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Inter-population variation in germination characteristics of *Plantago lanceolata* seeds: effects of temperature, osmotic stress and salinity

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Summary. *Plantago lanceolata* is a perennial herbaceous plant widely distributed throughout the Mediterranean region. Variation in germination requirements and tolerance to osmotic stress and salinity were tested using seeds from two wild populations. Germination experiments were conducted under controlled conditions at constant (15, 20 and 25ºC) and alternating (20/10 and 25/15ºC) temperatures, and under a 16 h/8 h light/dark photoperiod. Seeds of both populations were non-dormant and incubation temperature significantly affected the final germination percentage and germination rate. Germination percentages ranged from 27 to 85% for the different temperatures and populations. Significant inter-population variation was found for the germination percentages reached at alternate temperature regimes. To determine the effect of osmotic stress and salinity, four polyethylene glycol (PEG 6000: 10, 20, 30 and 40% w/v) and four NaCl concentrations (50, 100, 200 and 300 mmol·L⁻¹) were tested. Seed germination significantly decreased with increasing in PEG and NaCl concentration. Seed germination recovery when they were transferred to distilled water after PEG or salinity treatments was variable depending on population, suggesting that *P. lanceolata* seeds are moderately tolerant to osmotic and salt stresses at germination stage.
Keywords: Inter-population variability; intra-specific variability; *Plantago lanceolata*; seed germination.

Variación interpoblacional en las características germinativas de las semillas de *Plantago lanceolata*: efectos de la temperatura, estrés osmótico y salinidad

Resumen. *Plantago lanceolata* es una planta herbácea perenne ampliamente distribuida por toda la región Mediterránea. Se estudió la variación en los requerimientos germinativos y la tolerancia al estrés osmótico y la salinidad de las semillas de dos poblaciones silvestres. Los ensayos de germinación se realizaron en condiciones controladas, a temperaturas constantes (15, 20 y 25 ºC) y alternas (20/10 y 25/15 ºC) y bajo un fotoperiodo de 16 h/8 h luz/oscuridad. Las semillas de ambas poblaciones fueron no durmientes y la temperatura de incubación afectó significativamente al porcentaje final de germinación y a la velocidad de germinación. Los porcentajes de germinación variaron entre un 27 y un 85%, dependiendo de la temperatura y población. Se encontró una variación interpoblacional significativa para los porcentajes de germinación alcanzados a los regímenes de temperaturas alternas. Para determinar el efecto del estrés osmótico y de la salinidad, se ensayaron cuatro concentraciones de polietilenglicol (PEG 6000: 10, 20, 30 y 40% w/v) y de NaCl (50, 100, 200 y 300 mmol·L⁻¹). La germinación de las semillas disminuyó significativamente con el incremento de la concentración de PEG y ClNa. La recuperación de la germinación cuando las semillas se transfirieron a agua destilada después de estar bajo los tratamientos de PEG o salinidad fue variable, dependiendo de la población, lo que sugiere que las semillas de *P. lanceolata* son moderadamente tolerantes a los estreses osmótico y salino en la etapa germinativa.
Palabras clave: Germinación de semillas; *Plantago lanceolata*; variabilidad interpoblacional; variabilidad intraespecífica.

Introduction

Seed propagation strategies of Mediterranean plant species is key information for ecosystem conservation, especially in the present context of climate change (Cochrane, 2016). Seed germination is subject to strong selection pressure and, consequently, is likely to be highly sensitive to climatic changes (Walck & *al.*, 2011). Moreover, populations from similar habitats may have different germination responses to temperature and osmotic stress. These differences can arise from environmental variation during seed maturation and the effect of maternal genotype (Fenner & Tompson,

2005). Indeed, inter-population variation in germination characteristics is one of most important survival strategies for species growing under unpredictable environmental conditions, and is a common strategy in Mediterranean wild species (Kigel, 1995; Cruz & *al.*, 2003; Qaderi & *al.*, 2005; Pérez-García, 2009; Baskin & Baskin, 2014; Martínez-Fernández & *al.*, 2014). In this scenario, the source (origin) of seed samples should always be taken into account when defining plant conservation protocols, especially for *ex-situ* germplasm conservation of wild species with a high degree of physiological variability. A better understanding of plasticity in germination response among populations is, then, of high importance to both

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in-situ and *ex-situ* plant conservation (Pérez-García & *al.*, 2003; Hudson & *al.*, 2015).

