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Founded jointly by F. Korte and F. Coulston
Production by PSP - Vimy Str. 1e, 85354 Freising, Germany in
cooperation with PRT-Parlar Research & Technology
Vimy Str 1e, 85354 Freising
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Printed in Germany-ISSN 1018-4619

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DORMANCY BREAKING TREATMENTS FOR WILD *EREMURUS SPECTABILIS* M.BIEB SEEDS

Kader Keskiner, Burcu Tuncer*

Van Yuzuncu Yil University, Faculty of Agriculture, Department of Horticulture, 65080, Van, Turkey

ABSTRACT

Eremurus spectabilis M.Bieb seeds were subjected to moist-cold (4ºC) stratifications for different periods (0, 30, 40, 50, 60, 70, 80, 90, and 100 days), gibberellic acid (GA_3) (0, 250, 500, 750, 1000, 1500, and 2000 ppm), potassium nitrate (KNO_3) (0, 5 mM, 10mM, and 15mM), calcium chloride ($CaCl_2$) (0, 5 mM, 10 mM, and 15 mM) treatments and *in vitro* germinations in different nutrient media (MS, B5, and WH) to break seed dormancy. Treated seeds were germinated at 15ºC under two different photoperiod regimes (continuous dark and 16/8 hours light/dark). The greatest rate of germination was obtained from a combination of 100-day moist-cold stratification + continuous dark photoperiod (73.30%). For mean germination rates, the 5 mM dose of KNO_3 (10.00%) and $CaCl_2$ (3.33%) treatments yielded better outcomes than the other doses. The same treatments were also more successful in emergence parameters. With regard to germination and emergence parameters, positive outcomes were not able to be achieved from *in vitro* germination treatments in different nutrient media and GA_3 treatments.

KEYWORDS:
Eremurus spectabilis M.Bieb., germination, emergence, dormancy, seed

INTRODUCTION

Eremurus spectabilis belongs to the *Liliaceae* (*Asphodelaceae*) family and is known by the common names of 'ciris', 'gulluk', 'wild leek', and 'yellow lily'. It is a perennial, herbaceous wild species [1]. *Eremurus* species are widespread in Central Asia and the Middle East [2]. Fresh shoots and leaves are consumed as vegetables. Aboveground sections are also used in Van herby cheese, and leaves and roots are dried, ground, and sold as a medicinal plant [3]. Besides nutritional attributes, *E. spectabilis* species play significant roles in human health. Plants are quite rich in Na (148.7 mg/kg), K (15798.0 mg/kg), P (6497.4 mg/kg), Mg (1986.7 mg/kg), Ca (8769.1 mg/kg), Mn (48.9 mg/kg), Cu (11.6 mg/kg) and Zn (69.7mg/kg), and are used in different ways for the treatment of several diseases [4]. The extracts obtained through smashing the leaves are used in the remedy of fungal diseases and eczema; the boiled juice of the roots are used for diabetes, hepatitis, liver disorders, atrophic gastritis, acne, and fractures [5, 6].

Eremurus spectabilis M.Bieb seeds have dormancy, i.e., germination is not possible under normal conditions. Seed-propagation of *E. spectabilis* requires quite a long period (4-6 years) from sowing till flowering. Therefore, culture studies of *E. spectabilis* have not been achieved as yet. Efficient dormancy breaking methods should be identified for *E. spectabilis* seeds. In a previous study investigating the effects of different treatments on germination of *E. spectabilis*, the greatest rate of germination was reported for 90-day stratification treatments (65.3%) and the shortest mean germination time (4.34 day) was reported for 100-day stratification treatments [7].

Rahmanpour et al. [8] reported the greatest germination rate (53.3%) and speed in *E. spectabilis* seeds was for seeds with cortexes removed and washed with 35% sodium hypochlorite solution, then soaked in water for 1-2 days; for the seeds with cut-end and placed in 0.01 M GA_3 and 50 mg/L citric acid solution for 45 minutes and at 10-15ºC germination temperatures. Öztürk and Pirdal [9] achieved the greatest germination rate in *Asphodelus aestivus* Bioth L. seeds from 15 ºC temperature, 6 hours light/18 hours dark photoperiods, and 1 M sucrose treatments. In this study, the effects of different treatments in breaking seed dormancy of *Eremurus spectabilis*, a wild species belonging to *Liliaceae* family, were investigated.

