A comparative study of germination strategies of two species of genus *Allium* sect. *Allium*

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ABSTRACT

Germination experiments were performed with seeds of two species of genus *Allium* section *Allium,* a rare and endangered species *A. pyrenaicum* and a common *A. sphaerocephalon*. Different pre-treatments and a photoperiod of 24 h darkness were applied in order to simulate different germination conditions. Both species showed a high percentage of viable seeds a part of which were dormant. An elevate percentage of dormant seeds could be caused by a later collection time. Low altitude populations had more mortality than the others, possibly caused by the hard summer conditions during flowering and fruiting time. Comparisons between dates of species coexistence localities only show inter-population variability and it could be caused by the detected dormancy. Darkness accelerates germination, possibly for elongation radicle stimulation. Heat-shock pre-treatments decreased germination time in seeds from localities where fire is a probable event. The rarity of *A. pyrenaicum* not seems to be caused by restricted germination requirements but is attributable to distinct habitat preferences, related to his altitudinal range of distribution.

INTRODUCTION

Seeds are important structures to maintain populations of flowering plants and in natural environments germination is often restricted to locations that meet specific environmental conditions (Vandelook *et al.*, 2008). Although it happens it is possible that seeds do not germinate, because many species present dormancy mechanisms that avoid the germination under bad conditions (Fenner, 1993). This may be broken as a result of the exposure of the seed to a single factor at the required intensity for an appropriate period of time (Bradbeer, 1988). Dormancy is a barrier to the utilisation of many native plants in rehabilitation and revegetation programs (Cochrane & Probert, 2006), so the study of seed germination of endangered species is vital for conservation strategies (Fuentes & Estrelles, 2005; Lorite *et al.*, 2007).

To obtain an important quantity of information, many studies compare attributes of rare vascular plants with others common and closely phylogeny, morphology and phenology related (Bevill & Louda, 1999). In this work two taxa of genus *Allium* section *Allium*, *A. pyrenaicum* Costa & Vayreda in Costa and *A. sphaerocephalon* L. subsp. *sphaerocephalon* (Mathew, 1996; Kriminńska *et al.*, 2008) have been studied. The first taxon is endemic, distributed only in C & E Pyrenees (Pastor & Valdes, 1983) where lives in rocky places between (460)1000-1400 meters. It is an endangered and protected species (*Decret 172/2008, de 26 d'agost, de creació del Catàleg de la flora amenaçada de Catalunya* and *Orden de 4 de marzo de 2004 de modificación del Catálogo de la flora amenazada de Aragón*) with a surveying program (Oliver, 2008). The other taxon is widespread in Europe, except Scandinavia, and lives from sea level to 1550 meters. In Holland is produced commercially for ornamental purposes, but his natural forms live in dry stony or rocky slopes, waste places, cultivated fields, roadsides, beaches, scrublands (Mathew, 1996).

The objective of this study is determining the germination responses in order to detect the existence of inter-specific and inter-population differences in this two *Allium* species.

MATERIALS AND METHODS

Plant material

We collected at least ten umbels with mature capsules of two species, *A. pyrenaicum* (Apyr) and *A. sphaerocephalon* (Asph), between July and September 2007. These samples were collected in four sites of NE Iberian Peninsula along the altitudinal range of two species (Table I). Samples were then placed into a glasshouse for 2-3 weeks in order to obtain completely dried fruits (post-maturation). Cleaned seeds from each location were then mixed (Specht & Keller, 1997) and kept in glass vials with silica (1:1) (Bacchetta *et al.*, 2006) in room temperature (20ºC) until needed.

Table I. Origin of samples, coordinates, altitude situation, taxon, number of umbels and collection date.

