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Effects of Pre-Treatments on Seed Dormancy and Germination of Endemic *Muscari bourgaei* Baker

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Abstract: *M. bourgaei* Baker is an endemic plant that grows in Türkiye. It has the potential to be an ornamental plant, so it is important to know its germination characteristics and seed morphological characteristics. We evaluated the effects of moist chilling (3 to 12 months) and application of gibberellic acid (GA_3) (250 to 1000 mg/L^{-1}) on seed germination under two different light regimes (light phase and dark phase) and four temperature regimes (20, 20/10, 25/15, and 15/10 °C). Seeds were collected from the subalpine belt of Mount Uludağ at an altitude between 2200 and 2300 m. *M. bourgaei* seeds were dormant and reached the highest germination percentage after 12 months of moist chilling. GA_3 applications only have a limited effect on the breaking of dormancy in most cases. Maximum germination of 28 percent was obtained by the GA_3 application of 1000 mg/L under dark phase with incubation at 15/10 °C. Scarification with sulfuric acid did not result in any germination. Seed germination of above 80 percent was obtained after 6 months of moist chilling and above 90 percent after 9 and 12 months of moist chilling. Intermediate physiological dormancy was determined as the type of dormancy. Our findings on seed germination and dormancy characteristics of *M. bourgaei*, which is an endemic alpine meadow plant, will contribute to the protection and development of the germplasm of this species.

Keywords: *Muscari bourgaei*; germination; endemic; dormancy; seed characteristics; gibberellic acid

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1. Introduction

Germination is an important stage that initiates a plant's life cycle. Germination and seedling establishment are important for plant growth, development, and adaptation [1]. Germination behavior is deemed an adaptive trait in relation to habitat properties [1,2]. Seed dormancy is a complex process by which the seeds schedule their germination through the delay of germination until the next season [3,4]. Since germination properties and germination timing are critical phases of a plant's life cycle, the future of the species depends on these characteristics [5]. Seed dormancy is a process that prevents germination even under favorable conditions [6]. Baskin and Baskin (2004) [6] introduced a comprehensive dormancy classification system. There are five dormancy classes in this classification, and physiological dormancy (PD) is the most common form of dormancy among these classes. In temperate regions, the seeds are released from dormancy following winter conditions, and this strategy is advantageous as it protects the seedlings from adverse winter conditions [3,7].

Germination requirements are species- and habitat-specific. Germination and dormancy experiments are not complicated experiments, and they indicate the complex relationships between germination properties and environmental factors. Information regarding the germination characteristics of a plant species allows us to have a deeper and more critical understanding of a plant's life cycle [8]. At the beginning of a species' habitat establishment phase, having knowledge regarding the ecological conditions necessary for germination is also very important [8].

The knowledge of seed germination behavior is indispensable in developing effective procedures and protocols for *ex situ* conservation [9–11]. Furthermore, the study of seed germination provides a clue to the understanding of the survival strategy of the species, which can be defined as the timing of seed dispersal along with dormancy/germination characteristics that result in germination during the optimum period for seedling establishment [3].

Seeds adapt to various habitats by evolving in different ways [5], and seed dormancy is a way of avoiding harsh environmental conditions. Further, seed batches have different dormancy levels and types [3], and this trait is another survival strategy of the plants [2]. The geophytes are used in the pharmaceutical and perfumery industries due to the secondary metabolites they contain. [12]. *Muscari* species is an ornamental plant due to its flamboyant flowers, and it is preferred in rock gardens and landscaping applications such as borders [13]. Türkiye has a rich flora, and its endemism rate is around 34 percent [14]. *Muscari Mill*. is a genus represented by 41 taxa in Türkiye, 25 of which are endemic, and their endemism rate is 63 percent [15,16]. *Muscari Mill.* has a natural distribution in the Mediterranean, Europe, the Caucasus, Southwest Europe, and Central Asia. A total of 77 species in the world [17] represent the genus.

