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Effects of Moist-Cold Stratification and Gibberellic Acid Applications on Breaking Seed Dormancy in Foxtail Lily (*Eremurus spectabilis* M.Bieb.)*

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Abstract: *Eremurus spectabilis* is a wild edible perennial herbaceous geophyte species with medicinal and popular ornamental uses. In this study, gibberellic acid (GA_3) with moist-cold stratification treatments were applied to break the dormancy of *E. spectabilis* seeds. For this purpose, seeds were soaked in gibberellic acid solutions (GA_3: 500 and 750 ppm for 24 and 48 hours). Pretreated seeds were then subjected to moist-cold stratification treatments for different durations (30, 50, 80 and 100 days) at 4 ºC. In 24-hour GA_3 treatments, the germination and emergence ratios (21.04%) were obtained from the 500 ppm GA_3 dose and 100 days of stratification treatments. In 48-hour GA_3 treatments, the GA_3 doses did not have any significant effect on germination and emergence ratios; the greatest germination ratios were obtained from the 500 ppm (20.36%) and 750 ppm (20.00%) doses and 100 days of stratification treatments, and the best emergence ratios were obtained from the 500 ppm GA_3 dose and 100 days of stratification treatments (18.51%). It is considered that it is beneficial to try the combinations of moist-cold applications with different treatments in future studies.

Keywords: *Eremurus spectabilis, Liliaceae*, seed, germination, emergence

1. Introduction

Eremurus spectabilis is a wild perennial herbaceous species and belongs to the *Eremurus* genus of the *Liliaceae* family (Tuzlacı, 1985). Plant shoots and leaves are consumed as a vegetable. Apart from nutritional values, the plants are also used for medicinal purposes. Different parts of the plant are used to treat fungal diseases, diabetes, jaundice and liver disorders in folk medicine (Baytop, 1984; Tuzlacı and Doğan, 2010; Pourfarzad et al., 2014). *E. spectabilis* has recently been categorized as a popular ornamental geophyte plant and used in cut-flower production practices, especially in mild climate zones (Schiappacasse et al., 2013).

In order to break the seed dormancy, hormone treatments, in particular gibberellic acid (GA_3) applications, have been applied to different species (Rahman et al., 2006; Sarı et al., 2006; Rahnama-Ghahfarokhi and Tavakkol-Afshari, 2007; Hassani et al., 2009). In addition, moist-cold stratification applications are very effective in breaking the physiological and morphophysiological dormancy of the seeds (Sarı et al., 2006; Rouhi et al., 2010; Mancuso et al., 2012; Keskiner and Tuncer, 2019).

A review of the literature reveals that number of studies about the breaking of seed dormancy in *E. sepectabilis* is very limited (Güngör, 2002; Rahmanpour et al., 2005; Keskiner, 2017; Keskiner and Tuncer, 2019). In a previous study conducted with *E. spectabilis*, 0.0%-73.3% of germinations were achieved with moist-cold stratification treatments for different durations (Keskiner and Tuncer, 2019). In the study, 100 days of cold stratification treatments were found to be the most promising treatment, and very low germination rates were reported for single potassium nitrate (KNO_3), calcium chloride ($CaCl_2$) and GA_3 treatments without moist-cold

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stratification treatments (Keskiner and Tuncer, 2019).

There is also a limited number of studies conducted on different species within the same genus. Mamut et al. (2014) reported the greatest germination ratio of *E. anisopterus* for seeds dry stored at a low temperature (5/2 ºC) for 12-18 weeks followed by immersion in various GA_3 (0.1, 1 and 10 mmol L^{-1}) solutions. In another study conducted with *E. olgae*, the greatest germination ratio (80%) and germination speed (1.6 days) were reported for tip-cut and testa-abraded seeds immersed in a 0.08 M GA_3 solution for 45 minutes (Rahmanpour et al., 2007).

The aim of this study was to examine the effect of moist-cold stratification (4 ºC) and GA_3 treatments on breaking seed dormancy and enhancing germination and emergence of *E. spectabilis*.