The ability of seeds to germinate fast at low water potentials is an advantageous adaptation for species colonising water-stressed such as Mediterranean environments (Schütz & *al.*, 2002; Bochet & *al.*, 2007). There is a wide range of variability in salt tolerance among species (Khan & Gulzar, 2003; Khan & Gul, 2006), but it has been shown in many of them that an increase in salt concentration usually delays and reduces seed germination (Zia & Khan, 2004; El-Keblawy & *al.*, 2007; El-Keblawy & Al-Shamsi, 2008; Tlig & *al.*, 2008; Guma & *al.*, 2010). However, seeds of some species that are unable to germinate at high saline concentrations, might recover the ability to germinate when salinity levels decrease (Pujol & *al.*, 2000; Zia & Khan, 2008). Yet, for most species, little is known about the inter-population variability of the germination response to osmotic stress.

Several *Plantago* species are considered successful colonizers on road embankments, and are used in restoration programs and in revegetation projects in semiarid areas (Tormo & *al.*, 2006). Furthermore, some *Plantago* species have shown remarkable inter-population variability in seed germination. According to Puech & *al.* (1998) germination performance of *P. albicans* seeds is correlated with its biogeographical distribution. Braza & *al.* (2010) have shown the versatility of *P. coronopus* plants to combine fecundity traits in a flexible way, in order to increase fitness at each of the many possible habitats they occupy over heterogeneous environments. Bochet & *al.* (2007) found that *P. albicans* seeds reached high germination percentages (close to 90%) at a water potential of -0.05 MPa, and no germination occurred at -1.50 MPa. Veiga-Barbosa & Pérez-García (2014) have indicated that the presence of mucilage on *P. albicans* seeds significantly increased seed germination and seeds were highly tolerant to osmotic stress and salinity. Therefore, it is of great interest to study the effects of inter-population variability on germination requirements of *Plantago* species.

Plantago lanceolata L. (Plantaginaceae) is a perennial herbaceous plant widely distributed throughout the Mediterranean region (Pedrol, 2009). Generally, it grows on dry soils and sunny areas (Pedrol, 2009) and colonise arid and semiarid environments of the Mediterranean region (Puech & *al.*, 1998). However, little is known on the requirements for germination of *P. lanceolata*.

We hypothesised that *P. lanceolata* seeds might present variation between populations in germination requirements and in their tolerance to osmotic stress and salinity, making this species a good model for the study of inter-population variability of germination requirements under water-stressed environments. The specific objectives of this study were to: (1) determine the effect of different temperature regimes on seed germination of two wild populations of *P. lanceolata*; (2) determine the effect of osmotic potential and salinity on germination of seeds from these populations; and (3) establish if variability among populations exists in the germination requirements and tolerance to osmotic and saline stresses at the germination stage.

Material and Methods

Seed collection

Ripe seeds of *P. lanceolata* were collected in July 2014 from two wild populations (PL1 and PL2) growing in Central Spain (Table 1). After collection, seeds were manually cleaned, kept in paper bags, and stored dry under laboratory conditions (approximately at 23ºC in the dark, 35% relative humidity) until the start of the trials in November 2014. Empty seeds, seeds with symptoms of decay or seeds clearly undeveloped were excluded from the laboratory experiments.

Table 1. Habitat, collection site and climatic parameters of two *Plantago lanceolata* populations. Abbreviations are: total precipitation per year (P annual), precipitation June-August (P summer), number of drought months (D), annual mean temperature (T), mean temperature of the warmest month (Tw), mean temperature of the coolest month (Tc), annual mean of daily thermal oscillation (Osc), number of frost months (F). Universal Transverse Mercator (UTM). Climatic data from Spanish State Meteorological Agency (AEMET). All seeds were collected in July 2014.

