International Journal of Plant Developmental Biology ©2008 Global Science Books

Recent Advances in the Application of Plant Tissue Culture in *Dieffenbachia*

Xiuli Shen[1*] • Michael E. Kane[2]

[1] Citrus Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850, USA
[2] Department of Environmental Horticulture, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611-1067, USA

Corresponding author: * **xis300@ufl.edu**

ABSTRACT

Plant tissue culture has been shown to be a very important tool for the ornamental foliage plant industry. This is especially true for the foliage plant genus *Dieffenbachia*. The application of *in vitro* culture of *Dieffenbachia* has the potential to overcome some of the limitations associated with traditional methods of mass propagation, breeding and genetic manipulation. However, compared to other species, this approach has been limited in *Dieffenbachia* due to its recalcitrant nature *in vitro*. Recent advances in the application of plant tissue culture methods for the propagation and genetic manipulation of *Dieffenbachia* varieties are reviewed.

Keywords: clonal propagation, shoot organogenesis, somaclonal variation
Abbreviations: 2,4-D, 2,4-dichlorophenozyacetic acid; **2iP**, N^6-(Δ^2 – isopentenyl) adenine; **BA**, 6-benzyladenine; **CPPU**, *N*-(2-chloro-4-pyridyl)-*N*-phenylurea); **IAA**, indole-3-acetic acid; **IBA**, indole-3-butyric acid; **GA_3**, gibberellic acid; **MS**, Murashige and Skoog (1962); **NAA**, 1-naphthalene acetic acid; **SEM**, scanning electron microscopy; **TDZ**, thidiazuron

CONTENTS

DIEFFENBACHIA GENUS

Botany

The genus *Dieffenbachia* (commonly known as dumb cane) consists of about 30 monocot species contained in the family Araceae. Native to tropical regions of Central and South America (Chen *et al*. 2003b), the genus is comprised of herbaceous perennial evergreen species with thick stems bearing alternate leaves (Black 2002). The value of *Dieffenbachia* lies in its attractive foliar variegation. The leaves are broad and variegated with white markings or distinctive patterns with sheathed petioles resulting in a very striking appearance (Henny and Chen 2003). *Dieffenbachia* has a unique floral structure. Flowers are unisexual and contain only male or female parts consisting of a spadix and spathe. The spadix is the central fleshy spike, covered with many small staminate and pistillate flowers. The spathe is a modified bract and envelops the spadix until anthesis. In *Dieffenbachia*, separate male and female flowers are on the same plants with male flowers being on the upper one-or two thirds of spadix and female flowers on the lower one-third. Male flowers do not produce pollen until 2 to 3 days after the spathe initially opens while female flowers are only pollen receptive the same day the spathe unfurls. Thus, self-pollination is prevented in *Dieffenbachia* by this naturally occurring dichogamy as female flowers mature earlier than male flowers (Henny 1988). A special method of pollination is required in *Dieffenbachia* for seed production and breeding purposes.

Dieffenbachia requires low light levels for growth. For example, cv. 'Star Bright' and cv. 'Snow Flake' tolerate light levels as low as 50 foot candles (Chen *et al.* 2003b). In fact, *Dieffenbachia* grows significantly better in low rather than high light conditions. Plants maintained in high light levels may develop a washed-out appearance. As a result, *Dieffenbachia* is widely used as ornamental specimens in interiorscapes. Besides beautifying the environment, *Dieffenbachia* is also an ornamental plant with the ability to remove volatile organic compounds from air and thus functions to improve indoor air quality (Liu 2007). Not surprisingly, *Dieffenbachia* is among the most popular ornamen-

Received: 1 August, 2008. Accepted: 26 November, 2008.

Invited Review

tal foliage plants in the United States, continually ranking in the top five for annual wholesale value (McConnell *et al*. 1989; USDA 1999).

Traditional propagation

Dieffenbachia can be propagated by both sexual and asexual methods. Seed propagation is not usually used because seed set is very poor, viable seed production is low, and germination is erratic. Consequently, propagation by seeds is only used for breeding purposes. *Dieffenbachia* can be easily propagated by asexual methods, by tip, cane cuttings and divisions. It has been reported that cuttings require extremely long time periods for root initiation and axillary bud sprouting. Application of the plant growth regulators GA_3 (gibberellic acid), kinetin and IBA (indole-3-butyric acid) to stem cuttings either by soaking or direct application to axillary buds enhances propagation (More and Khalatkar 1988). Traditional asexual propagation can require significant labor inputs and result in the spread of pathogens. The advantage of propagation by cuttings or divisions is that the plants propagated are largely true-to-type.

Traditional breeding

Progress in breeding of *Dieffenbachia* has been slow due to the long breeding cycles and lack of basic information on breeding methodology. Hybridization has been the most common and widely used method for producing new cultivars in *Dieffenbachia*. Since the first hybrid *Dieffenbachia* cv. 'Bausei', obtained by a cross between *Dieffenbachia picta* and *Dieffenbachia weirii*, was released in 1870 in the garden of the Royal Horticultural Society of London at Chiswick (Birdsey 1951), about 100 new cultivars have been developed. Hybridization requires many crosses and careful selection. For example, the hybrid *Dieffenbachia* cv. 'Triumph' was selected from four crosses involving seven different parents; the hybrid *Dieffenbachia* cv. 'Star White' was generated from five crosses involving nine different parents; while the hybrid *Dieffenbachia* cv. 'Victory' was obtained from two crosses involving three parents. Naturally occurring dichogamy in this genus also makes this process very laborious, time consuming and usually requiring about 7-10 years for a new cultivar to be released. The *Dieffenbachia* breeding program at Mid-Florida Research and Education Center at the University of Florida (Apopka, FL) was initiated in 1976 and a series of important and popular *Dieffenbachia* hybrids including *Dieffenbachia* 'Sparkles' (Henny 1995a); *Dieffenbachia* 'Star Bright' (Henny 1995b); *Dieffenbachia* 'Triumph' (Henny *et al*. 1986, 1987a); *Dieffenbachia* 'Starry night' (Henny *et al*. 1991b); *Dieffenbachia* 'Star White' (Henny *et al*. 1991c); *Dieffenbachia* 'Sterling' (Henny 2006a); *Dieffenbachia* 'Tropic Honey' (Henny 2006b); *Dieffenbachia* 'Tropic Star' (Henny *et al*. 1988a, 1988b); *Dieffenbachia* 'Victory' (Henny *et al*. 1987b, 1991a), have been released from this program.

In addition to hybridization, new cultivars are also selected as a result of spontaneous mutation in *Dieffenbachia* cultivars that are prone to sport. For example, *Dieffenbachia* cvs. 'Carina', 'Honey Dew' and 'Rebecca' are sports of cv. 'Camille'; and cv. 'Camille' is a sport of cv. 'Marianne'. Cultivars 'Perfection Compacta' and 'Marianne' are sports of cv. 'Perfection' (Chen *et al*. 2003a).

Introduction of new species collected from the wild or private collectors is another way for new cultivar development. For instance, *Dieffenbachia* cv. 'Imperial' was discovered in eastern Peru in 1868 (Birdsey 1951). Regardless of origin, all new *Dieffenbachia* cultivars are selected for their distinctive leaf variegation and shape and plant form which differs from their parents. A summary of 30 *Dieffenbachia* cultivars and their origin are listed in **Table 1**.

Given that foliar variegation and color are the primary attractive characters of *Dieffenbachi*a, an understanding of genetic basis of leaf variegation patterns is important in *Dieffenbachia* breeding. In general, leaf variegation can have either a cell lineage or non-cell lineage origin. Cells in individual plant having different genotypes result in cell lineage variation. While non-cell lineage variation results from cells in an individual plant possessing the same genotypes but having differential gene expression. Leaf variation pattern in *Dieffenbachia* results from non-cell linage and follow simple Mendelian rules of inheritance (Henny and Chen 2003). It has been noted that a single dominant gene (*Pv*) (pattern of variation) controlled foliar variegation in the two *Dieffenbachia* cvs. 'Perfection' and 'Hoffmannil'. The difference in leaf variation between these two cultivars was caused by background modifying genes (Henny 1982). It was also found that a single dominant allele (Pv^1) controlled the foliar variegation pattern of *Dieffenbachia* cv. 'Camille'. Pv^1 was a mutated form of the *Pv* allele. The 'Camille' variegation pattern masks the 'Perfection' and 'Hoffmannil' pattern in plants carrying both $Pv^1$and *Pv* alleles (Henny 1986). A single dominant nuclear gene (*Wm*) (white midrib) controlled the inheritance of the white foliar midrib in three *Dieffenbachia* cultivars. The gene for white midrib (*Wm*) and the gene for foliar pattern of variegation (*Pv*) have also been shown to be linked (Henny 1983).

Table 1 30 *Dieffenbachia* cultivars and their origin.