On a global scale, drought, grazing, global warming, fires, and anthropogenic factors continue to have an impact on biodiversity [18] The ecosystems of the Mediterranean Mountains have important plant diversity in terms of taxonomy. On a global scale, the Mediterranean climate zone is one of the regions suffering species loss the most severely [19]. Thus, the conservation of plant species, especially rare or endemic species, is prominent in conservation biology. *Ex situ* preservation of seeds has an important role in the conservation and reestablishment of alpine species [8]. Data obtained from germination tests can and should be used to provide information about the germination requirements of threatened species [8]. In addition, *M. bourgaei* is not available as a seed collection in any seed bank in Türkiye (personal correspondence).

Muscari, as a geophyte genus from the Mediterranean basin, can propagate from both bulbs and seeds. Seed germination properties of some other *Muscari* genus members have been previously investigated [20–23]. It has been shown that stratification of seeds for 45 days and sulfuric acid scarification improve the germination of *M. neglectum* seeds [23]. *M. gussonei*, an endemic and endangered psammophyte species from Sicily, germinates almost completely at 10–15 °C [22]. The study on the germination ecophysiology of four *Muscari* species revealed that the seeds were not dormant and that they preferably germinated at cold temperatures [20]. Although there are studies on the phylogenetic, cytological, palynological, and ecological properties and seed morphology of *Muscari* species along with their natural distribution in Türkiye [24,25], there is no study on their germination and dormancy properties. *Muscari bourgaei* Baker, which is under the category of least concern (LC), still needs to be conserved as its habitat is suitable for grazing and tourism and is affected by anthropogenic factors.

Mount Uludağ is one of the prominent plant sites in Türkiye due to its high plant diversity and habitat and is known as one of the Important Plant Areas (IPA no 18) [26]. Mount Uludağ is also famous for its winter tourism activities and significant spring water systems. Thus, plant species at Mount Uludağ are at risk due to these anthropogenic factors.

Plant conservation requires deeper knowledge about the life cycle of plants. Seed germination is an important stage, and this is why the germination characteristics of rare and endemic plants have become important in recent years [27–30]. There is a need for knowledge associating the biology and habitat characteristics of threatened or endemic plants in order to develop guidelines for their conservation [31–33]. In this study, we aimed to understand the germination and dormancy properties and morphological characteristics of endemic *Muscari bourgaei* Baker seeds, which have not been studied before. Seed germination tests on endemic and/or rare species provide valuable basic information on the seed germination ecology of the studied species [8], and the determination of seed dormancy classes can help us understand the early plant life history, and this information can be used on seed propagation [34].

2. Materials and Methods

2.1. Plant Material

The bulbs of this species usually do not proliferate, and its tunics are violet–blue. It is raceme rather lax, broadly ovoid and oblong, shows no fruit elongation, and usually has only 15–40 imbricate flowers. Fertile flowers are obovoid to oblong–urceolate and strongly constricted. Fruit capsules are ovoid–orbicular with emarginate leaves. Flowering plants of this species can be observed among *Juniperus communis* dwarf shrub communities, and *Acantholimon ulucinum* communities dominated by cushion plants at alpine pastures between 1500 and 2450 m between May and July. *M. bourgaei*, which spreads in eight floristic regions and 11 localities around Northwest, West, and South Anatolia in the Republic of Türkiye, is also naturally observed in the subalpine region of Mount Uludağ. It is in the LC category, but its habitat is at risk due to its suitability for tourism and grazing [15].

2.2. Seed Material

Ripe seeds were collected from the Mount Uludağ region in August 2010 and 2013 at an altitude between 2200 and 2300 m (40°05′03″ N and 29°10′46″ E). The seeds were then removed from the dried capsules and stored under laboratory conditions (25 °C, 50% RH). Since the seeds were collected on two different dates and the experiments were carried out in different years, this study can be divided into two parts: the preliminary experiment and the main experiment. Although germination conditions were chosen differently, the same germination procedure was adopted in both experiments. All experiments were initiated within 1 month from the date of seed collection.

