Effect of Re-Drying and Storage after 10°C Wet Treatment on Germination and Growth of Five *Eustoma grandiflorum*（Raf.）Shinn Cultivars

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In *Eustoma* (*Eustoma grandiflorum* (Raf.) Shinn) cultivation in southwestern Japan, cold imbibition treatment of seeds is important to accelerate bolting and flowering of seedlings grown in the summer season. In order to facilitate the handling of cold imbibed seeds, re-drying treatment (RDT) after wet treatment at 10°C for 35 days in dark conditions (WT10°C) is considered to be an essential technique. The investigation of five *Eustoma* cultivars 'Exe Lavender', 'Orb Snow', 'Philia Lavender' , 'Dure Lavender' and 'Exe Light Pink' was to find the germinability and the growth characteristics of *Eustoma* when re-drying and storage at 10°C for 30 days were applied immedietely after ending the WT10°C. The results showed that germination and growth responses differed among *Eustoma* cultivars. RDT seeds and non-RDT seeds of 'Exe Lavender', 'Orb Snow' and 'Philia Lavender' had a similar germination rate. However, germination rate of RDT seeds in 'Dure Lavender' and 'Exe Light Pink' was lower than that of non-RDT seeds. There was no significant difference in cut-flower quality grown from RDT and non-RDT among 'Exe Lavender', 'Orb Snow' and 'Dure Lavender'. However, bolting and flowering rate of RDT in 'Dure Lavender' and 'Exe Light Pink' were lower than those of non-RDT. This suggested that it might not be useful to apply RDT after the cold-wet treatment of *Eustoma* seed due to lower germination or bolting rates in some cultivars. Further investigation of cold-wet exposure and dehydrating conditions is required to establish RDT procedures for *Eustoma* cultivars.

Key words : bolting, chilling, cut flower quality, dehydrate, flowering

Introduction

Eustoma（*Eustoma grandiflorum*（Raf.）Shinn）is a popular ornamental cut flower in Japan. In *Eustoma*, high temperatures in the summer season inhibit the bolting of many cultivars and induce rosette formation. This physiological disorder leads to the failure of commercial production of *Eustoma* cut flower from late autumn to early spring[5, 9, 11]. In common production, farmers usually treat with a cold exposure for hydrated seeds[8, 12]；the application of a wet treatment at 10℃ in dark conditions（WT10℃ from 4 to 5 weeks for *Eustoma* seeds has been widely used before sowing to prevent rosetting in growing *Eustoma* seedlings in the summer season[3, 12]. A recent study has shown that a combination of WT10℃ and intermittent low-temperature treatment on *Eustoma* young plant also improved the flower yield[10]. However, because most *Eustoma* seeds are traded as coated seeds, the farmers must conduct WT10℃ by using cell-trays and accompanying a large-sized refrigerator for cold treatment. If commercial *Eustoma* seed already treated at low temperature can be supplied for farmers, they can definitely skip the WT10℃ task. For this purpose, WT10℃ seed need to be dried and stored, but it is necessary to confirm the effects of low-temperature treatment and the re-drying of seed.

In a previous study, a re-drying treatment（RDT）at 10℃ for *Eustoma* 'King of Snow' seeds after 35 days of WT10℃ resulted in a reduction in the germination rate by 18.5% and a delay in germination time[4]. The authors speculated that a decrease in the germination rate after RDT might be due to protrusion of the radicle in WT10℃ seeds before dehydration. However, the effect of WT10℃ followed by RDT has not been tested in other *Eustoma* cultivars. In our present study, therefore, the effects of WT10℃ followed by RDT on the germination and subsequent growth of five *Eustoma* cultivars were investigated.

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Materials and Methods

General

Five *Eustoma* cultivars: 'Exe Lavender', 'Dure Lavender', and 'Philia Lavender' (Kaneko Seeds Co., Ltd., Tokyo, Japan), 'Orb Snow', and 'Exe Light Pink' (Sakata Seed Co., Tokyo, Japan) were investigated in this study (Fig. 1). Their naked seeds were treated WT10℃ for 35 days. After finishing such cold imbibition, 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 then put into a plastic bag with an aliquot of silica gel for moisture reduction and covered with aluminum foil to prevent light entry in re-drying treatment. The RDT seeds were re-dried and subsequently stored at 10℃ in the dark for 30 days. Seeds treated WT10℃ for 35 days and immediately sown (non-RDT) were also examined.

