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LEAVES OF CUT ROSE FLOWER CONVERT EXOGENOUSLY APPLIED GLUCOSE TO SUCROSE AND TRANSLOCATE IT TO PETALS

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ABSTRACT

To understand the role that the leaves play in the translocation of soluble carbohydrates in cut rose flowers, we first evaluated the effect of leaf removal on flower quality and the sugar content in petals. Cut rose flowers with leaves had higher soluble sugar content in petals compared with cut flower without leaves. Next, we treated cut flowers with radioactive glucose to clarify translocation routes of exogenously applied sugar. There was no significant difference between the specific radioactivity of sucrose and glucose in leaves, but specific radioactivity of sucrose in petals was much higher than that of glucose. These results suggested that most of the exogenously applied glucose first moved to the leaves, where it was converted into sucrose and then the synthesised sucrose was translocated to the petals. Our results showed that the leaves of cut rose flowers play an important role in the metabolism and transportation of exogenously applied soluble carbohydrates toward the petals, thus contributing to sustaining the post-harvest quality.

Key words: Cut flower quality, leaf function, sugar metabolism, radioactive glucose, *Rosa hybrida*

INTRODUCTION

In cut rose (*Rosa* × *hybrida*) flower stems, the leaves play an important role in maintaining rates of water uptake through their transpiration (Halevy & Mayak 1981), and removal of these leaves reduces water uptake rates and causes flowers to not fully open. Adverse water relations are associated with incomplete flower opening, premature petal wilting and bending of the pedicel in rose (Doi et al. 1999). The leaves and stems of cut flowers can also act as a source of soluble carbohydrates for the flower sink tissue. In chrysanthemum, soluble carbohydrates that accumulate in the leaves and stems were reported to affect vase life of cut chrysanthemum flower (Ishikawa et al. 2006) and Ichimura et al. (2005) reported that soluble carbohydrates accumulated in petals affect vase-life of cut roses.

The application of sugars to vase solutions improves the quality of some cut flowers, including rose (van Doorn et al. 1991; Ichimura et al. 2003). This suggests that soluble carbohydrates might act as respiratory substrates and may play a role in regulating osmotic pressure in petal cells (Ichimura et al. 2003). It is considered that sugar accumulation in petal cells reduces petal water potential, thereby promoting water influx for cell expansion, which might lead to flower opening (Ho & Nichols 1977). In addition, our previous research showed that the effect of soluble carbohydrate treatment on vase life of cut flower diminishes, if cut flower does not have leaves (unpublished data). Thus, it seems that leaves affect the metabolism and transportation of sugars applied to the cut flower.

Some reasons are conceivable as to why the effect of sugar treatment is enhanced, if cut flower has leaves. The first probable reason is that some amounts of sugars moved to petals directly from an applied solution are increased by their transpiration, leading to high soluble carbohydrate contents in petals. The second reason is that most of exogenously applied soluble carbohydrates first move to leaves by transpiration, and are then translocated to petals through phloem tissue. The distribution of exoge-

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nous carbon derived from externally applied sucrose has been studied using radioactive sucrose in cut rose flowers (Sacalis & Durkin 1972), but quantitative measurements of its movements have not been performed. In addition, the intra-plant movement of radioactive glucose applied to cut rose flowers has not been clarified.

Understanding the role of leaves of cut flowers in sugar metabolism and translocation would lead to improvement of storage conditions and to elaboration of holding solutions for prolonging the vase life.

In this study, we investigated the translocation route of exogenously applied radioactive glucose to cut rose flowers.

MATERIALS AND METHODS

Plant materials

Rose (*Rosa* × *hybrida* 'Meivildo') flower stems were harvested from a commercial nursery (Ooi farm) in Shiga Prefecture, Japan, bearing either tight flower buds (TB) or mature flower buds (MB) (commercial harvest stage). Cut flowers were transported in a dry, dark and cool conditions to our laboratory within a day.

Cut flower treatment

Flowers harvested at the TB stage were cut to 25-cm length and the stem ends were continuously placed in one of the four treatment solutions listed below to evaluate the effects of leaf removal and sugar treatment on petal fresh weight of cut flowers. Six cut flowers were sampled soon after arrival to the laboratory to measure petal fresh weight (0 day). Twelve flowers were used in each treatment and six flowers were sampled to measure petal fresh weight in each day on 2nd and 4th day). During all treatments, the cut flowers were maintained at 25 °C, 60% relative humidity and a 16 h photoperiod (photon flux density of 55-90 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The treatments were: (1) leaves were removed and the stems placed in de-ionised water (without leaves, water); (2) leaves were removed and 1% glucose added to holding solution (without leaves, 1% Glc); (3) leaves were removed, except for the upper two nodes and the cut flowers placed in de-ionised water (with leaves, water); (4) the same as variant 3 with addition of 1% glucose (with leaves, 1% Glc). Cut roses were treated with these solutions and the changes in the fresh weight of petals were recorded.