Pop. code	Collection site (Province)	Habitat	UTM	Altitude (m)	P annual (mm)	P summer (mm)	D (months)	T (ºC)	T_w (ºC)	T_c (ºC)	Osc (ºC)	F (months)
					Climatic parameters						
PL1	Alcocer (Guadalajara)	Wasteland over calcareous stony soil	30T0535142 4482707	805	410	43	3.5	13.4	23.6	4.8	15.2	1.8
PL2	Guadalix de la Sierra (Madrid)	Road embankment on calcareous substrate	30T0442495 4515675	876	545	53	3.3	13.3	23.7	4.8	9.3	0.7

Germination tests

Seeds were tested for germination at different temperature regimes with a 16-h light photoperiod (provided by cool white fluorescent tubes with an irradiance of 35 µmol·m^2·s^{-1}). Four replicates of 25 seeds each were tested for germination on top of two sheets of filter paper (previously moistened with 3.5 mL distilled water) in 7-cm-diameter glass Petri dishes. Filter papers were rewetted regularly with distilled water as required. Dishes were checked three times a week over a total 15-day test period. Seeds were considered germinated on emergence

of the radicle from the seed coat and germinated seeds were counted and removed. In all trials, seeds that had not germinated at the end of the incubation period were opened to check if the seed was empty or if the embryo looked healthy. Empty seeds and seeds with not healthy embryos were excluded from calculation of final germination percentage (Baskin & Baskin, 2014). The number of excluded seeds was, in all the Petri dishes, always less than 5% of the studied total seeds.

Effect of temperature regimes on seed germination

The aim of these trials was to determine optimal temperature for radicle emergence. Seeds were tested for germination at different constant temperatures (15, 20 and 25ºC), and at alternate temperature regimes of 20/10 and 25/15ºC (the highest temperature for 16 h in light and the lowest one for 8 h in dark).

Effect of osmotic stress on seed germination

Polyethylene glycol (PEG) 6000 was used to check the effect of osmotic stress on seed germination. Seeds were germinated in 10, 20, 30 and 40% (w/v) PEG 6000 solutions (-0.15, -0.49, -1.03, -1.76 MPa, respectively). Seeds germinated in distilled water were used as a control. Seeds were tested on top of two sheets of filter papers moistened with 3.5 mL of either distilled water or PEG solution. It must be taken into account that filter paper can slightly reduce the osmotic potential of the PEG solution (Hardegree & Emmerich, 1990; Emmerich & Hardegree, 1991). Petri dishes were sealed with Parafilm to minimize evaporation of water from the solutions. Seeds were germinated at 20ºC for a period of 15 days. This incubation temperature was chosen due to the good results reached in the previous germination trials.

For the recovery germination trials, non-germinated seeds from the PEG incubation tests were rinsed five times with distilled water, and then incubated for additional 30 days on top of two sheets of filter papers moistened with 3.5 mL distilled water at the same incubation temperature (20ºC). Recovery percentage (RP) was calculated by the following formula: RP = $(a-b)/(c-b)$ x 100, where a is the number of seeds germinated during the PEG treatment plus those that germinated after the 30-day period in distilled water, b is the number of seeds germinated during the PEG treatment and c is the total number of seed tested (Gul & Webber, 1999). The RP value will be between 0 and 100.

Effect of salinity on seed germination

To evaluate the effect of salt stress on seed germination of *P. lanceolata*, seeds were germinated in 50, 100, 200 and 300 mmol·L^{-1} NaCl solutions (-0.25, -0.50, -0.99, -1.49 MPa respectively). Distilled water served as control treatment. Seeds were germinated on top of two

sheets of filter papers moistened with 3.5 mL of either distilled water or NaCl solution. As above, Petri dishes were sealed with Parafilm to reduce loss of water, and seeds were germinated at 20ºC for a period of 15 days.