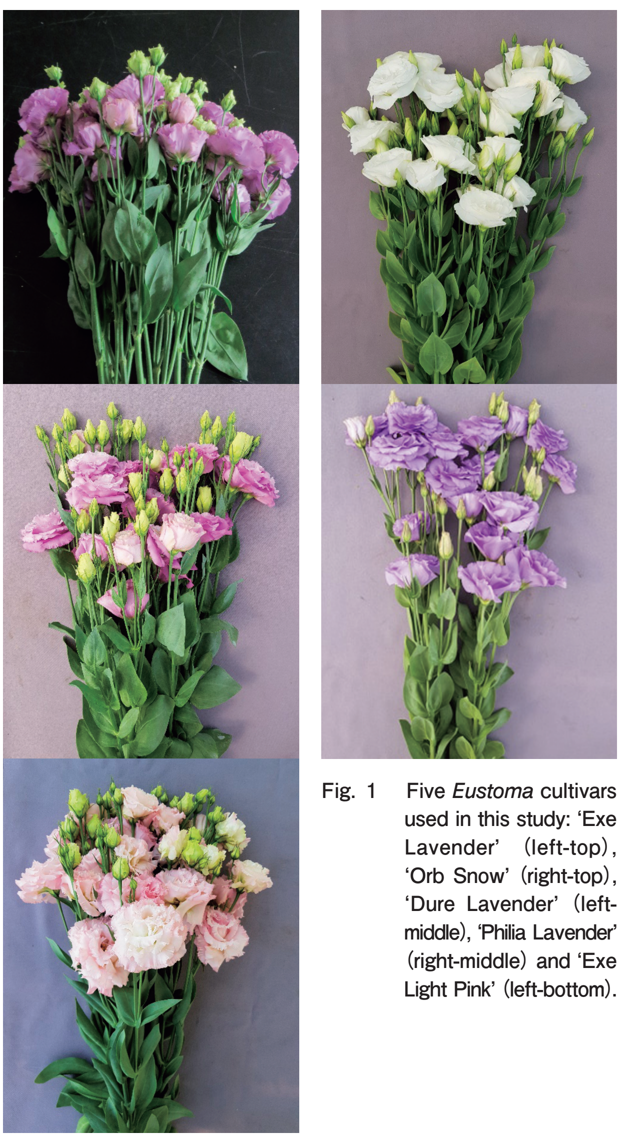

Fig. 1　Five *Eustoma* cultivars used in this study: 'Exe Lavender' (left-top), 'Orb Snow' (right-top), 'Dure Lavender' (left-middle), 'Philia Lavender' (right-middle) and 'Exe Light Pink' (left-bottom).

Raising seedling methods

All the seeds were sown on 288-cell trays on August 1, 2019 and each treatment had four replications and one replication had 24 seeds. Seeds were germinated using mist irrigation in a greenhouse until the cotyledons of most seedlings expanded completely. The cell-trays of seedlings were then transferred to another greenhouse and kept at a minimum of 15℃ and natural daylength on August 11, 2019. 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 on September 5, 2019. The air temperature in the greenhouse was recorded every 5 min using a datalogger (Ondotori, TR-71Ui; T&D Corporation, Matsumoto, Japan).

