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Elevated temperature inhibits anthocyanin biosynthesis in the tepals of an Oriental hybrid lily via the suppression of *LhMYB12* transcription

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ABSTRACT

High temperature often reduces the quality of anthocyanin colour in flowers, which is a problem in the commercial production of ornamental plants. Anthocyanins are the predominant pigments in flowers of the Oriental hybrid lily (*Lilium* spp.). We investigated the effects of elevated temperature on anthocyanin accumulation in the tepals of a pink-flowered cultivar Marrero. To clarify the flower stage most susceptible to elevated temperature, the flowers were divided into seven developmental stages. Potted plants with flowers at these stages were incubated at 35 °C or 20 °C for 2 days. The elevated temperature at 35 °C caused poor colouration in the tepals at stage (St) 2 and 3. At 20 °C, anthocyanin accumulation began at St 2 and anthocyanin content increased at St 3. The elevated temperature did not affect anthocyanin colouration at other stages. To clarify the mechanism for the poor colouration at St 2 and 3 at 35 °C, the transcription of *LhMYB12* (which regulates anthocyanin biosynthesis), *chalcone synthase* (*CHS*), *flavanone 3-hydroxylase* (*F3H*), and *dihydroflavonol 4-reductase* (*DFR*) as well as endogenous sugar content was evaluated in tepals. The transcription of *LhMYB12*, *CHS*, *F3H*, and *DFR* was suppressed at St 2 and 3 under the elevated temperature. The change in sugar content in the tepals at 35 °C was not correlated with the reduced anthocyanin accumulation. Thus, we conclude that elevated temperatures inhibit anthocyanin biosynthesis at St 2 and 3 via the suppression of *LhMYB12* transcription.

1. Introduction

The genus *Lilium* encompasses more than 90 species (Asano, 1989) and is classified into several sections (Comber, 1949; Smyth et al., 1989). The Oriental hybrid lily is derived from inter-specific crosses of the species of section Archelirion (Leslie, 1982). Oriental hybrid lily cultivars have been commercially predominant since the 1980s due to their outstanding flower shape and fragrance (Lim and van Tuyl, 2006). Oriental hybrid lily cultivars usually have white or pink flowers (Lim and van Tuyl, 2006). The predominant pigments in the tepals of Oriental hybrid lilies are anthocyanins; only a few cultivars accumulate carotenoid pigments. This is different from Asiatic hybrid lilies—many cultivars of which accumulate carotenoids in their tepals (Yamagishi et al., 2010a). Many Oriental hybrid lily cultivars contain cyanidin 3-*O*-β-rutinoside as a major anthocyanin and cyanidin 3-*O*-β-rutinoside-7-*O*-β-glucoside as a minor anthocyanin (Nørbæk and Kondo, 1999).

Anthocyanin colouring is commercially important to flowers and fruits. Temperature is one of the major environmental factors that affect anthocyanin accumulation; anthocyanin content is reduced under high temperatures in petunia (Shvarts et al., 1997), rose (Dela et al., 2003), kangaroo paw (Ben-Tal and King, 1997), chrysanthemum (Nozaki et al., 2006), carnation (Maekawa and Nakamura, 1977), apple (Lin-Wang et al., 2011), and grape (Mori et al., 2005). Anthocyanin concentrations increase under low temperatures in the flowers of *Plantago lanceolata* (Stiles et al., 2007) and the fruits of red orange (Lo Piero et al., 2005). The combination of cool temperature and UV irradiation stimulates anthocyanin pigmentation in apple fruits (Ubi et al., 2006). A long-term high-temperature condition applied to potted chrysanthemum resulted in pale flowers (Nozaki et al., 2006), while a short-term high-temperature condition for 3 days decreased anthocyanin concentration in potted rose by about 35% (Dela et al., 2003), indicating that anthocyanin accumulation is sensitive even to short-term exposure to elevated temperatures. The expression of anthocyanin biosynthesis genes such as *chalcone synthase* (*CHS*) and *dihydroflavonol 4-reductase* (*DFR*) in rose (Dela et al., 2003), and *CHS* and *leucoanthocyanidin dioxygenase* in apple (Lin-Wang et al., 2011) is suppressed by elevated temperatures, indicating that the low anthocyanin concentrations in flowers and fruits under elevated temperature