Sugar extraction

Five outermost petals from each flower were sampled, frozen in liquid nitrogen and stored at –80 °C for subsequent sugar extractions. The soluble carbohydrates were extracted and measured with liquid chromatography according to the method of Yamada et al. (2007). We calculated main soluble sugar content as the sum of sucrose, glucose and fructose. Three flowers were used to measure soluble carbohydrates in each 6 hours.

Radioactive glucose treatment

Flowers harvested at the MB stage were cut to 25-cm length and treated continuously with 2% glucose containing radioactive glucose (^{14}C glucose, 290 $Ci \cdot mmol^{-1}$, Amersham Bioscience, UK). Specific radioactivity of the treated glucose solution was 175 $cpm \cdot mg^{-1}$ glucose. Outer five petals and all leaves were taken from cut flower and frozen in liquid nitrogen, then stored at –80 °C for subsequent sugar extractions. The soluble carbohydrates were extracted and measured as above. Thirty-six flowers were used in this treatment.

Measurement of radiation levels

Glucose and sucrose fractions from leaves and petals were collected using liquid chromatography (Yamada et al. 2007). Collected fractions were dried with rotary evaporator and added to 1 ml of scintisol (Dojindo, Japan); then the amount of radiation was measured for 10 minutes by liquid scintillation counter (LSC-5100, Aloka, Japan). All leaves and five outermost petals per cut flower were used in this experiment. Four flowers were used soon after arrival to the laboratory and four flowers were used in each 6 h after treatment to measure radiation levels.

Experimental design and statistical analysis

Six TB flowers were used for fresh weight measurements. Three TB and four MB flowers were used for soluble carbohydrates measurements at every term and three flowers were used independently for measurements of radiation levels. All experiments were repeated twice. The data were subjected to analysis of variance and the significance of the differences across means were defined using Tukey's test at significance level of $p = 0.05$.

RESULTS

Petal fresh weights and total soluble carbohydrates content in tight flower buds

In all treatments, petal FW increased during the first 2 days in vase solutions and decreased during next 2 days (Fig. 1A). Cut flowers with leaves, treated with 1% glucose, maintained the significantly higher petal FW than those from three other treatments.

The amount of main soluble carbohydrates was kept higher in cut flowers with leaves compared with cut flowers without leaves (Fig. 1B). In cut flowers with leaves, main soluble carbohydrates content in petals decreased during 2 days and then increased during next 4 days but differences were not significant for time and treatment. Main soluble carbohydrates in petals of flowers maintained without leaves decreased significantly during 2 days and in the next 2 days remained constant (solution with glucose) or decreased significantly when were maintained in water

Carbohydrates content in mature flower buds

In cut flowers harvested at MB and maintained in solution containing 2% glucose, all carbohydrates contents in leaves increased constantly within 48 h (Fig. 2). In petals, each carbohydrate contents increased only after 12 h (Fig. 2B). The levels of sucrose and glucose were much lower in petals than in leaves but in fructose was the opposite; more fructose was in petals.

Amount of radiation in glucose and sucrose fractions

The amounts of radiation increased within 48 h in both glucose and sucrose fractions being more than twenty times higher in leaves than in petals (Figs. 3A, 3B). In petals, the radiation level in the sucrose fraction was significantly higher than that in the glucose fraction at 6, 36 and 48 h after treatment.

Figures 3C and 3D show changes in specific radioactivity of glucose and sucrose in leaves and