Transplanting methods

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

Germinated seed was recorded when the radicle protruded out of the seed coat and germination was observed daily with a magnifying glass LED-S3 (Terasaki Electric Co., Ltd., Osaka, Japan) until 15 days after sowing. Time to 50% germination (T50): $T50 = \left[\left(\frac{ta - tb}{na - nb}\right)(N - nb)\right] + tb$ (N = number of seeds when the population reached 50% of the final germination, ta = incubation day when the population reached 50% of the final germination, tb = incubation day before the population reached 50% of the final germination, na = number of germinated seeds on the day that the population reached 50% of the final germination, nb = number of germinated seeds on the day before the population reached 50% of the final germination). Times to 10% and 90% germination were measured as the same way with the above equation. The bolting date and bolting node were recorded when the length of an internode exceeded

5 mm. The flowering date was recorded when the first flower opened for all plants except the rosetted ones and the number of nodes to the first flower was also collected. Flowering shoots were harvested when the fourth flower opened up to January 31, 2020. Cut flower traits such as fresh weight (g) and length (cm), and the total numbers of visible flower buds and flowers were investigated. The mean values of each replication were subjected to one-way ANOVA and means were compared using Tukey's HSD test (Excel-toukei 2010; Social service Research, Information Co. Ltd., Japan).

Results

During seedling growth in 2019 (August-September), the daily mean temperature ranged from 24.0℃ to 32.9℃ and was mostly higher than 25℃ (Fig. 2). The average daily minimum temperature, daily mean temperature, and daily maximum temperature were 24.3℃, 28.4℃, and 34.6℃, respectively.

In Fig. 3 , when re-drying treatment (RDT) applied at

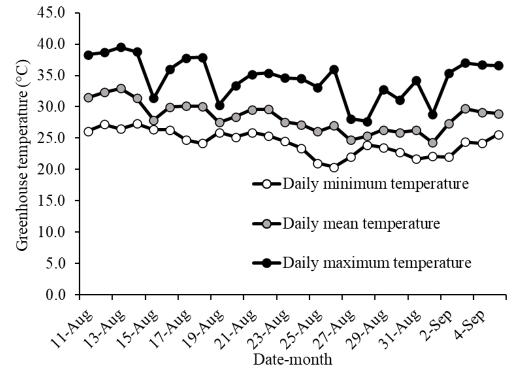

Fig. 2 Daily mean and daily maximum and minimum temperatures recorded during growing period from August 11 to September 5, 2019.