MATERIALS AND METHODS

Plant material and sterilization of seeds. Figure 1 presents in-situ original photos of *E. spectabilis* M.Bieb plants (Figure 1a-d), fruits (Figure 1e-f) and seeds (Figure 1g-h). In the present experiments, *E. spectabilis* M.Bieb seeds collected from Gürpınar town (Kirkgecit village) of Van province in the Eastern Anatolia region of Turkey were used. To remove fungal disease agents, the seeds were placed in 0.3% benomyl solution for an hour, then washed through distilled water and



FIGURE 1
Plants of *Eremurus spectabilis* M. Bieb.; leaves (a, b), roots and rhizomes (c), flowers (d), fruits (e, f), and seeds (g, h) (Photographs: B. Tuncer and K. Keskiner).

placed into distilled water for an hour. In the second stage of sterilization, the seeds were taken into sterile cabins and placed into Tween 20 containing 10% sodium hypochlorite solution for 10 minutes, then washed through bi-distilled water three times for 5 minutes each time.

Seed dormancy breaking treatments. Seeds were subjected to different treatments to break dormancy. In GA_3 treatments, the seeds were placed into 0, 250, 500, 750, 1000, 1500, and 2000 ppm solutions for 24 hours. Cold-moist stratification treatments were applied in glass petri dishes. For this purpose, two layers of filter paper were placed beneath the petri dishes, and drying papers were moistened with 5 ml bi-distilled water. Following sowing of the seeds, the petri dishes were closed, then placed in polyethylene bags and kept at 4ºC for various increments, including 30, 40, 50, 60, 70, 80, 90, and 100 days. The drying papers were remoistened as moisture content decreased. In the groups receiving potassium nitrate (KNO_3) and calcium chloride ($CaCl_2$) treatments, the seeds were moistened with a control (15 ml bi-distilled water), 5, 10, or 15 mM of KNO_3 or $CaCl_2$ solution in petri dishes with two-layers of filter papers until optimum germination was achieved [10]. In *in vitro* germinations in different nutrient media, sterilized seeds were germinated in hormone-free or 1500 ppm GA_3 including MS [11], B5 [12] and WH [13] media. About 7 g/L of agar was added to all media as a hardener, and 50 mg/L of citric acid was added to prevent browning in the tissue. While sucrose was supplemented with MS (30 g/L), and B5 (20 g/L) media, WH medium was not supplemented with sucrose.

Germination treatments. Treated seeds were geminated in a climate chamber at 15ºC temperature and with 2 different photoperiods regimes (continuous dark and 16/8 hours light/dark) until optimum germination was achieved. Germination (GR) and emergence (ER) ratios (%), mean germination (MGT), and emergence (MET) time (day) were determined. About 2 mm radicle elongation was taken as the basis for germination, and the period in which the cotyledon leaves laid fully parallel to the ground was taken as the basis for emergence. The following equations were used to calculate relevant parameters [14, 15, 16, 17]:

(GR) (ER) (%) = (G/T) x 100

(MGT) (MET) (day) = [(1.day G x 1. day) + (2. day G x 2.day) +…+ (n.day G x n.day)] / Total G

G=germinated or emerged seed number; T=total number of seeds

Statistical analysis. Each treatment had 3 replications with 25 seeds (3x25) in each replication. The resulting data were subjected to variance analysis in accordance with randomized plots experimental design. Significance level was considered as 5%. SPSS (v.13) statistical software was used for statistical analyses.

RESULTS AND DISCUSSION

Cold-moist stratification treatments. The results of cold-moist (4ºC) stratification treatments for different durations (0, 30, 40, 50, 60, 70, 80, 90, and 100 days) are given in Table 1. Germination rates varied between 0.00-73.30%. There were significant differences in germination rates (GR)

and mean germination times (MGT) of stratification durations, stratification duration x photoperiod interactions, and photoperiods ($p<0.05$). The greatest germination rates were obtained from 100 days + dark (73.30%) and 100 days + 16/8 hours light/dark combinations (70.00%), which were placed in the same statistical group (Table 1).

