Effects of Treatment with Trehalose and Sucrose on Sugar Contents, Ion Leakage and Senescence of Florets in Cut Gladiolus Spikes

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Abstract

Effects of pretreatments with 0.4 M sucrose for 24 h and consecutive post-treatments with 0.1 M trehalose on growth and senescence of the florets on cut gladiolus spikes were examined. Sugar contents and ion leakage in the perianths of 1st, 4th and 8th florets on the spikes were determined. Pretreatment with sucrose increased sugar contents and promoted unfolding of florets. Treatments with trehalose delayed both unfolding and senescence of florets. Trehalose treatments alleviated the decrement of soluble sugars in senescing perianths. The ratio of sucrose to hexose (fructose+glucose) in the perianths gradually raised concomitant with senescence; it was remarkably increased by trehalose, suggesting that trehalose inhibited exports of sucrose from the perianths to other flower parts. Ion leakage of 4th and 8th perianths treated with trehalose was higher than that of control until 4 days after harvest (DAH), while it became significantly lower than that of control 6 DAH. In marginal parts of trehalose-treated perianths, ion leakage was high, and water soaked tissues and slight wilting were observed. Trehalose is effective in delaying floret senescence and combined treatment with sucrose and treatments at lower trehalose concentration is recommended to improve quality of cut gladiolus spikes.

INTRODUCTION

Growth and senescence of florets determines quality of cut gladiolus spikes. Sensitivity to ethylene in gladiolus florets is relatively low (Woltering and van Doorn, 1988; van Doorn, 2001) and STS could not extend its vase life (Yamane et al., 1993; Serek et al., 1994) although pulsing with sucrose and STS improved the quality of mini-gladiolus spikes after MA storage (Meir et al., 1995). Cycloheximide (CHI), an inhibitor of protein synthesis, delayed onset of wilting of gladiolus perianths, but it also inhibited floret opening (Jones et al., 1994; Yamane and Ogata, 1995). In cells of senescing gladiolus perianths, some features of apotosis, such as DNA fragmentation, chromatin condensation and nuclear fragmentation, were detected (Yamada et al., 2003). Recently, trehalose is reported as an inhibitor of programmed cell death of ethylene-insensitive flowers such as gladiolus (Otsubo and Inoue, 2000; Yamada et al., 2003) and tulip (Inoue and Takata, 2001). Trehalose, a disaccharide consisting of two alpha-[1,1] linked glucose units, occurs in bacteria and it improves water retention and protects membranes from various stresses (Crowe et al., 1984; Lesile et al., 1995; Otsubo and Inoue, 2000). It is well known that sucrose improves vase life of many flower species including gladiolus (Bravdo et al., 1974; Marousky, 1969; Mayak et al., 1973), while interactive effects of trehalose and sucrose on vase life is yet unclear. In a preliminary experiment, 0.1 M trehalose in the vase solution tended to delay both unfolding and senescence of florets. In order to improve quality of cut gladiolus spikes, we tried to combine 0.4 M sucrose pretreatment and 0.1 M trehalose post-treatment in this study. Effects of the sugar treatments on sugar contents, ion leakage, unfolding and senescence of gladiolus florets were examined.

MATERIALS AND METHODS

Spikes of gladiolus (*Gladiolus* × *grandiflorus* 'Fujinoyuki') were harvested when a perianth emerged from the bract in the first florets on spikes. Cut spikes with 16-18 florets were held in 300 ppm 8-hydroxyquinoline sulfate (8-HQS) solution with or without 0.4 M

sucrose for 24 h as pretreatment and then continuously held in 8-HQS solution with or without 0.1 M trehalose as post-treatment. The state of growth and senescence of florets was evaluated under 20 ˚C, RH 60-80% and 15 mol m^{-2} s^{-1} of fluorescent lights. Developmental stages of florets were defined by the following 4 stages; folded perianths emerged out of bracts; half-unfolded perianths; fully unfolded perianths and wilting perianths.