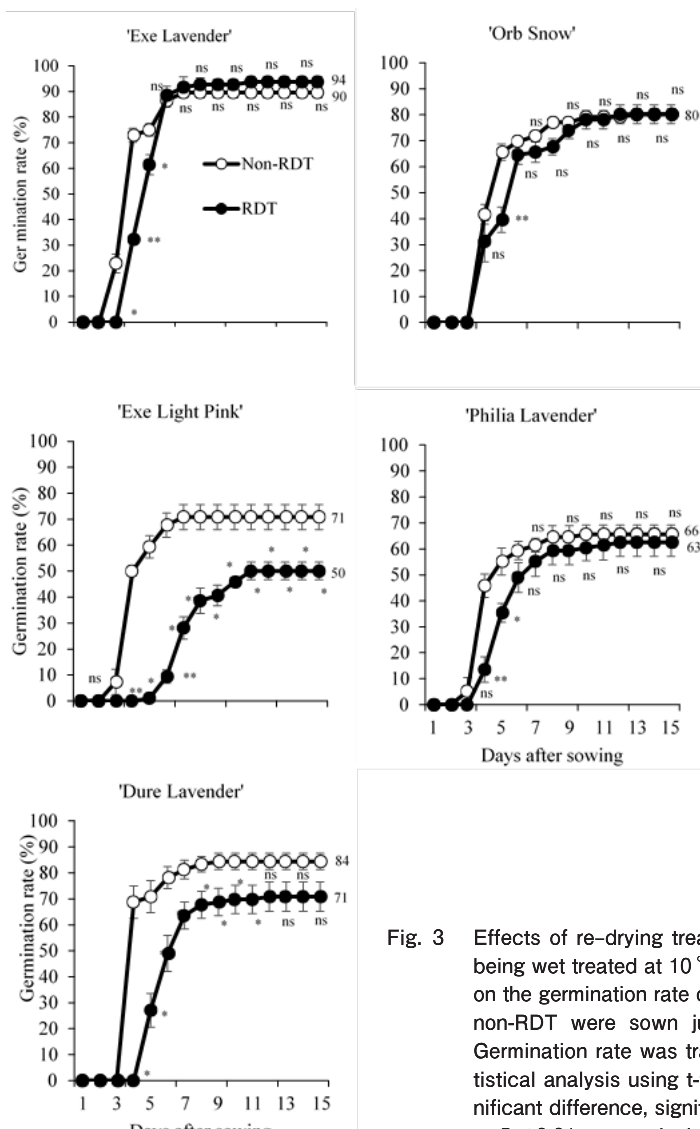

Fig. 3 Effects of re-drying treatment (RDT) for 30 days after being wet treated at 10 ℃ in dark conditions for 35 days on the germination rate of five *Eustoma* seeds. Seeds of non-RDT were sown just after their cold imbibition. Germination rate was transformed to arcsine to do statistical analysis using t-test: ns, * and ** mean no significant difference, significant difference at $P < 0.05$ and at $P < 0.01$, respectively. Bars represent ± SE (n = 4).

10℃ for 30 days storage after WT10℃ for 35 days, cultivars ‘Exe Lavender’, ‘Philia Lavender’ and ‘Orb Snow’ exhibited germination rate similar to with the non-RDT at 15 days after sowing. There was no significant difference in the germination rate of ‘Dure Lavender’, but it was slightly lower in RDT. However, ‘Exe Light Pink’ had a significant 21% reductions for RDT (Figs. 3). The WT10℃ followed by RDT delayed the time to 50% germination (T50) compared with non-RDT in most cultivars (Table 1). In RDT, ‘Exe Light Pink’ showed the highest T50 with 6.92 days whereas the others showed it less than 5 days. The time from 10% to 90% germination (T10-T90) differed significantly among either RDT or cultivars. The value of T10–T90 displayed the synchronicity of germinating performance of seeds. In ‘Dure Lavender’ and ‘Philia Lavender’, T10–T90 was quite similar between RDT and non-RDT. In RDT, there was no significant difference in T10–T90 but it was a little longer in ‘Orb Snow’ (4.92 days).

Table 2 shows the growth ability of five *Eustoma* cultivars treated with WT10℃ and subsequent RDT when their seedlings were raised under high-temperature conditions. Bolting rate and flowering rate did not change after cold imbibition and subsequent re-drying in ‘Exe Lavender’, ‘Orb Snow’, and ‘Dure Lavender’. However, ‘Exe Light Pink’ and ‘Philia Lavender’ showed a significant decline in the bolting rate when RDT was applied and the flowering rate of ‘Philia Lavender’ decreased for the plants grown from RDT seeds up to January 31, 2020. Bolting node did not differ among the cultivars whereas the number of nodes to the first flower differed in both cultivars and RDT. About flower characteristics, cut flower length was no different between RDT and non-RDT in every cultivar (Table 3). It was longest in ‘Orb Snow’ (65.2 cm) and it was less than 30 cm in ‘Exe Light Pink’. Similarly, cut flower weight was different among cultivars but was not different between RDT and

Table 1　Effect of RDT (re-drying treatment) for 30 days after wet treated at 10℃ in dark conditions for 35 days of five *Eustoma* seeds on time to 50% germination (T50), time from 10% to 90% germination (T10-T90).

Cultivar	RDT	T50 (day)		T10–T90 (day)	
‘Exe Lavender’	−	3.66	b[a]	3.05	ab
	+	4.52	c	2.65	a
‘Orb Snow’	−	4.01	bc	3.97	ab
	+	4.78	cd	4.92	b
‘Dure Lavender’	−	2.63	a	2.18	ab
	+	5.45	d	2.96	ab
‘Exe Light Pink’	−	3.66	b	2.48	a
	+	6.92	d	3.80	ab
‘Philia Lavender’	−	3.66	b	3.30	ab
	+	4.74	cd	3.94	ab
Re–drying (A)		**		*	
Cultivars (B)		**		**	
A × B		**		ns	

[a] Mean values (n = 3) followed by different letters indicate significant differences among treatments ($P < 0.05$, Tukey’s HSD test).
ns, *, ** indicate no significant and different significantly at $P < 0.05$ and $P < 0.01$ (Two-way ANOVA), respectively.