Considering the germination rates of stratification durations, the greatest value was obtained from 100-day (71.60%) stratification duration, and it was followed by 90-day (45.00%) and 80-day (41.60%) stratification durations, respectively, which were also placed in the same statistical group. The germination rates were quite low for stratification durations lower than 50 days, so these treatments were found to be ineffective (Table 1). Mean germination (MGT) and emergence times (MET) varied respectively between 0.00-20.00 days and between 0.00-23.30 days. These times exhibited an irregular distribution independent from the stratification durations. The same treatments of 100-day (73.30%), 90-day (45.00%), and 80-day (41.60%) stratification durations were also found to be successful in emergence rates. The differences in emergence rates of cold-moist stratification treatments are presented in Figure 2.

Cold-moist stratification treatments are commonly used to break seed dormancy of perennial plants [18]. Gungor [7] reported the greatest germination rate in *E. spectabilis* seeds for 90-day stratification treatments and the shortest mean germination time for 100-day stratification treatments. Our present findings align with those earlier ones.

Potassium nitrate (KNO_3) treatments. Effects of different KNO_3 doses on germination and emergence of *E. spectabilis* are provided in Table 2. The differences between the doses, photoperiods and dose x photoperiod interactions were found to be significant ($p<0.05$). Germination rates varied between 0.00 – 13.30% and emergence rates varied between 0.00–4.00%. The greatest germination (13.30%) and emergence rates (4.00%) were obtained from 5 mM KNO_3 + dark combinations. Mean germination times varied between 0-60 days and the mean emergence times varied between 0-43.33 days (Table 2).

TABLE 1
Comparison of germination and emergence parameters of *E. spectabilis* subjected to cold-moist stratification treatments.

