Nonuniform Coloration of Harvested Flower Buds of Double-flowered
Eustoma is Reduced by Methyl Jasmonate Treatment

Takayuki Mizuno, Naoko Fukuta and Hiroko Shimizu-Yumoto*

NARO Institute of Vegetable and Floriculture Science, Tsukuba 305-0852, Japan

Double-flowered Eustoma, a popular cut flower, sometimes shows nonuniform coloration of its petals when harvested at the flower bud stage. At the tips of the petals, pale greenish areas remain after the flowers open. This considerably reduces the value of cut Eustoma. In this study, to identify appropriate postharvest treatments leading to normal coloration of these cut flower buds, we investigated the influence of harvest stage, temperature, sugar, and methyl jasmonate (MeJA) on petal coloration using the Eustoma ‘Voyage (Type II) Blue’. Investigation of the harvest stage, which was compared at three flower bud stages (STG2, 3, and 4), revealed that detached pale greenish flower buds (STG2 and 3) had a high probability of becoming nonuniformly colored flowers, so these stages were used for subsequent investigations. Temperature, which was investigated at 20, 25, 30, and 35°C, did not show any influence on petal coloring. Investigation of sugar treatments, such as 1% or 3% glucose or sucrose, also showed no influence. However, MeJA treatment led to a significant difference in petal coloration. The nonuniform coloration of petals was reduced by continuous exposure to MeJA vapor. Furthermore, we performed shorter MeJA treatments, such as for 1 or 2 days after harvesting, which reduced the nonuniform coloration as well as continuous MeJA exposure. To evaluate the effect of MeJA treatment on petal coloration, we measured the rate of pale green areas of petals using digital images. The analysis revealed that the greenish areas in petals exposed to MeJA vapor were significantly smaller than in petals not exposed to MeJA. In addition, there were fewer days to flowering after MeJA treatment. We concluded that postharvest treatment with MeJA is effective for reducing nonuniform coloration of early harvested flower buds of the double-flowered Eustoma ‘Voyage (Type II) Blue’.

Key Words: flower coloring, image analysis, lisianthus, postharvest treatment.

Introduction

Eustoma grandiflorum (Raf.) Shinn. (Gentianaceae) is a popular cut flower native to the central and southern regions of the United States of America. The species was introduced to Japan more than 80 years ago (Okawa et al., 1991). Since then, it has been improved by breeding and selection. Statistics of the cut flower market in Japan in 2011 revealed that cut Eustoma has the fifth largest production volume, and that production is still increasing (Ichimura, 2013). The development of postharvest physiology and technology for cut Eustoma has contributed to its commercial value (Shimizu-Yumoto and Ichimura, 2010a). In Eustoma flowers, ethylene, a phytohormone related to leaf abscission and flower senescence that is mainly produced in the pistil, and sensitivity to ethylene are low at an early stage of anthesis, but increase with senescence (Ichimura and Goto, 2000; Ichimura et al., 1998). Ethylene production of cut Eustoma is reduced by sucrose pulse treatment or treatment with silver thiosulfate complex (Huang and Chen, 2002; Ichimura et al., 1998). To extend the vase life of cut Eustoma, sucrose with biocide treatment or pulse treatment with high concentrated sucrose is effective (Ichimura and Korenaga, 1998; Shimizu and Ichimura, 2005). Furthermore, a combination pulse treatment of a synthetic auxin, 1-naphthaleneacetic acid, and an ethylene synthesis inhibitor, aminooethoxyvinylglycine, is also reportedly effective (Shimizu-Yumoto and Ichimura, 2010b). For further development of the cut Eustoma market, improvements in shipping and handling techniques are expected.
Materials and Methods

Plant materials

A large, double-flowered Eustoma, ‘Voyage (Type II) Blue’ (Sakata Seed Co., Japan), with bluish-violet, fringed petals, was cultivated in a greenhouse of the National Institute of Floricultural Science, Ibaraki Prefecture, Japan. Each stage of flower buds (Fig. 1, STG2 to 4) was harvested in June or July, 2015. These flower buds were used for the following experiments after cutting their stems in air to a length of 5 cm.