Abbreviations: bHLH, basic helix-loop-helix; CHS, chalcone synthase; DFR, dihydroflavonol 4-reductase; dpa, days post anthesis; F3H, flavanone 3-hydroxylase; qRT-PCR, quantitative reverse transcription-PCR; St, stage.

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conditions are mainly due to a decreased rate of anthocyanin biosynthesis.

At present, the regulatory networks of anthocyanin biosynthesis are well understood. The coordinated expression of anthocyanin biosynthesis genes is controlled at the transcriptional level and regulated by a complex consisting of R2R3-MYB, basic helix-loop-helix (bHLH), and WD-repeat protein (Koes et al., 2005). *LhMYB12* and *LhbHLH2* have been identified from an Asiatic hybrid lily cultivar Montreux and control anthocyanin biosynthesis in lily tepals (Nakatsuka et al., 2009; Yamagishi et al., 2010b). In an Oriental hybrid lily cultivar Sorbonne, the orthologous gene *LhMYB12* controls the transcription of anthocyanin biosynthesis genes (Yamagishi, 2011). Anthocyanin accumulation is suppressed under elevated temperatures in fruits via the modulation of *MYB* anthocyanin regulatory genes; in grape berry skins, the transcription of *VvMYBA1* is lower at 30 °C than at 20 °C, which is responsible for the lower anthocyanin concentration at 30 °C (Yamane et al., 2006); in apple, elevated temperatures diminish the fruit skin colour via the suppression of *MdMYB10* transcription (Lin-Wang et al., 2011). However, the effect of elevated temperatures on the transcription of *R2R3-MYB* genes regulating anthocyanin biosynthesis has not been investigated in flowers.

Sugar is an important factor affecting anthocyanin accumulation. Sugar itself is the main component of anthocyanin; it also functions as an energy source, an osmotic regulator, and a physiological signal in plants (Smeekens, 2000; Weiss, 2000). Exogenously supplied sucrose stimulates the transcription of *PAP1/AtMYB75* resulting in anthocyanin accumulation in *Arabidopsis* seedlings (Teng et al., 2005; Solfanelli et al., 2006). In cut flowers whose colour is determined by anthocyanin, the addition of sugars to vase solutions improves the colour quality of sweet pea (Ichimura and Hiraya, 1999), lisianthus (Uddin et al., 2001; Shimizu and Ichimura, 2005; Shimizu-Yumoto and Ichimura, 2010), and an Oriental hybrid lily 'Stargazer' (Han, 2003), although the mechanism for this phenomenon is unknown. Because flowers accumulate large amounts of sugars along with bud development (Yamada et al., 2009), the endogenous sugars in flowers, whose concentrations may be altered under elevated temperatures, can affect anthocyanin accumulation. Thus, it is necessary to investigate the changes in sugar contents under an elevated temperature.

In this study, the effect of an elevated temperature on anthocyanin pigmentation in an Oriental hybrid lily cultivar Marrero was investigated. The flowers were divided into seven stages and exposed to an elevated temperature of 35 °C for 2 days to determine the stage most susceptible to the elevated temperature. To clarify the mechanism for the suppression of anthocyanin accumulation under the elevated temperature, the transcription of *LhMYB12*, *CHSa*, *flavanone 3-hydroxylase* (*F3H*), and *DFR* was investigated and sugar content was measured.

