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IMPROVING THE EFFICIENCY OF *Dieffenbachia picta* CV TROPICA MICROROPAGATION

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ABSTRACT

The best sterilizing treatment for *Dieffenbachia picta* cv Tropica buds was immersion for 30 min. in fungicide solution of Rizolex (2 g / L), followed by soaking in 0.3 % mercuric chloride solution for 20 min., then transferred to sodium hypochlorite solution at 1.0 % for 30 min. Establishment stage period could be decreased from 8 weeks to be only 4 weeks by culturing the buds on MS medium supplemented with (16 2ip + 2 IAA + 1 DPU mg/L) which also increased the number of shoot /explant. Different concentrations of triiodobenzoic acid (0.0, 1.0, 2.0, 4.0 or 8.0 mg/L), malt extract (0.0, 1.0, 2.0, 4.0 or 8.0 g\L), yeast extract (0.0, 1.0, 2.0, 4.0 or 8.0 g\L) or casein hydrolysate (0.0, 0.50, 1.0, 2.0 or 4.0 g\L) combined with (16 2ip + 2 IAA + 1 DPU mg/L) were tested during multiplication stage. The best treatment for shoot multiplication (10 shoots /explant) and rooting (4 roots /shoot with 6.48 cm root length) was 4 g/l malt extract combined with (16 2ip + 2 l IAA + 1 DPU mg/L). Plantlets were successfully acclimatized (95% survival percentage) in peat moss and sand (1:1, v/v).

INTRODUCTION

Dieffenbachia is one of the most important ornamental tropical foliage plant genera. It is highly prized for its decorative value, ease of growth and tolerance to interior environments (Henny *et al.,* 2000). Dieffenbachia can be propagated by cuttings, but the low rate of this conventional method of propagation has limited its multiplication and widespread use. Therefore, rapid in vitro propagation could be of great advantage, not only for quick clonal multiplication but also for elimination of pathogens and diseases transmission (Torres, 1989), especially *Erwinia spp.* infection which is not easily controlled by chemicals and causes restricted growth rate and loss of quality (Paola *et al.,* 1986). Another reason for preferring in vitro propagation of dieffenbachia is the tendency of obtained plants to initial branching more freely. This is a desirable feature that enhances the market value of plants (Voyiatzi and Voyiatzis, 1989).

In vitro propagation protocol of some dieffenbachia species has been established (Knauss, 1976; Kunisaki, 1977; Paola *et al.,* 1986; Voyiatzi and Voyiatzis, 1989; Genfa *et al.,* 1999 and Vardja and Vardja, 2001) but it has been hampered by two major problems. First, high contamination rate of initial explant which is a serious hindrance (Kunisaki, 1977). Second, is that initial culture grows slowly and takes up to 6 months to reach a suitable stage for a multiplication stage (Henny *et al.,* 2000).

Therefore, the objectives of this study were:

1) To assess the best sterilizing treatment to obtain the lowest contamination rate and the highest survival percentage of explants.
2) To decrease the initial culture period during establishment stage.

3) To study the effect of some factors affecting multiplication and growth rate of dieffenbachia shoots in order to obtain high multiplication rate. The main objective of this paper is to establish a micropropagation protocol of *Dieffenbachia picta* cv. Tropica.

MATERIALS AND METHODS

This study was carried out in Plant Tissue Culture Laboratory of Horticulture Department, Faculty of Agriculture, Zagazig University, throughout the period of 2005- 2007.