2.3. Germination Conditions and Tests

Sterile plastic 9 cm Petri dishes were used for germination. The surface sterilization of the seeds was achieved via the application of 5% NaOCI for 3 min, and the seeds were then rinsed with tap water. Then they were soaked in Petri dishes with Whatman No. 1 filter paper in the presence of 4 mL of distilled water or gibberellic acid (GA_3). In order to prevent evaporation, the Petri dishes were covered with stretch film for the light phase and aluminum foil for the dark phase during the incubations. Gibberellic acid potassium salt (Sigma Aldrich, St. Louis, MO, USA) was used for the hormone treatments. Gibberellic acid was applied as a pre-treatment for 24 h inhibition, and then the seeds were rinsed with distilled water. Incubation conditions varied according to the experimental procedure adopted. The photoperiodic conditions had an approx. 30 μmol m^{-2} s^{-1} photosynthetic photon flux density during the lighting phase provided by Philips TL-D 30W/54-764 cool fluorescent tubes. The moist chilling was carried out by soaking the seeds in Petri dishes in the presence of distilled water at +4 °C. All seeds were checked daily for germination and considered to have germinated following the emergence of the radicles. Four replicates of 25 seeds per Petri dish were used. Germinated seeds were counted and removed every second day for up to 40 days. Seeds incubated in the dark phase were checked under filtered red light.

2.4. Preliminary Treatments for the Breaking of Dormancy

Preliminary trials were conducted in 2010. According to the results obtained from the preliminary trials, some additional trials were carried out with longer moist chilling durations and additional incubations at different temperatures. The seeds collected in 2010 were initially tested for germination in the dark phase at 20 °C. Moist chilling was applied for 2 months. After two months of moist chilling, seeds were incubated at the same conditions with GA_3 (250, 500, and 1000 mg/L^{-1}). Since not enough germination was observed as per the results of the preliminary experiment, we planned to obtain the germination responses through further germination conditions and tests.

2.5. Main Experiment: Prolonged Moist Chilling, Scarification, Responses to GA_3, and Seed Morphological Characteristics

The conditions of the main experiment were designed as per the results of preliminary germination tests. Then, the same GA_3 concentrations (250, 500, and 1000 mg/L^{-1}), longer moist chilling durations (3, 6, 9, and 12 months) with a wider temperature range, and light phase and dark phase (25/15 °C; 20/10 °C; 15/10° C for 12 h/12 h) conditions were selected for the germination treatments.

Scarification was achieved by soaking the seeds in 80% H_2SO_4 for 10 min. The seeds kept in acid were then washed with running tap water, planted in Petri dishes by the method described above, and incubated at three different temperatures and under two light regimes. The untreated seeds were subjected to scarification.

For the scanning electron microscope, seed material was sputter coated with gold–palladium for 2 min in a BAL-TEC SCD 005. All observations were performed using a scanning electron microscope, and micrographs were taken at 20 kV. Macromorphological characteristics of the seeds, including color, shape, and size, were determined, and weighing of the seeds was performed by weighing a randomly selected 100 seeds (measurements were made on these seeds).

2.6. Statistical Analysis

Germination percentage and MGT were calculated (mean ± standard error). Germination percentage was calculated by the formula: G (%) = (A/B) × 100. In this equation, A is the total number of seeds that germinated within 40 days, and B is the total number of seeds (25 seeds). MGT is the mean germination time for the seeds, and it was calculated by the formula MGT = ΣDN/ΣN. In this equation, D is the number of days spent as of the sowing date and N is the number of seeds germinated on day D. Germination percentage and MGT results were evaluated with SPSS (Ver. 22 for Windows) and a two-way ANOVA. An arcsine transformation was performed before applying statistics to the germination percentage results. Untransformed data are presented in the tables. All tests were performed at a significance level of $\alpha = 0.05$.

