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Dormancy and Germination Requirements of Five Species From Brassicaceae

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ABSTRACT: The germination requirements of five plant species from Brassicaceae; *Thlaspi papillosum* Boiss. & Huet*, Thlaspi lilacinum* Boiss., *Draba brunifolia* ssp. *olympica* Sibth. ex DC*., Aubrieta olympica* Boiss*., Iberis spruneri* Jord*.,* from alpine site on Uludağ Mountain, Turkey, were investigated. The effects of moist chilling (+4 °C) for 15 and 30 days, and gibberellic acid (GA_3; 250, 500 and 1000 ppm), and combined GA_3 and moist chilling treatments were studied under darkness (20°C) and light (20/10 °C; 12/12 h, light/dark). *T.papillosum* seeds were found as non dormant, but the other four Brassicaceae seeds were found to have physiological dormancy which can be broken by moist chilling and GA_3 combinations. *T. lilacinum* and *D. bruniifolia* seeds, were resulted highest germination percentage at 1000 ppm GA_3 at darkness with 30 days of moist chilling and GA_3 combinations. *A. olympica* seeds germinated best at 15 days moist chilling and GA_3 combinations at darkness. *I. spruneri* seed germination increased at 15 days moist chilling and GA_3 combinations at both darkness and photoperiod conditions. Interactions among moist chilling and GA_3 were significant in *I. spruneri* and *A. olympica* seeds, but significant only at darkness in *T. lilacinum*, and non-significant in *D. bruniifolia* seeds. Thus, prolonged moist chilling of seeds combined by GA_3 under photoperiod conditions required to change the dormant status in these species. Our results suggest that these five Brassicaceae species, three of them are endemic, have different germination requirements to escape unfavorable habitat conditions.

Keywords: Acid, brassicaceae, endemic, gibberellic chilling, seed germination

Brassicaceae Familyasından Beş Türde Dormansi ve Çimlenme Gereksinimleri

ÖZET: Bu çalışmada Uludağ alpin bölgede yetişen Brassicaceae familyasına ait *Thlaspi papillosum* Boiss. & Huet*, Thlaspi lilacinum* Boiss., *Draba brunifolia* ssp. *olympica* Sibth. ex DC*., Aubrieta olympica* Boiss*., Iberis spruneri* Jord. türlerinin çimlenme gereksinimleri araştırıldı. Karanlık (20°C) ve fotoperiyot (20/10 °C; 12/12 h, ışık/karanlık) koşullarında, nemli soğuklama (15 ve 30 gün, +4 °C) ve gibberellik asit (GA_3; 250, 500 and 1000 ppm) ile nemli soğuklama ve GA_3 kombinasyonlarının etkileri araştırıldı. *T.papillosum* tohumlarının dormant olmadığı fakat diğer dört Brassicaceae *türünün tohumlarının* nemli soğuklama ve GA_3 kombinasyonları ile kırılabilen fizyolojik dormansiye sahip oldukları tespit edildi. *T. lilacinum* ve *D. bruniifolia türlerinde en yüksek çimlenme başarısı karanlıkta ve* 30 gün nemli soğuklama ile 1000 ppm GA_3 kombinasyonlarında bulundu. *A. olympica* tohumları en yüksek karanlıkta ve 15 gün gün nemli soğuklama ile GA_3 kombinasyonlarında çimlendi. *I. spruneri* çimlenmesi karanlık ve fotoperitotta 15 gün gün nemli soğuklama ile GA_3 kombinasyonlarında arttı. *I. spruneri* ve *A. olympica türlerinde nemli soğuklama ve* GA_3 interaksiyonu anlamlı, *T. lilacinum* türünde sadece karanlıkta anlamlı ve *D. bruniifolia* türünde ise anlamsız bulundu. Sonuç olarak *üçü endemik olan beş* Brassicaceae *türünde* dormansinin *kırılması için daha uzun süre nemli soğuklamaya veya* GA_3 kombinasyonlarına gerek duyulduğu, bu farklı *çimlenme davranışlarının türlerin* uygun olmayan çevre koşullarından kaçınabilmek için gerekli olduğu belirlenmiştir.