2. Materials and Methods

The mature seeds of *E. spectabilis* were collected from Gürpınar town (38º 8' 20.75" N, 43º 30' 55.15" E) in Van province of Turkey. This study was conducted at Van Yüzüncü Yıl University Faculty of Agriculture from 2017 to 2018. The seeds were kept in a 0.3% Benomyl (Fungicide) solution for an hour, washed with distilled water and kept in distilled water for 1 hour (Lee et al., 2015; Keskiner and Tuncer, 2019). For surface sterilization, 30% sodium hypochlorite solution (NaOCl) was applied to seeds for 10 minutes and rinsed three times with double distilled water for 5 minutes. Seed sowing and moistening practices were performed in a laminar flow cabinet and double distilled water was used in the irrigations.

For GA_3 treatments, the sterilized seeds were separated into groups for treatments and immersed in 500 ppm and 750 ppm GA_3 solutions for 24 and 48 hours. Then, the seeds were shaken and washed with double distilled water. Seeds were placed on drying papers to remove any excess water and seeded in Petri dishes placed in a laminar flow cabinet. The Petri dishes were closed and placed in polyethylene bags. For moist-cold stratification treatments, seeds in Petri dishes were treated at 4 °C for 30, 50, 80 and 100 days (Güngör, 2002; Keskiner and Tuncer, 2019). Then the seeds were germinated in an incubator at a temperature of 12-13 ºC under dark conditions until optimum germination was achieved (Keskiner and Tuncer, 2019).

At the end of the tests, the germination ratio (GR), emergence ratio (ER), germination speed (GS), emergence speed (ES), mean germination time (MGT), mean emergence time (MET), vigor index (VI), radicle length (RL), plumule length (PL) and seedling length (SL) (cm) were calculated. The following equations were used in the calculations (Abdul-Baki and Anderson, 1973; Murillo-Amador et al., 2002; Yıldırım and Güvenç, 2006; Keskiner and Tuncer, 2019):

$$(GR)\ (ER)\ (\%) = (G/T) \times 100 \quad (1)$$

$$(MGT)\ (MET)\ (day) = [(1.day\ G \times 1.\ day) + (2.\ day\ G \times 2.day) + \ldots + (n.day\ G \times n.day)] / Total\ G \quad (2)$$

$$Germination\ or\ emergence\ speed = n1/t1+n2/t2+\ldots\ldots\ldots+nn/tn \quad (3)$$

$$Vigor\ index = Ls \times GR\ /100 \quad (4)$$

In the equations; n1 and n2 denote number of germinated or emerged seeds, t1 and t2 denote number of days for germination or emergence, Ls means mean radicle + plumule length, G states number of germinated or emerged seeds, T denotes total number of seeds.

This experiment was established according to completely randomized design with 3 replications (25 seeds per replicate). Data for germination and emergence parameters were subjected to arcsine transformation before statistical analysis. The analysis was performed using Statgraphics statistical software (Statgraphics Centurion XVII Version 17.1.03) and means were separated by Duncan's multiple range test.

3. Results and Discussion

The effects of immersion in different doses of GA_3 solutions for 24 hours and moist-cold stratification treatments on germination and emergence rates are given in Tables 1 and 2.

The effects of different stratification durations on GR and MGT were found to be significant ($p< 0.01$). The greatest GR (21.04%) was obtained from 500 ppm GA_3 application with 100 days of stratification. It was followed by 500 ppm GA_3 with 80 days of stratification (17.53%) and 750 ppm GA_3 with 100 days of stratification (14.58%), respectively. The average of the GA_3 doses had significant effects only on MGT. MGT varied between 0.00 and 41.41 days depending on the dose. In the emergence experiments, the effects of different stratification durations ($p< 0.01$), doses ($p< 0.01$ at ER, RL and SL, $p< 0.05$ at other emergence parameters) and interactions ($p< 0.01$) on all parameters were found to be significant.

