

## The Relationship Between Endogenous Gibberellins and Rosetting in *Eustoma grandiflorum*

Tamotsu Hisamatsu<sup>1</sup>\*, Masaji Koshioka<sup>1</sup>, Naomi Oyama<sup>1</sup> and Lewis N. Mander<sup>2</sup>

<sup>1</sup> National Research Institute of Vegetables, Ornamental Plants and Tea, Ministry of Agriculture, Forestry and Fisheries, Ano, Age, Mie 514-2392

<sup>2</sup> Research School of Chemistry, Australian National University, Canberra, A.C.T. 0200 Australia

### Summary

Treatments with gibberellin A<sub>1</sub> (GA<sub>1</sub>), GA<sub>3</sub> and GA<sub>20</sub> promoted stem elongation in rosetted seedlings of *Eustoma grandiflorum* in the following order: GA<sub>1</sub> = GA<sub>3</sub> > GA<sub>20</sub>, whereas *ent*-kaurene (K), *ent*-kaurenoic acid (KA) and GA<sub>19</sub> did not. GA<sub>1</sub>, GA<sub>3</sub>, GA<sub>19</sub> and GA<sub>20</sub> treatments promoted leaf elongation in the order of GA<sub>1</sub> = GA<sub>3</sub> = GA<sub>20</sub> > GA<sub>19</sub>, although K and KA did not. Quantitative analysis of endogenous GAs suggested that GA-biosynthesis pathway prior to GA<sub>53</sub> might be blocked in the rosetted plants. Furthermore, the results of stem and leaf elongation by the GA treatment indicate that there was a difference in 20-oxidase activity between stem and leaves of rosetted plants. Quantitative analyses also provided evidence that there were tissue-specific 20-oxidase activities not only in the rosetted plants but also in the non-rosetted plants of *E. grandiflorum*. GA<sub>3</sub>-induced stem elongation, but not floral initiation in rosetted plants under 35°/30 °C. Uniconazole (UCZ) inhibited leaf expansion, stem elongation and flower bud development of the non-rosetted plants, but did not inhibit flower bud initiation. These results indicate that leaf expansion, stem elongation and flower bud development are apparently GA-dependent but flower bud initiation is not.

**Key Words:** *Eustoma grandiflorum*, flowering, gibberellin, rosetting.

### Introduction

*Eustoma grandiflorum* (Raf.) Shinn. is one of the major cut flowers in Japan. Stems of most *Eustoma* plants begin to elongate after the development of the third pair of leaves. When seedlings were subjected to high temperature, stems failed to elongate, and the plants became rosetted (Ohkawa et al., 1991; Harbaugh et al., 1992). Rosetting is a major problem in *Eustoma* production. Seedlings growing under high temperature, especially the first two weeks after sowing, is very sensitive to temperatures. The sensitivity to high temperature gradually decreases with developing leaves and is not apparent after the second leaf-pair stage (Ohkawa et al., 1991). Rosetted plants require 4 to 6 weeks exposure to 10-15 °C to induce stem elongation and flowering (Ohkawa et al., 1994). GA<sub>3</sub> treatment induces stems of rosetted plants to elongate, but the effect disappears after elongation of several internodes (Takeda, 1988).

We previously identified several endogenous GAs including GA<sub>1</sub>, GA<sub>19</sub>, GA<sub>20</sub>, GA<sub>44</sub>, and GA<sub>53</sub> in *E.*

*grandiflorum* which indicate that the early-13-hydroxylation pathway predominates in the plants (Hisamatsu et al., 1998b). Furthermore, we found that 1) endogenous GA<sub>1</sub> was physiologically active and regulated stem elongation and that 2) 3β-hydroxylation step from GA<sub>20</sub> to GA<sub>1</sub> occurred in rosetted seedlings.

However, the relationship between endogenous GAs and the rosetting of *E. grandiflorum* has not been fully understood. Therefore, we investigated the effect of several GAs and their precursors on stem and leaf elongation of rosetted seedlings and analyzed endogenous GAs in rosetted and non-rosetted seedlings. We also investigated the role of GA in the flowering process.

### Materials and Methods

#### *Effect of exogenous GAs and their precursors on growth of rosetted seedlings (Expt. 1)*

Seeds of *E. grandiflorum* 'Asuka no Nami' were sown and grown in plug trays [2.0 cm (L) × 2.0 cm (W) × 4.0 cm (D)] containing Metro Mix 350 (Scotts, OH, USA) in a growth chamber maintained at 30 °C under a 12-hr photoperiod with metal halide lamps (100 μmol · m<sup>-2</sup> · s<sup>-1</sup>, D-400, Toshiba Co., Tokyo, Japan) until the two-pair of leaf stage. Each seedling was transplanted to a 12 cm plastic pot, containing fertilized medium (Kureha Engeibaido, Kureha Chemical Industry Co., Ltd., To-

Received; June 5, 1998. Accepted; August 26, 1998.

