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EFFECTS OF ENVIRONMENTAL AND STORAGE CONDITIONS ON THE GERMINATION OF *ALLIUM* SPECIES

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ABSTRACT

In this study, we assessed the germination responses of *Allium flavum* var *minus, Allium guttatum* subsp *guttatum,* and *Allium olympicum* seeds to various treatments. Moist chilling, room and soil storage, GA_3 treatment and scarification were tested at three different temperatures (20/10, 15/10, 25/15 °C), four different durations (0, 3, 6 and 9 months) and under two light regimes (photoperiod/darkness) to assess the rates of breaking dormancy. All three *Allium* species reached the highest germination percentages after moist chilling. *A. guttatum* reached the highest germination after 9 months of moist chilling (80.00 %), 9 months of room storage (22.25 %) and 6 months of soil storage (54.75 %). *A. guttatum*, compared to the other species, also responded best to GA_3 and scarification. *A. flavum* was best germinated after moist chilling for 9 months (78.00 %), germinated at a rate of 20.00 % after 3 months of room storage, and exhibited lower germination than the control with soil storage. *A. guttatum* and *A. flavum* germinated after scarification at rates of 27.00 % and 24.00 %, but scarification was not successful on *A. olympicum. A. olympicum* responded only to moist chilling, and the other treatments were not successful at breaking the dormancy of this species. We suggest that all three species have physiological dormancy that may be exhibited at different levels.

KEYWORDS:
Allium flavum, Allium guttatum, Allium olympicum, seed dormancy, germination

INTRODUCTION

Allium is a highly variable and taxonomically difficult genus within the family Amaryllidaceae, and its species are widely distributed throughout the northern hemisphere [1] In Turkey, there are 190 *Allium* taxa, and 67 of which are endemic [2]. Many *Allium* species are used for food, spices and herbal remedies. The genus contains sulphur compounds, phenols and steroidal saponins. Additionally, some members are economically important or cultivated as ornamentals [3]. The three *Allium* species used in our study prefer to grow among limestone rocks and stones or in alpine meadows and flower from July to September [4].

Seed dormancy is a complicated feature of plants that aids in the avoidance of severe climate conditions; many seeds are dormant at maturity [5, 6,7]. Dormancy is likely controlled by environmental factors [8], and in alpine habitats, dormancy helps new seedlings to avoid freezing winter temperatures [9, 10]. After standing for a winter, a dormant seed will germinate in the next season and continue growing. Otherwise, seeds are unable to germinate the following spring, and they will enter the soil seed bank and wait for suitable conditions [11, 12]. The form of dormancy that continues in the seed bank is known as secondary dormancy [13] formerly.

If a non-dormant seed does not germinate in autumn due to unfavourable climatic conditions, it can enter secondary dormancy and wait for the next season [14, 15, 5]. Dormancy can be broken by cold and wet stratification in laboratory and/or natural habitats. Furthermore, if the cold and wet conditions continue longer, the seed can germinate more easily and break dormancy [5]. GA_3 can experimentally be replaced by cold wet stratification in most situations, which likely causes an increase in the seed's internal GA_3 content [16].

One important method for the conservation of plant biodiversity is to preserve rare or endemic plant seeds [17]. Some inhabitants of alpine plant communities may become extinct or rare [18] or may even vanish from mountains if their remaining habitats are lost [19]. Germplasm conservation of wild species with orthodox seeds through long term storage will only be truly effective if the germination processes of dormant seeds are also understood [20]. *Ex situ* conservation implications for rare and/or threatened plants are based on knowing germination-promoting applications [20, 21] although *in situ* protection is preferred [20, 21].

Germination requirements of native species, particularly for endemic or rare species, are very im-

portant for effective plant conservation [17]. Research on wild *Allium* germination is scarce and covers very few species. In a study on *Allium* species of Kazakhstan, lower temperatures promoted germination and scarification reduced dormancy [23]. Studies on the germination of *Allium* species were included; germination of *A. stracheyi* seeds [24], storage of 94 *Allium* species in a gene bank [25] and germination of *A. hirsutum* from Iran [26]. The seeds of the three *Allium* species used in this study were dormant, and their germination strategies have not been previously studied. *Allium guttatum* Steven subsp. *guttatum, Allium flavum* L. var *minus* Boiss., and *Allium olympicum* Boiss. grow on Mount Uludağ, Turkey. *A. flavum* is an endemic species. Aims of the study were to assess the type of dormancy exhibited by the selected *Allium* species and to determine the germination responses to different environmental factors such as temperature, moist chilling, and light regime, storage in soil and room conditions. It also aimed to determine responses to GA_3 as a stimulant, of the three *Allium* species from Mount Uludağ.