Lateral buds with small pieces (about 1×1 cm) of stem were excised from vigorously growing *Dieffenbachia picta* cv. Tropica plants grown in greenhouse. Buds were surface sterilized by immersion for 30 min. in fungicide solution of Rhizolex (2 g / L), followed by soaking in 0.3 % mercuric chloride solution for 20 min., then transferred to sodium hypochlorite solution at 0.5, 1.0 or 1.5 % for 10, 20, 30 or 40 min. Explants were thoroughly rinsed three times with sterile distilled water after each previous step. Explants were inoculated in glass tubes (25 ×150 mm) containing (Murashige and Skoog 1962) basal medium with 30 g/L sucrose, free from growth regulators. Each treatment was consisted of 15 tubes. Contamination and survival percentages were recorded after four weeks. The best sterilizing treatment (1.0 % sodium hypochlorite for 30 min) was used for the following experiments. Surface sterilized buds were cultured on MS medium supplemented with three different combinations of growth regulators treatments which commonly used with other dieffenbachia species to evaluate the efficiency of these treatments with *Dieffenbachia picta* cv. Tropica. These treatments were 16 2ip + 2 IAA mg/L (Paola *et al.*, 1986 on *Dieffenbachia amoena*), 4 mg/L BA (Genfa *et al.*, 1999 on three dieffenbachia species) or 1 mg/l Kinetin (Vardja and Vardja, 2001 on *Dieffenbachia sp*.) in addition to free growth regulators media (control). Data were recorded after 4 and 8 weeks from inoculation date. In order to improve the efficiency of the best treatment (16 2ip + 2 IAA mg/L) obtained from the previous experiment, diphenylurea (DPU) was added to MS medium at different concentrations (0.00, 0.25, 0.50, 1.00 or 2.00 mg/L) in combination with 16 2ip + 2 IAA mg/L. Data were recorded after 4 and 8 weeks from inoculation date. The best treatment (16 2ip + 2 IAA + 1 DPU mg/L) was repeated in order to obtain enough shoots for later multiplication experiments. Obtained shoots (about 2.0 cm length) were used as explants for the following experiments.

In order to obtain high multiplication rate of shoots with adequate roots, shoots were cultured on MS medium supplemented with different concentrations (0.0, 1.0, 2.0, 4.0 or 8.0 mg/L) of triiodobenzoic acid (TIBA) , malt extract (ME*) at 0.0, 1.0, 2.0, 4.0 or 8.0 g\L, yeast extract (YE*) at 0.0, 1.0, 2.0, 4.0 or 8.0 g\ L or casein hydrolysate (CH) at 0.0, 0.50, 1.0, 2.0 or 4.0 g\ L in combination with 16 2ip + 2 IAA + 1 DPU mg/L. Each treatment was consisted of 15 jars (60 × 120 mm), each one contained about 50 ml, and one shoot was inoculated in each jar. Cultures were incubated at 25 ±

2°C under 16 h. photoperiod. Data (i.e., No. of proliferated shoot/explant, shoot length, No. of leaf/shoot, shoot fresh weight, rooting %, No. of root/shoot and root length) were recorded after 12 weeks.

The best multiplication treatment 4 g/L ME + (16 2ip + 2 IAA +1 DPU mg/L) was repeated to obtain enough rooted shoots (about 2.8 cm length with about 4 roots) for adaptation experiment. These rooted shoots were acclimatized by transferring them to plastic pots (9 x 7 cm) containing peat moss, peat moss and sand (1:1, v/v) or peat moss and vermiculite (1:1, v/v). Each treatment consisted of 20 pots, and each one contained one plantlet.

*: ME and YE were obtained from Marine Chemicals Company, Indian

The cultured pots were covered with polyethylene bags for 4 weeks before removing them. The plantlets were hold in greenhouse at about 25 °C. The survival percentage was recorded after four weeks.

The statistical layout of all experiments was simple completely randomized design. The recoded data were statistically analyzed, and the means were compared using Duncan multiple range test according to Little and Hills (1978).

RESULTS AND DISSCUSION

1. Efficiency of sodium hypochlorite sterilization:

Data in Table 1 show that the explants of dieffenbachia sterilized with different sodium hypochlorite concentrations for different periods showed a gradual decrease in contamination percentage as the concentration of sodium hypochlorite increased to 1.5 % concomitant with the increase of exhibition time up to 40 minutes. However, the highest concentration (1.5%) and more exhibition time decreased explant survival percentage. The most efficient treatment was 1 % sodium hypochlorite for 30 minutes which resulted in 13.33 % contamination percentage and 92.30 % survival percentage.

