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# Sucrose treatment enlarges petal cell size and increases vacuolar sugar concentrations in cut rose flowers

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ABSTRACT

Treatment with sucrose promoted petal growth associated with flower opening in cut roses. We investigated the effect of sucrose treatment on cell size and subcellular concentration of soluble carbohydrates in petals of cut rose cv. Sonia flowers. Petals of sucrose-treated flowers, but not control flowers, markedly curved outward, resulting in complete reflection. Petal fresh weight (FW), petal area, and adaxial epidermal cell size in the control flowers increased with time, and treatment with sucrose accelerated this increase, indicating that sucrose promotes petal cell expansion. Glucose, fructose, sucrose, methyl glucoside, and xylose were detected in the petals. In the petals of control flowers, concentration of these carbohydrates, except fructose, decreased. Sucrose treatment markedly increased glucose and fructose concentrations in petals. Estimation of subcellular volumes based on transmission electron micrographs showed that volume of cell walls and vacuoles in the petals of control flowers increased in response to sucrose treatment. Sucrose treatment increased glucose and fructose concentrations in the vacuole and glucose, fructose, and xylose concentrations in the apoplast. We concluded that sucrose treatment increases glucose and fructose concentrations in the vacuole, which may reduce the osmotic potential of the symplast and increase water uptake leading to cell expansion during flower opening.

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## 1. Introduction

The vase life of cut roses is often short. The cut flowers wilt and the floral axis becomes bent just below the flower head, a condition called bent-neck. Development of such symptoms is considered to be caused by vascular occlusion, which inhibits water supply to flowers (Mayak et al., 1974; De Stigter, 1980). This explanation is supported by findings that continuous treatments with germicides, such as chlorine dioxide (Macnish et al., 2008), dichloroisocyanuric acid (Jones and Hill, 1993), 8-hydroxyquinoline sulfate (8-HQS) (Burdett, 1970; Ichimura et al., 1999a), and silver nitrate (Ohkawa et al., 1999), extend the vase life of cut roses. However, the petals do not grow sufficiently, resulting in incomplete reflection in cut rose flowers treated with germicides (Ichimura et al., 1999a). In contrast, combined treatment with sugars, including sucrose and glucose, and germicides promotes petal growth, leading to marked increase in flower diameter in cut roses (Ichimura et al., 2003). For many other cut flowers with many flower buds, including *Eustoma* (Shimizu-Yumoto and Ichimura, 2010), gypsophyla (Farnham et al., 1978), and snapdragon (Ichimura and Hisamatsu, 1999), treatments with sugars have been shown to promote flower opening and extend vase life (Halevy and Mayak, 1979; Pun and Ichimura, 2003). The positive effects of sugars on vase life are because of supplementation of carbon sources as substrates for respiration and osmotica that are required for petal growth (Halevy and Mayak, 1979).

Petal growth during flower opening is mainly due to cell expansion (Koning, 1984; Norikoshi et al., 2013; Yamada et al., 2009b). Accumulation of osmotica is required for cell expansion. In rose, soluble carbohydrates accumulate in the vacuole of petal cells, thereby reducing the osmotic potential of the symplast during flower opening (Yamada et al., 2009a). This reduction may facilitate water uptake, leading to cell expansion.

Although application of sugars promotes flower opening and extends the vase life of cut flowers, it remains unclear how applied sugars affect cell expansion and subcellular carbohydrate concentrations. Yamada et al. (2009a) developed a method using nonaqueous fractionation and infiltration for analysis of the subcellular distribution of carbohydrates. Subcellular carbohydrate concentrations can be determined accurately using this method.

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In the present study, we investigated the cell size of petals in cut rose flowers to determine whether applied sucrose promotes cell expansion during flower opening. We also investigated the effect of applied sucrose on subcellular carbohydrate concentrations to elucidate its effect on vase life.

## 2. Materials and methods

### 2.1. Plant material and treatment

Roses (*Rosa hybrida* L.) cv. Sonia, harvested at normal harvest maturity (stage 2 described by Yamada et al., 2009a), were obtained from a commercial grower in Namekata, Ibaraki Prefecture, Japan. After harvest, the cut ends of the flower stems were immersed in tap water within 1 h. The cut flowers were then transported to the laboratory and used for experiments within 2 h.

Flower stems were trimmed to 40 cm and all leaves except for the upper three to five leaflets were removed. Two cut flowers were placed in each of 500-mL beakers with 500 mL of 200 mg $L^{-1}$ 8-HQS (control) or 20 g $L^{-1}$ sucrose plus 200 mg $L^{-1}$ 8-HQS. Six or eight flowers were used for each time point for experiments. The beakers were then transferred to a temperature-controlled chamber at 23 °C under 70% relative humidity. A 12-h photoperiod was maintained with 10 $\mu$mol $m^{-2}$ $s^{-1}$ irradiance from cool-white fluorescence lamps. FWs of all petals and one petal out of the three outer petals were determined on days 0, 2, 4, and 7. The three outer petals were used for electron microscopy and carbohydrate analysis.

### 2.2. Measurement of petal area

Petals were photocopied and areas of photocopied petals were measured with LIA32 image analysis software (Yamamoto et al., 2010).

