Reversal of Heat-induced Resetting in *Eustoma grandiflorum* with Low Temperatures

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Abstract. The effects of seedling age and temperature regimes and durations on the reversal of *Eustoma grandiflorum* (Raf.) Shin. heat-induced rosette formation were clarified. When *E. grandiflorum* seedlings were grown in a natural-light phytotron ($600\text{--}800\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 4 weeks at 33/28C (12-h day/12-h night) from germination to the four true-leaf stage, the optimum temperature and duration required to break rosette formation was 15C for 4 weeks with continuous illumination ($35\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). However, when seedlings were grown for 12 weeks at 33/28C from germination to the eight true-leaf stage, shoot elongation required 6 weeks at 10C.

Seedlings of *Eustoma grandiflorum* 'Fukushihai' typically bolt after they develop three true-leaf pairs. However, when seedlings are germinated from the end of September to early October in an unheated greenhouse at a daily mean of 18.3–23.6C, rosette formation naturally occurs, and plants do not bolt until the following spring. This is because 5–8C nights are too cold for internode elongation to occur during the winter after resetting. Bolting, however, would occur at 20–25/15–20C after seedlings develop three true-leaf pairs. Seedlings germinated in July or August, when daily means are 25.3–26.7C, form rosettes because of high-temperature exposure early in their growth, and they do not bolt even if held at 20C after resetting. Internode elongation does not occur and is not accelerated by high temperature until after seedlings have encountered low temperatures in unheated greenhouses. *Eustoma grandiflorum* requires low temperatures to induce bolting once young seedlings form a rosette (Pergola, 1990; Takeda, 1988). Ohkawa et al. (1991) reported that *E. grandiflorum* seeds are sensitive to high temperature, from water absorption through germination during early growth and until two leaf pairs have formed. Also, bolting does not occur when seedlings are grown at a day temperature > 30C, if they receive a 20C night minimum. If plants are placed in high temperatures after more than two leaf pairs have formed, they do not rosette but develop a normal elongated shoot that rapidly flowers (Ohkawa et al., 1991). Our report defines the effects of seedling age and temperature regimes and durations on the reversal of heat-induced rosette formation in *E. grandiflorum* 'Fukushihai'.

Materials and Methods

On 13 Apr., 'Fukushihai' seeds were germinated in paper pots ($4 \times 4 \times 4$ cm) filled with a dry commercial peatmoss and vermiculite medium (Ibigawa Co., Gifu City, Japan). They grew for 4 or 12 weeks in a natural-light phytotron ($600\text{--}800\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (Hitachi Co., Tokyo) set at 33/28C (12-h day/12-h night). Seedlings were thinned and not transplanted. Then the seedlings were transferred to growth chambers and grown for another 4 or 6 weeks at a constant 5, 10, 15, 20, or 25C with continuous illumination ($35\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) from cool-white fluorescent lamps (Toshiba Co., Tokyo). After the respective 4- or 6-week temperature treatment, plants were transferred and grown in a natural-light phytotron at 28/23C (12-h day/12-h night). Shoot elongation was recorded weekly. Plants with first internodes longer than 3 mm were judged to have bolted. If the plant bolted, the number of true leaves that had formed before bolting was counted. There were 32 plants in four replications in each regime. This experiment was discontinued after 21 weeks.

Results and Discussion

If exposed to 5–20C after 4 weeks at high temperature, seedlings with four true rosette leaves (grown 4 weeks at 33/28C) elongated (Fig. 1A). The most effective temperature regime for reversing the high-temperature-induced resetting was 15C for 4 weeks; 5C was least effective. A total of 87% of the seedlings elongated after being treated at 5C for 4 weeks, and 98% elongated after 6 weeks. The bolting ratio at 25C was low—5% after 4 weeks and 17% after 6 weeks treatment.

When plants had eight true rosette leaves and were grown for 12 weeks at 33/28C, 4-week low-temperature treatments reversed resetting in the order of 10C > 5C > 15C. Bolting at 20C was 35% after 4 weeks of treatment and 52% after 6 weeks; no plants bolted at 25C (Fig. 1B).

