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GERMINATION REQUIREMENTS OF *ANDROSACE VILLOSA* L. (PRIMULACEAE)

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We investigated the germination requirements of *Androsace villosa* L. (Hairy Androsace), which spreads on limestone or granite screes or ledges of rocky or turfy slopes and hilltops of the alpine zone. With seeds collected from Uludağ Mt. (Bursa, Turkey, 2200–2300 m a.s.l.), germination was studied in fresh seeds, seeds subjected to short-time moist chilling (15 d, +4°C), to GA_3 (100, 150 and 250 ppm), and to chilling plus GA_3. The hormone and moist chilling treatments were carried out in continuous darkness (20°C) and under a 12 h photoperiod at 20/10°C. Seeds maintained in darkness gave higher germination percentages than seeds maintained under a photoperiod. Germination rates rose to 90–97% with 100–250 ppm GA_3 and short-time moist chilling in continuous darkness (20°C). Seeds germinated rapidly under a combination of GA_3 and short-time moist chilling in continuous darkness, generally giving the lowest mean germination times (4.4–5.0 d) among the treatments.

Key words: *Androsace villosa*, gibberellic acid (GA_3), scarification, moist chilling, dormancy, alpine plants.

INTRODUCTION

Seed germination is a critical early stage in the life cycle of the plant, controlling reproductive success and the persistence of populations (Grubb, 1977; Harper, 1977; Bu et al., 2008). Germination response patterns can vary depending on habitat, life history traits, phylogenetic relationships and geographic distribution. High-altitude ecosystems are controlled largely by climatic constraints, and many plants are found in environmental conditions that are close to their climatic limits of survival (Billings and Bliss, 1959). Some alpine taxa and alpine vegetation types might become extinct (Holten, 1990; Grabherr et al., 1995) and rare local species might disappear from some mountains if their refuge habitats are lost (Gottfried et al., 1999). The complicated microtopography of alpine and subalpine regions makes them good areas for studying germination timing and behavior, as environmental conditions change drastically within seasons and also spatially (Billings and Bliss, 1959; Körner, 1995, 1999). The growing season is very brief in alpine habitats, so the timing of germination is critical (Ellenberg, 1988; Körner, 1999). Seedlings emerging in spring will have greater fitness than those emerging in other seasons (Grime et al., 1981; Washitani and Masuda, 1990; Baskin and Baskin, 1998).

From an ecological perspective, dormancy can be defined as prevention of germination even when suitable conditions prevail. The dormancy mechanism allows a species to synchronize its germination with favorable environmental conditions, increasing its probability of survival and establishment (Baskin and Baskin, 1998).

In this study we evaluated the effects of several treatments on germination of *Androsace villosa* L. seeds. We tested their dormancy-breaking responses to GA_3 and short-time moist chilling treatments in continuous darkness or under photoperiods. The germination requirements of this species have not been studied before in this species, which, though not rare or endemic, is a component of alpine ecosystems under grazing and/or seasonal anthropopressure. Basic information on germination of *A. villosa* should add to our understanding of the germination mechanisms of alpine species, and assist restoration and conservation efforts in alpine ecosystems.

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TABLE 1. Final germination (mean % ±SE) and mean germination times (±SE MGT, days) of *A. villosa* seeds from the different treatment series. (n = 3)

Treatment Series		GA_3 (ppm)	Germination (%)	MGT (days)
Darkness (20°C)		0	24 ± 2	14.7 ± 0.8
		100	64 ± 4	10.9 ± 0.8
		150	63 ± 3	9.5 ± 1.1
		250	75 ± 6	9.2 ± 0.4
	Moist chilling 15 Days (+ 4°C)	0	56 ± 4	8.6 ± 0.9
		100	91 ± 5	5.0 ± 0.2
		150	97 ± 1	4.8 ± 0.1
		250	97 ± 1	4.4 ± 0.7
Photoperiod (20/10°C; 12/12h)		0	0	0
		100	20 ± 2	16.9 ± 1.5
		150	24 ± 2	16.1 ± 0.6
		250	29 ± 1	16.5 ± 1.1
	Moist chilling 15 Days (+ 4°C)	0	9 ± 1	12.2 ± 0.5
		100	64 ± 6	7.3 ± 0.6
		150	65 ± 3	10.0 ± 0.1
		250	68 ± 6	9.5 ± 0.0
Photoperiod (20/10°C; 12/12h)	Scarification (10 min. %80 H_2SO_4)	0	37 ± 6	17.6 ± 0.8