Perianths of 1st, 4th and 8th florets on the spikes were sampled at 0, 2, 4, 6 and 8 days after harvest (DAH). Perianths were weighed, lyophilized and weighed again. Soluble sugars were extracted with 80 % ethanol and aliquots of the extracts were analyzed by HPLC (Shimadzu, SCL-6A) with a TSK gel Amide-80 column (Tosoh). Ion leakage of outer perianth tissues was determined by the method of Yamane et al. (1993). In the trehalose-treated perianths, ion leakage of marginal and center parts of perianths were determined separately.

Data were analyzed by ANOVA using a software (StatView Ver.5.0 for Mac, SAS Institute Inc. Cary, NC, USA). Fisher's PLSD test was used to compare means.

RESULTS

Pretreatment with 0.4 M sucrose significantly increased the total number of growing of florets 2 DAH, and significantly increased number of fully unfolded florets but could not delay floret wilting 6 DAH (Fig. 1). Trehalose post-treatments with or without sucrose pulsing significantly increased number of folded florets and decreased number of fully unfolded florets 2 DAH (Fig. 1), while they significantly decreased number of wilting florets and increased that of fully unfolded florets 6 DAH (Fig. 1). The trehalose treatment in combination with sucrose significantly increased total number of developing florets and fully unfolded florets 6 DAH (Fig. 1). Total number of developed florets on spikes treated with trehalose alone was lowest among the treatments 8 DAH (Fig. 1).

Trehalose treatments slightly decreased fresh weight of perianths in 1st florets, but maintained it higher at senescing stage between 6 and 8 DAH (Fig. 2). First florets without trehalose treatment were completely wilted 8 DAH and therefore their data are not shown. In 8th florets, sucrose pulsing significantly increased fresh weight of perianth 4 and 6 DAH, while trehalose tended to decrease it until 6 DAH (Fig. 2).

The sucrose pretreatment significantly increased contents of total soluble sugar (TSS) per perianths until 4 DAH in 1st and 4th florets (Fig. 3). The trehalose treatment alleviated the decrement of TSS contents in the perianths in 1st and 4th florets (Fig. 3). The sucrose pretreatments significantly promoted uptake of trehalose into the perianths (Fig. 4). And trehalose treatments significantly increased sucrose contents in florets 6 and 8 DAH (Fig.4).

Ratio of sucrose to hexose (fructose+glucose) in perianths gradually raised concomitant with senescence; the ratio in trehalose-treated perianths with or without sucrose pulsing significantly increased after 4 DAH (Fig. 5). The ratio in control florets was less than 0.2 until 6 DAH but that in 1st and 4th florets treated with trehalose raised to more than 0.5 at the final day (Fig. 5).

Ion leakage of perianths in control florets gradually increased until 4 DAH and sharply increased at 6 DAH (Fig. 6). Ion leakage of central parts in 4th and 8th perianths treated with trehalose was relatively higher than that of control until 4 DAH, while it became significantly lower than that of control 6 DAH (Fig. 6). In marginal parts of trehalose-treated perianths, ion leakage was higher (Fig. 6), and water soaked tissues and slight wilting was observed (data not shown).

DISCUSSION

The facts that trehalose treatments with or without sucrose pulsing maintained fresh weight of perianths higher during senescing stage and delayed wilting of perianths (Fig. 2) support the finding that trehalose delayed senescence of perianths (Otsubo and Inoue, 2000; Yamada et al., 2003). Trehalose significantly decreased fully unfolded florets 2 DAH (Fig. 1) and slightly decreased fresh weight of perianths (Fig. 2), suggesting that trehalose

also delayed growth of gladiolus florets. Pretreatment with sucrose promoted floret unfolding and significantly increased fully unfolded florets (Fig. 1) just as the reports that sucrose promoted growth of florets and improved quality of cut gladiolus spikes (Bravdo et al., 1974; Marousky, 1969; Mayak et al., 1973). Trehalose and sucrose synergistically increased TSS levels of gladiolus perianths through growth and senescing (Fig. 3 and 4), resulting in delayed onset of wilting (Fig. 1). Since total number of developed florets was significantly lower in spikes treated with trehalose alone (Fig. 1), we recommend trehalose treatment in combination with sucrose to improve the quality of cut gladiolus spikes.

