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BALTIC FORESTRY

http://www.balticforestry.mi.lt
ISSN 1392-1355
eISSN 2029-9230

Baltic Forestry 2022 28(1): 591
Category: Research article
https://doi.org/10.46490/BF591

The effects of GA3 and storage time on the germination of *Epigaea gaultherioides* (Ericaceae) seeds

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Pulatkan, M., Çimen, N., Kurt, U. and Turna, İ. 2022. The effects of GA3 and storage time on the germination of *Epigaea gaultherioides* (Ericaceae) seeds. *Baltic Forestry* 28(1): article id 591. https://doi.org/10.46490/BF591.

Received 6 July 2021 **Revised** 20 June 2022 **Accepted** 10 July 2022

Abstract

Turkey is a country with diverse plant species and has significant potential in landscape planning. *Epigaea gaultherioides* (Boisse et Bal.) Takht. is a relic of the Black Sea (Euxine) province, aesthetic, and functional species indigenous to Northeastern Turkey and under the threat of extinction. The species should be cultured to ensure the permanence of the species with *ex situ* methods and employed in landscape planting design. The present study aimed to determine the effects of gibberellic acid (GA3) and cold storage time on seed germination performance in *E. gaultherioides.* Cold storage was applied to the seeds at +4°C for 16 and 28 months and subsequently the seeds were soaked in GA3 (0, 100, 500 and 1,000 ppm) solutions for 24 hours. The findings indicated that *E. gaultherioides* seeds that were not treated with GA3 solutions did not germinate. However, it was determined that germination was low in seeds treated with 500 ppm GA3, and 80% of the seeds germinated when treated with cold storage at 25°C (24 h dark) for 16 months. The ANOVA revealed that there were statistically significant differences between germination percentage and mean germination times for various gibberellic acid doses and cold storage – time processes.

Keywords: cold storage time, germination rate, gibberellic acid, landscape, relict

Introduction

The inclusion of nature in landscape design is essential for sustainable development. Thus, the significance of natural design approaches and spaces that could preserve the natural structure increase constantly (McHarg 1969). The use of indigenous taxa in planting design helps the preservation of the balance and beauty of natural ecosystems (Slattery et al. 2003). Several indigenous plant species could be preferred in landscape design. Several planting designs have been created with these species. The employment of indigenous species could lead to landscapes that imitate the traditional landscape and require less maintenance (Love et al. 2009).

Turkey has a quite rich floristic diversity. However, its potential is rarely utilized. Within the sustainability scope, native species that have an ornamental plant potential should be utilized in landscape design in urban and rural areas (Sarı and Acar 2015). Several taxa with high landscape potential remained in the rural landscape and could not be introduced to the urban landscape despite favourable temperature, rainfall and soil conditions.

Epigaea genus represents perennial woody plants in the Ericaceae family. They are indigenous to eastern Asia, North America and Transcaucasia (Rehder 1940, Yaltırık 1971, Bean 1973, Stevens 1978, Mackenzie 1997, Small and Catling 2007). *Epigaea* genus comprises three species: *Epigaea repens* L., indigenous to North America, *Epigaea asiatica* Maximowicz, indigenous to East Asia, and *Epigaea gaultherioides* (Boiss. et Bal.) Takht., indigenous to Caucasia (Stevens 1978, Small and Catling 2007). *Epigaea repens* and *Epigaea asiatica* exhibits myrmecochory (dispersal of seeds by ants) (Ōi et al. 1965, Clay 1983). Also, birds and snails consume *Epigaea repens* fruits and disperse its seeds (Everett 1980).

Epigaea gaultherioides is an evergreen prostrate shrub with a height of 40–50 cm that grows at elevations of 920–2,290 m a.s.l. (Yaltırık 1971, Stevens 1978). Its pink and white flowers bloom from May to July (Yaltırık 1971, Stevens 1978). These features can be qualified as a successful ground cover ornamental plant in landscape design.

It is a relic and rare species belonging to the elements of the Euxinian province (Stevens 1978, Ekim et al. 2014,

Eminağaoğlu 2014). It was included in the vulnerable category (VU) based on the IUCN Red List criteria (Ekim et al. 2014). Yaltırık (1971) reported that the species was distributed only in Northeast Anatolia in Turkey (TÜBIVES 2020) (Figure 1).

