.11	8.11				
L.S.D. (0.05)								1.21	1.08	2.41
(e) Mean number of roots formed/propagule:								**	**	**
	0.0	0.00	0.11	0.11	0.00	0.00	0.04			
	0.50	0.56	1.78	1.22	0.67	0.00	0.84			
	1.00	2.67	3.00	1.78	0.89	0.56	1.78			
	2.00	1.78	2.44	0.33	0.22	0.00	0.96			
Mean (BA)		1.25	1.83	0.86	0.44	0.14				
L.S.D. (0.05)								0.44	0.39	0.88

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

Fig. 3: Multiplication stage of *Dracaena draco* explants grown *in vitro* on MS medium augmented with BA at 4.00 mg/l and NAA at 1.00 mg/l over 60 days.

3. Rooting Stage:

Results in Table (3) and Fig (4) showed that the applied auxin levels exerted highly significant effects on the studied characters of *Dracaena draco*. The highest mean value was recorded at 1.00 mg/l IBA for the number of the shoot (1.50), shoot length (5.05 cm), the number of leaflets (10.22) and roots (3.23) formed per propagule. However, the highest mean values for callus formation percentage (53%) formed per propagule were always recorded at 2.00 mg/l IBA in the culture medium. On the other hand, NAA main effect disclosed that augmenting MS-basal medium with 0. 50 mg/l of it, gave the highest mean values of the above-mentioned traits. The above-mentioned results, generally, indicated that, the interaction between the used auxins IBA at 1.00 mg/l and NAA at 0. 50 mg/l resulted in the highest mean value of roots formed per propagule.

The obtained results showed that the used auxins (*viz*. NAA and IBA), in general, produced the best results in almost all studied traits. These results could be explained on the basis 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). Moreover, IBA at the above-mentioned level confirmed its superiority over NAA. The reason for these differences in root-inducing ability may be the slow and continuous release of IAA from IBA (Liu *et al*., 1998) and release of IBA through hydrolysis of conjugates (Epstein and Muller, 1993). These IBA conjugates were reported to be superior to free IBA in serving as an auxin source during later stages of rooting (Staswick *et al*., 2005).

These results are in agreement with those of Vinterhalter (1989), who reported that during root induction in *D. fragrans*, 0.5 mg/L IBA was optimal. Parallel with these results, Blanco *et al.,* (2004) reported that a hormone-less medium was convenient for rooting *D. deremensis*.

These results agreed with those obtained by Paek *et al.* (1985) on *Cordyline*. They concluded that using 2.0 or 3.0 mg\l IBA was more successful for increasing the rooting stage.Moreover, in vivo rooting offers several advantages over in vitro rooting as the former is cost-effective and the roots structurally and functionally contain more root hairs than the latter (Debergh and Read 1991; Preece and Sutter, 1991). Lowering the salt strength in the medium to half and the addition of 0.5 to 1 mg/L IBA yielded the highest rooting percentage for *D. fragrans* (Singh *et al.,* 2001). For in vitro rooting of *D. marginata*, IAA, IBA, and NAA were applied at 2 mg/L, and NAA induced the highest rooting percentage (80%) compared with IBA (50%) and IAA (48%) (El-Sawy *et al.,* 2000; Aslam *et al.*, 2013)Maximum rooting (75%) were observed in half-strength MS liquid medium supplemented with 0.5 mg/l IBA on *Dracaena fragrans* cv. Victoria (Jazib *et al.*, 2019).

