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In Vitro Micropropagation of the Ornamental Plant *Dieffenbachia* — A Review

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Abstract Ornamental industry has applied immensely in vitro propagation approach for large-scale plant multiplication at very high rates of elite superior varieties. As a result, hundreds of plant tissue culture laboratories have come up worldwide. *Dieffenbachia* species are popular foliage potted plants used in interiorescapes of homes, offices, and malls throughout the world. Most of *Dieffenbachia* species are now propagated by tissue culture for better utilize of species and expedite plant improvement. This review paper summarizes valuable literature on in vitro techniques including type of explants used, media optimized, ways of propagation and improvement through 45 years of research on *Dieffenbachia* spp. Which were provide basis for future studies such as genetic transformation for breeding aims, develop new cultivars, develop disease-resistant plants and overcome the environmental obstacles. There is a need for more application of the plant tissue culture techniques on *Dieffenbachia* to investigate the responses of different cultivars and explants to variable culture media.

Keywords *Dieffenbachia*, Review, Tissue culture, Multiplication, Indirect regeneration

1. Introduction

The genus *Dieffenbachia* consists of about 30 species and over 100 cultivars with spotted, striped or speckled with cream, white, yellow, gold, silver, or a combination of these colors leaves, 15 to 40 cm or so in length [1, 2]. *Dieffenbachia* is an ornamental perennial monocot plant native to tropical America [3], belonging to the family Araceae [4]. It is prized among interiorscapers for the attractive variegated foliage, tolerance of interior environments and easy production [5]. The most common species used in interior landscapes are: *Dieffenbachia maculate* (syn. *D. picta*), *D. amoena*, *D. seguine*.

Dieffenbachia has been produced as an ornamental foliage plant for interiorescaping since 1864 [6, 7] and consistently ranks among the top five most popular foliage plant genera based on annual wholesale value [7, 8]. Moreover, the number of *Dieffenbachia* cultivars increased form 7 in 1975 to 23 cultivars by 1999 [8]. In addition to its character as ornamental plant, this genus also reported to be used in biological control of some pests [9, 10] and as medicinal plant [11], since all parts of *Dieffenbachia* is poisonous and can be a source of antimicrobial activities [12].

Dieffenbachia conventionally propagated by seed, tip or cane cuttings, division and air layering. Seed is not commonly used except in breeding because it does not encourage the expansion of the species, moreover, seed production is limited [13, 14]. Conventional methods are very slow moreover, *ex vitro* vegetative propagation of *Dieffenbachia* is hampered by endogenous bacteria infection leads to total loss of vegetative parts [15, 16]. Besides conventional methods of propagation *in vitro* micropropagation contributed very much in the propagation of *Dieffenbachia*. *In vitro* techniques offers many unique advantages over conventional propagation methods such as disinfection, rapid multiplication of valuable genotypes, expeditious release of improved varieties, production of disease-free plants, non-seasonal production and facilitating their easy international exchange [17]. The main objective of this article is to overview literature covering the previously work done on *Dieffenbachia* tissue culture from 1976 until 2011, and brings forth some points that we consider as the major thrust of contemporary and future research. However, the works on *Dieffenbachia* is summarized in Table1. Figure 1 illustrated general scheme for *in vitro* micropropagation protocols of different *Dieffenbachia* cultivars.

Table 1. Summery of micropropagation studies on *Dieffenbachia* (chronologically 1976 - 2010)

