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Comparison of the dynamics of non-structural carbohydrate pools in cut tulip stems supplied with sucrose or trehalose

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ABSTRACT

The postharvest life of many cut flowers can be extended by adding sucrose to the vase solution. Recent reports indicate that trehalose, a non-reducing disaccharide consisting of two glucose molecules, is also effective in increasing the flower longevity in cut flower stems. To understand the physiological basis of trehalose on tulip, we investigated the changes in non-structural carbohydrate pools during the postharvest phase of cut tulip (*Tulipa gesneriana* L. cv. Ballerina) stems treated with sucrose or trehalose. Cut tulip stems were placed in water, 30 mmol L^{-1} and 60 mmol L^{-1} sucrose, or 30 mmol L^{-1} trehalose solutions, and kept at 22 °C for evaluation. Stems were harvested at 3-d intervals and non-structural carbohydrates in tepals and leaves were analyzed. Sucrose and trehalose increased flower longevity by 18–37%, depending on the experiment. Sucrose supplied in the vase solution increased the concentrations of glucose, fructose and sucrose in both leaves and tepals. When whole stems with leaves were used, exogenous trehalose caused trehalose accumulation in leaves (as high 80 g kg^{-1}) and to a much lesser extent in tepals (less than 10 g kg^{-1}). However, tepals of flowers with only a short stem (10 cm) accumulated trehalose up to 50 g kg^{-1}. Trehalose also exerted significant changes in other soluble sugars, notably increased concentrations of sucrose and fructose. The tips of trehalose-treated leaves became dry, and the trehalose concentration at the tip of the leaf was much higher compared to the base of the leaf.

1. Introduction

Developing flowers in cut flower stems are active sink organs that require a continuous supply of carbohydrates as an energy source, as building blocks for cell wall synthesis and to maintain osmotic potential. Photosynthesis in leaves of cut flowers under low light levels in interior environments is very limited and does not provide enough carbohydrate for proper opening and longevity of flowers. Thus, an exogenous supply of carbohydrates is usually beneficial in maintaining quality and longevity of cut flower stems. Common commercial preservative solutions typically contain a soluble carbohydrate source in addition to an acidifying agent (to improve water uptake), and a germicide (to prevent microbial growth).

The beneficial effects of exogenous sucrose have been documented in many cut flower species. These effects range from increased flower bud or floret (in the case of inflorescences) opening (Han, 1992; Kuiper et al., 1995; Ichimura and Hisamatsu, 1999; Su et al., 2001), delayed senescence of individual flowers or florets (Eason et al., 1997; Ichimura and Suto, 1999; Liao et al., 2000), increased flower size (Eason et al., 1997), increased intensity of petal color (Eason et al., 1997; Han, 2003), and suppression of ethylene production (Ichimura and Hisamatsu, 1999; Ichimura and Suto, 1999).

Recent research has indicated that trehalose (α-D-glucopyranosyl-(1 → 1)-α-D-glucopyranoside), a non-reducing disaccharide composed of two glucose units, may also be effective in extending flower longevity in cut stems. Trehalose is ubiquitous in bacteria, yeast, fungi, insects and invertebrates, but only occurs extremely rarely in higher plants (Muller et al., 1995). When included in the vase solution, trehalose extended the vase life of gladiolus spikes (Otsubo and Iwaya-Inoue, 2000) and tulip flowers (Iwaya-Inoue and Takata, 2001). It was suggested that trehalose-treated petals were able to retain water for a longer time, thereby delaying wilting and abscission. In addition, trehalose treatments decreased nuclear fragmentation in gladiolus petals, indicating that it may exert suppressive effects on apoptotic cell death (Yamada et al., 2003). However, trehalose treatment caused negative effects in other plant tissues such as increased wilting and leaf chlorosis in tulip (Iwaya-Inoue and Takata, 2001) and drying of bract edges in gladiolus (Otsubo and Iwaya-Inoue, 2000). These observations suggest either tissue

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sensitivity for trehalose is different between petals and leaves or trehalose is preferentially accumulated in leaves, thereby leading to injury.

Given the significant improvements in flower longevity caused by trehalose, which is not an endogenous sugar in most plants, and apparent differences between petals and leaves in terms of its toxicity, it was of interest to see the distribution of trehalose in different tissues of cut flower stems once it is taken up from the vase solution. This information will also provide some clues on how trehalose is translocated within the cut stem. The uptake, translocation and distribution of exogenous sucrose in cut flower stems have been studied in detail (Sacalis and Durkin, 1972; Kaltaler and Steponkus, 1974; Ho and Nichols, 1975; Sacalis and Chin, 1976; Chin and Sacalis, 1977) and will provide a basis to compare with trehalose. In this paper, we report a comparison of the dynamics of non-structural carbohydrate pools during the postharvest phase in tepals and leaves of cut tulip stems supplied with sucrose or trehalose in the vase solution.