\* To whom correspondence should be addressed:

National Research Institute of Vegetables, Ornamental Plants and Tea, Ministry of Agriculture, Forestry and Fisheries, Ano, Age, Mie, 514-2392

Tel: 059-268-1331 Fax: 059-268-1339

E-mail: tamotsu@nivot.affrc.go.jp

kyo, Japan), then grown in a natural-light phytotron maintained at 35°/30 °C (0600–1800 h / 1800–0600 h) under 16-hr photoperiod (0600–2200 h / 2200–0600 h) supplemented with fluorescent lamps (30  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , FL40SW, Mitsubishi Co. Ltd., Tokyo, Japan).

Ten  $\mu\text{l}$  of 1  $\mu\text{g} \cdot \mu\text{l}^{-1}$  (10  $\mu\text{g}$ )  $\text{GA}_1$ ,  $\text{GA}_3$ ,  $\text{GA}_{19}$  and  $\text{GA}_{20}$  in 50% (v/v) aqueous acetone containing 0.05% Tween 20 and 10  $\mu\text{l}$  of 0.1, 1.0, or 10  $\mu\text{g} \cdot \mu\text{l}^{-1}$  of *ent*-kaurene (K) and *ent*-kaurenoic acid (KA) in 75% aqueous acetone were administered to plants at the fifth leaf-pair stage. Fifty% aqueous acetone was applied to shoot tips of control plants. Each treatment consisted of 5 seedlings, and grown for 8 weeks after treatments.

#### *Quantitative analysis of endogenous gibberellins (Expt. 2)*

Seeds of 'Asuka no Nami' were sown and grown as in Expt. 1 until the second leaf-pair stage. Half of the plants (non-cold treatment) were transplanted to 9 cm plastic pots (one plant per pot), containing fertilized medium (Kureha Engeibaido), and grown in a natural-light phytotron described in Expt. 1. When the plants grew to the seventh leaf-pair stage, they were harvested. The other half of the plants were exposed to 5 °C (cold treatment), 8-hr photoperiod, and kept under with fluorescent lamps (30  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) for 9 weeks. They were grown then under the same condition as the non-cold treated plants. When the plants grew to the seventh leaf-pair stage, they were harvested and divided into leaves (upper third pair of leaves) and stems, including the apical meristems.

The samples which ranged from 46.9 – 75.0 gFW were homogenized and extracted three times with 80% aqueous methanol (MeOH), then 100 ng of  $[17,17\text{-}^2\text{H}_2]\text{GA}_1$ ,  $[17,17\text{-}^2\text{H}_2]\text{GA}_{19}$ ,  $[17,17\text{-}^2\text{H}_2]\text{GA}_{20}$ ,  $[17,17\text{-}^2\text{H}_2]\text{GA}_{44}$ , and  $[17,17\text{-}^2\text{H}_2]\text{GA}_{53}$  were added to the combined 80% aqueous MeOH extract as internal standards. Endogenous GAs were extracted and purified through several chromatographic steps according to Hisamatsu et al. (1998a). The purified GA fraction were bioassayed by a dwarf rice cv. Tan-ginbozu microdrop procedure (Nishijima and Katsura, 1989). The GA active fraction which were eluted from Develosil ODS column were:  $\text{GA}_1$ , fractions 4–7;  $\text{GA}_{20}$ , fractions 12–15;  $\text{GA}_{19}$ , and  $\text{GA}_{44}$ , fractions 17–19;  $\text{GA}_{53}$ , fractions 23–25. These fractions were separately passed through Nucleosil N ( $\text{CH}_3$ )<sub>2</sub>, a column as described previously (Hisamatsu et al., 1998a) and bioassayed. An aliquot of each GA fraction dissolved in 100  $\mu\text{l}$  of MeOH and methylated with ethereal diazomethane (20  $\mu\text{l}$ ) at room temperature; the methylated GAs were further purified using aminopropyl SPE columns (Varian Associates, USA). The eluates were then dried and trimethylsilylated with N-Methyl-N-TMS-trifluoroacetamide (10  $\mu\text{l}$ , MSTFA, Pierce, USA) in glass tubes at 75 °C. The silylated samples were analyzed by GC-MS using a gas chromatograph (model 5890, Hewlett-Pack-

ard, USA) coupled to a mass selective detector (model 5989B, Hewlett-Packard, USA). A 1- $\mu\text{l}$  subsample was injected into a fused silica chemically bonded capillary column (0.25 mm i.d. x 30 m, 0.25  $\mu\text{m}$  film thickness, DB-1, J & W, CA, USA). The oven temperature was initially set at 60 °C. The temperature was increased at 30 °C  $\text{min}^{-1}$  to 210 °C 2 min after the program started and then, increased at 2 °C  $\text{min}^{-1}$  to 280 °C. The electron energy was 70 eV and the source temperature was 250 °C. Samples were analyzed by selected ion monitoring (SIM). Ions monitored were as follows: for  $\text{GA}_1/[17,17\text{-}^2\text{H}_2]\text{GA}_1$ , m/z 506, 508, 491, 493, 448 and 450; for  $\text{GA}_{19}/[17,17\text{-}^2\text{H}_2]\text{GA}_{19}$ , m/z 434, 436, 402, 404, 374 and 376; for  $\text{GA}_{20}/[17,17\text{-}^2\text{H}_2]\text{GA}_{20}$ , m/z 418, 420, 403, 405, 375 and 377; for  $\text{GA}_{44}/[17,17\text{-}^2\text{H}_2]\text{GA}_{44}$ , m/z 432, 434, 373, 375, 238 and 240; for  $\text{GA}_{53}/[17,17\text{-}^2\text{H}_2]\text{GA}_{53}$ , m/z 448, 450, 416, 418, 389 and 391. The levels of endogenous  $\text{GA}_1$ ,  $\text{GA}_{19}$ ,  $\text{GA}_{20}$ ,  $\text{GA}_{44}$  and  $\text{GA}_{53}$  in the plant materials were calculated from the ratios of peak area at m/z 506/508, 434/436, 418/420, 432/434 and 448/450, respectively. The other ions were monitored to confirm the identity of the compounds analyzed.