Cultivars	Type	Origin
Bali Hai	Hybrid	Rex x unnamed rex hybrid
Bausei	Hybrid	Maculate x weirii
Corsii	Hybrid	Maculate x wallisii
GoldRush	Hybrid	Victory x Tropic Marianne
Paradise	Hybrid	Marianne x Wilson Delight
Sparkle	Hybrid	20 parents including Wilson Delight, Perfection, Perfection compacta
Star Bright	Hybrid	Several parents
Star White	Hybrid	5 crosses of 9 parents
Sterling	Hybrid	Victory x Tropic Marianne
Triumph	Hybrid	4 crosses of 7 different parents
Tropic Breeze	Hybrid	*Fourneri* x Angustior Lancifolia
Tropic Honey	Hybrid	Victory x Tropic Marianne
Tropic Marianne	Hybrid	Unidentified parent
Tropic Rain	Hybrid	*Daguensis x amoena*
Tropic Star	Hybrid	Perfection x Angustior Lancifolia
Victory	Hybrid	Wilson Delight x Perfection x AREC V-78
Camille	Sport	Perfection
Honey Dew	Sport	Camille
Parachute	Sport	Paradise
Snowflake	Sport	Tiki
Tike	Sport	Memeria-Corsii
Tropic Alix	Sport	Tropic Snow
Camouflage	Somaclonal variant	Panther
Carina	Somaclonal variant	Camille
Rebecca	Somclonal variant	Camille
Sarah	Somclonal variant	Camille
Jungle Giant	Wild collection	
Panther	unknown	
Gold Dust	unknown	
Octopus	unknown	

APPLICATION OF PLANT TISSUE CULTURE IN *DIEFFENBACHIA*

Plant tissue culture has been shown to be a very useful tool for both plant propagation and breeding. It can potentially overcome some limitations encountered when using traditional approaches to *Dieffenbachia* propagation and breeding including the efficient production of disease eradicated plants.

Pathogen-eradicated plant production

Since the early 1980s, *in vitro* culture has become an important method for the commercial propagation of *Dieffenba-*

chia. Establishment of contaminant-free and pathogen-eradicated cultures is the first goal for any *in vitro* based propagation protocol because contamination is the biggest hindrance for reliable *in vitro* propagation (Debergh and Maene 1981). Contaminants on the surface of explants taken from greenhouse or field can be removed by immersion in certain steriliants, such as ethanol, sodium hypochlorite or $HgCL_2$ for certain time durations. However, bacteria and fungi may also reside as endophytes in internal tissue (Kunisaki 1977; Kane 2000a). It is impossible to remove these internal contaminants by surface sterilization. Consequently, alternative *in vitro* culture techniques are required to achieve contaminant free plant production. An initial application of tissue culture for the propagation of *Dieffenbachia* was to eliminate systemic viral and bacterial pathogens.

Knauss (1976) described a method to produce *Dieffenbachia picta* cv. 'Perfection' plants free of cultivable fungal and bacterial contaminants. Lateral buds and meristem-tip explants (1-3 mm in length) were excised from plants grown in the greenhouse, surface sterilized and then cultured on a modified MS (Murashige and Skoog 1962) medium. Cultures exhibiting visible contamination were discarded. Cultures showing no sign of contamination were subject to indexing for cultivable contaminants. The indexing procedure consisted of 3 steps. In the first step, stems from *in vitro* grown plants were cut into 0.5–1.0 mm thick sections, then the cut sections were divided into groups of four and each section was cultured onto each of four indexing media. The remaining shoot tips were subcultured on the medium to promote continued shoot growth for the next indexing step. After three weeks culture on indexing media, plantlet lines showing any fungal and bacterial growth in any of the indexing media were destroyed. In Step 2, newly-developed shoot stems from subcultured shoot tips in Step 1 were indexed again using the same procedure. In Step 3, only internodes of stems of plantlets showing no sign of fungal and bacterial growth from Steps 1 and 2 were subject to indexing. Once again, plantlet lines showing fungal and bacterial growth in Step 3 were destroyed. Among 82 plantlets examined, Knauss (1976) observed that 32 were contaminated with bacteria and fungi, while 50 plantlet lines tested free of fungi and bacteria were retained for further propagation. These lines displayed vigorous growth and branched more freely in the absence of fungi and bacteria contaminants.

In addition to providing rapid and reliable propagation, pathogen eradicated plant lines can also serve as a source for selection of new cultivars. Chase *et al.* (1981) reported the release of the new *Dieffenbachia* cv. 'Perfection-137B' as a result of the selection from pathogen eradicated lines of *Dieffenbachia Maculata* generated *in vitro*. They used the same experimental procedure as developed by Knauss (1976), and more than two dozen pathogen-free lines were produced. Plants from these lines were transferred to soil and maintained under controlled condition. Plants were evaluated carefully for their growth habit and horticultural traits. Lines exhibiting slow cutting establishment in soil, or a tendency to produce sports were eliminated because of their inferior field performance compared to their parents and unreliable true-to-type plant production. Finally 'Perfection-137B' was selected according to its superior appearance and growth performance.

Clonal *in vitro* propagation

In vitro propagation (micropropagation), in general, is currently being used to commercially produce a large number of uniform and true-to-type healthy plants on a year-round basis. There are many factors affecting explant performance *in vitro*, including tissue related factors (genotype and explants) and non-tissue related factors (culture media and conditions). In order to maximize the efficiency of micropropagation of any species conditions to optimize the five micropropagation stages must be determined for each plant by manipulating the various culture and environmental factors affecting *in vitro* growth responses. Although *Dieffenbachia* cultivars are being commercially produced via micropropagation, surprisingly, there are very few publications on the *in vitro* propagation of *Dieffenbachia.* Voyiatzi and Voyiatzis (1989) were among the first to describe an *in vitro* culture *Dieffenbachia* protocol for true-to-type plant production through axillary shoot production. Shoot-tip and lateral bud explants excised from stock plants of *Dieffenbachia exotica* cv. 'Marianna' were surface sterilized in aqueous 2.8% sodium hypochlorite for 15 min, rinsed four times in sterile distilled water and then cultured in Erlenmeyer flasks containing 40 ml MS basal medium supplemented with different plant growth regulators. Media were solidified with 0.7% Difco Bacto agar. Factors examined included type and concentration of two cytokinins (kinetin at 0, 1, 2, and 4 mg/l, and 2iP [N^6-(Δ^2 – isopentenyl) adenine] at 0, 8, 16 and 32 mg/l), the number of recultures (1 to 4) of the initial basal shoot clump, and culture temperature (15, 20, 27 and 32°C). Basal medium supplementation with 16 mg/l 2iP was most effective in promoting shoot proliferation (6.2 shoots per flask). Shoot production increased with each successive reculture of the basal clumps at 6 week intervals, with 5.5 shoots at the 1st subculture, 8 at the 2nd, 14.4 at 3rd but then decreased to 0 shoot per flask by the 4th subculture. Shoot production increased with increasing temperature reaching a maximum of 6.5 shoots per flask at 27°C, then decreased to about 1.5 shoots per flask at 32°C when cultured on MS medium supplement with 16 mg/l 2iP. Their study showed that through manipulation of media and culture conditions, the shoot proliferation rate of *Dieffenbachia* can be significantly increased.

Shoot organogenesis

Besides clonal propagation from pre-existing meristems (Kane 2000b), plants can also be produced from adventitious shoot meristems induced to form on explants without pre-existing meristems via the process of shoot organogenesis (Kane *et al*. 1991). Shoots may form directly on the explant (direct shoot organogenesis) or indirectly on an intermediary callus which forms on the primary explant (indirect shoot organogensesis). Orlikowska *et al*. (1995) provided the first report of shoot organogenesis on cultured leaf petiole explants in *Dieffenbachia*. However, leaf petioles were the only responsive explants for indirect shoot organogenesis as induction of callus on leaf blades and root explants was not possible and the explants died after 3 to 4 weeks culture. The inductive period for direct shoot organogenesis in *Dieffenbachia* was very long, requiring 6 to 8 weeks incubation in the dark for small visible buds to be formed. MS basal medium supplemented with 1.0 mg/l TDZ (thidiazuron) plus 1.0 mg/l NAA (1-naphthalene acetic acid) or 1.0 mg/l BA (6-benzyladenine) plus 1.0 mg/l 2,4-D (2,4-dichlorophenozyacetic acid) were the most effective plant growth regulator combinations for shoot formation, with 15.4 and 10.2% petioles forming buds, respectively. The mean number of buds per responsive explant was 45. However, the specific cultivar used in this study was not stated and results were only described in the text, without detailed numeric data.