MATERIALS AND METHODS

Seed material. Seeds of three *Allium* species were collected from the alpine site of Mount Uludag in September 2013. Seeds were taken from their capsules, air dried for a week after collection, and stored until the experiments were started. Experiments were initiated one month after storage.

Seed germination. Sterile, plastic, 9-cm petri dishes were used for seed germination. Surfaces were sterilized using a 5 % Sodium hypochlorite solution for 3 minutes, and seeds were rinsed with tap water [27]. Seeds were placed on a sterilized Whatman no. 1 filter paper moistened with 4 ml of distilled water or GA_3 solution. K-salt of GA_3 was used for the hormone treatments. To reduce evaporation, petri dishes were wrapped with stretch film during photoperiods and with aluminium foil for dark incubations. We tested three different temperature regimes for germination experiments: 20/10 °C, 25/15 °C and 15/10 °C (darkness/photoperiod). Seeds were considered germinated when the radicle emerged from the testa. Germination was recorded for 70 days, and the germinated seeds were removed. Four replications of 25 seeds per petri dish were used.

Effects of moist chilling, room storage and storage in soil. Seeds were kept at +4°C in petri dishes after sowing for moist chilling. The samples were stored for 3, 6 and 9 months in a fridge. At the end of this period, seeds were incubated under three different temperature regimes and two light regimes.

Seeds were stored in paper bags at room conditions for 3, 6 and 9 months, and at the end of the storage period were sown in petri dishes and incubated with three different temperature regimes and two light regimes.

At the start of October in 2013, seeds were packed into 6 cm x 6 cm fine weaved cotton fabrics (25 seeds per packet) and tied with cotton rope. They were buried at 5 cm deep in pots filled with a soil mixture of 2/3 loam and 1/3 sand, with drainage holes at the bottom. Pots were placed outdoors at the Gemlik campus of Uludağ University. After standing for 3, 6 and 9 months in the soil, the packets were removed from the pots, sown in petri dishes and incubated under three temperature regimes and two light regimes. A data logger was used to monitor the outside temperature.

Scarification and responses to GA_3. Scarification of the testa was achieved by soaking the seeds in 80 % H_2SO_4 for 10 minutes. Seeds were then washed in running tap water, sown according to the procedure mentioned above, and incubated at 15/10 °C (darkness/photoperiod). GA_3 was applied as a 24-hours pre-treatment. GA_3 was applied in concentrations of 250, 500 and 1000 ppm, and distilled water was used for controls.

Statistical Comparisons. Final germination (arcsine transformed) was analysed by three-way ANOVA separately for each of the three species. All tests were carried out at a significance level of 0.05 using SPSS ver 22 for Windows.

RESULTS

***A. flavum*.** Moist chilling duration, incubation temperature and light significantly influenced seed germination in *A. flavum* (Table 1). The effects of the interactions of all three factors were also significant, except for duration and incubation temperature interactions in *A. flavum* (Table 1). *A. flavum* seeds preferred to germinate in darkness than in the photoperiod (Table 2). Among the control treatments, only 15/10 °C resulted in germination (11.00 ± 00.00) for darkness-incubated *A. flavum* seeds. Additionally, the highest germination percentage was found after 9 months of moist chilling and incubation at 15/10 °C in darkness (78.00± 03.31). The other incubation temperatures resulted in more or less the same levels between the darkness durations (Table 2). The photoperiod-incubated seeds reached a maximum of 62.75 ± 18.28 percent after 6 months of chilling at 20/10 °C.

TABLE 1
Three way analysis of variance results of the effects of duration, incubation temperature and Light regime and GA_3, incubation temperature and Light regime on three *Allium* species germination.