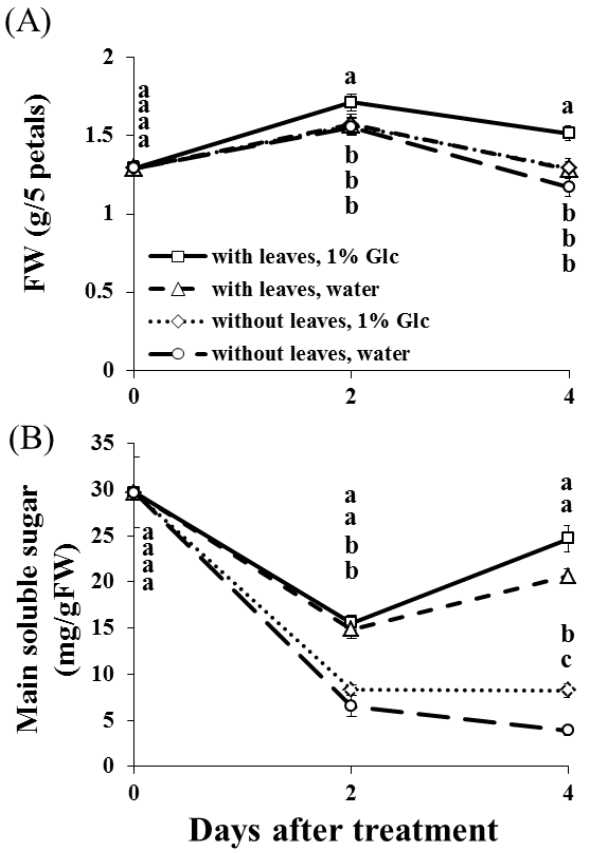

Fig. 1. Fresh weight (FW) of petals (A) and main soluble sugar in the petals (B) during flower opening of cut rose flower harvested at tight flower bud (TB) stage. Values are means of 6-12 flowers ± SE. Means followed by a different letters within each of the days after treatment are significantly different by Tukey's test at $p < 0.05$

petals. In leaves, specific radioactivity of glucose and sucrose markedly increased up to 6 h after treatment. Specific radioactivity of sucrose remained at almost the same level since 6th to 48th h but that of glucose decreased after 6 h treatment and remained at nearly the same level. In petals, specific radioactivity of sucrose was much higher than that of glucose and increased constantly up to 48 h. Specific radioactivity of glucose increased only slightly until 12 h and then remained at approximately the same low level (Figs. 3C, 3D).