### 2.3. Scanning electron microscopy

Petal adaxial epidermal cells were observed under a scanning electron microscope (SEM) (VE-7800, Keyence, Tokyo, Japan) according to the instruction manual. Fixation and scatter are unnecessary with this microscope model. The number of cells in two fields of view (404 × 302 $\mu$m) selected randomly from one petal were counted. The average area of individual cells was calculated by dividing the area of one field of view by the cell number, as described by Norikoshi et al. (2013).

### 2.4. Nonaqueous fractionation

Nonaqueous fractionation was performed using the method of Yamada et al. (2009a), which was modified from the method of Stitt et al. (1989). Petals were homogenized with a pre-cooled mortar and pestle in liquid nitrogen and the frozen powder was dried in a lyophilizer for more than four days. The dried powder was suspended in 20 mL of heptane and ultrasonicated (450D, Branson, Danbury, CT, USA) for 90 s, with alternating 5-s pulses and 15-s pauses. The suspension was then screened through an 80-$\mu$m nylon mesh and centrifuged at 2,000 × g for 10 min. The supernatant was discarded and the sediment was re-suspended in 2 mL of a tetrachloroethylene–heptane mixture (1.28 g $cm^{-3}$), and mixed well. The mixture was applied to a density gradient comprising a cushion of $CCl_4$ overlaid with a linear gradient of tetrachloroethylene–heptane mixture decreasing from 1.55 to 1.40 g $cm^{-3}$. The gradients were centrifuged at 15,000 × g for 15 h, and the content of the centrifuge tubes was removed from the top and separated into seven fractions. Each fraction was divided into two equal portions, diluted 3-fold with heptane, and centrifuged at 10,000 × g for 10 min, after which the sediments were evaporated to dryness *in vacuo* for 15 h, and assayed for markers and carbohydrates.

### 2.5. Collection of apoplastic carbohydrates by infiltration-centrifugation method

For collection of apoplastic fluids, 1 g FW of petals were cut into 5-mm squares and washed using deionized water to remove soluble proteins and carbohydrates from the cut surfaces. The petal pieces were then vacuum-infiltrated with 5 mM MES-NaOH buffer (pH 6.0) for 20 min. The petal pieces were blotted dry and placed vertically on 0.45-$\mu$m filters (Ultrafree CL, Millipore, Medford, MA, USA) in the upper compartments of disposable tubes to which 10 mM HEPES-NaOH buffer (pH 8.0) had been added to inhibit acid invertase activity. The tubes were centrifuged at 800 × g for 10 min. Soluble carbohydrate content of the resulting apoplastic fluid was determined using high-performance liquid chromatography (HPLC, JASCO, Tokyo, Japan). Recovery of apoplastic fluid was calculated by the method of Yamada et al. (2009a). The eluate was used to measure soluble marker enzyme activity.

### 2.6. Extraction and assay of NAD-glyceraldehyde-3-phosphate dehydrogenase and anthocyanin

NAD-glyceraldehyde-3-phosphate dehydrogenase (NAD-G3H; EC 1.2.1.12, cytoplasmic marker) was extracted from the dried sediments prepared using nonaqueous fractionation, and its activity was assayed as described by Yamada et al. (2009a). Anthocyanin (a vacuolar marker) was extracted from the dried sediments, and its content was determined as described by Ichimura and Hiraya (1999).

### 2.7. Carbohydrate extraction and determination

Fresh petals or dried sediments prepared using nonaqueous fractionation were immersed in 80% ethanol at 70 °C. For fresh petals, sorbitol was added to the sample as an internal standard. All samples were homogenized and centrifuged at 3,000 × g for 10 min. The pellet was re-extracted twice using 80% ethanol and the three supernatants were combined and dried *in vacuo* below 50 °C. The concentrate was dissolved in a minimum volume of water and applied to a Sep-Pak C-18 column (Millipore) with water. The eluate was separated using HPLC with refractive index detection on a Pb-loaded cation exchange column (Shodex SUGAR SP0810, Showa Denko, Tokyo, Japan) at 80 °C and eluted with water at a flow rate of 0.8 mL $min^{-1}$.

Water-soluble pectin was extracted by the method described by Terasaki et al. (2001), and uronic acid was estimated by the *m*-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen 1973).

### 2.8. Transmission electron microscopy

Petals were fixed in a mixture of 3% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for 5 h at room temperature. After washing with rinsing buffer (0.2 M phosphate buffer, pH 7.2), the samples were post-fixed in 1% osmium tetraoxide in 0.1 M phosphate buffer at 4 °C for 2 h. The samples were dehydrated in a graded alcohol series and embedded in epoxy resin. Ultrathin sections were prepared using diamond knives on SUPER NOVA microtome (LKB, Stockholm, Sweden). The sections on grids were stained with 2% aqueous uranyl acetate for 15 min followed by a lead electron staining solution for 10 min as described by Hanaichi et al. (1986). The sections were observed using a transmission electron microscope (JEM 1200EX, JEOL, Tokyo, Japan). Forty micrographs were taken for each petal stage.

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