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

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INTRODUCTION

Seeds are important structures to maintain populations of flowering plants, but dormancy and germination requirements influence his utilisation in rehabilitation and revegetation programs. In order to understand them many studies compare rare species with other common and closely related species. In this study germination experiments were performed with seeds of two species of genus *Allium* section *Allium*.

OBJECTIVES

Determining the germination responses in order to detect the existence of inter-specific and inter-population differences in the two studied species.

MATERIAL AND METHODS

Species: ***Allium pyrenaicum*** Costa & Vayreda is endemic of C & E Pyrenees (Pastor & Valdes, 1983), live in rocky places between (460)1000-1400 m, endangered, protected and with a surveying program (Oliver, 2008) (Fig. 1A).

Allium sphaerocephalon L. subsp. ***sphaerocephalon*** is common species, widespread in Europe, except Scandinavia, since sea level to 3000 m, in dry stony or rocky slopes, waste places, cultivated fields, roadsides, beaches, scrublands (Fig. 1B).

A. pyrenaicum Costa & Vayreda

A. sphaerocephalon L.

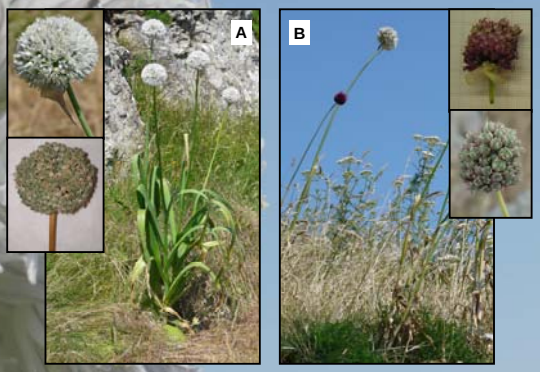


Fig. 1. Studied species.

Fig. 2. Situation of studied populations

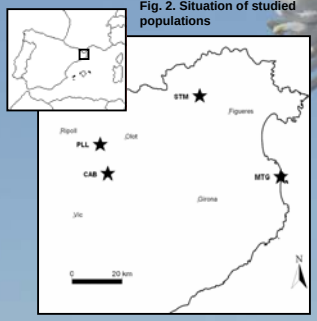


Table I. Origin of samples, coordinates, altitude situation, taxon, number of umbels and collection date

Site	Coordinates	Altitude (m)	Taxon	N	Date (dd/mm/yy)
Santuari de Cabrera [CAB]	2°24'31.86"E 42°4'30.57"N	1300	Apyr	11	08/08/07
			Asph	15	24/08/07
Puig Llandrics [PLL]	2°22'56.63"E 42°9'55.12"N	1100	Apyr	15	21/09/07
			Asph	11	
Santa Magdalena de Terradas [STM]	2°49'44.81"E 42°19'31.76"N	500	Asph	10	20/07/07
Massis del Montgrí [MTG]	3°8'51.26"E 42°3'35.72"N	175	Asph	10	25/07/07

Table II. Simulated germination conditions and pre-treatments

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

Plant material and germination experiment: Samples of mature capsules umbels from 4 localities (NE Iberian Peninsula) were collected (Fig. 2, Table I). 4 pre-treatments (Table II) and a photoperiod of 24h darkness (simulating burial) were applied (Narbona *et al.*, 2006). Seeds were sowed in Petri dishes with one filter paper disc inside (previously sterilised at 120ºC 20 min.). 25 seeds/dish and 4 replicates, 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). Dishes were checked every 3 days (during 46 days).
At the end of the germination experiment, tetrazolium tests were performed to check the viability of ungerminated seeds (Moore, 1985).

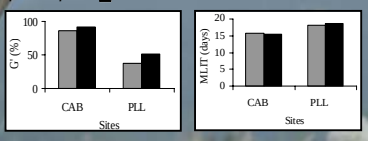
Data analysis: germinability or percentage of germinating viable seeds (G'), mean length of incubation time in days (MLIT) and, only for controls, percentage of death seeds (M), percentage of dormant seeds (D) and percentage of germinated seeds (G) (Ranal & Garcia de Santana, 2006) were calculated. Two-factor ANOVA, single-factor ANOVA and post-hoc Tukey HSD tests were applied using SPSS 1.5.

RESULTS

Seed viability: all studied populations have a high percentage of viable seeds (G+D) and, in all cases, a part of this viable seeds are dormant (Fig. 3). This portion of seeds is important in Puig Llandrics for both species. Santa Magdalena and Montgrí are the populations with the most important mortality (M).

Inter-population and intra-specific variability: G' and MLIT between Cabrera and Puig Llandrics show no significant species differences (F=2.704, p=0.126; F= 0.000, p=0.992). Significant differences have been found between sites (F=50.287, p=0.000; F= 5.464, p=0.038) but are not related to species (F=0.052, p=0.824; F= 0.179, p=0.680). Seeds from Cabrera has a major germinability in less incubation time (Fig. 4).

Fig. 4. Dates of percentage of viable seed germination (G') and mean length of incubation time (MLIT) of *A. pyrenaicum* ▒ and *A. sphaerocephalon* ■.



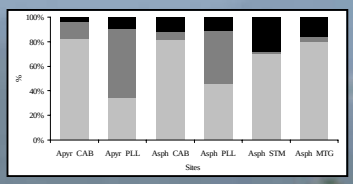


Fig. 3. Percentage of seed germination (G) ▒, percentage of seed dormancy (D) ▒ and percentage of seed mortality (M) ■ of control Petri dishes of all studied populations and species.

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 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. The effect of darkness incubation has been significant different for MLIT in *A. sphaerocephalon* from Cabrera, Santa Magdalena and Montgrí, reducing it. In case of *A. pyrenaicum* from Cabrera, both variables have shown significant differences, one increasing germinability and the other reducing incubation time. Another pre-treatment that reduces this time is which simulate a low intensity fast fire, it is statistically different in Santa Magdalena and Montgrí. Finally, significant differences occur in the simulation of a slowly low fire of Montgrí.

Table III. Percentage of germinating viable seeds (G') and mean length of incubation time (MLIT) of Apyr and Asph 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	Tukey HSD				
			p	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

Both studied species present various percentages of dormancy in the different populations and highest values, in both species, correspond to Puig Llandrics (Fig. 1). All samples, except Puig Llandrics umbels, were collected between July and August and maturated in laboratory conditions (post-maturation) and no on mother plant. This suggests an influence of collection time, that causes the entrance in a primary dormancy state (Bradbeer, 1988; Fenner, 1993; Baskin & Baskin, 2004). The high mortality corresponds to low altitude populations, Santa Magdalena and Montgrí, and it could be explained for the hard summer conditions during flowering and fruiting time.
Only inter-population variability between Cabrera and Puig Llandrics could be caused by dormancy.
Except in populations with an important dormancy, G' is high. 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 FIRE1 and reducing it in the case of FIRE2. 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.

aaA. pyrenaicum seeds could germinate in a wide diversity of conditions. Therefore, his rarity 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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