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# Micropropagation of Dieffenbachia Plants from a Single Stem-Nodes

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**Abstract:** This study was carried out during two successive years from 2001 to 2003 at the plant tissue culture laboratory of Horticulture Department, Faculty of Agriculture, Kafr El-Sheikh, Tanta University to determine the suitable methodology for *in vitro* propagation of *Dieffenbachia maculata* (Marianna and Exotica cvs.) and *D. amoena* (cv. Tropic Snow) from a single stem-node. The most effective sterilization method was by using HgCl at 0.1% for 20 min. Explants cultured on MS½ medium supplemented with 0.0, 2.0, 4.0, 6.0 and 8.0 mg $L^{-1}$ BA combined with 0.0, 0.1, 0.5 and 1.0 mg $L^{-1}$ NAA. The best cultivar was Exotica to produced the highest number of shoots 21.0 shoot/jar by 8 mg $L^{-1}$ BA. While, Marianna cv. cultured on MS½ without hormone produced the largest number of leaves/shoot (5.5). Also, explants of three cultivars cultured on MS½ medium supplemented with 0.5 mg $L^{-1}$ NAA gave the tallest shoot (3.1 cm). Explants from Exotica cv. cultured on MS medium supplemented with 1.0 mg $L^{-1}$ NAA produced the heaviest shoot fresh weight (2.34 g).

**Key words:** Dieffenbachia, Benzial adinine, 1-naphthaleneacetic acid, explants

## INTRODUCTION

The genus Dieffenbachia, a member of the Family Araceae, is native of South and Central America. Dieffenbachia is one of the most important ornamental tropical foliage plant genera (Trigiano and Gray, 2000). But all parts of the plant contain calcium oxalate crystals and proteolytic enzymes that is extremely poisonous for little children or pets. Once selected, new cultivars were propagated a sexually from stem cutting or stem node segments. Using this process 5 to 10 years were required for new cultivars to become commercially important (Trigiano and Gray, 2000). Also, the traditional propagation using cutting is sometimes encountered with various difficulties such as fungal, bacterial and viral diseases (Chase, 1987). The use of tissue culture technique in vegetative propagated plants is an alternative method to obtain rapid colonal multiplication. Subsequently, tissue culture was seen as a method whereby Dieffenbachia stock could be free from systemic viral and bacterial pathogens (Knauss, 1976, Taylor and Knauss, 1978). Systemic diseases made Dieffenbachia susceptible to deterioration and loss of quality, since plants in old stock beds were harvested continually over years using conventional cutting propagation. Dieffenbachia perfection-1378 B was the first improved cultivar free of systemic pathogens (Chase *et al*., 1981). Therefore, the aim of this study was determine the suitable methodology for *in vitro* propagation of three Dieffenbachia cultivars.

## MATERIALS AND METHODS

This study was carried out during the two successive years from 2001 to 2003 at the plant tissue culture laboratory of Horticulture Department, Faculty of Agriculture, Kafr El-Sheikh, Tanta University, to multipropagation of Dieffenbachia plants through *in vitro* propagation by using a single node that contains one bud.

**Plant materials:** Three cultivars of Dieffenbachia, i.e., Marianna, Exotica and Tropic Snow were used. The cultivars Marianna and Exotica belong to *Dieffenbachia maculata* Lodd, while the cultivar Tropic Snow belongs to *Dieffenbachia amoena* Bull.

**Surface sterilization:** Standard procedures of aseptic techniques were use in sterilization (Dodds and Roberts, 1985; Kyte, 1987; Torres, 1989).

Surface sterilization was carried out by washing Explants thoroughly with a mild liquid detergent under a running tap water for 30 min. Then under cabinet laminar flow, Explants were surface sterilized by dipping in 70% alcohol (ethanol) for two min. and later in 0.1% mercuric chloride with a few drops of wetting agent Tween-20 (poly oxyethylene-sorbitan monolaurate) for 20 min. After sterilization, Explants were rinsed four times with a sterilized distilled water, 5 min. for each time. Subsequently, 1/2-2 cm long Explants that contain one

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node were cut and taken from the stem and then Explants were cultured onto the fresh free- MS½ medium incubated at total darkness conditions for two weeks.