Anahtar kelimeler: Brassicaceae, çimlenme, endemik, gibberellik asit, nemli soğuklama

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INTRODUCTION

Seed germination and seedling establishment are critical early stages in the life cycle of plants and help control the plant's reproductive success and population persistence (Grubb, 1977; Harper 1977; Bu et al., 2008). Germination response patterns can vary depending on habitat, life history traits, phylogenetic relationships and geographical distribution. The plants in high elevations generally living close by their survival limits due to the climatic conditions, and some alpine taxa may eventually become extinct or rare (Holten, 1990; Grabherr et al., 1995). Alpine and subalpine regions can provide a valuable opportunity for the study of the timing and characteristics of germination because the environmental conditions change temporally with the season and spatially due to their topography (Billings and Bliss, 1959; Körner, 1999). Furthermore, the timing of germination plays a critical role in alpine habitats because the growing season is very short, and seedlings emerging during the spring will have greater fitness than those that emerge during other seasons (Grime et al., 1981; Washitani and Masuda, 1990; Baskin and Baskin, 1998).

A dormant seed is does not have the full germination capacity to during a specified period of time under any combination of normal physical environmental factors that are otherwise favourable for its germination (Baskin and Baskin, 2004). Cold stratification is regarded as the most important way to break dormancy in seeds of summer annuals and most temperate perennials (Baskin and Baskin, 1998). In addition, light and temperature are also important factors that influence germination (Hazerbroek and Metzger, 1990). Understanding the germination requirements for breaking of dormancy of a particular species is essential for those species needed for also restoration purposes (Urbanska, 1997).

In this study, we aimed to find out the basic data on germination and dormancy status of the five Brassicaceae species from an alpine site on Uludağ Mountain, Turkey, three of them were endemics. *Aubrieta olympica* is a rare and endemic species and also endangered, *Thlaspi lilacinum* is an endemic species, *Thlaspi papillosum* is also an endemic and critically endangered (IUCN, 2010). In addition to these endemics, we studied the germination requirements for *Draba bruniifolia* ssp. *olympica* and *Iberis spruneri*. We evaluated the germination by testing the responses to GA_3 (gibberellic acid) and short-duration moist-chilling treatments under darkness and light conditions. The germination requirements of these species have not been previously studied. In addition, data on the germination requirements of these species could be used for the ex situ conservation management of endemic species, currently, these five species facing threat by human activities in their natural environment (Arslan et al., 1999; Güleryüz et al., 1998, 2005).

MATERIALS AND METHODS

Germination tests

Mature seeds of plants were collected from the alpine belt of Uludağ Mountain between elevations of 1500-2200 m on September 2008. After collection, the seeds were air-dried for 1 week and then stored dry in a paper bag at room temperature for approximately 1 month (18-20 ºC) until they were used in the germination tests.

Seeds were classified into three groups according to the treatments made. The first group, of which controls were not germinated more than 10 %, as *T. lilacinum and D. bruniifolia ssp. olympica,* one month moist chilling and/or hormone treatments were applied. The second group, of which controls were germinated more than 10 %, as *A. olympica* and *I. spruneri,* 15 days of moist chilling and/or hormone treatments were applied. The third group as *T. papillosum*, seeds were not dormant, neither moist chilling nor hormone treatments were applied.

Seeds of the five species were tested for germination in darkness (20°C) and in light (12/12 h light/dark at 20/10 °C) in sterile plastic 9 cm Petri dishes and the GA_3 doses were 250, 500 and 1000 ppm with chilling/non-chilling and without GA_3 (distilled water) as control. Gibberellic acid was applied as a pre-treatment for a 24 hours imbibition. GA_3 was provided from Sigma. Moist chilling was achieved by keeping seeds soaked in Petri dishes with distilled water at +4º C for 15 or 30 days. All seeds were checked for germination daily and regarded germinated when the radicle emerged from the testa. For the application of the darkness treatment, the Petri dishes were wrapped with aluminum foil, and these seeds were monitored under safety red light. The final germination percentage and mean germination time were determined.