Endemic, rare and relict species should be strictly protected, and distribution should be ensured in a nation with high biological diversity in the world (Yıldırım et al. 2020). *E. gaultherioides* is exposed to the detrimental effects of road construction and forestry practices such as clear cutting. Because of its scarcity and disappearance of natural habitats conservation programmes should be implemented. For many species, propagation from seeds is the most common and the cheapest method employed in nurseries (Macdonald 2006). However, a major constraint to the sexual propagation of many species is the poor germination of their seeds. This is possibly due to low viability, although it is frequently due to seed dormancy (Mackay et al. 2002, Pipinis et al. 2017). Seed dormancy is a physiological condition in which the seeds cannot germinate even under the most suitable germination conditions that are in an environment with suitable temperatures, humidity and light conditions, or do not exhibit uniform germination. In general, seed germination is hampered by external factors (oxygen, temperature, humidity and light) and internal factors (e.g., seed coat, endosperm, embryo) (Bradbeer 1988, Bewley and Black 1994, Copeland and McDonald 2001, Koornneef et al. 2002, Baskin and Baskin 2004, Güney et al. 2015). Gibberellic acid has been reported to increase germination percentage and seedling growth and be one of the main regulators of plant growth and development (Hooley 1994, Kaur et al. 1998). In addition, it improves the growth potential of the embryo and eliminates the mechanical restrictions caused by the boll by weakening the tissues around the radicle (Ogawa et al. 2003).

The presented study aimed to determine the germination conditions of the groundcover *E. gaultherioides* species, indigenous to the eastern Black Sea region in Turkey. Experiments were conducted to determine the effects of cold storage time and GA3, i.e., storage combination on seed germination. In this study gibberellic acid and cold storage expected to increase germination.

Material and methods

Seed material

E. gaultherioides seed capsules were collected from Pokut village, Çamlıhemşin district (40°58'28" North, 40°59'29" East) in Rize province, Northeastern Turkey. In July 2018 and 2019 mature seeds were collected from at least 20 same plants with dry fruits from each population occurring in 1,820–1,960 m a.s.l. Then, the seeds were separated from the capsules manually, and empty and rotten