#### *Difference in response to $\text{GA}_1$ between leaves and stems of rosetted seedling (Expt. 3)*

Seeds of 'Asuka no Nami' were sown and grown under the same condition as in Expt. 1 until the end of the experiment.

Uniconazole-P [UCZ, ( $\pm$ )-(E)-1-(4-chlophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol, Sumitomo Chemical Co., Japan] was diluted with water to a concentration of 50  $\text{mg} \cdot \text{liter}^{-1}$ . The soil in each pot was drenched with 20 ml of UCZ three days after transplanting. When plants grew to the third leaf-pair stage, shoot tips of plants were administered 10  $\mu\text{l}$  of 0.1, 0.01, 0.001 and 0.0001  $\mu\text{g} \cdot \mu\text{l}^{-1}$   $\text{GA}_1$  dissolved in 10% aqueous acetone. Control plants were treated with 10  $\mu\text{l}$  of 10% aqueous acetone. Each treatment consisted of 12 plants, which were cultivated for 4 weeks after  $\text{GA}_1$  treatment.

#### *Effect of $\text{GA}_3$ on flowering of rosetted seedling (Expt. 4)*

Seeds of 'Asuka no Nami' were sown and grown under the same condition described in Expt. 1 until the second leaf-pair stage. After transplanting to 12cm plastic pots containing fertilized medium (Kureha Engeibaido), seedlings were grown as in Expt. 1, except that the phytotron was maintained at 30°/25 °C (0600–1800 h/1800–0600 h) under a natural photoperiod of 12–14h during experiment. When plants grew to the third leaf-pair stage, one half of the plants were transferred to 20°/15 °C and grown until flowering under natural photoperiod; the rest was grown at 30°/25 °C. Each treatment consisted of 15 seedlings, which were cultivated for 20 weeks after treatment started.

$\text{GA}_3$  was dissolved in 10% (v/v) aqueous acetone

(containing 0.05 % Tween 20) at concentrations of 0.1, 0.2 and 1.0  $\mu\text{g} \cdot \mu\text{l}^{-1}$ . When plants were transferred to 20°/15°C, GA<sub>3</sub> treatment started. Ten  $\mu\text{l}$  of each solution was treated to the shoot tips weekly interval for 10 weeks so that all shoot tips received 10  $\mu\text{g}$  of GA<sub>3</sub>. For examples, shoot tips were treated once with 1.0  $\mu\text{g} \cdot \mu\text{l}^{-1}$  (10  $\mu\text{g} \times 1$  time), 5 times with 0.2  $\mu\text{g} \cdot \mu\text{l}^{-1}$  (2  $\mu\text{g} \times 5$  time), and 10 times with 0.1  $\mu\text{g} \cdot \mu\text{l}^{-1}$  (1  $\mu\text{g} \times 10$  time) solution.

#### Effect of GA<sub>3</sub> and UCZ on flowering of non-rosetted seedling (Expt.5)

Seeds of 'Asuka no Nami' were sown and grown in as in Expt. 1 except the chamber was maintained at 20 °C. After transplanting to 12 cm plastic pots, seedlings were grown at 25°/20°C under a natural photoperiod of 12-14h during experiment. Each treatment consisted of 15 seedlings. Ten  $\mu\text{l}$  of GA<sub>3</sub> (0.1  $\mu\text{g} \cdot \mu\text{l}^{-1}$ ) were applied weekly for 10 weeks to shoot tips. Simultaneously, the soil was drenched with 20 ml of UCZ (50 mg  $\cdot$  liter<sup>-1</sup>) three days after transplanting.

### Results

#### Responses to exogenous GAs and their precursors on rosetted plants (Expt.1)

Stems of control plants remained rosetted for 8 weeks after the experiment was started (Fig. 1); whereas, those of rosetted seedlings treated with GA<sub>1</sub>, GA<sub>3</sub> and GA<sub>20</sub> elongated immediately; stems of plants treated with K, KA, and GA<sub>19</sub> failed to elongate. Stems of GA<sub>1</sub> -, GA<sub>3</sub> -, and GA<sub>20</sub> - treated plants followed a sigmoidal curve and reached a plateau 5 weeks after treatment started (data not shown). However, these plants re-formed rosettes at higher nodal positions and failed to flower. The order of activity for induction of stem elongation was GA<sub>1</sub> = GA<sub>3</sub> > GA<sub>20</sub>.