Influence of harvest stage

Flower buds (STG2 to 4, n=8) were placed in individual glass vessels containing 10 mL 1% glucose (Glc), 50 mg·L⁻¹ aluminum sulfate, and 0.5 mL·L⁻¹ antimicrobial agent (Kathon CG; Rohm and Haas Japan, Japan), which contained 11.3 g·L⁻¹ 5-chloro-2-methyl-4-isothiazolin-3-one and 3.7 g·L⁻¹ 2-methyl-4-isothiazolin-3-one as active ingredients. These flower buds were placed at 30°C in a chamber with light intensity of 80 μmol·m⁻²·s⁻¹ under continuous light irradiation. Observation of these flower buds was carried out until full opening or dehiscence, which was regarded as flowering (Fig. 1, STG6), or 10 days after treatment, whichever was earlier. After flowering, the rate of flowering, the rate of nonuniformly colored flowers, and the day of flowering after treatment were counted. Nonuniformly colored flowers with pale greenish parts were regarded as having clearly lost ornamental value.

Influence of temperature

STG3 flower buds, which were placed in individual glass vessels containing 1% Glc with 50 mg·L⁻¹ aluminum sulfate and 0.5 mL·L⁻¹ antimicrobial agent, were incubated at 20, 25, 30, and 35°C under continuous light irradiation with light intensity of 80 μmol·m⁻²·s⁻¹ until flowering or after 10 days, whichever was earlier. Rate of flowering, rate of nonuniformly colored flowers, and the number of days to flowering after treatment were counted.

Investigation of sugar treatments

Flower buds at STG3 were used for investigation of sugar treatments. Four treatments were prepared: 1 and 3% Glc and 1 and 3% sucrose (Suc). To each of these four solutions, 50 mg·L⁻¹ aluminum sulfate and 0.5 mL·L⁻¹ antimicrobial agent were added. The flower buds were placed at 30°C under continuous light irradiation with light intensity of 80 μmol·m⁻²·s⁻¹. After

and handling technique for cut flowers (Goszczyńska and Rudnicki, 1982; Koyama et al., 1995). Although this is a harvesting technique that can prevent wounding of flower parts during transport and reduce freight costs, immature flower buds often fail to flower satisfactorily, including petal coloration. Wild type Eustoma grandiflorum is single-flowered with five petals. Breeding of cut Eustoma has advanced from single-flowered to double-flowered cultivars with many petals (Sakata Seed Co., http://www.sakataseed.co.jp/special/toruko/history.html). In recent years, large, double-flowered cultivars of cut Eustoma with a fringe, such as the ‘Voyage’ series have become mainstream in the market, and these cultivars show nonuniform coloration of their petals when harvested at the immature flower bud stage. On the tips of these petals, pale greenish spots or larger areas remain until flowering. This reduces the commercial value of the inflorescences, so it is necessary to establish an appropriate postharvest treatment for early harvested Eustoma flower buds.

Flower color is an important factor affecting inflorescence quality, and some postharvest investigations have aimed to improve flower coloration. High temperature should be avoided when a detached inflorescence is temporarily stored, since it inhibits anthocyanin biosynthesis in some potted ornamental flowers, such as roses, carnations, and lilies (Dela et al., 2003; Lai et al., 2011; Maekawa and Nakamura, 1977). Sugar treatment improves flower coloring in the case of reddish flower cultivars, e.g. lily ‘Stargazer’ and tree peony ‘Luoyang Hong’ by promoting anthocyanin biosynthesis and accumulation (Han, 2003; Zhang et al., 2015). Methyl jasmonate (MeJA) is also useful for improving coloration (Rudell et al., 2002; Saniewski et al., 1998a, b). In the case of a fruit, the ‘Fuji’ apple, production of the anthocyanin cyanidin glucoside has been enhanced by MeJA treatment (Rudell et al., 2002). In radish sprouts, it has been reported that MeJA treatment enhances anthocyanin accumulation due to up-regulation of some flavonoid biosynthesis genes, such as those encoding 4-coumaroyl CoA ligase and dihydroflavonol reductase (Park et al., 2013). Although MeJA reportedly promotes anthocyanin accumulation in detached petunia flowers (Tamari et al., 1995), there have been no subsequent studies of its effects on coloration of detached flowers. In this study, to obtain information on effective treatments to improve flower coloration, we investigated the influence of harvest stage, temperature, sugar, and MeJA vapor using double-flowered Eustoma.
flowering, the rate of flowering, the rate of nonuniformly colored flowers, and the number of days to flowering after treatment were counted.