Treatment	Photoperiod	GR (%)	MGT (day)	ER (%)	MET (day)
Control	Continuous Dark	0.00 ± 0.00 g	0.00 ± 0.00 j	0.00 ± 0.00 h	0.00 ± 0.00 h
	16/8 h (light/dark)	0.00 ± 0.00 g	0.00 ± 0.00 j	0.00 ± 0.00 h	0.00 ± 0.00 h
Average		0.00 ± 0.00 F	0.00 ± 0.00 F	0.00 ± 0.00 F	0.00 ± 0.00 F
30-day	Continuous Dark	1.66 ± 0.33 g	20.00 ± 8.66 a	16.60 ± 5.77 e	23.30 ± 2.88 a
	16/8 h (light/dark)	3.33 ± 0.57 g	10.16 ± 2.46 cd	3.33 ± 1.15 h	9.67 ± 1.42 e
Average		2.50 ± 1.04 F	15.08 ± 7.80 A	10.00 ± 2.2 E	16.50 ± 7.21 A
40-day	Continuous Dark	0.00± 0.00 g	0.00 ± 0.00 j	0.00 ± 0.00 h	0.00 ± 0.00 h
	16/8 h (light/dark)	1.66 ± 0.33 g	5.00 ± 1.00 f-i	1.66 ± 0.33 h	8.00 ± 1.0 ef
Average		0.83 ± 0.70 F	2.50 ± 1.60 EF	0.83 ± 0.01 F	4.00 ± 1.77 E
50-day	Continuous Dark	10.00 ± 2.10 f	16.00 ± 2.00 b	10.00 ± 4.80 fg	19.60 ± 0.33 b
	16/8 h (light/dark)	20.00 ± 5.00 e	3.66 ± 1.15 g-j	5.00 ± 1.80 gh	6.50 ± 1.32 f
Average		15.00 ± 7.42 E	9.83 ± 4.91 B	7.50 ± 2.18 E	13.08 ± 7.26 B
60-day	Continuous Dark	28.30 ± 2.88 d	4.57 ± 0.98 f-i	28.30 ± 2.88 d	8.00 ± 1.10 ef
	16/8 h (light/dark)	13.30 ± 2.88 f	3.22 ± 0.14 g-j	13.30 ± 2.88 ef	7.05 ± 1.08 f
Average		20.80 ± 8.50 D	3.90 ± 1.00 DE	20.83 ± 8.61 D	7.53 ± 1.06 D
70-day	Continuous Dark	33.30 ± 2.88 d	6.44 ± 0.60 d-h	33.30 ± 2.88 d	14.06 ± 0.16 c
	16/8 h (light/dark)	31.60 ± 2.88 d	5.81 ± 0.18 e-h	31.6 ± 2.89 d	8.47 ± 0.60 ef
Average		32.50 ± 2.73 C	6.12 ± 0.66 CD	32.50 ± 2.88 C	13.06 ± 1.41 B
80-day	Continuous Dark	51.60 ± 2.88 b	1.49 ± 0.18 ij	51.60 ± 5.77 b	6.99 ± 0.08 f
	16/8 h (light/dark)	31.60 ± 2.88 d	2.30 ± 0.05 h-j	31.60 ± 2.89 d	6.75 ± 0.25 f
Average		41.60 ±2.44 B	1.89 ± 0.29 EF	41.60 ± 10.2 B	6.87 ± 0.15 D
90-day	Continuous Dark	50.00 ± 5.00 b	8.64 ± 0.02 d-f	50.00 ± 5.00 b	12.05 ± 1.33 d
	16/8 h (light/dark)	40.00 ± 4.20 c	12.75 ± 1.73 bc	40.00 ± 5.10 c	4.42 ± 0.05 g
Average		45.00 ± 6.32 B	10.70 ± 2.40 B	45.00 ± 7.07 B	6.45 ± 2.24 D
100-day	Continuous Dark	73.30 ± 5.77 a	7.30 ± 0.01 d-g	73.30 ± 5.77 a	9.44 ± 1.35 e
	16/8 h (light/dark)	70.00 ± 5.00 a	9.31 ± 0.94 c-e	68.3 ± 2.88 a	9.10 ± 1.29 e
Average		71.60 ± 5.16 A	8.30 ± 1.27 BC	70.83 ± 4.91 A	9.27 ± 1.11 C
Photoperiod Average.	Continuous Dark	27.60 ± 4.45 [1]	7.16 ± 2.51 [1]	29.26 ± 8.22 [1]	9.99 ± 2.56 [1]
	16/8 h (light/dark)	23.50 ± 3.31 [2]	5.80 ± 2.09 [2]	21.66 ± 5.67 [2]	7.06 ± 1.22 [2]

Different capital letters in the same column indicate the differences among the treatments, different numbers indicate the differences among photoperiods and different small letters indicate significant the differences between treatment x photoperiod interactions ($p<0.05$).

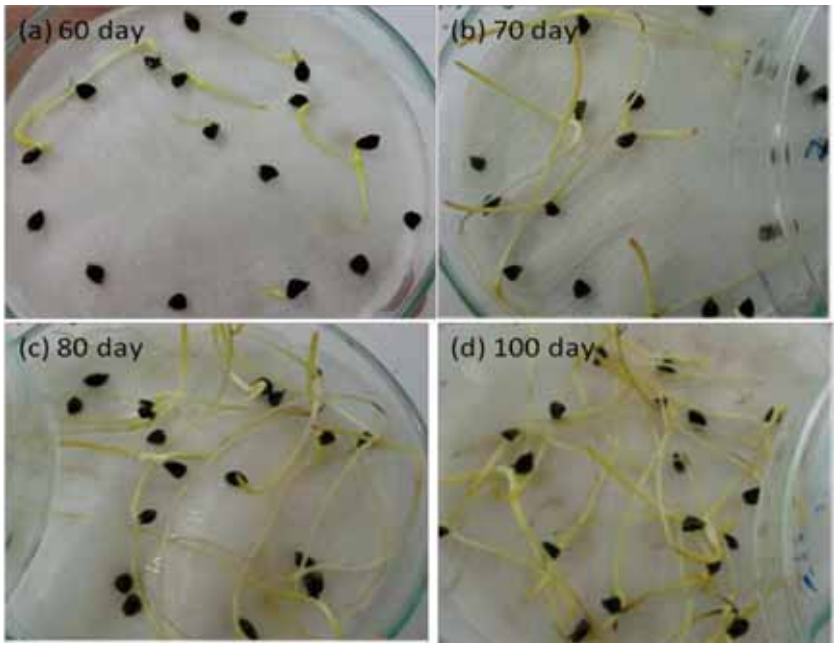

FIGURE 2
Emergence rates of cold-moist stratification treatments, 60-day (a), 70-day (b), 80-day (c), and 100-day (d).

