Evaluating Some Plant Growth Regulators, Medium Strength and Supporting Method for Optimum Micropropagation of Cordyline 'Atom'

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ABSTRACT

A series of *in vitro* experiments were conducted during the 2022/2023 season at the Tissue Culture Laboratory, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. The aim was to develop an efficient micro-propagation protocol for *Cordyline fruticosa* 'Atom' while maintaining high quality and productivity. The experiments tested different cytokinin types and concentrations with or without adding NAA (for the first multiplication experiment), varying medium salt strengths and explant support methods (for the second multiplication experiment) during the multiplication stage. Also, different medium salt strengths, IBA concentrations, and explant support methods were studied during the rooting stage. For acclimatization, various potting mixtures were used. The results showed that using BA at 3.0 mg/l without NAA for the first multiplication experiment and full or ¾-strength MS medium with a solid medium for the second multiplication experiment yielded the highest values for shoot formation percentage, number of shoots per explant, shoot length, number of leaves per shoot, and pigment content. For rooting, a ¾-strength medium combined with 1.0 mg/l IBA and a solid medium was most effective. Coco peat alone or mixed with sand was the best potting mixture for acclimatizing. The study found BA to be more effective than kinetin, diluted strength medium could be used without reducing productivity or quality, there was no need for NAA during the multiplication stage, low concentrations of IBA were important for rooting, the solid medium outperformed liquid medium (supported with filter paper bridge) and coco peat surpassed peat moss based mixtures.

Keywords: *Cordyline fruticosa* 'Atom'- Micropropagation- Multiplication- Rooting- Acclimatization.

INTRODUCTION

Cordyline fruticosa, syn. *C. terminalis* (Asparagaceae) known as good luck tree or Ti tree is an erect, suckering, clump-forming shrub with generally unbranched stems and strap-shaped, deep green leaves, 30-60 cm long (Brickell, 1997). There are about 20 species of cordyline, mostly native to India and Australia. *C. fruticosa* is the principal cultivated species native to moist tropical forests of Eastern Asia. The cultivars of *C. fruticosa* can include purple, maroon, rose, pink or yellow in addition to green the highly variegated foliage gives them a somewhat painted appearance (Griffith, 2006).

For the micropropagation technique, both auxins and cytokinin groups are the basic plant growth regulators required for directing growth. Auxins induce cell division and expansion and are mainly used during the rooting stage. Indole and naphthalene compounds are the common auxins used in tissue culture. Besides auxin compounds are often used alongside cytokinins, most of which are derivatives of adenine (aminopurine). Cytokinins play a crucial role in shoot induction and plant regeneration across many species, and they can also promote cell division. By controlling the concentrations of both auxins and cytokinins, organogenesis in many species could be induced. A high concentration of cytokinin relative to auxin induces shoot formation, while on the contrary, rooting is induced by applying a high auxin concentration relative to cytokinin or auxin alone without cytokinin (Gamborg and Phillips, 1995).

Depending on plant species, a diluted tissue culture medium could be used with optimal growth. Previous studies have highlighted the beneficial effects of using diluted MS medium for all *in vitro* stages of a wide range of plants (Tauqeer et al., 2006

on banana 'Basrai', Ray et al., 2006 on *Cordyline terminalis*, Tauqeer et al., 2007 on *Zingiber officinale* and Dewir et al., 2015 on Cattleya).

In static liquid cultures, the tissue remains submerged, which can lead to anaerobic conditions and potentially cause the tissue to die. To address this issue, the medium is solidified using an appropriate gelling agent. The semi-solid consistency of the medium allows the explants to be placed on its surface, ensuring they remain properly aerated. Agar is the most gelling agents used in plant tissue cultures (Bhojwani and Dantu, 2013). The liquid medium could be used by various methods to increase its efficiency, for cordyline, Ray et al. (2012) provide support with filter paper bridge in the culture tubes for liquid root elongation medium. Esuola and Amoran (2023) stated that using a liquid medium eliminates the need to remove gelling agents from rooted shoots during the hardening process, which also reduces both costs and labor.

In addition to environmental conditions during the acclimatization stage, the choice of growing medium plays a significant role. Peat moss and vermiculite are commonly used substrates during this phase. Research on the acclimatization media required by plantlets from *in vitro* culture has been documented for various species, i.e. Manjusha and Sathyanarayana (2008) on stevia (*Stevia rebaudiana* Bert.), Patra and Beura (2016) on some gerbera cultivars, Pérez-Pazos et al. (2023) on sweet potato and Dwiyani et al. (2024) on strawberry (*Fragaria x ananassa* Duch.).

The aim of this study was to develop an efficient micro-propagation protocol for *Cordyline fruticosa* 'Atom' while maintaining high quality and productivity.

MATERIALS AND METHODS

A series of *in vitro* experiments at the Tissue Culture Laboratory, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt was carried out during the 2022/2023 season to introduce an efficient micro-propagation protocol of *Cordyline fruticosa* 'Atom' while maintaining high quality and productivity. These experiments included multiplication, rooting and acclimatization stages.

Explant source and preparation:

Homogeneous actively growing micro-shoots, 1.0-1.5 cm long, of *C. fruticosa* 'Atom' were excised from *in vitro* micro-propagated shootlets grown in the growth room of Tissue Culture Laboratory, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt in April 2022 and were used as explants for the upcoming trials.

Medium preparation and culture condition:

Murashige and Skoog (1962) medium (MS) containing macro, micronutrients, with vitamins and glycine (produced by Caisson Laboratories, Inc., USA, product number: MSP09-50LT) supplemented with 3.0% sucrose and 7.0 g/l agar for solid, or without agar for liquid medium and enriched with different concentrations of cytokinins or auxins.

After mixing the different components, the medium was adjusted to 5.8 pH and then heated to dissolve the agar (in case of the solid medium) and distributed in 200 ml glass jars then the filter paper bridge was set up (in case of the liquid medium) then covered with polypropylene caps and autoclaved for 20 minutes under a pressure of 1.05 kg/cm^2 at 121 °C. The jars were left to cool and stored for one week before being used at 25±2 °C. The cultures were incubated till data were collected, about 4 weeks later, in a growth room at 25 ºC under 16 hrs photoperiod at a light intensity of 3000 lux (about 36.3 µmol/m^2/s) supplemented by 120 cm long Phillips cool-white fluorescent tubes.

Experimental procedures:

1. Multiplication stage:

a. Effect of cytokinins and NAA:

This experiment aimed to study the influence of cytokinins i.e. benzyladenine (BA) and kinetin (kin) at 1.0, 3.0 or 5.0 mg/l for each and the addition of 1-naphthaleneacetic acid (NAA) at 0.0 or 0.5 mg/l during the multiplication stage. Solid MS basal medium at full strength, containing 7.0 g/l agar and 30 g/l (3%) sucrose, was utilized in this experiment. Under aseptic

conditions, the previously mentioned micro-shoot explants (1.0-1.5 cm long) were prepared by removing the leaves from the base and then cultured on the medium described above.