This experiment was conducted to study the effect of different levels of BA, NAA and their combination on multipropagation of the three Dieffenbachia cultivars to determine the suitable balance between cytolainin and auxin which gave the maximum rate of shoot multiplication. Therefore numerous single nodes were singled out from establishment media after two weeks and cultured on MS½ medium supplemented with BA at five levels; i.e. 0.0, 2, 4, 6 and 8 mg $L^{-1}$. Each BA level was supplemented with four levels of NAA (1-naphthaleneacetic acid); i.e., 0.0, 0.1, 0.5 and 1.0 mg $L^{-1}$. Therefore, the experiment consists of 20 treatments. Each treatment consists of 4 jars. pH media was adjusted to 5.7. Agar 8 g $L^{-1}$ was added before autoclaving. The media were autoclaved for 15 min. at 121°C and 1.1 kg $cm^{-3}$. Each treatment consisted of four jars. The tested treatments were arranged in a factorial experiment in randomized complete design.

The Explants were transferred to a fresh medium every one month; cultures were incubated in the growth room at 26±2°C under light intensity of 2000 lux. After four months the following data were recorded.

- Number of shoots/explant.
- Number of leaves/shoot.
- Shoot length (cm).
- Shoot fresh weight (g).

Data were tested by analysis of variance and Duncan's multiple range test was used for the comparison among the treatment means (Duncan, 1955).

After shoots were obtained from micropropagation experiments in jars they were transplanted individually into jars containing MS½ medium without hormones and kept in 16 h photoperiod (2000 lux) for one month. Plant acclimatization was as described by Elmahrouk (2005).

## RESULTS AND DISCUSSION

**Number of shoots/explant and number of leaves/shoot:** Table 1 and Fig. 1 show that, Exotica cv. produced the largest number of shoots/explant (10.6) as well as the highest number of leaves/shoot (4.1), followed by Marianna cv. with significant differences among them. Many investigators reported that, success in culturing the explants is influenced by many factors, i.e., genotype, size of explant, physiological age and the tissue or organ source of explant. These results are in harmony with those obtained by Mederous and Rodriguez (1987) on rose.

Concerning, the effect of BA and NAA at the different combinations. Data recorded that Explants cultured on MS½ medium supplemented with 8 mg $L^{-1}$ BA produced the largest number of shoots/explant (14.12) when compared with control and other treatments. On the other hand MS½ without hormone produced the highest number of leaves/shoot (4.43) followed by all treatments which included al levels of NAA with zero of BA. In this study, the results referred to that the higher capacity for

Table 1: Effect of BA and NAA at different concentrations on number of shoots/explant and number of leaves/shoot from a single stem-node of the three *Dieffenbachia* cultivars after four months of incubation

