Effect of seed cold PEG-priming and subsequent long storage on germination, growth and flowering of *Eustoma grandiflorum* (Raf.) Shinn 'Exe Lavender'

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Cold wet treatment is frequently applied to *Eustoma* seeds to enhance the bolting rate of plants grown under high temperatures. Our previous study indicated that cold PEG-primed *Eustoma* seeds could maintain their germination rate and bolting rate even after being re-dried for 30 days and grown under high temperatures. The present study aimed to investigate whether prolonged storage after cold PEG-priming affect the germination, growth, and flowering of *Eustoma* 'Exe Lavender' seedling. Seeds were initially cold-primed with water or PEG-6000 at -1.5 MPa for 5 weeks at 10°C in the dark and were then subjected to re-drying and storage for 30, 60, 90 and 360 days at 10°C. After 360 days of storage, cold PEG-primed seeds germinated earlier and more effectively than cold hydro-primed seeds. Compared to the results after 30 days of storage, plants grown from 360 days exhibited similar bolting rate, days to bolting, bolting node and flowering rate, cut flower length and number of flower node. These results suggest that 'Exe Lavender' seeds can germinate and develop well even after 360 days of storage and under high temperature conditions when subjected to cold PEG-6000 treatment.

Key words : bolting, chilling, dehydrate, long storage, PEG-6000

Introduction

Eustoma is well known for its sensitivity to high temperatures, which can result in rosetted plant and limit flowering[8]. Cold imbibition of seeds has demonstrated positive effects on elongating Eustoma plant internodes and promoting flower growth in many cultivars when grown under such conditions[5,6,13]. Futhermore, seeds or seedlings of the strong-rosette type require additional treatments to prevent short plant growth or the absence of flowers, such as intermittent low-temperature storage[15]. Supplying commercially treated *Eustoma* seeds already treated with low temperature treatment makes it easier for growers to sow the seeds in the summer and harvest cut flowers in the winter. To achieve this goal, our research was conducted to evaluate the germination, bolting, and flowering abilities of *Eustoma* seeds subjected to cold wet treatment followed by dehydration. Some of the *Eustoma* seeds were imbibed at 10℃ in dark conditions for 5 weeks, and subsequent dehydration resulted in good germination and the development of healthy seedlings[16].

In our previous report, we found that *Eustoma* seeds could undergo cold imbibiton for up to 8 weeks, resulting in faster and higher germination rates when Polyethylene glycol (PEG) was used for priming instead of water[17]. Cold PEG-priming for such an extended duration did not induce chilling injury in *Eustoma* seeds. On the contrary, immersing the seeds in a lower osmotic potential, like PEG -1.5 Mpa, helped prevent the embryonic axis from protruding out of the seed coat, while maintaining the bolting and flowering abilities of *Eustoma* seeds, even after re-drying and growth under high temperatures. PEG is commonly used in osmopriming due to its large molecular size, chemical inertness, and lack of damage to embryos[9,18]. This priming method has been shown to provide support for optimal germination and seedling quality under temperature stress conditions[10,12].

To assist seed companies in providing high-quality *Eustoma* seeds to farmers, further studies on cold PEG-

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priming and subsequent storage are necessary. Therefore, the present study aimed to investigate the effects of cold PEG-priming and storage for at least one year on *Eustoma* seed germination, growth and flowering.

Materials and Methods

Eustoma 'Exe Lavender' (Kaneko Seeds Co., Ltd., Tokyo, Japan) seeds were cold-primed with water (0 MPa) and PEG-6000 (-1.5 MPa) at 10℃ in the dark for 35 days before being entered for re-drying. After finishing cold-priming, the seeds were collected using a plankton net and quickly re-dried at room temperature for about 15 min. Next, they were put into a paper bag, which was put in a plastic bag with an aliquot of silica gel for moisture reduction and then covered with aluminium foil to prevent light entry in re-drying and storage. After finishing cold priming and quick re-drying, the cold-primed seeds were re-dried and stored at 10℃ in the dark for 30, 90, 180, and 360 days in the incubator. Cold-primed seeds without re-drying were also investigated as non-RDT. Seeds were sown in a 288-cell tray on July 03 2019 and placed in a greenhouse. Seedlings were transplanted on August 11 2019.

