In Vitro Induction of Tetraploids in *Dieffenbachia* × ‘Star Bright M-1’ by Colchicine

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Abstract. Colchicine application successfully induced tetraploids from in vitro-cultured diploid *Dieffenbachia* × ‘Star Bright M-1’. Shoot clumps, each with six to eight small, undifferentiated shoot primordia, were cultured in liquid Murashige and Skoog (MS) medium and treated with colchicine at rates of 0, 250, 500, or 1000 mg·L⁻¹ for 24 h. In vitro survival of shoot clumps significantly decreased as colchicine concentrations increased. Shoot clumps that survived were transferred to colchicine-free MS medium containing 2.0 mg·L⁻¹ N⁶-isopentenyl adenine and 0.10 mg·L⁻¹ indole-3-acetic acid. Shoots were harvested during four subsequent subcultures and planted in a soilless substrate in a shaded greenhouse. The number of plants that survived 6 months after ex vitro planting was 690, 204, 59, and 69 for colchicine treatments at 0, 250, 500, and 1000 mg·L⁻¹, respectively. The 332 plants from colchicine treatments along with 90 control plants (selected from 690 in the control treatment) were evaluated morphologically in a shaded greenhouse. Overall plant growth, including crown height, plant canopy, and leaf size, of colchicine-treated plants was significantly less than controls. Based on the growth data, 10, 32, 15, and 16 plants from the 0, 250, 500, and 1000 mg·L⁻¹ colchicine rates, respectively, were selected and analyzed by flow cytometry. Flow cytometry confirmed the presence of 13 tetraploids and 29 mixoploids among the 63 colchicine-treated selections; all 10 plants from the control were diploid. A colchicine rate of 500 mg·L⁻¹ produced a higher percentage of tetraploids (10.2%) than did the 250 (2.9%) or 1000 mg·L⁻¹ (1.4%) rates. Subsequent comparisons showed tetraploids had significantly smaller and thicker leaves, greater specific leaf weights, and longer stomata than diploids. Tetraploids also showed increased net photosynthetic rate, decreased g_s, decreased intercellular CO₂ concentration, decreased transpiration rate, and increased water use efficiency. Tetraploids appeared robust and their smaller size could make them potentially more durable plants used as living specimens for interior decoration.

Cultivars of the aroid genus *Dieffenbachia* are valued as ornamental plants for their attractive foliage, ease of production, and their durability as living specimens for interior decoration. Since 1980, with the control of flowering and pollination techniques, many commercial *Dieffenbachia* cultivars have resulted from breeding programs that select for both aesthetics and tolerance of abiotic and biotic stresses (Henny, 2000). To facilitate commercial production, tissue culture methods have been used as a tool for fast and reliable increase of hybridized *Dieffenbachia* selections.

At least 80 commercial foliage plant cultivars have originated from somaclonal

variation in tissue culture propagation (Chen and Henny, 2008). *Dieffenbachia* × ‘Star Bright M-1’ is a somaclonal variant of a commercial cultivar *D.* × ‘Star Bright’ (U.S. patent PP9051; Henny, 1995). The M-1 variant was selected out of a population of tissue culture-derived plants because its shorter internodes gave a more compact appearance and the lower leaves were wider than the parent cultivar. In addition, it showed improved adaptability to interior low-light conditions because older leaves were held longer on the plant. A strategy for enhancing plant adaptability to stressful environments is chromosome doubling (Levin, 1983). Gene redundancy leads to genome buffering by increasing allelic diversity (Udall and Wendel, 2006), thus increasing plant tolerance to environmental stress. Polyploid plants can be more robust, have thicker leaves, larger fruit, a greater degree of drought and disease tolerance, improved adaptability, and resistance to environmental stress (Chakraborti et al., 1998; Eeckhaut et al., 2004). Additionally, chromosome doubling may provide an opportunity for novel