Factor		Germination Percentage		
		A. flavum	*A. guttatum*	*A. olympicum*
Moist chilling	*df*	F	F	F
Duration (A)	3	121.329 ***	203.534 ***	169.570 ***
Incubation temperature (B)	2	3.938 *	2.851 ns	13.775 ***
Light (C)	1	23.866 ***	10.797***	4.770 *
interaction A x B	6	1.343 ns	10.942***	2.687 *
interaction B x C	2	19.432 ***	15.548 ***	24.404 ***
interaction A x C	3	3.065 *	4.391 **	6.252 **
interaction A x B x C	6	2.260 *	7.202 ***	6.735 ***
Error			72	
Room storage	*df*	F	F	F
Duration (A)	3	21.064 ***	54.848 ***	32.599 ***
Incubation temperature (B)	2	5.708 **	12.105 ***	14.036 ***
Light (C)	1	23.528 ***	14.235 ***	0.061 ns
interaction A x B	6	19.956 ***	37.504 ***	12.702 ***
interaction B x C	2	23.139 ***	15.583 ***	1.714 ns
interaction A x C	3	31.004 ***	1.646 ns	0.879 ns
interaction A x B x C	6	8.218 ***	9.586 ***	4.932 ***
Error	72			
Soil storage	*df*	F	F	F
Duration (A)	3	6.479 **	47.258 ***	37.760 ***
Incubation temperature (B)	2	11.136 ***	7.120 **	18.322 ***
Light (C)	1	0.036 ns	6.878 **	0.139 ns
interaction A x B	6	20.928 ***	2.335 *	8.362 ***
interaction B x C	2	4.368 *	1.297 ns	0.191 ns
interaction A x C	3	0.042 ns	7.032 ***	4.164 *
interaction A x B x C	6	7.303 ***	3.804 *	2.870 *
Error	72			
GA_3	*df*	F	F	F
GA_3 (A)	3	4.032*	22.496***	2.651 ns
Incubation temperature (B)	2	120.296***	27.248***	15.553***
Light (C)	1	74.948***	1.605 ns	4.613*
interaction A x B	6	1.533 ns	5.170***	4.039*
interaction B x C	2	73.789***	11.563***	3.835*
interaction A x C	3	1.322 ns	5.740***	4.125*
interaction A x B x C	6	20.748***	8.401***	7.914***
Error	72			

*P<0.05, ** P<0.01, ***P<0.001, ns no significant difference.

Storage at room temperature also stimulated germination slightly after 3 months in *A. flavum* seeds. Germination reached 20.00± 04.42 percent in 20/10 °C in darkness. The room storage duration, the incubation temperature and light significantly influenced *A. flavum* seed germination (Table 1). The effect of the interactions between all three factors was also significant (Table 1).

The mean monthly outside temperature was given in Figure 1. Storage in soil did not stimulate *A. flavum* germination, except in the case of storage for 6 months, where the germination reached 09.25±03.31 percent at 25/15 °C in darkness (Table 2). The soil storage duration and the incubation temperature significantly influenced *A. flavum* seed germination (Table 1). The effect of the interactions of all the three factors was also significant, except for the interaction between the soil storage duration and the incubation temperature (Table 1).

Scarification resulted in 24.00 percent germination in darkness-incubated *A. flavum* seeds (Table 5). GA_3 treatment was ineffective at 25/15 °C and 20/10 °C. However, germination was slightly stimulated at 500 ppm GA_3 incubated at 15/10 °C in darkness (18.75± 04.21, Table 5).

***A. guttatum*.** Moist chilling duration and light significantly influenced *A. guttatum* seed germination (Table 1). The effects of the interactions of all three factors were also significant (Table 1). The highest germination percentages occurred in photoperiod-incubated *A. guttatum* seeds, mainly with moist chilling and soil treatments. In general, the germination percentage increased with the duration of moist chilling treatment (Table 3). Moist chilling for 3 and 6 months stimulated germination and changed the dormant status of the seeds. The highest germination percentages were observed for seeds chilled for 6 months at 15/10 °C and incubated in darkness (73.25 ± 11.32 percent) and for seeds chilled for 9 months at 25/15 °C and incubated in darkness (80.00 ±04.42 percent).