TABLE 2
Comparison of germination and emergence parameters of *E. spectabilis* subjected to KNO_3 treatments.

Treatment	Photoperiod	GR (%)	MGT (day)	ER (%)	MET (day)
Control	Continuous Dark	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
	16/8 h (light/dark)	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Average		0.00 ± 0.00 B	0.00 ± 0.00 C	0.00 ± 0.00 C	0.00 ± 0.00 C
5 mM	Continuous Dark	13.30 ± 2.30 a	27.36 ± 3.42 b	4.00 ± 1.23 a	19.00 ± 4.21 b
	16/8 h (light/dark)	6.66 ± 1.33 b	60.00 ± 4.21 a	2.66 ± 1.15 b	43.33 ± 9.17 a
Average		10.00 ± 3.22 A	43.68 ± 9.22 A	3.33 ± 1.02 A	31.16 ± 7.42 A
10 mM	Continuous Dark	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
	16/8 h (light/dark)	1.33 ± 1.15 cd	26.60 ± 8.22 b	0.00 ± 0.00 c	0.00 ± 0.00 c
Average		0.66 ± 0.12 B	13.33 ± 4.25 B	0.00 ± 0.00 C	0.00 ± 0.00 C
15 mM	Continuous Dark	2.66 ± 1.33 c	18.33 ± 2.88 c	2.66 ± 0.96 b	23.33 ± 5.63 b
	16/8 h (light/dark)	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.0 c
Average		1.33 ± 1.15 B	9.16 ± 2.14 B	1.33 ± 0.45 B	11.66 ± 3.53 B
Photoperiod Average.	Continuous Dark	4.00 ± 1.23 [1]	11.42 ± 2.34 [1]	1.66 ± 0.11 [1]	10.58 ± 2.89 [1]
	16/8 h (light/dark)	2.00 ± 0.01 [2]	21.60 ± 3.53 [2]	0.66 ± 0.33 [2]	10.83 ± 3.12 [2]

Different capital letters in the same column indicate the differences among the treatments, different numbers indicate the differences among photoperiods and different small letters indicate significant the differences between treatment x photoperiod interactions ($p<0.05$).

Gungor [7] was not able to achieve germination of *E. spectabilis* seeds with 0.2% KNO_3 treatments. Contrary to Gungor [7], promising outcomes were achieved for germination and emergence of *E. spectabilis* with 5 mM KNO_3 dose in present study. In another study, 50% germination was achieved in wild rhubarb (*Rheum ribes* L.) seeds with 10 mM KNO_3 treatments [10].

Calcium chloride ($CaCl_2$) treatments. Considering the effects of $CaCl_2$ treatments on parameters, the greatest germination (4.00%) and emergence rates (4.00%) were obtained from the same dose (5 mM) and continuous dark photoperiods regime (Table 3). $CaCl_2$ treatments are also applied to stimulate germination of dormant seeds and the rate of success usually varies with the species. Farzami Sepeher and Ghorbanli [10] achieved better outcomes from $CaCl_2$ treatments than from KNO_3 treatments for wild rhubarb (*Rheum ribes* L.) seeds and reported the greatest germination rate for 10 mM and 5 mM $CaCl_2$ treatments. In our study, 5 mM dose was found to be more successful. Although different species used in present experiments, contrary to the findings of Farzami Sepeher and Ghorbanli [10], better outcomes were achieved from KNO_3 treatments than from $CaCl_2$ treatments both for germination and emergence parameters.

TABLE 3
Comparison of germination and emergence parameters of *E. spectabilis* subjected to $CaCl_2$ treatments.