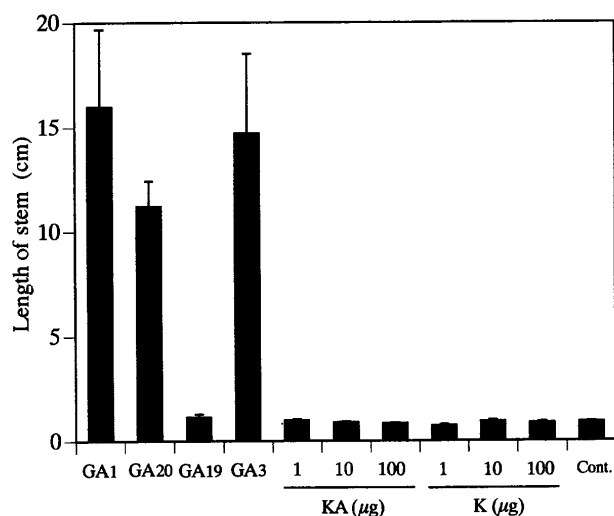


Fig. 1. Effect of GAs and their precursors on stem elongation in the rosetted plants of *E. grandiflorum* 8 weeks after treatment. Values are Mean  $\pm$  SE (n=5).

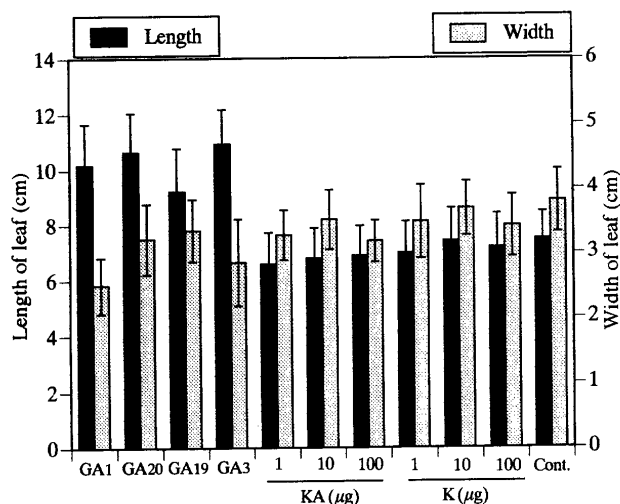


Fig. 2. Effect of GAs and their precursors on length and width of leaves in the rosetted plants of *E. grandiflorum*. Data were obtained from the 6th and the 7th node leaves. Values are Mean  $\pm$  SE (n=20).

Leaves of the rosetted seedlings treated with GA<sub>1</sub>, GA<sub>3</sub>, GA<sub>19</sub> and GA<sub>20</sub> elongated (Fig. 2); The order of the activity was GA<sub>1</sub>=GA<sub>3</sub>=GA<sub>20</sub>>GA<sub>19</sub>. Contrarily, leaves of K- and KA- treated plants failed to elongate.

#### Endogenous GAs levels (Expt.2)

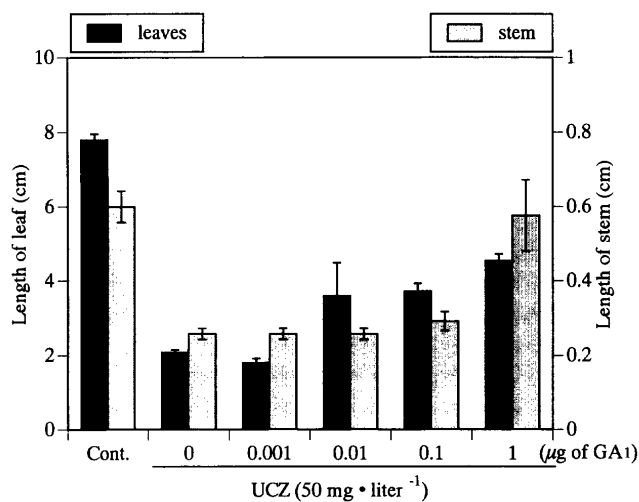
Stems of the untreated control plants remained rosetted and compressed (< 0.5 cm) until harvest, but their leaves expanded to the same size as those of cold-treated ones. Stems of the cold-treated plants elongated (5-7 cm) but did not initiate any flower buds.

Endogenous levels of GA<sub>1</sub>, GA<sub>20</sub>, GA<sub>19</sub>, GA<sub>44</sub> and GA<sub>53</sub> in the stems of the non-rosetted plants were 14.3, 20.0, 13.8, 16.1 and 6.7 times higher than those of the rosetted plants, respectively (Table 1). The ratios of [GA<sub>53</sub>]:[GA<sub>44</sub>], [GA<sub>44</sub>]:[GA<sub>19</sub>], [GA<sub>19</sub>]:[GA<sub>20</sub>] and [GA<sub>20</sub>]:[GA<sub>1</sub>] were 1.37, 1.17, 13.52 and 0.95 in the non-rosetted plants, and 3.26, 1.00, 19.63 and 0.66 in the rosetted plants, respectively.