Transplanting methods

The seedlings were daily supplied with 1/6 strength OAT A solution (N, 17.7 mM ; P, 1.7 mM ; K, 7.8 mM ; Ca, 4.1 mM ; Mg, 1.86 mM ; standard solution, OAT Agrio Co., Ltd, Tokyo, Japan) by sub-irrigation until transplanting time. The air temperature in the greenhouse was recorded every 5 min using a datalogger (Ondotori, TR-71Ui ; T&D Corporation, Matsumoto, Japan). At transplanting time, six seedlings were transplanted to a planter (64 cm × 22 cm × 18 cm, soil capacity 15.0 L) as one replication and there were three replications per treatment. The planters were placed in the greenhouse kept at a minimum of 15℃ and day length of 16 h (natural daylength plus lighting by incandescent lamp from 04 : 00 to 09 : 00 and from 16 : 00 to 20 : 00). Ten days after transplanting, 1.5 liters of 1/2 strength OAT A solution was applied to each planter once a week.

Data collection

A germinated seed was recorded when the radicle emerged from the seed coat, and germination was observed daily until 15 days after sowing. The bolting date and the number of nodes to the first bolting were recorded when the length of an internode exceeded 5 mm. The flowering date and number of nodes to the first flowering were recorded when the first flower opened for all plants except the rosette ones. Flowering shoots were harvested when the fourth flower opened and cut flower length (cm) was recorded at the harvesting. The mean values of each replication were subjected to one-way ANOVA and means were compared using Tukey's HSD test or subjected to *t-test* (Excel-Toukei 2010 ; Social service Research, Information Co. Ltd., Japan).

Results and discussion

During the seedling growth in 2019 (July-August), Eustoma 'Exe Lavender' was exposed to high daily temperatures ranging from 24.0℃ to 33.0℃, with most days exceeding 25℃ (Fig. 1). The average daily minimum temperature, daily mean temperature, and daily maximum temperature were 24.1℃, 28.7℃, and 35.5℃, respectively. This plant belongs to mild-strong rosette type, which requires cold wet treatment to accelerate bolting and flowering when grown under such hot conditions. The effects of PEG-priming on the germination and growth of 'Exe Lavender' was previously published [17]. The bolting rate was maintained even when the seeds were immersed in a PEG-6000 solution and subsequently re-dried for 30 days. In the current study, the results of treating with cold PEG-priming and subsequent storage for up to 360 days were reported.

Regarding storage, a significant reduction in seed germination was observed between 180 days and 360 days storage for *Eustoma* seed treated with cold hydro-priming. However, the germination rate of seeds treated with cold PEG-priming remained unchanged even after 360 days storage (Fig. 2). Cold PEG-primed seeds exhibited a final germination rate of 91% after storage

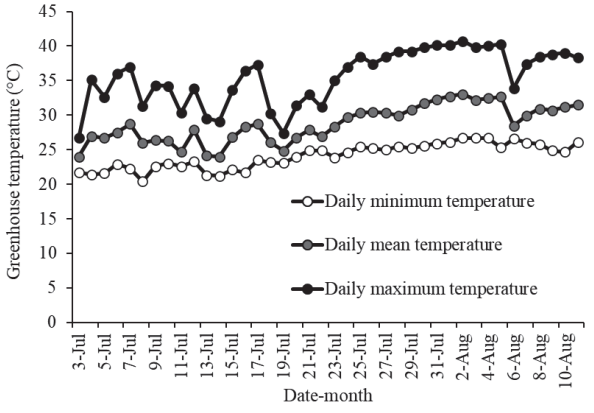

Fig. 1　Daily mean and daily maximum and minimum temperatures recorded during 2019 growing period from July 03 to August 11.

for 360 days, whereas hydro-primed seeds only reached 73% (Fig. 2, Fig. 3). Treating *Eustoma* 'Exe Lavender' with cold PEG-priming at −1.5 MPa (10℃) for 35 days had no impact on the germination rate of seeds stored for up to 360 days. PEG at −1.5 MPa demonstrated the most effective priming results by reducing the time to germinate and achieving a germination rate of over 80% within just 6 days of sowing. Similar to our finding, osmotic priming did not affect the storage stability of onion[3] and spinach seeds[2]. However, priming did accelerate seed deterioration during storage in the seeds of lettuce[14], muskmelon[11] or tomato[1]. Even within a single genus, different species that treated by osmopriming may exhibit varying responses to abiotic stress, with some successfully germinating and growing while others show strong germination but susceptibility at the seedling stage[4].