phenotypic variation resulting from gene duplications (Udall and Wendel, 2006). Thus, an approach to further enhance the adaptability of the M-1 variant to interior low-light conditions could be chromosome doubling. Colchicine is the most widely used chemical agent for chromosome doubling. Tetraploids at frequencies of 83.3% and 80.0% were induced in *Xanthosoma sagittifolium* when in vitro-grown plants were treated with 1.25 mM or 2.5 mM of colchicine, respectively (Tambong et al., 1998). Colchicine has been reported to induce tetraploidy in nine *Zantedeschia* cultivars (Cohen and Yao, 1996). Rapidly multiplying in vitro shoot cultures were exposed to 0.05% (w/v) colchicine on solid Murashige and Skoog (MS) media for 1, 2, or 4 d resulting in a recovery of tetraploids ranging from 12.9% to 41.8%. Colchicine along with oryzalin and trifluralin also successfully induced tetraploids of *Alocasia micholitziana* ‘Green Velvet’ (Thao et al., 2003) and *Spathiphyllum wallisii* Regal (Eeckhaut et al., 2004). However, chemical induction of polyploidy has not been reported in *Dieffenbachia*.

The objectives of this study were to use colchicine to induce tetraploids of *Dieffenbachia* × ‘Star Bright M-1’ in vitro and determine if chemically induced tetraploids were stable and showed better adaptability to low-light conditions for interiorscaping.

Materials and Methods

In vitro culture establishment. Shoot tips, 10 to 15 mm in length, were dissected from *Dieffenbachia* × ‘Star Bright M-1’ grown in a shaded greenhouse at the University of Florida, Mid-Florida Research and Education Center in Apopka, FL. Lateral buds from the shoots were excised, sterilized, and placed onto culture media using established procedures (Knauss, 1976). Explants were placed aseptically on a culture medium consisting of MS salts (Murashige and Skoog, 1962) supplemented with Gamborg B₅ vitamins (Gamborg et al., 1968), 30 g·L⁻¹ sucrose, 49.2 μmol·L⁻¹ N⁶-(Δ²-isopentenyl) adenine (2iP), 5.7 μmol indole-3-acetic acid (IAA), and 8 g·L⁻¹ of tissue culture-grade agar. Explants were placed in a growth room equipped with cool-white fluorescent lamps under a 16-h light photoperiod at 40 μmol·m⁻²·s⁻¹ and a constant temperature of 26 ± 2 °C and were subcultured every 6 weeks. At the fifth subculture, proliferating explant tissue was transferred to 177-mL baby food jar culture vessels fitted with Magenta® B-Cap closures (Magenta Corp., Chicago, IL). A total of eight subcultures were performed, which resulted in more than 120 shoot clumps. Shoots from the clumps over 2 cm in length were excised and discarded. The resultant clumps had six to eight small, undifferentiated shoot primordia, which were subjected to colchicine treatments.

Colchicine application. Colchicine solutions of 0, 250, 500, and 1000 mg·L⁻¹ were made by dilutions of a 10 g·L⁻¹ colchicine stock solution with liquid MS medium

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containing full-strength MS salts, 30 g·L⁻¹ sucrose, and no growth regulators. Forty empty baby food jars were filled with 50 mL of these solutions, 10 jars per treatment, autoclaved for 30 min at 15 psi, and 121 °C. The 120 shoot clumps were aseptically transferred into the jars containing 0, 250, 500, and 1000 mg·L⁻¹ colchicine/MS liquid medium, three clumps per jar, and placed on a shaker at 80 rpm for 24 h. Shoot clumps then were removed aseptically from the colchicine/MS treatments, rinsed in autoclaved, deionized water, and then placed onto a colchicine-free medium containing MS salts, 9.8 µmol 2iP, 0.11 µmol IAA, 30 g·L⁻¹ sucrose, Gamborg B₅ vitamins, and 8 g·L⁻¹ tissue culture-grade agar and cultured under a 16-h light photoperiod at 40 µmol·m⁻²·s⁻¹ provided by cool-white fluorescent lamps. The cultures were transferred to fresh medium at 6-week intervals. At the time of the second and third transfers, all developed shoots exceeding 2 cm in length were harvested, counted, removed from culture vessels, and transferred to a shaded greenhouse. In vitro survival was determined 12 weeks after colchicine treatment by counting the number of surviving shoot clumps. At 26 weeks after colchicine treatment, all developed shoots were harvested, counted, and planted. Because the number of control plants exceeded a manageable amount, 90 control plants were randomly selected for transfer to the greenhouse (three plants from each of 10 controls for each of the three harvest dates).