Title	Explant	Purpose	Taxa*	References [ref. no.]
Tissue culture method for producing *Dieffenbachia picta* cv. Perfection free of fungi and bacteria	Shoot tip	*In vitro* disease elimination	*D. picta* cv. 'Perfection'	Knauss, 1976 [36]
Tissue culture propagation of some foliage plants	Shoot tip\ node	Organogenesis	*D. spp.*	Litz and Conover, 1977 [18]
Tissue culture multiplication and subsequent handling of known pathogen-free *Dieffenbachia* 92*maculate* cv. Perfection	Shoot tip\ node	Shoot proliferation	*D.92maculate* cv. 'Perfection'	Taylor and Knauss, 1978 [19]
In vitro shoot proliferation rate of *Dieffenbachia exotica* cultivar Marianna as affected by cytokinins, the number of recultures and the temperature	-	Shoot proliferation	*D. exotica* cv. 'Marrianna'	Voyiatzi and Voyiatzis, 1989 [49]
Isolation and characterization of bacterial contaminants from *Dieffenbachia amoena* Bull, *Anthurium andraeanum* Linden and *Spathiphyllum sp.* shoot *cultured in vitro*	Leaf	Microorganisms isolation	*D. amoena*	Brunner *et al.*, 1995 [38]
Adventitious shoot regeneration on explants of *Anthurium*, *Codiaeum*, *Dieffenbachia*, *Gerbera*, *Rosa* and *Spathiphyllum* for breeding purpose	Petiole	Organogenesis	*D. spp.*	Orlikowska *et al.*, 1995 [51]
Propagation of *Dieffenbachia* through tissue culture	Shoot tip	Shoot proliferation	*D. picta* cv. 'Tropica'	El-Sawy and Bakheet, 1999 [26]
Bud proliferation and regeneration in Dumb-cane (*Dieffenbachia seguine*) leaf callus.	Leaf	Shoot proliferation and breeding	*D. seguine*	Iqbal *et al.*, 1999 [29]
Role of benzyladenine and activated charcoal in optimizing the culture media of *in vitro* cultured *Dieffenbachia exotica* cv. Tropic-Snow	Node	Shoot proliferation	*Dieffenbachia exotica* cv. 'Tropic-Snow'	Arafa *et al.*, 1999 [39]
Tissue culture and rapid propagation of *Dieffenbachia*	-	Organogenesis	*D. amoena* cv. 'Mars', *D. maculate* cv. 'Camila', cv. 'Rudolph Roehrs', *D. seguine* cv. 'Wilson's Delight'	Genfa *et al.*, 1999 [41]
Shoot apex culture and rapid propagation of *Dieffenbachia* Tropic Marianne	Shoot tip / axillary bud	Organogenesis	*D.* hybrid cv. 'Tropic Marianne'	Feng *et al.*, 2000 [20]
Effect of three cytokinins and the location of the explant *in vitro* multiplication of the *Dieffenbachia maculate* Schott. Cv. Sublime	Stem node	Shoot proliferation	*D. maculata* cv. 'Sublime'	Mogollon, 2000 [27]
Production of *Dieffenbachia amoena* cv. Tropic plants by *in vitro*	Node	Organogenesis	*D. amoena* cv. 'Tropic'	Schroeder, 2000 [48]
Micropropagation of *Dieffenbachia picta*	Axillary bud	Shoot proliferation	*D. picta*	Sierra *et al.*, 2001 [21]
Tissue culture for rapid propagation of *Dieffenbachia amoena* cv. Green Sea and *Philodendron erubescens* cv. Emerald King	Leaf/ node	Organogenesis and shoot proliferation	*D. amoena* cv. 'Green Sea'	Jun *et al.*, 2001[25]
The effect of cytokinin type and concentration and the number of subcultures on the multiplication rate of some decorative plants	-	-	*D. spp.*	Vardja and Vardja, 2001 [42]
Studies on contamination control in stem culture of *Dieffenbachia amoena* cv. Camilla	Stem segment	Contamination control	*D. amoena* cv. 'Camilla'	Hui *et al.*, 2002 [37]
Micropropagation and mutation of *Dieffenbachia maculate* Rudolph Roehrs-86-A	Leaf	Organogenesis and *in vitro* breeding	*D. maculate* 'Rudolph Roehrs-86-A'	Chu, 2002 [47]
Micropropagation of *Dieffenbachia* plants from a single stem –nodes	Node	Organogenesis	*D. maculate* (cv. 'Marianna', 'Exotica') and *D. amoena* cv. 'Tropic Snow'	El-Mahrouk *et al.*, 2006 [3]
Indirect shoot organogenesis and plantlets regeneration from stem of ornamental *Dieffenbachia maculate* cv. Marianna	Stem segment	Organogenesis	*D. maculate* cv. 'Marianna'	El-Mahrouk *et al.*, 2007 [14]
Indirect shoot organogenesis from leaves of *Dieffenbachia* cv. Camouflage	Leaf	Organogenesis	*D.* cv. 'Camouflage'	Shen *et al.*, 2007 [31]
Assessment of somaclonal variation in *Dieffenbachia* plants regenerated through indirect shoot organogenesis	Leaf	Organogenesis and somaclonal variation	*D.* cv.: 'Camouflage', 'Camille' and 'Star Bright'	Shen *et al.*, 2007 [32]