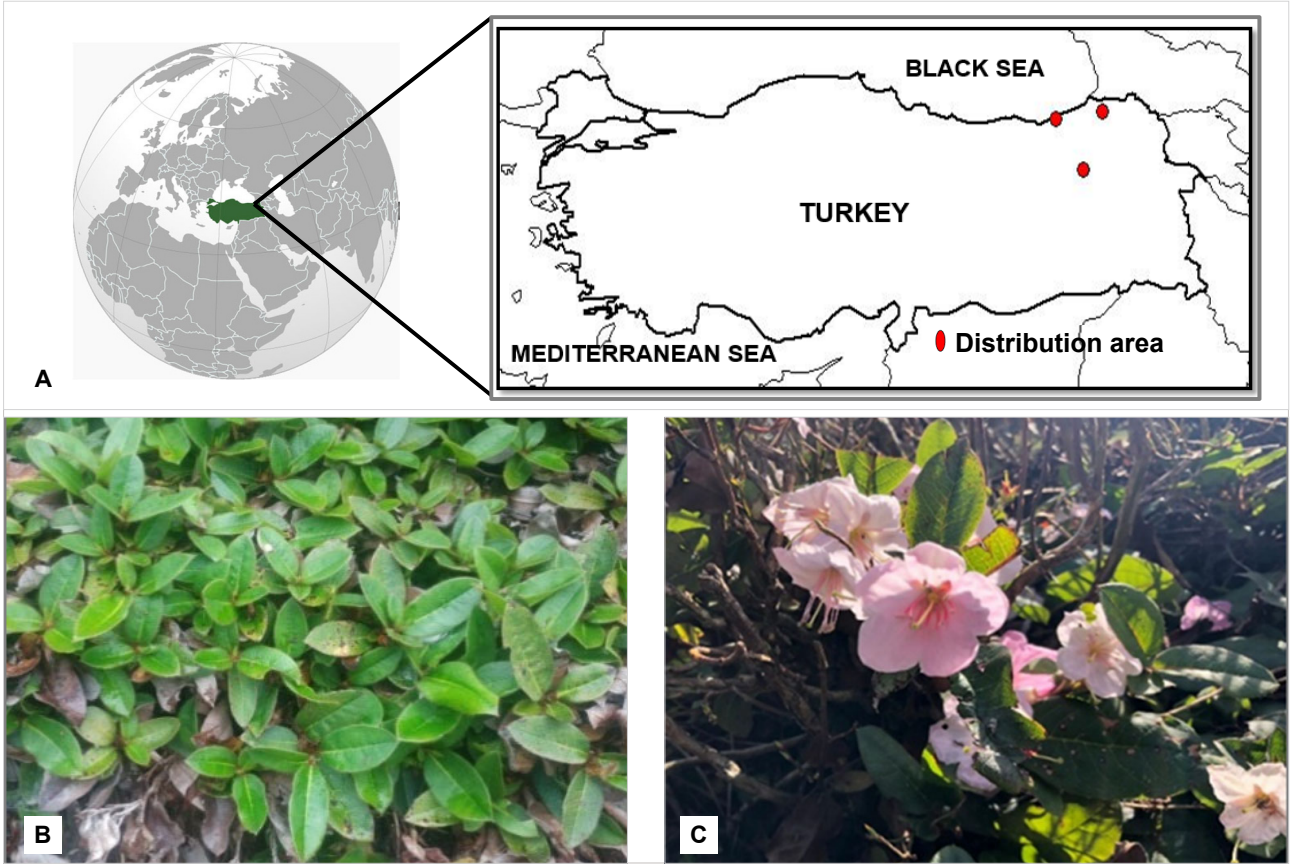


Figure 1. Distribution of *Epigaea gaultherioides* in Turkey (A), the form (B) and flowers (C)

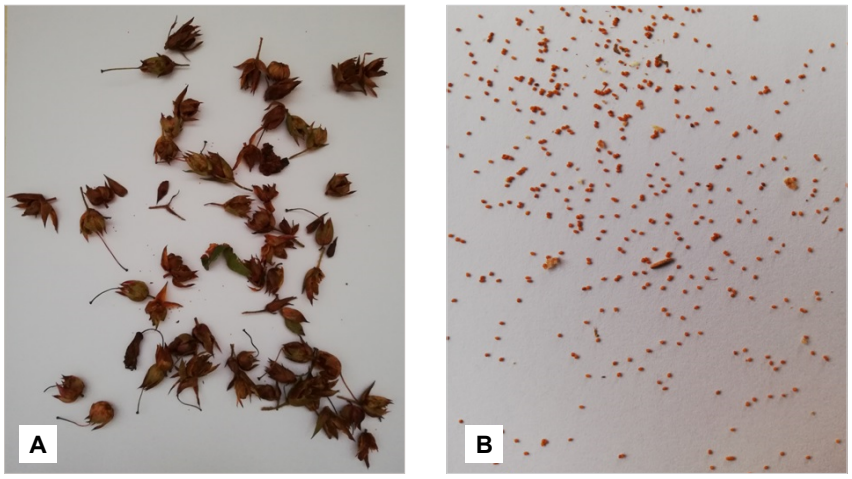


Figure 2. *Epigaea gaultherioides* seed capsules (A) and seeds (B)

seeds were removed. Potentially viable seeds were stored in the refrigerator (+4°C, 50% relative humidity) until the experiment (Figure 2).

Since the seeds were very small, viability tests were not conducted on them, and healthy seeds were selected under a magnifying glass (Barrows 1936, Pulatkan and Kamber 2019). Randomly selected 8 × 100 seeds were counted with a magnifying glass and then weighed on a precision scale. 1,000 seed weight was calculated based on these values (ISTA 1993).

Seed germination treatments

The seeds that were stored for 16 and 28 months were placed in Petri dishes in November 2020. The seeds with different cold storage time applications were treated with GA3 (0 (control), 100, 500, 1,000 ppm) for 24 hours. Seeds were allowed to germinate at 25°C in the 24 h dark in a growth chamber. The Petri dishes were covered and randomly placed in the growth chamber. Every three days the germinated seeds were counted and removed from the Petri dishes. Germination tests were conducted in 3 replications (Petri dishes) per treatment (4), and storage variants (2). There were 30 seeds in each Petri dish and a total of 720 seeds germinated. The seeds were placed on filter paper in the Petri dishes and were periodically watered with distilled water to maintain moisture.

Germination parameters

Seed germination was described as the appearance of an at least 1 mm long radicle based on the methods proposed by Oliva et al. (2009) and Vera et al. (2010). After no further seeds had germinated, the germination experiments were ended (Tremblay et al. 1996, Tsuyuzaki and Miyoshi 2009). Therefore, in this study, germination tests were terminated after 38 days. Final germination percentage, mean germination time and germination rate were determined at the end of the 38-day incubation period (Bewley and Black 1994).