2. Effect of different growth regulators treatments on dieffenbachia shoot proliferation:

Data in Table 2 clear the effect of different growth regulators chosen treatments on dieffenbachia shoot proliferation. It worth to mention that these treatments were previously investigated with other dieffenbachia species and recommended by some investigators (Paola *et al*., 1986; Genfa *et al*., 1999 and Vardja and Vardja, 2001). The herein investigated plant (*Dieffenbachia picta* cv. Tropica) did not show any response to any treatment after 4 weeks ,while after 8 weeks, shoot proliferation percentages were 73.33, 40 and 33.33 % with 16 2ip + 2 IAA, 4 BA and 1 Kin mg/L, respectively. The most efficient treatment in this regard was 16 2ip + 2 IAA mg/L which resulted in the highest values of shoot proliferation percentage (73.33%), shoot length (1.06 cm), while shoot number/explant was of similar magnitude for the three growth regulators treatments. Also, less callus formation was observed with (16 2ip + 2 IAA mg/L) treatment.

3. Effect of different diphenylurea (DPU) concentrations combined with 16 2ip + 2 IAA (mg/L) on shoot proliferation:

Table 3 shows application of several concentration of DPU in addition to 16 2ip + 2 IAA mg/L (the best treatment obtained from the previous experiment) with the aim of enhancing more shoot proliferation and shoot number/explant. Data clear that early shoot proliferation (after 4 weeks) was enhanced due to DPU addition. Increasing of DPU concentration up to 1.0 mg/L was the most effective treatment. As the time of incubation increased to 8 weeks, 0.25, 0.5 and 1.0 mg/L DPU resulted in similar proliferation percentage (100%), while 2.0 mg/L showed depressive effect comparing with 16 2ip + 2 IAA mg/L alone. The striking effect of DPU was the early shoot proliferation which decreased the initial culture period during establishment stage. Shoot length was increased by using DPU especially at 0.5 and 1.0 mg/L, while shoot number/ explants was at the highest value (2.1) with 1.0 mg/L DPU. This was concomitant with high callus formation. The enhancing effect of diphenylurea and its derivatives was previously reported by Genkov and Ivanova (1999).

Table 1: Effect of different concentrations (%) and exhibition time (min) of sodium hypochlorite on contamination and survival percentages of dieffenbachia explants.

Treatments		Contamination %	Survival % *
Sodium hypochlorite (%) (%)concentration	Exhibition time (min.)		
0.5	10	93.33	100
	20	86.66	100
	30	73.33	100
	40	66.66	100
1.0	10	60	100
	20	40	100
	30	13.33	92.30
	40	13.33	76.92
1.5	10	46.66	75
	20	33.33	50
	30	6.66	28.57
	40	0	6.66

*** Calculated for uncontaminated explants**

On *Funaria hygrometrica*, Ricci *et al*. (2001) on *Malus pumila* rootstock M 26 and Srinivasan *et al*. (2006) on coleus plant. On the other hand, the depressive effect of high concentration DPU was demonstrated by Christianson and Hornbuckle (1999) on *Funaria hygrometrica* and Srinivasan *et al*. (2006). This may be due to that relatively low concentrations of DPU would bind all the available receptor sites and increased concentration of DPU would lead to the same low level of stimulation of bud formation as exposure to the lower concentration (Christianson and Hornbuckle, 1999).

Table 2: Effect of different growth regulators treatments on shoot proliferation of dieffenbachia after 4 and 8 weeks during establishment stage.