| | | Cultivar | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Marianna | | Exotica | | Tropic snow | | | |
| BA (mg $L^{-1}$) | NAA (mg $L^{-1}$) | No. of shoots/explant | No. of leaves/shoot | No. of shoots/explant | No. of leaves/shoot | No. of shoots/explant | No. of leaves/shoot | Mean | |
| 0.0 | 0.0 | 2.04s | 5.53a | 3.03rs | 5.13ab | 3.00rs | 2.63r | 2.69I | 4.43a |
| 2.0 | 0.0 | 9.33g-j | 3.70g-q | 9.33g-j | 4.33b-i | 5.33m-r | 3.03o-r | 8.00ef | 3.69c-g |
| 4.0 | 0.0 | 7.33j-m | 4.00d-n | 13.33b-e | 3.70g-q | 7.00j-n | 3.30mr | 9.22de | 3.67c-g |
| 6.0 | 0.0 | 14.00b-d | 3.80e-q | 14.33bc | 4.57b-g | 5.00m-r | 3.00o-r | 11.11b | 3.79c-f |
| 8.0 | 0.0 | 15.33b | 3.50i-r | 21.03a | 3.33l-r | 6.00m-q | 3.10n-r | 14.12a | 3.31f-h |
| 0.0 | 0.1 | 3.03rs | 3.87e-o | 5.03m-r | 5.00a-c | 3.33rs | 4.07d-m | 3.80I | 4.31ab |
| 2.0 | 0.1 | 7.33j-m | 3.37k-r | 9.03h-k | 3.63h-q | 4.33o-s | 3.23m-r | 6.90f-h | 3.41e-h |
| 4.0 | 0.1 | 10.00f-i | 3.30m-r | 11.33e-h | 3.20m-r | 5.00m-r | 2.87qr | 8.78de | 3.12h |
| 6.0 | 0.1 | 9.33h-j | 3.90e-o | 9.00h-k | 4.63b-f | 6.33l-p | 2.67r | 8.22df | 3.73c-f |
| 8.0 | 0.1 | 12.00c-f | 4.00d-n | 14.00b-d | 4.07d-m | 6.40l-p | 3.10n-r | 10.80bc | 3.72c-f |
| 0.0 | 0.5 | 3.67q-s | 3.50i-r | 4.67n-r | 4.30b-j | 2.03s | 4.50b-h | 3.46I | 4.10a-c |
| 2.0 | 0.5 | 6.67k-o | 4.00d-n | 7.33j-m | 4.70b-e | 4.33o-s | 3.40j-r | 6.11gh | 4.03a-c |
| 4.0 | 0.5 | 9.33g-j | 3.10n-r | 13.00b-e | 3.83e-p | 4.34o-s | 3.30m-r | 8.89de | 3.41e-h |
| 6.0 | 0.5 | 11.67d-g | 3.70g-q | 14.33bc | 3.77f-q | 6.00m-q | 2.90p-r | 10.67bc | 3.46e-h |
| 8.0 | 0.5 | 14.67b | 3.30m-r | 14.00b-d | 3.40j-r | 3.33rs | 3.40j-r | 10.67bc | 3.37f-h |
| 0.0 | 1.0 | 3.67q-s | 3.67g-q | 3.04rs | 4.83a-d | 3.07rs | 3.20m-r | 3.26I | 3.90b-e |
| 2.0 | 1.0 | 7.03j-n | 4.27b-k | 7.00j-n | 4.23c-l | 4.00p-s | 3.40j-r | 6.01h | 3.97a-d |
| 4.0 | 1.0 | 8.67i-l | 3.20m-r | 9.33g-j | 4.00d-n | 4.07o-s | 3.40j-r | 7.36fg | 3.53d-h |
| 6.0 | 1.0 | 9.00H-k | 3.00o-r | 15.33b | 3.53i-r | 4.33o-s | 3.73f-q | 9.56cd | 3.42e-h |
| 8.0 | 1.0 | 13.00b-e | 3.30m-r | 15.00b | 3.20m-r | 5.07m-r | 3.10n-q | 11.02b | 3.21gh |
| Mean | | 8.86b | 3.70b | 10.63a | 4.07a | 4.615c | 3.27c | 8.03 | 3.68 |

Means with the same letter(s) did not different significantly according to Duncan (1955)

shoot proliferation was obtained on MS medium with a high cytokinin/auxin ratio. Also, this may be due to the effect of BA on promoting shoot proliferation which have a little internodes and leaves, while NAA promoted the shoot growth elongation which has more internodes and leaves. A similar trend of results was obtained by Lemos and Blake (1996) they found the increasing amounts of BAP added to the medium significantly stimulated the number of usable shoots, while the addition of NAA promoted bud elongation. Chalupa (1987) found that multiplication occurred as a result of the release of axillary buds from apical dominance by BA in the culture medium. These results are in line with others obtained with different plant species like Limonium (Casazza *et al*., 2002) and *Bombax malabaricum* (Atta *et al*., 2003).

Regarding the interaction effects between the three Dieffenbachia cultivars and the different combinations of BA and NAA, data recorded that Exotica cv. grown on $MS^{½}$ medium supplemented with 8.0 mg $L^{-1}$ BA produced the highest number of shoots/jar (21.0). While, Marianna cv. cultured on hormone-free $MS^{½}$ medium produced the largest number of leaves/shoot (5.5) followed by Exotica cv. on the same medium which produced 5.1 leaves/shoot.

**Shoot length and shoot fresh weight:** Data presented in Table 2 show that Exotica and Marianna cultivars produced the longest shoots (2.02 and 1.93 cm, respectively) when compared with Tropic Snow. While Marianna cv. produced the heaviest shoot fresh weight (0.96 g) followed by Exotica cv. (0.79 g). This may be due to the differences of cultivars in response to nutrients and growth regulators concentrations in the media, which create suitable conditions for cell division and elongation.