Statistical analyses

The final germination percentages (arcsine transformed) and mean germination time (MGT) values were analyzed by two-way ANOVA for two germination test environments. The independent factors were the GA_3 concentration, presence of chilling and their interaction. All of the tests were performed at a significance level of α= 0.05 using SPSS ver. 16.0 for Windows (SPSS Inc., 2007) packet program.

RESULTS

The germination rate of *T. papillosum* seeds was 83.0% in both dark and light, thus, GA_3 and moist-chilling were not applied (Table 1). The MGTs of the *T. papillosum* seeds were similar to the MGT values for the GA_3 and moist-chilled and dark incubated seeds of *T. lilacinum*. Germination was more rapid in the light (MGT, 7.0±0.3) than in the darkness (MGT, 8.1±0.3) (Table 1).

T. lilacinum seeds were not germinated unless they were treated (Table 1). The highest germination percentage was found at the 1000 ppm GA_3 + 30 days of moist chilling in darkness. A maximum of 12 % of the seeds geminated in the darkness with 1000 ppm GA_3 under non-chilled conditions, but the value was increased to 63.0% after 30 days of moist chilling (Table 1).

Table 1. Final germination (%) and MGTs; days of *T. lilacinum, T. papillosum* and *D. bruniifolia* seeds for different treatment series.

Treatment Series			Germination		MGT	
		GA_3 (ppm)	***T. lilacinum***	***D. bruniifolia***	***T. lilacinum***	***D. bruniifolia***
Darkness (20°C)	Without Moist chilling	Control	0.0±0.0	10.0±2.6	-	-
		250	24.0±1.6	10.0±2.6	19.2±3.6	-
		500	25.0±1.9	24.0±8.2	22.4±1.5	15.6±1.6
		1000	12.0±4.3	19.0±5.5	13.8±2.5	15.2±1.3
	Moist chilling 30 Days (+4°C)	Control	0.0±0.0	23.0±3.4	-	16.2±2.8
		250	46.0±7.7	37.0±9.5	13.2±1.0	7.2±0.9
		500	48.0±4,9	58.0±2.6	13.5±1.9	9.1±0.1
		1000	63.0±3,4	60.5±7.4	8.3±0.9	12.1±1.0
Photoperiod (20/10°C;12/12h)	Without Moist chilling	Control	0.0±0.0	9.0±1.0	-	-
		250	27.0±3.4	25.0±2.6	19.3±1.5	18.3±4.3
		500	44.0±5.4	36.0±7.1	16.1±0.6	15.0±2.3
		1000	54.0±3.8	37.0±3.4	21.1±0.8	16.0±1.3
	Moist chilling 30 Days (+4°C)	Control	0.0±0.0	11.0±4.1	-	5.0±0.4
		250	34.0±7.0	37.0±3.4	14.7±2.1	6.9±2.0
		500	38.0±3.5	36.0±9.1	8.4±0.7	7.6±1.4
		1000	58.0±5.3	44.0±5.4	6.3±0.6	3.7±0.9
T. papillosum						
Darkness		Control	83.0±1.9	nd	nd	8.1±0.3
Photoperiod		Control	83.0±2.2	nd	nd	7.0±0.3

Control (Distilled water) the values shown are the mean followed by the standard error (n = 4). nd. not determined

GA_3, chilling and their interaction were all significant for the final germination in darkness, but only GA_3 was significant for light (P<0.005) (Table 2).

The lowest MGT values were found for the *T. lilacinum* seeds given a combination of 1000 ppm GA_3 and 30 days of moist chilling (Table 1). The MGT values were also found to be significant almost under all of the treatments (P<0.05), with the exception of the chilling x GA_3 interaction in the darkness. The decreases in the MGT values were in accord with the two-way comparison results (Table 2).

D. brunifolia germination was 10.0% for darkness and 9.0% for light without GA_3 (Table 1). Germination percentage was slightly increased after moist chilling in controls. Among the treatments, only GA_3 affected germination significantly at light and darkness. Highest germination was found for 1000 ppm GA_3 and moist chilled seeds in darkness (60.0±7.4). One month moist chilling has significant effect on both germination percentage and MGT at darkness (P<0.005). The lowest MGT values were (3.7±0.9) at moist chilling + GA_3 at light and their interaction was significant (P<0.005) (Table 2).