Endogenous levels of GA<sub>1</sub>, GA<sub>20</sub>, GA<sub>19</sub>, GA<sub>44</sub> and GA<sub>53</sub> in the leaves of the non-rosetted plants were 4.2, 11.6, 11.6, 3.6 and 17.1 times higher than those of the rosetted plants, respectively (Table 1). The ratios of [GA<sub>53</sub>]:[GA<sub>44</sub>], [GA<sub>44</sub>]:[GA<sub>19</sub>], [GA<sub>19</sub>]:[GA<sub>20</sub>] and

Table 1. GA levels (ng g<sup>-1</sup> fresh weight) of the non-rosetted and the rosetted plants of *E. grandiflorum*.

	non-rosetted		rosetted	
	stems	leaves	stems	leaves
GA <sub>53</sub>	11.66	2.73	1.73	0.16
GA <sub>44</sub>	8.51	2.19	0.53	0.61
GA <sub>19</sub>	7.30	4.05	0.53	0.35
GA <sub>20</sub>	0.54	2.20	0.03	0.19
GA <sub>1</sub>	0.57	0.54	0.04	0.13



**Fig. 3.** Interaction between UCZ and GA<sub>1</sub> on leaf and stem elongation in rosetted plants of *E. grandiflorum* 4 weeks after GA<sub>1</sub> treatment. Data were obtained from the 4th node leaves. Values are Mean ± SE (leaves: n=24, stem: n=12).

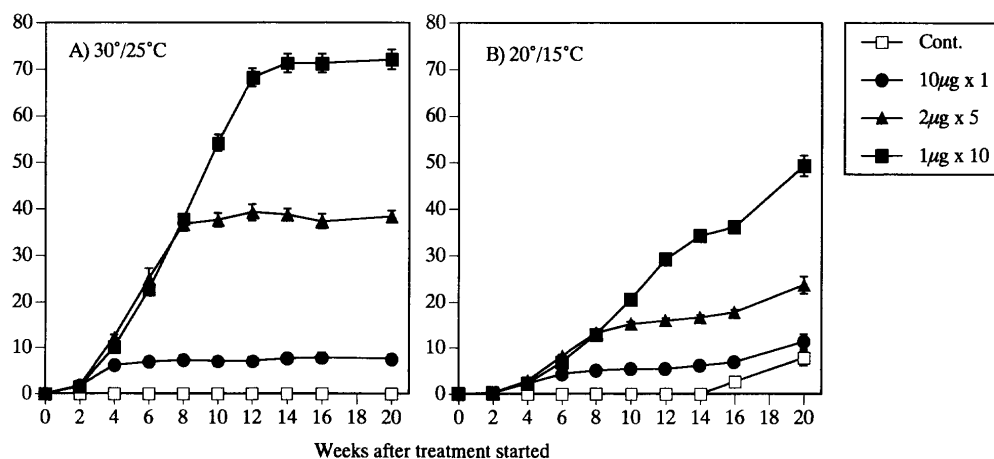
[GA<sub>20</sub>]:[GA<sub>1</sub>] were 1.25, 0.54, 1.84 and 4.07 in the non-rosetted plants, and 0.26, 1.74, 1.84 and 1.46 in the rosetted plants, respectively.

#### Difference in response to GA<sub>1</sub> between leaves and stem of rosetted seedling (Expt. 3)

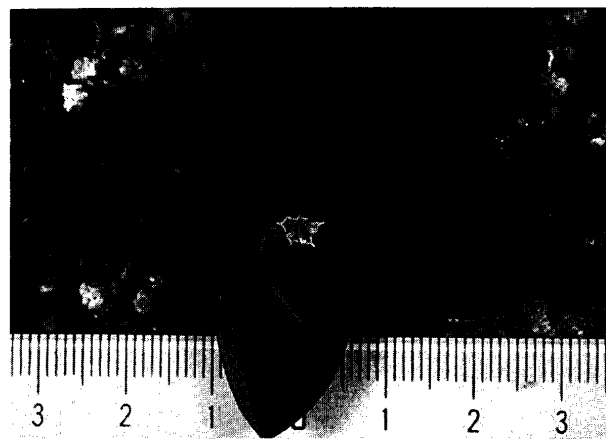
Elongation of leaves and stems of UCZ treated plants was markedly inhibited, compared to those of control plants (Fig. 3). The inhibition of leaf elongation by UCZ was not reversed by 0.001 μg GA<sub>1</sub> but it was partially reversed at higher dosages of GA<sub>1</sub>. UCZ inhibition of stem elongation was reversed only by the highest dose of 1.0 μg GA<sub>1</sub>; lower dosages of GA<sub>1</sub> had no reversible effect.