Growth regulators (mg/L)	Shoot proliferation %		Proliferated shoot length (cm)		No. of shoot/ explant		Callus size	
	Weeks							
	4	8	4	8	4	8	4	8
0.0	0.0	0.0	0.0	0.0 a	0.0	0.0 a	–	–
16 2ip + 2 IAA	0.0	73.33	0.0	1.06 c	0.0	1.0 b	–	+
4 BA	0.0	40	0.0	0.60 b	0.0	1.0 b	+	++
1 Kin.	0.0	33.33	0.0	0.50 b	0.0	1.0 b	–	+

*** Data with the same letter vertically are not significant**

Table 3: Effect of different concentrations of diphenylurea (DPU ppm) 2ip + 2 IAA (ppm) on shoot proliferation of combined with 16 during establishment stage dieffenbachia after 4 and 8 weeks

Treatments (mg/L)		Shoot proliferation %		Proliferated shoot length (cm)		No. of shoot/ explant		Callus size	
Growth regulators	DPU concentration	weeks							
		4	8	4	8	4	8	4	8
16 2ip + 2 IAA + 1 DPU	**0.00**	0.00	80	0.0 a	1.20 b	0.00 a	1.0 a	–	+
	0.25	40.00	100	0.5 b	1.50 c	1.00 b	1.0 a	+	++
	0.50	86.66	100	1.8 c	2.0 d	1.10 b	1.2 a	+	++
	1.00	100	100	2.1 d	2.10 d	1.53 c	2.1 b	+++	++++
	2.00	0.00	73.33	0.0 a	1.02 a	0.00 a	1.0 a	++	+++

*** Data with the same letter vertically are not significant**

4. Effect of different triiodobenzoic acid (TIBA) concentrations combined with 16 2ip + 2 IAA + 1 DPU (mg/L) on shoot proliferation:

Table 4 shows another attempt to enhance shoot proliferation by using different concentrations of TIBA combined with 16 2ip + 2 IAA + 1 DPU (mg/L). Addition of TIBA up to 2 mg/L was very effective in enhancing number of shoot/explant, as it resulted in 11.1 or 10.0 shoots /explant with 1 or 2 mg/L TIBA, respectively (Fig. 1). On the other hand, increasing TIBA concentration to 4 or 8 mg/L showed less significant effect as 5 or 4 shoots /explant, respectively.

The most effective concentration of TIBA for shoot length was 1 mg/L which also resulted in the highest values of leaf number/shoot and shoot fresh weight. The advantages of TIBA addition to the media at concentration range 0.5 – 5.0 µM for enhancing embryos and shoot buds regeneration has been reported with some species such as *Colocasia esculenta* (Nyman and Arditti, 1984), *Lilium formosanum* (Nakano *et al*., 2000) and oncidium plant (Chen and Chang, 2004). Katekar and Geissler (1980) suggested that addition of TIBA to regeneration media might inhibit the transport of endogenous IAA to the regeneration sites, so that an

auxin/cytokinin balance becomes more favorable for the regeneration of shoot buds.

It worth to mention that rooting process took place after 12 weeks for all applied treatments by the same efficiency (100%) in either omitted TIBA treatment or that has it, but number of root/shoot was the highest (6.0 roots/shoot) at 4 mg/L TIBA concomitant with the high root length (6.09 cm).

Table 4: Effect of different concentrations of triiodobenzoic acid (TIBA) combined with 16 2ip + 2 IAA + 1 DPU (mg/L) on shoot proliferation of dieffenbachia after 12 weeks during multiplication stage.