Ex vitro transfer. Shoots were transferred into 288-celled trays containing Vergro Container Mix A (Verlite Co. Inc., Tampa, FL). The components of the mix were 2:1:1 (v/v) of Canadian peat:vermiculite:perlite. After acclimatization for 2 weeks under a light intensity of 160 to 200 µmol·m⁻²·s⁻¹, plants were moved to a light intensity of 240 to 300 µmol·m⁻²·s⁻¹. Thereafter, plants were fertilized weekly with Peter's 20:20:20 (N:P₂O₅:K₂O; The Scotts Co., Marysville, OH) at 250 mg·L⁻¹ nitrogen and hand-watered as needed. After 17 weeks in the shaded greenhouse, plants were repotted into 50-celled trays and at 26 weeks, they were stepped up to 12.5-cm pots for an additional 26 weeks. After the 52 weeks in the shaded greenhouse, plants had developed eight to 10 mature leaves and were subjected to morphological analysis. Ex vitro survival was determined 26 weeks after transfer to the shaded greenhouse by calculating the number of surviving plants per treatment. A total of 422 plants (332 colchicine-treated and 90 controls) survived ex vitro and were grown for analysis.

Morphological characterization. Two separate morphological characterizations were made on plants after growing for 1 year in the shaded greenhouse. The first included all 332 surviving plants from colchicine treatments and 90 from control plants. Measurements included primary stem height (centimeters), canopy height (centimeters), canopy width (centimeters), growth index (meters cubed), largest leaf length (centimeters), and largest

leaf width (centimeters). Primary stem height was the length of the primary stem from the soil line to the point at where newly emerging leaves form an apex (crown) at the top of the plant. Canopy height was determined by an approximate measurement from the base of the plant to a point where the upper most leaves formed an arch and began to curve downward. Canopy width was recorded as an average of two horizontal lengths (≈90° from one another) across the canopy of the plants. Growth index (meters cubed) was calculated by the multiplying the product of two canopy widths by canopy height.

The second morphological data included 63 colchicine-treated plants (32, 15, and 16 from 250, 500, and 1000 mg·L⁻¹ colchicine treatments, respectively) and 10 plants from the control. Compared with control plants, these 63 treated plants were shorter, had thicker leaves and stems, and the nonvariegated leaf areas were darker green. In addition, the leaves had a hard leathery feel, and the foliar variegation across the entire leaf blade was less defined and appeared diffuse. In addition to the aforementioned morphological evaluation, the 73 plants were evaluated by measuring stomata length and then analyzed by flow cytometry. To measure stomata length, stomata of plants were "fixed" onto a glass slide using an epidermal peel. One drop of Super Glue[®] (Pacer Technology, Rancho Cucamonga, CA) was placed on a 75 × 25-mm slide, pressed against the underside of a leaf, held for 30 s, and then removed to give a permanent impression of the epidermis from the underside of the leaf. Under a light microscope at 400× magnification, stomata were measured in micrometers by placing the scale end to end along the lengths of the guard cells in the longitudinal direction. Ten stomata per leaf were measured.

Flow cytometry. The 73 plants used for stomata measurements were subsequently examined for ploidy levels using a PARTEC PA flow cytometer (Partec GmbH, Münster, Germany). Leaf squares, 5 × 5 mm, were cut from edges of newly matured leaves, placed in a 55-mm diameter plastic petri dish along with 0.4 mL nuclei extraction buffer (Cystain ultraviolet Precise; Partec GmbH), and chopped using a razor blade into ≈100 pieces. The contents of the petri dish were then filtered (pore size = 50 µm) into a 3.5-mL test tube to which 1.6 mL of staining buffer (Cystain ultraviolet Precise) was added. The flow cytometer's gain value was set at 363.0 and speed was set at 0.40 µL·s⁻¹. A minimum of 2000 nuclei was analyzed per sample. The standard peak of a known diploid *Dieffenbachia* was calibrated to appear at approximately 100 of fluorescence intensity (channel number).