Effect of auxin concentration on somatic embryogenesis from male inflorescence culture of *Dieffenbachia* 'Tiki	Inflorescene	Somatic embryogenesis	*D.* hybrid cv. 'Tiki'	Shen and Lee, 2007 [34]
Production of indole acetic acid (bioauxin) from *Azobacter sp.* isolated and its effect on callus induction of *Dieffenbachia maculate* cv. Marianne	Shoot tip / stem segment	Organogenesis and production of biouxin	*D. maculate* cv. 'Marianna'	El-Mahrouk *et al.*, 2007 [43]
Study on tissue culture and rapid propagation of Dieffenbachia amoena cv. Kiki	Node/ axillary bud	Shoot proliferation	*D. amoena* cv. 'Kiki'	Chao and Li-si, 2008 [22]
Increasing lateral bud production with 6-benzylaminopurine and antibiotics pretreatments on establishment of aseptic culture for axillary explants in *Dieffenbachia*	Axillary bud	Shoot proliferation and contamination control	*D. spp.*	Shen and Lee, 2008 [23]
Effects of genotype, explant source, and plant growth regulators on indirect shoot organogenesis in *Dieffenbachia* cultivars	Leaf / root	Organogenesis	*D.* cv.: 'Camouflage', 'Camille', 'Octopus', and 'Star Bright'	Shen *et al.*, 2008 [33]
In vitro induction of Tetraploids in *Dieffenbachia* x 'Star Bright M-1' by Colchicine	Lateral bud	Shoot proliferation and Tetraploid	*Dieffenbachia* x 'Star Bright M-1'	Henny *et al.*, 2009 [28]
Cytokinins stimulate somatic embryogenesis and plant regeneration from male inflorescene of *Dieffenbachia* 'Tiki	Inflorescene	Somatic embryogenesis	*D.* hybrid cv. 'Tiki'	Shen and Lee, 2009 [35]
In vitro shoot micropropagation and plant establishment of an ornamental plant dumb cane (*Dieffenbachia* Compacta)	Node	Shoot proliferation	*D. maculate* cv. 'Compacta'	Elsheikh and Khalfalla, 2010 [24]
In vitro induction of mutations in *Dieffenbachia picta* CV. Tropica	Shoot	*In vitro* mutation induction	*Dieffenbachia picta* cv. 'Tropica'	Abass *et al.*, 2011 [44]

* Dieffenbachia species names as provide in the articles.

Figure 1. General scheme for micropropagation protocols of Dieffenbachia. Determination the aim of micropropagation verifies the appropriate technique and explant type. (A): D. maculata cv. Compacta healthy mother plant source for explants. (B): Surface disinfection then done for explants selected: 1. auxiliary buds/ stem node, 2. shoot tip/ apical bud, 3. shoot, for adventitious shoot induction or shoot proliferation, 4. inflorescence, 5. whole leaf blade or petiole, 6. stem segment, 7. root, for organogenesis, embryogenesis or callusing. (C): Explants cultured on initiation media (mostly MS+BA±NAA) develop 1. organogenic meristems, 2. calli, or 3. induced shoots. (D): shoots formation and elongation on same initiation medium or subcultured to multiplication medium for 6 weeks depending on cultivar. Shoots cluster may used to repeat step D. (E): Rooting of shoots within 2-8 weeks either in vitro (½ MS±IBA/NAA) or ex vitro that micro-cuttings transferred directly to soil or soil-less medium. (F): Acclimatized Dieffenbachia plantlets under greenhouse condition on potting mixture soil or soil-less medium with higher rate 90-100% for 60 days.