Non-germinated seeds from the NaCl incubation tests were rinsed five times with distilled water, and then incubated for additional 30 days on top of two sheets of filter papers moistened with 3.5 mL distilled water at the same temperature regimes. RP values were calculated as previously detailed

Data analysis

For all experiments where different incubation temperatures were assayed, final germination percentage and mean germination time (MGT) were calculated. The latter was determined according to the following formula (Ellis & Roberts, 1981): MGT = $\Sigma DN / \Sigma N$; where D is the number of days counted from the date of sowing and N is the number of seeds germinated on day D. In all germination trials, the number of empty seeds in each replicate was always excluded when calculating the final germination percentage.

The values of final germination percentages were arcsine square-root transformed and then subjected to analysis of variance (ANOVA) using SPSS. A two-way factorial ANOVA was applied to test the effect of temperature regimes, and concentrations of PEG or NaCl on the final germination percentage. The statistical analysis of MGT values was also carried out with two-way factorial ANOVA. When ANOVA indicated a significant effect, a comparison of mean values was carried out through the least significant difference test (l.s.d.) at 0.05 level of probability.

Results

Effect of temperature regimes

Germination percentages ranged from 27 to 85%, depending on temperature and population (Table 2). The lowest germination percentages were obtained at 25ºC for PL1 and at 25ºC and 25/15ºC for PL2. The highest germination was reached at 20ºC in both populations.

Significant differences were found between the MGT values reached at the different temperature regimes (Table 3). The germination period was very short and ranged from 2.8 to 7.7 days depending on population and temperature. The highest germination rate (i.e. the lowest MGT value) for all two populations was recorded at 20ºC and the lowest at 25/15ºC for PL1 and at 20/10ºC for PL2 (Table 3).

Temperature and population factors had significant ($P<0.001$) effects on final germination percentage and the two-way interaction between both factors was not significant ($P=0.476$). Germination rate was significantly ($P<0.001$) affected by temperature and population and the interaction between both factors was also significant ($P=0.032$).

Table 2. Final germination percentage at different temperature regimes for two *Plantago lanceolata* populations. Mean values within a row followed by the same letters are not significantly different. Abbreviations are: $P^{(a)}$: For each population, significance level among the final germination percentages from temperatures; $P^{(b)}$: For each temperature, significance level among the final germination percentages from populations; ***$P<0.001$; **$P<0.01$; *$P<0.05$; ns, not significant.

| Population | Germination (% ± SE) | | | | | |
	15 °C	20 °C	25 °C	20/10 °C	25/15 °C	$P^{(a)}$
PL1	78 ± 3 c	85 ± 4 c	34 ± 6 a	64 ± 4 bc	46 ± 3 ab	***
PL2	71 ± 2 b	79 ± 4 b	27 ± 3 a	46 ± 5 a	27 ± 4 a	***
$P^{(b)}$	ns	ns	ns	**	*	---

Table 3. Mean germination time (MGT, mean ± SE) at different temperature regimes for two populations of *Plantago lanceolata*. Mean values within a row followed by the same letters are not significantly different. Abbreviations are: $P^{(a)}$: For each population, significance level among the MGT values from temperatures; $P^{(b)}$: For each temperature, significance level among MGT values from populations; ***$P<0.001$; **$P<0.01$; *$P<0.05$; ns, not significant.

| Population | MGT (days) | | | | | |
	15 °C	20 °C	25 °C	20/10 °C	25/15 °C	$P^{(a)}$
PL1	3.6 ± 0.5 ab	2.9 ± 0.1 a	6.4 ± 0.9 bc	6.6 ± 0.7 bc	7.8 ± 1.0 c	**
PL2	3.2 ± 0.0 ab	2.8 ± 0.1 a	3.3 ± 0.4 ab	5.9 ± 0.2 c	4.6 ± 0.3 bc	***
$P^{(b)}$	ns	ns	*	ns	*	---

Table 4. Germination percentage of *Plantago lanceolata* seeds from two populations (PL1 and PL2) after incubation at 20 °C in different PEG or NaCl concentrations for 15 days, and germination percentages (values shown in parenthesis) when non germinated seeds were incubated for other 30 days in distilled water (total incubation period of 45 days). For each treatment (PEG or NaCl), mean values within a column followed by the same uppercase letter are not significantly different from each other ($P>0.05$), and mean values within a row followed by the same lowercase letter are not significantly different ($P>0.05$).