The greatest ER was obtained from 500 ppm with 100 days of stratification treatments (21.04%) and 50 days of stratification treatments (17.53%)

Table 1. Effects of different GA_3 doses (24 hours) and moist-cold stratification treatments (MCST) on germination rate (GR), germination speed (GS) and mean germination time (MGT)

GA_3 doses	MCST (day)	GR (%)	GS (day)	MGT (day)
500 ppm	30	0.00 e	0.00 b	0.00 f
	50	0.00 e	0.00 b	0.00 f
	80	17.53 b	0.24 ab	18.66 d
	100	21.04 a	0.46 a	37.98 b
Average		9.64	0.17	14.16^{2}
750 ppm	30	4.16 d	0.04 b	4.66 e
	50	2.08 de	0.02 b	4.66 e
	80	12.41 c	0.08 b	22.08 c
	100	14.58 bc	0.05 b	41.41 a
Average		8.31	0.05	18.20^{1}
Avarage of MCST	30	2.08 C	0.02 B	2.33 C
	50	1.04 C	0.01 B	2.33 C
	80	14.97 B	0.16 AB	20.37 B
	100	17.81 A	0.25 A	39.70 A
		P Value		
GA_3		ns	ns	0.001^{**}
MCST		0.0001^{**}	ns	0.0001^{**}
GA_3 x MCST		0.0001^{**}	ns	0.0001^{**}

Different capital letters in the same column indicate differences among the moist-cold stratification time, different numbers$^{(1,2)}$ indicate differences among GA_3 doses and different small letters indicate significant differences between interactions, **: Significant at p<0.01 level, ns: Not significant

(Table 2). For the ER and the MET, 500 ppm dose was more successful than other treatments. MET varied between 0.00 and 51.33 days, RL varied between 0.00 and 0.60 cm, PL varied between 0.00 and 5.66 cm, SL varied between 0.00 and 6.10 cm, and VI values varied between 0.00 and 137.33. Increases were observed in RL, PL, and SL at an average of 750 ppm and increases were observed in VI at 500 ppm dose average (Table 2).

The effects of 48-hour immersion in GA_3 solutions are given in Table 3. The average of the stratification durations was found to be significant for all germination parameters, and the average of the doses was found to be significant only on GS ($p< 0.01$). The greatest GR was observed in 500 ppm (20.36%) and 750 ppm GA_3 (20.00%) with 100 days of stratification treatments. The MGT varied between 0.66 and 55.58 days (Table 3).

The emergence parameters are summarized in Table 4. While stratification durations were found to be significant for all parameters except RL, average of the doses was found to be significant for PL, SL and VI. ER varied between 1.85% and 18.51%. ER was more successful in 500 ppm with 100 days of stratification treatments (18.51%), 750 ppm with 100 days of stratification treatments (15.00%) and 80 days of stratification treatments (13.33%) (Table 4).

Güngör (2002) was unable to get any *E. spectabilis* seeds to germinate with different GA_3 doses (250, 500, 750 and 1000 ppm). Keskiner and Tuncer (2019) were not able to get any positive outcomes from single GA_3 treatments (without moist-cold stratification treatments) and reported the greatest germination (1.66%) for a 750 ppm GA_3 dose.

Table 2. Effects of different GA_3 doses (24 hours) and moist-cold stratification treatments (MCST) on emergence rate (ER), emergence speed (ES), mean emergence time (MET), radicle length (RL), plumule length (PL), seedling length (SL) and vigor index (VI)

GA_3 doses	MCST (day)	ER (%)	ES (day)	MET (day)	RL (cm)	PL (cm)	SL (cm)	VI
500 ppm	30	0.00 e	0.00 b	0.00 e	0.00 c	0.00 d	0.00 d	0.00 f
	50	0.00 e	0.00 b	0.00 e	0.00 c	0.00 d	0.00 d	0.00 f
	80	17.53 b	0.96 a	11.25 c	0.60 a	5.00 a	5.60 a	94.36 b
	100	21.04 a	1.09 a	22.11 b	0.43 a	5.66 a	6.10 a	137.33 a
Average		9.64^{1}	0.51^{1}	8.34^{2}	0.25^{2}	2.66^{2}	2.92^{2}	57.92^{1}
750 ppm	30	4.16 d	0.08 b	2.66 d	0.43 a	3.33 bc	3.76bc	23.54 e
	50	2.08 de	0.04 b	2.33 de	0.20 bc	3.50 bc	3.70 c	23.12 e
	80	12.50 c	0.05 b	23.33 b	0.40 ab	2.16 c	2.56 c	45.00 d
	100	14.58 c	0.04 b	51.33 a	0.53 a	4.66 ab	5.20ab	74.16 c
Average		8.33^{2}	0.05^{2}	19.91^{1}	0.39^{1}	3.41^{1}	3.80^{1}	41.45^{2}
Average of MCST	30	2.08 C	0.04 B	1.33 C	0.21 B	1.66 C	1.88 C	11.17 C
	50	1.04 C	0.02 B	1.16 C	0.10 B	1.75 C	1.85 C	11.56 C
	80	15.01 B	0.51 A	17.29 B	0.50 A	3.58 B	4.08 B	69.68 B
	100	17.81 A	0.56 A	36.72 A	0.48 A	5.16 A	5.65 A	105.75A
				P Value				
GA_3		0.020^{*}	0.001^{**}	0.000^{**}	0.031^{*}	0.004^{**}	0.031^{*}	0.000^{**}
MCST		0.000^{**}	0.006^{**}	0.000^{**}	0.003^{**}	0.006^{**}	0.004^{**}	0.000^{**}
GA_3 x MCST		0.000^{**}	0.004^{**}	0.000^{**}	0.010^{**}	0.000^{**}	0.000^{**}	0.000^{**}

