Variation in Chilling Sensitivity among Eight *Dieffenbachia* Cultivars

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Abstract. This study evaluated chilling sensitivity of eight popular Dieffenbachia cultivars. Tissue culture liners were potted in 15-cm diameter pots using Vergro Container Mix A and grown in a shaded greenhouse under maximum photosynthetically active radiation of 285 µmol·m⁻²·s⁻¹ for 5 months. After determining growth indices, the plants were chilled in walk-in coolers at 2, 7, or 12 °C for 6, 12, or 24 h. Chilled plants were placed back in the shaded greenhouse for chilling injury and growth evaluation. Visible symptoms of injury included chlorosis, necrosis, water-soaked patches on leaves, or complete wilting. In addition to leaf injury, stems of some cultivars chilled at 2 °C for 24 h became water-soaked at the base, which resulted in the death of either entire shoots or entire plants depending on cultivars. Leaf injury occurred in all cultivars chilled at 2 °C, except for 'Panther'; and the longer the exposure at this temperature, the greater the injury. No visual injury was observed among plants chilled at 7 and 12 °C except 'Tropic Honey' that had 26% of leaves injured at 7 °C. Based on the percentage of injured leaves 12 days after chilling at 2 °C for 24 h, the sensitivity of the eight cultivars ranked as follows: Tropic Honey > Sterling > Carina ≥ Octopus > Camille > Camouflage > Star Bright > Panther. In addition to visual injury, plant growth was also affected by chilling during the subsequent 3 months of growth. All 'Tropic Honey' chilled at 2 °C died regardless of the tested chilling duration. Growth indices of all other cultivars except for 'Panther' chilled at 2 °C for 24 h significantly decreased compared with those of controls. 'Camille', 'Camouflage', 'Carina', and 'Sterling' also exhibited significant growth reduction after chilling at 2 °C for 12 h. This study showed that genetic variation in chilling sensitivity exists among cultivated Dieffenbachia. The identified chilling-tolerant cultivars could be used for breeding of new chilling-tolerant cultivars. The use of chillingtolerant cultivars in production may reduce the chance of injury during heating outages and shipment.

Chilling has been a significant cause of foliage plant injury not only in production, but also in transportation, retail display, and interiorscaping (Chen et al., 2005). In an attempt to exploit genetic potentials of foliage plants for chilling tolerance, Qu et al. (2000) and Chen et al. (2001) evaluated 15 cultivars of *Spathiphyllum* Schott and 12 cultivars of *Aglaonema* Schott and found significant differences in chilling tolerance among respective cultivars. The identified chilling-tolerant cultivars are widely used in commercial production, which helps reduce chilling injury (CI) incidences in *Aglaonema* and *Spathiphyllum* and also conserves energy

normally used for greenhouse heating. Additionally, chilling-tolerant cultivars have been used in breeding programs for developing new cultivars with chilling tolerance (Henny and Chen, 2004).

Dieffenbachia, commonly known as dumb cane, is a member of the family Araceae and ranks among the top five most popular foliage plant genera produced and sold in the United States based on the annual wholesale value (U.S. Department of Agriculture, 1999). There are ≈30 recognized species native to South and Central America (Mayo et al., 1997). In addition to some introductions collected from the wild, commercially available cultivars are largely derived from D. amoena Hort. ex Gentil and D. maculata (Lodd.) G. Don. Methods of cultivar development for Dieffenbachia mainly include selection from progenies of interspecific hybridization and from somaclonal variation produced by tissue culture (Henny and Chen, 2004). Since the first hybrid release in 1870, ≈100 cultivars have been introduced, but commercial production has been limited to \approx 20 cultivars (Chen et al., 2002).

Because of its lowland tropical origin, Dieffenbachia is considered one of the most

chilling-sensitive foliage plant genera. Root fresh weights of Dieffenbachia 'Exotica Perfection' cuttings initially chilled at 7 °C for 6 d were only 59% of those of the control 2 months after rooting (McConnell et al., 1981). Leaves of Dieffenbachia maculata 'Rudolph Roehrs' were severely injured after chilling at 5 °C for 48 h (Semeniuk et al., 1986). Visible foliar injury of 'Rudolph Roehrs' began to appear 27 h after chilling at 1.2 °C, and old leaves became watersoaked in 75 h (McMahon et al., 1994). However, there has been no report on Dieffenbachia cultivar differences in chilling sensitivity. The objectives of this study were to evaluate chilling responses of eight popular Dieffenbachia cultivars and determine CI symptoms and chilling effects on subsequent growth.