Evaluation of tetraploids versus diploids. Leaf morphology and photosynthetic parameters of 13 identified tetraploids were evaluated in the shaded greenhouse against 16 diploid control plants. Leaf morphological characterization included leaf width to length ratio, leaf area, leaf thickness, leaf midrib

thickness, and leaf-specific weight. Using an average of three leaves and three observations in each case, leaf area was measured using a Li-3100 area meter (LI-COR, Inc., Lincoln, NE). Leaf thickness was measured at points along the flat side of the leaf blade in the approximate center to the right or left of the midrib. Midrib thickness was measured by the thickness of the midrib when a leaf is cut at a 90° angle to the midrib and a micrometer is placed on the edge of the cut midrib. Leaf-specific weight was measured as the fresh weight per unit area.

Net photosynthesis (*P_n*), *g_s*, intercellular CO₂ concentration, and transpiration (*E*) of three recent fully expanded leaves of 2× and 4× plants were determined with an LI-6400 portable photosynthesis system (LI-COR BioSciences, Inc., Lincoln, NE) between 1000 and 1200 hr in the shaded greenhouse. During such measurements, the CO₂ concentration of reference varied from 360 to 370 µmol CO₂/mol (air) within the leaf chamber, and temperature ranged from 26 to 28 °C. Ten readings were taken per leaf. Water-use efficiency (WUE) was calculated as *P_n/E*.

Experimental design and statistical analysis. In vitro survival rates were analyzed based on four colchicine treatment levels and 10 baby food jar replications per treatment. Morphological, stomatal, flow cytometry, and photosynthetic data were analyzed using the statistical program MINITAB Release 14 (2005; Minitab Inc., State College, PA). Means that were significant were separated by Fisher's protected least significant difference.

Results

Colchicine effects on shoot cultures. Colchicine effects were apparent between 12 and 26 weeks after treatment (Fig. 1). Shoot clumps not exposed to colchicine grew rapidly with a mean survival rate of 93.3%, whereas mean survival rates of shoot clumps treated by 250, 500, and 1000 mg·L⁻¹ colchicine were 63.3%, 30.0%, and 23.3%, respectively (Table 1). Number of shoots produced from control clumps was 690 compared with 306, 73, and 88 from colchicine-treated at 250, 500, and 1000 mg·L⁻¹, respectively (Table 1). In the greenhouse, survival rates of ex vitro transfer of shoots resulted from colchicine treatments of 0, 250, 500, and 1000 mg·L⁻¹ were 100.0%, 66.4%, 80.2%, and 80.4%, respectively.

Morphological changes. In all morphological observations (Table 2), control plants were significantly larger than plants treated with colchicine. Primary stem height, canopy height, and width of control plants were the greatest values, whereas plants exposed to 1000 mg·L⁻¹ colchicine yielded the smallest values. Similarly, controls had the greatest growth index value, largest leaf length, and largest leaf width. Plants exposed to 1000 mg·L⁻¹ colchicine had the smallest growth index value in growth index, leaf length, and leaf width (Table 2). Statistically, however, there were no significant differences in these

Fig. 1. Shoots of *Dieffenbachia* × 'Star Bright M-1' produced 26 weeks after in vitro treatment with colchicine at 0, 250, 500, or 1000 mg·L⁻¹ for 24 h in liquid Murashige and Skoog medium in which A = 0, B = 250, C = 500, and D = 1000 mg·L⁻¹.

Table 1. In vitro percent survival of tissue cultured *Dieffenbachia* × 'StarBright M-1' shoot clumps 12 weeks after treatment with four rates of colchicine in vitro and total number and mean percent survival of shoots harvested 6 months after transfer to the shaded greenhouse.

Colchicine (mg·L ⁻¹)	Total no. of clumps survived	Percent clump survival at 12 wk	Total no. of shoots harvested	Total no. of shoots survived ex vitro	Mean percent survival ex vitro
0	28	93.3 a ^z	690	690	100.0 a
250	19	63.3 b	306	204	66.4 b
500	9	30.0 c	73	59	80.2 b
1,000	7	23.3 c	88	69	80.4 b

^zValues followed by the same letter are not significantly different using Fisher's least significant difference at $P \leq 0.05$.