#### Effect of GA<sub>3</sub> on flowering of rosetted seedling (Expt. 4)

At 30°/25 °C, stems of the control plants remained rosetted until the end of the experiment (Fig. 4A),



**Fig. 4.** Cumulative growth curves of stems of rosetted *E. grandiflorum* plants after a weekly administration of GA<sub>3</sub> for 1, 5, and 10 weeks and grown under 30°/25 °C (A) and 20°/15 °C (B). Values are Mean ± SE (n=15).



**Fig. 5.** Flowering of the non-rosetted plant of *E. grandiflorum* treated with UCZ.

whereas, GA<sub>3</sub> treatment induced stem elongation. Stem elongation of the plants treated with 10 μg × 1 time, 2 μg × 5 times and 1 μg × 10 times of GA<sub>3</sub> continued for 4, 8, and 12 weeks after treatment started, respectively. However, those plants reformed rosettes at the higher nodal positions and failed to flower. Stems of control plants kept at 20°/15 °C (Fig. 4B) began to elongate 14 weeks after the experiment started. Stems of plants treated with 10 μg × 1 time, 2 μg × 5 times and 1 μg × 10 times of GA<sub>3</sub> elongated rapidly for 6, 10, and 14 weeks, respectively, and then re-formed rosettes. But these plants grew out of the rosettes on the 14th week as did those of the control. All plants kept at 20°/15 °C formed flower buds by the end of the experiment.

#### Effect of GA<sub>3</sub> and UCZ on flowering of non-rosetted seedling (Expt. 5)

UCZ completely inhibited stem elongation (Table 2), whereas GA<sub>3</sub> tended to overcome this inhibition. Control plants initiated flower buds after forming 16.4 pair-leaves under our experimental conditions; flower bud initiation was delayed by GA<sub>3</sub> treatment until 18.6 pair-leaves were formed, whereas it was accelerated by UCZ

**Table 2.** Effect of GA<sub>3</sub> and UCZ on stem length and flowering in the non-rosetted plants of *E. grandiflorum*.

Treatment	Control	GA <sub>3</sub>	UCZ	UCZ + GA <sub>3</sub>
Stem length (cm)	60.6 ± 2.18 <sup>z</sup>	94.7 ± 2.66	1.9 ± 0.32	84.3 ± 1.85
Number of flowering node	16.4 ± 0.36	18.6 ± 0.41	14.7 ± 0.42	16.6 ± 0.50

<sup>z</sup> mean ± SE (n=15)

treatment and occurred after 14.7 pair-leaves developed. UCZ inhibited leaf expansion, stem elongation and flower bud development (Fig. 5).

### Discussion

In a previous paper (Hisamatsu et al., 1998b), we proposed that one major GA biosynthesis pathway, the early 13-hydroxylation pathway ( $\rightarrow$ GA<sub>53</sub>  $\rightarrow$  GA<sub>44</sub>  $\rightarrow$  GA<sub>19</sub>  $\rightarrow$  GA<sub>20</sub>  $\rightarrow$  GA<sub>1</sub>  $\rightarrow$  GA<sub>8</sub>), functioned in shoots with flower buds of *E. grandiflorum*, and that endogenous GA<sub>1</sub> could regulate elongation of stems and leaves. Differences in the levels of endogenous GAs between non-rosetted plants and the rosetted plants indicate that the GA-biosynthesis step prior to GA<sub>53</sub> is blocked in the rosetted plants (Table 1). Because K and KA had no effect on stem elongation (Fig. 1) and leaf elongation (Fig. 2), it can be construed that activities of *ent*-kaurenoic acid 7  $\alpha$ -hydroxylase and/or *ent*-kaurene oxidase may be low in the rosetted plants. Their activities were observed to be increased by cold treatment in *Thraspi arvense* (Hazebroek and Metzger, 1990, Hazebroek et al., 1993). K and KA are highly hydrophobic compounds, so that further investigation on their absorption and metabolism in the plant is required to explain these results.

Complementary DNAs of 20-oxidases have been cloned from some plant species, and the products of heterologous expression in *E. coli* that 20-oxidases catalyze the multiple steps (GA<sub>53</sub>  $\rightarrow$  GA<sub>44</sub>  $\rightarrow$  GA<sub>19</sub>  $\rightarrow$  GA<sub>20</sub>) in the early 13-hydroxylation pathway was demonstrated (Hedden and Kamiya, 1997). In *Arabidopsis*, genes of 20-oxidase were found to be tissue-specific with transcripts detected (Phillips et al., 1995). The number of isoenzymes may vary between species so that GA<sub>53</sub>-oxidase and GA<sub>19</sub>-oxidase may differ from GA<sub>44</sub>-oxidase in spinach (Gilmour et al. 1986). That stems of rosetted plants failed to respond to GA<sub>19</sub>, whereas leaves did, indicating that the conversion of GA<sub>19</sub> to GA<sub>20</sub> through 20-oxidation may not be active in the stem of rosetted plants. Leaf elongation responding to GA<sub>19</sub> in the rosetted plants indicates that the conversion activity may be higher in leaves than in stems (Figs. 1 and 2). Furthermore, the ratio of [GA<sub>19</sub>]:[GA<sub>20</sub>] in the stems was greater than that in the leaves in rosetted as well as non-rosetted plants (Table 1). Such a difference between stems and leaves also occurred in *Lathyrus odoratus* (Ross et al., 1993). In *Pisum sativum*, applied [<sup>3</sup>H<sub>2</sub>]GA<sub>19</sub> was more rapidly converted to [<sup>3</sup>H<sub>2</sub>]GA<sub>20</sub> in leaflets than in stems

(Smith, 1991). Thus, differences in the ratio of [GA<sub>19</sub>]:[GA<sub>20</sub>] between stems and leaves suggest that there are tissue-specific activities of 20-oxidases in plants.