This experiment was laid out as a completely randomized design in a factorial experiment with two factors, cytokinin type represented factor A (6 treatments) and NAA addition represented factor B (2 treatments), so this experiment containing 12 treatments with three replicates/treatment and 4 glass jars/ replicate.

At the end of the incubation period, after 4 weeks, shoot formation percentage, number of shoots/explant, shoot length (cm) and number of leaves/shoot were calculated. To calculate the shoot formation % the following equation was applied:

Shoot formation %= number of explants produced new shoots/total number of cultured explants × 100

b. Effect of medium salt strength and explants supporting method:

This experiment investigates the effects of varying MS medium salt concentrations (full, ¾, or ½ strength) and the method used to support the explants in the medium (either agar in the medium or a filter paper bridge in a liquid medium) during the multiplication stage. A solid medium was prepared using 7.0 g/l of agar, while the liquid medium used a bridge made from Whatman No. 9 filter paper. Both types of the medium were supplemented with 3.0 mg/l of benzyladenine (BA), identified as the optimal concentration from the first experiment, and 30 g/l (3%) sucrose. The culture techniques and incubation conditions were similar to those applied in the first experiment.

This experiment was laid out as a completely randomized design in a factorial experiment with two factors, MS medium salt strength represented factor A (3 treatments) and explants supporting method factor B (2 treatments), so this experiment containing 6 treatments with three replicates/treatment and 4 glass jars/replicate.

Four weeks after the incubation period, shoot formation percentage, number of shoots/ explant, shoot length (cm), number of leaves/shoot, chlorophylls a, b and carotenoids content (mg/g f.w.) were calculated. Chlorophylls and carotenoids were determined according to the method described by Lichtenthaler and Wellburn (1983).

2. Effect of medium salt strength, IBA concentration and explants supporting method during the rooting stage:

To induce rooting on the explants *in vitro* varying MS medium salt concentrations (full, ½, or ¾ strength), IBA concentrations (0.0, 1.0 and 3.0 mg/l) and the method used to support the explants in the medium (either agar in a medium or a filter paper bridge in a liquid medium) were evaluated. As in the previous experiment, a solid medium was prepared using 7.0 g/l of agar, while the liquid medium used a bridge made from Whatman No. 9 filter paper. Both media types were free of cytokinins and were supplemented with 30 g/l (3%) sucrose. The culture techniques and incubation conditions were similar to those applied in the previous experiments.

This experiment was laid out as a completely randomized design in a factorial experiment with three factors, MS medium salt strength represented factor A (3 treatments), IBA concentrations as factor B (3 concentrations) and explants supporting method for factor C (2 treatments), so this experiment containing 18 treatments with three replicates/ treatment and 4 glass jars/replicate.

After 4 weeks, root formation percentage (rooting %), number of roots/shoot, root length (cm), plantlet height (cm), chlorophylls a, b and carotenoids content (mg/g f.w.) were calculated (Lichtenthaler and Wellburn, 1983). The rooting % was calculated as follows:

Rooting %= number of shoots produced roots/total number of cultured shoots × 100.

3. Effect of growing media during the acclimatization stage:

Well-developed plantlets with uniformly, healthy growth were chosen from the rooting experiment for acclimatization evaluation. After removing the culture medium, the plantlet roots were gently rinsed with distilled water to eliminate any remaining medium and agar. The plantlets were then transferred to the greenhouse at the National Gene Bank, ARC, Giza, Egypt, and

individually placed in 200 ml pots filled with different sterilized moist potting media e.g. peat moss, peat moss + vermiculite (1:1, v/v), peat moss + sand (1:1, v/v), coco peat, coco peat + vermiculite (1:1, v/v) and coco peat + sand (1:1, v/v) were used. They were irrigated with water containing 0.1% Topsin fungicide and kept in the greenhouse (average temperature of 25 °C and relative humidity of 65%) for 6 weeks before data collection. During this period, all plantlets were watered regularly and fertilized with NPK at 1.0 g/l as a soil drench, applied once two weeks after planting.

This experiment was laid out as a completely randomized design containing 6 treatments with three replicates/ treatment and 5 pots/replicates.

After 6 weeks from planting, plantlet height (cm), number of leaves/plantlet, root length (cm) and fresh weight of vegetative growth (aerial parts) and roots (g) were recorded.

Statistical analysis:

The appropriate analysis of variance (ANOVA) model for each experiment was applied to analyze the collected data using the MSTAT Computer Program (MSTAT Development Team, 1989). Mean comparisons were conducted using the least significant difference (L.S.D.) test, as described by Gomez and Gomez (1984), to determine significant differences between the means of various treatments.

RESULTS

1. Multiplication stage:

a. Effect of cytokinins and NAA:

Regarding the influence of cytokinins, the highest percentage of shoot formation on cordyline micro-shoots was obtained by applying BA at 3.0 mg/l (89.50%). This was associated with the highest number of leaves/shoot (4.64) and shoot length (1.65 cm). The highest number of shoots/explant was achieved with kin at 3.0 mg/l (2.11). In general, the effect of cytokinins on the number of shoots was insignificant **(Fig. 1).**