**Investigation of exposure to MeJA vapor**

These experiments were conducted in sealed 9 L acrylic boxes within the growth chamber. The temperature in the chamber was adjusted to 30°C, and the temperature inside of the acrylic boxes stabilized at 32 to 33°C. The boxes were continuously irradiated with light under light intensity of 95 μmol·m⁻²·s⁻¹. Flower buds were placed in individual glass vessels containing 1% Glc with 50 mg·L⁻¹ aluminum sulfate and 0.5 mL·L⁻¹ antimicrobial agent. For MeJA (Wako Pure Chemical Industries Ltd., Japan) treatment, vapor was prepared by placing MeJA/ethanol (EtOH) (1:9) onto a filter paper in the central region of the boxes. The MeJA vapor was introduced to a final concentration of 4 μM·L⁻¹ of air. The control was exposed to the same volume of 100% EtOH. A continuous exposure experiment was performed using STG2 and 3 flower buds (n = 6). A shorter exposure experiment was carried out using STG3 flower buds (n = 6) and was comprised of three treatments: 0 days (as control), 1 day, and 2 days of MeJA vapor exposure. After exposure, these flower buds were moved into another acrylic box containing a filter paper saturated with 100% EtOH. The air and the filter paper containing MeJA or EtOH in the chambers were exchanged every day. The experiments were performed until flowering or up to 10 days. The rate of flowering and the number of days to flowering after treatment were counted. In addition, the rate of petals that were pale green was recorded using a scanner. Flowers that had partially wilted before flowering were omitted from this measurement.

**Measurement of pale greenish area of petals**

In the investigation of MeJA vapor exposure using STG2 and STG3 flower buds, the pale greenish areas of the flowers were measured. All petals were carefully separated and immediately captured as digital images using a flatbed scanner (CanoScan 9000F; Canon Inc., Japan). The images were captured in TIFF format at a resolution of 400 bpi and 48-bit color. In the captured images, the bottom side of each petal with a brown color and 3 mm around the brown area were eliminated. Color distribution of the petals was determined using the histogram in Adobe Photoshop CC 2015 software (Adobe Systems, USA) in the Photoshop LAB color space. The values of a can be converted to a*, which is a parameter with values from greenness (negative) to redness (positive) using formula (1) (Yam and Papadakis, 2004). In this study, pixels having a negative a* value (a < 128) were regarded as pale green. In addition, our preliminary results indicated that the pale greenish areas (average a = 120.17) contained almost no anthocyanin based on high performance liquid chromatography (HPLC), although the white and violet areas (average a values of 129.47 and 145.39, respectively) did contain anthocyanin (unpublished data). The rate of pale greenish area was calculated using equation (2). Furthermore, we verified the light stability of the scanner and the color precision of captured images using Color Separation Guides and Gray Scales (Eastman Kodak Co. Ltd., USA) and the Mini ColorChecker (X-Rite, USA) before experimental measurements.

\[
a* = \frac{240a}{255} - 120 \tag{1}
\]

Rate of pale greenish area (%)

\[
= \frac{\text{Pixels of pale greenish area}}{\text{Pixels of overall area}} \times 100 \tag{2}
\]

**Statistical analysis**

The values for flowering day after treatment and rate of pale greenish area are expressed as mean values ± standard errors (SE). Tukey-Kramer’s multiple range test or t-test was used to compare the means of these values (Tables 1, 4, and 5). P values < 0.05 were regarded as statistically significant in Tukey-Kramer’s multiple range test. Two-way ANOVA was used to reveal differences in flowering day and rate of pale greenish area between flower bud stages (STG2 and 3) and treatments (EtOH and MeJA) in the investigation of continuous treatment with MeJA vapor (Table 4). Data obtained as percentages, such as the rate of pale greenish areas, were converted to the corresponding arc sine value before statistical calculations. These statistical analyses were performed using StatView software v. 5.0 (SAS Institute Inc., USA).

**Results**

**Influence of harvest stage**

Rate of flowering, rate of nonuniform colored flowers, and day of flowering after treatment of STG2 to 4 flower buds are shown in Table 1. Four of the STG2 flower buds flowered (50%), while all of the STG3 and 4 flower buds flowered. Nonuniformly colored flowers were observed in all of the STG2 and 3 flower buds, and their petals showed pale greenish patches over

| Stage | Rate of flowering (%) | Rate of nonuniform colored flowers (%) | Flowering day after treatment |
|-------|-----------------------|----------------------------------------|------------------------------|
| STG2  | 50                    | 100                                    | 8.0 ± 0.6 a*                 |
| STG3  | 100                   | 100                                    | 5.3 ± 0.4 b                  |
| STG4  | 100                   | 0                                      | 1.6 ± 0.2 c                  |

Values are the means of 8 replications.