2. Micropropagation of *Dieffenbachia*

2.1. Culture Initiation

Generally culture initiation involves explant selection and isolation, surface sterilization and establishment on an appropriate culture medium.

2.1.1. Explant Selection

Culture initiation depends on explants type or the physiological stage of the donor plant at the time of excision. The objective of micropropagation determines the nature of explant. *Dieffenbachia* was *in vitro* propagated by direct or indirect organogenesis using various explants. Axillary branching using axillary bud and stem node, as in other plants, is the majority common explant type utilized for direct shoot propagation of *Dieffenbachia*. Therefore, shoot induction was reported by many authors on axillary buds [18-24] and stem nodes [3, 22, 25]. However, other types of explants were also utilized for micropropagation of *Dieffenbachia*. Shoot tip explants reported with rapid propagation results [19, 20, 26], and Mogollon [27] found that sub-apical stem segments gave the highest number of shoots in comparison with apical stem section in *Dieffenbachia* sublime. Lateral buds excised from shoot tips of *Dieffenbachia* 'Star Bright M-1' were implemented to initiate shoots clump utilized later to induce polyploidy plantlets [28]. Moreover, for somatic embryogenesis and shoot organogenesis induction, leaf explants were mainly selected [29-33]. However, other plant parts such as inflorescence [34, 35], stem segment [14] and root [33] were also utilized as explants. Furthermore, axillary buds and shoot tip culture was employed by Knauss [36], Taylor and Knauss [19] for indexing *Dieffenbachia* stock for the presence of fungi and bacteria and stem culture from contamination control [37].

In *Dieffenbachia* micropropagation effects of time and explants orientation should be taken in consideration. Therefore, Mogollon [27] evaluated the effect of explant location in the multiplication. He used stem sections, apical and sub-apical, 1.0 to 1.5 cm in length as explants, cultivated vertically and horizontally. He found that the highest number of shoots was obtained with sub-apical segments placed vertically, while the height of the shoots was higher in apical explants.

2.1.2. Surface Sterilization

Culture initiation of *Dieffenbachia* described as difficult stage because it is encountered with excessive endogenous contamination. It was reported that the rate of microbial contamination was very high up to 80% [38]. Age of material and season of collection are important factors determining the success in establishing aseptic cultures. Selection of appropriate disinfectant with supreme concentration sufficient to destroy any microbial contamination without harming the explant tissue is critical. Therefore, variable detergents were used for surface sterilization of *Dieffenbachia* initial explants. Several studies necessitate using highly effective and extremely toxic detergent like mercuric chloride [24]. Moreover, other studies apply double sterilizing method [20] which is means of sterilising explant using deferent detergents in two steps processes. For example, using even mercuric chloride followed by commercial bleach [39, 40], or the highly phytotoxic agent viz.: ethanol followed by commercial bleach [40], chloramine [41] or mercuric chloride [3, 40, 42]. However, this may indicate that the surface sterilization of *Dieffenbachia* explant depends on the cultivar used and mother plant more than explant nature. Therefore, other authors such as Knauss [36], Litz and Conover [18], El-Sawy and Bakheet [26], Henny *et al.* [2], Sierra *et al.* [21] and Shen *et al.* [31] reported that commercial bleach alone was sufficient in disinfection of various *Dieffenbachia* cultivars. Other authors reported use of methods such as depressurizing disinfection or augmented culture medium [37] or pre-treatment explants [23] through using microorganism inhibitors or antibiotics, were found to be very effective in reducing contamination rate.