Statistical analysis

Statistical analyses were conducted with SPSS 23 software package (IBM 2015). To test the normality of the data, the Kolmogorov-Smirnov test of normality was applied, and there was no need for transformation because the data showed normal distribution. Data were analysed with a one-way analysis of variance (*F* test). Duncan's multiple range test was applied to the significant test results to determine the groups that were different based on the impact of GA3 and cold storage time on germination (Duncan 1955, Klockars and Sax 1986, Ozdamar 2010).

Results

We calculated the 1,000 seed weight in the laboratory based on the International Seed Testing Association regulations (ISTA 1993). It was determined that the 1,000 seed weight of seeds collected in 2018 was 0.036 g and 0.042 g in 2019. The initial germination was observed after 17 days, and it was completed after 38 days.

In the study, analysis of variance was conducted to determine whether various gibberellic acid doses and cold storage time processes led to a difference in germination percentage and mean germination time in *E. gaultherioides* species (Table 1).

As seen in Table 1, there were statistically significant differences ($p < 0.05$) between germination percentage and mean germination times in storage time and GA3 treatments, while there was no statistically significant difference ($p > 0.05$) with storage time and GA3 interaction. The analysis of variance, statistical differences in germination percentage and mean germination time were determined based on GA3 and cold storage time applications (Table 2).

The highest germination percentage (80%) was identified in seeds treated with 500 ppm GA3 hormone and 16-month storage time. The lowest germination percentage was observed in the control group seeds that were not treated with GA3 (0%) and the seeds treated with 100 ppm GA3 hormone and 28-month storage time (17.78%). In general, higher germination percentages were observed in seeds treated with storage time for 16 months. The shortest mean germination time was observed in the control group, the longest germination time was observed in seeds treated with 100 ppm GA3 and 16 months storage time. Based on Duncan's test results, the first group was the GA3 1,000 ppm group, and the last group was the GA3 500 ppm and 1,000 ppm concentrations group.

Based on the mean germination time, seeds that were cold stored for 16 months and exhibited the shortest germination time, initially, a high germination rate were observed with 1,000 ppm GA3 application, and then the highest ger-

Table 1. The analysis of variance findings on germination percentage and mean germination time

Trait	Source	Sum of Squares	Degree of Freedom	Mean Square	*F*-test	Significance level (*p*)
Germination percentage (%)	Model	16322.924	7	2331.846	10.472	< 0.05
	Error	3562.770	16	222.673		
	Total	19885.695	23			
	Storage	1837.850	1	1837.850	8.254	< 0.05
	GA3	12439.091	3	4146.364	18.621	< 0.05
	Storage x GA3	2045.983	3	681.994	3.063	0.06
Mean germination time (days)	Model	2790.553	7	398.650	66.220	< 0.05
	Error	96.321	16	6.020		
	Total	2886.875	23			
	Storage	78.048	1	78.048	12.965	< 0.05
	GA3	2680.123	3	893.374	148.399	< 0.05
	Storage x GA3	32.382	3	10.794	1.793	0.19

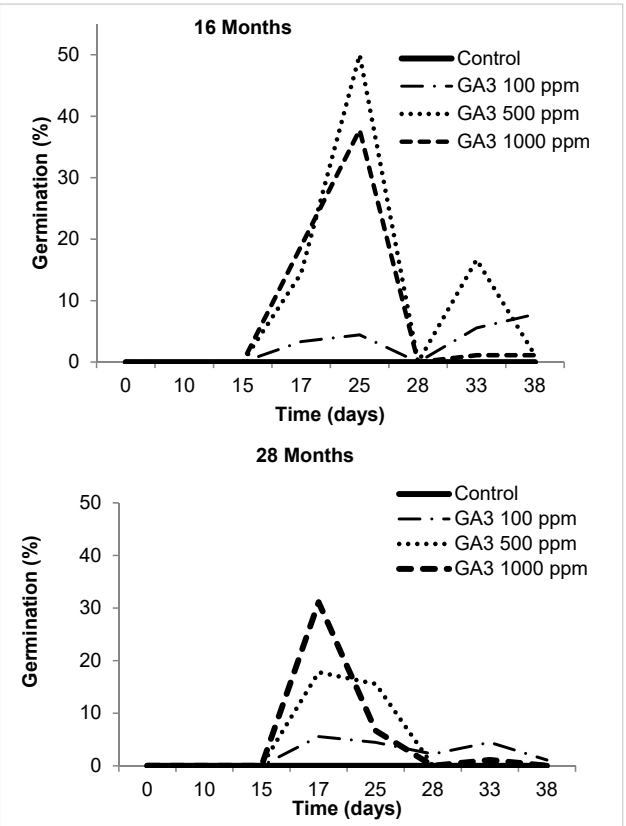


Figure 3. The effect of gibberellic acid (GA3) and storage time on *Epigaea gaultherioides* germination rate

mination rate was observed with 500 ppm GA3 treatment. Similarly, the germination rate was higher in seeds with short germination time and treated with 1,000 ppm GA3 and stored for 28 months (Figure 3).