Table 2　Effect of RDT (re–drying treatment) for 30 days after wet treated at 10℃ in dark conditions for 35 days of five *Eustoma* seeds on bolting and flowering up to January 31, 2020.

Cultivar	RDT	Bolting rate (%)		Bolting node		Flowering rate (%)		Number of nodes to the first flower	
‘Exe Lavender’	−	100.0	a[a]	4.9	a	100.0	a	8.3	a
	+	100.0	a	4.9	a	94.4	a	8.5	ab
‘Orb Snow’	−	100.0	a	4.6	a	100.0	a	10.7	cde
	+	100.0	a	4.4	a	94.4	a	11.0	de
‘Dure Lavender’	−	83.3	ab	4.8	a	88.9	ab	9.6	abc
	+	83.3	ab	4.9	a	83.3	ab	10.5	cde
‘Exe Light Pink’	−	94.4	a	4.8	a	88.9	ab	9.4	abc
	+	50.0	b	4.9	a	44.4	bc	9.7	bcd
‘Philia Lavender’	−	100.0	a	4.9	a	88.9	ab	10.3	cd
	+	33.3	b	5.1	a	33.3	c	12.0	e
Significant difference									
Re-drying (A)		**		ns		**		**	
Cultivars (B)		**		*		**		**	
A × B		**		ns		ns		*	

[a] Percentage values were transformed to arcsin values. Mean values (n = 3) followed by different letters indicate significant differences among treatments ($P < 0.05$, Tukey’s HSD test).
ns, *, ** indicate no significant and different significantly at $P < 0.05$ and $P < 0.01$ (Two-way ANOVA), respectively.

Table 3 Effect of RDT (re-drying treatment) for 30 days after wet treated at 10℃ in dark conditions for 35 days of five *Eustoma* seeds on cut flower traits up to January 31, 2020.

Cltivar	RDT	Cut flower length (cm)	Cut flower weight (g)	Total number of flower and visible flower bud
'Exe Lavender'	−	42.9 b[a]	42.4 bcd	6.0 ab
	+	41.6 b	36.5 cd	5.0 bc
'Orb Snow'	−	63.4 a	68.6 a	5.0 abc
	+	65.2 a	73.2 a	4.4 bcd
'Dure Lavender'	−	47.5 b	43.9 bcd	7.6 a
	+	46.0 b	52.5 abc	7.3 a
'Exe Light Pink'	−	26.7 c	29.5 d	2.6 d
	+	25.2 c	35.7 cd	3.2 cd
'Philia Lavender'	−	42.7 b	53.1 abc	5.8 abc
	+	44.0 b	67.0 ab	6.0 abc
Significant difference				
Re-drying (A)		ns	ns	ns
Cultivars (B)		**	**	**
A × B		ns	ns	ns

[a] Mean values (n = 3) followed by different letters indicate significant differences among treatments ($P < 0.05$, Tukey's HSD test).
ns, *, ** indicate no significant and different significantly at $P < 0.05$ and $P < 0.01$ (Two-way ANOVA), respectively.

non-RDT. In RDT, the highest weight was in 'Orb Snow' (73.2 g) and the lowest weight was in 'Exe Light Pink' (29.5 g). Most examined cultivars exhibited a higher number of flower and visible flower buds more than four when their seeds were treated re-drying and storage, excepted for 'Exe Light Pink'.