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

Characters	NAA Levels (mg/l)	IBA levels (mg/l)				Mean (NAA)	Significance		
		0.0	0.5	1.0	2.0		IBA	NAA	IBA X NAA
(a) Mean number of shoots formed/propagule:							**	**	**
	0.0	1.00	1.00	1.00	1.00	1.00			
	0.25	1.00	1.67	2.11	1.00	1.44			
	0.50	1.00	1.67	1.89	1.89	1.61			
	1.00	1.11	1.67	1.00	1.00	1.19			
Mean (IBA)		1.03	1.50	1.50	1.22				
L.S.D. (0.05)							0.30	0.29	0.58
(b) Mean shoot length (cm)/propagule:							**	**	**
	0.0	2.33	3.28	3.62	2.34	2.89			
	0.25	3.12	4.47	5.32	3.79	4.18			
	0.50	3.07	6.74	8.74	5.76	6.08			
	1.00	2.87	3.04	2.52	1.88	2.58			
Mean (IBA)		2.85	4.38	5.05	3.44				
L.S.D. (0.05)							0.76	0.77	1.56
(c) Mean number of leaflets formed/propagule:							**	**	**
	0.0	4.78	5.78	6.67	4.89	5.53			
	0.25	6.00	9.67	11.33	7.89	8.72			
	0.50	7.00	15.11	16.67	9.00	11.94			
	1.00	6.00	8.56	6.22	4.44	6.31			
Mean (IBA)		5.94	9.78	10.22	6.56				
L.S.D. (0.05)							1.46	1.45	2.91
(d) Mean number of roots formed/propagule:							**	**	**
	0.0	0.11	0.56	1.67	1.22	0.89			
	0.25	0.44	2.11	2.78	1.44	1.69			
	0.50	1.33	3.89	5.33	1.22	2.94			
	1.00	0.44	1.78	3.33	0.33	1.47			
Mean (IBA)		0.58	2.08	3.28	1.06				
L.S.D. (0.05)							0.59	0.59	1.18
(d) Mean of callus formation percentage/propagule:							**	**	**
	0.0	0%	0%	0%	22%	6%			
	0.25	0%	0%	0%	33%	8%			
	0.50	0%	0%	11%	56%	17%			
	1.00	22%	33%	89%	100%	61%			
Mean (IBA)		6%	8%	25%	53%				
L.S.D. (0.05)							0.15	0.14	0.29

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

Fig. 4: Rooting stage of *Dracaena draco* shoots grown *in vitro* on MS medium plus IBA at 1.00 mg/land NAA at 0.50 mg/l over 35 days.

4. Callus formation:

Data presented in Table (4) and Fig (5) exhibit that both applied growth regulators (BA and NAA) and their combinations exerted highly significant effects on the studied characters on caulogensis of *Dracaena draco* using internodal segment.

Concerning the percentage of explants formed callus and callus size, the main effect of BA declared that BA at 0.50 mg/l, had a highly significant effect on the setraits (67%) for callus formation percentage, and for callus size formed per propagule (2.64) with intensive size and compact green colour callus, respectively, compared to the control treatment which didn't show any callus formation. On the other side, the presence of NAA at 2.00 mg/l, gave the highest callus percentage (100%) and the highest callus size (3.50) respectively. Meanwhile, the interaction between BA and NAA at any applied levels except nil levels had a significant effect on the given traits. Regarding the percentage of direct organogenesis and number of shoots formed per explants, the main effect of BA revealed that providing the MS medium with BA at 0.250 mg/l, gave the highest percentage of direct organogenesis (36 %) and the highest number of shoots formed per explants (0.78) respectively. On the other side, the presence of NAA in the culture medium led to inhibition of direct organogenesis, while the absence of it gave the highest percentage of this trait (64% and 1.28) respectively. With respect to the interaction between BA at either 0.250 or 0.50 mg/l, and the absence of NAA resulted in the highest percentage (100%) of direct organogenesis per explants and the highest number of shoots (2.78 and 1.78) formed per explants.

Respecting the number of roots formed per propagule, the main effect of the different levels of BA had asignificant effect on this trait and the absence of BA from the culture medium resulted in the highest number of roots (1.97). On the other hand, the main effect of NAA showed that there was a directly proportional relationship between NAA levels and the mean number of roots. However, the highest value (1.36) was recorded due to the presence of NAA at 2.00 mg/l in the culture medium. The interaction between BA at 0.00 mg/l and NAA at 2.00 mg/l brought about the highest number of roots per propagule.