Growth regulators (mg/L)	TIBA concentration (mg/L)	No. of shoot/ explant	Shoot length (cm)	No. of leaf/ shoot	Shoot fresh weight (g)	Rooting %	No. of root/ shoot	Root length (cm)
16 2ip + 2 IAA + 1 DPU	0.0	4.5 b	2.49 b	2.5 bc	0.60 b	100	2.4 a	4.60 a
	1.0	11.1 c	3.22 c	3.3 c	0.91 c	100	2.5 a	4.54 a
	2.0	10.0 c	2.71 b	2.6 bc	0.72 b	100	3.6 b	5.02 a
	4.0	5.0 b	2.61 b	2.2 b	0.61 b	100	6.0 c	6.09 b
	8.0	3.2 a	1.90 a	1.5 a	0.41 a	100	4.1 b	6.22 b

- **Data with the same letter vertically are not significant**

5. Effect of different malt extract (ME) concentrations combined with 16 2ip + 2 IAA + 1 DPU (mg/L) on shoot proliferation:

Table 5 shows significant gradual increase in shoot number/explant due to increasing malt extract up to 4 g/L (Fig. 1). Many workers have been also reported the stimulatory effect of ME on embryonic callus induction and somatic embryo development in *Morus alba* (Agarwal *et al.*, 2004) and citrus species (Ricci *et al.,* 2002 and Singh *et al.*, 2006). Increasing the concentration of malt extract to 8 g/L had a depressive effect on shoot number/explant. This depressive effect of high ME concentration was also reported by Agarwal *et al*. (2004), since they found that 1 g/L ME gave the highest percentages of embryogenesis and cotyledonary embryos of *Morus alba*, while higher concentration (2 g/L) decreased these percentages. Also, shoot length recorded similar significant increase at 2 and 4 g/L ME. On the other side, leaf number/shoot did not show significant response and tended to show an increase at 4 g/L ME. Shoot fresh weight recorded the highest value (0.86 g) with 4 g/L ME. This stimulatory effect of addition ME to the media on shoot quantity and quality was also recorded by Komalavalii and Rao (2000) on *Gymnema sylvestre*. This may be due to the constituents of malt extract, since Staden (1974) found that malt extract activity is due to a compound that co-chromatographed with zeatinriboside and other extremely active undefined substances, while Dix and Staden (1981) have been detected auxin-like and gibberellin- like activity substances in ME. Malt extract also contains amino acids and vitamins (Pierik, 1987).

Rooting percentage showed similar magnitude with all ME treatments (100%), while root number/shoot and root length recorded the highest values at 4 g/l ME.

Table 5: Effect of different concentrations of malt extract (ME) combined with 16 2ip + 2 IAA + 1 DPU (mg/L) on shoot proliferation of dieffenbachia after 12 weeks during multiplication stage.

Growth regulators (mg/L)	ME concentration (g\L)	No. of shoot/ explant	Shoot length (cm)	No. of leaf/ shoot	Shoot fresh weight (g)	Rooting %	No. of root/ shoot	Root length (cm)
16 2ip + 2 IAA + 1 DPU	0.0	4.5 a	2.49 a	2.5 a	0.60 a	100	2.4 a	4.80 a
	1.0	6.5 b	2.55 a	2.5 a	0.58 a	100	3.2 b	5.43 b
	2.0	8.0 c	2.72 b	2.7 a	0.70 b	100	3.4 bc	5.31 b
	4.0	10.0 d	2.82 b	3.0 a	0.86 c	100	4.0 c	6.48 c
	8.0	5.0 a	2.50 a	2.8 a	0.67 ab	100	3.9 bc	5.63 b