3. Results

3.1. Preliminary Treatments for the Breaking of Dormancy

M. bourgaei seeds did not germinate during the control treatments with distilled water. However, the germination rate increased to 30.4 ± 4.7 percent after 2 months of moist chilling. By the GA_3 application following moist chilling, 31.0 ± 3.8 percent germination occurred at a 250 mg/L^{-1} concentration (Table 1). MGT values for preliminary treatment were found to be between 4.9 and 5.3 days (Table 1). Moist chilling for two months, by also considering the GA_3 and distilled water (control) treatments, was not significant in terms of germination percentage and MGT.

Table 1. Germination percentage and MGT of *M. bourgaei* seeds incubated under three GA_3 concentrations at 20 °C in darkness following two months of moist chilling (mean ± SE) (preliminary experiment).

GA_3 Concentration (mg/L^{-1})	**% Germination**	**MGT**
Distilled water	30.4 [a] ± 4.7	4.9 [a] ± 0.8
250	31.0 [a] ± 3.8	5.3 [a] ± 0.5
500	21.0 [a] ± 8.9	5.1 [a] ± 0.8
1000	21.0 [a] ± 1.0	5.0 [a] ± 0.2

Lowercase letters were used to compare the germination rates among the GA_3 concentrations.

3.2. Main Experiment: Prolonged Moist Chilling, Scarification, Responses to GA_3, and Seed Morphological Characteristics

M. bourgaei seeds did not again germinate during incubations performed with distilled water at other temperatures during the trials in 2013 (the main experiment), and so it was concluded that the seeds were dormant. Moist chilling at four different durations, and then incubation under different temperatures, increased the germination rate, and the dormancy was thus broken (Table 2). Among the moist chilling treatments that continued for 3 months, the incubations at 15/10 °C gave better results on the germination rates compared to incubations at 25/15 °C and 20/10 °C. Three months of moist chilling and then incubation under 15/10 °C resulted in 40.00 ± 4.60 percent germination for dark phase conditions and 25.00 ± 3.40 percent germination for light phase conditions. The germination rate was around 80.00 to 93.00 percent after 6 months of moist chilling at all incubation temperatures. Nine months of moist chilling generally resulted in more than 90.00 percent germination, and dormancy was completely broken except at 15/10 °C. The germination rate was 83.00 ± 1.00 percent in the dark phase and 89.00 ± 3.80 percent in the light phase at 15/10 °C.

Table 2. Effects of moist chilling on the germination rate of *M. bourgaei* at three different temperatures and under two light regimes (mean ± SE) (main experiment). Capital letters were used to compare the germination rates among the temperature series, while lowercase letters were used to compare the germination rates among the moist chilling durations.

Temperature	Moist Chilling Duration	Darkness	Photoperiod
20/10 °C	0 m	00.00 ^{dD} ± 00.00	00.00 ^{dD} ± 00.00
	3 m	26.00 ^{cC} ± 06.80	09.00 ^{cC} ± 03.00
	6 m	81.00 ^{abAB} ± 07.30	80.00 ^{bB} ± 07.80
	9 m	90.00 ^{abB} ± 03.50	95.00 ^{aA} ± 03.00
	12 m	96.00 ^{aA} ± 01.60	99.00 ^{aA} ± 01.00
15/10 °C	0 m	00.00 ^{dD} ± 00.00	00.00 ^{cD} ± 00.00
	3 m	40.00 ^{cCB} ± 04.60	25.00 ^{bC} ± 03.40
	6 m	85.00 ^{abAB} ± 07.10	87.00 ^{aB} ± 02.50
	9 m	83.00 ^{abAB} ± 01.00	89.00 ^{aB} ± 03.80
	12 m	98.00 ^{aA} ± 02.00	94.00 ^{aA} ± 03.90
25/15 °C	0 m	00.00 ^{dD} ± 00.00	00.00 ^{cC} ± 00.00
	3 m	13.00 ^{cC} ± 01.90	18.60 ^{bB} ± 03.50
	6 m	80.00 ^{abAB} ± 02.30	93.00 ^{aA} ± 01.90
	9 m	97.00 ^{aA} ± 03.00	98.00 ^{aA} ± 02.00
	12 m	98.00 ^{aA} ± 01.50	94.00 ^{aA} ± 03.50

Incubation temperature as a single factor was found to be non-significant, but the moist chilling duration was found to be significant alone ($p < 0.05$) under both dark and light phases (Table 3). The interaction of temperature and moist chilling duration was found to be significant under the light phase ($p < 0.05$), but it was not significant in the dark phase ($p > 0.05$) (Table 3).