At the University of Florida, extensive research was conducted on shoot organogenesis in *Dieffenbachia* from 2003 to 2008. For the first time, a protocol for indirect shoot organogenesis in *Dieffenbachia* cv. 'Camouflage' was established (Shen *et al*. 2007a). Lateral buds taken from plants grown in the greenhouse served as the explant source to initiate *in vitro* shoot cultures. Lateral buds were sterilized in 1.2% sodium hypochlorite for 10 min, then rinsed 3 times with sterile water, and cultured in baby food jars (4.4 × 7.0 cm^2) containing 40 ml of MS medium supplemented with 80 µM 2iP and 2 µM IAA (indole-3-acetic acid). Cultures were maintained at 22 ± 3°C under a 16-h light photoperiod provided by cool white fluorescent lights. The axillary shoots formed were transferred to the same fresh medium every 8 weeks to increase *in vitro* stock shoot cultures. To detect any cultivable contaminants, established cultures

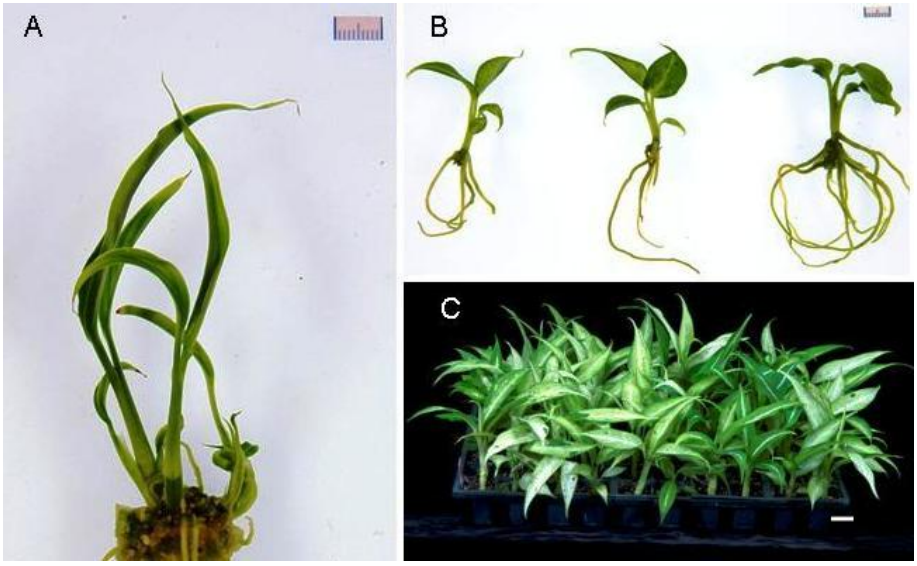

Fig. 1 ***In vitro*** **regenerated** ***Dieffenbachia*** **cv. 'Camouflage' plants via indirect shoot organogenesis.** (**A**) Shoots developed from calli after 8 weeks culture on MS medium supplemented with 40 µM 2iP and 2 µM IAA. (**B**) Plantlets with fully developed leaves and roots after 8 weeks cultures on MS medium supplemented with 40 µM 2iP and 2 µM IAA. (**C**) Acclimatized plants grown in the greenhouse for 8 weeks. Scale bars = 1 cm.

Table 2 Characterization of calli and their shoot regeneration ability in 4 *Dieffenbachia* cultivars.

Cultivar	Callus structure	Callus color	Growth rate	Organogenic	Regenerative capacity
Camouflage	nodular	green	medium	yes	> 24 months
Camille	nodular	brown	medium	yes	16 months
Octopus	friable	light yellow	high	no	no
Star Bright	compact	light green	low	yes	2 months

were routinely indexed using the procedure developed by Kane (2000a). Leaf explants excised from these *in vitro* shoot cultures were cultured on the MS medium supplemented with TDZ at 0, 1, 5, 10 µM and 2,4-D at 0, 0.5 and 1 µM for callus induction, initially in dark for 8 weeks and then transferred to the 16-h light photoperiod for another 4 weeks. The type and concentration of plant growth regulators had a significant effect on callus induction and shoot differentiation. The greatest frequency of callus formation (96%) was obtained on medium supplemented with 5 µM TDZ and 1 µM 2, 4-D.

An adventitious shoot regeneration medium was selected by transferring calli onto MS medium supplemented with 2iP at 0, 20, 40, or 80 µM and IAA at 0 or 2 µM and then assessing indirect shoot organogenesis after 8 weeks culture (**Fig. 1A**). A maximum of 7.9 shoots/callus were produced on the medium supplemented with 40 µM 2iP and 2 µM IAA. Roots formed spontaneously with shoot formation in most of the *Dieffenbachia* cultures (**Fig. 1B**). Shoots (some with roots) longer than 20 mm with 2-3 leaves were easily acclimatized to greenhouse conditions after transplanted in plug trays containing a 2:1:1 (v/v/v) mixture of Canadian peat: vermiculite: perlite. Plantlets were maintained under shade cloth with a maximum irradiance of 345 µmol m^{-2} s^{-1}, natural photoperiod (10-14.5 h light), and a temperature range 20-31°C. An *ex vitro* survival rate of 100% was obtained (**Fig. 1C**).

The capacity for indirect shoot organogenesis in three other *Dieffenbachia* cvs. 'Camile', 'Octopus' and 'Star Bright' were also examined (Shen *et al*. 2008). Results indicated that the capacity for indirect shoot organogenesis was clearly genotype-dependent. Using the same experimental procedure developed for *Dieffenbachia* cv. 'Camouflage', we observed distinct differences in callus morphology, callus forming ability, and subsequent shoot differentiation among the three additional *Dieffenbachia* cultivars examined. Callus formation frequencies of 96, 62, 54 and 52% were obtained from cvs. 'Camouflage', 'Camille', 'Octopus' and 'Star Bright', respectively.

Four distinct callus types, varying in structure and color, green nodular, brown nodular, yellow friable and green compact calli, were produced from cultured leaf explants of cvs. 'Camouflage', 'Camile', 'Octopus' and 'Star Bright', respectively (**Table 2**). These different callus types displayed different potentials for shoot organogenesis. Yellow friable calli, produced from cv. 'Octopus' leaf explants exhibited no shoot regeneration capacity. While green compact calli from cv. 'Star Bright', having a very limited capacity for indirect shoot organogenesis, exhibited no capacity for sustained callus culture as calli lost their shoot regeneration ability after 2 months culture. Calli of cv. 'Camille' retained the capacity for shoot regeneration for up to 16 months. 'Camouflage' calli retained the capacity for shoot regeneration after 24-month culture using 8 week subculture intervals (**Table 2**). The cultivar 'Camouflage' also exhibited the highest shoot production capacity with a maximum of 6.7 shoots/callus, followed by 4.4 shoots/callus from cv. 'Camille' and 3.5 shoots/callus from cv. 'Star Bright' (Shen *et al*. 2008). Consistent with the report of Orlikowska *et al*. (1995), we also observed that root explants displayed no capacity for callus induction regardless of cultivar or plant growth regulator combination. The *in vitro* culture procedures for sustained callus culture and indirect shoot organogenesis in *Dieffenbachia* are illustrated in **Fig. 2**.

Dieffenbachia is a naturally-slow-growing plant and this characteristic is also manifested during *in vitro* culture, probably indicative of a slow cell division rate. Even when leaf explants, taken from *in vitro* produced plants, were cultured on callus induction media, they responded slowly. At least 4 weeks culture was required for the first sign of callus production to be observed. The slow response of leaf tissue explants *in vitro* might also be attributed to the need for a rejuvenation period. It has been noticed that juvenile tissues responded more quickly than mature tissues when cultured *in vitro* (Greenwood 1987; Webster and Jones 1989).

The developmental sequence of indirect shoot organogenesis in *Dieffenbachia* was investigated using light microscopy. Calli were observed from leaf explants on MS medium supplemented with 5 µM TDZ and 1 µM 2,4-D after 28 days of culture. Two types of cells were observed in calli: 1) regenerative cells which were smaller in size and more compact with more densely stained cytoplasm, thinner cell walls, more prominent nuclei and no visible vacuoles (**Fig. 3A**); and 2) non-regenerative cells which were larger and not as compact with less cytoplasm and smaller nuclei, thicker cell walls and larger vacuoles (**Fig. 3B**). Early mitotic activity was observed after 31days culture (**Fig. 3C**). The first cell division was usually anticlinal followed by