TABLE 2
Effects of moist chilling, room and soil storage on the germination percentage of *A. flavum* at three different temperatures (Means ± SE) m. month

Moist chilling	Duration	Darkness	Photoperiod
20/10 °C	0 m	00.00 ± 00.00	00.00±00.00
	3 m	51.00 ± 06.37	27.50±07.80
	6 m	46.00±00.00	62.75±09.14
	9 m	52.00±06.48	53.00±07.11
15/10 °C	0 m	11.00±00.00	00.00±00.00
	3 m	72.25±08.75	34.25±03.09
	6 m	68.00±08.96	30.50±04.92
	9 m	78.00±03.31	42.70±10.05
25/15 °C	0 m	00.00±00.00	00.00±00.00
	3 m	38.75±01.70	35.25±09.45
	6 m	51.00±02.91	50.00±04.04
	9 m	50.75±04.00	52.50±01.66
Room storage			
20/10 °C	0 m	00.00±00.00	00.00±00.00
	3 m	20.00±04.42	02.25±01.03
	6 m	00.00±00.00	00.00±00.00
	9 m	01.00±00.58	02.00±00.00
15/10 °C	0 m	11.00±00.00	00.00±00.00
	3 m	11.00±00.00	01.00±01.15
	6 m	00.00±00.00	00.00±00.00
	9 m	00.00±00.00	01.00±00.58
25/15 °C	0 m	00.00±00.00	00.00±00.00
	3 m	00.00±00.00	00.00±00.00
	6 m	00.00±00.00	04.50±02.51
	9 m	01.00±00.58	05.50±02.02
Soil storage			
20/10 °C	0 m	00.00±00.00	00.00±00.00
	3 m	04.50±01.66	03.50±00.87
	6 m	00.00±0.00	06.50±00.96
	9 m	01.75±01.18	01.00±00.58
15/10 °C	0 m	11.00±00.00	11.00±00.00
	3 m	02.50±01.45	03.00±01.23
	6 m	01.75±01.75	03.00±01.23
	9 m	01.00±01.15	01.00±00.58
25/15 °C	0 m	00.00±00.00	00.00±00.00
	3 m	01.50±0.05	02.25±01.03
	6 m	09.25±03.30	05.00±00.05
	9 m	01.00±00.58	01.50±01.05

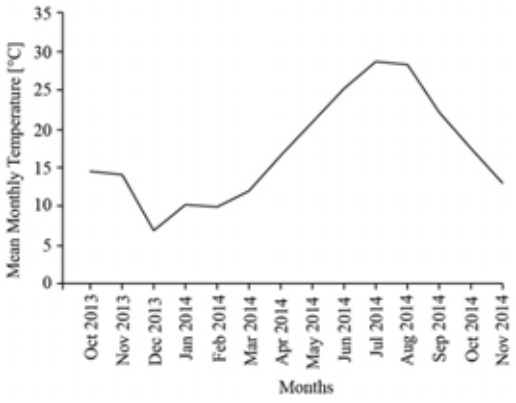

FIGURE 1
Mean monthly temperature of the Gemlik campus of Uludağ University during the experimental period

TABLE 5
Germination percentages of scarified and 15/10 °C incubated seeds of three *Allium* species

Treatment	*A. flavum*		*A. guttatum*		*A. olympicum*	
	D	P	D	P	D	P
Control	11.00 ±00.00	00.00 ±00.00	11.25±01.03	08.50 ±01.05	02.00 ±01.16	02.25 ±01.03
Scarified	24.00±01.23	08. 50±04.35	27.00±00.41	26.00±02.86	00.00±00.00	00.00±00.00

D. Darkness, P: Photoperiod

TABLE 3
Effects of moist chilling, room and soil storage on the germination percentage of *A. guttatum* at three different temperatures (Means ± SE) m. month

Moist chilling	Duration	Darkness	Photoperiod
20/10 °C	0 m	05.50 ± 02.02	07.75±02.14
	3 m	41.00 ± 09.23	24.00±02.38
	6 m	72.25±06.35	74.50±02.24
	9 m	70.00±04.89	66.00±05.90
15/10 °C	0 m	11.25±01.03	08.50±01.50
	3 m	48.00±04.95	42.00±03.46
	6 m	73.25±05.66	58.25±02.39
	9 m	68.25±08.00	14.50±01.66
25/15 °C	0 m	02.75±00.29	15.50±02.60
	3 m	43.00±02.22	36.50±03.77
	6 m	60.75±07.34	59.50±07.96
	9 m	63.25±02.39	80.00±02.00
Room storage			
20/10 °C	0 m	05.50±02.02	07.75±04.27
	3 m	05.00±01.78	00.00±00.00
	6 m	00.50±00.05	05.50 ±00.58
	9 m	22.25±04.25	20.00±02.12
15/10 °C	0 m	11.25±01.03	08.50±01.50
	3 m	11.25±01.03	08.50±01.50
	6 m	00.50±00.05	05.00 ±00.05
	9 m	06.00±00.58	10.75±02.36
25/15 °C	0 m	02.75±01.50	15.50±02.60
	3 m	05.00±00.05	13.50±01.44
	6 m	01.75±01.18	00.50±00.05
	9 m	01.93±00.88	03.50±00.80
Soil storage			
20/10 °C	0 m	05.50±02.02	07.75±02.14
	3 m	21.75±04.89	22.75±01.18
	6 m	17.75±01.18	33.50±02.40
	9 m	00.00±00.00	04.00±04.69
15/10 °C	0 m	11.25±01.03	08.50±01.50
	3 m	20.25±02.52	21.00±02.20
	6 m	22.00±02.72	54.75±01.74
	9 m	10.25±03.12	04.00±01.44
25/15 °C	0 m	02.75±00.58	15.50±02.59
	3 m	20.66±03.25	10.75±00.72
	6 m	20.16±02.05	20.75±04.15
	9 m	04.33±02.81	01.50±00.05