Responses of stem and leaf elongation to GA<sub>1</sub> and GA<sub>20</sub> in the rosetted plants were the same as previously reported (Hisamatsu et al., 1998b) which reconfirms that the metabolic pathway from GA<sub>20</sub> to GA<sub>1</sub> through 3  $\beta$ -hydroxylation is functioning in rosetted stems and leaves.

Hydroxylation at position 2  $\beta$  of gibbane skeleton is a deactivation step of biologically active GAs (Graebe, 1987). GA<sub>3</sub> has a double bond between carbon positions 1 and 2 in ring A of gibbane skeleton, which diminishes 2-hydroxylation. That the response of stem and leaf elongation to GA<sub>1</sub> in the rosetted plants is the same as that to GA<sub>3</sub> (Fig. 1) indicates that 2  $\beta$ -hydroxylation activity of the metabolic pathway from GA<sub>1</sub> to GA<sub>8</sub> may not be active in the rosetted plants. If the rosetted plants have a high 2  $\beta$ -hydroxylation activity, GA<sub>3</sub> should be more biologically active than GA<sub>1</sub>, as observed in *Matthiola incana* (Hisamatsu et al., 1997).

Blockage of GA-biosynthesis is not restored by exogenous GAs in the rosetted plants since plants treated with GA<sub>1</sub>, GA<sub>3</sub> and GA<sub>20</sub> re-formed rosetted growth at higher nodal positions after transitional internode elongation under high temperature conditions (in Expt. 1 and 4).

Stems did not elongate but leaves expanded in the rosetted plants of *E. grandiflorum* as well as in other plants species. Although we suggested that the endogenous GA<sub>1</sub> level in the rosetted plants was sufficient for leaf expansion (Hisamatsu et al., 1998b), it has not been proven whether the difference in growth between stems and leaves in the rosetted plants was caused by a tissue-specific GA biosynthesis or by differences in responsiveness to GA<sub>1</sub> among the different tissues. Leaves of the rosetted plants expanded as well as the non-rosetted plants, although the level of endogenous GA<sub>1</sub> in the former was about a quarter of the latter (Table 1). Although inhibition of leaf and stem elongation by UCZ treatment was partially reversed by GA<sub>1</sub>, the minimum level of GA<sub>1</sub> required for elongation between the stems and the leaves, was different and this suggests that of leaves requires lower level of GA<sub>1</sub> than that for stems (Fig. 3). Thus, the rosetting in *E. grandiflorum* may be caused by a complex interaction that involves GA-biosynthesis inhibition prior to GA<sub>53</sub>, different 20-oxidases activities between stems and leaves, and different requirement of GA<sub>1</sub> amount for development be-

tween stems and leaves.

Day/night fluctuation between 20°/15 °C induced, stems of control plants and those treated with GA<sub>3</sub> to elongate 14 weeks after treatment. All had flower buds at the end of experiment, although GA treated plants passed through a transitional internode elongation (Fig. 4). Ohkawa et al. (1994) reported that temperatures between 5 and 20 °C were effective for induction of stem elongation and flowering of rosetted plants of *E. grandiflorum*. Therefore, stem elongation and flowering of the rosetted plants in this study were probably induced by 20°/15 °C treatment. However, flowering of the rosetted plants was not recovered by GA<sub>3</sub> treatment at 30°/25 °C, although the stems elongated (Fig. 4). Reduction of endogenous GA levels by UCZ treatment did not inhibit but accelerated flower bud initiation of the non-rosetted plants (Table 2), which we also found under greenhouse conditions (data not shown). Although UCZ treatment inhibited flower bud development, stem elongation and leaf expansion (Fig. 5), we observed that these inhibition by UCZ was overcome by a combined treatment with GA<sub>3</sub>. Thus, our results indicate that the stem and leaf elongation and the flower bud development of *E. grandiflorum* are apparently GA-dependent but flower bud initiation is not.

#### Acknowledgment

This work was supported by a research grant from Ministry of Agriculture, Forestry and Fisheries (Japan). We are grateful to Prof. R. P. Pharis (Univ. of Calgary, Canada) for supplying of GA<sub>1</sub>, GA<sub>19</sub> and GA<sub>20</sub>, Dr. M. S. Roh (USDA, ARS, USA) for his useful comments and valuable suggestions on the manuscript, Ms. S. Maeda and Ms. T. Yamato (NIVOT, Japan) for their assistance in the experiments.