*a* Significant difference based on Tukey-Kramer’s multiple range test (P < 0.05) is indicated by different letters.
more than one-third of the petal area. On the other hand, detached flower buds at the stage when petals became colored (STG4) reached normal flowering. Significant differences in the day of flowering after treatment were revealed. In subsequent experiments, we used pale greenish flower buds to develop a technique for reducing the nonuniform coloration of the double-flowered Eustoma cultivar.

Influence of temperature

The rate of flowering, rate of nonuniform colored flowers, and day of flowering after treatment at four temperatures were revealed (Table 2). The rate of flowering was the lowest at 20°C (50%), while all flower buds flowered at 35°C. No difference in the rate of nonuniform colored flowers was observed among the four temperatures. More than one-third of the petal surface was occupied by pale greenish areas. In addition, no difference in the day of flowering after treatment was observed, but flowering days of STG3 buds treated at 30°C tended to be earlier compared to the other three treatments (Table 2). Therefore, subsequent experiments were carried out at 30°C.

Effects of sugar treatments

In the investigation of sugar treatments, the rate of flowering, rate of nonuniformly colored flowers, and day of flowering after treatment are shown in Table 3. No significant differences were observed among treatments. In the 1% Glc and Suc treatments, all of the nonuniformly colored flowers showed pale greenish areas over more than one-third of all petals. In the 3% sugar treatments, one flower bud in Glc became colored except for the tips of the petals and one flower bud in Suc showed normal flowering. These two flowers opened earlier than the other flower buds (at 3 and 2 days, respectively). We assumed that there was a mistake in sample selection because the buds reached STG4 within one day after treatment, which indicated that the effects of postharvest treatment were not at the expected stage.

Effect of MeJA treatment

The appearance of Eustoma flowers after continuous exposure to MeJA is shown in Figure 2A to D. The petals exposed to MeJA turned violet (Fig. 2B, D). On the other hand, petals exposed to EtOH showed only a slight change to violet (Fig. 2A, C). STG2 flower buds exposed to MeJA reached flowering, although pale green areas were observed in the central region of the flowers (Fig. 2B). The appearance of flowers after a briefer exposure is also shown in Figure 2E to G. The petals in the shorter MeJA treatment (1 and 2 days) turned as violet as those in the continuous MeJA treatment (Fig. 2F, G). In contrast, the color change of the petals in the control treatment (0 days) was slight (Fig. 2E).

In these experiments, we used a scanner to record pale greenish areas of petals. A flatbed scanner and glass sheet can be used to capture the petal color in a flat configuration, although the light source, which is one of the important factors for capturing proper color images, cannot be controlled. We preliminarily tested the light stability of the scanner using 3-color patches (green, yellow, and red) of a Color Separation Guide.

| Table 2. The influence of temperature on harvested STG3 flower buds in the Eustoma ‘Voyage (Type II) Blue’. |
| --- |
| Temperature (°C) | Rate of flowering (%) | Rate of nonuniform colored flowers (%) | Flowering day after treatment* |
| 20 | 50 | 100 | 8.8 ± 0.6 |
| 25 | 88 | 100 | 6.6 ± 0.8 |
| 30 | 75 | 100 | 5.7 ± 0.8 |
| 35 | 100 | 100 | 7.1 ± 0.8 |

Values are the means of 8 replications.

* Significant differences were not detected.

| Table 3. The effect of sugar treatment on harvested STG3 flower buds of the Eustoma ‘Voyage (Type II) Blue’. |
| --- |
| Treatment | Rate of flowering (%) | Rate of nonuniform colored flowers (%) | Flowering day after treatment* |
| 1% Glc | 100 | 100 | 6.5 ± 0.9 |
| 3% Glc | 100 | 100 | 6.0 ± 1.0 |
| 1% Suc | 100 | 100 | 6.5 ± 1.0 |
| 3% Suc | 100 | 83 | 4.8 ± 0.8 |

Values are the means of 6 replications.