MGT, the mean germination time, was also evaluated during moist chilling at three different temperatures (Table 4). The lowest value of MGT was found to be 3.9 ± 0.9 days at 20/10 °C under the dark phase following 3 months of moist chilling (Table 4). After 9 months of moist chilling, germination time was shorter among seeds incubated at 15/10 °C and 25/15 °C, compared to 3 and 6 months of moist chilling. When the factors were evaluated in view of MGT, the moist chilling duration was significant in both the dark and light phases, the temperature was significant only in the dark phase, and the interaction of these two factors was significant in all the treatments (Table 5).

Table 3. Results of the two-way ANOVA regarding the effects of incubation temperature and moist chilling duration on the germination rate of *M. bourgaei* seeds. Values are two-way ANOVA F-ratios. $p < 0.05$ indicates a significant difference among the treatments.

Factor	Germination Percentage				
		Darkness		Photoperiod	
	df	F	*p*	F	*p*
Temperature (A)	2	2.017	0.145	0.650	0.527
Duration (B)	4	378.899	0.000	148.212	0.000
A × B	8	6.122	0.000	0.855	0.561
Error	45				

Table 4. Effects of moist chilling on the MGT of *M. bourgaei* seeds at three different temperatures and under two light regimes (mean ± SE) (main experiment). MGT for a 12-month moist chilling duration was not calculated as most of the seeds had already germinated in the meantime. Capital letters were used to compare germination rates among temperature series, while lowercase letters were used to compare germination rates among the moist chilling durations.

Moist Chilling Duration	Temperature	Darkness	Photoperiod
3 months	20/10 °C	13.6 ± 1.9^{bD}	3.9 ± 0.9^{bB}
	15/10 °C	10.6 ± 1.2^{bCB}	7.5 ± 3.2^{bB}
	25/15 °C	21.1 ± 0.9^{bB}	17.1 ± 3.9^{aA}
6 months	20/10 °C	9.3 ± 1.1^{aA}	27.1 ± 3.3^{bB}
	15/10 °C	11.6 ± 2.3^{aB}	15.8 ± 1.2^{bB}
	25/15 °C	13.3 ± 0.5^{aB}	12.7 ± 0.3^{bA}
9 months	20/10 °C	22.5 ± 0.5^{abC}	8.7 ± 0.1^{aA}
	15/10 °C	7.2 ± 0.8^{abCB}	6.0 ± 1.4^{abB}
	25/15 °C	2.0 ± 0.0^{abD}	2.0 ± 0.0^{cB}

Table 5. Comparison by two-way ANOVA of the main effects (temperature and light regime treatments) on the mean germination time of *M. bourgaei* seeds incubated at three temperature series and under two light regimes. Values are two-way ANOVA F-ratios.

Factor	Germination Percentage				
		Darkness		Photoperiod	
	df	F	*p*	F	*p*
Temperature (A)	2	5.260	0.012	29.751	<0.001
Duration (B)	2	13.453	<0.001	4.268	0.25
A × B	4	45.586	<0.001	21.640	<0.001
Error	27				

3.3. Scarification and GA_3 Pre-Treatment

Scarification with H_2SO_4 did not produce any germination. Since the treatment was ineffective, it was not included in the tables. GA_3 pre-treatment was found to be effective only at 15/10 °C. Seeds did not germinate at 20/10 °C and 25/15 °C at the same GA_3 concentrations. The highest germination rate was found to be 28 ± 2.3 percent for the 1000 mg/L^{-1} GA_3 application under the light phase as significantly different (Table 6) ($p < 0.05$). The fastest germination time of 6.1 ± 0.8 days was also found for the 1000 mg/L^{-1} GA_3 application. The two-way ANOVA was applied only for GA_3 applications at 15/10 °C (Table 7). The effect of light, as a single factor, was found to be significant for both the germination rate and MGT ($p < 0.05$). The interaction of light and GA_3 was also significant for both the germination rate and MGT ($p < 0.05$).