2.1.3. Culture Medium

Most of the tissue culture works, for different purposes, in *Dieffenbachia* were successfully achieved on agar based MS [43] medium (full or half-strength salt formulations). El-Sawy and Bakheet [26], Feng *et al.* [20], Hui *et al.* [37], Iqbal *et al.* [29], Mogollon [27], Jun *et al.* [25] El-Mahrouk *et al.* [14], El-Mahrouk *et al.* [42], Shen and Lee, [23] Elsheikh and Khalfalla [24] and Abass *et al.*, [44] employed full MS for bud proliferation, callus induction and rapid multiplication of *Dieffenbachia*. However, some modifications on MS medium were stated to improve multiplication such as Shen and Lee [34] which used full MS with 2% sucrose and 1% glucose, El-Mahrouk *et al.* [3] and Chao and Li-si [22] exploited half-strength MS medium and Shen and Lee [35] as well as added 2% glucose. However, previously, Taylor and Knauss [19] developed DM basic medium for tissue culture of *Dieffenbachia* which is identical to MS medium except for the addition of adenine sulphate 80 mg/L, and NaH2PO4.H2O 170 mg/L. Also their experimentation showed that doubling the concentration of adenine sulphate and NaH2PO4-H2O did not increase development of the tissue cultures. Moreover the addition of nicotinic acid, 0.5 mg/L, pyridoxine-HCl, 0.5 mg/L, or glycine, 2 mg/L, alone or in combination, to be unnecessary for explant development. More recent, Sierra *et al.* [21] and Henny *et al.* [2] used DM for micropropagation of *Dieffenbachia*. Even though, no study demonstrates the difference between MS and DM media formulas. Beside MS and DM media, LS medium [45] was as well reported for micropropagation of *Dieffenbachia* spp. [41]. Moreover, other media including B5 [46] and N6 [47] were used for rapid propagation but as modifications to MS medium such as macro elements [40] or B5 vitamins [28].

Various *Dieffenbachia* species including their different cultivars were in vitro propagated through direct adventitious

shoots induction or through intervention of callus formation as specified below.

2.2. Direct Regeneration

In general *Dieffenbachia* was fond to have slow bud proliferation *in vitro*. That the initial pattern of shoot induction and development mostly occurs after approximately 4 months in culture [18, 21]. Therefore, *In vitro* shoot formation of various *Dieffenbachia* species and cultivars was achieved using variable levels of different cytokinins alone or in combination with auxins (mainly BAP+NAA). Multiplication rates depend on species, cultivar, auxin/cytokinin ratio and explant type. Iqbal *et al.* [29] employed 1.0 mg/L each of BA/BAP and NAA for *Dieffenbachia* buds (apical and axillary buds) proliferation on MS medium. Whereas, Chao and Li-si [22] showed that 1.5 mg/L BA + 0.05 mg/L NAA was the appropriate medium for axillary bud development. While Schroeder [48] added 1.0 mg /L NAA to 3.0 mg/L 2iP and 10 mg/L kin instead of BAP to obtain plantlets from aaxillary buds of *D. amoena* cv. Tropic. Other work replaced NAA with IAA (0.1 and 1.9 mg/L) in combinations with BAP (0.5 and 1.0 mg/L) as effective for establishment of *Dieffenbachia* buds [21]. Henny *et al.* [28] cultured buds on media supplemented with combination of 2iP and IAA to produce shoots. However, using BA alone (3-5 mg/L) for multiple shoot induction reported for the proliferation of 9 species of *Dieffenbachia* [25, 41].

