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Hydrogen peroxide in the vase solution increases vase life and keeping quality of cut Oriental × Trumpet hybrid lily 'Manissa'

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ABSTRACT

Hydrogen peroxide (H_2O_2) is an important signal molecule involved in diverse plant developmental processes. Oriental × Trumpet hybrid lily, *lilium* 'Manissa' was used to investigate the effects of H_2O_2 in the vase solution on the vase life and keeping quality of cut flowers. The results show that the optimum concentration (600 μM) caused an increase in vase life, from 9.8 days in control to about 12.8 days. Concentrations of 800 and 1200 μM resulted in negative effects. Concurrently, 600 μM H_2O_2 resulted in the maximum flower diameter and the maximum number of days for full flower opening. Moreover, the H_2O_2 scavenger ascorbic acid (ASA) or the H_2O_2 inhibitor diphenylene iodonium (DPI) reversed the positive effects of 600 μM H_2O_2 on vase life and flower diameter. Treatments with 600 μM H_2O_2 delayed leaf and petal senescence and flower opening for some days. Additionally, 600 μM H_2O_2 treatments significantly reduced the relative degree of decline of leaf relative water content (RWC), leaf chlorophyll, petal water-soluble carbohydrate and cut flowers fresh weight. However, petal electrolyte leakage in 600 μM H_2O_2 treatments showed the minimum variations. Together, these results indicated that H_2O_2 treatments at the proper dosage prolonged the vase life of cut lily and enhanced postharvest quality by retarding the degradation of RWC, chlorophyll, carbohydrate as well as cut flowers fresh weight and simultaneously keeping membrane integrity.

1. Introduction

Postharvest senescence is a limiting factor in the marketing of many species of cut flowers. Flower senescence is associated with changes in the membrane permeability, leakage of ions (Ezhilmathi et al., 2007; Singh et al., 2008), regulation of oxidative enzymes (Ezhilmathi et al., 2007), degradation of proteins, lipids, carbohydrates and nucleic acids (Hussein, 1994; Langston et al., 2005; Elgimabi and Ahmed, 2009), imbalance of plant hormones, polyamines and Ca^{2+} (Mutui et al., 2006; Zeng et al., 2011). Although dramatic progress has been made in recent years in understanding flower senescence and its associated events, more data are needed to reveal the mechanism of flower senescence.

Hydrogen peroxide (H_2O_2) is one of the most important reactive oxygen species. It is continually generated from via electron transport reactions both in mitochondria and chloroplasts. There is evidence for H_2O_2 production in plants from several enzymes including nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, cell wall peroxidases, amine oxidases, as well as other flavin containing enzymes (Neill et al., 2002). As H_2O_2 causes oxidative stress at high concentrations in plants, it was still viewed mainly as toxic cellular metabolites until recently. However, increasing lines of evidence support the idea that H_2O_2 may act as a signal molecule with multiple functions in plants due to its relatively high stability and long half-life (Delledonne et al., 2001; Liao et al., 2011). It is well known that H_2O_2 is involved in the plant response to various environmental stresses, such as salinity (Zhang et al., 2007), drought (Luna et al., 2005), heat stress (Uchida et al., 2002), ozone (Rao and Davis, 2001), UV radiation (He et al., 2005), disease (Delledonne et al., 2001), and mechanical wounding (Orozco-Cárdenas and Ryan, 1999). In addition, H_2O_2 mediates plant growth and influences various developmental processes, including programmed cell death (PCD; Fath et al., 2001), seed germination (Schopfer et al., 2001), senescence (Hung et al., 2006), flowering (Potocký et al., 2007), adventitious root development (Liao et al., 2011), and many others. However, there has been no suggestion of positive effects of H_2O_2 in cut flower senescence.

Lilies (*Lilium* spp.), a bulbous plant with large trumpet-shaped and typically fragrant flowers, are one of the most important ornamental plants world-wide. They have long been cultivated as flowering potted plants or cut flowers. In this paper we report on the ability of H_2O_2 in the vase solution to extend the postharvest life and quality of cut Oriental × Trumpet hybrid lily, *lilium* 'Manissa'. Therefore, the objectives of this study are to determine the

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effects of H_2O_2 on flower senescence during the postharvest life of cut flowers.

2. Materials and methods

2.1. Plant material

Experiments were conducted with Oriental × Trumpet hybrid *lilium* 'Manissa'. Flowering stems were harvested in the early morning from a local commercial grower (Longhai Florist, Lanzhou, China) at the tight, green bud (puffy bud) stage (commercial harvest stage). Stems were graded for uniform quality, combined into bunches of multiple stems, and then re-cut to 60–70 cm-length in accordance with commercial practice. Bunches were packed dry into commercial flower boxes and were transported from the cultivation area to the refrigeration laboratory within 2–3 h.