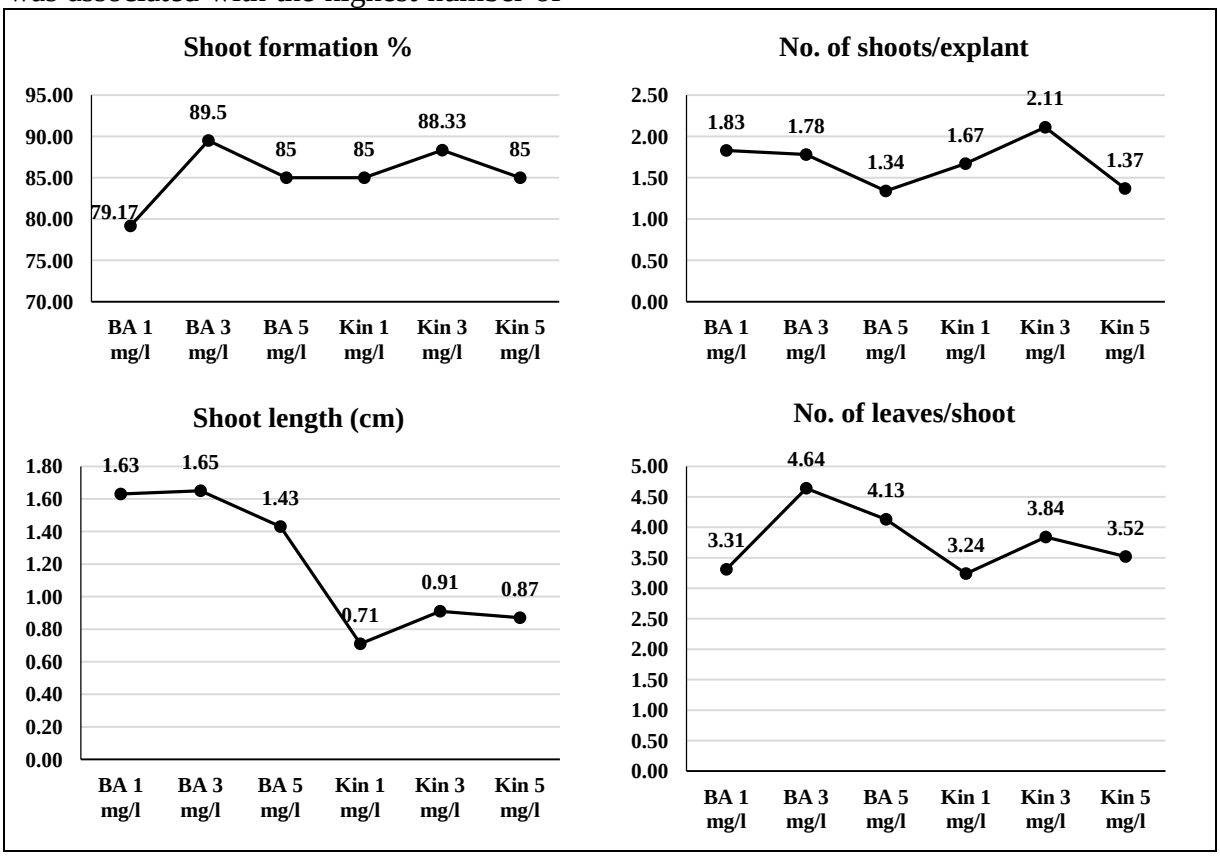

Fig. (1). Effect of type and concentration of cytokinins on multiplication of *C. fruticosa* 'Atom'.

In terms of NAA addition, excluding NAA from the medium seems to be more useful for the *in vitro* multiplication of cordyline, as this procedure produced the highest values for all the studied parameters as recorded 95.28% for shoot formation percentage, 2.21 shoots/explant, 1.56 cm for shoot length and 4.08 for number of leaves/shoot **(Fig. 2).**

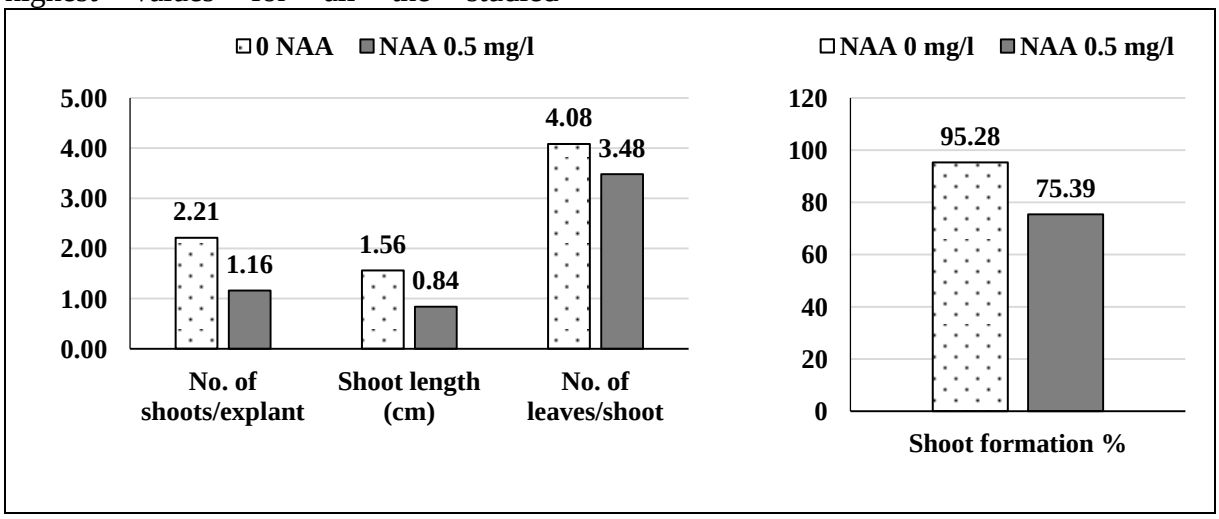

Fig. (2). Effect of NAA addition on multiplication of *C. fruticosa* 'Atom'.

As for the effect of the interaction between cytokinins and NAA **(Table 1),** the highest values were obtained by BA at 3.0 mg/l + NAA at 0.0 mg/l. This treatment produced the highest shoot formation percentage, the highest shoot length and the highest number of leaves/explant as recorded 100%, 2.76 cm and 5.19 leaves, respectively. Kin at 3.0 mg/l + NAA at 0.0 mg/l resulted in the highest value in the case of the number of shoots/explant as recorded 3.11 shoots. It is worth mentioning that, there were no significant differences between the interaction treatments of BA at 3.0 mg/l + NAA at 0.0 mg/l, BA at 1.0 mg/l + NAA at 0.0 mg/l and kin at 3.0 mg/l + NAA at 0.0 mg/l, and that applying NAA at 0.5 mg/l greatly declined the parameter values compared with unapplied.

Table (1). Effect of interaction between type and concentration of cytokinins and NAA addition on multiplication of *C. fruticosa* 'Atom'.

