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Chilling injury in *Dendrobium* inflorescences is alleviated by 1-MCP treatment

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ABSTRACT

The effects of cold storage were studied in cut *Dendrobium* inflorescences. Two cultivars were used, one (cv. Princess) being more cold-sensitive than the other (cv. Sakura). Inflorescences were stored, for various intervals, at 5 °C and were then placed at 25 °C. Visible chilling injury (CI) mainly developed at 25 °C. Water-soaking, the most prominent CI symptom, was present more in closed floral buds and less in open flowers. Electrolyte leakage (EL) increased considerably more in floral buds than in open flowers, and more so during cold storage than during subsequent placement at 25 °C. Total (water-soluble) antioxidant capacity (TAC) also increased during 5 °C storage, but did not change during the period after cold storage. TAC was higher in cv. Sakura than in cv. Princess. No clear effects were found on activities of lipoxygenase (LOX), catalase and superoxide dismutase. A pulse treatment with 1-MCP prior to storage at 5 °C was studied in cv. Princess. 1-MCP reduced CI and resulted in less increase in EL, but had no effect on TAC and LOX activity. The data suggest that CI symptoms of *Dendrobium* floral buds and open flowers were related to membrane damage and antioxidant capacity, and that the chilling injury signal is mediated by ethylene.

1. Introduction

Storage of cut flowers at low temperature is used widely in commercial practice. Only a few cut flowers, such as *Anthurium*, *Strelitzia*, and several orchids, cannot be stored at the conventional 1 °C (Carow, 1981; Ketsa and van Doorn, 2009). The optimum temperature for storage of some tropical orchids is 7–10 °C, if held at this temperature for at most 2 weeks (Lutz and Hardenburg, 1968).

Membranes are thought to be the primary sensing sites for development of chilling injury. The immediate effect of low temperature seems the phase transition of membrane fatty acids, from fluid liquid-crystalline to a solid-gel state. This leads to a cascade of physiological effects (Marangoni et al., 1996; Sevillano et al., 2009). Low temperature also often increases the levels of reactive oxygen species: oxygen free radicals and other oxidative oxygen compounds such as hydrogen peroxide (Yang et al., 2011). Reactive oxygen species can contribute to the loss of cellular functions through direct peroxidation of membrane lipids (Wismer, 2003). The membrane lipids can become degraded by enzymes such as lipoxygenase (LOX; Liavonchanka and Feussner, 2006), which can be activated by reactive oxygen species but can also act independently of oxidative stress (Stark, 2005).

The formation of reactive oxygen species can be prevented by anti-oxidative compounds such as vitamin C and phenolics. These compounds are part of the total antioxidant capacity (TAC). There are several methods that estimate TAC, one being FRAP (Ferric Reducing Ability of Plasma; Moon and Shibamoto, 2009). Once formed, the action of reactive oxygen species can become inhibited by enzymes that render them less harmful or innocuous, for example superoxide dismutase and catalase.

The objectives of the present research were: (1) to investigate the degree of chilling injury in some *Dendrobium* cultivars, whereby cut inflorescences were stored at low temperature and then placed at room temperature (25 °C); (2) to study the possible role of total antioxidant capacity (using the FRAP assay), that of catalase, SOD, and LOX in the development of chilling injury; and (3) to find out if a pulse treatment with an anti-ethylene compound might reduce chilling injury.

2. Materials and methods

2.1. Plant material

Preliminary experiments with five *Dendrobium* cultivars (Sonia Bom #17, Sakura, Princess, Jacky and Burana Jade) established that