Materials and Methods

Tissue culture liners of eight popular Dieffenbachia cultivars—Camille, Camouflage, Carina, Octopus, Panther, Star Bright, Sterling, and Tropic Honey-were obtained from a commercial tissue culture laboratory in Aug. 2006. With the exception of 'Panther' that was presumably introduced as a collection from the wild, these cultivars were developed from the selection of sports, somaclonal variants, or interspecific hybrids; and some were genetically related (Table 1). Uniform liners were potted immediately into 15-cm diameter plastic pots using a sphagnum peat-based medium (Vergro Container Mix A; Verlite Co., Tampa, FL) in which Canadian peat, vermiculite, and perlite were in a 3:1:1 ratio based on volume. Potted plants were grown in a shaded greenhouse under maximum photosynthetically active radiation of 285 µmol·m⁻²·s⁻¹ and a relative humidity of 50% to 100%. Temperatures in the shaded greenhouse ranged from 20 to 32 °C. Two weeks after potting, a 15N-7P-15K controlled-release fertilizer (Multicote; Haifa Chemicals Ltd., Haifa Bay, Israel) was applied to the substrate surface at a rate of 5 g per pot.

Plant canopy heights and widths were measured 5 months after potting. The growth index of each plant was calculated as [(canopy widest width + width perpendicular) ÷ 2] × plant height (Chen et al., 2003). Plants of each cultivar with similar growth indices were selected and labeled for chilling treatments. The chilling treatments were conducted in 3.1 m \times 3.1-m walk-in coolers with temperatures preset at 2, 7, or 12 °C until they stabilized. Plants were chilled in the dark with the duration of 6, 12, or 24 h per temperature setting. Individual plants were the experimental unit and they were replicated six times. Additionally, six plants of each cultivar were left in the shaded greenhouse as controls. After the 6, 12, or 24 h of chilling, plants were moved back to the shaded greenhouse for injury evaluation and continuing growth.

CI was indicated by visual foliar blemishes. Because the aesthetic appearance of

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Dieffenbachia is directly related to foliage color and quality, any damage on leaves, regardless of severity, can greatly reduce its ornamental value in the marketplace. Thus, the percentage of injured leaves was used as an important parameter for determining the sensitivity of cultivars to chilling. Numbers of injured leaves were counted 5 and 12 d after chilling. The percentages of injured leaves relative to total numbers of leaves per plant were calculated. To determine the effects of chilling on subsequent growth, plant heights and widths were measured monthly after chilling for 3 months, and growth indices were calculated. The experiments were arranged in a completely randomized design. Data, including the percentage of injured leaves and growth indices, were analyzed by analysis of variance (SAS Institute, 1996), and means separations were determined using Fisher's protected least significant differences at the 5% and 1% levels (Fisher, 1951).

Results

Chilling injury symptoms. CI was observed in leaves, stems, and roots. Leaf injury was the most notable symptom, including chlorosis, necrosis, water-soaked patches, and wilting (Fig. 1A–D). Stem injury occurred either at the base, manifested by reddish-brown color, or to the entire stem, which became water-soaked and collapsed (Fig. 1E–F). The browning of root systems was the typical symptom of root injury and was only observed in *Dieffenbachia* 'Tropic Honey'.