* Significant differences were not detected.
which was recorded with the petal samples. Standard deviation (SD) and differences between minimum and maximum values (Max-Min) of the $a$ values in these patches revealed that the values were very stable (green: SD = 0.07, Max-Min = 0.36, yellow: SD = 0.17, Max-Min = 0.56, red: SD = 0.15, Max-Min = 1.07, n = 42). Color precision of captured images was also verified using the 24-color patches of the Mini ColorChecker. The relationship of the $a$ values between the color standards and the captured images of the 24-color patches had a positive correlation and high variance ($y = 0.9867x$, $R^2 = 0.97$). These preliminary examinations confirmed that the method is suitable.

We used the Photoshop $a$ value to measure the pale greenish area of the *Eustoma* petals, since the histograms of this parameter for greenish and other areas of the petals were distinguishable. As one example, we showed the captured images of the STG3 petals treated with EtOH and MeJA vapor (Fig. 3). Furthermore, the $L$ value (lightness) of the captured images was digitally removed (Fig. 3A, B, lower right), since the $L$ value has high sensitivity to curvature and is not involved in the hue (Mendoza et al., 2006). The histograms of $a$ values in the captured images are shown (Fig. 3, left). In the petals exposed to EtOH, the histogram revealed two peaks, one distributed around greenish values ($a < 128$) and the other around redness values ($a > 127$). On the other hand, the histogram of MeJA-treated flower petals showed one peak, with values distributed in the redness region.

The rate of flowering, the day of flowering after treatment, and the rate of pale greenish areas of petals are shown after continuous MeJA treatment in Table 4. Flowering day was significantly accelerated by continuous MeJA treatment for both stages (Table 4). The rate of pale greenish areas in flower buds continuously treated with MeJA vapor was lower than that of EtOH-exposed flower buds at both stages (Table 4). For the briefer exposure treatment, the rate of flowering, day of flowering after treatment, and rate of pale greenish areas are shown in Table 5. Flowering day was accelerated by 1 or 2 days after exposure to MeJA (Table 5). In addition, the rate of pale greenish areas was significantly reduced by 1 or 2 days of exposure to MeJA (Table 5).

**Discussion**

Understanding the appropriate stage of harvest for a

---

**Fig. 3.** Evaluation of nonuniform coloring in cut *Eustoma* by the $a$ value in Photoshop. Effects of continuous exposure to EtOH (A, as control) and MeJA: EtOH (1:9) vapor (B) on petal coloring of STG3 flower buds are shown. Histogram (left) shows the proportion of $a$ values. Right, captured rows of images (upper) and images with L value removed (lower) are shown.
flower bud is important for inflorescence quality. First, we examined the relationship between harvest stage of the flower bud and occurrence of nonuniform coloration in the double-flowered *Eustoma* cultivar ‘Voyage (Type II) Blue’. Detached pale greenish flower buds (such as those at STG2 and 3) showed a high probability of becoming nonuniformly colored flowers, although buds whose petals had already turned violet before harvesting reached normal flowering. This suggests that once the petals turn violet, nonuniformly colored flowers do not occur. However, the day of flowering after cutting of STG4 flower buds was 1.6 days (Table 1), which is too early and inappropriate for flower bud harvesting. To develop an appropriate postharvest technique for development of normal coloration of the pale greenish flower buds of double-flowered *Eustoma*, we performed subsequent investigations.

The influence of incubation temperature was checked because high temperature inhibits anthocyanin biosynthesis in some ornamentals (Dela et al., 2003; Lai et al., 2011; Maekawa and Nakamura, 1977). The results for day of flowering suggest that 30°C is suitable for opening of *Eustoma* flower buds, but the number of nonuniformly colored flowers did not decrease. We revealed that the nonuniform coloration of the flower buds of double-flowered *Eustoma* is not improved by controlling the incubation temperature.

Sugar treatment is known as an effective method for improving flower coloring due to its promotion of anthocyanin biosynthesis (Han, 2003; Zhang et al., 2015). In detached flower buds of single-flowered *Eustoma* ‘Royal Purple’ treated with sucrose, the petal color becomes more intense as the concentration increases (Kawabata et al., 1999). In this study, although we applied higher concentrations, such as 3% glucose or sucrose, no significant difference in the rate of nonuniformly colored flowers was observed (Table 2). This suggested that sugar application does not improve the nonuniform coloration of double-flowered *Eustoma* and that a different approach is necessary.