Table 2. Two-way ANOVA results for the arcsine-transformed germination percentage and mean germination time (MGT) under dark and photoperiodic conditions of *T. lilacinum* and *D. bruniifolia.* The means of the germination percentage and MGT were analyzed for GA_3 x chilling interaction.

Source of variation	Germination Percentage			MGT		
	df	F	*P**	*df*	F	*P**
T.lilacinum						
Photoperiod						
Chilling	1	0.229	0.637	1	86.099	**0.000**
GA_3	3	141.025	**0.000**	3	103.472	**0.000**
Chilling x GA_3	3	0.854	0.478	3	18.430	**0.000**
Error	24			24		
Darkness						
Chilling	1	58.026	**0.000**	1	15.164	**0.000**
GA_3	3	59.046	**0.000**	3	38.165	**0.000**
Chilling x GA_3	3	9.239	**0.000**	3	2.002	0.141
Error	24			24		
D. bruniifolia						
Photoperiod						
Chilling	1	1.0	0.316	1	175.1	**0.000**
GA_3	3	13.7	**0.000**	3	0.8	0.505
Chilling x GA_3	3	0.7	0.558	3	2.4	**0.090**
Error	24			24		
Darkness						
Chilling	1	61.4	**0.000**	1	11.9	**0.002**
GA_3	3	8.7	**0.000**	3	1.3	0.313
Chilling x GA_3	3	1.7	0.187	3	4.8	**0.009**
Error	24			24		

*P**<0.05 indicate the significant difference among treatment series

A. olympica seeds were germinated 54.0% at darkness and 52.0% at light conditions with distilled water (Table 3). Germination percentages show a decrease from 54.0% to 34.0% at darkness and from 52.0 % to 48.0 % at light with moist chilling. After 15 days moist chilling with GA_3 application showed highest germination percentage (79.0 % with 500 ppm GA_3) in light, whereas GA_3 and GA_3 x moist chilling interaction was significant ($P<0.005$). The GA_3, moist chilling and their interaction were significant at darkness.

The lowest MGT was found 250 ppm $GA_3$with moist chilling (1.8 days) at light, and the interaction was also significant ($P<0.005$). Germination of *A. olympica* was found more rapid at moist chilling with GA_3 in both darkness and light (Table 4).

Table 3. Final germination (%) and mean germination times (MGTs; days) of *A. olympica I. spruneri* seeds for different treatment series.

Treatment Series			**Germination**		**MGT**	
		GA_3 (ppm)	***A. olympica***	***I. spruneri***	***A. olympica***	***I. spruneri***
Darkness (20°C)	Without Moist chilling	Control	54.0±4.2	17.0±3.8	6.5±0.6	7.3±1.0
		250	74.0±6.0	91.0±4.2	7.2±0.44	6.5±0.2
		500	77.0±3.8	96.0±1.7	7.6±0.5	4.5±0.4
		1000	67.0±4.4	96.0±1.6	8.1±0.72	5.1±0.5
	Moist chilling 15 Days (+4°C)	Control	34.0±3.9	8.0±0.0	6.2±0.5	-
		250	59.0±4.7	73.0±2.5	6.1±1.2	3.5±0.5
		500	55.0±3.8	91.8±0.1	3.2±0.1	1.8±0.1
		1000	69.0±4.4	97.0±1.8	4.2±0.4	1.8±0.5
Photoperiod (20/10°C;12/12h)	Without Moist chilling	Control	52.0±5.8	69.0±3.0	8.7±0.5	7.4±0.7
		250	61.0±3.4	83.0±1.0	12.5±0.6	8.9±1.7
		500	69.0±2.5	93.0±3.2	9.7±0.43	7.3±0.5
		1000	70.0±6.8	95.0±2.6	10.4±0.6	6.4±0.2
	Moist chilling 15 Days (+4°C)	Control	48.0±6.0	80.0±0.0	7.5±0.6	-
		250	73.0±3.8	53.0±1.2	1.8±0.3	6.4±0.2
		500	79.0±7.7	94.0±1.7	2.6±0.7	4.4±0.7
		1000	73.0±3.0	91.0±1.9	3.0±0.4	1.4±0.2