Treatment	Photoperiod	GR (%)	MGT (day)	ER (%)	MET (day)
Control	Continuous Dark	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00± 0.00 c	0.0 0± 0.00 d
	16/8 h (light/dark)	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 d
Average		0.00 ± 0.00 C	0.00 ± 0.00 B	0.00 ± 0.00 C	0.00 ± 0.00 C
5 mM	Continuous Dark	4.00 ± 0.10 a	8.33 ± 1.52 b	4.00 ± 1.00 a	21.33 ± 2.31 a
	16/8 h (light/dark)	2.66 ± 1.33 b	6.66 ± 2.88 c	1.33 ± 0.33 b	11.66 ± 2.88 b
Average		3.33 ± 1.13 A	7.50 ± 2.25 A	2.66 ± 1.62 A	16.50 ± 5.78 A
10 mM	Continuous Dark	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 d
	16/8 h (light/dark)	2.66 ± 1.33 b	18.33 ± 2.88 a	0.00 ± 0.00 c	0.00 ± 0.00 d
Average		1.33 ± 0.01 B	9.16 ± 2.13 A	0.00 ± 0.00 C	0.00 ± 0.00 C
15 mM	Continuous Dark	1.33 ± 0.33 c	5.33 ± 1.15 c	1.33 ± 0.33 b	8.66 ± 2.31 c
	16/8 h (light/dark)	1.33 ± 0.33 c	13.33± 5.77 a	0.00 ± 0.00 c	0.00 ± 0.00 d
Average		1.33 ± 0.01 B	9.33 ± 2.25 A	0.66 ± 0.33 B	4.33 ± 1.41 B
Photoperiod	Continuous Dark	1.33 ± 0.01 [1]	3.42 ± 1.11 [1]	1.33 ± 1.10 [1]	7.50 ± 3.41 [1]
Average.	16/8 h (light/dark)	1.66 ± 0.02 [1]	9.58 ± 2.10 [2]	0.33 ± 0.21 [2]	2.92 ± 1.01 [2]

Different capital letters in the same column indicate the differences among the treatments, different numbers indicate the differences among photoperiods and different small letters indicate significant the differences between treatment x photoperiod interactions ($p<0.05$).

TABLE 4
Comparison of germination and emergence parameters of *E. spectabilis* subjected to GA_3 treatments.

Treatment	Photoperiod	GR (%)	MGT (day)	ER (%)	MET (day)
Control	Continuous Dark	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
	16/8 h (light/dark)	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00± 0.00 c
Average		0.00 ± 0.00 C	0.00 ± 0.00 C	0.00± 0.00 B	0.00 ± 0.00 C
250 ppm	Continuous Dark	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
	16/8 h (light/dark)	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Average		0.00 ± 0.00 C	0.00 ± 0.00 C	0.00 ± 0.00 B	0.00 ± 0.00 C
500 ppm	Continuous Dark	1.33 ± 0.12 a	20.00 ± 5.20 a	1.33 ± 0.33 a	8.00 ± 2.00 b
	16/8 h (light/dark)	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Average		0.66 ± 0.33 B	10.00 ± 2.50 A	0.66 ± 0.01 A	4.00 ± 1.00 B
750 ppm	Continuous Dark	1.33 ± 0.10 a	10.00 ± 3.20 b	0.00 ± 0.0 c	0.00± 0.00 c
	16/8 h (light/dark)	1.66 ± 0.21 a	20.00 ± 4.20 a	0.00 ± 0.00 c	0.00 ± 0.00 c
Average		1.33 ± 1.02 A	15.00 ± 6.32 A	0.00 ± 0.00 B	0.00 ± 0.00 C
1000 ppm	Continuous Dark	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00± 0.00 c
	16/8 h (light/dark)	1.00 ± 0.20 a	8.33 ± 2.11 b	0.66 ± 0.33 b	13.30 ± 3.21 a
Average		0.66 ± 0.33B	4.16 ± 1.21 B	0.33 ± 0.01 A	6.66 ± 1.67 A
1500 ppm	Continuous Dark	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
	16/8 h (light/dark)	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Average		0.00 ± 0.00 C	0.00 ± 0.00 C	0.00 ± 0.00 B	0.00 ± 0.00 C
2000 ppm	Continuous Dark	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
	16/8 h (light/dark)	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Average		0.00 ± 0.00 C	0.00 ± 0.00 C	0.00 ± 0.00 B	0.00 ± 0.00 C
Photoperiod	Continuous Dark	0.38 ± 0.22 [1]	4.28 ± 2.31 [1]	0.19 ± 0.01 [1]	4.28 ± 1.43 [1]
Average.	16/8 h (light/dark)	0.38 ± 0.22 [1]	4.76 ± 1.92 [1]	0.19 ± 0.01 [1]	1.90 ± 0.21 [2]