The ratio of sucrose to hexose (fructose+glucose) remarkably increased in the trehalose-treated perianths 4 DAH (Fig. 5). In wilting perianths, invertase activity become lower (Halaba and Rudnicki, 1989; Yamane et al., 1991) and the ratio of sucrose to hexose become higher and can be exported to other plant parts (Yamane et al., 1993; 1995). These results suggest that trehalose could not maintain invertase activity in perianths but inhibit exports of sucrose from the perianth to other parts of the spikes.

Ion leakage of perianths in control florets gradually increased until 4 DAH and sharply increased at 6 DAH (Fig. 6). Ion leakage of central parts in 4th and 8th perianths treated with trehalose was relatively higher than that of control until 4 DAH, while it was significantly lower than that of control 6 DAH (Fig. 6). These results suggest that trehalose partially could increase ion leakage until unfolded stage but could maintain membrane integrity during wilting stage. Bakaltcheva et al. (1994) reported that trehalose protects biological membranes, such as the plasma membrane and tonoplast, under drought stress. Trehalose may reduce loss of water and soluble sugars of perianth tissues through protecting its membranes at senescing stage. However, ion leakage was higher (Fig. 6) and water soaked tissues were observed in marginal parts of trehalose-treated perianths (data not shown). By sugar analysis, trehalose concentration in marginal parts of perianths was higher than that of central parts of perianth (data not shown). These results suggest that trehalose was not metabolized and accumulated in perianth margin, thus water soaked symptom could occur just as Otsubo and Inoue (2000) reported that trehalose maintained gladiolus florets at slightly wilting stage. Since Inoue and Takata (2001) reported that 50 mM trehalose delayed the senescence of tulip tepals, lower concentration or shorter period of trehalose treatments may reduce this symptom.

Since senescence of ethylene-insensitive flower petals is genetically programmed, it is difficult to delay that; there is almost no effective and commercial inhibitor. CHI delayed the senescence of gladiolus florets but it also inhibited the growth and opening of upper florets on spikes (Jones et al., 1994; Yamane and Ogata, 1995). Trehalose is safer than CHI and delaying effects of florets development was much less than that of CHI (Otsubo and Inoue, 2000). In wilting perianths, their browning seemed to be alleviated by trehalose (data not shown). Thus, trehalose treatment seems to be effective in delaying floret senescence, and it in combination with sucrose will improve quality of gladiolus spikes. However, trehalose should be applied carefully at lower concentration so as not to induce slight wilting of perianths.