2. Materials and methods

2.1. Plant material

An Oriental hybrid lily (*Lilium* sp.) cultivar Marrero was used in this study. The bulbs were purchased from Takii & Co., Ltd. (Kyoto, Japan) and were kept at 4 °C for more than 2 months to break dormancy. The bulbs were planted into pots (21 cm in diameter, 2 bulbs per pot) filled with commercial compost soil, Biogreen II (Hokkaido Aurace Co., Ltd, Sapporo, Japan). The plants were grown in an incubator at 20 °C under a 16 h light/8 h dark condition until anthesis.

2.2. Elevated temperature treatments

Flowers were divided into seven developmental stages: stage (St) 1, St 2, St 3, St 4, 0 days post anthesis (dpa), 1 dpa, and 2 dpa. St 1–4 were determined on the basis of the pigmentation on bud surfaces; St 1 buds contained no anthocyanin pigments, St 2 buds began pigmentation, St 3 buds were pigmented in half of bud surfaces, and St 4 buds exhibited full colouration in bud surfaces (Fig. 1a–d). The potted plants were subsequently treated by an elevated temperature of 35 °C for 2 days. Meanwhile, control plants were maintained at 20 °C. Buds or flowers were harvested immediately after the temperature treatment. Inner tepal segments 4 cm long and 2 cm wide that included some spots were cut alongside midribs and were used for anthocyanin determination, RNA isolation, and sugar extraction. The tepal segments did not include midribs because the abaxial side of the midribs accumulate anthocyanins earlier than other tepal parts.

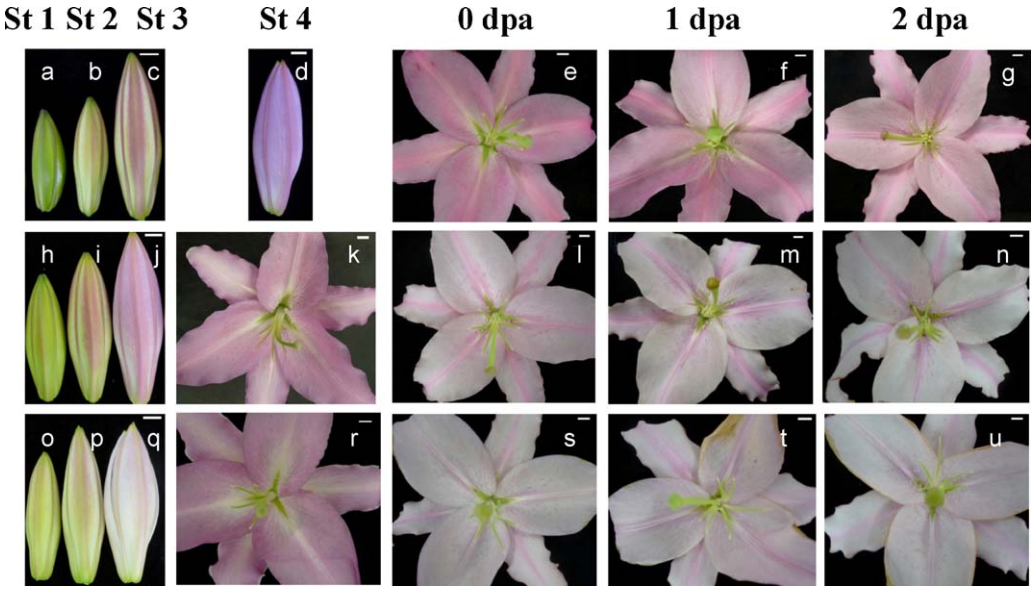

Fig. 1. Effects of an elevated temperature at 35 °C for 2 days on the colouration of buds and flowers at different flower developmental stages. Potted plants with buds at St 1–4 and with flowers at 0, 1, and 2 dpa were cultured at 35 °C or 20 °C for 2 days. (a–g) At the beginning of the temperature treatment. (h–n) 2 days after the treatment at 20 °C. (o–u) 2 days after the treatment at 35 °C. Bar = 1 cm.