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Methyl jasmonate treatment promotes flower opening of cut *Eustoma* by inducing cell wall loosening proteins in petals

Masaki Ochiai[a,b], Shogo Matsumoto[a], Kunio Yamada[c,*]

[a] *Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan*
[b] *Japan Society for the Promotion of Science, Japan*
[c] *College of Bioscience and Biotechnology, Chubu University, Kasugai 487-8501, Japan*

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ABSTRACT

Establishing the technique for controlling the rate of cut flower opening is important to maintain appropriate cut flower supplies to meet consumer demand. Cut flowers of *Eustoma grandiflorum* (Raf.) Shinn. were held in a vase solution containing (±)-abscisic acid (ABA), 6-benzylaminopurine (BA), gibberellic acid-3 (GA), methyl jasmonate (MeJA) or 1-naphthaleneacetic acid (NAA) at 100 μM. MeJA accelerated flower opening. Only the timing of flowering was earlier, and there was no change in maximum flower diameter at the fully open stage. Expansin and xyloglucan endotransglycosylase/hydrolase (XTH), regarded as cell wall loosing proteins, participate in petal growth from bud stage to the fully open stage in *Eustoma*. MeJA also accelerated the expression of *EgEXPA2*, *EgEXPA3* and *EgXTH1* mRNA and the accumulation of expansin and XTH protein in petals. Meanwhile, the acceleration of both flower opening and expression of these genes was not observed by ABA, BA or GA treatment. It was proposed that early flower opening by JA treatment resulted from petal cell wall loosening by accelerated expression of expansin and XTH.

1. Introduction

It is important to control the supply of cut flower crops with quantities appropriate to demands. In general, cut flower crops are harvested at the bud stage when the buds will be able to open fully after distribution and be displayed in retail outlets at the flowering stage. Though the demand for cut flowers changes from day to day, the duration from the bud stage to the flowering stage is constant. Thus, establishing techniques for controlling the rate of flower opening is needed. Flower opening is mainly due to the expansion of petal cells. However, cell walls are normally rigid in order to maintain cell form. Therefore, loosening of cell walls will be required before reduction of water potential and promotion of water influx by sugar accumulation, for cell expansion. In elongating organs, such as coleoptiles and roots, mechanical properties of the cell walls have been studied by evaluating auxin-, gibberellin-, and acid-induced increases in extensibility (Nakamura et al., 1975; Tanimoto et al., 2000). Recently, it was reported that flower opening is accompanied by an increment of cell wall extensibility in rose (Yamada et al., 2009) and *Eustoma* (Ochiai et al., in press). Therefore, it has been proposed that cell wall extensibility is also a limiting factor for petal growth and flower opening.

Expansin and xyloglucan endotransglycosylase/hydrolase (XTH) are candidates for cell wall loosening proteins (Cosgrove, 2001). Expansins are believed to disrupt hydrogen bonding between cellulose microfibrils and matrix glucans (Cosgrove, 2000). XTHs are capable of catalyzing either the molecular grafting or disassembly of xyloglucan cross-links within the cellulose-xyloglucan framework (Nishitani and Tominaga, 1992; Okazawa et al., 1993). In petals, it is confirmed that both mRNAs and proteins of expansin and XTH increased accompaning petal growth in several species (O'Donoghue et al., 2002; Gookin et al., 2003; Yamada et al., 2009; Harada et al., 2011; Ochiai et al., in press). A number of expansins and XTHs are controlled by plant growth regulators, such as auxin, cytokinin, ethylene and gibberellic acid (Downes and Crowell, 1998; Catalá et al., 2000; Chen et al., 2001; Belfield et al., 2005; Cui et al., 2005; Ookawara et al., 2005). Several reports have noted effects of plant growth regulators on cut flower opening. Gibberellic acid promotes flower opening of rose (*Rosa x hybrida* 'Mercedes') and statice (*Limonium sinuatum* L.) (Steinitz, 1982; Sabehat and Zieslin, 1995). Absence of jasmonate biosynthesis delays petal growth and flower opening in *Arabidopsis thaliana* (Ishiguro et al., 2001). Petal curving of citrus flowers is stimulated by exogenous ethylene treatment (Zacarias and Tudela, 1991). On the other hand, flower opening of *Gladiolus* spp. is inhibited by ethylene (Serek et al., 1994). In cut roses, the effect of ethylene on flower opening is cultivar-dependent. There are 3 types of rose cultivars, those with inhibited petal opening, accelerated petal