The analysis of the impact of GA3 concentrations on total germination percentage revealed that three groups were formed based on Duncan's test (Figure 4). The control was the first group with 0.00% germination percentage. The second group was the 100 ppm GA3 application group with 19.45% germination percentage, while 500 ppm and 1,000 ppm GA3 applications were included in the last group with 56.67% and 48.89% germination percentages.

Table 2. Germination percentages and mean germination time of *Epigaea gaultherioides* seeds after various combinations of GA3 and cold storage time treatment (homogenous groups determined with Duncan's test, $p = 0.05$)

Cold storage time (months)	GA3 dose (ppm)	Germination percentage (%, ± S.D.)	Mean germination time (days ± S.D.)
16	0	0.00 ± 0.00 a	-
	100	21.11 ± 7.70 a	30.68 ± 1.61 c
	500	80.00 ± 20.52 b	25.10 ± 0.50 b
	1000	58.89 ± 19.53 b	22.73 ± 0.82 a
28	0	0.00 ± 0.00 a	-
	100	17.78 ± 6.94 ab	24.19 ± 6.34
	500	33.33 ± 16.67 b	21.18 ± 2.04
	1000	38.89 ± 24.12 b	18.71 ± 0.50

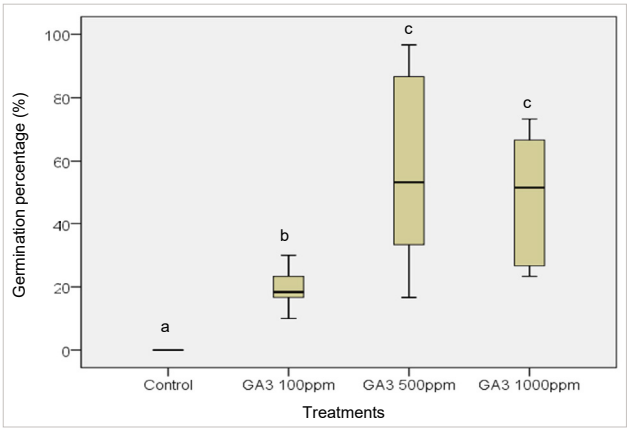


Figure 4. Graphical representation of total germination percentages (%), median and Duncan's test results

Discussion and conclusions

Germination requirements for native species, particularly for rare and/or endemic species, are important in conservation biology (Cerabolini et al. 2004). In our study, certain pre-treatment applications affected the germination of *E. gaultherioides,* a relict species. Physiologically non-deep dormant seeds are responsive to exogenously application of gibberellic acid and, depending on the spe-

cies, require warm or cold stratification to break dormancy (Baskin and Baskin 2004). Also, physiologically intermediate and deep dormant seeds require cold stratification to alleviate dormancy (Finkelstein et al. 2008). Previous studies reported that seed treatment with gibberellins increased germination and the germination rate (West et al. 1970, Genç 2007). Similarly, in the present study, GA3 affected germination percentage and germination rate. Also, Vecino-Bueno et al. (2009) determined that seeds with low average germination time had a high germination rate.

E. gaultherioides seed germination process has not been reported before. In a study, Kurt (2020) attempted to germinate *E. gaultherioides* seeds under greenhouse conditions (25 ± 2°C temperature and 70 ± 2% humidity) and peat + soil + sand (4 : 4 : 2), peat + soil (7 : 3), peat + sand (7 : 3), soil + sand (7 : 3) media; however, the seeds did not germinate after 60 days. But as our results suggest, seeds might germinate after pre-treatment with GA3. Barrows (1936) germinated *Epigaea repens* seeds in moist filter paper over peat and soil mixture and achieved a germination percentage between 0.3 and 87.3%. In the present study conducted in Petri dishes, the highest germination percentage was 80%, which was gained with 500 ppm GA3 treatment and 16-month cold storage time.