MeJA treatment of various parts of plants such as fruits, seedlings, bulbs, and seeds has been attempted for anthocyanin accumulation (Park et al., 2013; Rudell et al., 2002; Saniewski et al., 1998a, b). These attempts show the utility of MeJA treatment for agricultural applications. In this study, we treated pale green flower buds with MeJA. The flower buds exposed to MeJA showed essentially normal coloring on opening

### Table 4. The effect of continuous MeJA vapor treatment on harvested flower buds of the *Eustoma* ‘Voyage (Type II) Blue’.

| Treatment | Rate of flowering (%) | Flowering day after treatment | Rate of pale greenish area (%) |
|-----------|-----------------------|-------------------------------|-------------------------------|
| STG2      |                       |                               |                               |
| EtOH 100 | 7.7±0.6               | 62.35±7.86                    |
| MeJA 100 | 4.8±0.2               | 16.54±3.83                    |
| t-test    | ***                   | **                            |
| STG3      |                       |                               |                               |
| EtOH 100 | 5.3±0.2               | 48.90±5.69                    |
| MeJA 100 | 4.2±0.2               | 8.92±3.18                     |
| t-test    | **                    | ***                           |
| ANOVA     |                       |                               |                               |
| STG       | ***                   | NS                            |
| Treatment | ***                   | ***                           |
| STG × Treatment | *     | NS                            |

Values are the means of 6 replications, except for the rate of the pale greenish area in MeJA treatments of STG2 flower buds (n=5).

NS, not significant; *, **, and *** indicate significance at $P<0.05$, 0.01, and 0.001, respectively.

### Table 5. Effect of temporal MeJA vapor treatment on harvested flower buds of the *Eustoma* ‘Voyage (Type II) Blue’.

| Duration of treatment | Rate of flowering (%) | Flowering day after treatment | Rate of pale greenish area (%) |
|-----------------------|-----------------------|-------------------------------|-------------------------------|
| 0 days (control)      | 100                   | 8.0±0.7 a                     | 36.45±2.62 a'                |
| 1 day                 | 100                   | 4.5±0.2 b                     | 4.23±1.14 b                  |
| 2 days                | 100                   | 4.5±0.2 b                     | 2.16±0.72 b                  |

Values are the means of 6 replications, except for the rate of pale-greenish area of MeJA treatment for 0 days as control (n=4).

* Significant difference based on Tukey-Kramer’s multiple range test ($P<0.05$) is indicated by different letters.
(Fig. 3A, B), although there were still a few pale greenish areas. This result indicated that MeJA reduces the nonuniform coloration of the petals of *Eustoma*.* Even 1 and 2 day MeJA treatments showed this effect, meaning that MeJA exposure for only one day is effective.

To quantitatively and objectively evaluate flower coloration, we estimated the area of petals that were pale green using a scanner and Photoshop software. The software is powerful and affordable, and has been used to analyze the colors of food samples and agricultural products (Yam and Papadakis, 2004; Yoshioka et al., 2004, 2006). Digital images enabled detection of differences between petals exposed to MeJA vapor and those not exposed (Tables 4 and 5), and thus demonstrated the effectiveness of MeJA in reducing the nonuniform coloring of the *Eustoma* petals. Furthermore, the histograms of the distribution of *a* values showed a decrease in the ratio of areas of greenness in the MeJA-exposed petals, which suggested that MeJA mediates chlorophyll degradation. In the same way, an increase in areas with higher redness values in the MeJA-exposed petals suggested that MeJA also mediates the initiation of anthocyanin biosynthesis. Chlorophyll degradation by MeJA treatment has been reported in ‘Golden Delicious’ apple peel (Pérez et al., 1993). Furthermore, the gene expression encoding CHLOROPHYLLASE1 is induced by MeJA in *Arabidopsis thaliana* (Tsuchiya et al., 1999). Regulation of anthocyanin biosynthesis is also a known function of the jasmonates. In the seedlings of *A. thaliana*, the molecular mechanism for jasmonate function in regulating anthocyanin accumulation has been reported (Qi et al., 2011). Physiological analysis is necessary to understand the role of jasmonates during flower coloration in *Eustoma*.