Table 6. Germination rate and MGT of *M. bourgaei* seeds incubated under three different GA_3 concentrations at 15/10 °C (means ± SE). nd: Not determined due to a low germination rate. Lowercase letters were used to compare the germination rates and MGT among the GA_3 concentrations.

Temperature	GA_3 Concentration (mg/L^{-1})	% Germination		MGT	
		Darkness	Photoperiod	Darkness	Photoperiod
15/10 °C	Control	00.00 ^{c} ± 00.00	00.00 ^{c} ± 00.00	nd	nd
	250	16.60 ^{ab} ± 03.33	14.60 ^{b} ± 05.33	09.20 ^{b} ± 02.81	12.10 ± 01.26
	500	18.00 ^{b} ± 01.30	22.60 ^{a} ± 03.50	21.40 ^{a} ± 02.70	19.30 ± 03.66
	1000	28.00 ^{a} ± 02.30	05.30 ^{ab} ± 01.30	06.10 ^{b} ± 00.80	nd

Table 7. Comparison by two-way ANOVA of the main effects (light and GA_3 concentration) on the germination rate and MGT of GA_3-treated *M. bourgaei* seeds. Values are two-way ANOVA F-ratios.

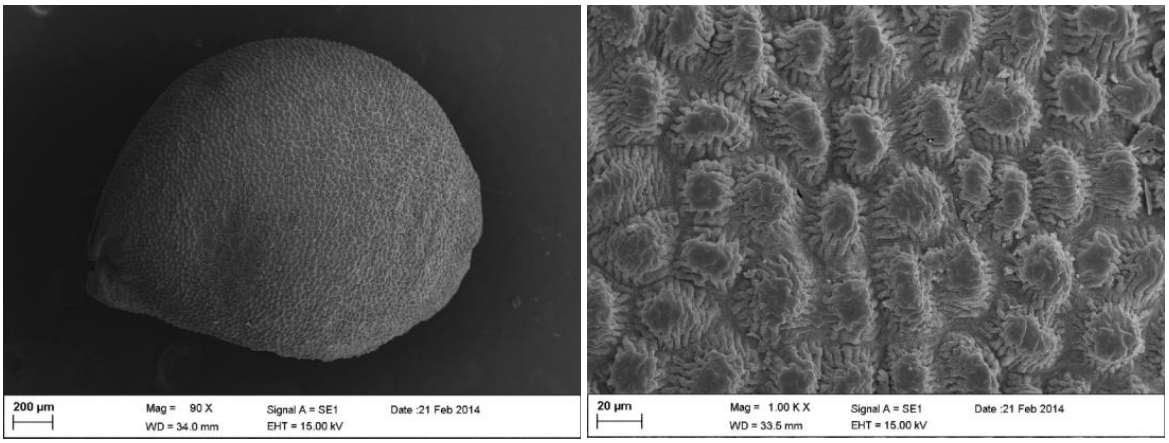
Factor	Germination Percentage			MGT	
	Darkness			Photoperiod	
	df	F	*p*	F	*p*
Light (A)	1	2.006	0.170	78.067	<0.001
Hormone (B)	3	18.801	<0.001	35.109	<0.001
A × B	3	24.153	<0.001	21.076	<0.001
Error	24				

3.4. Seed Morphological Characteristics

The morphological characteristics of seeds were determined through measurements and SEM. The mean seed weight was found to be 3.2 mg (Table 8). The seed's shape was orbicular, its size was 1.17 × 1.04 mm, and its color was black (Figure 1a,b). Figure 2 shows the dried inflorescence with seeds.

Table 8. Seed characteristics of *M. bourgaei* (mean ± SE) n = 100 seeds.