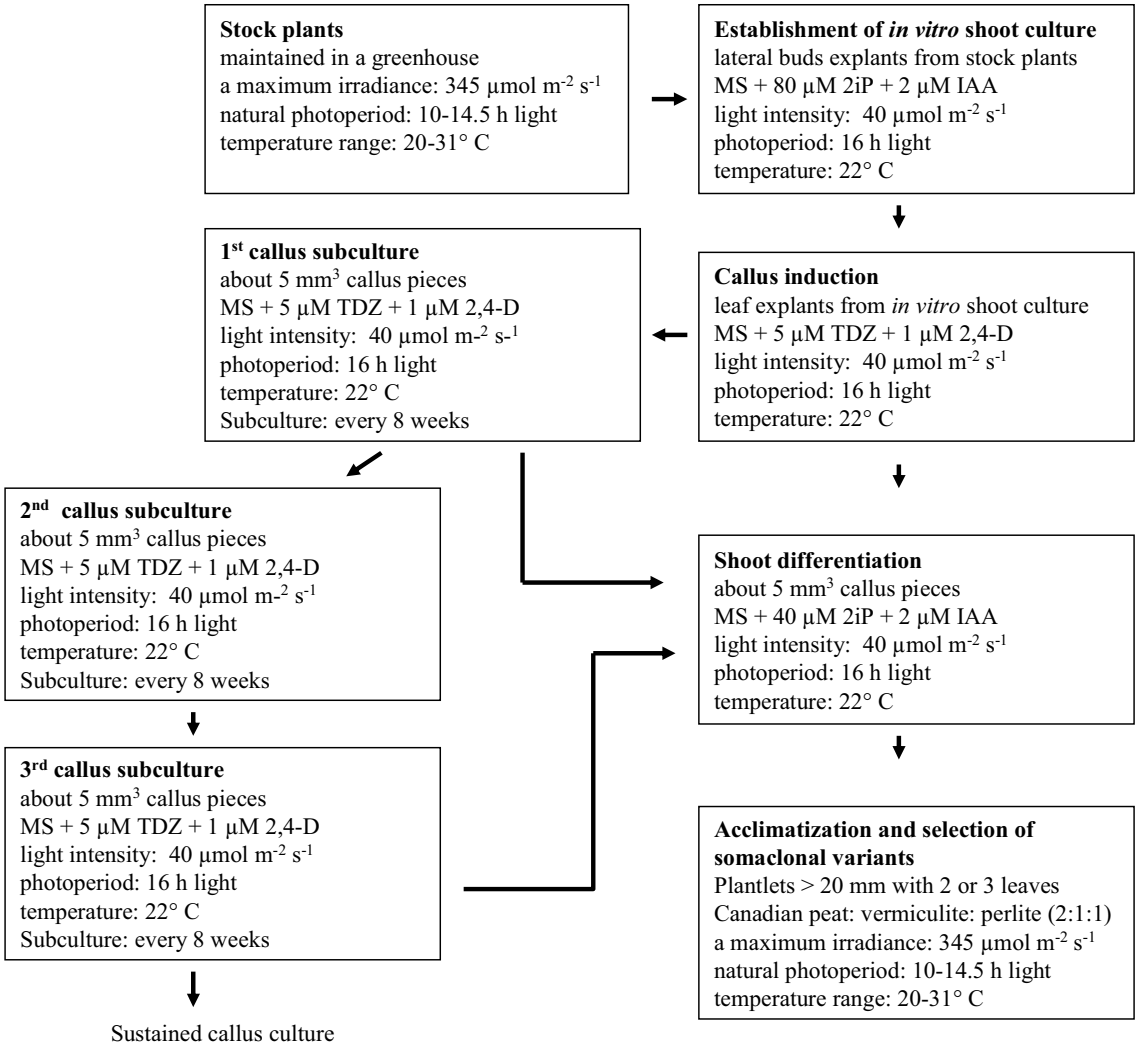

Fig. 2 Indirect shoot organogenesis and sustained callus subculture for continued shoot production for selection of somaclonal variants.

periclinal cell division (**Fig. 3D**). After several mitotic division, the differentiation of a meristematic zone occurred (**Fig. 3E**). By continuous anticlinal and periclinal cell division, bigger meristematic cell masses composed of actively dividing cells were formed by 43 days culture. Each meristematic mass was characterized by cells with thick walls (**Fig. 3F**). Meristematic cell masses may also develop into globular shapes, assuming an appearance similar to globular somatic embryos (**Fig. 3G, 3H**). Cell divisions usually were initiated from superficial callus cells (**Fig. 3D**), but a cell or a group of cells within the callus may also give rise to a meristematic mass (**Fig. 3I**). The meristematic mass became progressively more organized forming a meristematic dome which developed into a shoot apical meristem after 12 days culture on shoot induction medium (**Fig. 3J**). Cell divisions along the flanks of the apical meristem resulted in leaf primordia formation after 18 days following shoot induction (**Fig. 3K**). A well-developed adventitious bud with apical shoot meristem and leaf primordia were formed after 27 days culture (**Fig. 3L**). Multiple shoots were occasionally formed (**Fig. 3M**). Root formation occurred after 39 days of culture (**Fig. 3N**). A complete plantlet was regenerated after 8 weeks of culture on shoot induction medium. A vascular connection between a developing shoot and callus tissue was detected by day 24 (**Fig. 3O**). Scanning electron microscopy (SEM) revealed stomata were present on the epidermis of developing leaves by day 36 (**Fig. 3P**).

The formation of meristemoids was prerequisite for shoot regeneration which was in agreement with the findings of previous studies in other plants (Choffe *et al*. 2000; Budimir 2003). In our study, meristemoids from both surface and internal cells can develop into shoots. However, Hu *et al*. (2005) reported only meristemoids formed in superficial cell layers could develop into plants while those developed from inner cell layers of calli mostly developed into abnormal adventitious shoots meristems due to the structural restriction from peripheral cells. Differences in the species used in these two studies may explain this variation.

We have observed that starch content generally was lower in cells undergoing intense mitotic activity. Starch content could be an indicator of the degree of cell differentiation. A cell degeneration process also occurred in some cells characterized by initial expansion, loss of cytoplasm and formation of large intercellular spaces. Cell degeneration results in tissue shrinkage and necrosis in other species (Benelli *et al*. 2001; Quiroz-Figueroa *et al*. 2002).

Somatic embryogenesis

Somatic embryogenesis is the production of embryos from somatic cells and not resulting from gametic fusion (Merkle 1997; Von Arnold *et al.* 2002). Somatic embryos are bipolar, morphologically and anatomically similar to zygotic embryos and have no vascular connection to the original tissue. Somatic embryogenesis is known to occur naturally in the ovule of many plant species and many different terms are used by different authors to describe this phenomenon in different species, such as apomixis, polyembryony, adventive, sporophytic and nucellar embryony. Pollen and many tissues, such as the nucellus, inner integument, synergids, antipodals, endosperm, and suspensor have been observed to naturally give rise to asexual embryos (Tisserat *et al*. 1979). Given the diverse types of tissues from which

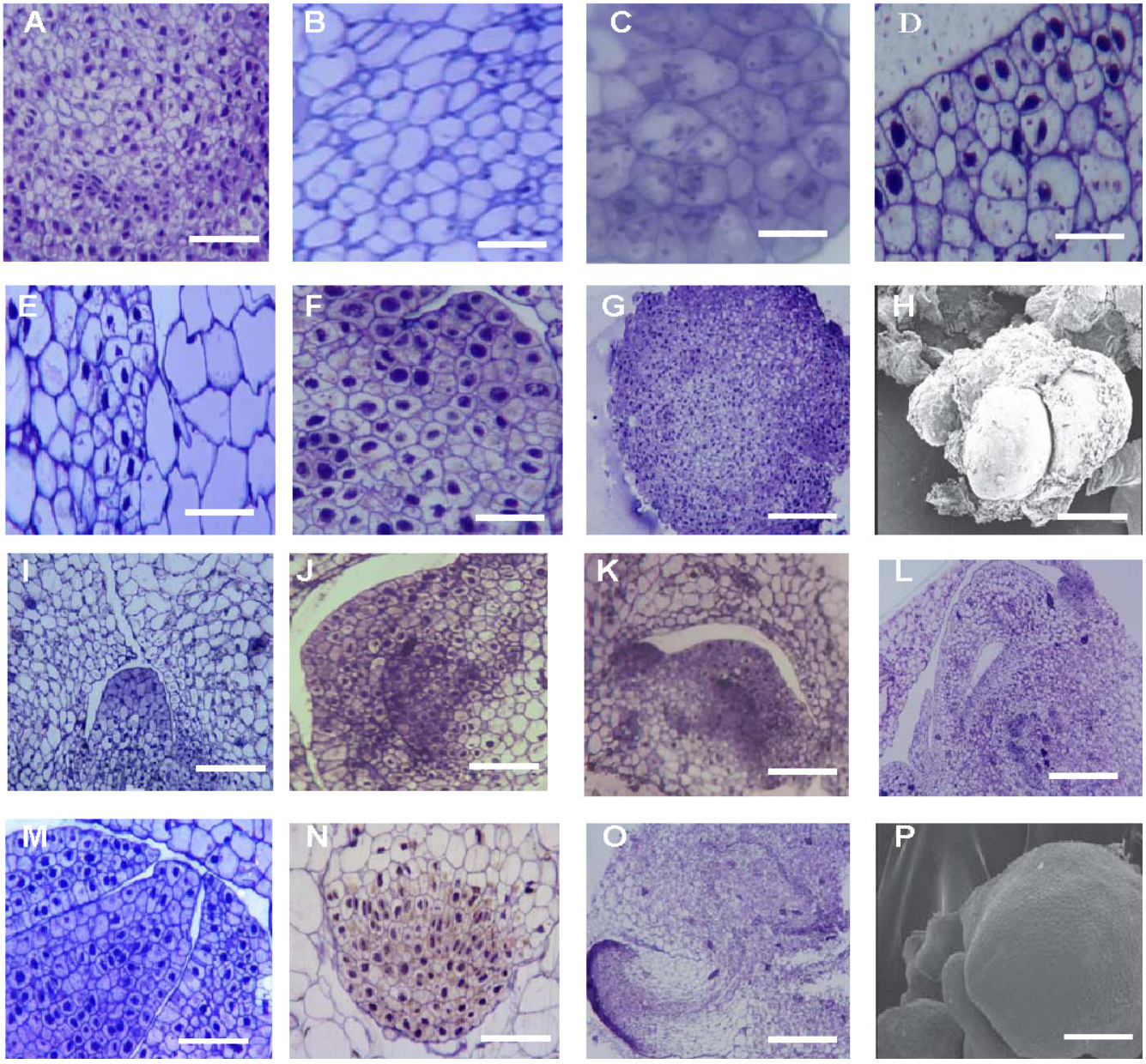

Fig. 3 Histological evidence of indirect shoot organogenesis in *Dieffenbachia* at different developmental stages when cultured on MS medium supplemented with 5 µM TDZ and 1 µM 2,4-D for callus induction and on MS medium supplemented with 40 µM 2iP and 2 µM IAA for shoot differentiation. (**A**) Regenerative cells in calli originated from leaf explants. Bar = 250 µm. (**B**) Non-regenerative cells in calli. Bar=250 µm. (**C**) Early mitotic activity observed at day 37 on callus induction medium. Bar = 167 µm. (**D**) Initial anticlinal division on the surface of calli. Bar = 250 µm. (**E**) Initiation of meristematic zone by continued anticlinal and periclinal cell division. Bar = 250 µm. (**F**) Development of meristematic mass by day 43. Bar = 250 µm. (**G**) Formation of a globular shaped meristematic mass. Bar = 500 µm. (**H**) Appearance of a globular shaped meristematic mass. Bar = 1 mm. (**I**) Meristematic cell mass formed within calli. Bar = 250 µm. (**J**) Meristematic dome formation after 12 days of culture on shoot differentiation medium. Bar = 250 µm. (**K**) Development of shoot meristem and leaf primordia by day 18. Bar = 500 µm. (**L**) Well-developed shoot bud enclosed by leaves at day 27. Bar = 500 µm. (**M**) Multiple shoot formation. Bar = 250 µm. (**N**) Root formation by day 39. Bar = 250 µm. (**O**) Vascular connection between a developing shoot and callus tissue. Bar = 500 µm. (**P**) SEM depicting a developing shoot with stomata on the leaf epidermis at day 36 culture on shoot differentiation medium. Bar = 750 µm. (Photos K, N, O from **Shen X, Chen J, Kane ME** (2007a) Indirect shoot organogenesis from leaves of *Dieffenbachia* cv. Camouflage. *Plant Cell, Tissue and Organ Culture* **89**, 83-90, with kind permission of Springer Science + Business Media, ©2007).