After 9 months of room storage, germination was slightly stimulated, with 22.25 ± 02.98 percent germination at 20/10 °C for darkness incubations and 20.00 ± 04.24 percent for photoperiod incubations. Room storage duration, incubation temperature and light significantly influenced seed germination, except for the interaction between the room storage duration and light for *A. guttatum* seed germination (Table 1).

Soil storage duration, incubation temperature and light significantly affected *A. guttatum* seed germination (Table 1). Soil storage resulted in similar germination percentages at 3 and 6 months; however, the germination percentage was decreased in all cases, and almost no germination occurred after 9 months of burial (Table 3).

A. guttatum seeds responded to GA_3 treatments more than the other two *Allium* species. There was

32.50 ± 05.33 % germination at 1000 ppm GA_3 for incubations at 20/10 °C and at darkness (Table 6). The difference level of GA_3 treatment was highest in *A. guttatum* ($P<0.001$) among the other two *Alliums* (Table 1).

Scarification also permitted germination to some extent. The germination was 27.00 ± 00.00 percent for darkness- and 26.00 ± 00.00 percent for photoperiod-incubated *A. guttatum* seeds. *A. guttatum* responded to scarification more than the other two *Allium* species (Table 5).

***A. olympicum*.** Moist chilling duration, incubation temperature and light significantly influenced the seed germination of *A. olympicum* (Table 1). The effect of the interactions of all the three factors was also significant (Table 1). *A. olympicum* germination favoured photoperiods more than darkness. Moist chilling treatment for 6 and 9 months resulted in the highest germination percentages for photoperiod incubations at 20/10 °C and 25/15 °C. The highest germination percentage was found when seeds were chilled for 6 months and incubated at 25/15 °C under photoperiod (74.00 ± 05.71) (Table 4).

Room storage also stimulated the germination percentage of *A. olympicum* seeds to some extent. The highest germination percentage was 15.75 ± 01.97 after 9 months of room storage (20/10 °C, photoperiod) (Table 4). Room storage duration and incubation temperature significantly influenced the seed germination of *A. olympicum* (Table 1). The effects of the interactions between factors were significant for room storage duration and incubation temperature and for the interaction between all three factors (Table 1).

TABLE 4
Effects of moist chilling, room and soil storage on the germination percentage of *A. olympicum* at three different temperatures (Means ± SE) m. month