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Figures

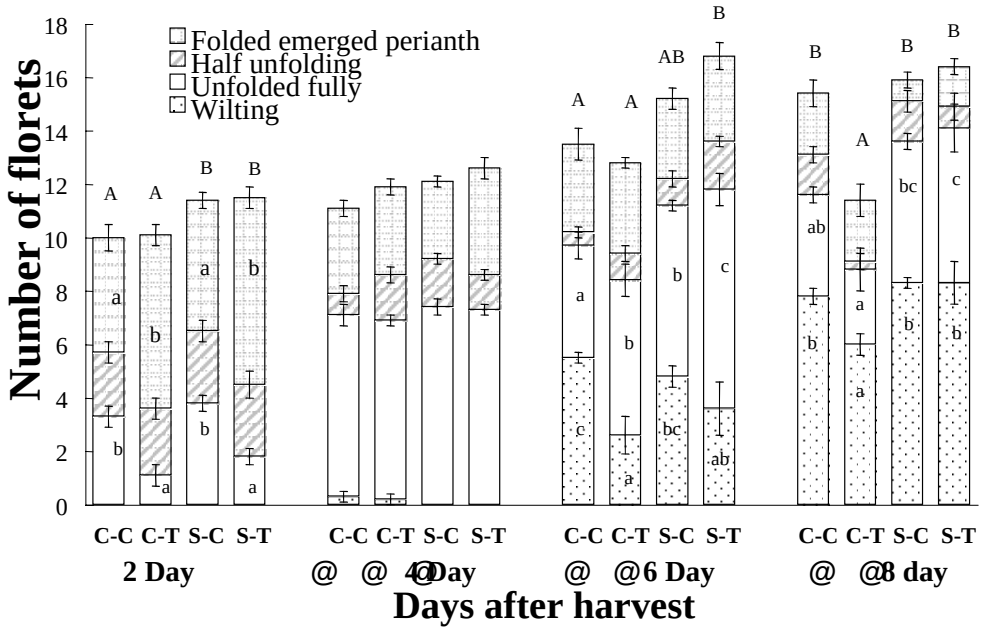

Fig 1. Effects of pre-treatment with 0.1 M sucrose (S) for 24 h and continuous post-treatment with 0.1 M trehalose (T) on development of florets on cut gladiolus spikes. C: Control solution contained 300 ppm 8-HQS. Mean±SE. Mean separation within treatments by Fisher's PLSD test ($P<0.05$). Lowercase letters show the difference in numbers of each stage; Capital letters show the difference in total numbers of all stages.

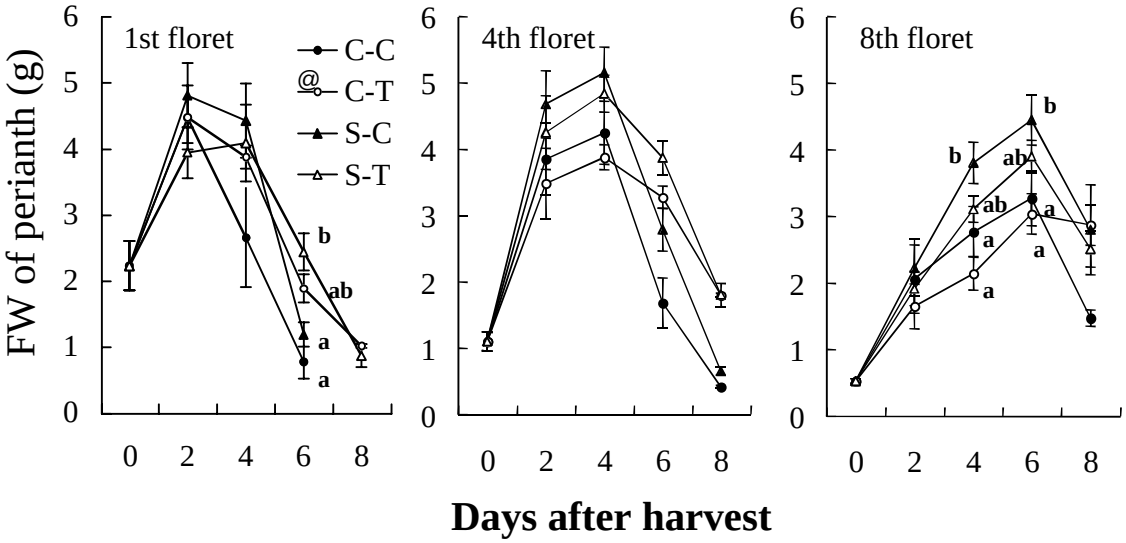

Fig. 2. Effects of pretreatment with 0.1 M sucrose (S) for 24 h and continuous post-treatment with 0.1 M trehalose (T) on fresh weight of perianth of florets on cut gladiolus spikes. C: Control solution contained 300 ppm 8-HQS. Mean±SE. Mean separation within treatments by Fisher's PLSD test ($P<0.05$).