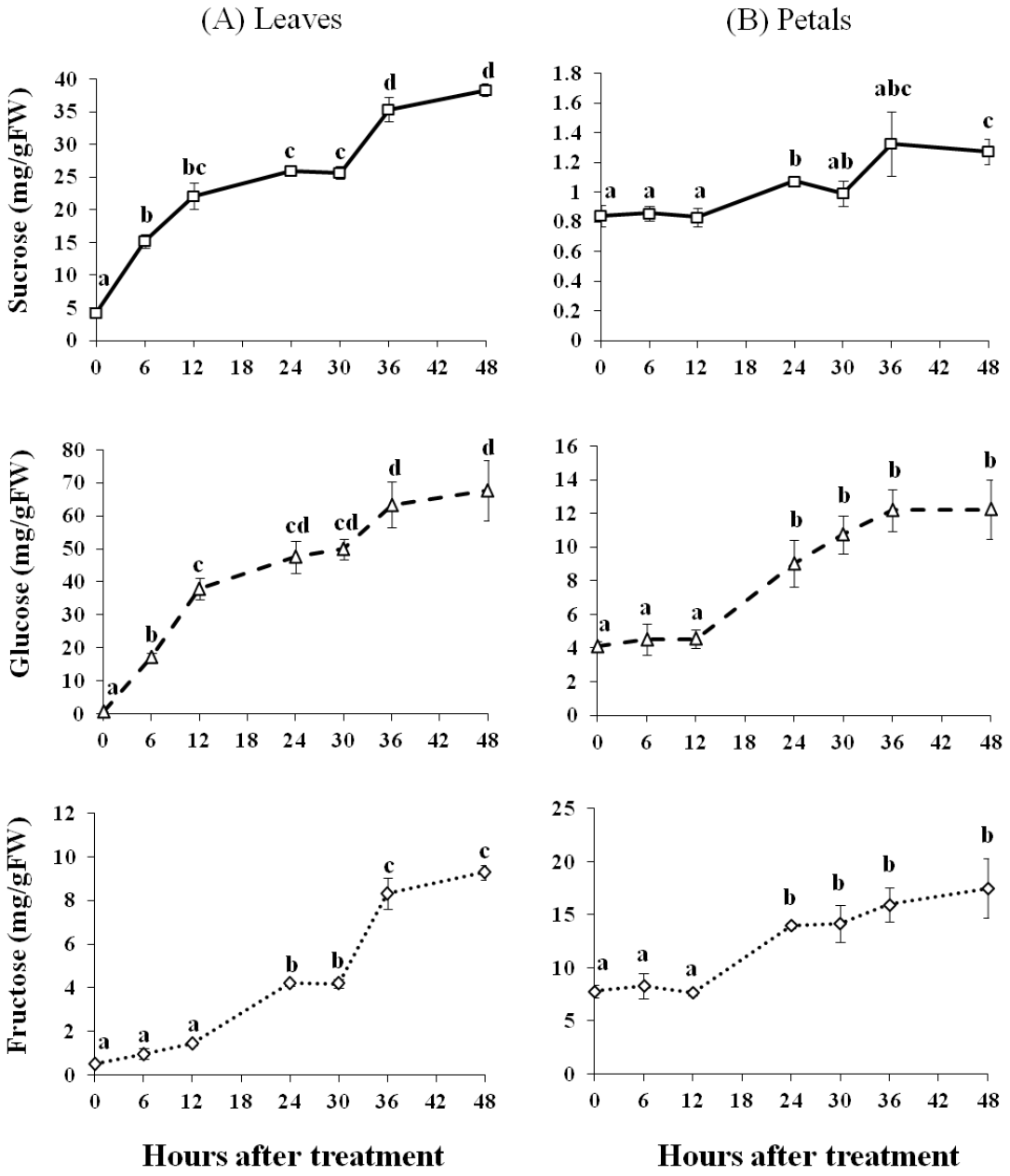

Fig. 2. Soluble carbohydrates in leaves (A) and petals (B) during flower opening. Cut flowers were harvested at mature bud (MB) stage and treated with 2% glucose. Values are means of eight flowers ± SE. Means followed by a different letters within each set of experiments are significantly different by Tukey's test at $p < 0.05$

DISCUSSION

FW of petals was higher in cut flowers with leaves than in cut flowers without leaves, indicating that leaves affect cut flowers quality. Soluble carbohydrate contents were also kept high in petals of cut flowers with leaves, while those without leaves were low even when treated with 1% glucose. Soluble carbohydrates accumulation in petal cells leads to the reduction of petal water potential and promotes water influx for cell expansion (Ichimura et al. 2003; Yamada et al. 2009). Thus, it seems that the translocation of soluble carbohydrates from leaves to petals is important for flower development of cut flower.

Cut flowers harvested at MB stage were treated with radioactive glucose to clarify sugar

