BA and Sucrose Increase Vase Life of Cut *Eustoma* Flowers

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Abstract. An experiment was conducted to measure the effects of pulse treatments of BA, sucrose, and BA before, after, or with sucrose, on the vase life of cut *Eustoma* flowers. A BA pulse at 50 mg·L⁻¹ before 4% sucrose promoted the longevity of cut *Eustoma* flowers better than other treatments. Simultaneously, sucrose, glucose, and mannose concentrations in flowers during vase periods were maintained at higher levels in double pulse treatments than in the single pulses. Ethylene production in flowers 2 days after vase treatment was highest in the BA-treated flowers; intermediate in flowers pulsed with BA before, after, or with sucrose; and lowest in sucrose-treated flowers. Although a BA pulse increased ethylene production over that of controls, it inhibited senescence in cut *Eustoma* flowers. Respiration in flowers pulse-treated with sucrose or with BA before, after, or with sucrose, was significantly higher than that in controls. Results suggest that the vase life of cut *Eustoma* flowers is improved by either BA or sucrose in vase solution and especially when BA was pulsed before the sucrose pulse. Chemical name used: N⁶-benzyladenine (BA).

Exogenous application of cytokinin to detached leaves delays the onset of senescence (Binns, 1994; Letham and Palni, 1983), and promotes the transport of solutes from older to younger parts of a plant (Letham and Palni, 1983). Kinetin application at low concentrations delayed senescence in tobacco leaf discs, but at the same time stimulated ethylene production in them (Fuchs and Lieberman, 1978; Lau and Yang, 1973). Sugar promotes flower bud opening in many cut flowers (Ichimura and Korenaga, 1998; Kuiper et al., 1995; Mayak et al., 1973). Ichimura and Hiraya (1999) reported that a sucrose pulse treatment extended the vase life and inhibited ethylene production in cut sweet pea florets regardless of their stage of development. In addition, 8-hydroxyquinoline sulfate (HQS) inhibits the occurrence of bent-neck in cut rose flowers (Burdett, 1970) and improves water relations by inhibiting both vascular blockage and transpiration (Halevy and Mayak, 1981). Climacteric ethylene production in cut sweet pea flowers was unaffected by HQS (Ichimura et al., 1998). Since HQS is a germicide, its effectiveness could be partially due to inhibition of bacterial proliferation (Marousky, 1969).

Our studies were undertaken to investigate the effects of pulse treatments of BA, sucrose, BA before or after sucrose, and BA with sucrose on vase life, changes in sugars and ethylene and respiration in cut *Eustoma* flowers during vase treatments.

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Materials and Methods

Eustoma grandiflorum Shinn. cv. Heide flowers with stems initially ≈40 cm long were used. Flower stems were packed in corrugated cardboard boxes and transported to the National Chiayi Univ. ≈2 h after harvesting in Chia-Yi County, Taiwan. Cut flowers were immediately recut in deionized water, and placed in 1000-mL flasks containing 800 mL of vase solution. The cut flowers were kept at 25 °C, 15 μmol·m⁻²·s⁻¹ photosynthetic photon flux using cool-white fluorescent lamps (Philips, Taoyung County, Taiwan) with a 14-h photoperiod. The cut stems were treated with water controls, BA at 50 mg·L⁻¹, 4% sucrose, BA at 50 mg·L⁻¹ before or after 4% sucrose, and BA at 50 mg·L⁻¹ + 4% sucrose. All solutions contained 200 mg·L⁻¹ of HQS. Either the sucrose pulses, BA pulses, or both, were given once for 24 h each. Vase life was recorded as the number of days from the end of the pulse treatments until the day when the last flower senesced. A randomized complete-block design was used with four cut stems per flask, and four replicate flasks per treatment. Data were subjected to analysis of variance (ANOVA) (SAS Institute, 1989), and the means were separated using Duncan's multiple range test.

Determination of sugar concentrations in flowers. Sugar analysis (sucrose, glucose, mannose, and ribose) was done as described by Chen et al. (1994). Open flowers were ground in a laboratory blender with 95% ethanol, and filtered through Whatman No. 1 papers. The residues were re-extracted three times with the same solvent. The combined solvent was centrifuged at 10,000 g for 15

min. The resulting supernatant was evaporated at 35 °C to dryness under vacuum. The dried sample was dissolved in 1 mL distilled water, and filtered through a 0.45-μm millipore filter. The filtrate was used for HPLC (Hitachi-L-7100, Tokyo) analysis. Sugars were separated with a Sugar Pak 1 column (300 × 6.5 mm i.d., Waters Associates, Milford, Mass.) at 90 °C using water (0.5 mL/min) as eluant. The sugars were identified by their retention times vs. pure standards and quantified by integrating peak areas. Three replicate flowers were used in each sampling date. Means of sugar contents were compared using ANOVA.