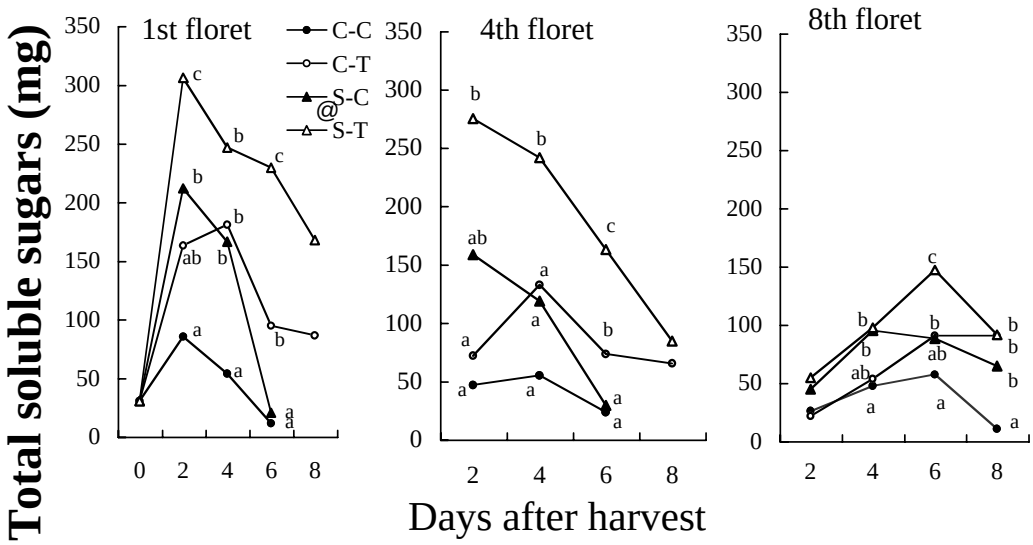

Fig. 3. Effects of pretreatment with 0.4 M sucrose (S) for 24 h and continuous post-treatment with 0.1 M trehalose (T) on contents of total soluble sugars per perianth in florets on cut gladiolus spikes. C: Control solution contained 300 ppm 8-HQS. Mean separation within treatments by Fisher's PLSD test ($P<0.05$).

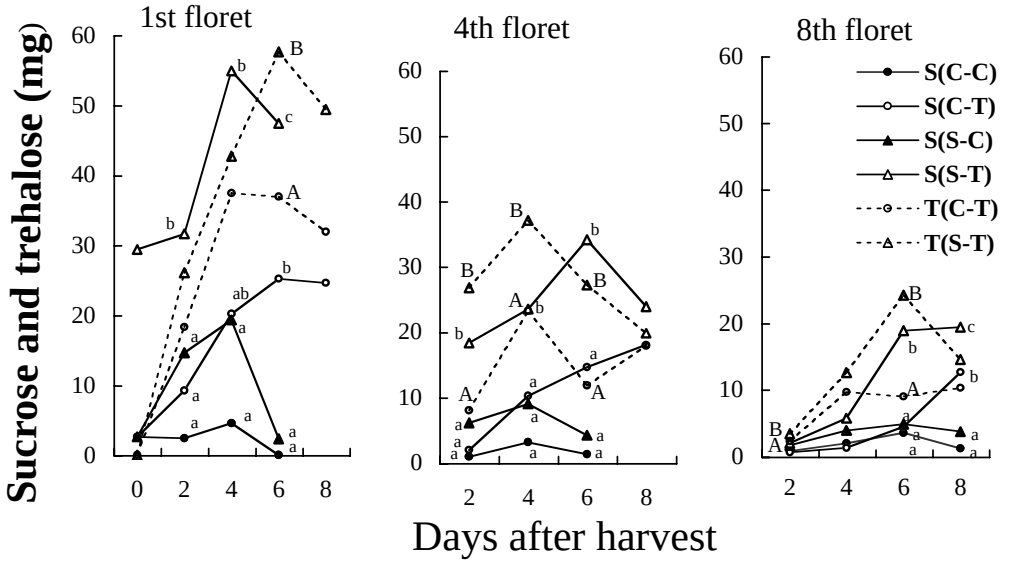

Fig. 4. Effects of pretreatment with 0.4 M sucrose (S) for 24 h and continuous post-treatment with 0.1 M trehalose (T) on sucrose (line) and trehalose (dotted line) per perianth of florets on cut gladiolus spikes. C Control solution contained 300 ppm 8-HQS. Mean separation within treatments by Fisher's PLSD test ($P<0.05$). Lowercase letters show the difference in sucrose; Capital letters show the difference in trehalose.