Cultivar differences in visible injury. Cultivars differed significantly in leaf, stem, and root injury. After chilling at 2 °C for 24 h, young leaves of Dieffenbachia 'Tropic Honey' wilted within 3 h. Water-soaked patches appeared in 2 d, all young leaves became necrotic, and older leaves wilted 3 d later. The entire shoot collapsed 10 d after chilling (Fig. 1F). On the contrary, no injury was observed in Dieffenbachia 'Panther' irrespective of the tested chilling temperatures and duration. Twelve days after chilling at 2 °C for 24 h, the percentages of injured leaves varied from 0% in 'Panther' to 100% in 'Tropic Honey' (Fig. 2). Stem injury occurred in 'Camille', 'Carina', 'Sterling', and 'Tropic Honey' but not in 'Camouflage', 'Octopus', 'Panther', or 'Star Bright' after chilling at 2 °C for 24 h. The injury characterized by the reddish-brown color at the stem base initially caused wilting of some leaves but eventually resulted in the collapse of the entire shoots of 'Camille', 'Carina', and 'Sterling'. However, because no root injury occurred to these three cultivars, lateral shoots grew from the stem base, resulting in pots with multiple shoots. The stem injury in 'Tropic Honey' differed from the aforementioned three cultivars because the entire stem became water-soaked and then collapsed. In connection with root injury, all 'Tropic Honey' chilled at 2 °C died regardless of the tested chilling durations.

Table 1. A brief genetic background of the eight *Dieffenbachia* cultivars and their critical chilling temperatures and durations based on visual leaf injury.

Cultivar	Genetic background ^z	Critical chilling temp	Chilling duration
Sterling	Interspecific hybrid	2 °C	6 h
Carina	Somaclonal variant of 'Camille'	2 °C	6 to 12 h
Octopus	Somaclonal variant of 'Camouflage'	2 °C	12 h
Camille	Sport from D. maculate 'Perfection'	2 °C	12 h
Camouflage	Somaclonal variant of 'Panther'	2 °C	12 to 24 h
Star Bright	Interspecific hybrid	2 °C	24 h
Panther	Unknown (likely a collection from the wild)	2 °C	>24 h
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^zBased on Chen et al. (2004).

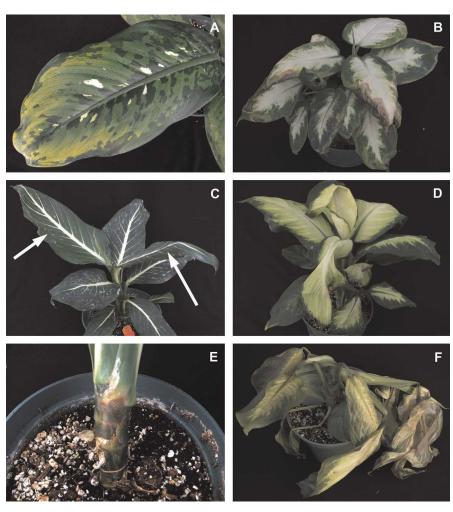


Fig. 1. Chilling injury symptoms of *Dieffenbachia* cultivars after exposure to 2 °C for 24 h. (A) Occurrence of chlorosis on young leaves of 'Octopus' 24 h after chilling; (B) necrotic leaves of 'Camille' 5 d after chilling; (C) water-soaked lesion (indicated by arrows) on leaf margin of 'Sterling' 5 d after chilling; (D) complete wilting of leaves of 'Tropic Honey' 10 d after chilling; (E) stem injury of 'Sterling' indicated by the reddish-brown color at the base; and (F) collapse of stem and entire shoot of 'Tropic Honey' 12 d after chilling.

Cultivars also varied in response to chilling durations. In contrast to the seven cultivars injured after chilling at 2 °C for 24 h, leaf injury occurred only in 'Camille', 'Carina', 'Octopus', 'Sterling', and 'Tropic Honey' chilled at 2 °C for 12 h and only in 'Octopus', 'Sterling', and 'Tropic Honey' chilled at 2 °C for 6 h (Fig. 2). The percentages of injured leaves were also markedly decreased as duration of chilling decreased except for 'Tropic Honey'. No cultivars exhibited leaf injury after chilling at 7 or 12 °C except

'Tropic Honey', which had 26% of leaves injured after chilling at 7 °C. Additionally, cultivars differed in leaf symptom development. There was no increase in injured leaves in 'Camille' and 'Star Bright' from 5 d to 12 d after chilling 2 °C for 24 h. However, the percentages of injured leaves in 'Camouflage', 'Carina', 'Octopus', and 'Sterling' significantly increased from 5 d to 12 d after chilling. Leaf ages appeared to vary in chilling sensitivity as well; chlorosis and necrosis or water-soaked patches appeared first or