The above-mentioned results could be attributed to the role of exogenous cytokinin in enhancing plant cell division. It has been suggested that cytokinins might be required to regulate the synthesis of proteins involved in the formation of new cells (Pasternak *et al.*, 2002). Regarding the main effect of auxin, it represents the important growth regulator for the induction of callus in the majority of angiosperms (Roy and Banerjee, 2003). NAA acts as an auxin to induce cell division and enlargement at low concentrations. Cell enlargement has been associated with an increase in activities of autolytic and synthetic enzymes, which affect cell wall plasticity and synthesis of new wall materials (Cleland, 1981). Hence, a balance between auxin and cytokinin growth regulators is most often required for the formation caulogenesis and subsequent events (Johri and Mitra, 2001).However, in Dracaena fragran young stem segments produced calli on medium supplemented with 2,4-D alone or in combination with BA (Vinterhalter, 1989). plantlets were produced from shoot culture of *D. deremensis*, *D. fragrans*, and *D. surculosa* on media containing kinetin and naphtoxyacetic acid or IAA (Badawy *et al.*, 2005; Blanco *et al.*, 2004; Miller and Murashige, 1976), or calluses were induced from the shoot or nodal explants of *D. deremensis*, *D. fragrans*, *D. marginata*, and *D. sanderiana* by 2,4-D only (Chua *et al.*, 1981; Junaid *et al.*, 2008; Vinterhalter, 1989) or 2,4-D with BA (Lu, 2003) and shoots were induced using BA or kinetin only or BA with NAA.Nodal explants of *Dracaena fragrans* cv. Victoria showed high callus induction potentiality (80%) on MS medium supplemented with 1.5 mg/l 2,4-D (Jazib *et al.*, 2019).

Table 4: Effect of different levels of BA and NAA (mg/l) and their combinations on the caulogenesis stage of *Dracaena draco* cultured *in vitro* for 60 days

Characters	NAA Levels (mg/l)	BA levels (mg/l)				Mean (NAA)	Significance		
		0.0	0.125	0.250	0.50		BA	NAA	BA X NAA
(a) Percentage of explants formed callus/propagule:							**	**	**
	0.0	0%	0%	0%	0%	0%			
	0.50	0%	0%	56%	67%	31%			
	1.00	44%	78%	100%	100%	81%			
	2.00	100%	100%	100%	100%	100%			
Mean (BA)		36%	44%	64%	67%				
L.S.D. (0.05)							0.14	0.14	0.28
(b) Percentage of direct organogenesis formed/propagule:							**	**	**
	0.0	0%	56%	100%	100%	64%			
	0.50	0%	0%	44%	0%	11%			
	1.00	0%	0%	0%	0%	0%			
	2.00	0%	0%	0%	0%	0%			
Mean (BA)		0%	14%	36%	25%				
L.S.D. (0.05)							0.19	0.19	0.38
(c) Callus size/propagule:							**	**	**
	0.0	0.00	0.00	0.00	0.00	0.00			
	0.50	0.00	0.00	2.33	2.78	1.28			
	1.00	1.67	2.67	3.00	3.78	2.78			
	2.00	2.56	3.44	4.00	4.00	3.50			
Mean (BA)		1.06	1.53	2.33	2.64				
L.S.D. (0.05)							0.46	0.45	0.90
(d) Mean number of shoots formed/propagule:							**	**	**
	0.0	0.00	0.56	2.78	1.78	1.28			
	0.50	0.00	0.22	0.33	0.22	0.19			
	1.00	0.00	0.00	0.00	0.00	0.00			
	2.00	0.00	0.00	0.00	0.00	0.00			
Mean (BA)		0.00	0.19	0.78	0.50				
L.S.D. (0.05)							0.42	0.42	0.84
(d) Mean number of roots formed/propagule:							**	**	**
	0.0	0.00	0.00	0.00	0.00	0.00			
	0.50	1.44	0.44	0.00	0.00	0.47			
	1.00	2.56	0.89	0.00	0.00	0.86			
	2.00	3.89	1.56	0.00	0.00	1.36			
Mean (BA)		1.97	0.72	0.00	0.00				
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.

Fig. 5: Caulogensis stage of *Dracaena draco* internodal segments (a and b) and shoots regenerations grown *in vitro* on MS medium augmented with BA at 0.5 mg/l and NAA at 2.00 mg/l over 60 days.

5. *Ex vitro* and *in vivo* Acclimatization:

Data presented in Fig (6) and Table (5) exhibit that both applied mixtures of perlite and peatmoss (v/v) and their combinations, in addition to fixed volume (1 portion) of sand had highly significant effects on acclimatization of neoformed plantlets of *Dracaena draco* grown *ex vitro* for four weeks.

Concerning the average survival percentage per plant, perlite and peatmoss have a highly significant effect on this trait. The highest mean value was recorded at levels (2v/v perlite) 67% and (3v/v peatmoss) 81%. Meanwhile, the interaction between peatmoss and perlite exerted a highly significant effect. However, the combination of peatmoss and perlite at either (1:1) or (2: 2) or (2:3) and (3: 3), respectively, resulted in the highest mean value (100%).