Site	Coordinates	Altitude (m)	Taxon	N	Date (dd/mm/yy)
Santuari de Cabrera [CAB]	2º24'31.86"E 42º4'30.57"N	1300	Apyr	11	08/08/07
			Asph	15	24/08/07
Puig Llandrics [PLL]	2º22'56.63"E 42º9'55.12"N	1100	Apyr	15	21/09/07
			Asph	11	
Santa Magdalena de Terradas [STM]	2º49'44.81"E 42º19'31.76"N	500	Asph	10	20/07/07
Massís del Montgrí [MTG]	3º8'51.26"E 42º3'35.72"N	175	Asph	10	25/07/07

Germination experiment

In order to simulate different seed germination conditions four pre-treatments (Table II) and a photoperiod of 24h darkness (Narbona *et al.*, 2006), obtained covering each Petri dishes with two aluminium foil layers, were applied. Experiments were performed using 55 x 14 mm Petri dishes with one filter paper (ALRM13054252, Filter Anoia, Spain) disc inside, which was previously sterilised at 120ºC during 20 min. Each dish contained 25 seeds, and 4 replicates for pre-treatment and control were made. At the sowing moment 2 ml of deionised water were added to each dish using a micropipette, and the dishes were randomly placed on shelves in a germination chamber (PGA-180). Germination conditions were: 16ºC (optimal temperature) and a photoperiod of 12 h light/12 h darkness (Specht & Keller, 1997).

Petri dishes were checked every 3 days, during a period of 46 days, and rearranged randomly. When checked, germinated seeds were counted and removed, in the case of darkness dishes under green light. A seed was considered germinated when the radicle penetrated the seed coat and was observed (radicle protrusion) (Salvador & Lloret, 1995; Albert *et al.*, 2002; Lorite *et al.*, 2007). Finally, additional water (0.25-0.5 ml) was added if necessary.

Table II. Simulated germination conditions and pre-treatments applied.

Germination conditions	Pre-treatments
Spring germination after winter (cold stratification)	17-39 days at a temperature of $+ 4 \pm 0.6ºC$ [WINT]
Fast fire of low intensity	$+100 \pm 5ºC$ during 1 minute [FIRE1]
Slowly fire of low intensity	$+100 \pm 5ºC$ during 5 minutes [FIRE2]
Intense fast fire	$+120 \pm 5ºC$ during 1 minute [FIRE3]

Viability

At the end of the germination experiment, tetrazolium tests (using 5% dissolution of 2,3,5-triphenyltetrazolium chloride) were performed to check the viability of ungerminated seeds (Moore, 1985; Specht & Keller, 1997; Narbona *et al.,* 2006).

Data analysis

Different variables were calculated: germinability or percentage of germinating viable seeds (G'), mean length of incubation time in days (MLIT) and, only for controls, percentage of dead seeds or mortality (M), percentage of dormant seeds or dormancy (D) and percentage of germinated seeds (G) (Ranal & Garcia de Santana, 2006; Karlsson & Milberg, 2007). MLIT data were arcsine square root transformed and G' data were naperian logarithmic transformed. Differences between G' and MLIT were tested using a two-factor ANOVA and pre-treatments and darkness effects were tested (excluding *A. sphaerocephalon* dates from Puig Llandrics) using a single-factor ANOVA and a post-hoc Tukey HSD tests (Narbona *et al.,* 2006). All of data analyses were performed with SPSS 1.5.

RESULTS

Seed viability

All the studied populations have a high percentage of viable seeds (G+D) but in all cases a part of them have not germinated and are dormant (Figure 1). This portion of seeds is important in Puig Llandrics both in *A. pyrenaicum* as in *A. sphaerocephalon*. In Santa Magdalena the percentage of death seeds (D) is the biggest of all the studied populations, followed by Montgrí.
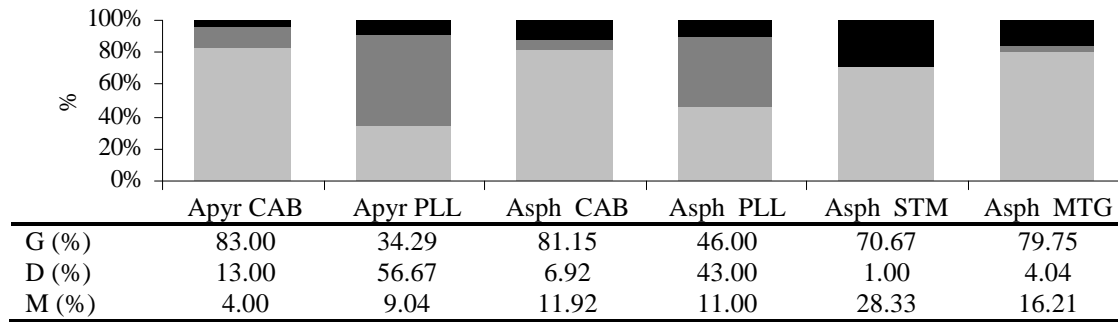
	Apyr CAB	Apyr PLL	Asph CAB	Asph PLL	Asph STM	Asph MTG
G (%)	83.00	34.29	81.15	46.00	70.67	79.75
D (%)	13.00	56.67	6.92	43.00	1.00	4.04
M (%)	4.00	9.04	11.92	11.00	28.33	16.21

Figure 1. Percentage of seed germination (G) ▨, percentage of seed dormancy (D) ▨ and percentage of seed mortality (M) ■ of control Petri dishes of all studied populations and species.