* Corresponding author. Tel.: +81 568 51 9749; fax: +81 568 51 9749.
E-mail address: yamakuni@isc.chubu.ac.jp (K. Yamada).

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opening and those with petal opening not affected by ethylene (Reid et al., 1989; Yamamoto et al., 1994). Thus, the effects of application of plant growth regulators on cut flowers varies widely among species and cultivars.

Eustoma grandiflorum (Raf.) Shinn. (also known as Lisianthus or Prairie Gentian) has a wide range of flower color, size and shape. Moreover, cut *Eustoma* flowers, which have a relatively long vase life, are produced throughout the year. Thus, *Eustoma* is one of the important ornamental plants for cut flowers in Japan. Furthermore, both expansin and XTH reportedly participate in petal growth from bud to opening flower in *Eustoma* (Ochiai et al., in press). In this study, we clarified the effects of plant growth regulators on expression of expansin and XTH protein and flower opening in cut *Eustoma*.

2. Materials and methods

2.1. Plant material and treatment

Cut flowers of *E. grandiflorum* (Raf.) Shinn. 'King of Orchid' (Sakata Seed, Yokohama, Japan) were obtained commercially from the Sakuma Shoukaen flower shop. Stems with one flower bud, with petals still tightly closed and the perianth with the same length as the calyx (T0), were recut in water 8 cm below the base of the calyx and all leaves were removed. Cut flowers were divided into 6 groups and held in solutions containing (±)-abscisic acid (ABA) (Wako, Osaka, Japan), 6-benzylaminopurine (BA) (Wako), gibberellic acid-3 (GA) (Wako), methyl jasmonate (MeJA) (Wako) or 1-naphthaleneacetic acid (NAA) (Wako) at 100 μM. Solutions without plant growth regulators were used as controls. All solutions contained 4% (w/v) sucrose, 0.05% (v/v) dimethyl sulfoxide (DMSO) and 0.02% (w/v) 8-hydroxyquinoline sulphate (HQS). Treatments were carried out in a temperature-controlled chamber at 23 °C with a 16 h photoperiod. The diameter of each flower was measured daily. Petals of 5 replicates from each treatment were sampled after 3 day of treatment for quantitative real-time RT-PCR and Western blotting.

2.2. Quantitative real-time RT-PCR

Total RNA was isolated from petals with TRIzol reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. The first strand of cDNA was synthesized by PrimeScript RT reagent Kit with gDNA Eraser (Perfect Real Time) (Takara-Bio, Shiga, Japan). Gene-specific mRNA quantification was performed using SYBR Premix Ex Ta II (Tli RNaseH Plus) (Takara-Bio) in a Thermal Cycler Dice Real-Time System (Takara-Bio), according to the protocol provided by the supplier. Paralog-specific primers for each gene were mainly designed based on the 3′ untranslated region sequence of each cDNA. Relative gene expression levels were calculated using the comparative Ct method, including normalization against constitutively expressed ubiquitin (*EgUbi*: accession no. AB049409.1) and against a control sample. Primer pairs for each paralog were described in a previous study (Ochiai et al., in press).