Respecting the average number of neoformed shoots per plant and the number of newly formed leaves (true leaves), peatmoss and perlite mixture and their interactions exerted significant effects on the given trait. In the case of perlite main effect at (2 v/v), brought about the highest mean value (2.25, and 13.08), respectively, and (1 v/v) for plant height (13.17). On the other side, peatmoss had a highly significant effect on the number of neoformed shoots per plant, the number of newly formed leaves (true leaves) and plant height per plant at (3 v/v) (2.64, 15.47 and 15.61), respectively. However, the interaction between both added levels of perlite and peatmoss at (3:3), resulted in the highest mean value (4.22, 18.44 and 19.44), respectively.

In this respect, material as peatmoss is one of the most important constituents of media due to its capacity in affecting plant growth either indirectly or directly. Indirectly, improves the physical conditions of media by enhancing aggregation, aeration (8%) and water retention (77%), thereby creating a suitable environment for root growth (Sensi and Loffredo, 1999). On the other hand, perlite is known to have a moderate capacity to retain water (38%) and provide' aeration (25%) and its neutral pH and the fact that it is sterile and weed-free. Hence, it is ideal for use in container growing substratum. Also, it is known that perlite decreases the bulk density of the soils and increases the porosity (Preece and Sutter, 1991, Abido, 2016). These results are in agreement with Vinterhaler (1989) for *D. fragrans*, Blanco *et al.*, (2004) for *D. deremensis*, and Miller and Murashige (1976) for *D. godseffiana*, Aslam *et al.*, (2.13) for *D. sanderiana* Sander ex Mast and by Liu *et al.*, (2010) for *D. surculosa*, Junaid *et al.*, (2010) for *D. sanderiana* Sander ex Mast and for *Dracaena fragrans* cv. Victoria (Jazib *et al.*, 2019).

Fig. 6: Acclimatization stage of *Dracaena draco*shoots grown on rooting medium of peat moss andperlite at (1:1, v/v) over 4 weeks (a), and over 8 weeks (b), and over 6 months (c).

Table 5: Effect of different levels of peat moss and perlaite (v: v) and their combinations on the acclimatization stage of *Dracaena draco* cultured *in vitro* for 4 weeks

Characters	Peat Levels (v: v)	Perlaite levels (v:v)				Mean (Peat)	Significance		
		0.0	1.0	2.0	3.0		Perlaite	Peat	Perlaite X Peat
(a) Mean number of neoformed shoots:							**	**	**
	0.0	0.00	0.33	0.33	0.33	0.25			
	1.00	1.67	3.89	1.67	1.33	2.14			
	2.00	1.56	2.44	4.11	1.22	2.33			
	3.00	1.33	2.11	2.89	4.22	2.64			
Mean (Perlaite)		1.14	2.19	2.25	1.78				
L.S.D. (0.05)							0.77	0.77	1.56
(b) Mean plant height (cm):							*	**	**
	0.0	0.00	2.94	2.50	2.97	2.10			
	1.00	9.79	17.90	11.06	8.68	11.86			
	2.00	10.97	15.20	19.07	11.78	14.25			
	3.00	11.20	16.27	15.54	19.44	15.61			
Mean (Perlaite)		7.99	13.08	12.04	10.72				
L.S.D. (0.05)							1.98	1.98	3.96
(c) Mean number of neoformed leaves:							**	**	**
	0.0	0.00	4.22	4.67	4.67	3.39			
	1.00	11.33	15.67	15.11	9.44	12.89			
	2.00	12.89	16.33	17.00	11.56	14.44			
	3.00	12.56	15.00	15.89	18.44	15.47			
Mean (Perlaite)		9.19	12.81	13.17	11.03				
L.S.D. (0.05)							1.57	1.57	3.02
(d) Mean of plant servaival percentage %:							**	**	**
	0.0	0%	11%	11%	11%	8%			
	1.00	44%	100%	56%	22%	56%			
	2.00	44%	78%	100%	44%	67%			
	3.00	44%	78%	100%	100%	81%			
Mean (Perlaite)		33%	67%	67%	44%				
L.S.D. (0.05)							0.18	0.18	0.37