For Eustoma, a 35-day cold treatment was sufficient to maintain the bolting ability of 'Exe Lavender' even after storage for up to one year (Table 1). In PEG-priming, there was no significant influence on days to bolting, flowering, bolting rate, flowering rate, bolting node and flowering node across different storage periods. In hydro-priming, however, the flowering rate slightly decreased after 360 days storage compared to the other periods. The number of flower and flower-bud and cut flower length ranged from approximately 5.6 to 7.6 and from 44.8 to 52.0 cm, respectively, for both priming agents. One intriguing aspect of this study is that the positive effect of pre-sowing cold treatment on *Eustoma* seeds persisted even after storage of 360 days. Plants grown from seeds stored for 360 days exhibited a high bolting rate, even when subjected to high temperatures. Remarkably, re-drying and storing 'Exe Lavender' seeds

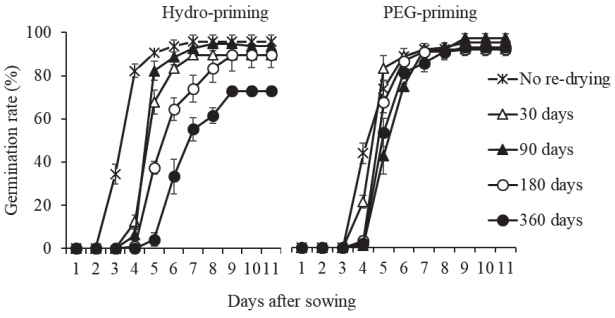

Fig. 2 Effect of cold imbibition and storage period on germination rate of *Eustoma* 'Exe Lavender'. Bars indicate standard errors in each day after sowing (n = 4, 24-seed/replication).

Fig. 3 Germination and seedlings of 'Exe Lavender' grown from seeds treated with cold hydro-priming (left) and cold PEG-priming (right) and following 360-day storage.

Table 1 Effect of seed cold-priming and different storage period on bolting and flowering ability of Eustoma 'Exe Lavender'

Water potential in cold imbibition	Storage days	Days to bolting	Bolting rate (%)	Bolting node	Days to flowering	Flowering rate (%)	Flowering node	Cut flower length (cm)	Number of flower bud
0 MPa	0	22.8 ab[y]	94.4 a[z]	5.4 ab	67.8 ab	77.8 b	9.9 a	49.0 a	7.1 a
	30	18.7 ab	100.0 a	4.7 a	65.0 ab	100.0 a	9.7 a	50.7 a	6.2 a
	90	21.9 ab	100.0 a	5.3 ab	68.2 ab	100.0 a	10.2 a	51.3 a	7.1 a
	180	17.6 a	100.0 a	4.8 a	61.8 a	94.4 a	9.7 a	50.9 a	6.9 a
	360	25.8 b	88.9 a	5.6 b	74.4 b	77.8 b	10.1 a	49.1 a	7.6 a
−1.5 MPa	0	18.5 a	94.4 a	5.1 a	67.4 a	88.9 a	9.7 a	48.5 a	6.2 a
	30	18.3 a	100.0 a	4.7 a	65.8 a	100.0 a	9.8 a	48.7 a	6.9 a
	90	23.6 a	94.4 a	5.5 a	73.4 a	88.9 a	10.4 a	44.8 a	6.2 a
	180	21.5 a	77.8 a	5.3 a	69.5 a	100.0 a	10.0 a	48.5 a	5.8 a
	360	19.9 a	94.4 a	5.3 a	66.2 a	88.9 a	9.9 a	52.0 a	7.3 a

[y] Different letters among treatments in each water potential indicate significant difference (Tukey's HSD test, n = 3, 6-plant/replication)
[z] Values were transformed to arcsine for statistical analysis

presented no issues for up to one year, as the growth improvement achieved from 35 days of cold imbibition at 10℃ was maintained. Utilizing cold PEG-treatment not only prevented radicle protrusion and maintained the germination rate but also prserved the cold effect observed during cold imbibition, even after long-term storage for *Eustoma* 'Exe Lavender'[17]. Therefore, PEG can be considered as a suitable priming agent for futher research on cold priming *Eustoma* seeds and their storage.

Conclusion

In this work, the effects of cold priming plus storage periods were investigated on *Eustoma* seeds. The use of PEG-priming enabled 'Exe Lavender' seeds to germinate at an rate of over 80%, even after 360-day storage. The application of cold pre-sowing treatment for *Eustoma* seeds proved to be essential for improving the growth of seedlings under high temperatures. The beneficial effects of cold treatment in hydro-priming or PEG-priming were sustained, resulting in *Eustoma* plants that bolted and flowered even after one year of storage. These results suggest the possibility for seed companies to produce cold priming *Eustoma* seeds which be able to be store for 6 month or more. Further studies should be conducted to clarify the influence of cold PEG-priming on other *Eustoma* cultivars.

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