2.3. Western blotting

Frozen petals were homogenized in 10 mM sodium phosphate buffer (pH 6.0) containing 1 M sodium chloride and 1% (v/v) glycerol. After centrifugation at 20,000 × *g* for 10 min, 80% (w/v) ammonium sulphate was added to the supernatant and kept at 4 °C overnight. The mixture was centrifuged at 20,000 × *g* for 20 min, and the pellet was dissolved in 8 M urea. Protein amount was determined by Bio-Rad Protein Assay (BIO-RAD, CA, USA). Twenty μg of extracted protein was separated by SDS-PAGE using 12% (w/v) polyacrylamide gel and electrotransfered to Hybond-P (GE Healthcare, UK). The protein was probed with an antibody raised against recombinant EgEXPA1 or EgXTH1 (Ochiai et al., in press) followed by a horseradish peroxidase-conjugated secondary antibody. The blot was detected with ECL Prime Western Blotting Detection System (GE Healthcare).

Table 1
Effects of continuous plant growth regulator treatment on flower opening in cut Eustoma.

Treatment	Time to reach a particular stage (d)	
	Bud unfolding	Blooming
Ctrl	3.5[a]	5.8[ab]
ABA	3.1[ab]	5.5[ab]
BA	3.6[a]	5.9[a]
GA	3.4[ab]	6.4[a]
MeJA	2.3[b]	4.0[b]
NAA	3.5[a]	4.9[ab]

Means with different letters within a column are significantly different among treatments by Tukey's multiple range test at $P < 0.05$ ($n = 8$).

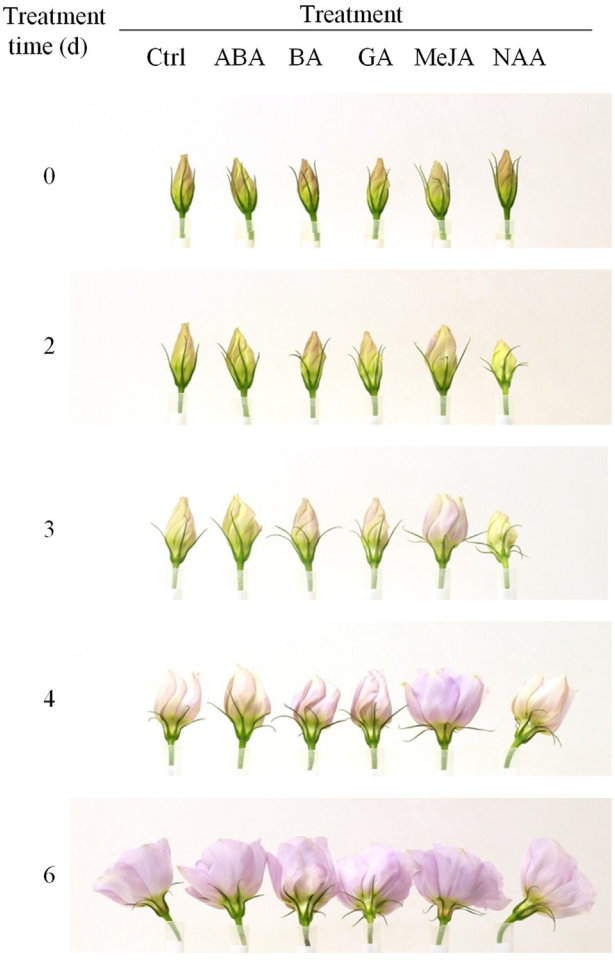

Fig. 1. Effects of continuous plant growth regulator treatment on flower opening in cut *Eustoma*. Cut flowers were placed in vase solutions containing abscisic acid (ABA), 6-benzylaminopurine (BA), gibberellic acid-3 (GA), methyl jasmonate (MeJA) or 1-naphthaleneacetic acid (NAA) at 100 μM. A vase solution without plant growth regulator was used as a control (Ctrl). Vase solutions contain 4% (w/v) sucrose, 0.05% (v/v) DMSO and 0.02% (w/v) HQS. Flowers after 0-, 2-, 3-, 4- or 6-day treatment were shown.