embryos can be generated, the more general term non-zygotic embryogenesis has currently been widely adopted. Somatic embryogenesis can also be induced during *in vitro* culture. Since the first report of somatic embryogenesis in carrot callus cultures in 1958 (Steward *et al*. 1958a, 1958b), somatic embryogenesis *in vitro* has been reported in over 100 species (Merkle and Sommer 1986; Merkle and Wiecko 1989; Merkle *et al*. 1990; Krishnaraj and Vasil 1995; Merkle *et al*. 1995). To date, there have been no reports of somatic embryogenesis in *Dieffenbachia*. Our unsuccessful attempts to induce somatic embryogenesis, either directly or indirectly in *Dieffenbachia*, were partially the results of high contamination rates (>70%) when culture establishment was attempted using leaf explants taken directly from greenhouse-grown donor plants. Leaves excised from greenhouse grown stock plants of *Dieffenbachia* cvs. 'Camouflage' and 'Camille' were rinsed in running water for 10 min, and then sterilized in aqueous 1.2% sodium hypochlorite (20%, v/v) for 10 min followed by three 5-min rinses with sterile water. Leaf explants were then cut into about 5-mm^2 sections and cultured on induction media for somatic embryogenesis. Induction media were composed of MS basal medium supplemented with different concentrations and combinations of PGRs (plant growth regulators). PGRs tested were: (1) BA at 0, 1, 10, 50 µM and 2,4-D at 0, 1, 10, 50 µM; (2) CPPU [N-(2-chloro-4-pyridyl)-N-phenylurea] at 0, 1, 2.5, 5 µM and 2,4-D at 0, 2, 4, 8, 10 µM; (3) CPPU at 0, 1, 2.5, 5 µM and NAA at 0, 2, 4, 8, 10 µM ; (4) kinetin at

0, 1, 5, 10 μM and IAA at 0, 1 μM ; (5) dicamba at 0, 1, 3, 9 μM and 2,4-D at 0, 1 μM; (6) picloram at 0, 1, 3, 9 μM and 2, 4-D at 0, 1 μM; (7) TDZ at 0, 1, 10, 50 μM and NAA at 0, 1, 10, 50 μM; (8) TDZ at 0, 1, 10, 50 μM and 2,4-D at 0, 1, 10, 50 μM; and (9) TDZ at 0, 1, 5, 10 μM and 2,4-D at 0, 0.5, 1 μM. Explants were cultured in 100 × 15 mm sterile Petri plates containing 20 ml medium. There were 5 explants per Petri plate and 5 replicate plates per treatment. Cultures were initially maintained in dark for 8 weeks and then transferred to the 16 h photoperiod for another 4 weeks. Unfortunately, all PGRs screened failed to induce somatic embryogenesis (unpublished data).

Dieffenbachia is mainly propagated vegetatively by cuttings and divisions and is maintained under moist and shaded condition. Conceivably, under these cultural conditions more bacteria and fungi may accumulate on the surface of plants or even inside plants as endophytes. This may account for such high contamination rates experienced when attempting to establish *in vitro* cultures. Furthermore, leaf explants, even when not contaminated, were not responsive on any of the media tested. Using leaf explants from established shoot cultures is an alternative means to reduce contamination rate and increase explant response. However this approach has not been attempted.

We have been successful in inducing regeneration of nodular structures from leaf explants of both cvs. 'Camouflage' and 'Camille'. Morphologically, these nodules resemble somatic embryos. We also found that these nodular structures were comprised of actively dividing cells. However, the absence of bipolar structure (shoot and root meristems) in more developed nodules indicates that these nodules were not somatic embryos.

The factors and mechanisms determining the capacity for cellular regenerative as well as the subsequent developmental pathway are largely unknown, but undoubtedly, the type and concentration of plant growth regulators in the medium play a role in the determination of cell differentiation (Onay 2000). Ma and Xu (2002) reported that the same cells may give rise to either somatic embryogenesis or shoot organogenesis depending on the duration of induction and plant growth regulators included in media. This suggests that plant growth regulators could determine the developmental pathway of competent cells. However, attainment of competence is not clearly understood. The presence of inductive signals, for instance, plant growth regulators in culture medium, was necessary for a cell to become competence (Rugini and Muganu 1998). Compete cells may subsequently form meristematic cells. Meristematic cells may give rise to the formation of meristemoids. Once meristemoids are produced, some of them may develop into adventitious shoots, roots or somatic embryos. It has also been demonstrated that the *in vitro* regeneration pathway could be shifted by manipulation of plant growth regulators in culture media in other species. It was possible in some species to switch regeneration from shoot organogenesis to somatic embryogenesis by increasing the TDZ concentration in the medium. TDZ induced shoot organogenesis at low concentration (< 2.5 μM) and somatic embryogenesis at high concentration (5-10 μM) in African violet (Mithila *et al*. 2003). In our study we also tried to induce somatic embryogenesis from leaf derived calli of *Dieffenbachia* by increasing concentration of TDZ from 5 to 10, 20, 40 and 80 μM in media. Unfortunately, there was no evidence for the occurrence of somatic embryogenesis.

Selection of somaclonal variation

The genetic variation among plants regenerated from *in vitro* culture has been termed somaclonal variation (Larkin and Scowcroft 1981). Plants with the deviant phenotypes are known as somaclones or somaclonal variants. Somaclonal variation is a random phenomenon that can occur at any location in the genome (De Schepper *et al*. 2003). From its origin, it can be deduced that somaclonal variation occurs in the period before the formation of meristematic tissues and terminated with formation of meristematic tissues (Bouman and De Klerk 1997). Compared to natural sport production, somaclonal variation occurs at a much higher rate. Somaclonal variation can result from either pre-existing variation in explant tissues or induced variation during tissue culture (Skirvin *et al*. 1994).

In addition to facilitating clonal propagation, *in vitro* culture can also result in production of off-type plants. In the early application of tissue culture for commercial propagation of *Dieffenbachia*, all off-type plants were rouged out to maintain the genetic fidelity of the plants produced. It was later realized that these off-type plants could be a source for selection of somaclonal variation for new cultivar development.

Among the factors affecting *in vitro* regeneration of somaclonal variants, genotype plays an important role. The potential for and frequency of somaclonal variation is genotype-dependent (Merkle 1997). During the assessment of somaclonal variation in *Dieffenbachia* regenerated through indirect shoot organogenesis, Shen *et al*. (2007b) noted that the rates of somaclonal variation were 40.4 and 2.6% among regenerated cvs. 'Camouflage' and 'Camille' plants, respectively. Cultivar 'Star Bright' displayed no potential for producing somaclonal variants while all regenerated 'Star Bright' plants were true-to-type. It was also found that duration of callus culture had no effect on somaclonal variation rates of cv. 'Camouflage' as the somaclonal variation rates between plants regenerated from 8 months and 16 months of callus culture were similar. This is inconsistent with the general belief that somaclonal variation increases with the length of time that a culture has been maintained *in vitro.* Orton (1985) noted that if calli, derived from immature petiole segment, were maintained for 6 months by a series of repeated subculture transfers, 84% of the callus cells were karyologically indistinguishable from the control. The remaining 16% exhibited chromosome loss or fusion with only 1 regenerated plant out of 95 displaying an abnormal phenotype. After 12 months in culture, 97% of the callus cells were karyologically distinguishable from the control. Most cells were aneuploids and all callus cells lost the capacity to produce embryoids.

Somaclonal variation is often associated with indirect shoot organogenesis or somatic embryogenesis, each of which involves an interviewing callus stage. During this period, differentiated cells undergo dedifferentiation, induction, redifferentiation (Rout 1999). Bouman and De Klerk (2001) showed that the rate of somaclonal variation among *Begonia* regenerated via somatic embryogenesis was 1.5% for direct but 10.6% for regenerants derived from the callus stage. Since Larkin and Scowcroft (1981) advocated that somaclonal variation could be used as a promising tool for breeding to produce novel genetic variation, foliage plant somaclones with commercially desirable characteristics have been generated by this method (Chen *et al*. 2006).

