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Effects of auxin and methyl jasmonate on cut rose petal growth through activation of acid invertase

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ABSTRACT

Petal growth associated with flower opening depends on cell expansion caused by water influx. To understand the mechanism of flower opening and to develop a method for improving cut flower quality, we investigated the changes in the amount of soluble carbohydrates and invertase activities in rose (*Rosa* 'Meivildo') petals using attached and cut flowers, and the effects of α-napthylacetic acid (NAA) and methyl jasmonate (MeJA) on cut flowers. Cut rose flowers were harvested at the tight bud stage (TB), or mature bud stage (MB), which is the commercial harvest stage, and 2 days after TB. Cut flowers were immediately treated with deionized water or 1% (w/v) glucose. Fresh weight (FW) and the levels of soluble carbohydrates in petals in cut flowers were lower under postharvest conditions than those of attached flowers during flower opening. Although invertase activities in petals of attached flowers increased drastically during TB and MB, those in the petals harvested at TB did not increase under postharvest conditions. These results suggested that sucrose metabolism, including invertase activity, could be an important factor in cut rose flower opening to a greater extent after harvest. In addition, we tried to control flower opening by affecting invertase activities in petals of cut rose flowers. Cut flowers treated with NAA opened faster and those treated with MeJA opened later than in controls. Levels of soluble carbohydrates and invertase activity in petals were also changed by these treatments. In flowers treated with NAA, activities of both vacuolar and cell wall invertases increased 1 day after treatment and then decreased through to the end of the treatment, although activities in control flowers never increased after harvested. By contrast, cell wall invertase activity in MeJA treated flowers increased 1 day later than with the NAA treatment and remained at a relatively high level until 4 days after treatment compared to the controls. Our results suggest that inducing invertase activity in postharvest conditions may important for the quality of cut roses.

1. Introduction

Understanding the mechanisms of flower opening will benefit commercial horticulture as well as plant science generally. The process of flower bud development and subsequent flower opening is related to petal growth, including cell division and expansion. In *Gaillaradia grandiflora*, cell division of the petals seems to stop at an early stage of flowering, and no increase in the number of abaxial epidermal cells has been observed (Koning, 1984). In carnation flowers, the amount of DNA in the petals does not increase once petals emerge from the calyx, suggesting that cell division also stops at an early stage (Kenis et al., 1985). These findings indicate that petal growth associated with flower opening mainly depends on cell expansion. Large amounts of soluble carbohydrates accumulate in rose petals (van Doorn et al., 1991; Ichimura et al., 2003), especially in vacuoles in the petal cells (Yamada et al., 2009a) during flower opening. As well, the application of sugars improves the quality of cut flowers (Ichimura et al., 2003), suggesting that soluble carbohydrates play a key role in regulating osmotic pressure in petal cells. It is thought that sugar accumulation in petal cells reduces petal water potential, therefore promoting water influx for cell expansion, which might lead to flower opening (Ho and Nichols, 1977).

The enzymes that metabolize sucrose translocated from leaves to sink tissues are sucrose synthase (EC 2.4.1.13) and acid invertase (β-fructofuranosidase, EC 3.2.1.26), which are present in vacuoles (soluble form) and cell walls (insoluble form) in many higher plants. Cell wall acid invertase plays a role of converting sucrose into hexoses after sucrose is translocated from the phloem to the apoplast (Roitsch and Gonzalez-Garcia, 2004), and this enables petals to take up more sucrose from the phloem. Vacuolar invertase also plays an important role in biological function associated with sucrose

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metabolism. It presumably hydrolyzes sucrose to supply hexoses necessary for cell growth and development (Tymowska and Kreis, 1998; Tang et al., 1999).

We have reported changes in the levels of soluble carbohydrates and the invertase activities during the growth of rose petals (Ito et al., 2007; Yamada et al., 2007). These studies showed that invertase activities in the petals of attached flowers increased markedly during petal growth, but not in cut flowers, even when cut flowers were treated with sucrose. On the other hand, sucrose synthase activity was very weak compared with acid invertase activities in rose petals (Kumar et al., 2008). The invertase activity seems to limit petal growth and associated flower opening. Some studies have shown that certain phytohormones affect invertase activity (Miyamoto et al., 1993; Balibrea Lara et al., 2004; Trouverie et al., 2004; Pan et al., 2006; Gonzalez-Garcia and Cejudo, 2007). Thus, we presume that the quality of cut rose flowers might be improved by controlling the invertase activity under postharvest conditions.

In our previous study, we found that NAA and MeJA could affect invertase activities of petal discs (Horibe et al., 2010). In the present study, we further investigated the role of invertase in flower opening using attached and cut flowers, and the relationship between invertase activities and the quality of cut flowers treated with these plant growth regulators.

2. Materials and methods

2.1. Plant materials

Roses (*Rosa* 'Meivildo') were harvested at a commercial nursery (Ooi farm) in Shiga Prefecture, Japan at two bud stages (Fig. 1). At the tight bud stage (TB), although their sepals were completely reflected, the petals were not yet unfolding yet. At the mature bud stage (MB), which is 2 days after TB and a commercial harvesting stage, the petals had just started unfolding. Cut flowers were transported in a dry, cool condition to the laboratory within a day.