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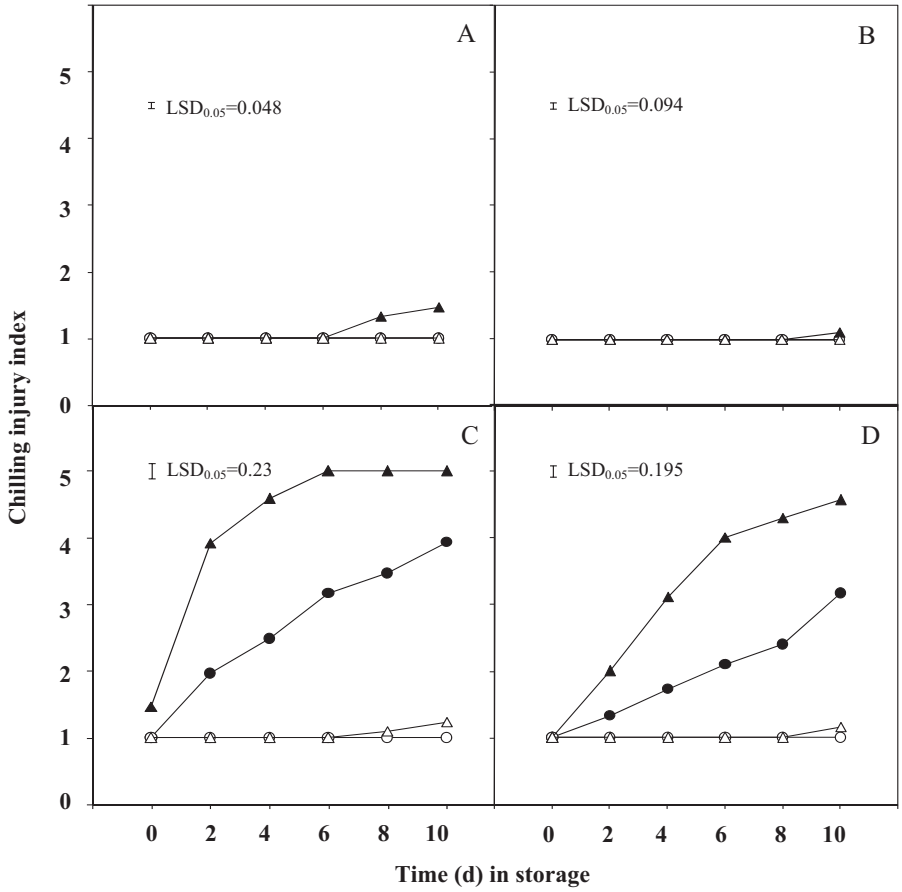

Fig. 1. Chilling injury of flower buds (A, C) and open flowers (B and D) in *Dendrobium* inflorescences cvs. Princess (▲, △) and Sakura (●, ○) during storage at 5 °C (▲, ●) or 15 °C (△, ○). CI was recorded immediately upon removal from low temperature (A, B) and after holding at 25 °C for 3 d, subsequent to storage at 5 °C (C, D). The flowers in C and D are thus three days older than those in A and B, but have been placed at the same time on the *X*-axis as they were removed out of 5 °C storage at that time. Data are means of 10 replicate inflorescences.

cv. Sakura was less CI sensitive than cv. Princess. We therefore used these two cultivars for further investigation. Export grade inflorescences were purchased from a commercial grower in Suphanburi province, Thailand. Inflorescences were shipped by truck, with the stem ends in distilled water. Upon arrival in the laboratory, on the same day, inflorescences with 5–7 open flowers and 5–10 closed floral buds were selected for freshness and uniformity. Peduncles of individual inflorescences were cut off in air, at 10 cm from the lowermost open flowers. The stem ends of individual inflorescences were inserted into plastic centrifuge tubes containing distilled water. The inflorescences were placed into cardboard boxes and stored at 5 or 15 °C (85 ± 5% RH) for 10 d, and were transferred 25 °C at various 2-d intervals. After removal from storage the inflorescences were individually held in new centrifuge tubes containing 15 mL of water. The CI symptoms were monitored both during storage and during the subsequent period at room temperature. Flower tissues were frozen in liquid N_2 and stored at −70 °C for further experimentation.

2.2. Assessment of chilling injury

The main chilling injury (CI) symptom was tepal water soaking. Less common (and considerably less pronounced) symptoms were flower drop, tepal venation, tepal wilting, and tepal fading. Water soaking was observed before any of these other symptoms. A chilling injury index was used, based on water soaking of the tepals in closed buds and open flowers. CI symptoms were assessed daily, using the following scale: (1) no chilling injury; (2) slight injury (visible in <20% of closed buds and open flowers on the inflorescence); (3) moderate injury (visible in 20–30% of the buds and open flowers on the inflorescence); (4) severe injury (visible in 30–40% of the buds and flowers on the inflorescence); and (5) very severe injury (visible in >40% of the buds and flowers in the inflorescence). The chilling injury index was calculated for each individual inflorescence, as follows:

$$\text{Chilling injury index} = \frac{\sum \text{Injury classification level} \times \text{Number of buds and open flowers at that level}}{\text{Total number of buds and open flowers}}$$

2.3. Vase life

The end of vase life was assessed daily during storage at low temperature and after holding at room temperature (25 °C). Vase life was considered to be terminated when in the open flowers the CI index was higher than 4.0, or when the open flowers showed senescence symptoms.

2.4. Electrolyte leakage

Electrolyte leakage (EL) was measured according to Campos et al. (2003), with slight modification. Segments (0.5 cm^2) of tepals were excised with a razor blade and washed in deionizer water. Half a gram FW was placed in an Erlenmeyer flask containing 30 mL of 0.3 M mannitol. The flasks were shaken at 100 rpm for 1 h. The solution electric conductivity was measured using a Consort model C831 (Turnhout, Belgium). Maximum conductance was measured after incubating flasks in an autoclave at 121 °C for 30 min and