Inter-population and intra-specific variability

Comparisons of percentage of germinating viable seeds and mean length of incubation time from Cabrera and Puig Llandrics show no significant differences between studied *Allium* species. Also, exist significant differences between sites but not are related to species (Table III). The germinability in Cabrera is 86.57% and 91.96%, and in Puig Llandrics is 37.78% and 51.77% for *A. pyrenaicum* and *A. sphaerocephalon* respectively (Figure 2). The mean germination time in Cabrera is 15.72 and 15.35 days, and in Puig Llandrics is 18.10 and 18.5 days for *A. pyrenaicum* and *A. sphaerocephalon* respectively. Therefore, Cabrera seeds show higher germinability than those from Puig Llandrics and require less incubation time.

Table III. ANOVA results comparing percentage of germinating viable seeds (G') and mean length of incubation time (MLIT) between species and sites. *p<0.05

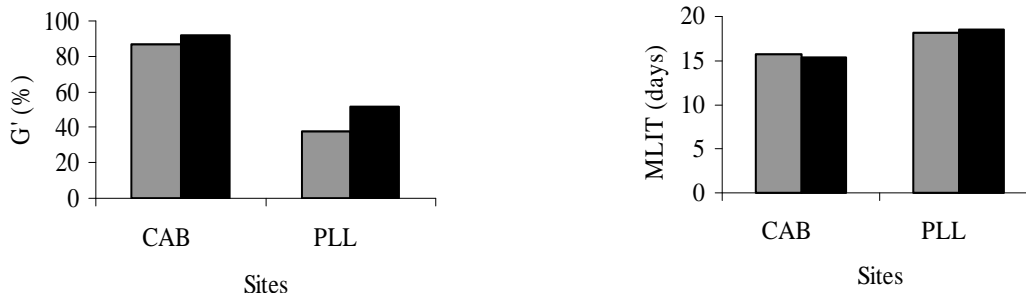|	df	G' (%)			MLIT (days)		
		MS	F	p	MS	F	p
Species	1	0.630	2.704	0.126	2.15E-6	0.000	0.992
Site	1	1.179	50.287	0.000*	0.106	5.464	0.038*
Site * Species	1	0.001	0.052	0.824	0.003	0.179	0.680
Error	12	0.281			0.233		

Figure 2. Dates of percentage of viable seed germination (G') and mean length of incubation time (MLIT) of *A. pyrenaicum* ▨ and *A. sphaerocephalon* ■.

Pre-treatments and darkness effect

In *A. pyrenaicum* exist significant differences between sites and pre-treatments for G' (F=255.433, p=0.000; F=7.492, p=0.000) and for MLIT (F=31.432, p=0.000; F=10.296, p=0.000), but only in the case of MLIT the site differences are caused by pre-treatments (F=3.891, p=0.006). In *A. sphaerocephalon* exist significant differences between pre-treatments and darkness for G' (F=3.092, p=0.540; F=2.528, p=0.040) and for MLIT (F=2.457, p=0.950; F=23.215, p=0.000), but pre-treatments have the same effect in all the sites (F=1.141, p=0.351).

Respects to control, any pre-treatment and darkness incubation have produced a negative effect in germination (Table IV). The effect of darkness incubation has been significant different for MLIT in *A. sphaerocephalon* from Cabrera (10.26 days ± 0.76 SE), Santa Magdalena (11.72 days ± 1.44 SE) and Montgrí (10.50 days ± 2.02 SE), reducing it. In case of *A. pyrenaicum* from Cabrera, both variables have shown significant differences, one increasing germinability (100.00 % ± 0.000 SE) and the other reducing incubation time (9.05 days ± 0.24 SE). Another pre-treatment that reduces this time

3

is which simulate a low intensity fast fire, it is statistically different in Santa Magdalena (12.45 days ± 0.76 SE) and Montgrí (13.40 days ± 1.48 SE). Finally, significant differences have occurred in the simulation of a slowly low fire of Montgrí (17.61 days ± 0.84 SE).