Moist chilling	Duration	Darkness	Photoperiod
20/10 °C	0 m	01.00 ± 00. 58	00.00±00.00
	3 m	47.75 ± 07.92	62.00±02.89
	6 m	55.75±10.18	60.00±08.08
	9 m	30.00±04.22	63.25±02.39
15/10 °C	0 m	01.00 ± 00. 58	01.75±01.18
	3 m	50.75±04.19	19.25±03.94
	6 m	48.25±06.69	47.25±04.31
	9 m	51.25±06.10	25.50±03.80
25/15 °C	0 m	01.00 ± 00. 58	05.25±01.18
	3 m	49.25±04.48	43.75±01.89
	6 m	51.50±06.40	74.00±02.89
	9 m	37.75±07.34	72.25±01.80
Room storage			
20/10 °C	0 m	01.00 ±00. 58	00.00±00.00
	3 m	02.75±00. 58	01.25±01.30
	6 m	00.00±00.00	01.00 ±00. 58
	9 m	08.50±00.50	15.75±01.97
15/10 °C	0 m	01.00 ±00. 58	01.75±01.18
	3 m	01.00 ±00. 58	01.00±00. 58
	6 m	00.00±00.00	05.00 ±00.50
	9 m	04.50±02.72	05.00 ±00.50
25/15 °C	0 m	01.00 ±00. 58	05.25±01.18
	3 m	00.00±00.00	00.00±00.00
	6 m	08.75±01.80	05.00±02.35
	9 m	08.50±02.90	06.50±00.95
Soil storage			
20/10 °C	0 m	01.00±00. 58	00.00±00.00
	3 m	14.75±03.11	16.50±02.50
	6 m	08.50±00.95	20.50±02.47
	9 m	17.50±05.12	06.25±01.89
15/10 °C	0 m	01.00±00. 58	01.75±01.18
	3 m	04.50±02.60	06.00±00. 58
	6 m	05.25±01.18	02.75±01.49
	9 m	11.75±00. 58	10.50±03.50
25/15 °C	0 m	01.00±00. 58	05.25±01.18
	3 m	09.50±00.96	07.50±00.50
	6 m	10.50±02.87	14. 50±03.66
	9 m	25.50±02.06	23.50±04.77

TABLE 6
Effects of GA_3 on the germination of three *Allium* species. (Means ± SE)

Incubation temperature	GA_3 (ppm)	*A. flavum*		*A. guttatum*		*A. olympicum*	
		D	P	D	P	D	P
20/10 ° C	0	00.00 ±00.00	00.00 ±00.00	05.50 ±02.02	07.75 ±02.14	01.00±00.58	00.00 ±00.00
	250	01.50±00.50	00.00 ±00.00	10.00 ±00.58	23.00 ±01.78	01.00±00.58	00.00 ±00.00
	500	00.00 ±00.00	01.00±00.58	21.75 ±03.61	19.00 ±02.89	00.00 ±00.00	02.00 ±00.00
	1000	01.50±00.50	01.75±01.18	20.00 ±05.49	32.50 ±05.33	05.00 ±05.00	00.00 ±00.00
15/10 ° C	0	11.00±00.00	00.00 ±00.00	11.25±01.03	08.50 ±01.50	02.00 ±01.16	02.25 ±01.03
	250	13.75±01.88	04.75±01.03	10.25±01.11	21.50 ±02.10	00.00 ±00.00	04.00 ±01.23
	500	18.75 ±04.21	00.50±00.50	17.25±03.15	20.50 ±02.47	03.50 ±00.87	02.00 ±00.00
	1000	13.75±00.94	04.00±00.61	21.00±00.41	19.75 ±02.14	03.50 ±00.87	01.75 ±01.18
25/15 ° C	0	00.00 ±00.00	00.00 ±00.00	02.75±00.58	15.50 ±02.60	01.00±00.58	07.00 ±00.81
	250	01.50±00.50	01.00±00.58	10.25±01.11	05.00 ±01.78	01.00±00.58	01.50±00.50
	500	01.00±00.58	01.50±00.50	13.00±01.78	01.50±00.50	01.00±00.58	01.00±00.58
	1000	00.00 ±00.00	00.00 ±00.00	21.00±00.41	04.50±01.44	01.00±00.58	01.00±00.58

D: Darkness, P: Photoperiod

Soil storage for 6 and 9 months resulted in the stimulation of germination under both photoperiod and darkness for all temperatures tested. The highest germination percentage, 25.50 ± 02.06, was reached after 9 months storage in soil at 25/15 °C in darkness. Soil storage duration and incubation temperature significantly influenced *A. olympicum* seed germination (Table 1).

Scarification was not successful on breaking dormancy (Table 5). Additionally, *A. olympicum* seeds did not respond to GA_3 treatments (Table 6) and the effect of GA_3 treatments was found as non-significant (Table 1).

DISCUSSION AND CONCLUSION

Allium species from different taxonomic groups and habitats have different responses to environmental conditions and variety of germination mechanisms [26].