Different capital letters in the same column indicate differences among the moist-cold stratification time, different numbers$^{(1,2)}$ indicate differences among GA_3 doses and different small letters indicate significant differences between interactions, *: Significant at p<0.05 level, **: Significant at p<0.01 level

Table 3. Effects of different GA_3 doses (48 hours) and moist-cold stratification treatments (MCST) on germination rate (GR), germination speed (GS) and mean germination time (MGT)

GA_3 doses	MCST (day)	GR (%)	GS (day)	MGT (day)
500 ppm	30	1.85 d	0.04 b	2.33 f
	50	5.55 cd	0.09 b	7.16 e
	80	7.40 c	0.03 b	40.83 b
	100	20.36 a	0.06 b	57.24 a
Average		8.79	0.06[2]	26.89
750 ppm	30	3.33 d	0.66 a	0.66 f
	50	5.00 cd	0.04 b	18.66 d
	80	13.33 b	0.07 b	26.44 c
	100	20.00 a	0.11 b	55.58 a
Average		10.41	0.22[1]	25.33
Avarage of MCST	30	2.59 C	0.35 A	1.50 D
	50	5.27 C	0.06 B	12.91 C
	80	10.36 B	0.05 B	33.63 B
	100	20.18 A	0.08 B	56.41 A
		P Value		
GA_3		ns	0.003**	ns
MCST		0.000**	0.008**	0.000**
GA_3 x MCST		ns	0.004**	0.000**

Different capital letters in the same column indicate differences among the moist-cold stratification time, different numbers[(1,2)] indicate differences among GA_3 doses and different small letters indicate significant differences between interactions, **: Significant at p<0.01 level, ns: Not significant

Present findings for 24-hour GA_3 treatments were greater at 500 ppm average values for GR and ER (9.64%), MGT (14.16 days), MET (8.34 days) and VI (57.92) values. On the other hand, RL (0.39 cm), PL (3.41 cm) and SL (3.80 cm) values were greater at the dose of 750 ppm. For 48-hour GA_3 treatments, an average of 750 ppm was more successful for the GR (10.41%), ER (8.33%), MGT (25.33 days) and MET (25.40 days) whereas 500 ppm was more remarkable for RL (0.42 cm), PL (5.25 cm), SL (5.67 cm) and VI (65.32) parameters. Keskiner and Tuncer (2019) reported that they applied only moist-cold stratification without any other application to *E. spectabilis* seeds for 30-100 days and maximum germination (73.30%) was obtained from the 100 days moist cold application. Present germination and emergence values were lower than the values reported by Keskiner and Tuncer (2019). At low germination and low emergence values, GA_3 and moist-cold stratification applications are thought to have an antagonistic effect on each other. Rouhi et al. (2010) applied moist-cold stratification to *Tulipa kaufmanniana* Regel (*Liliaceae*) seeds for 7 weeks and indicated 500 ppm GA_3 dose as a promising treatment for germination parameters.