	NAA addition							
Cytokinins	**0 NAA**	**NAA 0.5 mg/l**	**0 NAA**	**NAA 0.5 mg/l**	**0 NAA**	**NAA 0.5 mg/l**	**0 NAA**	**NAA 0.5 mg/l**
	Shoot formation %		**No. of shoots/ explant**		**Shoot length (cm)**		**No. of leaves/explant**	
BA 1.0 mg/l	96.67	61.67	2.33	1.33	2.40	0.85	3.96	2.67
BA 3.0 mg/l	100.00	79.00	2.56	1.00	2.76	0.53	5.19	4.08
BA 5.0 mg/l	95.00	75.00	1.67	1.00	1.56	1.30	4.33	3.92
Kin 1.0 mg/l	91.67	78.33	2.00	1.33	0.83	0.58	3.65	2.83
Kin 3.0 mg/l	95.00	81.67	3.11	1.11	1.04	0.78	4.17	3.51
Kin 5.0 mg/l	93.33	76.67	1.56	1.17	0.74	1.00	3.15	3.89
LSD 0.05	12.13		1.269		0.566		1.223	

These results aligned with the findings of Evaldsson and Welander (1985) on *Cordyline terminalis* 'Atoom', Atta-Alla et al. (1996) on *Cordyline terminalis* 'Atoom', and Youssef (2012) on *Cordyline fruticose*. Akter et al. (2022) on gerbera (*Gerbera jamesonii* Bolus) reported that the highest frequency of shoot multiplication and the greatest number of shoots were achieved in MS medium supplemented with 2.0 mg/l BAP.

b. Effect of medium salt strength and explants supporting method:

The effect of medium strength **(Table 2)** was significant on all parameters except for shoot length only. Full-strength medium produced the highest values of shoot formation % (89.50%), number of shoots/explant (6.11), chlorophyll a (0.116 mg/g f.w.) and chlorophyll b (0.074 mg/g f.w.). This treatment reduced shoot length and number of leaves/shoot to the lowest values (1.60 cm and 3.56 leaves, respectively). In this regard, the longest shoots were obtained by using half-strength medium (2.62 cm), while the highest number of leaves/shoot was obtained by three-quarters strength medium (9.39) which produced the highest carotenoids content (0.042 mg/g f.w.).

Table (2). Effect of medium salt strength and explants supporting on multiplication of *C. fruticosa* 'Atom'.

Treatments	Shoot formation %	No. of shoots/ explant	Shoot length (cm)	No. of leaves/shoot	Ch 'a" (mg/g f.w.)	Ch 'b' (mg/g f.w.)	Carotenoids (mg/g f.w.)
Medium salt strength							
MS full	89.50	6.11	1.60	3.56	0.116	0.074	0.035
MS ¾	84.00	4.72	2.41	9.39	0.109	0.055	0.042
MS ½	60.17	2.61	2.62	6.19	0.071	0.050	0.027
LSD 0.05	5.467	1.332	NS	3.200	0.012	0.011	0.010
Explants supporting method							
Agar	90.33	6.67	2.51	9.93	0.117	0.077	0.034
Bridge	65.44	2.30	1.91	2.83	0.080	0.042	0.035
LSD 0.05	4.464	1.088	NS	2.613	0.010	0.009	NS

Solidifying the medium with agar seems to have more effects than a filter paper bridge in a liquid medium as presented in **Table (2).** Agar increased shoot formation %, number of shoots/explant, shoot length, number of leaves/explant, chlorophyll a and chlorophyll b to the highest values (90.33%, 6.67, 2.51 cm, 9.93, 0.117 mg/g f.w. and 0.077 mg/g f.w., respectively). The highest carotenoids content was obtained by filter paper bridge supporting method (0.035 mg/g f.w.).

For interaction between MS medium strength and medium supporting method, three-quarters-strength medium shared full-strength medium when both of them were solidified by agar in producing the highest percentage of shoot formation (100.0 and 97.33%), number of shoots/explant (9.0 and 7.22 shoots), and chlorophyll b (0.081 and 0.081 mg/g f.w.)-without significant differences between them. Also, three-quarters-strength medium solidified with agar produced the highest significant value for the number of leaves/shoot (14.56). No significant influence was observed in case of the shoot length but the three-quarters-strength produced the longest shoot (3.00 cm) when solidifying by agar and the highest value of carotenoids content was obtained by applying filter paper bridge (0.046 mg/g f.w.). In general, all combinations involving the filter paper bridge resulted in the lowest values, except for carotenoids content **(Table 3).**

Similar results were obtained by Ray et al. (2006) who found that the best elongation of *Cordyline terminalis* (L.) Kunth. shoots was found on $^{1}/_{2}$ MS basal medium and Stamenković et al. (2012) on *Campanula velebitica* Borbás reported that the highest multiplication rate was achieved on ½ MS medium containing 1.0 mM BA. In the same context, Sakai and Imai (2007) reported that a solid medium was a superior supporting method for explant growth compared to a filter paper-bridge with a liquid medium of *Canna edulis* Ker-Gawl.

Table (3). Effect of interaction between medium salt strength and explants supporting method on multiplication of *C. fruticosa* 'Atom'.

	Shoot formation %		No. of shoots/ explant		Shoot length (cm)		No. of leaves/shoot	
Medium salt strength	**Explants supporting method**							
	Agar	**Bridge**	**Agar**	**Bridge**	**Agar**	**Bridge**	**Agar**	**Bridge**
MS full	100.00	79.00	9.00	3.22	1.64	1.56	6.00	1.11
MS ¾	97.33	70.67	7.22	2.22	3.00	1.82	14.56	4.22
MS ½	73.67	46.67	3.78	1.44	2.89	2.36	9.22	3.17
LSD 0.05	7.732		1.884		NS		4.525	
	Ch 'a" (mg/g f.w.)		**Ch 'b' (mg/g f.w.)**		**Carotenoids (mg/g f.w.)**			
	Agar	**Bridge**	**Agar**	**Bridge**	**Agar**	**Bridge**		
MS Full	0.122	0.110	0.081	0.067	0.035	0.035		
MS ¾	0.144	0.074	0.081	0.028	0.037	0.046		
MS ½	0.084	0.057	0.069	0.030	0.029	0.025		
LSD 0.05	0.017		0.016		NS			

2. Effect of medium salt strength, IBA concentration, explants supporting method during the rooting stage:

Table (4) showed that there were no significant differences between full-strength and three-quarters-strength medium on all studied parameters resulting in high values of 81.11 and 79.76 rooting %, 4.26 and 3.99 roots, 2.27 and 2.46 cm root length, 3.38 and 2.98 cm plantlet height, 0.308 and 0.189 mg/g f.w. chlorophyll a, 0.143 and 0.110 mg/g f.w. chlorophyll b and 0.123 and 0.088 mg/g f.w. carotenoids, respectively. Half-strength medium produced the lowest values at all showing significant differences with the other medium salt strengths.

Table (4). Effect of medium salt strength, IBA concentration and explants supporting method during the rooting stage of *C. fruticosa* 'Atom'.