Color	1000 Seed Weight (g)	Length (mm)	Width (mm)	Weight (g)	Shape	Shape of Testa Cells
Black	03.20 ± 00.23	01.17 ± 00.03	1.04 ± 00.02	0.0034 ± 0.0001	orbicular	Stellate-grooved



(**a**) (**b**)

Figure 1. Scanning electron micrographs of *M. bourgaei* seeds (**a**) and seed testa cells (**b**).

Figure 2. Dried inflorescence of *M. bourgaei* with seeds.

4. Discussion

In alpine regions, seeds cannot germinate because their temperature needs cannot be fulfilled in the autumn [35,36]. Each species has particular seed germination requirements due to adaptive radiation in rugged and variable environments [37]. Moist chilling is deemed one of the most effective methods for breaking dormancy [35]. Further, moist chilling affects many processes such as the promotion of embryo maturation, alteration of hormone levels in seeds, degradation of inhibitory compounds, stimulation of numerous enzyme activities, and switching on some specific gene regions [38].

Dormancy and the depth of dormancy can be determined by the germination and germination rate of fresh and mature seeds under optimum conditions and within a certain period of time [3]. *M. bourgaei* seeds are dormant and do not germinate if left untreated. In the present study, moist chilling for more than 3 months significantly increased the germination, and thus, the seed dormancy was broken (Table 2). This may indicate that *M. bourgaei* seeds have physiological dormancy (PD). PD is common among woodland herbaceous plants and among numerous plant families [3]. In terms of alpine plants, the seeds are usually dispersed in the fall, spend the winter in the soil seed bank, and germinate the following spring [39]. Moist chilling practices at low temperatures can also extend the temperature range that the seeds need for germination [5,6]. *M. bourgaei* seeds are most

likely to have PD, but since many Liliaceae seeds have immature linear embryos [3], the possibility of morphophysiological dormancy should not be ignored.

Temperature is one of the significant factors in the breaking of dormancy and seed germination [35]. If the moist chilling was not performed, seeds would not have germinated at all three temperatures, and thus, after more than 9 months of moist chilling, most of the seeds germinated (Table 2). Seed dormancy in *M. bourgaei* seeds was broken by at least six months of moist chilling. Salmeri and Trubia (2019) [22] studied the endemic and endangered *M. gussonei* which prefers lower temperatures for germination. *M. gussonei* seeds germinate almost completely at 10–15 °C, whereas germination decreases with increased temperature (20 °C). In addition, Doussi and Thanos (2002) [20] studied four *Muscari* taxa and found that the seeds were not subject to dormancy but that they preferred a temperature of 10–15 °C and dark conditions for germination *M. neglectum* also prefers low temperatures (15 °C) and dark conditions for germination. Moreover, another Liliaceae species also seems to prefer lower temperatures for germination [21]. For example, *Allium schoenoprasum* seeds achieve more than 90 percent germination through incubation at 15 °C [40]. Specht and Keller (1997) [41] tested 97 *Allium* species' germination in terms of their temperature preferences and found that the species often preferred 5–11–16 °C ranges for germination. Kamenetsky and Gutterman (2000) [40] studied *A. altissimum* seeds' dormancy and found that its seeds require a minimum of 70 days of moist chilling for germination.

Gibberellic acid is a significant stimulator for germination, and it is considered an important factor in the breaking of dormancy [2,42]. It also has effects such as weakening the tissues surrounding the embryo [5], increasing its growth potential, overcoming inhibitors such as ABA [38], and stimulating the mobilization of reserves [43]. In the present study, *M. bourgaei* seeds responded to GA_3 pre-treatment, but only at 15/10 °C. Thus, we can specify that GA_3 application without moist chilling causes only a slight increase in germination. Jang et al. (2022) [34] studied the dormancy mechanism of *Veronicastrum sibiricum* seeds and found that GA_3 treatment at 2887 mM resulted in 90% seed germination during four incubation weeks at 20/10 °C. Kaya et al. (2015) [44] investigated four endemic *Sideritis* taxa and found that 200 mg/L^{-1} GA_3 treatment and/or the combination of GA_3 and moist chilling treatment were the most effective resulting in more than 60% germination. In our preliminary experiment, we determined 21.0 percent germination through 1000 mg/L^{-1} GA_3 treatment following two months of moist chilling (Table 1). In addition, in the main experiment, we determined 28.0 percent germination through 1000 mg/L^{-1} GA_3 pre-treatment without moist chilling (Table 2). It seems that gibberellic acid has a slight effect on the breaking of dormancy in *M. bourgaei* seeds. The type of dormancy among *M. bourgaei* seeds is probably physiological dormancy (PD). According to Baskin and Baskin (2004) [6], in terms of seed dormancy types, intermediate PD is broken through cold stratification for at least two months and through GA_3 treatment. Scarification by sulfuric acid did not result in germination and was therefore found to be ineffective, suggesting that there was no physical component with respect to the dormancy type.