Concerning, the effect of BA and NAA at the different combinations. Data declared that Explants grown on MS medium supplemented with 0.5 mg $L^{-1}$ NAA gave the longest shoots (3.1 cm) followed by Explants grown on MS medium supplemented with 1.0 mg $L^{-1}$ NAA which produced shoots with 2.72 cm. While Explants grown on MS medium supplemented with 1.0 mg $L^{-1}$ NAA gave the heaviest shoot fresh weight (1.53) followed by Explants grown on MS medium supplemented with 0.5 mg $L^{-1}$ NAA which produced 1.25 g shoot fresh weight. It is known 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 increased the apical dominance as there are essential and rapid processes involved in growth and elongation (Wilkins, 1989). These finding are in agreement with Lee (1992) on *Salicornia brigelovii*, Atta *et al*. (2003) on *Bombax malabricum*, Parthasarathy and Nagaraja (1999) on *Gerbera jamesonii* and Ault (1992) on silence.

Table 2: Effect of BA and NAA at different combinations on shoot length (cm) and shoot fresh weight (g) from a single stem-node of the three *Dieffenbachia* cultivars after four months of incubation

| | | Cultivar | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Marianna | | Exotica | | Tropic snow | | | |
| BA (mg $L^{-1}$) | NAA (mg $L^{-1}$) | Shoot length (cm) | Shoot fresh weight | Shoot length (cm) | Shoot fresh weight | Shoot length (cm) | Shoot fresh weight | Mean | |
| 0.0 | 0.0 | 1.90d-l | 0.799f-p | 2.50b-d | 0.609k-v | 1.37lm | 0.405t-v | 1.92de | 0.604f-h |
| 2.0 | 0.0 | 1.70g-m | 0.823e-m | 1.90d-l | 0.745f-r | 1.60h-m | 0.550m-v | 1.74e-h | 0.706d-h |
| 4.0 | 0.0 | 1.50i-m | 0.924d-i | 1.80e-l | 0.697h-s | 1.68g-m | 0.544m-v | 1.66e-h | 0.723d-h |
| 6.0 | 0.0 | 1.50i-m | 0.729g-r | 1.63g-m | 0.684i-t | 1.15m | 0.454r-v | 1.43h | 0.622e-h |
| 8.0 | 0.0 | 1.60h-m | 0.696h-s | 1.50i-m | 0.520p-v | 1.44j-m | 0.513p-v | 1.51h | 0.576gh |
| 0.0 | 0.1 | 2.00c-k | 1.095de | 2.60bc | 1.015d-f | 2.19c-h | 0.652i-t | 2.26c | 0.921c |
| 2.0 | 0.1 | 1.83e-l | 1.171cd | 2.03c-j | 0.840e-l | 1.72f-m | 0.506q-v | 1.86d-g | 0.839cd |
| 4.0 | 0.1 | 2.00c-k | 0.850e-l | 1.60h-m | 0.627j-u | 1.16m | 0.473r-v | 1.59e-h | 0.650e-h |
| 6.0 | 0.1 | 2.10c-i | 0.865e-l | 2.10c-i | 0.981d-g | 1.38k-m | 0.329v | 1.86d-g | 0.725d-g |
| 8.0 | 0.1 | 1.60h-m | 0.731f-r | 1.60h-m | 0.589l-v | 1.44j-m | 0.362uv | 1.55gh | 0.560h |
| 0.0 | 0.5 | 2.80b | 1.431b | 2.53bc | 0.878e-k | 3.88a | 1.429b | 3.07a | 1.246b |
| 2.0 | 0.5 | 2.50b-d | 0.977d-h | 2.40be | 0.897e-j | 1.39k-m | 0.401t-v | 2.09cd | 0.759d-f |
| 4.0 | 0.5 | 1.70g-m | 0.934d-i | 1.43jm | 0.481r-v | 1.90d-l | 0.724g-r | 1.68e-h | 0.713d-g |
| 6.0 | 0.5 | 1.77f-m | 0.812f-n | 2.00c-k | 0.774f-q | 1.47j-m | 0.540n-v | 1.75e-h | 0.709d-h |
| 8.0 | 0.5 | 1.40k-m | 0.619j-u | 1.63g-m | 0.524o-v | 2.24b-gh | 0.703g-s | 1.76e-h | 0.616f-h |
| 0.0 | 1.0 | 2.80b | 1.491b | 3.63a | 2.366a | 1.74f-m | 0.723g-r | 2.72b | 1.527a |
| 2.0 | 1.0 | 2.40b-e | 1.394bc | 2.03c-j | 0.673i-t | 2.33b-f | 0.809f-o | 2.26c | 0.958c |
| 4.0 | 1.0 | 2.00c-k | 1.176cd | 2.20c-h | 0.717g-s | 1.50i-m | 0.431s-v | 1.90d-f | 0.774e |
| 6.0 | 1.0 | 1.77f-m | 0.855e-l | 1.63g-m | 0.675i-t | 1.30lm | 0.589l-v | 1.57f-h | 0.706d-h |
| 8.0 | 1.0 | 1.67g-m | 0.729g-r | 1.53i-m | 0.583l-v | 1.40k-m | 0.546m-v | 1.53gh | 0.619f-h |
| Mean | | 1.93a | 0.955a | 2.02a | 0.794b | 1.71b | 0.584c | 1.89 | 0.778 |