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

Conclusion

In conclusion, the present study established an efficient protocol for in vitro propagation, caulogensis and acclimatization for *Dracaena draco*, which required 12 weeks from initiation culture to plant regeneration, and 4 weeks for *ex vitro* acclimatization. The highest number of shoots (31.65) obtained from the combination of BA and NAA at 4.00 and 1.00 mg/l augmented in cultured medium. The interaction between IBA at 1.00 mg/l and NAA at 0.50 mg/l resulted in the highest mean value of roots formed per propagule (533). The highest percentage of callus formation per explants (100%) resulted from NAA and BA at 2.00 and 0.50 mg/l. the combination of peatmoss and perlite at either (1:1) or (2: 2) or (2:3) and (3: 3), respectively, resulted in the highest mean value (100%) of survival percentage per plant. Micropropagation would ensure a continuous supply of plants in exclusive time and space. This protocol will be helpful for rapid and large-scale propagation for *Dracaena draco* to enrich and valorization the ornamental industry and increasing awareness for its conservation.

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ARABIC SUMMARY

الإكثار المعملى الدقيق وتكوين الكالس فى نباتات الدراسينا دراكو

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نبات الدراسينا دراكو هو نبات شبه استوائى إحادى الفلقة من جنس الدراسينا و الذى يعد من أكبر المجموعات النباتية في العائلة الهليونية، و يعتبر نبات الدراكو من النباتات المعمرة و الموجود فى جميع الحدائق النباتية فى دول المتوسط و على مستوى العالم و قد تم إدراجة فى القائمة الحمراء للنباتات المعرضة لخطر الإنقراض التابعة للإتحاد العالمى للحفاظ على البيئة (IUCN, 1998) .و كان الهدف من هذه الدراسة هو تطوير بروتوكول فعال للإكثار المعملى الدقيق وتكوين الكالس في المختبر لنبات الدراسينا دراكو لمحاولة الحفاظ علية من خطر الإنقراض عن طريق تشجيع نمو البراعم الإبطية الموجودة فى القطع البرعمية الساقية المفردة و المزروعة على بيئة موراشيج وسكوج كاملة القوة و التى تحتوى على 30 جم / لتر سكروز و 4 جم / لتر جيل رايت للتصلب و مدعمة بتركيزات مختلفة من منظمات نمو النبات مثل البنزيل ادنيين وحامض النفثالين اسيتك اسيد واندول بيوتريك اسيد. بشكل عام ، خلصت الدراسة الحالية إلى أن أفضل النتائج لمرحلة التنشئه تم تسجيلها على بيئة موراشيج وسكوج مدعمة بـحامض النفثالين اسيتك اسيدوالبنزيل ادنيين عند 2.00 و 1.00 مجم / لتر بالتتابع. في حين أن البيئة المضاف اليها بنزيل ادنيين وحامض النفثالين اسيتك اسيدعند تركيز 4.00 و 1.00 مجم / لتر على التوالي أعطت أفضل النتائج لمرحلة التضاعف. فيما يتعلق بمرحلة تكوين الجذور ، تم تسجيل أفضل النتائج عندما تم تقسيم البراعم الناتجه من مرحلة التضاعف و زراعتها على بيئة موراشيج وسكوج مدعمة بالإندول بيوتريك اسيدوحامض النفثالين اسيتك اسيدعند 1.00 و 0.50 مجم / لتر بالتتابع ، مما أعطى أعلى متوسط لعدد الجذور المتكونة لكل نبات. لتشجيع نمو الكالس ، تمت زراعة أجزاء من السلميات في بيئة موراشيج وسكوج مدعومة بتركيزات مختلفة من البنزيل ادنيينوحامض النفثالين اسيتك اسيد و كانت أفضل النتائج للنسبة المئوية للكالس المتكون وحجم الكالس عند تركيز 0.50 و 2.00 مجم / لتر على التوالي ،أما النسبة المئويه لعدد البراعم النامية على الكالس و عدد البراعم المتكونة لكل جزء نباتى فإن أفضل النتائج التي تم الحصول عليها من البنزيل ادنيين وحامض النفثالين اسيتك اسيدعند تركيز 0.250 مجم / لتر و 0.00 مجم / لتر على التوالي. تم أقلمة النبيتات حديثة التكوين بقوة خارج المختبر في خليط من البيرلايت والبيتموس فى خلطات نمو كان أفضلها عند (1: 1) أو (2: 2) أو (2: 3) و (3: 3) على التوالي ، بالإضافة إلى لحجم ثابت (جزء واحد) من الرمل المعقم ؛ و التى أعطت أعلى متوسط لنسبة البقاء على قيد الحياة / نبات (100٪).