Control (Distilled water) The values shown are the mean followed by the standard error (n = 4)

I. spruneri seeds were germinated 17.0% at darkness and 69.0% at light in controls (Table 3). Germination percentages were decreased to 8 at both darkness and light incubated moist chilled controls. The germination without GA_3 was very low at darkness, but it increased to above 90.0% when GA_3 applied (above the 500 ppm). Seeds were germinated nearly full at 500 ppm GA_3 in both light and darkness, with or without moist chilling. The lowest MGT was found at moist chilling and 1000 ppm GA_3 (1.4 days).All the treatments showed significance ($P<0.005$) except MGT results in darkness (Table 4).

Table 4. Two-way ANOVA results for the arcsine-transformed germination percentage and mean germination time (MGT) under dark and photoperiodic conditions of *A. olympica* and *I. spruneri* The means of the germination percentage and MGT were analyzed for GA_3 x chilling interaction.

Source of variation	Germination Percentage			MGT		
	df	F	*P**	*df*	F	*P**
A. olympica						
Photoperiod						
Chilling	1	0.330	0.571	1	295.852	0.000
GA_3	3	5.735	0.004	3	4.679	0.010
Chilling x GA_3	3	3.225	0.040	2	26.127	0.000
Error	24			24		
Darkness						
Chilling	1	12.128	0.002	1	43.738	0.000
GA_3	3	7.695	0.001	3	1.233	0.319
Chilling x GA_3	3	3.695	0.026	2	5.920	0.004
Error	24			24		
I. spruneri						
Photoperiod						
Chilling	1	60.131	0.000	1	15.6	0.001
GA_3	3	96.9	0.000	3	20.3	0.000
Chilling x GA_3	3	20.6	0.000	2	18.4	0.000
Error	24			24		
Chilling	1	60,131	0,000	1	15.6	0,001
GA_3	3	96.9	0.000	3	20.3	0.000
Chilling x GA_3	3	20.6	0.000	2	18.4	0.000
Error	24			24		
Darkness						
Chilling	1	10.9	0.003	1	1.1	0.315
GA_3	3	185.2	0.000	3	1.1	0.376
Chilling x GA_3	3	3.2	0.042	2	0.9	0.436
Error	24			24		

*P**<0.05 indicate the significant difference among treatment series.

DISCUSSION

Dormancy and germination responses of the seeds were differed between the two endemic *Thlaspi* species. *T. papillosum* seeds have little or no dormancy. Conversely, T. *lilacinum* seeds were exhibited dormancy (Table 1). *Thlaspi caerulescens* from Scottish mountains germinated at 78.0 % and were not dormant (Cummings and Miller, 2000). The germination behavior can change within a single species from one population to another, like two endemic *Thlaspi* species, from year to year and among individuals (Urbanska and Shütz, 1986; Karlsson et al., 2008).

GA_3 treatment has been used to overcome low seed germinability in many plant species (Kırmızı et al., 2011; Güleryüz et al., 2011; Arslan et al., 2011; Dar et al., 2009; Golmohammadzadeh et al., 2015).

T. lilacinum seeds exhibited non-deep physiological dormancy (PD), and it was broken by moist chilling and GA_3 treatments. The effects of moist-chilling and GA_3 treatments and their interaction were found to be significant under the darkness and almost all of the treatments were significantly important for the MGT (Table 2). Physiological dormancy is the most abundant kind of seed dormancy among the Angiosperms (Baskin and Baskin, 1998). Baskin and Baskin (2004) have noted that species regarded as having a long-term, persistent seed bank may or may not be characterized by dormancy behavior. In addition, *Thlaspi* seeds can remain viable in the soil for several years (Dorph-Peterson, 1924; Kjaer, 1940).

Baskin and Baskin (1970, 1979), studied *Draba verna* germination. They found, *D. verna* seeds have conditional dormancy and can be broken after ripening. *D. verna* seeds were found to prefer lower temperatures and so that they germinate in autumn temperatures in the nature. We found a very limited germination without application of GA_3 in *D. bruniifolia* seeds. The highest germination percentage was 60.0% with 1000 ppm GA_3 in darkness (Table 1), suggesting that seeds were in physiologically dormant state.