The multiplication of *Dieffenbachia* is known to be a demanding stage: requiring high cytokinin, successive recultures, poor multiplication rate and time consuming stage. Voyiatzi and Voyiatzis [49] studies showed that 16 mg/L of 2iP resulted in 6.2 shoots / flask. Sierra *et al.* [21] obtained multiple shoots of *Dieffenbachia* using 16.0 mg/L of 2iP. Elsheikh and Khalfalla [24] used 10.0 mg/L BA to gain 6.7 shoots/nodal explants of *D. compacta*. Litz and Conover [18] employed 8.0 mg/L BA for rapid *in vitro* propagation of *Dieffenbachia sp.* El-Mahrouk *et al.* [3] fortified MS media with 8.0 mg/L BA and 0.1 mg/L NAA to multiply stem nodes of *D. amoena* cv. Tropic snow. Elsawy and Bakheet [26] multiplied shoot tip of *D. picta* cv. Tropica on MS medium supplemented with 4.0 mg/L BA. Mogollon [27] multiplied shoot tips of *D. sublime* with 0.5mg/L 2ip. Clearly, cytokinin concentrations employed in *Dieffenbachia* multiplication depend on the cultivar used. Also it is noticeable that 2ip and BA are the most effective cytokinins used for multiplication of *Dieffenbachia* this refers to the poor effect of kin on *Dieffenbachia* explant and the callus formation affect of TDZ addition [24].

Other factors, including number of subcultures and environmental conditions, were also considered in regeneration of *Dieffenbachia in vitro*. Subcultures found to be essential to increase the number of shoots. Voyiatzi and Voyiatzis [49] demonstrate that successive recultures of the basal clump of tissue remaining after the first culture, resulted in an increase in the number of new shoots. Mogollon [27] noticed significant differences in the in the number of shoots, length of shoots and roots between two subcultures. The highest values were obtained at second subculture. The effects of temperature [49] and light quality [50] on aseptically growth of *Dieffenbachia* were regarded to lesser extent.

2.3. Indirect Regeneration

Many indirect shoot organogenesis protocols were established for at least eight *Dieffenbachia* cultivars. Most of theses protocols were achieved using combination of BAP and NAA in deferent concentrations in MS media for callus formation on leaf explant and then shoot induction after subculture. However, stem segment explant were also reported using the same media. For example, shoot organogenesis induced on *D. seguine* leaf through callus formation with 0.5 mg/L BAP + 1.0 mg/L NAA then shoot stimulation on medium supplemented with equal amount (0.5 mg/L) of both regulators [29]. Likewise, Chu [28] successfully induced shoot regeneration through callus culture from young expanded leaves of *D. maculata* on medium containing 5.0 mg /L BA and 0.5 mg / L NAA. The shoots were proliferated when callus explant of 5mm in cubic was cultured on the medium containing 5.0 mg / L BA and 0.125 mg / L NAA. Moreover, El-Mahrouk *et al.* [14] reported that high concentrations (15 mg/L) of BA and NAA were required for callus formation and shoot induction on stem segment of *D. maculata* cv. Marianna. Using the same *Dieffenbachia* cultivar and explant, El-Mahrouk *et al.* [43], found that combination of 10 mg/L IAA+ 5 mg/L BA produces the highest callus formation and 2 mg/L+0.06 mg/L IBA for differentiation. However, Jun *et al.*, [25] utilized one step media as 1.0 mg/L BA and 0.1 mg/L NAA for production organogenic shoots on *D. amoena* cv. Green Sea leaves. Other studies reported using combination of other growth hormones such as TDZ and 2,4-D or NAA. Shen *et al.* [32, 33] examined the effect of 5 μM TDZ and 1 μM 2,4-D on indirect shoot organogenesis from leaf explants. The results showed that the combination produced the greatest callus frequency among *Dieffenbachia* cultivars tested. In addition to leaf and stem segment, other types of explants were also utilized for callus formation and shoot regeneration on *Dieffenbachia*. Orlikowska *et al.* [51] utilized 4.5 μM TDZ in combination with 5.4 μM NAA to induce direct shoot organogenesis on petiole explants of *Dieffenbachia*. Feng et al. [20] employed the shoot apex and root tip to determine the optimum medium for callus induction and speed growth of callus. MS medium with BA at 4.0 mg/L was found to be the best. The callus differentiated into plenty of buds on medium containing 2.0mg/L BA. Nevertheless, root explant presents no action against callus induction. Unresponsiveness of root explants of Dieffenbachia cultivars to in vitro cultures was also reported in other works [33, 51].