MATERIALS AND METHODS

SPECIES DESCRIPTION AND SEED COLLECTION

Androsace villosa L. is a widespread species on mountains, reported from 16 sites ranging from 1400 to 4000 m a.s.l. in Turkey (Davis, 1975). It is a perennial herb forming dense cushions or lax mats. The inflorescences are 1–4(-9)-flowered. The flowers are white with a yellow eye becoming rosy pink with age. Flowering specimens can be observed between May and September (Davis, 1978). The fruits are capsules. The species is distributed on limestone or granite screes or cliffs of rocky or turfy slopes and hilltops.

Freshly mature seeds of *A. villosa* L. were collected from the alpine belt of Uludağ Mt. between 2200 and 2300 m a.s.l. in September 2009. Seeds were collected from 50 randomly chosen individuals from one population. A hundred seeds were weighed separately to determine mean seed weight. The mature, dry seeds were further air-dried for 1 week immediately after collection and then stored dry in a paper bag at 18–20°C for about a month until used in the germination tests.

GERMINATION TESTS

Sterile plastic Petri dishes (9 cm diam.) were used for the germination experiments. The seeds were surface-sterilized for 3 min with 5% sodium hypochlorite and then rinsed with tap water and sown on two layers of sterile filter paper. Test solutions included 100, 150 and 250 ppm GA_3, and distilled water as the control. The hormone solutions were analytical grade. The hormone was applied as pre-treatment for 24 h imbibition and then the seeds were rinsed with distilled water. Moist chilling was achieved by incubating seeds under wet and cold (+4°C) conditions for 15 days. For scarification, seeds were treated with 80% H_2SO_4 for 10 min and then rinsed several times with tap water. Three replicates of 25 seeds per Petri dish were prepared. The seeds were germinated in an incubator (Nüve GC400) under 20 W cool white fluorescent lamps (Phillips). Half of the plants were germinated under a 12 h photoperiod at 20/10°C, and the others were incubated at 20°C in continuous darkness. The seed germination percentage was checked by preliminary experiments in darkness with daily short-time dim light (DSDL). The seeds that germinated were counted and removed every day for up to 25 days. For application of DSDL the Petri dishes were wrapped with aluminum foil. Seeds were recorded as having germinated when the radicle emerged from the testa. Mean germination times (MGT) were calculated from the germination counts and used to determine the speed of germination. Final germination percentages and mean germination times were determined.

STATISTICAL ANALYSES

The results for the two germination test environments and for final germination (arcsine-transformed) and MGT were analyzed by two-way ANOVA. Independent factors were GA_3 concentration, chilling, and their interaction. All statistical tests were performed with