| Treatment | Germination (% ± SE) | | Recovery (%, RP) | |
	PL1	PL2	PL1	PL2
PEG (% w/v, [osmostic potential, MPa])				
Control	84 ± 4 aC	80 ± 5 aC	---	---
10 [-0.15]	64 ± 5 aC (75 ± 1)	52 ± 6 aBC (59 ± 6)	30.5	14.6
20 [-0.49]	32 ± 6 aB (73 ± 5)	25 ± 5 aAB (54 ± 5)	60.3	38.7
30 [-1.03]	19 ± 3 aB (70 ± 4)	8 ± 3 aA (46 ± 5)	63.0	41.3
40 [-1.76]	0 aA (61 ± 3)	2 ± 2 aA (59 ± 7)	61.0	58.2
NaCl (mmol L^{-1}, [osmostic potential, MPa])				
Control	84 ± 4 aC	80 ± 5 aC	---	---
50 [-0.25]	45 ± 2 aB (77 ± 4)	43 ± 4 aB (51 ± 3)	58.2	14.0
100 [-0.50]	24 ± 5 aB (58 ±4)	32 ± 8 aB (39 ± 9)	44.7	10.3
200 [-0.99]	2 ± 1 aA (45 ± 5)	3 ± 2 aA (27 ± 7)	43.9	24.7
300 [-1.49]	0 A (47 ± 6)	0 A (23 ± 2)	47.0	23.0

Effect of PEG concentration

For both populations, the highest germination was obtained in distilled water (control seeds). Germination percentages of *P. lanceolata* seeds decreased with increasing PEG concentrations (Table 4), and in the case of 20%, 30% and 40% PEG solutions the differences were significant when compared to control seeds (Table 4). A proportion of *P. lanceolata* seeds that did not germinate after incubation in PEG solution were able to germinate when they were then incubated in distilled water (Table 4). Recovery percentages (RP) ranged from 15 to 63% depending on population and PEG concentration (Table 4). Besides, RP values of PL2 were lower than those of PL1 for all PEG tested concentrations.

Treatment (incubation in PEG solutions of different concentration) factor had significant ($P<0.001$) effects on final germination percentages. However, population factor and the two-way interaction between both factors (population x treatment) were not significant ($P=0.072$ and $P=0.408$, respectively).

Effect of NaCl concentration

The highest germination percentages were obtained in distilled water. The response to increasing NaCl concentrations was a reduction in the final germination percentage (Table 4). Significant ($P<0.05$) differences were found between the germination of control seeds and in all four tested NaCl concentrations (Table 4). Besides, the highest NaCl concentration inhibited germination.

A proportion of non-germinated, salt-treated seeds were able to germinate when they were transferred to distilled water. RP of *P. lanceolata* seeds ranged from 14 to 58% depending on population and NaCl concentration (Table 4). Besides, as in the above PEG trial, RP values of PL2 were lower than those of PL1 for all NaCl tested concentrations.

As occurs with the above PEG experiment, treatment (incubation in NaCl solutions of different concentration) factor had significant ($P<0.001$) effects on final germination percentages. Population factor and the two-way interaction between both factors were not significant ($P=0.908$ and $P=0.762$, respectively).

Discussion

It has been shown that the optimal germination temperature for *Plantago* seeds varies depending on species, and it ranges from 15 to 25ºC (Arnold, 1973; Blom, 1992; Zaady & *al.*, 1997; Fons & *al.*, 2008). Thus, in *P. algarbiensis* and *P. almogravensis* (Martins & *al.*, 2012) and in *P. ovata* (Hammouda & Bakr, 1969) best germination results were obtained at 15ºC. However, the highest seed germination of *P. albicans* (Puech & *al.*, 1998; Veiga-Barbosa & Pérez-García, 2014) and *P. major* (Pons & Van Der Toorn, 1988) was reached at 25ºC and 25/15ºC.