2.2. General processing

Upon arrival in the laboratory, flowering stems were cut to a uniform length of 45 cm under distilled water to avoid air embolism. The blades of cutters were surface sterilized by rinsing in 95% (v/v) ethanol prior to use. Leaves on the bottom 20 cm of stems that would otherwise be submerged in vase water were removed by hand. The stems were placed randomly in 2500 ml glass vases containing various concentration of 1000 ml distilled water (control) or 1000 ml of test solutions. Glass vases were surface sterilized with ethanol as described above for cutters. The test solutions were as follows: (1) various concentration of H_2O_2 (0, 200, 400, 600, 800 and 1200 μM; Sigma, St. Louis, MO, USA); (2) 6 μM ascorbic acid (ASA, Sigma); (3) 1 μM diphenylene iodonium (DPI, Sigma); (4) 600 μM H_2O_2 + 6 μM ASA; (5) 600 μM H_2O_2 + 1 μM DPI. Flowers were put in a controlled environment room at 20 ± 1 °C, 60 ± 5% relative humidity, and 12 h photoperiod (08:00–20:00 h) provided by cool fluorescent lamps (15 μmol photons m^{-2} s^{-1} irradiance). Vase solutions were replaced with freshly made solutions for every day.

2.3. Vase life and flower diameter

The vase life of each flower was determined by the number of days from the day that the flowers were placed in the test solutions until flowers had no decorative value (underwent color change; wilt and loose turgidity). The flower diameter was defined as the maximum width of each flower and measured by vernier caliper. For each treatment, 10 flowers were selected for evaluation, and the means of vase life and flower diameter were determined.

2.4. Leaf senescence, leaf relative water content and total chlorophyll content

Leaf senescence was determined by daily observation of cut flower leaves. Leaf water status was expressed as relative water content (RWC). Immediately after sampling, fresh leaves were weighed (W_f) and then immersed in distilled water for 8 h at room temperature. The leaves were then blotted dry and weighed (W_t). Leaf samples were oven dried at 60 °C to a constant weight for 72 h, and then re-weighed (W_d). RWC was calculated by the formula: RWC (%) = $[(W_f - W_d)/(W_t - W_d)] \times 100$. To determine chlorophyll content, 0.5 g of leaves was frozen in liquid nitrogen, ground to a powder, and extracted in 5 ml of 80% (v/v) acetone until complete bleaching. Total chlorophyll was quantified by measuring the absorption at 652 nm and its concentration was calculated according to Arnon (1949). RWC and chlorophyll content were measured at 2 days intervals.

2.5. Flower opening and senescence

The visual quality of lily flowers was assessed daily with a rating scale during vase life. A seven-category rating scale was designed for assessing senescence symptoms of lily flowers (Fig. 1): Grade 1, tight, green bud; Grade 2, lightly coloured bud; Grade 3, newly open; Grade 4, half-open; Grade 5, fully open (optimum quality); Grade 6, incipient senescence; Grade 7, senescence.

2.6. Petal electrolyte leakage and water-soluble carbohydrate content

Fresh petals (1 g) were cut into 25 mm^2 pieces and were immersed in test tubes with 10 ml of double distilled water for 3 h at room temperature. The initial conductivity (C_0) was determined, and the test tubes were placed in the boiling water for 15 min. After cooling to room temperature, the conductivity (C) was again determined. The electrolyte leakage was expressed as percent value according to the formula: electrolyte leakage (%) = $(C_0/C) \times 100$.

Water-soluble carbohydrate (WSC) content of petal was estimated using the anthrone method (Van Handel, 1968). Petal samples (0.1 g), which were oven dried at 60 °C for 72 h, were extracted for 12 h in water at 70 °C. The carbohydrate extract was analyzed by reacting 0.5 ml of the supernatant with 0.5 ml freshly prepared anthrone reagent (1 g anthrone in 50 ml ethyl acetate) and 1.5 ml distilled water, and then placed in boiling water bath for 1 min. After cooling to room temperature, the absorbance at 630 nm was measured. Electrolyte leakage and WSC content were measured at 2 days intervals.

2.7. Changing of fresh weight

The original fresh weights of cut flowering stems ($W_{t=0}$) were measured immediately after cutting flowers and before placing them in test solutions. Fresh weights were recorded individually daily (W_t; where t = day 0, 1, 2, etc.) and changing of fresh weight of each stems was calculated as: $[(W_t - W_0)/W_0] \times 100$.

2.8. Statistical analysis

Where indicated, the results are expressed as mean values (±SE) from three independent experiments (n = 10). In all experiments, flowering stems were arranged using a completely randomized design. Mean comparisons were performed using Duncan's multiple range test to determine whether the differences between the variables were significant at $P < 0.05$. All statistical analyses were performed using the Statistical Package for Social Sciences for Windows (version 13.00; SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Effects of various concentrations of H_2O_2 on vase life, maximum flower diameter, and days until full flower opening

Compared with the control, various concentrations of H_2O_2 had significant effects on the vase life of cut lily, and the effects were dose dependent (Table 1). The vase life of lily was extended by lower doses of H_2O_2 (200, 400, and 600 μM), whereas a higher dose of H_2O_2 (1200 μM) resulted in significantly decreased vase life. The maximum vase life was 12.8 days in 600 μM H_2O_2 in comparison to 9.8 days for the control.

When compared with the control, 400 or 600 μM H_2O_2 significantly increased maximum flower diameter (Table 1). There was no significant difference in maximum flower diameter between 0 (control), 200, and 800 μM H_2O_2 treatments. However, flowers placed in the highest H_2O_2 concentration (1200 μM) registered a