RESEARCH NOTE

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Research Note

Analysis of sucrose metabolism during petal growth of cut roses

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Abstract

To clarify the mechanism of flower opening, we investigated sucrose metabolism in rose petals using attached and detached flowers. The petal fresh weight of sucrose-treated detached flowers was lower than for attached flowers, and hexose levels of these detached-flower petals were also lower. Invertase activities in attached flowers increased markedly during petal growth, but these activities in detached flowers decreased, even when detached flowers were treated with sucrose. These different invertase activities might be the cause of the different growth between attached flowers and sucrose-treated detached flowers. Our results suggest that inducing invertase activity in postharvest conditions might be important for the quality of some rose cultivars.

Keywords: Flower opening; Invertase; Petal growth; *Rosa hybrida*; Sucrose metabolism

1. Introduction

Roses are important cut flowers and many studies have therefore focused on their quality after harvest. Vase life mainly depends on development of adverse water relations, which results in a lack of flower opening, premature petal wilting and bending of the pedicel. Relatively little is known about the additional effects of lack of carbohydrates and sugar metabolism during flower opening, which results from expansion growth of petal cells. Sugar accumulation in petal cells is believed to be a mechanism to reduce petal water potential, promoting water influx for cell enlargement and flower opening (Ho and Nichols, 1977). Adding sucrose to cut flowers increases the levels of glucose and fructose, but has little affect on the sucrose content in petals, indicating that sucrose translocated to petals from other organs is metabolized to glucose and fructose and accumulates in petal cells (Kaltaler and Steponkus, 1974). The enzyme that metabolizes sucrose translocated from leaves to sink tissues is mainly acid invertase (β-fructosidase, EC 3.2.1.26), which is present in many higher plants in the vacuole (soluble form) and cell wall (insoluble form). Insoluble acid invertase has a role in converting sucrose into hexoses, after its translocation from the phloem to the apoplast (Roitsch and Gonzalez, 2004), and this allows hexose uptake by the petals. Soluble acid invertase also has an important biological function related to sucrose metabolism. It presumably hydrolyzes sucrose to supply hexoses necessary for cell growth and development (Tymowska and Kreis, 1998; Tang et al., 1999). We have accordingly studied changes in soluble sugars and related these to the activities of invertases during the growth of rose petals.

2. Materials and methods

Roses (*Rosa hybrida* L.) 'Febesa', locally known by the Japanese equivalent of 'Pretty Woman', were obtained from our university farm in autumn 2004 and 2005 and were used for all experiments. The flowers were harvested at three stages (Fig. 1A): ST I, sepals were closed and petals were pigmented (younger than the commercial harvesting stage); ST II, sepals were separated completely from each other; ST III, outer petals were expanded and started to bend back. For the cut flower experiments, rose buds at ST I were harvested, and their stems were recut at their peduncle under water.

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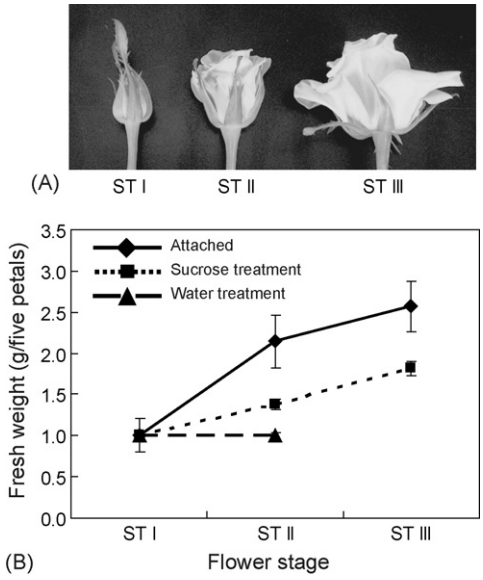

Fig. 1. Flower opening stages and petal fresh weight after treatments given to flowers at stage I. (A) Stages as observed in attached flowers. (B) Flowers were harvested at ST I, placed in water or treated with sucrose solution. The petals were sampled when the flowers had opened to ST II or III. Data refer to FW of five outer petals per flower. Values are means of seven or nine replications ±S.E.

These buds were treated with deionized water or 90 mM sucrose solution at 25 °C and 70%RH with a 16 h photoperiod (PAR; 55–90 μmol/m^2/s). Five outermost petals from each bud or flower were sampled. After their fresh weight was measured, the petals were frozen in liquid nitrogen for subsequent enzyme and sugar extractions.