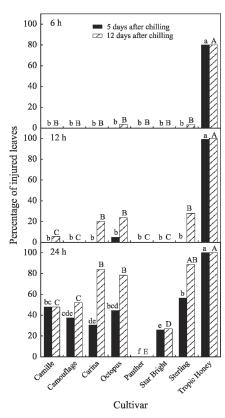


Fig. 2. Mean percentages of injured leaves of eight Dieffenbachia cultivars chilled at 2 °C for 6 h, 12 h, and 24 h. Different lowercase letters indicate significant differences in percentages of injured leaves 5 d after chilling and the different uppercase letters indicate significant differences in percentages of injured leaves 12 d after chilling analyzed by Fisher's least significant difference test at $P \le 0.05$ level.

most in young leaves rather than mature and old leaves.

Cultivar differences in growth indices. Plant growth indices differed significantly among cultivars after chilling at 2 °C (Fig. 3). All 'Tropic Honey' died 1 month after chilling at 2 °C (Fig. 4). However, there were no significant differences in growth indices of 'Panther' chilled at 2, 7, or 12 °C against the control. Growth indices of 'Camille', 'Carina', 'Camouflage', 'Octopus', 'Star Bright', and 'Sterling' chilled at 2 °C for 24 h were significantly reduced compared with those of the controls or those chilled at 7 or 12 °C during the subsequent 3-month growth period. The reduction of growth indices of 'Camille', 'Carina', and 'Sterling' chilled at 2 °C for 24 h was different from that of 'Octopus', 'Camouflage', and 'Star Bright'. Stems collapsed 1 month after chilling in the former three, and the recorded growth indices were actually lateral shoots. There was no stem injury in 'Octopus', 'Camouflage', or 'Star Bright'; the difference in growth indices may simply be attributed to growth retardation caused by chilling. Similarly, the reduction in growth indices of 'Camille', 'Camouflage', 'Carina', or 'Sterling' chilled at 2 °C for 12 h might also be the result of sublethal CI. It is interesting to note that there

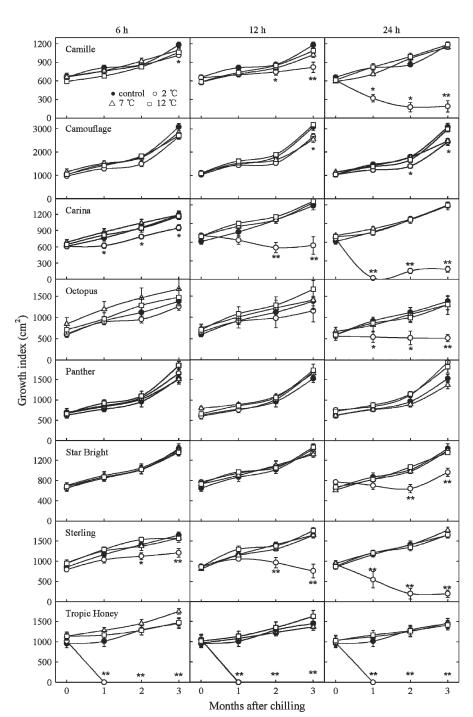


Fig. 3. Mean growth indices of eight *Dieffenbachia* cultivars before and after chilling at 2, 7, and 12 °C for 6, 12, and 24 h; * and ** indicate significant differences from the control based on Fisher's least significant difference test at $P \le 0.05$ and $P \le 0.01$ levels, respectively. Bars are standard errors with n = 6.

was no visible injury to 'Carina' and 'Sterling' chilled at 2 °C for 6 h, but growth indices of the two cultivars decreased significantly. Plant growth indices were not significantly different from respective controls after chilling at 7 or 12 °C except 'Tropic Honey' that had 30% less growth index after chilling at 7 °C than the control.

In summary of visible injury and growth index data, it appeared that plants chilled at 2 °C for 24 h showed great variation. Apparently, 'Panther' was the most tolerant cultivar because it had no leaf injured 5 d or 12 d after chilling, and 'Tropic Honey' was the most

sensitive cultivar because all plants died after being chilled at 2 $^{\circ}$ C. The relative sensitivity of the eight cultivars ranked as follows: Tropic Honey > Sterling > Carina \geq Octopus > Camille > Camouflage > Star Bright > Panther. The critical chilling temperatures and durations are summarized in Table 1.