Table IV. Percentage of germinating viable seeds (G') and mean length of incubation time (MLIT) of *A. pyrenaicum* and *A. sphaerocephalon* from studied sites and the significance level of post-hoc Tukey HSD test. Asph dates from Puig Llandrics have been excluded.

Sp	Sites	Var.	ANOVA p	Tukey HSD					
				CONTROL	DARK	WINT	FIRE1	FIRE2	FIRE3
Apyr	CAB	G'	0.001	86.57±11.86	*	n.s.	n.s.	n.s.	n.s.
		MLIT	0.000	15.72±1.47	*	n.s.	n.s.	n.s.	n.s.
	PLL	G'	0.180	37.78±7.28	n.s.	n.s.	n.s.	n.s.	n.s.
		MLIT	0.206	18.10±2.97	n.s.	n.s.	n.s.	n.s.	n.s.
Asph	CAB	G'	0.213	91.96±8.10	n.s.	n.s.	n.s.	n.s.	n.s.
		MLIT	0.000	15.35±2.21	*	n.s.	n.s.	n.s.	n.s.
	STM	G'	0.190	98.53±2.94	n.s.	n.s.	n.s.	n.s.	n.s.
		MLIT	0.000	15.00±2.45	*	n.s.	*	n.s.	n.s.
	MTG	G'	0.685	95.17±0.30	n.s.	n.s.	n.s.	n.s.	n.s.
		MLIT	0.000	14.13±1.47	*	n.s.	*	*	n.s.

Means ± standard errors are represented. n.s. = not significant. *p<0.05

DISCUSSION

Viability results show that both, *A. pyrenaicum* and *A. sphaerocephalon*, present various percentages of dormancy in the different populations (Figure 1). The highest percentage of dormant seeds in both species corresponds to Puig Llandrics. Dormancy permits, generally, seeds to remain for long periods in the soil, generating a seed bank (Fenner, 1993; Bradbeer, 1988). So the fact that all samples, except Puig Llandrics, were collected between July and August and matured in laboratory conditions (post-maturation) suggests the influence of collection time. Puig Llandrics umbels were collected at the end of September and seeds maturate on mother plant, so it is possible that they enter in a primary dormancy state (Bradbeer, 1988; Fenner, 1993; Baskin & Baskin, 2004). If it is correct, collection time could influence in the posterior requirements to induce the germination. In order to prove this, in next years, germination experiments have to be performed with samples of the same populations collected in two different moments, at the beginning and middle (July-August) and at the end (September) of summer. In the other hand, the high percentage of death seeds corresponds to low altitude populations, Santa Magdalena and Montgrí, and it could be explained for the hard summer conditions during flowering and fruiting time.

Inter-population variability observed in G' and MLIT of Puig Llandrics and Cabrera (Table III; Figure 2) have showed that seeds from first site germinate in minor percentage and in more time than in Cabrera, possibly caused by dormancy. Therefore, if dormancy really were caused by the collection moment and not were exclusively of this site, inter-population variability would not exist.

Except in populations with an important dormancy, G' is high (Table IV). Darkness incubation accelerates germination, possibly because conditions are more favourable fore radicle development because of elongation stimulation (Azcón-Bieto & Talon, 1999). Fire pre-treatments also modify MLIT, increasing it in the case of a fast and low intensity fire and reducing it in the case of a slowly low intensity fire. This effect is only present in seeds from localities where fire is a probable event, and this response is related to a fast recruitment in rich germinant species environments (Salvador *et Lloret*, 1995).

In conclusion, *A. pyrenaicum* seeds could germinate in a wide diversity of conditions, after different types of fire, after a winter temperatures and in darkness conditions. Therefore, the rarity of this species is not caused by restricted germination requirements but is attributable to distinct habitat preferences (Vandelook *et al.*, 2008) related to his altitudinal range of distribution. So, his seeds could be used in conservation programs but future studies have to be focused in discover which factors induces dormancy.

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