Total protein was extracted at 4 °C or on ice by homogenizing frozen petals in liquid nitrogen with a mortar and a pestle. The frozen powder was mixed well in twice the volume of extraction buffer (0.1 M K phosphate buffer, pH 7.4 containing 2 mM ethylenediaminetetraacetate, 10 mM 2-mercaptoethanol and 10 mM sodium ascorbate). The mixture was centrifuged at 15,000 × *g* for 10 min, and the pellet was washed with extraction buffer and centrifuged again. This wash step was repeated twice. After centrifugation, the supernatant that contained soluble protein and the residue that contained insoluble protein were separated. Soluble proteins in the supernatant were precipitated with 80% saturated ammonium sulfate, centrifuged at 15,000 × *g* for 10 min, and then dissolved in a small volume of extraction buffer (soluble fraction). The well-washed residue, which contained insoluble protein, was mixed with a small volume of extraction buffer (insoluble fraction). Both soluble and insoluble fractions were dialyzed against dialysis buffer (10 mM K phosphate buffer at pH 7.4 containing 2 mM 2-mercaptoethanol) overnight. The invertase activity was assayed by the method of Yamada et al. (2006). The assay mixture consisted of an aliquot of dialyzed extract, 100 mM sucrose and 100 mM acetate buffer (pH 5.0) for acid invertase or 100 mM Tris–HCl buffer (pH 7.5) for neutral invertase. The amount of glucose produced in the assay mixture was measured by using the Glucose CII-Test (Wako Pure Chemical Industries Ltd., Osaka, Japan).

The soluble sugars were extracted and each sugar was measured as described in detail by Ichimura et al. (2005). The soluble sugars were separated using a high-pressure liquid chromatography system (655A-11 LC; Hitachi High-Technologies Corporation, Tokyo, Japan) equipped with a refractive index detector, a Shodex Sugar SP0810 column (Showa Denko Co., Tokyo, Japan), and milliQ water (Millipore, Milford, MA, USA) as the carrier solvent at 80 °C.

3. Results and discussion

The fresh weight of each group of five outer petals was measured soon after sampling (Fig. 1B). The petals of attached buds grew markedly from ST I to ST II and increased their fresh weight from 1.0 g/five petals ($n = 9$) at ST I to 2.6 g/five petals ($n = 7$) at ST III. The petals of sucrose-treated buds, which were detached at ST I, increased their fresh weight linearly during petal growth, but their fresh weight at ST III (1.8 g/five petals, $n = 9$) was lower than for attached flowers. The sepals of flower buds treated with water separated, but the petals did not expand or bend back, and the fresh weight of the petals did not increase (1.0 g/five petals, $n = 9$). Flower bud opening is a process of irreversible petal growth and reflection, in which existing cells expand and fresh and dry weights increase (Faragher et al., 1984; Reid and Evans, 1986; Evans and Reid, 1988). For the expansion of the cells, the osmotic potential is important to promote water influx. Thus, sugar accumulation in cells could be a mechanism to reduce petal water potential and promote water uptake. Starch hydrolysis is also important for promoting rose petal growth (Hammond, 1982), and it is suggested that flower opening may be due to a combination of sugar uptake and degradation of various polysaccharides (Van Doorn and van Meeteren, 2003). Petal starch levels at harvest are high in many cultivars, but are low in a cultivar such as 'Madelon' and can limit the rate of opening in this cultivar (Van Doorn et al., 1991). Kuiper et al. (1995) reported that adding sucrose to cut 'Madelon' rose buds induced proper flower opening, even though cut roses of this variety frequently fail to open completely under postharvest conditions.

Soluble sugars were measured at each stage of attached, sucrose-treated and water-treated flowers (Fig. 2). The main soluble carbohydrates in rose petals are fructose, glucose, sucrose and *myo*-inositol, with xylose and methyl-β-glucopyranoside as minor sugars (Ichimura et al., 1997). The levels of soluble sugars, except for *myo*-inositol, increased during petal growth in attached flowers: glucose and fructose particularly, increased about 2.6 and 2.7 times, respectively, at ST III compared with ST I. Fructose in detached buds treated with sucrose increased about 1.9 times by ST III compared with ST I, but sucrose and glucose increased only a little. Thus, the hexose levels at ST III in sucrose-treated flowers (93.0 mmol/g FW) were lower ($P < 0.05$) than in attached