*** Data with the same letter vertically are not significant**

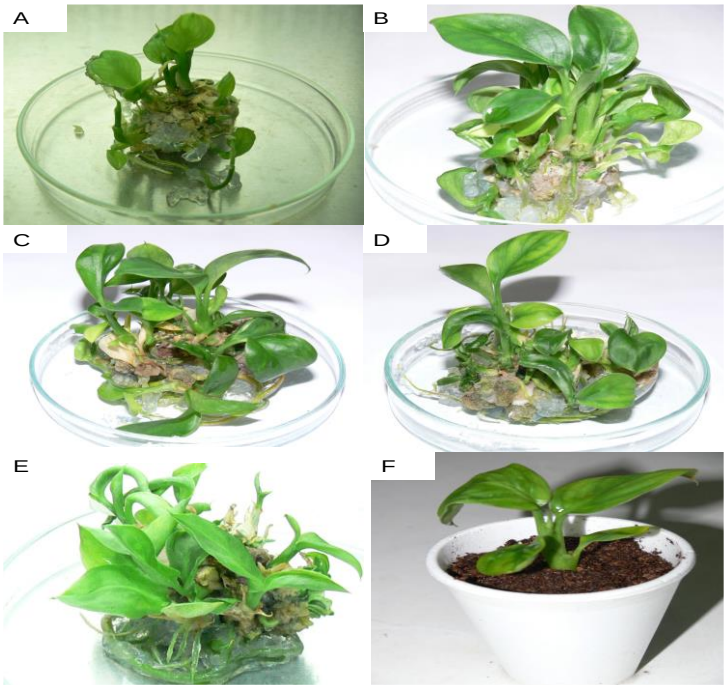

Fig. 1: Micropropagation of *Dieffenbachia picta* cv. Tropica:

- **A. Control (16 2ip + 2 IAA + 1 DPU mg/L)**
- **B. 1 mg/L TIBA combined with 16 2ip + 2 IAA + 1 DPU mg/L.**
- **C. 4 g/L ME combined with 16 2ip + 2 IAA + 1 DPU mg/L.**
- **D. 1 g/L YE combined with 16 2ip + 2 IAA + 1 DPU mg/L.**
- **E. 1 g/L CH combined with 16 2ip + 2 IAA + 1 DPU mg/L.**
- **F. Acclimatized plantlet of *Dieffenbachia picta* cv. Tropica after 4 weeks from adaptation.**

6. Effect of different yeast extract (YE) concentrations combined with 16 2ip + 2 IAA + 1 DPU (mg/L) on shoot proliferation:

Data in Table 6 show that yeast extract at 1 g/L combined with 16 mg/l 2ip + 2 mg/l IAA + 1 mg/l DPU was effective treatment in enhancing shoot number/explant, since it resulted in 6 shoots/explant (Fig. 1). In harmony with this result, Gras and Calvo (1996) demonstrated that YE has a promotive effect on shoot proliferation of Lavandula latifolia. Also, Parabia *et al*. (2007) reported that supplementation of media with 5 % w/v YE without any hormones increased the percentage of responded explant and the number of shoot/explant of Leptadenia reticulata. This stimulatory effect may be due to cytokinin activity of YE, since Staden (1974) found that most of the activity of YE is due to a compound that co-chromatographed with zeatinriboside. Moreover, Pierik (1987) mentioned that YE is used because of high quality of B vitamins On the other side, increasing YE concentration up to 8 g/L resulted in gradual significant reduction in shoot number/explant being of highest depressive effect at 8 g/L. This inhibitory effect of increasing the concentration of YE on shoot proliferation was previously observed by Komalavalii and Rao (2000) on *Gymnema sylvestre*.

Shoot length, number of leaf/shoot and shoot weight were also decreased as YE concentration increased. On the other side, rooting percentage was not affected by YE addition, while a positive effect on root number/shoot was noticed especially at the highest concentration of YE (8 g/L). Root length was enhanced by low concentrations (1.0 and 2.0 g/L) and significantly decreased at 8 g/L.