With seedlings at the four-leaf stage, the time that elapsed before 80% of plants bolted ranged from 4.5 at 15C to 6.8 weeks at 5C for plants held for 4 weeks (Table 1). Consequently, 4 weeks of lower temperatures was enough to reverse heat-induced resetting. In contrast, when seedlings were at the eight-leaf stage at treatment, the time that elapsed before 80% of plants bolted was 7.0 and 10.5 weeks for plants kept at 10 or 5C for 4 weeks, respectively (Table 1). However, 6 weeks at 10 or 5C hastened bolting to a level similar to plants at the four-leaf stage treated for 4 weeks at 15C.

The fewest true leaves developed at 10 or 15C before bolting when plants were treated at the four-leaf or eight-leaf stage before they were transferred to the lower temperatures (Table 2).

We found that the effective low temperature to induce bolting was between 5 to 20C (Fig. 1). The most effective temperature was

Fig. 1. Effect of seedling age, temperature, and duration (○ = 4 weeks; ● = 6 weeks) on shoot elongation of *Eustoma grandiflorum*. (A) Plants with four true leaves. (B) Plants with eight true leaves. Standard error bars were smaller than the symbols and are not shown.

Table 1. Effect of *Eustoma grandiflorum* seedling age, temperature, and duration on the number of weeks elapsed before 80% of plants had bolted after low-temperature treatment.

		Seedling age (true leaves)			
		4		8	
		Temp duration (weeks)			
Low-temp regime (°C)	4	6	4	6	
	No. weeks				
5	6.8	5.0	10.5	5.0	
10	6.0	4.7	7.0	4.0	
15	4.5	3.8	---	6.0	
20	5.8	4.0	---	---	
Significance					
Linear	**	**	**	**	
Quadratic	*	*	NS	NS	

*With 15C for 4 weeks and 20C for 4 and 6 weeks, the eight true-leaf plants did not reach 80% bolting until the experiment was discontinued.

NS * **Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

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Table 2. Effects of *Eustoma grandiflorum* seedling age, duration of temperature exposure, and temperature on seedlings with four or eight rosette leaves on the number of true leaves that developed before bolting.

Low-temp regime (°C)	Seedling age (true leaves)			
	4		8	
	Temp duration (weeks)			
	4	6	4	6
	No. leaves			
5	8.0	7.5	12.4	11.0
10	7.7	7.1	11.2	11.2
15	7.6	7.0	13.0	11.8
20	8.5	7.9	14.0	13.0
25	11.1	10.5	---	---
Significance				
Linear	**	**	*	*
Quadratic	NS	*	NS	NS

^zNo bolting occurred.

NS, *, **Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

15C for young resetting seedlings with four true leaves and 10C for seedlings with eight true leaves. Four weeks of cold treatment were

needed to induce bolting for young seedlings, but 6 weeks were needed for older seedlings.

Chrysanthemum [*Dendranthema* × *grandiflorum* (Ramat.) Kitamura] (Schwabe, 1950) and statice (*Limonium sinuatum* Mill.) (Shillo and Zamski, 1985) exhibit rosette formation and elongated shoots only after being exposed to low temperature. However, for *E. grandiflorum* the low-temperature requirement for bolting is minimal; the optimum temperature is high (10-15 C), and the duration is short (4-6 weeks). From our results, we assume that current commercial cultivars (progenitors in Texas, New Mexico, and Oklahoma) still have ecological characteristics of heat-induced rosette formation for overwintering. The natural species of *E. grandiflorum* from Colorado, a cold climate area, requires low temperatures (2.5-7.5C) for 90 days to break heat-induced rosette formation (Ohkawa, 1990, unpublished data). Also, Harbaugh et al. (1992) have found that high-temperature-induced resetting is a characteristic that can be eliminated by selection, thus allowing cultivars to be developed

specifically for autumn and winter production.

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