Apart from moist-cold stratification treatments, GA_3 treatments were combined with different treatments in previous studies. Rahmanpour et al. (2005) reported a GR of 53% for mechanically abraded *E. spectabilis* seeds immersed in a 0.01 M GA_3 solution for 45 minutes. Rahmanpour et al. (2005), for *E. spectabilis*, and Rahmanpour et al. (2007), for *E. olgae*, reported that the mechanical abrasion of seeds with some chemicals and tip-

Table 4. Effects of different GA_3 doses (48 hours) and moist-cold stratification treatments (MCST) on emergence rate (ER), emergence speed (ES), mean emergence time (MET), radicle (RL), plumula (PL) and seedling length (SL) and vigor index (VI)

GA_3 doses	MCST (day)	ER (%)	ES (day)	MET (day)	RL (cm)	PL (cm)	SL (cm)	VI
500 ppm	30	1.85 e	0.33 a-c	0.33 g	0.16 d	2.83 de	3.00 de	16.65 f
	50	5.55 cd	0.40 ab	4.83 f	0.40 a-c	4.66 bc	5.06 bc	37.57 d
	80	7.40 c	0.03 c	37.33 c	0.53 ab	6.83 a	7.36 a	55.71 c
	100	18.51 a	0.05 bc	55.37 b	0.60 a	6.66 a	7.26 a	151.36 a
Average		8.33	0.20	24.46	0.42	5.25[1]	5.67[1]	65.32[1]
750 ppm	30	3.33 de	0.66 a	0.66 g	0.33 b-d	4.30 b-d	4.66 b-d	23.33 ef
	50	3.66 de	0.05 bc	9.33 e	0.26 cd	3.30 c-e	3.60 c-e	24.66 e
	80	13.33 b	0.06 bc	24.11 d	0.40 a-c	2.33 e	2.73 e	56.66 c
	100	15.00 b	0.05 bc	67.51 a	0.40 a-c	5.16 ab	5.56 ab	126.83 b
Average		8.83	0.20	25.40	0.35	3.79[2]	4.14[2]	57.87[2]
Avarage of MCST	30	2.59 D	0.50 A	0.50 D	0.25 C	3.58 B	3.83 B	19.99 D
	50	4.61 C	0.22 B	7.08 C	0.33 BC	4.00 B	4.33 B	31.12 C
	80	10.3 B	0.05 B	30.72 B	0.46 AB	4.58 B	5.05 AB	56.19 B
	100	16.7 A	0.05 B	61.44 A	0.50 A	5.91 A	6.41 A	139.10A
				P Value				
GA_3		ns	ns	ns	ns	0.044*	0.005**	0.001**
MCST		0.000**	0.003**	0.000**	0.019*	0.009**	0.008**	0.000**
GA_3 x MCST		0.000**	ns	0.000**	ns	0.003**	0.002**	0.001**

Different capital letters in the same column indicate differences among the moist-cold stratification time, different numbers[(1,2)] indicate differences among GA_3 doses and different small letters indicate significant differences between interactions, *: Significant at p<0.05 level, **: Significant at p<0.01 level, ns: Not significant

cutting treatments had positive impacts on germination and emergence parameters. At the end of 3 weeks, Rahmanpour et al. (2005) reported the greatest germination rate (53.3%) and germination speed (0.88 days) for tip-cut seeds of *E. spectabilis* subjected to a pretreatment of immersion in a 35% sodium hypochlorite solution for 24 and 48 hours and 0.01 M GA_3 and citric acid (50 mg L^{-1}) solutions for 45 minutes.

In another study, Rahmanpour et al. (2007) reported a 70% germination rate for citric acid-treated tip-cut seeds of *E. olgae* and 80% germination ratio for different combined treatments (immersion in water for 24–48 hours plus tip-cutting plus immersion in a 0.08 M GA_3 solution for 45 minutes). Such findings do not comply with the present ones because of the different species used.

4. Conclusions

This study was the first report to investigate the effect of combined GA_3 applications with moist-cold stratification on *E. spectabilis* in order to break seed dormancy. In conclusion, it was determined that the combination of GA_3 and moist-cold stratification applications together had an antagonistic effect in breaking the dormancy of *E. spectabilis* seeds. For this reason, it is considered that it is beneficial to evaluate various combinations of moist-cold applications with different treatments (especially seed tip cutting) in future studies.

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