7. Effect of different casein hydrolysate (CH) concentrations combined with 16 2ip + 2 IAA + 1 DPU (mg/L) on shoot proliferation:

Data in table 7 show that CH up to 4 g/L combined with 16 2ip + 2 IAA + 1 DPU mg/L resulted in significant increase in shoot number/explant being the highest at 1 g/L (7.4 shoots/explant) against 4.5 shoots/explant for control treatment (16 2ip + 2 IAA + 1 DPU mg/L). Also, the same concentration of CH appeared to be effective in enhancing shoot length (Fig. 1), where higher concentrations (2.0 and 4.0 g/L) were not significantly effective. This promotive effect of CH on shoot proliferation was also demonstrated by Gras and Calvo (1996) on *Lavandula latifolia* and Ainsley *et al.* (2000) on *Prunus dulcis.* This is consistent with the knowledge that CH is a milk protein product composed of amino acids and other substances that can be incorporate in basal media to provide plant cells with a source of organic nitrogen, calcium, phosphate and vitamins (George, 1993).On the contrary, leaf number/shoot did not show any significant response.

Shoot fresh weight responded positively and attained significant increase at 0.5 and l.0 g/L CH.

Root percentage did not show any response due to addition of CH to the medium, while root number was increased as CH concentration increased up to 2.0 g/L and this was concomitant by the maximum significant increase in root length.

Table 6: Effect of different concentrations of yeast extract (YE) combined with 16 2ip + 2 IAA + 1 DPU (mg/L) on shoot proliferation of dieffenbachia after 12 weeks during multiplication stage.

Growth regulators (mg/L)	YE concentration (g\L)	No. of shoot/ explant	Shoot length (cm)	No. of leaf/ shoot	Shoot fresh weight (g)	Rooting %	No. of root/ shoot	Root length (cm)
16 2ip + 2 IAA + 1 DPU	0.0	4.5 d	2.49 b	2.5 ab	0.60 ab	100	2.4 a	4.80 b
	1.0	6.0 e	2.53 b	2.6 ab	0.76 b	100	3.5 bc	5.81 c
	2.0	4.0 c	2.47 b	2.7 b	0.70 b	100	3.0 ab	6.05 c
	4.0	2.9 b	2.02 a	1.9 a	0.56 a	100	3.2 b	4.58 b
	8.0	1.2 a	2.04 a	2.2 ab	0.6 ab	100	4.0 c	2.51 a

*** Data with the same letter vertically are not significant**

Table 7: Effect of different concentrations of casein hydrolysate (CH) combined with 16 2ip + 2 IAA + 1 DPU (mg/L) on shoot proliferation of dieffenbachia after 12 weeks during multiplication stage.

Growth regulators (mg/L)	CH concentration (g\L)	No. of shoot/ explant	Shoot length (cm)	No. of leaf/ shoot	Shoot fresh weight (g)	Rooting %	No. of root/ shoot	Root length (cm)
16 2ip + 2 IAA + 1 DPU	0.0	4.5 a	2.49 a	2.5 a	0.60 a	100	2.4 a	4.8 a
	0.5	5.1 ab	2.71 b	2.7 a	0.78 b	100	3.1 a	5.5 b
	1.0	7.4 c	2.91 b	2.9 a	0.79 b	100	3.0 a	6.1 c
	2.0	6.1 b	2.50 a	2.7 a	0.67 ab	100	4.1 b	7.5 d
	4.0	5.7 b	2.45 a	2.6 a	0.59 a	100	2.8 a	5.1 ab

*** Data with the same letter vertically are not significant**

8. Over all evaluation:

In order to obtain concise results from the abovementioned experiments, the best treatments from each experiment during multiplication stage were subjected to statistical analysis and Data were presented in Table 8.

Table 8: Evaluation of the best treatments obtained from multiplication stage experiments of dieffenbachia.