Measurement of ethylene and CO₂ production in flowers. One flower was sealed in a 125-mL Erlenmeyer flask and kept at 25 °C. After 2 h, 1-mL gas samples were withdrawn with a hypodermic syringe for ethylene determination. Ethylene content in 1-mL gas samples was measured using a gas chromatograph equipped with an activated alumina column fitted with a flame ionization detector. Carbon dioxide concentration was determined using an infrared gas analyzer. Ethylene and CO₂ production rates of three replicate flowers were calculated and were compared using ANOVA.

Results and Discussion

Both BA and sucrose pulse treatments prolonged vase life, inhibiting *Eustoma* senescence (Table 1). BA also stimulated ethylene production, whereas sucrose suppressed it (Fig. 1). When cut *Eustoma* flowers were placed in a sucrose solution, ethylene production in the initial stages of vase treatment was significantly lower than that of flowers in BA or control solutions (Fig. 1). Therefore, the improved vase life of BA-treated flowers cannot be attributed to suppression of ethylene production. Cytokinins may have a complex role in delaying senescence, probably acting on different systems. When cut *Eustoma* flowers were treated with BA pulses before or after sucrose, or in BA + sucrose, the effectiveness on longevity was: BA before sucrose > BA + sucrose = BA after sucrose (Table 1). Furthermore, flowers treated with both BA and sucrose (double pulse) were better in maintaining longevity than those treated with BA or sucrose. How-

Table 1. Effects of a BA pulse at 50 mg·L⁻¹, 4% sucrose, or both on longevity of *Eustoma* flowers. BA and/or sucrose were pulsed for 24 h, respectively, and then transferred to water. All solutions contained HQS at 200 mg·L⁻¹. Data are means of 16 replications ± SD.

Treatment	Flower longevity (days)
Control	7.1 ± 0.2 d ^a
BA→water	10.2 ± 0.4 c
Sucrose→water	10.5 ± 0.3 c
BA→sucrose→water	15.8 ± 0.4 a
Sucrose→BA→water	12.2 ± 0.4 b
BA + sucrose→water	12.5 ± 0.5 b

^aMean separation within column by Duncan's multiple range test, *P* ≤ 0.05.

ever, ethylene production was significantly greater in double pulse treatments than in sucrose alone (Fig. 1). Ethylene production in the initial stages following the double pulse treatments was higher in BA before sucrose than in BA after sucrose. However, except for day 2, no significant difference in ethylene production was found between pulses of BA before sucrose and BA + sucrose (Fig. 1). It seems likely that the high ethylene production in double pulse treatments was due to BA rather than sucrose. CO_2 produc-

tion in controls was significantly lower than that in all other treatments (Fig. 2).

In the single pulse study, the concentrations of sucrose, glucose, and mannose were highest in sucrose-treated flowers, intermediate in BA-treated flowers, and lowest in controls (Fig. 3). Mayak and Dilley (1976) reported that kinetin was effective in delaying wilting in carnation flowers. The positive effect of kinetin was increased when sucrose was also included in the vase solution. In the double pulse study, sugar (sucrose, glucose, mannose, and ribose) levels

were not significantly different (Fig. 3), suggesting that a pulse of BA either before or after sucrose, or BA + sucrose increased sink strength in flowers and accelerated translocation of sugar from the vase solution to the flower organs. The sugar contents in florets were: BA before sucrose = BA + sucrose = BA after sucrose > sucrose > BA > controls (Fig. 3). We suggest that the relatively high levels of flower carbohydrate concentrations coupled with BA effects produced longer vase life in *Eustoma*. The data are consistent with the finding that sugars

Fig. 1. Effects of pulse treatments of BA at $50 \text{ mg}\cdot\text{L}^{-1}$, 4% sucrose, or both, on ethylene production ($\text{nL}\cdot\text{g}^{-1}$ fresh weight/h) in *Eustoma* flowers. All solutions contained HQS. Each value is the mean of three replicate samples. Vertical bars indicate sd when larger than the symbol.

Fig. 3. Effects of pulse treatments of BA at $50 \text{ mg}\cdot\text{L}^{-1}$, 4% sucrose, or both, on sugar content ($\text{mg}\cdot\text{g}^{-1}$ fresh weight) in *Eustoma* flowers. All solutions contained HQS. Each value is the mean of three replicate samples. Vertical bars indicate sd when larger than the symbol.

Fig. 2. Effects of pulse treatments of BA at $50 \text{ mg}\cdot\text{L}^{-1}$, 4% sucrose, or both, on CO_2 production ($\mu\text{L}\cdot\text{g}^{-1}$ fresh weight/h) in *Eustoma* flowers. All solutions contained HQS. Each value is the mean of three replicate samples. Vertical bars indicate sd when larger than the symbol.

such as sucrose extend the vase life of cut roses (Halevy and Mayak, 1981). Sucrose and glucose were the main carbohydrates, followed by mannose and ribose in *Eustoma* flowers (Fig. 3). In the controls, concentrations of sucrose and glucose rapidly decreased during vase life.

In conclusion, the experimental results suggest that the vase life of cut *Eustoma* flowers is improved by either BA or sucrose in vase solution, and especially when BA is pulsed before the sucrose pulse.

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