2. Materials and methods

2.1. Plant materials

Tulip (*Tulipa gesneriana* L. cv Ballerina) bulbs were received from a commercial source, and held dry at 17 °C until planting. Bulbs were planted in crates filled with a commercial potting mix, and subjected to precooling for 16 weeks. Precooling began at 9 °C and gradually dropped to 0.5–1.0 °C as rooting and sprouting progressed. Then, plants were transferred to a glass greenhouse, and forced at 17 °C in natural sunlight using standard cultural practices.

2.2. Postharvest treatments, evaluation and tissue sampling

Tulip stems were harvested when the buds showed full color and were within 0.5 d of opening. Uniform and healthy stems were selected and the stems were cut about 1 cm above the base plate. Stems were recut in water before placing in vase solutions. In the first experiment (whole stem experiment), whole stems with four intact leaves were used. The average length of a stem was 25 cm. In the second experiment (flower-only experiment), flower buds with stems cut to 10 cm in length were used (no leaves). The stems for the two experiments came from two different batches of plants (the flower-only experiment was conducted 2 weeks after the whole stem experiment). Stems were placed individually in jars containing 400 mL of solution. Vase solutions (treatments) in the whole stem experiment consisted of distilled water (control), or 30 mmol L^{-1} sucrose or trehalose. Vase solutions in the flower only experiment were distilled water, 30 mmol L^{-1} and 60 mmol L^{-1} sucrose, or 30 mmol L^{-1} trehalose. All solutions were prepared with distilled water. Stems were kept in a postharvest evaluation room maintained at 22 °C with 15 μmol m^{-2} s^{-1} of light from cool white florescent lamps (12 h/d). Eight replicate stems were used for weight or longevity determinations. Flower stems were monitored daily to determine flower longevity. A flower was considered senesced when more than half of the tepal area became severely wilted. Eight replicate stems were used for longevity determination.

Harvests were made on day 3 and day 6 for weight measurements and carbohydrate analyses. The tepals of each flower were individually collected in both experiments, and the second leaf (from the top) was collected in the whole stem experiment. To determine carbohydrate distribution along the leaf, four replicate leaves of the second leaf (from the top) was harvested on day 3 and sectioned into five parts of equal length along the longitudinal axis. Sections were numbered from the base, i.e., S1 was the basal section, and S5 was the distal section. Fresh weight was recorded, and tissues were immediately frozen in liquid nitrogen and stored at −80 °C until freeze-drying. After freeze-drying, the dry weight was recorded, and tissues were ground to a powder for carbohydrate extraction. There were eight replicate stems for weight determinations, and four replicates for carbohydrate analyses.

2.3. Carbohydrate extraction and analysis

Tissue samples (50 mg) were extracted at 70 °C with 80% ethanol (three extractions of 3 mL each, 1 h per extraction). Tissue suspensions were centrifuged at 4000 × *g* for 10 min after each extraction, and the supernatants were pooled. The extracts were passed through ion exchange columns consisting of 1 mL each of Amberlite IRA-67 (acetate form) (Sigma) and Dowex 50W (hydrogen form) (Sigma) to remove charged material. The extracts were evaporated to dryness at 55 °C, and dissolved in 10 mL of HPLC-grade water. After appropriate dilution, samples were subjected to high-performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD) with a Dionex DX-500 series chromatograph, equipped with a Carbopac PA-1 column, a pulsed amperometric detector and a gold electrode (Dionex, Sunnyvale, CA, USA). Carbohydrates were eluted with 200 mmol L^{-1} NaOH at a flow rate of 16.7 μL s^{-1} for 15 min at about 11 MPa. The amounts of glucose, fructose, sucrose, and trehalose in chromatograms were determined by comparison with calibration curves derived from standard authentic sugars. Results are expressed on a dry weight basis.

The residue after ethanol extraction was dried in an oven at 55 °C overnight to remove ethanol. The residue was then boiled for 30 min in 4 mL of 100 mmol L^{-1} Na-acetate buffer (pH 4.5) to gelatinize starch. After cooling, 50 U of amyloglucosidase (in 1 mL Na-acetate buffer, above) were added to each sample and incubated for 48 h at 55 °C. The amount of glucose released was determined by HPAEC-PAD using an aliquot of the digested sample and starch was estimated according to the amounts of glucose released.

2.4. Statistical analysis

Analysis of variance was performed to detect treatment effects, and Duncan's Multiple Range test was performed for mean comparison using SAS software.

3. Results

3.1. Postharvest performance of tulip stems treated with sucrose or trehalose

The longevity of flowers was improved by both sucrose (ca. 30–35% increase) and trehalose (ca. 18–30% increase, Table 1). The effect of trehalose on flower longevity was more pronounced in the flower only experiment compared to the whole stem experiment. Tepal dry weight in control stems increased from day 0 to day 3, then decreased by day 6. Either exogenous sugar prevented this decline in dry weight, but sucrose was more effective than trehalose. The amount of water per unit of dry weight followed the same pattern as the dry weight (increased by day 3, and decreased thereafter). Trehalose was particularly effective in retaining water in tepals on day 6.

In both experiments, the visual intensity of the tepal color of flowers treated with sucrose or trehalose was greater compared with the controls. Phytotoxicity was observed in all leaves of trehalose-treated stems in the whole stem experiment, where leaf tips became dry and crispy-brittle beginning from around day