Treatments	Rooting %	No. of roots/ plantlet	Root length (cm)	Plantlet height (cm)	Ch 'a" (mg/g f.w.)	Ch 'b' (mg/g f.w.)	Carotenoids (mg/g f.w.)
Medium salt strength							
MS full	81.11	4.26	2.27	3.38	0.308	0.143	0.123
MS ¾	79.76	3.99	2.46	2.98	0.189	0.110	0.088
MS ½	66.52	2.67	1.59	2.38	0.232	0.116	0.084
LSD 0.05	2.361	0.384	0.287	0.313	0.021	0.021	0.006
IBA concentration							
IBA 0.0 mg/l	66.79	2.82	1.72	2.61	0.239	0.123	0.111
IBA 1.0 mg/l	85.20	4.34	2.53	2.87	0.280	0.135	0.107
IBA 3.0 mg/l	75.39	3.76	2.07	3.25	0.209	0.112	0.077
LSD 0.05	2.361	0.384	0.287	0.313	0.021	0.020	0.006
Explants supporting method							
Agar	94.11	5.25	2.56	3.28	0.287	0.133	0.125
Bridge	57.48	2.03	1.65	2.54	0.199	0.114	0.071
LSD 0.05	1.927	0.314	0.234	0.255	0.017	0.016	0.005

The effect of IBA concentrations was significant **(Table 4).** IBA at 1.0 mg/l produced the highest values for rooting % (85.20%), number of roots/plantlet (4.34), root length (2.53 cm), chlorophyll a (0.280 mg/g f.w.) and chlorophyll b (0.135 mg/g f.w.). IBA at 3.0 mg/l increased the plantlet height which recorded 3.25 cm. While the medium free of IBA produced the highest carotenoids content (0.111 mg/g f.w.).

Data in **Table (4)** revealed that the impact of solid medium produced the highest values of rooting % (94.11%), number of roots/plantlet (5.25 root), plantlet height (2.56 cm), chlorophyll a (0.287 mg/g f.w.), chlorophyll b (0.133 mg/g f.w.) and carotenoids content (0.125 mg/g f.w.) when compared with filter paper bridge in liquid medium.

The interaction between medium salt strength and IBA concentrations was

significant. It could be noticed that three-quarters-strength without IBA or with IBA at 1.0 mg/l seems to be more effective in producing the highest rooting %, number of roots and root length (88.91 and 88.37%, 4.39 and 5.56 and 2.50 and 3.17 cm, respectively), such values were insignificant in case of rooting %. The highest values of plantlet height were obtained by three-quarters-strength without IBA (3.28 cm), half-strength without IBA (3.65 cm) and three-quarters-strength in addition to IBA at 1.0 mg/l (3.28 cm) without significant differences between them. On the other hand, three-quarters-strength without IBA addition induced pigments content and produced the highest values (0.431, 1.97 and 0.151 mg/g f.w. for chlorophyll a, b and carotenoids, respectively) as shown in **Table (5) and Fig. (3).**

Table (5). Effect of interaction between medium salt strength and IBA concentration during the rooting stage of *C. fruticosa* 'Atom'.

Medium salt strength	Rooting %			No. of roots/plantlet		
	IBA concentration					
	IBA 0.0 mg/l	IBA 1.0 mg/l	IBA 3.0 mg/l	IBA 0.0 mg/l	IBA 1.0 mg/l	IBA 3.0 mg/l
MS full	73.67	71.50	55.22	4.11	2.11	2.25
MS ¾	88.91	88.37	78.33	4.39	5.56	3.08
MS ½	80.75	79.42	66.01	4.28	4.31	2.68
LSD 0.05		4.089			0.666	
	Root length (cm)			**Plantlet height (cm)**		
MS full	2.15	1.77	1.23	3.19	2.61	2.03
MS ¾	2.50	3.17	1.93	3.28	3.04	2.31
MS ½	2.17	2.45	1.61	3.65	3.28	2.81
LSD 0.05		0.498			0.542	

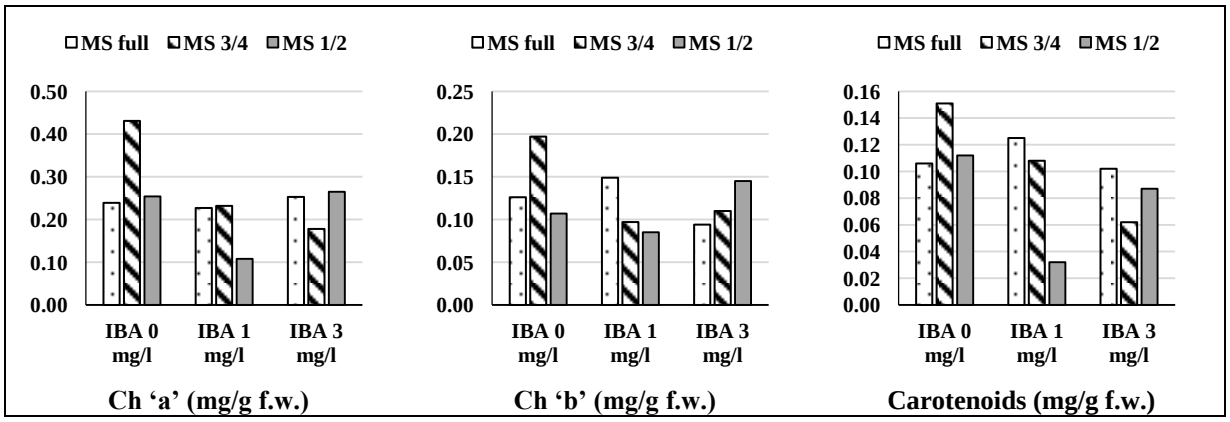

Fig. (3). Effect of interaction between medium salt strength and IBA concentration on pigments content of *C. fruticosa* 'Atom' during the rooting stage.

As for the influence of the interaction between medium salt strength and explant supporting method **(Table 6 and Fig. 4),** full-strength medium + agar solid medium produced the highest values followed by three-quarters-strength medium + agar solid medium (99.11 and 97.78% for rooting, 6.41 and 5.30 for number of roots, 2.88 and 2.84 cm for root length, 3.80 and 3.26 cm for plantlet height and 0.135 and 1.39 mg/g f.w. for carotenoids, respectively). The differences between these two treatments were significant for the number of roots/plantlet and plantlet height only. The highest chlorophyll a content was obtained by full-strength medium + agar solid medium (0.332 mg/g f.w.), while the highest chlorophyll b was obtained by full-strength medium + filter paper in liquid medium (0.153 mg/g f.w.)

followed without significant difference by three-quarters-strength medium + agar solid medium (0.144 mg/g f.w.).

Table (6). Effect of interaction between medium salt strength and explant supporting method during the rooting stage of *C. fruticosa* 'Atom'.