Discussion

In this study, the effect of re-drying and short-term storage treatment after WT10℃ on germination and subsequent growth of five *Eustoma* cultivars was investigated. In germination, the 50% germination times of 'Exe Lavender', 'Orb Snow', and 'Philia Lavender' were slightly delayed (Table 1), but their germination rates were not significantly affected by RDT (Fig. 3). It can be postulated that these *Eustoma* seeds had tolerance to dehydration and storage up to 30 days. However, in RDT in 'Dure Lavender' and 'Exe Light Pink' the 50% germination times increased and the germination rate reduced. In particular, the seed performance after RDT of 'Exe Light Pink' was much different from other cultivars; the germination of RDT seeds reduced 21% when compared to that of non-RDT seed. There was no re-dried 'Exe Light Pink' seed that sprouted from 10 days to 15 days after sowing, thus 50% could be the final germination percentage. RDT also significantly lengthened the mean germination times. Dehydration at low temperature could be suitable for some cultivars but it might still cause embryo damage and specifically here decreased the vigor ability of 'Exe Light Pink' seed. In addition, a longer period of seed hydropriming might cause irreversible embryo damage during re-drying and storage[6]. A lower germination percentage of under 80% found in imbibed seeds of 'Exe Light Pink' and 'Philia Lavender' could be associated with the long duration of imbibition. Similar to 'King of Snow' in the previous experiment[4], viability loss in 'Exe Light Pink' might be correlated with the increasing number of seeds with a protruding root prior before RDT. It is suggested that the reduction of germination after re-drying in 'Exe Light Pink' might be due to the combination of overhydrating, immediate dehydration and poor desiccation tolerance.

There is no doubt that cold imbibition of *Eustoma* seed can promote bolting and flowering ability. Without RDT, *Eustoma* cultivars showed a higher percentage of flowering more than 80%. However, the growth response of *Eustoma* seeds differed among cultivars when subsequent re-drying and storage were applied. 'Exe Lavender', 'Orb Snow', and 'Dure Lavender' still maintained the cold effects to improve bolting and flowering obtained from WT10℃ until re-drying and storage for 30 days (Table 2). However, re-dried seeds of 'Exe Light Pink' and 'Philia Lavender' were disadvantaged when grown under high-temperature conditions. Their bolting and flowering was late and had a lower percentage less than 50%. In 'Philia Lavender', node number of RDT significantly increased compared with non-RDT.

Re-drying and storage at 10℃ for 30 days after WT10℃ may not be suitable for such cultivars. On the other hand, when the plants of these *Eustoma* cultivars were grown from seeds without pre-treated cold imbibition they exhibited completely rosetting under high-temperature ambient (data not shown). This result suggest that they are very sensitive to growth under high temperatures. Lower cut flower length less than 30 cm and lower number of total flowers less than 4 (Table 3) in 'Exe Light Pink' could point out that only pre-treated cold imbibition for 35 days might be not sufficient to improve the normal bolting and shoot length of this rosetting cultivar. For cultivars in which occurred rosettes even raising seeds of WT10℃ for 35 days, intermittent low-temperature storage treatment[10] should be considered to get high yield of bolting and flowering. On the other hand, Fukushima et al.[4] suggested that growth promotion in *Eustoma* was based on the total number days of WT10℃. If we want to produce high quality re-dried seed, it is thought that prolonged cold imbibition is required to improve the bolting, lengthening stem and the flowering of such a high-temperature sensitive cultivar. As mentioned above, however, over-priming can occur and cause embryo damage or root emergence before entering dehydration and storage, leading to viability loss in seeds. In other studies, priming seeds of some vegetables and ornamental plants at lower water potential has shown a beneficial effect on seed vigor after dehydration or long-term storage[1, 2, 7]. Therefore, the effects of dehydrated conditions after cold-wet treatment and priming conditions on germinability and flower yield of *Eustoma* should be further investigated.

In conclusion, the germination and growth responses of *Eustoma* seeds treated with cold imbibition and following re-drying and storage differed among cultivars. Applying re-drying and storage at 10℃ for 30 days after WT10℃ was appropriate for some imbibed *Eustoma* seeds such as 'Exe Lavender', 'Orb Snow' and 'Dure Lavender'. Such re-drying condition as this could maintain the cold effect that *Eustoma* seed obtained from the cold imbibition to improve the subsequent bolting and flowering of *Eustoma* grown under high temperatures. However, decreasing germinability occurred in 'Exe Light Pink', which it is suggested was due to seed damage during dehydration process. Therefore, further investigation should be focused on both the cold-wet conditions and the following re-drying conditions.

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