In studies conducted on Ericaceae family species such as *Erica cinerea* (Luna et al. 2008), *Andersonia heterophylla*, *Astroloma serratifolium*, *Astroloma xerophyllum*, *Conostephium minus*, *Conostephiu pendulum*, *Croninia kingiana*, *Leucopogon polymorphus*, and *Lysinema pentapetalum* (Just 2018), control seeds did not germinate after cold stratification, however, GA3 treatment affected germination. Cold storage (4°C for 30 days) broke dormancy in *Arctostaphylos pungens* Kunth (Jurado et al. 2011). Similarly, 30, 130, 200 and 360-day cold storage were effective in increasing seed germination of *Scorpiurus subvillosus* (Gresta et al. 2007). In the present study, GA3 positively affected germination percentage. But longer storage time treatment resulted in a decrease in germination.

According to Ricardo and Veloso (1987), about 90% and 50% of *Arbutus unedo* seeds treated only with 500 ppm GA3 germinated at 20°C and 25°C, respectively. In a study by Pipinis et al. (2017), non-stratified seeds of three *Arbutus unedo* varieties that were not treated with GA3 solutions exhibited very low germination (0.83–7.50%). In the present study, like the above-mentioned studies, the highest germination percentage was observed with 500 ppm GA3 treatment, while the lowest germination was observed in the control group (without GA3 addition).

Gibberellic acid (GA3) pre-treatments broke dormancy successfully in *Corema album*, and about 40% of the seeds germinated with 200 ppm and 400 ppm GA3 (Álvarez-Cansino et al. 2017). GA3 medium enhanced germination regardless of achene maturity, storage time, or storage temperature (Seiler 1998). Our study demonstrated that 48.89–56.67% germinated with 1,000 and 500 ppm GA3 treatments.

Vaccinium myrtillus seeds that were stratified for 60 days reached maximum germination percentage (80.67%), and the highest cumulative germination percentage was observed on the sixth week and reached 45.67% in *Vaccinium arctostaphylos* seeds (Karabulut and Çelik 2013). In *Astroloma xerophyllum* (Ericaceae), maximum germination was achieved after only 24 days of incubation with seeds pre-treated with GA3 for 24 hours (Turner et al. 2009). In contrast, the maximum germination rate was obtained after only 17 days of 16-month long cold storage in the present study, while the highest germination rate was achieved on the 25-h day in seeds that were cold storage for 28 months. Upon the seed dormancy breaking of *Jasione supina* Sieber subsp. *supina*, which is an endemic to Mount Uludağ, the effects of GA3, the combination of hormone series, short-term moist chilling (1-month) and long-term moist chilling (4-month) related to the germination percentage and mean germination time were investigated by Güleryüz et al. (2021). After 4-month moist chilling treatment, 27% of seeds germinated in a light/dark and 80% in a dark regime. In *E. gaultherioides*, dormancy is broken by cold storage time and gibberellins in dark (25°C).

The 1,000 seed weight is an important measure of seed quality, which affects sprouting, seed potential, seedling growth, and plant performance (Afshari et al. 2011). Thus, 1,000 seed weight was determined in our study. The 1,000 seed weight findings in previous studies included *Rhododendron ponticum* (0.063 g) (Cross 1975), *R. thomsonii* (0.0792 g), *R. aganniphum* var. *flavorufum* (0.0932 g), *R. cerasinum* (0.0704 g) (Wang et al. 2014), and *Epigaea repens* (0.044 g) (Bonner and Karrfalt 2018), where the seeds were as small as *E. gaultherioides* (0.036– 0.042 g).

In conclusion, *E. gaultherioides* is ecologically important for relict diversity in Turkey (Ozturk et al. 2020), blooms very attractive pink flowers that could be used for ornamental purposes and fruits eaten by small ants. *Ex situ* conservation of the seeds is considered a viable and inexpensive method for endangered plant species; however, germination requirements of alpine species are poorly researched with a few exceptions (Cerabolini et al. 2004, Gimenez-Benavides et al. 2005). The germination requirements determined in our study could be beneficial for future *ex situ* conservation of *E. gaultherioides*. Findings demonstrated that germination pre-treatment was a powerful tool to produce this valuable species. This species could be propagated with this method and employed in landscape architecture. When the lean seed years and the difficulty of obtaining seeds in the limited natural range of the species is considered, the improvement of germination percentage for seedling growing is essential.

Acknowledgements

This study was a part of a research project funded by the Karadeniz Technical University Scientific Research Projects (No FYL-2018-7818).

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