Medium salt strength	Rooting %		No. of roots/plantlet		Root length (cm)		Plantlet height (cm)	
	Explant supporting method							
	Agar	Bridge	Agar	Bridge	Agar	Bridge	Agar	Bridge
MS full	99.11	63.10	6.41	2.11	2.88	1.67	3.80	2.95
MS ¾	97.78	61.74	5.30	2.69	2.84	2.08	3.26	2.69
MS ½	85.44	47.60	4.06	1.29	1.97	1.20	2.78	1.98
LSD 0.05	3.339		0.544		0.406		0.443	

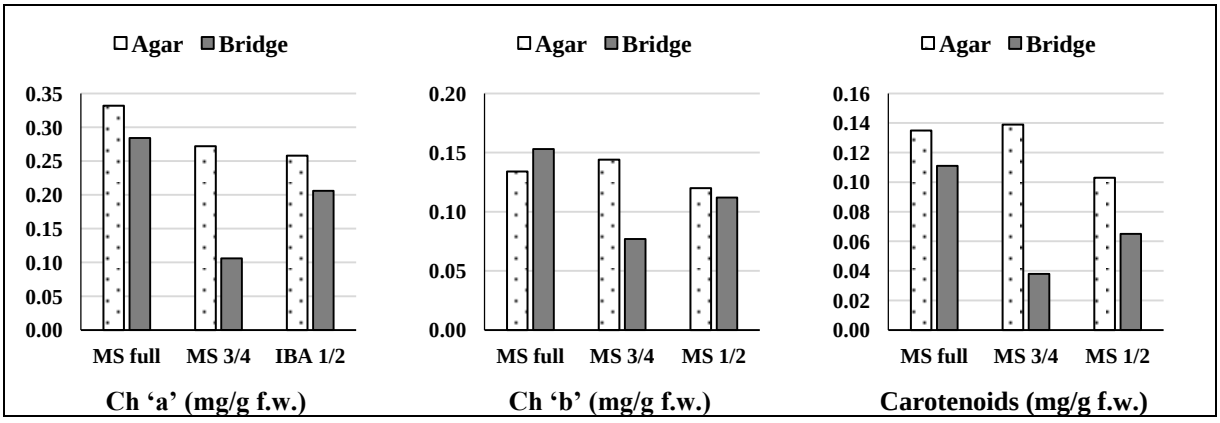

Fig. (4). Effect of interaction between medium salt strength and explant supporting method on pigments content of *C. fruticosa* 'Atom' during the rooting stage.

Regarding the influence of the combined treatments between IBA concentrations and explant supporting method **(Table 7 and Fig. 5),** IBA at 1.0 and 3.0 in addition to agar solid medium produced the highest values for rooting percentage (98.11 and 96.11%), number of roots/plantlet (6.17 and 5.44), root length (2.99 and 2.69 cm) and plantlet height (3.35 and 3.59 cm), the only significant difference was obtained for number of roots/plantlet. The highest values for chlorophyll a (0.430 mg/g f.w.), chlorophyll b (1.68 mg/g f.w.) and carotenoids (1.73 mg/g f.w.) were recorded by IBA at 1.0 mg/l + agar solid medium.

Table (7). Effect of interaction between IBA concentration and explant supporting method during the rooting stage of *C. fruticosa* 'Atom'.

IBA concentration	Rooting %		No. of roots/plantlet		Root length (cm)		Plantlet height (cm)	
	Explant supporting method							
	Agar	Bridge	Agar	Bridge	Agar	Bridge	Agar	Bridge
IBA 0.0 mg/l	88.10	45.48	4.15	1.50	2.01	1.42	2.89	2.33
IBA 1.0 mg/l	98.11	72.29	6.17	2.52	2.99	2.07	3.35	2.40
IBA 3.0 mg/l	96.11	54.67	5.44	2.07	2.69	1.45	3.59	2.90
LSD 0.05	3.339		0.544		0.406		0.443	

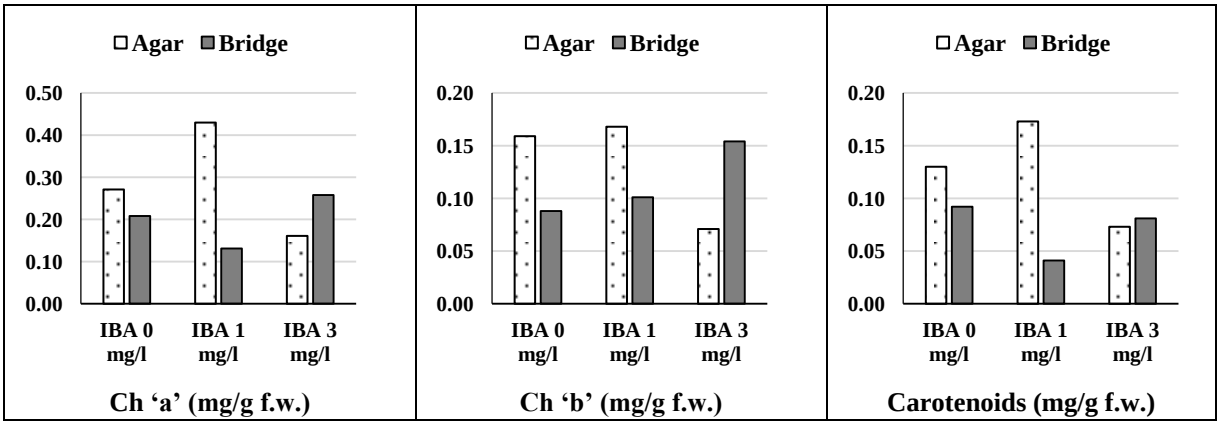

Fig. (5). Effect of interaction between IBA concentration and explant supporting method on pigments content of *C. fruticosa* 'Atom' during the rooting stage.

The combination of medium salt strength, IBA concentration and the explant supporting method shows notable variations in the results obtained. The superior treatments for rooting parameters were full-strength medium + IBA at 1.0 mg/l + agar solid medium and three-quarters-strength medium + IBA at 1.0 mg/l + agar solid medium as recorded the highest values for rooting %, number of roots and root length (100 and 100%, 6.78 and 7.22 root and 2.78 and 3.78 cm, for these two combined treatments, respectively), the differences in this regard were insignificant except for root length only. It could be also mentioned that full-strength and three-quarters-strength medium produced a 100% rooting percentage when combined with IBA at 3.0 mg/l. The obtained data reveal that increasing IBA concentrations when combined with full-strength medium in agar solid medium gradually produced the tallest plantlet (3.67, 3.83 and 3.89 cm, for IBA at 0.0, 1.0 and 3.0 mg/l, respectively) without significant differences between them. The combined treatment of three-quarters-strength medium + IBA at 1.0 mg/l + agar solid medium shared the previously mentioned treatments without significant differences (3.56 cm). In the same context, full-strength medium + IBA at 1.0 mg/l + agar solid medium resulted in the highest chlorophyll a (0.640 mg/g f.w.), chlorophyll b (0.208 mg/g f.w.) and carotenoids (0.252). All combinations including liquid medium produced the lowest values compared to agar solid medium **(Table 8).**

These results were consistent with Sarhan et al. (2010) on *Polygala myrtifolia*, Stamenković et al. (2012) on *Campanula velebitica* Borbás, Dewir et al. (2015) on Cattleya, Uddin et al. (2016) on *Cestrum nocturnum* L., Thakur and Kanwar (2018) on *Dianthus caryophyllus* L. 'Master', Alsoufi et al. (2021) on Chrysanthemum plant (*Chrysanthemum indicum* L.) and Suraya et al. (2021) on *Phyllanthus niruri*. Ray et al. (2006) reported that *Cordyline terminalis* (L) Kunth. shoots were rooted on ½ MS medium supplemented with IBA.