Treatment with MeJA accelerated flowering compared to the control, but there was little difference in flower diameter at the fully open stage in detached flower buds of the double-flowered *Eustoma* ‘King of Orchid’ (Ochiai et al., 2013). It has been reported that MeJA accelerates the expression of the cell wall loosening-related genes *EgEXP12*, *EgEXP13*, and *EgXTH1*, and the accumulation of expansin and xyloglucan endotransglycosylase/hydrolase, regarded as a cell wall loosening protein, in petals (Ochiai et al., 2013). In this study, flower buds exposed to MeJA showed accelerated flowering (Tables 4 and 5). This means that MeJA application to cut *Eustoma* harvested at the flower bud stage has an effect on both petal coloration and acceleration of flowering. We suggest that MeJA is a useful postharvest treatment, and further investigation of its practical application is necessary.

In conclusion, the nonuniform coloration of *Eustoma* flowers developing from early harvested flower buds, which have pale green petals in the fringed, double-flowered cultivar ‘Voyage (Type II) Blue’, was reduced by MeJA treatment. On the other hand, it was not reduced by control of temperature or sugar application. In addition, 1 day of MeJA treatment was sufficient to achieve the effect. We measured the rate of pale greenish areas of the petals using a scanner and Photoshop software as an objective evaluation of flower coloration. The data showed the effectiveness of MeJA for normal flower coloration. We propose that MeJA postharvest treatment as a useful method for inducing normal flowering, including petal coloration, of cut pale green flower buds of double-flowered *Eustoma*.

**Literature Cited**

Dela, G., E. Or, R. Ovadia, A. Nissim-Levi, D. Weiss and M. Oren-Shamir. 2003. Changes in anthocyanin concentration and composition in ‘Jaguar’ rose flowers due to transient high-temperature conditions. Plant Sci. 164: 333–340.

Goszczyńska, D. and R. M. Rudnicki. 1982. Long-term storage of carnations cut at the green-bud stage. Sci. Hortic. 17: 289–297.

Han, S. S. 2003. Role of sugar in the vase solution on postharvest flower and leaf quality of oriental lily ‘Stargazer’. HortScience 38: 412–416.

Huang, K. L. and W. S. Chen. 2002. BA and sucrose increase vase life of cut *Eustoma* flowers. HortScience 37: 547–549.

Ichimura, K. 2013. Recent trends in floral distribution. Bull. Natl. Inst. Flor. Sci. 13: 1–15 (In Japanese).

Ichimura, K. and R. Goto. 2000. Acceleration of senescence by pollination of cut ‘Asuka-no-nami’ *Eustoma* flowers. J. Japan. Soc. Hort. Sci. 69: 166–170.

Ichimura, K. and M. Korenaga. 1998. Improvement of vase life and petal color expression in several cultivars of cut *Eustoma* flowers using sucrose with 8-hydroxyquinoline sulfate. Bull. Natl. Res. Veg., Ornam. Plant & Tea, Japan 13: 31–39.

Ichimura, K., M. Shimamura and T. Hisamatsu. 1998. Role of ethylene in senescence of cut *Eustoma* flowers. Postharvest Biol. Technol. 14: 193–198.

Kawabata, S., Y. Kusuhara, Y. H. Li and R. Sakiyama. 1999. The regulation of anthocyanin biosynthesis in *Eustoma grandiflorum* under low light conditions. J. Japan. Soc. Hort. Sci. 68: 519–526.

Koyama, Y., A. Uda, O. Wada and M. Fujiino. 1995. Effects of pre-conditioning budded carnation shoots prior to long-term cold storage on flower quality. J. Japan. Soc. Hort. Sci. 63: 835–842 (In Japanese with English abstract).

Lai, Y. S., M. Yamagishi and T. Suzuki. 2011. Elevated temperature inhibits anthocyanin biosynthesis in the tepals of an Oriental hybrid lily via the suppression of *LhMYB12* transcription. Sci. Hortic. 132: 59–65.

Maekawa, S. and N. Nakamura. 1977. Studies on the coloration of carnation flowers. VII. The effects of temperature on the coloration and pigmentation for the intact flower and plant growth. J. Japan. Soc. Hort. Sci. 45: 375–382.

Mendoza, F., P. Dejmek and J. M. Aguilera. 2006. Calibrated color measurements of agricultural foods using image analysis. Postharvest Biol. Technol. 41: 285–295.

Ochiai, M., S. Matsumoto and K. Yamada. 2013. Methyl jasmonate treatment promotes flower opening of cut *Eustoma* by inducing cell wall loosening proteins in petals. Postharvest Biol. Technol. 82: 1–5.