Any change at the phenotypic level, such as foliar variegation pattern, alterations in leaf shape and texture, or variation in overall plant form, can be a desirable trait in *Dieffenbachia* because the value of *Dieffenbachia* lies in its aesthetic appearance (Chen *et al*. 2003a). Three types of somaclonal variants with novel and distinct foliar variegation patterns differing from the parental plants have been obtained in *Dieffenbachia* 'Camouflage' plants regenerated from leaf-derived calli via indirect shoot organogenesis (**Fig. 4**). One type of somaclonal variant bearing lanceolate leaves instead of oblong leaves of the parent has been identified from regenerated cv. 'Camille' plants (**Fig. 5**).

There are two major advantages of indirect shoot organogenesis *in vitro*. A large number of shoots can be produced from an explant following callus induction and shoot formation. It also has great potential for regenerating somaclonal variants due to intervening callus phase and resultant genetic instability. Selection of somaclonal variants from *in vitro* cultures has become an important method for new cultivar development since it can hasten the breeding process. It generally requires from 2 to 3 years to develop a new cul-

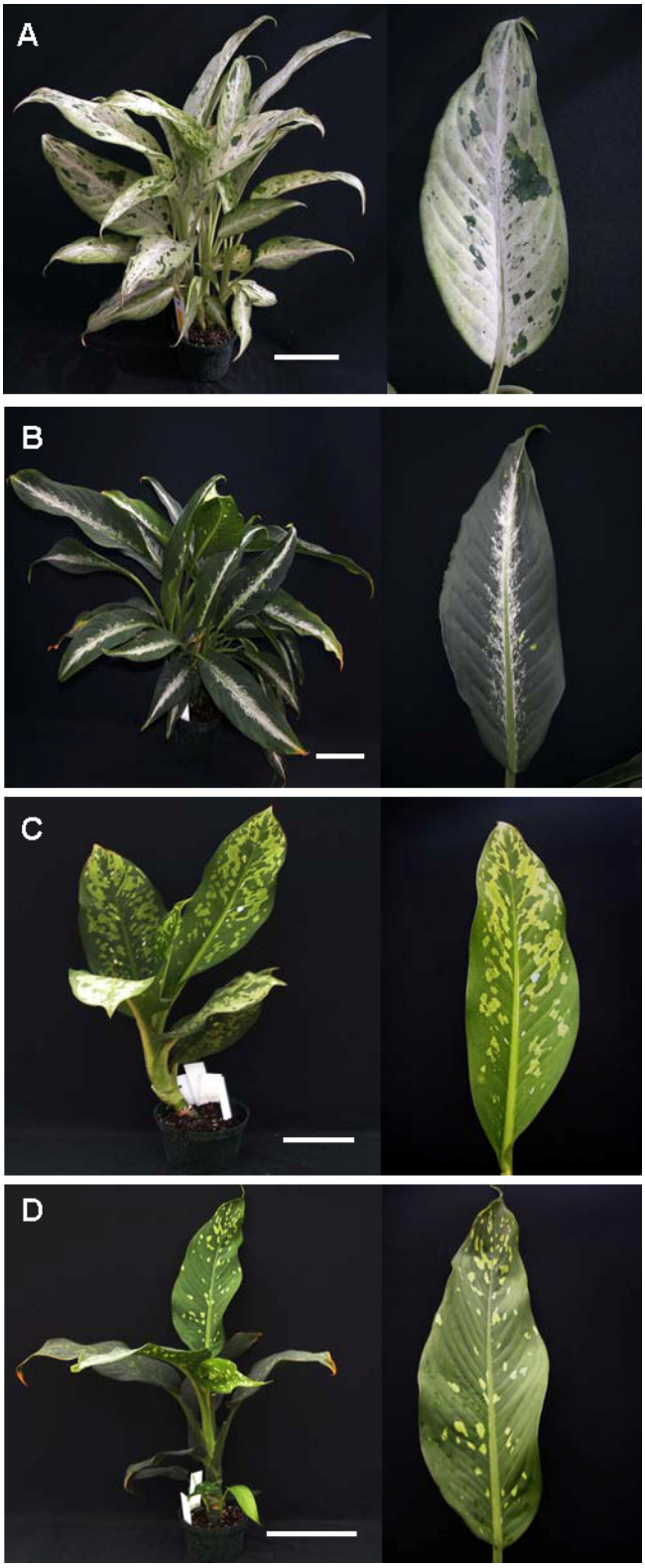

Fig. 4 *Dieffenbachia* cv. 'Camouflage' plants regenerated by indirect shoot organogenesis showing variation in leaf variegation and color. (**A**) Parental plant: creamy, camouflaged leaves with random green batches of different size. Bar = 1 cm. (**B**) SV1 (Somaclonal Variation): solid dark green leaves with whitish variegation along the midvein. Bar = 1 cm. (**C**) SV2: light green leaves with many yellowish spots, and connections among spots resulted in large yellowish blotches. Bar = 1 cm. D) SV3: green leaves with few scattered yellowish spots. Bar = 1 cm. (From **Shen X, Chen J, Kane ME** (2007b) Assessment of somaclonal variation in *Dieffenbachia* plants regenerated through indirect shoot organogenesis. *Plant Cell, Tissue and Organ Culture* **91**, 21-27, with kind permission of Springer Science + Business Media, ©2007).

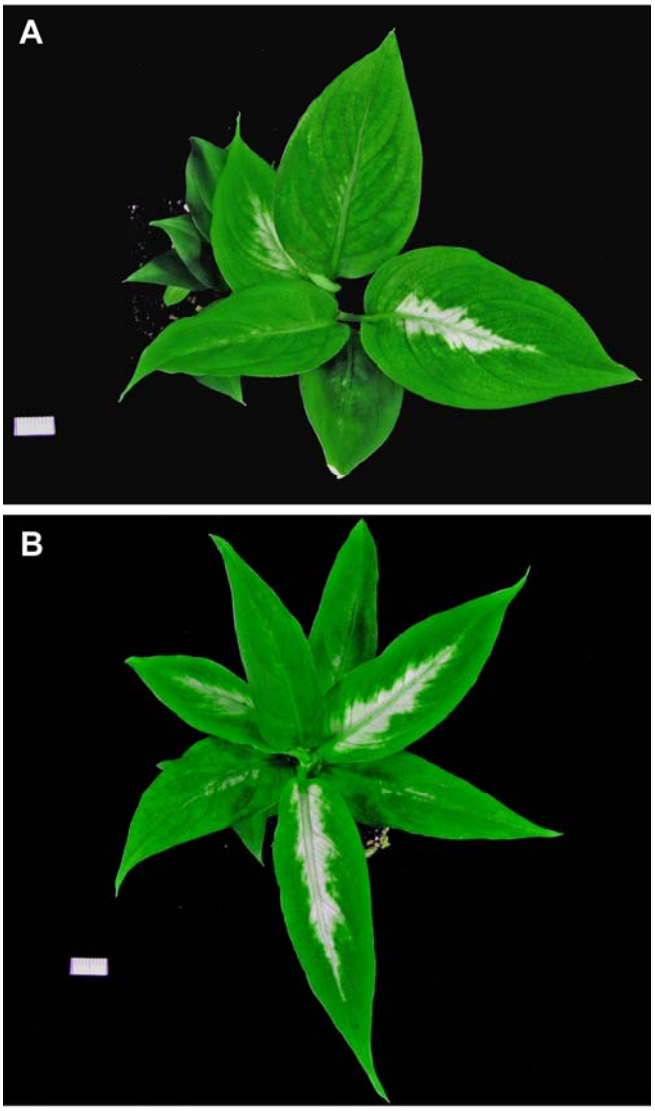

Fig. 5 *Dieffenbachia* cv. 'Camille' plants regenerated by indirect shoot organogenesis showing variation in leaf shape. (**A**) Parental type plants with oblong shaped leaves. (**B**) Somaclonal variants with lanceolate leaves. Bars = 1 cm. (From **Shen X, Chen J, Kane ME** (2007b) Assessment of somaclonal variation in *Dieffenbachia* plants regenerated through indirect shoot organogenesis. *Plant Cell, Tissue and Organ Culture* **91**, 21-27, with kind permission of Springer Science + Business Media, ©2007).

tivar via somaclonal variation compared to 7 to 10 years using traditional breeding techniques (Henny *et al*. 2000).

Polyploidy production

Polyploidy occurs naturally in some plant species and can also be induced *in vitro*. Since Murashige and Nakano (1966) first reported the successful induction of polyploidy in tobacco *in vitro*, it has been employed as a breeding tool to overcome sexual sterility (Von Aderkas and Anderson 1993). Production of tetraploids in *Dieffenbachia* cv. 'Star Bright' was reported by Holm (2007). Cultivar 'Star Bright M-1', a somaclonal variant of cv. 'Star Bright', was selected among the regenerated plants. Cultivar 'Star Bright M-1' is a desirable breeding parent for its unique leaf variegation pattern and bushy appearance, however, it is sterile. The establishment of *in vitro* culture of *Dieffenbachia* 'Star Bright M-1' was achieved by culturing shoot tips on MS medium supplemented with 2iP at 10 mg/l and IAA at 0.1 mg/l. Shoot cultures were subcultured at 6 week interval to regenerate sufficient plants for colchicine treatment. Four colchicine concentrations of 0, 250, 500 and 1000 mg/l were screened. Shoot clumps were soaked in colchicine solution

for 24 hours on a shaker, then transferred onto the same MS medium used for shoot culture. Following colchicine treatment, shoot clumps were subcultured at 6-week intervals. Regenerated shoots, longer than 2 cm, were removed from the shoot clumps and acclimatized to greenhouse condition for further evaluation. Among 422 surviving plants following colchicine treatment, 63 plants displayed visible traits of polyploidy. These polyploid plants were potential candidates as parents for breeding.