Temperature is one of the very important environmental factors controlling seed germination, and plays an important role on the emergence of seeds in *Iberis pectinata* and *Ziziphora aragonensis* (Copete et al., 2009). Furthermore, the seeds of both species were in conditional dormancy and were facultative winter annuals so that seeds do not prefer to germinate after summer, but prefer to germinate in autumn and next spring. *I. spruneri* and *A. olympica* seeds germinated more than 50.0 % at least in controls of one of the light conditions, and germinated more than 70.0 % at moderate GA_3 concentration (500 ppm). And also, *I. spruneri* seeds germinated higher at photoperiod than darkness and germination percentage was rose when GA_3 applied in darkness conditions (Table 3). Germination was also found lower in darkness than photoperiod in *Z. aragonensis* and *I. pectinata* (Copete et al., 2009). *I. pectinata* seeds were germinated 100 % after 24 years of storage (-10º C, 3 % moisture content) in germaplasm bank (Maselli et al., 1999). This study approved that *I. pectinata* seeds have both longer viability and a long term seed bank in their habitat.

The sensitivity to GA is known to increase in seeds during the breaking of non-deep physiological dormancy (Finch Savage and Leubner-Metzger, 2006), and chilling is known to induce changes in hormone levels (Benech-Arnold et al., 2002; Yamauchi et al., 2004, Finkelstein et al., 2008). GA application to dormant seeds was found to be effective in many cases such as in *Pedicularis* (Kırmızı et al., 2010) and *Papaver* (Golmohammadzadeh et al., 2015). In the present study, GA_3 was the most effective treatment for the breaking of dormancy in four dormant Brassicaceae seeds. Seeds from alpine sites generally require long moist chilling periods (Jaganathan et al., 2015).

(Baskin and Baskin (1986) reported that after-ripening of the seeds of *Thlaspi perfoliata* occurred between spring and autumn at high temperatures, but the seeds remained dormant when stored at lower temperatures *T. lilacinum* seeds stored at one year on room temperature, did not germinate (data not shown) and *T. lilacinum* seeds may require low winter temperatures for breaking dormancy. *T. arvense* seeds were also dormant at following dispersal (Pelton 1956; Hazerbroek and Metzger, 1990; Baskin and Baskin, 1989), and dormancy was lost during a 1 month dry storage; few seeds would germinate following the winter (Baskin and Baskin, 1989, Hazerbroek and Metzger, 1990). Baskin and Baskin (1989) found that *T. arvense* germinated fully after 4 weeks of cold treatment and that the seeds germinated at 80-100 % after burial for several weeks. In another study, *T. arvense* seeds showed significant germination when overwintered outdoors, but the seeds maintained for 11 months at 8 ºC germinated poorly (Pelton, 1956). Conversely, Hazerbroek and Metzger (1990) suggested that the induction of a secondary dormancy is prevented in seeds maintained outdoors. If the conditions unfavourable, seeds can cycle back to dormancy instead of germination (Baskin and Baskin, 1998).

The habitat of these Brassicaceae species is under risk due to land use changes, overgrazing and recreational activities in the Uludağ Mountain (Arslan et al., 1999;, Güleryüz et al., 1998; 2005). Seeds of these dormant species could germinate at the beginning of the next growing seasons. *T. papillosum* seeds were not dormant, whereas *T. lilacinum* seeds were found as dormant. Further, *T. lilacinum* and *D. bruniifolia*

seeds might have non-deep physiological dormancy, because one month of moist chilling was not enough to change the dormant status of seeds, and GA_3 was promoted the germination to some extent. *A. olympica* and *Iberis spruneri* seeds might also have non-deep physiological dormancy, since germination promoted by GA_3. *I. spruneri* seeds seemed to prefer light for germination and, in the darkness conditions, seeds germinated more than 90 % with GA_3.The use of 1000 ppm GA_3 and moist chilling for several weeks might be efficient for the termination of seed dormancy in these four Brassicaceae seeds.

Our results constitute the initial knowledge on the germination requirements of these five Brassicaceae species.

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