Ohkawa, K., A. Kano, K. Kanematsu and M. Korenaga. 1991. Effects of air temperature and time on rosette formation in seedlings of *Eustoma-grandiflorum* (Raf.) Shinn. Sci. Hortic. 48: 171–176.
Park, W. T., Y. B. Kim, J. M. Seo, S. J. Kim, E. Chung, J. H. Lee and S. U. Park. 2013. Accumulation of anthocyanin and associated gene expression in radish sprouts exposed to light and methyl jasmonate. J. Agric. Food Chem. 61: 4127–4132.

Pérez, A. G., C. Sanz, D. G. Richardson and J. M. Olias. 1993. Methyl jasmonate vapor promotes β-carotene synthesis and chlorophyll degradation in Golden Delicious apple peel. J. Plant Growth Regul. 12: 163–167.

Qi, T., S. Song, Q. Ren, D. Wu, H. Huang, Y. Chen, M. Fan, W. Peng, C. M. Ren and D. Xie. 2011. The Jasmonate-ZIM-domain proteins interact with the WD-Repeat/bHLH/ MYB complexes to regulate Jasmonate-mediated anthocyanin accumulation and trichome initiation in Arabidopsis thaliana. Plant Cell 23: 1795–1814.

Rudell, D. R., J. P. Mattheis, X. Fan and J. K. Fellman. 2002. Methyl jasmonate enhances anthocyanin accumulation and modifies production of phenolics and pigments in ‘Fuji’ apples. J. Amer. Soc. Hort. Sci. 127: 435–441.

Saniewski, M., A. Miszczak, L. Kawa-Miszczak, E. Wegrzynowicz-Lesiak, K. Miyamoto and J. Ueda. 1998a. Effects of methyl jasmonate on anthocyanin accumulation, ethylene production, and CO₂ evolution in uncooled and cooled tulip bulbs. J. Plant Growth Regul. 17: 33–37.

Saniewski, M., K. Miyamoto and J. Ueda. 1998b. Methyl jasmonate induces gums and stimulates anthocyanin accumulation in peach shoots. J. Plant Growth Regul. 17: 121–124.

Shimizu, H. and K. Ichimura. 2005. Effects of silver thiosulfate complex (STS), sucrose and their combination on the quality and vase life of cut Eustoma flowers. J. Japan. Soc. Hort. Sci. 74: 381–385.

Shimizu-Yumoto, H. and K. Ichimura. 2010a. Postharvest physiology and technology of cut Eustoma flowers. J. Japan. Soc. Hort. Sci. 79: 227–238.

Shimizu-Yumoto, H. and K. Ichimura. 2010b. Combination pulse treatment of 1-naphthaleneacetic acid and aminoethoxyvinylglycine greatly improves postharvest life in cut Eustoma flowers. Postharvest Biol. Technol. 56: 104–107.

Tamari, G., A. Borochov, R. Atzorn and D. Weiss. 1995. Methyl jasmonate induces pigmentation and flavonoid gene expression in petunia corollas: a possible role in wound response. Physiol. Plant. 94: 45–50.

Tsuchiya, T., H. Ohta, K. Okawa, A. Iwamatsu, H. Shimada, T. Masuda and K. Takamiya. 1999. Cloning of chlorophyllase, the key enzyme in chlorophyll degradation: Finding of a lipase motif and the induction by methyl jasmonate. Proc. Natl. Acad. Sci. USA 96: 15362–15367.

Yam, K. L. and S. E. Papadakis. 2004. A simple digital imaging method for measuring and analyzing color of food surfaces. J. Food Engineering 61: 137–142.

Yoshioka, Y., H. Iwata, R. Ohsawa and S. Ninomiya. 2004. Quantitative evaluation of flower colour pattern by image analysis and principal component analysis of Primula sieboldii E. Morren. Euphytica 139: 179–186.

Yoshioka, Y., R. Ohsawa, H. Iwata, S. Ninomiya and N. Fukuta. 2006. Quantitative evaluation of petal shape and picotee color pattern in lisianthus by image analysis. J. Amer. Soc. Hort. Sci. 131: 261–266.

Zhang, C., J. Fu, Y. Wang, S. Gao, D. Du, F. Wu, J. Guo and L. Dong. 2015. Glucose supply improves petal coloration and anthocyanin biosynthesis in Paeonia suffruticosa ‘Luoyang Hong’ cut flowers. Postharvest Biol. Technol. 101: 73–81.