Table 2. Morphological characteristics of 422 *Dieffenbachia* × 'Star Bright M-1' plants 18 months after treatment with four rates of colchicine in vitro or 12 months after transfer to the shaded greenhouse.

Colchicine (mg·L ⁻¹)	No. of plants	Primary stem ht (cm)	Canopy ht (cm)	Canopy width (cm)	Growth index (m ³)	Largest leaf length (cm)	Largest leaf width (cm)
0	90	13.8 a ^z	30.6 a	30.0 a	918.0 a	22.4 a	6.0 a
250	204	10.5 b	24.0 b	24.1 b	578.4 b	18.0 b	5.3 b
500	59	9.2 c	21.9 c	22.6 bc	494.9 bc	16.9 bc	5.0 bc
1,000	69	8.6 c	20.1 c	21.0 c	422.1 c	15.3 c	4.6 c

^zValues followed by the same letter are not significantly different using Fisher's least significant difference at $P \leq 0.05$.

Table 3. Morphological characteristics of *Dieffenbachia* × 'Star Bright M-1' selected based on visual indicators of polyploidy 18 months after treatment with four rates of colchicine in vitro and 12 months after transfer to the greenhouse.

Colchicine (mg·L ⁻¹)	No. of plants	Crown ht (cm)	Canopy ht (cm)	Canopy mean width (cm)	Growth index (m ³)	Largest leaf length (cm)	Largest leaf width (cm)
0	10	13.4 a ^z	29.9 a	29.2 a	873.1 a	22.1 a	5.7 a
250	32	7.5 b	17.9 b	19.5 b	349.1 b	13.8 b	4.1 b
500	15	6.5 bc	16.1 bc	17.9 b	288.2 bc	12.8 bc	4.1 b
1,000	16	6.0 c	14.7 c	17.5 b	257.3 c	11.6 c	3.8 b

^zValues followed by the same letter are not significantly different using Fisher's least significant difference at $P \leq 0.05$.

three indices between plants treated by 500 or 1000 mg·L⁻¹ colchicine.

A second morphological evaluation of these 63 plants, plus 10 randomly selected control plants, substantiated the effects of colchicine concentration on morphology (Table 3). Control plants had significantly greater values in crown height, canopy height and width, growth index, largest leaf length, and width than treated plants. Overall, the assessment of morphological traits confirmed that increasing colchicine concentration significantly affected morphology of *Dieffenbachia* × 'Star Bright M-1', which might indicate polyploidy among treated plants.

Stomata observation. Stomata observation showed that mean stomata length of control plants was 37.8 μm. Mean stomata lengths of the 63 colchicine-treated plants was 40.2 μm but varied from 38.3 to 47.0 μm.

Flow cytometry analysis. Flow cytometry screening of these 73 plants confirmed that 21 treated and 10 control plants were diploid, whereas 13 treated plants were tetraploid and 29 were mixoploid. Histograms with excitation peaks of florescent intensity at channel 100 corresponded to diploids (Fig. 2A) and channel 200 corresponded to tetraploids (Fig. 2B). Histograms with excitation peaks at florescent intensity of both channels 100 and 200 corresponded to mixoploids (Fig. 2C). The number and percentage per treatment of diploids, tetraploids, and mixoploids are represented in Table 4. Colchicine at 500 mg·L⁻¹ produced more tetraploids by percent (40.0%) than all other treatment levels. Colchicine at 1000 mg·L⁻¹ produced more mixoploids by percent (75.0%). These data suggest that a colchicine concentration of 500 mg·L⁻¹ is the optimum concentration under the conditions of this experiment for induction of tetraploidy in *Dieffenbachia* × 'Star Bright M-1'.

Evaluation of tetraploid versus diploid leaves. All leaf morphological comparisons of tetraploids to diploids were significantly different except for the midrib thickness (Table 5; Fig. 3). Leaves of tetraploids were broader than diploids. Leaf area of tetraploids was 53% smaller than diploids. Leaves of tetraploids averaged 83% thicker than diploids and felt sturdier than leaves of the diploids. Leaf-specific weight of tetraploids was 53% greater than diploids.