#### Literature Cited

- Gilmour, S. J., J.A.D. Zeevaart, L. Schwenen and J. E. Graebe. 1986. Gibberellin metabolism in cell-free extracts from spinach leaves in relation to photoperiod. *Plant Physiol.* 82: 190–195.
- Graebe, J.E. 1987. Gibberellin biosynthesis and control. *Ann. Rev. Plant Physiol.* 38: 419–465.
- Harbaugh, B. K., M. S. Roh, R. H. Lawson and B. Perberton. 1992. Rosetting of *Lisianthus* cultivars exposed to high temperature. *HortScience* 27: 885–887.
- Hazebroek, J.P. and J.D. Metzger. 1990. Thermoinductive regulation of gibberellin metabolism in *Thlaspi arvense* L. I. Metabolism of [<sup>2</sup>H]-ent-Kaurenoic acid and [<sup>14</sup>C] Gibberellin A<sub>12</sub>-aldehyde. *Plant Physiol.* 94: 157–165.
- Hazebroek, J.P., J.D. Metzger and E.R. Manserger. 1993. Thermoinductive regulation of gibberellin metabolism in *Thlaspi arvense* L. II. Cold induction of enzymes in gibberellin biosynthesis. *Plant Physiol.* 102: 547–552.
- Hedden, P. and Y. Kamiya. 1997. Gibberellin biosynthesis: enzymes, genes and their regulation. *Annu. Rev. Plant Physiol. Plant. Mol. Biol.* 48: 431–460.
- Hisamatsu, T., M. Koshioka, S. Kubota, R. W. King and L. N. Mander. 1997. The role of GA biosynthesis in the control of flowering in *Matthiola incana* (L.) R. Br. *Proceedings of PGRSA* 24th: 54.
- Hisamatsu, T., M. Koshioka, S. Kubota, T. Nishijima, H. Yamane, R. W. King and L. N. Mander. 1998a. Isolation and identification of GA<sub>112</sub> (12 β-hydroxy-GA<sub>12</sub>) in *Matthiola incana*. *Phytochemistry* 47: 3–6.
- Hisamatsu, T., M. Koshioka, T. Nishijima and L. N. Mander. 1998b. Identification of endogenous gibberellins and their role in rosetted seedlings of *Eustoma grandiflorum*. *J. Japan. Soc. Hort. Sci.* 67: 866–871.
- Nishijima, T. and N. Katsura. 1989. A modified micro-drop bioassay using dwarf rice for detection of femtomol quantities of gibberellins. *Plant Cell Physiol.* 30: 623–627.
- 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. *Scientia Hort.* 48: 171–176.
- Ohkawa, K., T. Yoshizumi, M. Korenaga and K. Kanematsu. 1994. Reversal of heat-induced rosetting in *Eustoma grandiflorum* with low temperatures. *HortScience* 29: 165–166.
- Phillips, A. L., D. W. Ward, S. Uknes, N. E. J. Appleford, T. Lange, A. K. Huttly, P. Gaskin, J. E. Graebe and P. Hedden. 1995. Isolation and expression of three gibberellin 20-oxidase cDNA clones from *Arabidopsis*. *Plant Physiol.* 108: 1049–1057.
- Ross, J. J., I. C. Murfet and J. B. Reid. 1993. Distribution of gibberellins in *Lathyrus odoratus* L. and their role in leaf growth. *Plant Physiol.* 102: 603–608.
- Smith, V. A. 1991. Gibberellin A<sub>1</sub> biosynthesis in *Pisum sativum* L. II. Biological and biochemical consequences of the *le* mutation. *Plant Physiol.* 99: 372–377.
- Takeda, T. 1988. Rosette formation of *Eustoma grandiflorum*. *J. Japan. Soc. Hort. Sci.* 57 (Suppl. 2): 574–575 (In Japanese).

## トルコギキョウのロゼット化と内生ジベレリンとの関係

久松 完<sup>1</sup>・腰岡政二<sup>1</sup>・大山直美<sup>1</sup>・L. N. Mander<sup>2</sup><sup>1</sup> 農林水産省 野菜・茶業試験場 514-2392 三重県安芸郡安濃町草生 360<sup>2</sup> Research School of Chemistry, Australian National University, Canberra, A.C.T. 0200, Australia

## 摘 要

トルコギキョウのロゼット化と内生ジベレリンとのかかわりを明らかにするために、ロゼット化した実生に対する数種ジベレリンおよび前駆物質の影響ならびにロゼット化および非ロゼット化実生の内生ジベレリン含量を調査した。また、それらの開花に及ぼすジベレリン生合成阻害剤と GA<sub>3</sub> の影響を調査した。その結果、ロゼット化した実生では初期 13 位

水酸化経路上の GA<sub>53</sub> より上流で生合成がブロックされている可能性が示された。また、ロゼット化および非ロゼット化実生にかかわらず、葉と茎において GA<sub>19</sub> から GA<sub>20</sub> に至る 20 位の酸化活性に違いがあることが示唆された。葉の伸展、茎の伸長および花芽発達は活性型 GA により制御されているが、花芽分化は制御されていない可能性が示された。