Chilling in moist conditions was the most effective method for overcoming dormancy in the three *Allium* species in this study; 6 and 9 months of moist chilling were found to be the most effective treatments. *A. flavum* and *A. guttatum* reached the highest germination percentages after 9 months of moist chilling (Tables 2 and 3). Moist chilling in the laboratory can replace the overwintering requirement. *Allium schoenoprasum* seeds also germinated at rates of more than 90 % at 15° C and 20° C after 3 months of cold and wet stratification [28]. Specht and Keller (1997) [25], tested the temperature requirements of 94 *Allium* species from 4 subgenera stored in the Gatersleben gene bank in Germany. They found that among the 4 constant test temperatures, species mostly preferred to germinate at 5, 11 and 16 ° C, suggesting that temperature requirements for germination could be related to geographical origin. *Allium* species from different habitats might also have a variety of germination mechanisms. For example, a minimum of 60 days of low temperature is required for *A. suworowii* and 70 days of low temperature for *A. altissimum* [23] but 6 weeks of low temperature improved germination in *A. hirsutum* [26].

Dormant alpine seeds that are generally produced in autumn and held in seed banks are expected to germinate after overwintering in spring [5]. Most of the alpine species do not readily germinate after maturation because of a high temperature requirement, which is believed to be species specific (Reviewed by Jaganathan et al., 2015) [29]. Moist chilling can reduce the temperature requirements for germination [5, 8].

After ripening under dry conditions may also help reduce physiological dormancy (PD) [30]. On the other hand, storage at room temperature and in soil was not found to be as successful as moist chilling in our study. However, there was still stimulation of germination in some of the seeds stored in room and soil conditions. The highest germination was found for room stored *A. guttatum* seeds, with rates of 22.5 ± 04.25 percent after 9 months (Table 3).

Gibberellic acid (GA_3) is very important for seed germination and is accepted as one of the most effective factors for breaking dormancy [30]. The type of dormancy and the quantity of dormant seeds may change from year to year within a species [30]. In intermediate PD, seeds can germinate after 2-3 months of cold stratification. In deep PD, seeds require longer periods of cold stratification, scarification and GA_3 failed to induce germination [5, 33]. Among the three *Allium* species, 1000 ppm GA_3 resulted in 32.50 ± 05.33 percent germination in *A. guttatum*. Responses to GA_3 were weak in *A. flavum* and *A. olympicum*. Germination of *A. stracheyi* seeds was stimulated by 100 ppm GA_3 [24].GA_3 is a well-known stimulator of germination in dormant seeds [30] and has been shown to induce the germination of many dormant species, such as *Papaver* [16], *Syagrus coronata* [32], and *Capsella bursa-pastoris* [33]. In our study, we found slightly positive responses to GA_3 in *A. flavum* and *A. olympicum*, which was probably due to the deep PD of those two

species. Scarification was not successful in stimulating germination in *A. olympicum*, though it did stimulate germination in *A. flavum* and *A. guttatum*. Scarification and GA_3 application were ineffective at releasing deep PD [5, 30].

It has been suggested that dormant seeds are used to establish seed banks in alpine regions [34, 12] and it is affected by seed size and shape [28]. Alpine seeds have been shown to have smaller seeds [35, 36], which can be more easily adapted to seed banks [37]. Soil storage for 3 and 6 months stimulated germination in *A. guttatum,* but 9 months of soil storage did not. However, 6 and 9 months of soil storage promoted germination in *A. olympicum,* which has smaller seeds than the other two *Allium* species. *A. flavum* did not respond to soil storage for any of the three durations, suggesting that it is not suitable for a seed bank.

Classification of dormancy can help to identify requirements for breaking dormancy. Our findings suggest that the type of dormancy in these three *Allium* species is PD. However, the level of PD might be different. Longer durations of moist chilling broke the dormancy in all three species, whereas scarification, GA_3, and room and/or soil storage did not successfully change the dormant status in most cases. *A. olympicum* probably exhibits a deeper form of physiological dormancy because it did not respond to either GA_3 or scarification. Thus, we suggest the three *Allium* species have deep PD according to the classification system proposed by Baskin and Baskin (2004) [30].

Alpine plants are vulnerable to current threats to plant diversity, such as land management practices, tourism, and climate change [38]. *Ex situ* conservation is becoming increasingly important for safeguarding alpine species and can provide materials for *in situ* conservation and restoration purposes [38]. *A. flavum* var. *minus* is endemic to Uludağ and a rare species. *A. olympicum* is not endemic but listed as near threatened. The germination data on wild *Allium* species is scarce and limited to those stored in seed banks [25]. Our study is the first study dealing germination of these three *Allium* species.

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