Photosynthetic comparison showed that tetraploids had significantly higher net photosynthetic rate but lower g_s , intercellular CO₂ concentration, and transpiration rate than diploids (Table 6). As a result, WUE of tetraploids was 83.4% higher than the diploids.

Discussion

This study showed that tetraploids could be induced by treating rapidly growing in vitro shoot cultures of *Dieffenbachia* × 'Star Bright M-1' with colchicine at concentrations of 250, 500, or 1000 mg·L⁻¹. Treatment of cultures at 500 mg·L⁻¹ colchicine produced more tetraploids (10.2%) than did all other

Fig. 2. Flow cytometry histogram of a diploid, tetraploid, and mixoploid plant from tissue cultured *Dieffenbachia* × 'Star Bright M-1' 18 months after treatment with 500 mg·L⁻¹ colchicine in vitro and 12 months after transfer to the greenhouse. The diploid control plant (A), induced tetraploid (B), and mixoploid (C).

treatment levels. High colchicine concentrations increased the proportion of mixoploids in *Alocasia* (Thao et al., 2003). Similarly, higher levels of colchicine tended to produce mixoploids in *Morus alba* (Chakraborti et al., 1998). However, these authors showed that

low concentrations of colchicine decreased the efficiency of converting diploid plants to tetraploids. In this study, both 250 and 1000 mg·L⁻¹ treatments produced higher percentages of mixoploids than 500 mg·L⁻¹ treatment (Table 4).

Colchicine concentrations at 500 mg·L⁻¹ or 1000 mg·L⁻¹ significantly reduced in vitro survival compared with 250 mg·L⁻¹. Similarly, colchicine reduced ex vitro survival at all treatment rates compared with untreated plants. Although the significance of ex vitro survival affected by colchicine concentration was low, any decrease in survival of colchicine-treated shoots over nontreated controls is likely the result of a carryover effect of colchicine ex vitro as seen in *Pyrus pyrifolia* (Kadota and Niimi, 2002). Some clumps exposed to 250 mg·L⁻¹ colchicine showed growth retardation and a small amount of necrosis. The percent of treated clumps that died was 36.7%, 70.0%, and 76.7% at 250, 500 and 1000 mg·L⁻¹, respectively. In *Dieffenbachia*, there appears to be a threshold level between 500 and 1000 mg·L⁻¹ rates as the percent of dead clumps leveled off. This contrasts with *Alocasia*, in which colchicine was lethal at concentrations greater than 500 mg·L⁻¹ (Thao et al., 2003). In future experimentation, improved success of colchicine-induced polyploidization and survival in vitro could be facilitated by more frequent transfer of explants to fresh media as observed in *Buddleia globosa* (Rose et al., 2000) or shorter exposure times and smaller dosages as seen in *Spathiphyllum* (Eeckhaut et al., 2004). Nevertheless, this study showed that a 24-h exposure to 500 mg·L⁻¹ colchicine was the optimum in vitro treatment method and concentration range of colchicine for inducing polyploidization of *Dieffenbachia* hybrids.

This study also suggests that morphological data alone is insufficient to confirm the presence of polyploids but can be valuable tool to select candidates for flow cytometry analysis. The tetraploid plants obtained from all colchicine treatment levels had similarities in plant height, plant width, and leaf shape. Stomata length comparison between tetraploid plants from colchicine treatment and control plants indicated that diploid plants had an average stomata length of 37.5 μm, whereas 13 colchicine-treated plants had a stomata length of 47.0 μm. Stomata size is another source of data that can be used as a tool to prescreen for polyploids. This is in agreement with results from *Xanthosoma* (Tambong et al., 1998), *Alocasia* (Thao et al., 2003), and *Alstromeria* (Lu and Bridgen, 1997) that stomata in polyploids were found to be significantly larger than diploids.