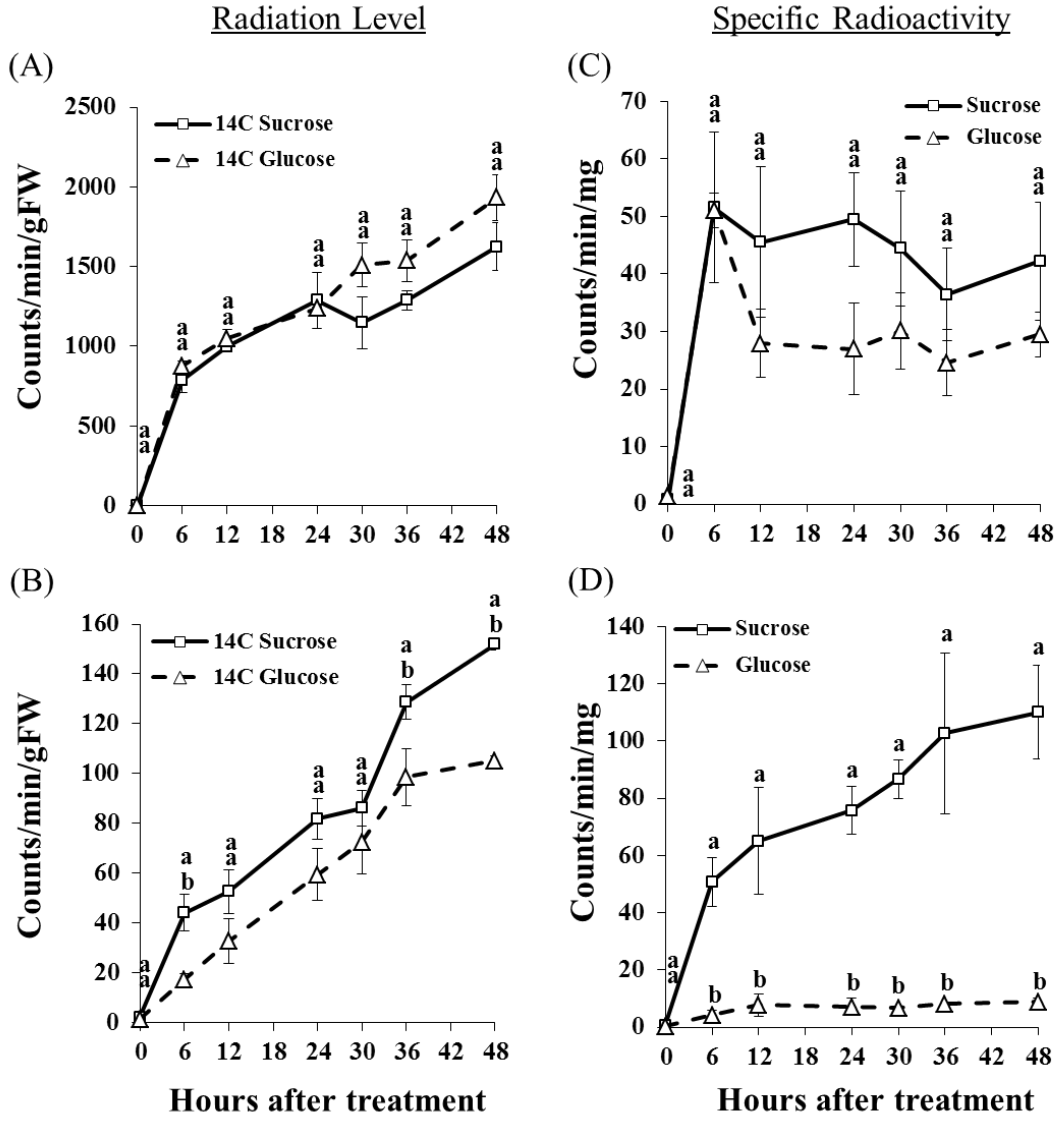

Fig. 3. Radiation levels of glucose fraction (open square) and sucrose fractions (open triangle) extracted from leaves (A) and petals (B). Specific radioactivity of glucose (open square) and sucrose (open triangle) extracted from leaves (C) and petals (D). Values are means of 6 flowers ± SE. Means followed by a different letters within each hour after treatment are significantly different by Tukey's test at $p < 0.05$

translocation routes during time when flowers are in trade conditions. A large amount of glucose was accumulated in leaves compared with petals. In addition, radiation levels in glucose and sucrose fractions extracted from leaves were much higher than those extracted from petals. These results indicate that most of the glucose moved to leaves first as reported in *Eustoma* (Shimizu-Yumoto & Ichimura 2007). Moreover, radioactive sucrose was detected in both petals and leaves. This means that leaves not only act as a source of soluble carbohydrates, but also synthesise sucrose using exogenously applied glucose.

We also compared the specific radioactivity of sugars in leaves and petals. There was no significant difference between specific radioactivity of sucrose and glucose in leaves, but specific radioactivity of sucrose in petals was much higher than that of glucose. If radioactive sucrose in petals was synthesised from radioactive glucose transported directly from the applied solution to petals, specific radioactivity of glucose should have been much higher than

that of sucrose. However, the results showed that the specific radioactivity of sucrose was nearly 10 times higher than that of glucose in petals. Some reports have shown that the activity of sucrose phosphate synthase (SPS), an enzyme which synthesises sucrose 6-phosphate from UDP-glucose and fructose 6-phosphate, was quite low in rose petals (Kumar et al. 2008). Therefore, our results suggested that most of the applied glucose is moved first to leaves where is converted to sucrose and then the synthesised sucrose is translocated to petals.

Specific radioactivity of sucrose in petals was higher than in leaves and kept increasing. We consider that radioactive glucose accumulated in the cytosol in leaf cells was used for synthesis of sucrose there, and the synthesised sucrose was transported to petals whereas the rest of it was stored in vacuoles. In vacuoles, large amounts of soluble carbohydrates containing sucrose are stored, so that the specific activity of sucrose is weakened by dilution (Yamada et al. 2009). Meanwhile in petals, the amount of accumulated sucrose is lower than that in leaves, so that translocated sucrose would not be diluted much. Therefore, we consider that specific radioactivity of sucrose in petals was higher than that in leaves and kept increasing.

In this study, we showed the importance of leaves in sugar translocation. Without leaves, cut flower cannot accumulate soluble carbohydrates abundantly in petals; even in shoots treated with glucose, FW of petals was low. Plenty of soluble carbohydrates accumulate in leaves after sugar treatment, and only a part of them seems to translocate to petals. Promoting sugar translocation from leaves to petals would improve cut flower quality and elevate the positive effects of sugar treatment. More studies are still necessary to further understand the functions of leaves of cut flowers in sugar metabolism and sucrose translocation.

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