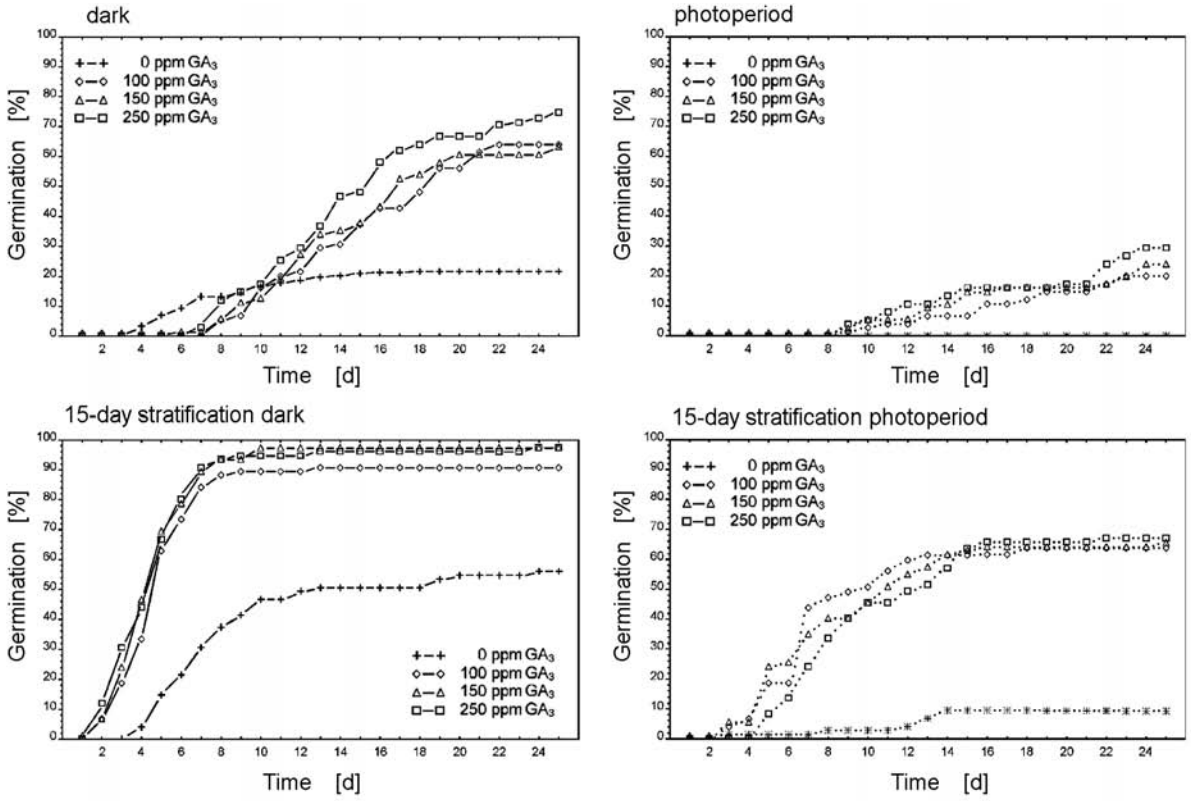

Fig. 1. Cumulative germination percentages for *Androsace villosa* seeds from the different hormone treatment series incubated in darkness at 20°C and under a 12 h photoperiod at 20/10°C.

SPSS 16.0 for Windows (SPSS Inc. 2007), with significance assumed at $P < 0.05$.

RESULTS

The mean weight of *A. villosa* seeds was 0.0016 ± 0.001 g/seed. Germination significantly increased in the GA_3 treatments (Tabs. 1, 2). In the non-chilled treatments, seed germination without GA_3 treatment (control) was very low (24%) in darkness, and under photoperiod conditions the seeds failed to germinate (Tab. 1, Fig. 1). Moist chilling increased the germination rate from 24% to 56% in darkness (Tab. 1), a significant effect (Tab. 2). Germination exceeded 90% when moist chilling was combined with GA_3 doses in darkness (Tab. 1). That combination also stimulated germination in photoperiod conditions, increasing it from 20% to more than 60% (Tab. 1). Germination was faster and MGT values were lower under combined GA_3 and moist chilling (Tab. 1). MGT values were higher and germination was slower when GA_3 was applied alone. Except for GA_3 combined with moist chilling in darkness, all the treatments significantly affected MGT (Tab. 2). Application of 80% sulphuric acid gave 37% germination and 17.6 days MGT, suggesting that the dormancy type was not connected with characteristics of the testa (Tab. 1).

DISCUSSION

Many montane species are non-dormant (Baskin and Baskin, 1998) but remain quiescent, as the growth period is too short to allow germination directly after dispersal (Washitani and Masuda, 1990). Physiological dormancy is the most common form, found in seeds of gymnosperms and all angiosperm clades; it is the most prevalent form of dormancy in temperate zone seed banks (Baskin and Baskin, 1998, 2004). GA_3 treatment has been used to overcome low seed germinability in many plant species (Kırmızı et al., 2011; Güleryüz et al., 2011; Koyuncu, 2005; Białecka and Kępczyński, 2010), suggesting that the fresh seeds may be physiologically dormant. Such a dormancy mechanism may play an important role (together with temperature) in preventing premature germination during summer or early autumn, before conditions are suitable for plant growth (Bell et al., 1993). In our study, scarification of *Androsace villosa* seeds gave a low germination rate (Tab. 1), further indicating that its dormancy type is physiological. There are few investigations of the germination ecology of alpine plants, and the factors and mechanisms regulating germination in alpine habitats are poorly known (Baskin and Baskin, 1998; Kırmızı et al., 2010). Many arctic and alpine species have dormant