Most alpine species are not ready to germinate after maturation due to temperature requirements, which are believed to be species-specific [45]. *M. bourgaei* seeds can germinate under both dark and light conditions following the break of their dormancy, and it is probably because the seeds remain at the soil seed bank (short-term), and thus they are classified as transient type II [34]. In addition, if the particle size in the soil is large, the seeds can easily sink into the soil [46]. The mean weight of *M. bourgaei* seeds was found to be 3.2 mg (Table 5), and this suggests that it is possible to store the seeds temporarily in the soil seed bank for 1 to 5 years [47]. In the present study, the viability of the seeds was not directly tested by the tetrazolium test. But the results of moist chilling suggested that the seeds were completely viable and maintained their viability for at least 1 year (Table 2). The seeds' anatomical characteristics are suggested to be useful for classification. *M. bourgaei* seeds' test in terms of anatomical characteristics consists of one layer of sclerenchymatous large flat cells and two layers of sclerenchymatous flat cells [24]. The micromorphological

characteristics of the *Muscari* seeds have variations, but the seed color is black among them. It was previously found that the color of the seed coat has an effect on dormancy in legume seeds and that colored seeds imbibe more slowly [48].

Our findings indicated that *M. bourgaei* seeds are physiologically dormant and do not germinate. In its natural habitat, this species can potentially remain for a while without germination. In nature, *M. bourgaei* seeds become mature at the end of the summer season and can germinate in the next spring by staying under moist and cold conditions for a sufficient period of time throughout the winter. The seed bank data provide valuable information on germination ecology, which can be used for *in situ* conservation and as a baseline for further germination studies [8]. Seed storage in gene banks and enabling regeneration are the most economical means of *ex situ* conservation of endemic species [27]. Moreover, for *ex situ* conservation of plant genetic resources, it is required to perform cultivation operations on wild and endemic species [49,50].

5. Conclusions

According to the results of the present study, a germination protocol can be proposed for *M. bourgaei* seeds covering physiological dormancy following dispersal. Optimally, 9 months of moist chilling treatment results in more than 90% germination and faster germination, while GA_3 application has a limited effect on the breaking of dormancy, so it is not recommended. The addressed habitat of the *M. bourgaei* is likely to face risks in the future due to its location as Mount Uludağ is a point of attraction for winter sports and tourism. These kinds of studies are gaining importance due to the gradual decrease in biological diversity in our country and around the world [51].

Propagation of endemic or rare plant species from seeds is considered an ideal method for the *ex situ* conservation of plants. The European Convention on Biological Diversity emphasized the priority of *in situ* conservation for long-term biodiversity conservation, but it also recognized the significance of the supporting role of *ex situ* conservation. *Ex situ* conservation acts as an insurance against extinction in the wild and provides material for re-introduction, plant breeding, and sustainable use programs, as well as education and research. Techniques include seed and gene banks, in vitro field gene banks, and pollen banks [52]. Important issues related to seed conservation efforts will be addressed as more seeds of wild species are collected and stored in the seed banks [51]. Finally, *M. bourgaei* may also be suggested as an ornamental plant for landscape projects prepared by the local flora. Plants of local origin will not only grow and survive best in their own locality but will also support the full range of native fauna [52].

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