Ovule culture

In some species, especially in interspecific and intergeneric crosses, embryo abortion occurs at an early stage, and difficulties in excising embryo are encountered. Following fertilization ovules were excised and cultured on medium *in vitro* to obtain mature embryos, seeds or plantlets. This method has been successfully used in many species for breeding and propagation (Van Creij *et al*. 1999; Honda *et al*. 2003). *Dieffenbachia* cv. 'Tropic Star' is a hybrid generated from a cross of *Dieffenbachia maculata* cv. 'Perfection' and *Dieffenbachia maculata angustior lancifolia*. To overcome premature inflorescence abortion, ovules can be excised and cultured on MS basal medium free of plant growth regulators. Survival rates of cultured ovules were about 25%. *Dieffenbachia* cv. 'Tropic Star' was selected among the *in vitro* regenerated plants following evaluation under greenhouse conditions (pers. comm. Richard J Henny, University of Florida).

FUTURE PROSPECTS

Somatic embryogenesis has many advantages over shoot organogenesis. A higher multiplication rate can often be achieved in somatic embryogenesis than with shoot organogenesis. Somatic embryos, being bipolar, contain both shoot and root meristems and, as such, the rooting stage can be omitted which saves labor, time and production costs. Somatic embryogenesis, especially indirect somatic embryogenesis, is associated with higher rates of somaclonal variation which provides a means for new cultivar development (Kohlenbach 1985; Mooney and Van Staden 1987). Therefore, somatic embryogenesis can serve a dual purpose: for large scale of propagation and selection of somaclonal variants. Development of a somatic embryogenesis protocol is also prerequisite for somatic hybridization, synthetic seed production and genetic transformation (Rout *et al*. 1999). The establishment of a protocol for somatic embryogenesis is clearly a future goal for *Dieffenbachia* improvement. This possibly can be achieved by selecting responsive cultivars, explants, manipulating media and culture conditions.

Dieffenbachia is mainly propagated by vegetative means (cuttings and division) but only a limited number of propagules can be produced from each plant, even *in vitro*. Similarly, seed production is very poor and seed viability is very low. The application of synthetic seed technology, which involves processing, handling and delivery of somatic embryos as artificial seed, could represent a novel means to enhance *Dieffenbachia* propagation (Senaratna 1992; Standardi and Piccioni 1998). This method could also be used to propagate polyploids with elite traits.

Recent advancements in somatic hybridization offer a new means to produce hybrids within or between closely related species which otherwise would be impossible by sexual crosses due to various reproductive barriers. This method also provides a tool for producing polyploid plants (Waara and Glimelius 1995). New plant varieties derived from somatic hybridization have been produced in many other species which have been used for hybrid production or as parental lines in various breeding program (Grosser *et al*. 2000; Johnson and Veilleux 2001). Undoubtedly, somatic hybridization technology will also facilitate new cultivar development and broaden gene pool for breeding in *Dieffenbachia*.

Gene transfer technology (transgenics) has been shown to be a powerful tool for plant improvement including phenotypic and production traits of many species (Rego and Faria 2001). Given the ability to regenerate plants from callus via shoot organogenesis, there is no doubt that the application of genetic transformation techniques will prove beneficial to the genetic improvement of *Dieffenbachia* in the future.

REFERENCES

Benelli C, Fabbri A, Grassi S, Lambardi M (2001) Histology of somatic embryogenesis in mature tissue of olive (*Olea europaea* L.). *Journal of Horticultural Science and Biotechnology* **76**, 112-119

Birdsey MR (1951) *The Cultivated Aroids*, The Gillick Press, Berkeley, CA, 14 pp

Black RJ (2002) Florida 4-H horticulture identification and judging study manual: flowers and foliage plants. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, 24 pp

Bouman H, De Klerk G (1997) Somaclonal variation. In: Geneve RL, Preece JE, Merkle SA (Eds) *Biotechnology of Ornamental Plants*, CAB International, Wallingford, UK, pp 165-183

Bouman H, De Klerk G (2001) Measurement of the extent of somaclonal variation in begonia plants regenerated under various conditions, comparison of three assays. *Theoretical and Applied Genetics* **102**, 111-117

Budimir S (2003) Developmental histology of organogenic and embryogenic tissue in *Picea omorika* culture. *Biologia Plantarum* **47**, 467-470

Chase AR, Zettler FW, Knauss JF (1981) Perfection-137B: a pathogen-free selection of *Dieffenbachia Maculata* derived through tissue culture. In: *Circular S-280*, Florida Agricultural Experiment Stations, Institute of Food and Agricultural Sciences, University of Florida, 7 pp

Chen J, Henny RJ, Chao CCT (2003a) Somaclonal variation as a source for cultivar development of ornamental aroids. *Recent Research and Development in Plant Science* **1**, 31-43

Chen J, Henny RJ, Devanand PS, Chao CT (2006) AFLP analysis of nephthytis (*Syngonium podophyllum* Schott) selected from somaclonal variants. *Plant Cell Reports* **24**, 743-749

Chen J, McConnell DB, Henny RJ, Everitt KC (2003b) Cultural guidelines for commercial production of interiorscape *Dieffenbachia*. Institute of Food and Agricultural Sciences, University of Florida, *ENH880*, 5 pp

Choffe KL, Victor JMR, Murch SJ, Saxena PK (2000) *In vitro* regeneration of *Echinacea purpurea* L.: direct somatic embryogenesis and indirect shoot organogenesis in petiole culture. *In Vitro Cellular and Developmental Biology – Plant* **36**, 30-36

Debergh PC, Maene LJ (1981) A scheme for commercial propagation of ornamental plants by tissue culture. *Scientia Horticulturae* **14**, 335-345

De Schepper S, Debergh P, Van Bockstaele E, De Loose M, Gerats A, Depicker A (2003) Genetic and epigenetic aspects of somaclonal variation: flower color, bud sports in azalea, a case study. *South African Journal of Botany* **69**, 117-128

Greenwood MS (1987) Rejuvenation of forest trees. *Plant Growth Regulator* **6**, 1-12

Grosser JW, Ollitrault P, Olivares-Fuster O (2000) Somatic hybridization in citrus: an effective tool to facilitate variety improvement. *In Vitro Cellular and Developmental Biology – Plant* **36**, 434-449

Henny RJ (2006a) 'Sterling' *Dieffenbachia*. *HortScience* **41**, 1356

Henny RJ (2006b) 'Tropic Honey' *Dieffenbachia*. *HortScience* **42**, 398

Henny RJ (1995a) 'Sparkles' *Dieffenbachia. HortScience* **30**, 163

Henny RJ (1995b) 'Star Bright' *Dieffenbachia*. *HortScience* **30**, 164

Henny RJ (1988) Ornamental aroids: culture and breeding. *Horticultural Reviews* **10**, 1-33

Henny RJ (1986) Inheritance of foliar variegation in *Dieffenbachia maculata* 'Camille'. *The Journal of Heredity* **77**, 285-286

Henny RJ (1983) Inheritance of the white foliar midrib in *Dieffenbachia* and its linkage with gene for foliar variegation. *The Journal of Heredity* **74**, 483-484

Henny RJ (1982) Inheritance of foliar variegation in two *Dieffenbachia* cultivars. *The Journal of Heredity* **73**, 384

Henny RJ, Chen J (2003) Foliage plant cultivar development. *Plant Breeding Reviews* **23**, 245- 290

Henny RJ, Conover CA, Poole RT (1991a) 'Victory' a hybrid *Dieffenbachia* for foliage producers. *Circular S-378*, Florida Agricultural Experiment Stations, Institute of Food and Agricultural Sciences, University of Florida, 5 pp

Henny RJ, Conover CA, Poole RT (1988a) 'Tropic Star' *Dieffenbachia*. *HortScience* **23**, 919-920

Henny RJ, Conover CA, Poole RT (1987a) 'Triumph' *Dieffenbachia*. *HortScience* **22**, 965-966

Henny RJ, Conover CA, Poole RT (1987b) 'Victory' *Dieffenbachia*. *HortScience* **22**, 967-968

Henny RJ, Conover CA, Poole RT (1986) Triumph, a hybrid *Dieffenbachia* for foliage producers. *Circular S-334*, Florida Agricultural Experiment Stations, Institute of Food and Agricultural Sciences, University of Florida, 6 pp

Henny RJ, Goode L, Ellis W (2000) Micropropagation of *Dieffenbachia*. In: Trigiano RN, Gray DJ (Eds) *Plant Tissue Culture Concepts and Laboratory*

Exercises (2nd Edn), CRC Press, Boca Raton, pp 97-102

Henny RJ, Poole RT, Conover CA (1991b) Starry Night: a hybrid *Dieffenbachia* for foliage producers. *Circular S-377*, Agricultural Experiment Stations, Institute of Food and Agricultural Sciences, University of Florida, 2 pp

Henny RJ, Poole RT, Conover CA (1991c) Star White- a hybrid *Dieffenbachia* for foliage producers. *Circular S-379*, Florida Agricultural Experiment Stations, Institute of Food and Agricultural Sciences, University of Florida, 2 pp