TABLE 2. Two-way ANOVA results for arcsine-transformed germination percentage and mean germination time (MGT) of *A. villosa* seeds in darkness and under a 12 h photoperiod [Mean germination percentage and MGT were analyzed for GA_3 × chilling interaction at α; 0.05 significance level]

Factor	Germination Percentage			MGT		
	df	F	P	*df*	F	P*
			Photoperiod			
Chilling	1	176,092	0.000	1	24,710	0.000
GA_3	3	93,139	0.000	3	38,968	0.000
Chilling x GA_3	3	1,087	0.383	3	86,923	0.000
Error	16			24		
			Darkness			
Chilling	1	97,424	0.000	1	130,086	0.000
GA_3	3	39,456	0.000	3	22,190	0.000
Chilling x GA_3	3	1,096	0.379	3	0,640	0.600
Error	16			24		

*significant difference between treatment series at $P < 0.05$

seeds exhibiting mainly physiological dormancy (PD) or, to a much smaller extent, physical dormancy (PY) (Baskin and Baskin, 1998, 2004). Despite earlier studies on alpine seed germination (Körner, 2003; Amen, 1966) there is still insufficient knowledge of the mechanisms underlying dormancy in alpine species (Baskin and Baskin, 1998; Gimenez-Benavides et al., 2005; Dar et al., 2009).

Darkness favored *A. villosa* seed germination (Tab. 1). Wesche et al. (2006) found that 26% of the seeds of *Androsace maxima*, which grows on Asian steppes, germinated under photoperiod conditions. *A. villosa* did not germinate under photoperiod conditions; this behavior has been described in other species (Martin et al., 1995; Nishitani and Masuzawa, 1996; Navarro and Guitián, 2003), and has been related to control of germination by phytochromes (Probert et al., 1985). Increased germination in the absence of light could favor seeds that fall into cracks or crevices in the rockface. Seeds that reach a rock crevice would have a greater chance of germinating, and would do so more rapidly (in view of the absence of light), and seedlings growing at such microsites would be subject to less herbivory and less competition from other plant species than seedlings growing in the soil (Navarro and Guitián, 2003). High germinability in darkness has also been reported in other species such as *Lysimachia minoricensis*, a species from the Primulaceae which is extinct in the wild (Rosello and Mayol, 2002).

Moist chilling significantly affected germination (Tab. 1) and increased the final germination percentage in darkness (Tab. 2). Moist chilling also significantly increased germination in two populations of *Primula modesta* (Shimono and Washitani, 2004). Such physiology is adaptive in habitats where seedlings emerge in other seasons (Grime et al., 1981; Washitani and Masuda, 1990; Baskin and Baskin, 1998). Germinability of *Androsacea maxima*, a species of the Central Asian steppes, is low (26%) with no treatment (Wesche et al., 2006). The dormancy pattern can be similar for closely related taxa (Karlsson and Milberg, 2007) but can differ substantially within a family even among co-occurring species (Karlsson et al., 2008).

Studies of many species have shown that seed weight and/or size very often have significant effects on the final germination rate, seedling survival and seedling growth, and even on resistance to intraspecific or interspecific competition (Navarro and Guitián, 2003). A general correlation between seed size and the light requirement for germination has been suggested (e.g., Grime et al., 1981; Karlsson et al., 2008). Our results support that hypothesis: *A. villosa* has relatively heavy seeds and can germinate in the absence of light. Seed weight-related germinability in darkness could be an adaptation to poor insolation of the rocky substrate in its habitat.

The habitat of *A. villosa* is subjected to intense overgrazing and recreational activities in the spring and summer (Arslan et al. 1999; Güleryüz et al. 1998; 2005). Application of 100–250 ppm GA_3 combined with moist chilling is an effective way to increase its germination percentage. Our data on *A. villosa* germination should prove useful in developing strategies for restoring and conserving this and other species in alpine ecosystems such as Uludağ Mountain.

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