Flow cytometry is a valuable tool in confirming ploidy level of the 63 plants. This method is simple and convenient; more than 20 leaf samples can be run in 1 h to confirm the ploidy levels with *Dieffenbachia*. However, as a result of the expense of running large numbers of samples, reducing sample size based on morphology can significantly lessen the cost of screening using flow cytometry and increase the chance of identifying tetraploids. In this study, 66.7% of plants selected based on morphological screening had a ploidy change (either tetraploid or mixoploid) compared with 12.7%

Table 4. Ploidy level of 63 *Dieffenbachia* × ‘Star Bright M-1’ plants initially selected as potential polyploids based on morphological traits 18 months after treatment with four rates of colchicine in vitro.^z

Colchicine (mg·L ⁻¹)	No. selected for flow cytometry ^y	Ploidy levels determined by flow cytometry					
		2×		4×		2× + 4×	
		No.	Percent	No.	Percent	No.	Percent
0	10	10	100.0	0	0.0	0	0.0
250	32	13	40.6	6	18.8	13	40.6
500	15	5	33.3	6	40.0	4	26.7
1,000	16	3	18.8	1	6.3	12	75.0
Total	73	31	42.5	13	17.8	29	39.7

^zPlants had grown for 12 months after transfer to the greenhouse.

^yTen plants were randomly selected from control treatment and the rest of plants were selected based on visual characteristics of polyploidy, i.e., thicker leaves, deeper green color, and slower growth rate.

Table 5. Morphological comparison of tetraploids to diploids of *Dieffenbachia* × ‘Star Bright M-1’ at 20 months after treatment with four rates of colchicine in vitro or 14 months after transfer to the greenhouse.^z

Ploidy	No. of plants	Leaf width: length ratio	Leaf area (cm ²)	Leaf thickness (mm)	Leaf midrib thickness (mm)	Specific wt (mg·cm ⁻²)	Stomate length
2×	16	0.2 a ^z	115.0 a	0.3 a	2.1 a	36.7 a	37.5 a
4×	13	0.3 b	67.4 b	0.6 b	2.3 a	56.3 b	47.0 b

^zPlants examined were selected based on flow cytometry results.

^yValues followed by the same letter are not significantly different using Fisher’s least significant difference at $P \leq 0.05$.

Fig. 3. An 18-month-old diploid (left) and tetraploid (right) *Dieffenbachia* × ‘Star Bright M-1’.

for the entire population. It is generally agreed that polyploids have larger cell size, thus larger leaves, fruits, and overall larger plant forms (Levin, 1983; Sparnaaij, 1979). Characterization of the identified *Dieffenbachia* tetraploids, however, showed that tetraploids were miniaturized (Table 6; Fig. 3), exhibited smaller leaf area, thicker and more leathery leaves, and increased specific leaf-specific weight. It is unclear why this exception occurred in the colchicine-induced tetraploids of *Dieffenbachia* × ‘Star Bright M-1’. On the other hand, these altered mor-

phological characteristics might suggest that tetraploids could be more robust and could be more tolerant to stressful environments. Photosynthetic evaluation showed that tetraploids had higher net photosynthetic rate, lower stomata conductance, intercellular CO₂ concentration, and transpiration rate. As a result, WUE of tetraploids was 83.4% higher than the diploids. The higher net photosynthesis is another exception in *Dieffenbachia* × ‘Star Bright M-1’ in which net photosynthetic rate of tetraploids was 39.6% higher than the diploids. This exception is not unique; high rates were found in tetraploids of *Beta vulgaris* (Beysel, 1957) and *Hippocrepis comosa* (Guern et al., 1975). The higher net photosynthetic rate could be attributable in part to the thicker leaves because net photosynthetic rate is measured based on unit leaf surface area. The higher net photosynthetic rate and higher WUE in tetraploids were found to increase adaptation to interior low-light conditions and tolerance to drought in our preliminary interiorscape study, which may suggest that chromosome doubling could be a strategy for increasing plant tolerance to stressful environments

Table 6. Net photosynthetic rate (P_n , $\mu\text{mol CO}_2/\text{m}^2\cdot\text{s}^{-1}$), g_s (g_s , $\text{mol H}_2\text{O}/\text{m}^2\cdot\text{s}^{-1}$), intercellular CO₂ concentration (C_i , $\text{cm}^3\cdot\text{m}^{-3}$), transpiration rate (E , $\text{mmol H}_2\text{O}/\text{m}^2\cdot\text{s}^{-1}$), and water use efficiency (WUE, $\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$) of 2× and 4× *Dieffenbachia* × ‘Star Bright’ M-1 grown in the shaded greenhouse.^z