Table (8). Effect of the interaction between medium salt strength, IBA concentration and explants supporting method during the rooting stage of *C. fruticosa* 'Atom'.

IBA concentration	Explant supporting method	Rooting %			No. of roots/plantlet		
		Medium salt strength					
		MS full	MS ¾	MS ½	MS full	MS ¾	MS ½
IBA 0.0 mg/l	Agar	97.33	93.33	73.65	5.89	3.22	3.33
	Bridge	50.00	49.67	36.79	2.33	1.00	1.17
IBA 1.0 mg/l	Agar	100.00	100.00	94.33	6.78	7.22	4.50
	Bridge	77.81	76.73	62.33	2.00	3.89	1.67
IBA 3.0 mg/l	Agar	100.00	100.00	88.33	6.56	5.44	4.33
	Bridge	61.50	58.83	43.68	2.00	3.17	1.03
LSD 0.05			5.782			0.942	
		Root length (cm)			Plantlet height (cm)		
IBA 0.0 mg/l	Agar	2.63	2.11	1.28	3.67	2.61	2.39
	Bridge	1.67	1.43	1.17	2.72	2.61	1.67
IBA 1.0 mg/l	Agar	2.78	3.78	2.42	3.83	3.56	2.67
	Bridge	2.22	2.56	1.44	2.72	2.52	1.94
IBA 3.0 mg/l	Agar	3.22	2.64	2.22	3.89	3.61	3.28
	Bridge	1.11	2.25	1.00	3.42	2.94	2.33
LSD 0.05			0.704			0.767	
		Ch 'a' (mg/g f.w.)			Ch 'b' (mg/g f.w.)		
IBA 0.0 mg/l	Agar	0.187	0.377	0.250	0.118	0.242	0.116
	Bridge	0.290	0.076	0.256	0.134	0.057	0.072
IBA 1.0 mg/l	Agar	0.640	0.341	0.309	0.208	0.111	0.186
	Bridge	0.222	0.124	0.046	0.186	0.083	0.034
IBA 3.0 mg/l	Agar	0.168	0.098	0.216	0.076	0.078	0.059
	Bridge	0.340	0.118	0.315	0.137	0.092	0.231
LSD 0.05			0.052			0.051	

IBA concentration	Explant supporting method	Carotenoids (mg/g f.w.)		
		MS full	MS ¾	MS ½
IBA 0.0 mg/l	Agar	0.066	0.221	0.104
	Bridge	0.146	0.029	0.100
IBA 1.0 mg/l	Agar	0.252	0.164	0.103
	Bridge	0.050	0.052	0.021
IBA 3.0 mg/l	Agar	0.086	0.031	0.101
	Bridge	0.138	0.033	0.073
LSD 0.05			0.016	

3. Effect of growing media during the acclimatization stage:

Data in **Table (9)** showed that among the 6 tested growing media mixtures both coco peat and coco peat + sand produced the highest values for plantlet height (20.07 and 19.03 cm), number of leaves/plantlet (9.00 and 8.33), root length (20.73 and 24.23 cm), fresh weight of vegetative growth (2.38 and 2.02 g) and roots fresh weight (1.15 and 1.17 g), respectively. The differences between these two treatments were significant in case of root length and fresh weight of vegetative growth only.

In this regard, Manjusha and Sathyanarayana (2008) on stevia (*Stevia rebaudiana* Bert.) reported that the survival rate of the micropropagated plantlets increased to over 50% with the incorporation of 75% cocopeat into the hardening medium. Successfully use of cocopeat-based mixtures was reported by many authors e.g. Patra and Beura (2016) on some gerbera cultivars, Pérez-Pazos et al. (2023) on sweet potato and Dwiyani et al. (2024) on Strawberry (*Fragaria* × *ananassa* Duch.).

Table (9). Effect of growing media during the acclimatization stage of *Cordyline fruticosa* 'Atom'.

Medium mixture	Plant height (cm)	No. of leaves/plant	Root length (cm)	Vegetative growth f.w. (g)	Roots f.w. (g)
Peat moss	16.55	7.67	13.62	1.37	0.81
Peat moss + Vermiculite	17.10	8.00	13.14	1.67	0.55
Peat moss + Sand	18.90	7.33	13.70	1.91	0.87
Coco peat	20.07	9.00	20.73	2.38	1.15
Coco peat + Vermiculite	16.83	6.67	18.20	1.57	1.08
Coco peat + Sand	19.03	8.33	24.23	2.02	1.17
LSD 0.05	NS	0.940	2.540	0.410	0.199

DISCUSSION

The results of the present study emphasized that BA is more effective than kinetin for micropropagation of *Cordyline fruticosa* 'Atom'. In general, Vardja and Vardja (2001) classified *Cordyline terminalis* in a group of plants that can be propagated on media containing either BA or Kin, within a wider concentration range (0.5-4 mg/l) without negative side effects. However, many authors have proved the advantage of BA e.g. Youssef (2012) on *Cordyline fruticosa*. The response to cytokinin type and the addition of NAA during the multiplication stage seems to be influenced by plant species, Thakur and Kanwar (2018) on *Dianthus caryophyllus* L. 'Master' reported that for *in vitro* multiplication of shoots, MS medium containing 2.0 mg/l kinetin and 0.25 mg/l NAA was more effective. Also, Barakat (2021) on *Dracaena draco* showed that 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. In contrast, Alawaadh *et al.* (2020) reported that BAP alone at 1.0 mg/l significantly increased shoot multiplication compared with other cytokinins for *Philodendron bipinnatifidum.* Benzyladenine is more effective than other cytokinins because it is the more stable cytokinin as reported by Mok (1994). Also, BA has a stronger binding affinity to cytokinin receptors in plant tissues than other cytokinins (Brinegar, 1994). George *et al.* (2008) reported that in different plants, BA is first converted into several metabolites, the formation of these conjugates does not necessarily indicate that BA is inactivated, as one or more of these conjugates may still retain cytokinin activity or serve as storage products.