Somatic embryogenesis was also induced indirectly on many *Dieffenbachia* species. Shen and Lee [34, 35] use 4.0

mg/L 2,4-D to encourage callus on male inflorescence of *Dieffenbachia* 'Tiki' and then stimulate embryogenesis on 2.0 to 5.1 mg/L 2,4-D and 0.5 mg/L Kinetin or 1.0 mg/L TDZ alone.

2.4. Rooting

Dieffenbachia is known as an easy rooting plant. Virtually the rooting of shoot depends on rooting medium. Adventitious roots initiation without growth substances both was observed on proliferation media and after transfer to basal medium [3, 14, 18, 24]. That encourages the *ex vitro* rooting possibility as described by Taylor and Knauss [19], Feng *et al.*, [20] and Shen *et al.* [32].

The elimination of a laboratory rooting phase makes costs more reasonable although specific environmental requirements must be recognized for the critical greenhouse acclimatization phase. On the other hand, Feng *et al.* [20], Jun *et al.* [25], El-Sawy and Bakheet [26], Chu [30] and Genfa *et al.* [40] found that implement ½ MS with NAA was essential for *in vitro* rooting of *Dieffenbachia* shoots. While Chao and Li-si [22] found ½ MS + IBA 0.2 mg/L was the most suitable medium for rooting of *D. amoena* cv. Kiki shoots.

2.5. Acclimatization

Transfer of *in vitro* regenerants to outside conditions is a very important stage in *Dieffenbachia* micropropagation work. Production of healthy plants and new varieties are the main objective of commercial industry of species. Successful acclimatization of regenerated plants in greenhouse on potting mixture was reported. Various cultivars reported to growth well in pots containing soil-less medium of vermiculite, peat, perlite or compost [3, 19, 26, 32] and other on salt: sand soils [24]. High survival rates of *Dieffenbachia* transplanted plantlets, after *in vitro*/ *ex vitro* rooting, were recorded by many authors. El-Mahrouk *et al.* [3], Elsheikh and Khalfalla [24] and Shen *et al.* [32] successfully acclimatized plantlets in greenhouse with 100% survival rate. Whereas, Feng *et al.* [20], Chao and Li-si [22] and El-Sawy and Bakheet [26] reported 95% survival rate of *ex vitro* rooted plantlets for 42-50 days, Jun *et al.* [25] and Mogollon [27] reported survival ratio of over 90%, but for 60 days.

3. Clonal Stability through *in Vitro* Culture

Clonal stability of the micropropagated plants is essential for *in vitro* germplasm conservation [52]. The long term maintenance (2 - 3 years or more) of the *D. maculata* cv. Perfection lines employed in Taylor and Knauss [19] research suggests that once indexed, *Dieffenbachia* lines can be kept *in vitro* to eliminate the risks of re-infection and can later be used as stock for tissue culture multiplication. The plants developed throughout these studies from these lines appear to be similar to the parental types, thus indicating satisfactory genetic stability.

Clonal stability could be hampered by somaclonal variation. The occurrence of somaclonal variations in regenerated *Dieffenbachia* plants had been proved to be induced *in vitro* through indirect shoot organogenesis [53]. However, somaclonal variation found to be an effective way to induce new cultivars among *Dieffenbachia* plants. Shen *et al.* [31] demonstrated the potential for new cultivar development by selecting callus-derived somaclonal variants of *Dieffenbachia* in 3 - 4 years compared to 7 - 10 years through traditional breeding methods. Chen *et al.* [4] analyzed genetic relatedness of some cultivated *Dieffenbachia* using amplified fragment length polymorphism and found that cultivars selected from somaclonal variants differ genetically from their parents.