2.2. Cut flower treatment

Soon after arrival at the laboratory, the stems, with the leaves removed except for the upper two nodes, were re-cut in water to 25 cm lengths. They were held in 1% (w/v) glucose plus 0.02% (w/v) 8-hydroxyquinoline monohydrate (Wako Pure Chemical Industries Ltd., Japan) at 25 °C, 60% relative humidity, and a 16 h photoperiod (55–90 μmol m^{-2} s^{-1}). Five outermost petals and all leaves at the upper two nodes from each flower were sampled. After their fresh weight was measured, petals were frozen in liquid nitrogen and stored at −80 °C for subsequent enzyme and sugar extractions.

2.3. Enzyme and sugar extraction and assay

Total proteins were prepared by the method of Yamada et al. (2007). 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). The amount of glucose produced in the assay mixture during a 30 min reaction was measured using the Glucose CII-Test (Wako Pure Chemical Industries Ltd., Japan). The soluble sugars in petals were extracted and measured by a liquid chromatography (LaChrom Elite, Hitachi High-Technologies Corporation, Japan) with a column (Shodex SUGAR SP0810, Showa Denko K.K., Japan) according to the method of Yamada et al. (2007).

2.4. Plant growth regulator treatments on cut flowers

Flowers harvested at TB were re-cut in water to 25 cm lengths and then held in 1% (w/v) glucose (control), 1% (w/v) glucose-containing 100 μM α-naphthylacetic acid (Wako Pure Chemical Industries Ltd., Japan), or 1% (w/v) glucose-containing 100 μM methyl jasmonate (Wako Pure Chemical Industries Ltd., Japan) at 25 °C, 60% relative humidity, and a 16 h photoperiod (55–90 μmol m^{-2} s^{-1}) in a plant growth chamber (BioTron; Nippon

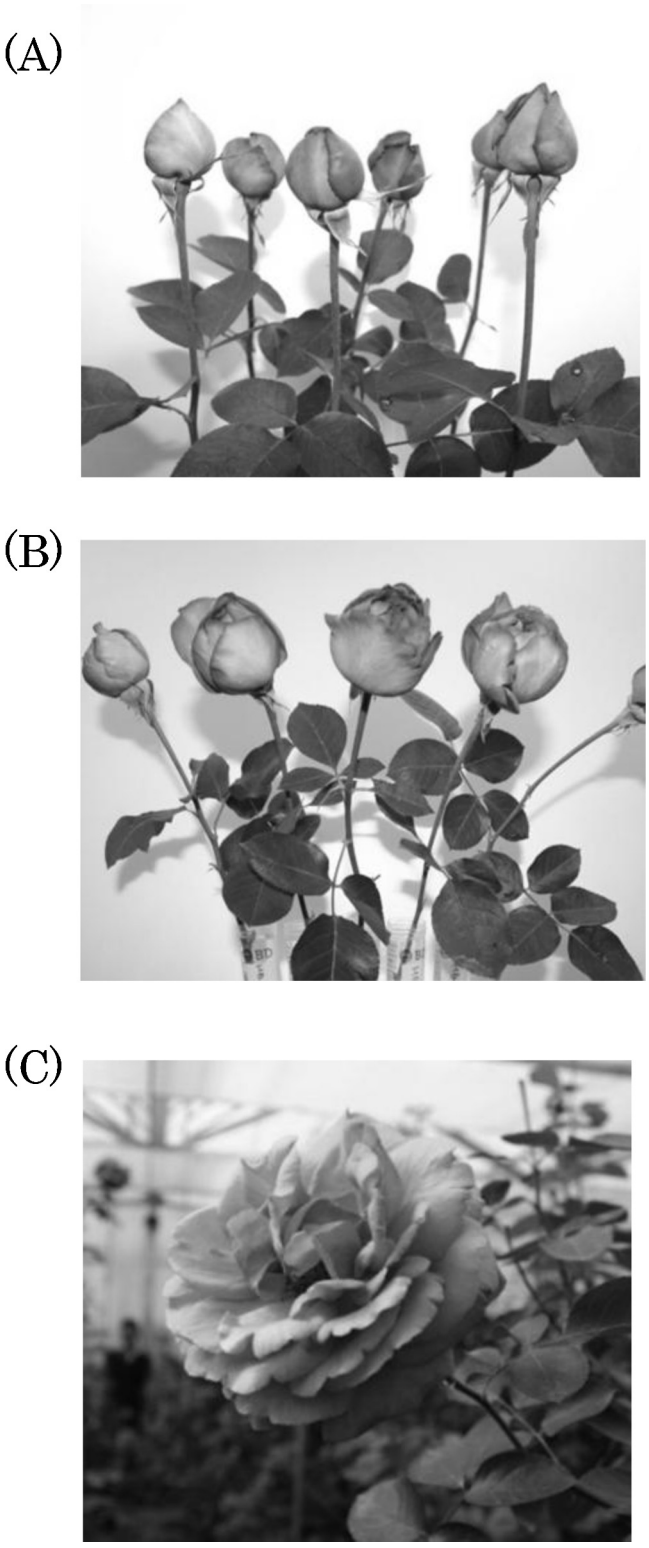

Fig. 1. Flower opening stage of *Rosa* 'Meivildo'. (A) Cut flowers harvested at the tight bud stage (TB); (B) cut flowers harvested at the mature bud stage (MB, 2 days after TB); (C) flower opened fully on the bush (6 days after TB).