Different capital letters in the same column indicate the differences among the treatments, different numbers indicate the differences among photoperiods and different small letters indicate significant the differences between treatment x photoperiod interactions ($p<0.05$).

Gibberellic acid (GA_3) treatments. In GA_3 treatments, germination and emergence rates varied respectively between 0.00-1.66% and between 0.00-1.33%; germination and emergence times varied respectively between 0.00-20.00 days and between 0.00-13.30 days (Table 4). Positive outcomes were reported for GA_3 treatments on species of different families [19, 20, 21]. Contrary to earlier findings, single GA_3 treatments of the present study were not found to be effective in breaking dormancy of *E. spectabilis* seeds. Such a case may be resulted from differences in species and more persistent dormancy of *E. spectabilis*.

***In vitro* germination treatments.** Positive outcomes for germination and emergence were not also achieved from *in vitro* trials with different growth media. Germination was not achieved on the MS [11] and B5 [12] medium, but low rate germination was obtained on WH medium [13]. (Table 5).

TABLE 5
Comparison of germination and emergence parameters of *E. spectabilis* for *in vitro* treatments.

Treatment	Photoperiod	GR (%)	MGT (day)	ER (%)	MET (day)
WH	Continuous Dark	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 c
	16/8 h (light/dark)	1.66 ± 0.33 a	10.00 ± 1.50 b	0.00 ± 0.00 b	15.00 ± 5.00 b
Average		0.83 ± 0.11 B	5.00 ± 1.21 B	0.00 ± 0.00 B	7.50 ± 3.00 B
WH + GA_3	Continuous Dark	1.66 ± 0.42 a	18.33 ± 2.88 a	1.66 ± 0.81 a	21.66 ± 2.88 a
	16/8 h (light/dark)	1.33 ± 0.33 a	18.33 ± 3.51 a	1.66 ± 0.33 a	21.66 ± 2.88 a
Average		1.50 ± 0.30 A	18.33 ± 2.88 A	1.66 ± 0.33 A	18.33 ± 3.20 A
Photoperiod	Continuous Dark	0.83 ± 0.12 [1]	9.16± 0.02 [1]	0.83 ± 0.15 [1]	10.83± 0.01 [1]
Average.	16/8 h (light/dark)	1.50 ± 0.24 [2]	14.16 ± 1.02 [2]	0.83 ± 0.15 [1]	18.33 ± 1.20 [2]

Different capital letters in the same column indicate the differences among the treatments, different numbers indicate the differences among photoperiods and different small letters indicate significant the differences between treatment x photoperiod interactions ($p<0.05$).

CONCLUSION

In present study, positive outcomes were not achieved from GA_3 treatments and *in vitro* trials in breaking dormancy of *E. spectabilis* seeds. The greatest success was achieved from cold-moist stratification treatments and 5 mM KNO_3 and $CaCl_2$ treatments also yielded promising outcomes. The present study is a kind of preliminary study. Further research is recommended to research the effects of different applications and combinations apart from the present cold-moist stratification and promising treatments on seed dormancy, germination and emergence of dormant seeds. Combined treatments may also reduce stratification durations of *E. spectabilis* seeds requiring long-term cold-moist stratification durations.

ACKNOWLEDGEMENTS

Authors would like to thank Van Yuzuncu Yil University, Scientific Research Projects Department for the financial support (Project No: FYL-2016-5400). This study was a part of M.Sc thesis of the first author.

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Received: **08.06.2018**
Accepted: **21.12.2018**

CORRESPONDING AUTHOR

Burcu Tuncer
Van Yuzuncu Yil University
Faculty of Agriculture
Department of Horticulture
Van – Turkey

e-mail: brctuncer@gmail.com

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