Shen *et al.* [32] evaluated the occurrence of somaclonal variation among regenerants derived through indirect shoot organogenesis from leaf explants of three *Dieffenbachia* cultivars Camouflage, Camille and Star Bright. Three types of somaclonal variants (SV1, SV2, and SV3) were identified from regenerated plants of cv. Camouflage, one type from cv. Camille, but none from cv. Star Bright. Consequently, the rate of somaclonal variation was cultivar correlated. The highest variation percentage (40.4%) reported among the regenerants, was recorded by cv. Camouflage whereas a rate of 2.6% occurred with cv. Camille. Generally the duration of callus culture is a factor contributing somaclonal variation induction and ratio [54, 55]. Shen *et al.* [30] found that the duration of callus culture had no effect on somaclonal variation rates of cv. Camouflage as the rates between plants regenerated from 8 months to 16 months of callus culture were similar.

Variations between *Dieffenbachia* cultivars in response to plant tissue culture applications were reported. Shen *et al.* [32] investigate the capacity of four *Dieffenbachia* cultivars (Camouflage, Camille, Octopus, and Star Bright) for indirect shoot organogenesis from leaf explants. Significant differences in callus and shoot formation was observed among cultivars. Cultivars Camouflage, Camille, Octopus, and Star Bright produced green nodular, brown nodular, yellow friable, and green compact calli with corresponding maximum callus formation frequencies of 96%, 62%, 54%, and 52%, respectively. A maximum of 6.7 shoots / callus was observed in cv. Camouflage, followed by cultivars Camille and Star Bright at 3.7 and 3.5, respectively. Calli of cv. Octopus displayed no capacity for shoot organogenesis.

Moreover, *in vitro* mutagenesis for breeding and environmental tolerant can be induced using different chemicals. Henny *et al.*, [28] successfully induced tetraploids in *in vitro* culture of diploid *Dieffenbachia* x 'Star Bright M-1' using colchicine. The polyploid plants showed morphological variation compared to control. Flow cytometry confirmed presence of tetraploids among the colchicine-treated plants. Additionally, Abass *et al.*, [44] stimulated mutation on *D. picta* cv. Tropica shoots when

cultured *in vitro* with different concentrations of NMU.

4. Conclusion

Tissue culture allows for large amounts of material to be produced, therefore facilitating intensive selection [56]. Tissue culture techniques have been applied to a wide range of ornamental species (about 156 ornamental genera) such as *Begonia*, *Ficus*, *Anthurium*, *Codiaeum*, *Chrysanthemum*, *Rosa*, *Saintpaulia*, *Gerbera* and *Spathiphyllum*. Moreover, tissue culture is an important role in ornamentals breeding programs with respect to improving the efficiency and the quality of plantlets produced. Consequently, based on the results noted in Table 1, there is no reason why tissue culture should not be applied to the propagation of *Dieffenbachia* commercially. However, multiplication of *Dieffenbachia* is still difficult to establish and to enhance optimal growing conditions in vitro. Therefore, there prolongs to be an urgent need for extensive work in the basic tissue culture protocols for *Dieffenbachia* spp. plants.

Abbreviations

BA	benzyladenine
BAP	6-benzyl aminopurine
N6	Chu's medium
2,4-D	2,4-dichlorophenoxy acetic acid
DM	*Dieffenbachia* medium
B_5	Gamborg's medium
IAA	indole-3-acetic acid
IBA	indole-3-butyric acid
Kin	kinetin; 6-furfurylaminopurine
LS	Linsmaier and Skoog's medium
MS	Murashige and Skoog's medium
NAA	α-naphthalene acetic acid
NMU	N-nitroso-N-methylurea
2iP	N^6-(2-isopentenyl).adenine
$NaH_2PO_4.H_2O$	Sodium dihydrogen phosphate dihydrate
TDZ	thidiazuron

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