Henny RJ, Poole RT, Conover CA (1988b) Tropic Star, a hybrid *Dieffenbachia* for foliage producers. *Circular S-349*, Florida Agricultural Experiment Stations, Institute of Food and Agricultural Sciences, University of Florida, 4 pp

Holm JR (2007) *In vitro* application of colchicine to induce tetraploids in *Dieffenbachia* x 'Star Bright M-1'. MSc thesis, University of Florida, 75 pp

Honda K, Watanabe H, Tsutsui K (2003) Use of ovule culture to cross between *Delphinium* species of different ploidy. *Euphytica* **129**, 275-279

Hu JB, Liu J, Yan HB, Xie CH (2005) Histological observations of morphogenesis in petiole derived callus of *Amorphophallus rivieri* Durieu *in vitro*. *Plant Cell Reports* **24**, 642-648

Johnson AAT, Veilleux RE (2001) Somatic hybridization and application in plant breeding. *Plant Breeding Reviews* **20**, 167-225

Kane ME (2000a) Culture indexing for bacterial and fungal contaminant. In: Trigiano RN, Gray DJ (Eds) *Plant Tissue Culture Concepts and Laboratory Exercise* (2nd Edn), CRC Press, Boca Raton, pp 427-431

Kane ME (2000b) Propagation from pre-existing meristems. In: Trigiano RN, Gray DJ (Eds) *Plant Tissue Culture Concepts and Laboratory Exercises* (2nd Edn), CRC Press, Boca Raton, pp 75-86

Kane ME, Gilman EF, Jenks MA (1991) Regenerative capacity of *Myriophyllum aquaticum* cultured *in vitro*. *Journal of Aquatic Plant Management* **29**, 102-109

Knauss JF (1976) A tissue culture method for producing *Dieffenbachia picta* cv. 'Perfection' free of fungi and bacteria. *Proceedings of the Florida State Horticultural Society* **89**, 293-296

Kohlenbach HW (1985) Fundamental and applied aspects of *in vitro* plant regeneration by somatic embryogenesis. In: Schafer-Menche A (Ed) *In Vitro Technique: Propagation and Long Term Storage*, Marfenus Adhoff, Boston, pp 101-109

Krishnaraj S, Vasil IK (1995) Somatic embryogenesis in herbaceous monocots. In: Thorpe TA (Ed) *In Vitro Embryogenesis in Plants*, Kluwer Academic Publishers, Dordrecht, the Netherlands, pp 155-203

Kunisaki JT (1977) Tissue culture of tropic ornamental plants. *HortScience* 12, 141-142

Larkin PJ, Scowcroft WR (1981) Somaclonal variation – a novel source of variability from cell cultures for plant improvement. *Theoretical and Applied Genetics* **60**, 197-214

Liu Y (2007) Which ornamental plant species effectively remove benzene from indoor air? *Atmospheric Environment* **41**, 650-654

Ma G, Xu W (2002) Induction of somatic embryogenesis and adventitious shoots from immature leaves of cassava. *Plant Cell, Tissue and Organ Culture* **70**, 281-288

McConnell DB, Henley RW, Kelly CB (1989) Commercial foliage plants: twenty years of changes. *Proceedings of the Florida State Horticultural Society* **102**, 297-303

Merkle SA (1997) Somatic embryogenesis in ornamentals. In: Geneve JE, Preece JE, Merkle SA (Eds) *Biotechnology of Ornamental Plants*, CAB, International, pp 13-33

Merkle SA, Parrott WA, Flinn BS (1995) Morphogenic aspects of somatic embryogenesis. In: Thorpe TA (Ed) *In Vitro Embryogenesis in Plants*, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 155-203

Merkle SA, Parrott WA, Williams EG (1990) Application of somatic embryogenesis and embryo cloning. In: Bhojwani SS (Ed) *Plant Tissue Culture: Application and Limitations*, Elsevier Science Publishers B. V. Amsterdam, the Netherlands, pp 67-101

Merkle SA, Sommer HE (1986) Somatic embryogenesis in tissue cultures of *Liriodendron tulipifera*. *Canadian Journal of Forest Research* **16**, 420-422

Merkle SA, Wiecko AT (1989) Regeneration of *Robinia psuedoacacia* via somatic embryogenesis. *Canadian Journal of Forest Research* **19**, 285-288

Mithila J, Hall JC, Victor JMR, Saxena PK (2003) Thidiazuron induces shoot organogenesis at low concentrations and somatic embryogenesis at high concentrations on leaf and petiole explants of African violet (*Saintpaulia ionantha* Wendl.). *Plant Cell Reports* **21**, 408-414

Mooney PA, Van Staden J (1987) Induction of embryogenesis in callus from immature embryos of *Persea american*. *Canadian Journal of Botany* **65**, 622-626

More VN, Khalatkar AS (1988) Effect of gibberellic acid, kinetin and indolebutyric acid on propagation in *Dieffenbachia picta*. *Acta Horticulturae* **226**, 473-478

Murashige T, Nakano R (1966) Tissue culture as a potential tool in obtaining polyploidy plant. *Journal of Heredity* **57**, 114-118

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarium* **15**, 473-497

Onay A (2000) Histology of somatic embryogenesis in cultured leaf explants of pistachio (*Pistacia vera* L). *Turkish Journal of Botany* **24**, 91-95

Orlikowska T, Sabala I, Nowak E (1995) Adventitious shoot regeneration on explants of *Anthurium*, *Cadiaeum*, *Dieffenbachia*, *Gerbera*, *Rosa* and *Spathiphyllum* for breeding purpose. *Acta Horticulturae* **420**, 115-117

Orton TJ (1985) Genetic instability during embryogenic cloning of celery. *Plant Cell, Tissue and Organ Culture* **4**, 159-169

Quiroz-Figueroa FR, Fuentes-Cerda CFJ, Rojas-Herrera R, Loyola-Vargas VM (2002) Histological studies on the developmental stages and differentiation of two different somatic embryogenesis systems of *Coffea arabica*. *Plant Cell Reports* **20**, 1141-1149

Rego LDV, Faria RTD (2001) Tissue culture in ornamental plant breeding: A review. *Crop Breeding and Applied Biotechnology* **3**, 283-300

Rout GR, Samantaray S, Mottley J, Das P (1999) Biotechnology of rose: a review of recent progress. *Scientia Horticulturae* **81**, 201-228

Rugini E, Muganu M (1998) A novel strategy for the induction and maintenance of shoot regeneration from callus derived from established shoots of apple (*Malus* × *Domestica* Borkh.) cv. Golden Delicious. *Plant Cell Reports* **17**, 581-585

Senaratna T (1992) Artificial seeds. *Biotechnology Advances* **10**, 379-392

Shen X, Chen J, Kane ME (2007a) Indirect shoot organogenesis from leaves of *Dieffenbachia* cv. Camouflage. *Plant Cell, Tissue and Organ Culture* **89**, 83-90

Shen X, Chen J, Kane ME (2007b) Assessment of somaclonal variation in *Dieffenbachia* plants regenerated through indirect shoot organogenesis. *Plant Cell, Tissue and Organ Culture* **91**, 21-27

Shen X, Kane ME, Chen J (2008) Effects of genotypes, explant source, and plant growth regulators on indirect shoot organogenesis in *Dieffenbachia* cultivars. *In Vitro Cellular and Developmental Biology – Plants* **44**, 282-288

Skirvin RM, McPheeters KD, Norton M (1994) Sources and frequency of somaclonal variation. *HortScience* **29**, 1232-1236

Standardi A, Piccioni E (1998) Recent perspective on synthetic seed technology using nonembryogenic *in vitro*-derived explants. *International Journal of Plant Science* **159**, 968-978

Steward FC, Mapes MO, Mears K (1958a) Growth and organized development of cultured cells. II. Organization in culture grown from freely suspended cells. *American Journal of Botany* **45**, 705-708

Steward FC, Mapes MO, Smith J (1958b) Growth and organized development of cultured cells. I. Growth and division of freely suspended cells. *American Journal of Botany* **45**, 693-703

Tisserat B, Esan EB, Murashige T (1979) Somatic embryogenesis in angiosperms. *Horticultural Reviews* **1**, 1-78

USDA (United States Department of Agriculture) (1999) 1998 census of horticultural specialties. United States Department of Agriculture, Washington, D.C.

van Creij MGM, Kerckhoffs DMFJ, Van Tuyl JM (1999) The effect of ovule age on ovary- slice culture and ovule culture in intraspecific and interspecific crosses with *Tulipa gesneriana* L. *Euphytica* **108**, 21-28

Von Aderkas P, Anderson P (1993) Aneuploidy and polyploidization in haploid tissue cultures of *Larix decidua*. *Physiologia Plantarium* **88**, 73-77

Von Arnold S, Sabala I, Bozhkov P, Dyachok J, Filonova L (2002) Developmental pathways of somatic embryogenesis. *Plant Cell, Tissue and Organ Culture* **69**, 233-249

Voyiatzi C, Voyiatzis DG (1989) *In vitro* shoot proliferation rate of *Dieffenbachia exotica* cultivar 'Marianna' as affected by cytokinins, the number of recultures and the temperature. *Scientia Horticulturae* **40**, 163-169

Waara S, Glimelius K (1995) The potential of somatic hybridization in crop breeding. *Euphytica* **85**, 217-233

Webster CA, Jones OP (1989) Micropropagation of the apple rootstock M9: effect of sustained subculture on apparent rejuvenation *in vitro*. *Journal of Horticulture Science* **64**, 421-428