Growth regulators (mg/L)	Additives concentration	No. of shoot/ explant	Shoot length (cm)	No. of leaf/ shoot	Shoot fresh weight (g)	Rooting %	No. of root/ shoot	Root length (cm)
16 2ip + 2 IAA + 1 DPU	0.0	4.5 a	2.49 a	2.5 a	0.60 a	100	2.4 a	4.80 a
	1.0 mg/L TIBA	11.1 d	3.22 c	3.3 b	0.91 c	100	2.5 a	4.54 a
	4.0 g/l ME	10.0 d	2.82 b	3.0 ab	0.86 bc	100	4.0 b	6.48 c
	1.0 g/l YE	6.0 b	2.53 a	2.6 ab	0.76 b	100	3.5 b	5.81 b
	1.0 g/l CH	7.4 c	2.91 b	2.9 ab	0.79 b	100	3.0 ab	6.11 bc

*** Data with the same letter vertically are not significant**

Data indicated that, the treatments of 1.0 mg/l TIBA combined with 16 2ip + 2 IAA + 1 DPU (mg/L) or 4 g/l ME combined with 16 2ip + 2 IAA + 1 DPU (mg/L) were the most effective treatments in enhancing shoot

number/explant after 12 weeks without significant differences between them. They were also promising for shoot growth characters, but number of root/shoot and root length responded better for 4 g/l ME than 1 mg/l TIBA.

In conclusion, it could be advised to use 4 g/l ME combined with 16 2ip + 2 IAA +1 DPU (mg/L) as the best treatment for shoot multiplication of *Dieffenbachia picta* cv. Tropica.

9. Effect of different planting medium on survival percentage of dieffenbachia plantlets during acclimatization stage:

Data presented in Table 9, indicate that survival percentage of plantlets after four weeks recorded the highest value of survival percentage (95%) when plantlets were cultured in mixture of Peat and Vermiculite (1:1, v/v) followed by using Peat : Sand (1:1, v/v) and Peat moss, respectively.

Table 9: Effect of some planting medium on survival percentage of dieffenbachia plantlets during acclimatization stage after 4 weeks.

Planting medium	Survival %
Peat moss	80
Peat moss : Vermiculite (1:1 v/v)	90
Peat moss : Sand (1:1 v/v)	95

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تحسين فاعلية الاكثار الدقيق لنبات الدفينباخيا

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ثبت أن أفضل معاملة لتعقيم براعم الديفنباخيا هي النقع في محلول مطهر فطري (ريزولكس) بتركيز 2 جم/ لتر يعقبها النقع في كلوريد الزئبقيك بتركيز ٠,3 ٪ لمدة 20 دقيقة ثم في هيبوكلوريت الصوديوم بتركيز 1٪ لمدة 30 دقيقة. كما أمكن تقليل الفترة اللازمة لمرحلة الانشاء لاربعة أسابيع بدلا من ثمانية أسابيع و ذلك بزراعة البراعم على بيئة موراشيج و سكوج تحتوى على 16 مجم/ لتر 2ip + 2 مجم/ لتر IAA + 1 مجم/ لتر DPU . أما خلال مرحلة التضاعف فقد تم اختبار اضافة كلا من حمض التراي ايودوبنزويك بتركيزات : صفر ، 1 ، 2 ، 4 أو 8 مجم/ لتر او مستخلص الشعير بتركيزات: صفر ، 1 ، 2 ، 4 أو 8 جم/ لتر أو مستخلص الخميرة بتركيزات: صفر ، 1 ، 2 ، 4 أو 8 جم/ لتر أو الكازين هيدروليزيت بتركيزات: صفر ، ٠,5 ، 1 ، 2 أو 4 جم/ لتر بالاشتراك مع 16 مجم/ لتر 2ip + 2 مجم/ لتر IAA + 1 مجم/ لتر DPU ، حيث وجد أن أفضل معدل تضاعف للأفرخ (10 أفرخ/ منفصل نباتي) و كذلك أفضل تجذير (4 جذور/ فرخ بمتوسط طول جذر 6,48 سم) قد امكن الحصول عليهما باستخدام معاملة 4 جم/لتر مستخلص شعير + 16 ملليجم/ لتر 2ip + 2 مجم/ لتر IAA + 1 مجم/ لترDPU. كما أقلمت النباتات الناتجة بنجاح (95٪ نسبة بقاء) عند زراعتها فى مخلوط من البيت موس و الرمل (1:1 بالحجم).