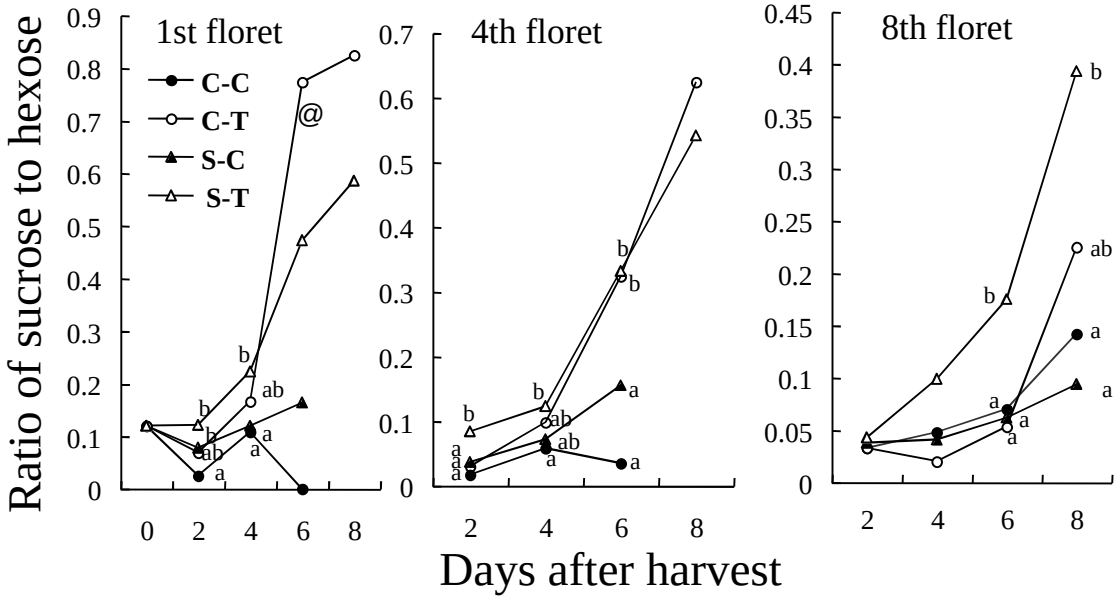

Fig 5. Effects of pretreatment with 0.4 M sucrose (S) for 24 h and continuous post-treatment with 0.1 M trehalose (T) on the ratio of sucrose to hexose (fructose + glucose) in perianth of florets on cut gladiolus spikes. C: Control solution contained 300 ppm 8-HQS. Mean separation within treatments by Fisher' s PLSD test ($P<0.05$).

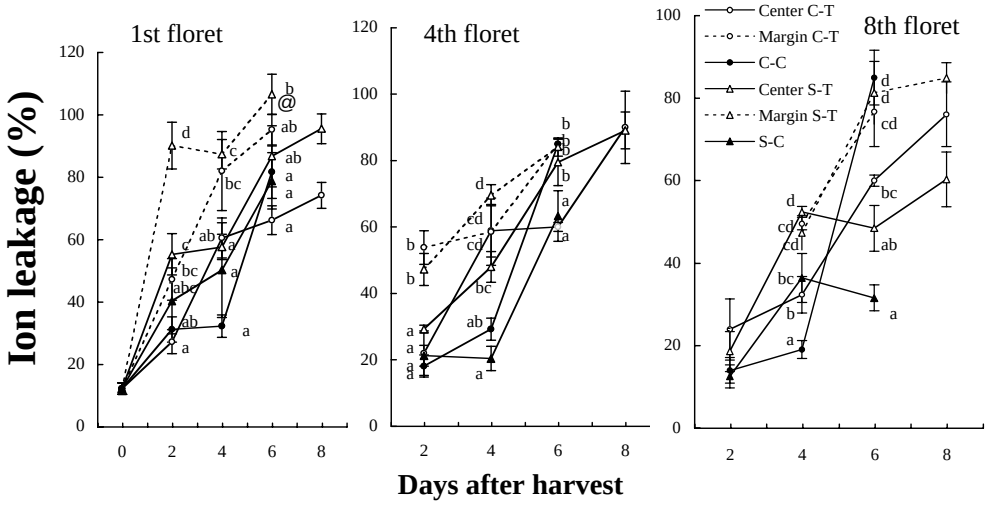

Fig. 6. Effects of pretreatment with 0.4 M sucrose (S) for 24 h and continuous post-treatment with 0.1 M trehalose (T) on ion leakage of perianth of florets on cut gladiolus spikes. C: Control solution contained 300 ppm 8-HQS. Mean±SE. Mean separation within treatments by Fisher's PLSD test ($P<0.05$).