Ploidy	P_n	g_s	C_i	E	WUE
2×	1.438 b	0.016 a	203.167 a	0.486 a	2.95 b
4×	2.007 a	0.011 b	127.283 b	0.372 b	5.41 a

^zThe value of P_n , g_s , C_i , E , and WUE was mean of nine leaves (three leaves per replicate). The value of canopy height and width, root, shoot, and total dry weights was mean of three containerized plants. Means followed by different letters within columns were significantly different based on Fisher’s least significant difference test at $P \leq 5\%$.

such as interior low light and low humidity conditions.

Literature Cited

- Beysel, D. 1957. Assimilations und atemungsmessungen an diploiden und polyploiden. Zuckerruben Zuchter 27:261–272.
- Chakraborti, S.P., K. Vijayan, B.N. Roy, and S.M.H. Qadri. 1998. In vitro induction of tetraploidy in mulberry (*Morus alba* L.). Plant Cell Rpt. 17:799–803.
- Chen, J. and R.J. Henny. 2008. Ornamental foliage plants: Improvement through biotechnology, p. 140–156. In: Kumar, A. and S.K. Sopory (eds.). Recent advances in plant biotechnology and its application. I.K. International Publishing House Pvt. Ltd., New Delhi, India.
- Cohen, D. and J.L. Yao. 1996. In vitro chromosome doubling of nine *Zantedeschia* cultivars. Plant Cell Tissue Organ Cult. 47:43–44.
- Eeckhaut, T.G.R., S.P.O. Werbrugg, L.W.H. Leus, E.J. Van Bockstaele, and P.C.P. Debergh. 2004. Chemically induced polyploidization in *Spathiphyllum wallisii* Regal through somatic embryogenesis. Plant Cell Tissue Organ Cult. 78:241–246.
- Gamborg, O., R. Miller, and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50:151–158.
- Guern, M., R. Bourdu, and M. Roux. 1975. Polyploidie et appareil photosynthétique chez l’*Hippocrepis comosa* L. Photosynthetica 9: 40–51.
- Henny, R.J. 1995. ‘Star Bright’ *Dieffenbachia*. HortScience 30:164.
- Henny, R.J. 2000. Breeding ornamental aroids, p. 121–132. In: Callaway, D., and B. Callaway (eds.). Breeding ornamental plants. Timber Press, Portland, OR.
- Kadota, M. and Y. Niimi. 2002. In vitro induction of tetraploid plants from a diploid Japanese pear cultivar (*Pyrus pyrifolia* N. cv. Hosui). Plant Cell Rpt. 21:282–286.
- Knauss, J.F. 1976. A tissue culture method of producing *Dieffenbachia picta* cv Perfection free of fungi and bacteria. Proc. Fla. State Hort. Soc. 89:293–296.
- Levin, D.A. 1983. Polyploidy and novelty in flowering plants. Amer. Nat. 122:1–25.
- Lu, C. and M. Bridgen. 1997. Chromosome doubling and fertility study of *Alstroemeria aurea* × *A. caryophyllaea*. Euphytica 94:75–81.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473–497.
- Rose, J.B., J. Kubba, and K.R. Tobutt. 2000. Induction of tetraploidy in *Buddleia globosa*. Plant Cell Tissue Organ Cult. 63:121–125.
- Sparnaaij, L.D. 1979. Polyploidy in flower breeding. HortScience 14:496–499.
- Tambong, J.T., V.T. Sapra, and S. Garton. 1998. In vitro induction of tetraploids in colchicine-treated cocoyam plantlets. Euphytica 104: 191–197.
- Thao, N.T., K. Ureshino, I. Miyajima, Y. Ozaki, and H. Okubo. 2003. Induction of tetraploids in ornamental *Alocasia* through colchicine and oryzalin treatments. Plant Cell Tissue Organ Cult. 72:19–25.
- Udall, J.A. and J.F. Wendel. 2006. Polyploidy and crop improvement. Crop Sci. 46:S3–S14.