Means with the same letter(s) did not different significantly according to Duncan (1955)

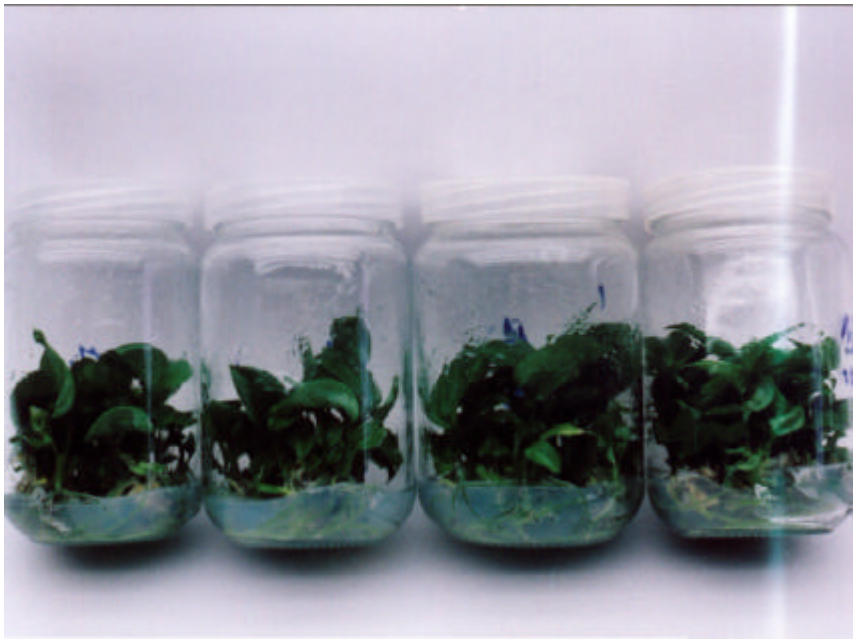

Fig. 1: Multiple shoots produced from a single node of Exotica cv

Fig. 2: *Dieffenbachia* plantlet in a jar containing $MS^{1/2}$ medium without hormones suitable for acclimatization

Regarding the interaction effect between the three Dieffenbachia cultivars and the different combinations of BA and NAA. data cleared that explants from Tropic Snow and Exotica cvs. cultured on MS medium supplemented with 0.5 mg $L^{-1}$ NAA or 1.0 mg $L^{-1}$ NAA gave the highest values of shoot length (3.88 and 3.63 cm, respectively). While Explants from Exotica cv. cultured on MS medium supplemented with 1.0 mg $L^{-1}$ NAA produced the heaviest shoot fresh weight (2.37 g), followed by Explants from Marianna and Tropic Snow cv. cultured on MS medium supplemented with 0.5 mg $L^{-1}$ NAA which produced 1.43 and 1.42 g/shoot, respectively.

Fig. 3: A regenerated *Dieffenbachia* plant in a cup after successful acclimatization

Shoots had a little proliferation when BA was omitted from the medium; instead, rooting was observed in all of the shoots, so that shoots were transplanted individually into jars (Fig. 2) containing $MS^{½}$ medium without hormones and kept in 16 hours photoperiod (2000 Lux) for one month. After root development, plantlets were transplanted to pots containing a medium of 1 vermiculite; 1 peat (v/v) and placed under high humidity and low light intensity for acclimatization. After 4 weeks, plants were transferred to greenhouse conditions (Fig. 3).

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