In addition, this study also revealed that a diluted strength medium could be used without reducing the productivity or quality of *in vitro* cultured *C. fruticosa* 'Atom'. The enhanced effect of the lower salt concentration in MS may be due to the decreased intensity of nitrogen, especially the ammonium salt (El-Esawi, 2016). Nitrogen is presented in MS medium in two forms nitrate (NO_3^-) and ammonium (NH_4^+), the ratio between them 66:34, respectively (George et al., 2008). Reducing the total nitrogen concentration resulted in a decrease in both NO_3^- and NH_4^+ levels, but the amount of NH_4^+ in the medium will be significantly lower compared to NO_3^-, as the NO_3^- concentration is twice that of NH_4^+ in the medium composition. The impact of ammonium salts can range from inhibitory to essential, depending on the specific tissue type and the intended purpose of the culture (Lindsey, 1997). Bhojwani and Dantu (2013) reported that ammonium ions are toxic to the protoplasts of various species. High levels of ammonium in the medium inhabit some essential enzyme systems (George et al., 2008), also the proportion of ammonium ions in the

medium can influence the way in which growth regulants control morphogenesis (Walker and Sato, 1981). Bhojwani and Dantu (2013) reported that nutrient salts account for about 20–50% of the osmotic potential in the medium, with the remainder being maintained by sucrose. Therefore, lowering the salt concentration in the medium reduces the osmotic potential, which in turn promotes the growth of microshoots cultured in the medium.

Although this study revealed that there was no need for NAA addition during the multiplication stage, this was contrary to the results obtained by other works on cordyline e.g. Meshkova et al. (1999), Chinnu et al. (2012) and Hassan and Abdallah (2015). The negative role of NAA addition exerted from the present study could be interpreted by George *et al.* (2008) who reported that synthetic auxins such as NAA are often converted, after uptake into plant tissues, to conjugates, mainly glucosyl esters. This reversible conjugation may regulate levels of free active substances needed by plant tissues. Also, they reported that reducing the external NAA concentration resulted in a significant increase in internal free IAA concentration and cytokinin.

Low concentrations of IBA were important for rooting. In some species, the continued presence of auxin will inhibit root elongation, in which case root development can occur in low concentrations or even hormone-free medium (Gamborg, and Phillips, 1995) .

This study showed that a solid medium proved to be more effective than a liquid medium supported by a filter paper bridge. Although, Maitra et al. (2009) on Cymbidium aloifolium (L.) Sw., Sarhan et al. (2010) on *Polygala myrtifolia* and Naz et al. (2011) on *Bauhinia tomentosa* L. proved a positive influence of liquid MS medium using filter-paper bridge for support, this method was not preferred for *Cordyline fruticosa* 'Atom', likely due to the insufficient contact between the explant tissues and the medium. The poor anchorage of the explants on the filter paper bridge may have hindered the optimal absorption of nutrients from the medium.

In the same manner, the findings of this study showed that coco peat surpassed peat moss-based mixtures. This could be interpreted as that cocopeat has several advantages as a planting medium, including neutral pH, micronutrient contents such as Fe, K, Mn, Cu, and Zn in high amounts, capability of facilitating root growth and distribution, high degree of aeration, high porosity, and high level of water holding capacity. Another advantage of using cocopeat as planting media is reducing agriculture waste (Dwiyani et al., 2024).

CONCLUSION

In conclusion, there was no need for NAA, BA at 3.0 mg/l + ¾-strength solid MS medium could be applied in the multiplication stage, ¾-strength solid MS medium + 1.0 mg/l IBA was most effective for the rooting stage and coco peat alone or mixed with sand was the best potting mixture for the acclimatization of *C. fruticosa* 'Atom' *in vitro*-produced plantlets.

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الملخص العربي

تقييم بعض منظمات النمو النباتية، قوة تركيز البيئة و طريقة التدعيم للإكثار الدقيق الأمثل لنبات الكوردالين أتوم

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سلسلة من التجارب المعملية تم اجرائها بمعمل زراعة الأنسجة، معهد بحوث البساتين، مركز البحوث الزراعية، الجيزة، مصر، متبوعة بتجربة خارج المعمل خلال عام 2023/2022. هدف هذه التجارب تطوير بروتوكول فعال للإكثار الدقيق لنباتات الكوردالين 'أتوم' مع الحفاظ على الجودة والانتاجية العالية. في هذا الخصوص فقد تم اجراء ثلاث تجارب معملية وواحدة داخل الصوبة البلاستيكية. درست هذه التجارب انواع السيتوكينينات وتركيزها مع اضافة نفثالين حمض الخليك من عدمها (تجربة التضاعف الأولى)، تركيزات متنوعة من أملاح بيئة الزراعة وطريقة تثبيت المنفصلات النباتية (تجربة التضاعف الثانية) وذلك خلال مرحلة التضاعف. تراكيز متنوعة من أملاح بيئة الزراعة، تركيز اندول حمض البيوتريك وطريقة تثبيت المنفصلات النباتية خلال مرحلة التجذير. اما خلال مرحله الاقلمه فقد تم دراسة تأثير مخاليط بيئة الزراعة المختلفة. أظهرت النتائج أن استخدام البنزايل آدينين بتركيز 3 ملجم/لتر بدون اضافة نفثالين حمض الخليك (في تجربة التضاعف الأولى) و بيئة موراشيج وسكوج كاملة التركيز أو ثلاث أرباع قوة الأملاح كلاهما مع بيئة صلبة (تجربة التضاعف الثانية) أحدثت أعلى القيم للنسبة المئوية لتكوين الأفرع، عدد الأفرع/منفصل نباتي، طول الفرع، عدد الأوراق/فرع وكذلك محتوى الأوراق من الصبغات. بالنسبة للتجذير فقد وجد أن بيئة بثلاث ارباع قوة تركيز الأملاح + 1.0 ملجم/لتر اندول حمض البيوتريك مع البيئة الصلبة كانت أكثر كفاءة. الكوكوبيت بمفرده أو مع الرمل كان أفضل مخلوط أصص خلال مرحلة الأقلمة. أظهرت هذه الدراسة أن البنزايل آدينين أكثر كفاءة من الكينيتين، البيئة ذات قوة تركيز أملاح مخففة يمكن استعمالها بدلاً من البيئة كاملة القوة بدون أي خفض في الجودة، لا داعي لإضافة نفثالين حمض الخليك خلال مرحلة التضاعف، التركيز المنخفض من اندول حمض البيوتريك كان ضرورياً للتجذير، البيئة شبه الصلبة تفوقت على البيئة السائلة المدعومة بورق الترشيح و أن الكوكوبيت تفوق على البيتموس خلال مرحلة الأقلمة.