Discussion

This study shows clearly that variation in chilling sensitivity exists among the eight popular cultivars of *Dieffenbachia*. The variation ranged from no visible injury symptom

















Fig. 4. Morphological appearance of eight *Dieffenbachia* cultivars grown in the shaded greenhouse 3 months after chilling at 2 °C for 6, 12, and 24 h (from left to right: 24 h, 12 h, 6 h, and control).

in 'Panther' to complete death of 'Tropic Honey' chilled at 2 °C (Fig. 2). Cultivars differed in critical chilling temperatures and durations (Table 1), which are important in *Dieffenbachia* production because growers

could manage their greenhouse temperatures based on cultivar-dependent chilling temperature sensitivity ranges. Cultivars also varied temporally in chilling symptom expression. The percentage of injured leaves in 'Camouflage', 'Carina', and 'Octopus' were significantly greater 12 d after chilling than those at 5 d after chilling, but there was no such change in 'Camille' and 'Star Bright' (Fig. 2). The temporal variation in symptom development may suggest that cells were injured in different degrees, and more severely injured cells exhibited symptoms sooner than the less severely injured cells. Young leaves injured first and most in Dieffenbachia; such leaf age effects were different from those observed in Spathiphyllum (Qu et al., 2000) and Aglaonema (Chen et al., 2001) in which more mature or old leaves were visibly injured after chilling. It is unknown why the three genera from the same family (Araceae) differed in leaf age sensitivity to chilling temperatures. A possible explanation could be that roots of Dieffenbachia are more sensitive to chilling than those of Aglaonema and Spathiphyllum. The sensitivity may result in temporal shortage of water transported from roots to shoots, thus causing injury to young leaves first.

In addition to visual injury, chilling affected the growth of 'Carina' and 'Sterling' 3 months after treatment at 2 °C for 6 h (Fig. 3). Such phenomenon was referred to as latent effects by Qu et al. (2000), i.e., plants with no visible injury were actually injured, which affected subsequent growth. McMahon et al. (1994) reported that chilled Dieffenbachia 'Rudolph Roehrs' showed reduced chlorophyll content and transpiration rate and increased stomatal diffusive resistance. Thus, the latent effects could significantly delay plant growth. For example, the growth index of 'Sterling' chilled at 2 °C for 6 h was only 73% of the control. Latent effects, however, have been largely ignored or misdiagnosed as improper nutrient management in production (Ou et al., 2000).

Cultivar differences in chilling sensitivity have substantial genetic bases (Table 1). The exact origin of 'Panther' is unknown, but it is the most tolerant cultivar so far identified. 'Camouflage' is a selection of somaclonal variants of 'Panther', and 'Octopus' is a cultivar selected from somaclonal variants of 'Camouflage' (Chen et al., 2004). The chilling-tolerant characteristic of 'Panther' has been largely maintained in 'Camouflage' but moderately lost in 'Octopus'. 'Camille' is a cultivar selected from somaclonal variants of D. maculata 'Perfection', and 'Carina' was selected from somaclonal variants of 'Camille'. Both show sensitivity to chilling. 'Star Bright' is an interspecific hybrid (Henny, 1995), which might have chillingtolerant cultivars as parent(s) and is the second most chilling-tolerant cultivar next to 'Panther'. 'Sterling' and 'Tropic Honey' were selected from the progeny of the same cross between 'Victory' and 'Marianne' (Henny et al., 2006, 2007). Although both

are chilling-sensitive, 'Sterling' is more tolerant than 'Tropic Honey' because the critical temperature and duration for the former is 2 °C for 6 h compared with 7 °C for 12 h for the latter, which suggests that differences in chilling sensitivity occurred within progeny of the same cross. Nevertheless, this study showed that chilling-tolerant cultivars occurred in the progeny of interspecific hybridization or somaclonal variants derived from chilling-tolerant cultivars. New cultivar development should not only focus on aesthetic appearance, but also physiological traits such as chilling tolerance so that chilling-tolerant cultivars can be identified and selected.

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