In the present study, seeds reached maximum germination percentages of 79% (PL2) and 85% (PL1) without any treatment and therefore they can be considered non-dormant seeds (according to Baskin and Baskin, 2014). Germination percentages were highly variable depending on the incubation temperature and population. As it occurs in several Mediterranean species (Galmés & *al.*, 2006; Chaneze & *al.*, 2010; Martínez-García & *al.*, 2012; Veiga-Barbosa & Pérez-García, 2014), *P. lanceolata* seeds are able to germinate in a wide range of temperatures.

For both populations of *P. lanceolata*, the highest germination percentages were obtained at 15 and 20ºC, and the lowest at 25ºC, thereby, it seems logical to predict that most *P. lanceolata* seeds from the two studied populations will germinate in late autumn and throughout winter, when temperatures are lower than 25ºC. However, inter-population variability could be detected for alternate germination temperatures; seeds from PL2 had lower germination than seeds from PL1 at the alternate temperatures studied. Seeds from PL1 seem to be better adapted to daily temperature oscillations, which is supported by the climatic data from the habitat (Table 1), showing that habitat of PL1 has a higher thermal oscillation (15.2ºC annual mean) than PL2 (9.3ºC).

In our study, the germination of *P. lanceolata* seeds was very fast at optimum temperatures (15 and 20ºC). However, variability between populations was detected in MGT. While less seeds from PL2 germinated at sub-optimal temperatures (25ºC) and alternate temperatures regimes (25/15ºC), germination was significantly faster than for PL1 seeds. Rapid germination increases the likelihood of rapid establishment in a habitat with intermittent precipitation and could be a useful character for plant species (Verdú & Traveset, 2005). In dry environments, a strategy of fast germination can ensure sufficient water availability to complete germination and achieve establishment (Montes-Recinas & *al.*, 2012).

There was a significant negative correlation between PEG concentration and the final germination percentage, i.e. seed germination decreased as PEG concentration increased. These decreases were no significant at concentrations lower than 20%, ceasing germination at 40% PEG. Tolerance for osmotic stress of *P. lanceolata* seeds from PL1 was always higher than those of PL2. Besides, these seeds showed a high recovery of germination when they were transferred from the tested PEG concentrations to distilled water. As above, the recovery percentages were always higher for PL1. These results are in accordance with the climatic parameters of both populations.

Similarly, seed germination of *P. lanceolata* decreased as saline concentration increased. Final germination percentages reached with saline solutions were significantly lower than germination percentages reached in distilled water. Moreover, seed germination was totally inhibited at the highest concentration. Although the germination percentages reached under the different NaCl concentrations were very similar for both populations, the recovery of germination was

always higher for seeds from PL1. Therefore PL1 seeds are more tolerant to salt stress than PL2 seeds.

It is possible to compare the effect of PEG and NaCl solutions on seed germination in those conditions with similar osmotic potential. PEG solutions of 20 and 30% w/v had a similar osmotic potential to NaCl solutions of 100 and 200 mmol·L^{-1}, respectively. Seeds showed similar germination percentages under similar osmotic potentials; however, the recovery percentage was higher when seeds had been submitted to the PEG treatment in comparison with the salt treatment. Therefore, despite what was indicated in other species (Lawlor, 1970; Macar, 2009), PEG did not have a permanent negative effect on *P. lanceolata* seed germination, at the studied concentrations.

The ability of species to germinate under osmotic stress could be highly useful under semiarid conditions, and pioneer species are usually able to germinate fast at low water potentials (Bochet & *al.*, 2007). Several studies have reported that salt stress negatively affected seed germination of some species (Khan & Gulzar, 2003; Zia & Khan, 2008; El-Keblawy & *al.*, 2007; El-Keblawy & Al-Shamsi, 2008; Mohammad Esmaeili & *al.*, 2009; Vallejo & *al.*, 2010; Veiga-Barbosa & Pérez-García, 2014). High saline concentrations can slow the rate of water uptake by seeds, and therefore inhibit germination and radicle elongation (Mohammad Esmaeili & *al.*, 2009; Yang & *al.*, 2010; Western, 2012). Our results indicate that *P. lanceolata* seeds are moderately tolerant to saline conditions when compared to other species (Khan & Gul, 2006; El-Keblawy & *al.*, 2007; El-Keblawy & Al-Shamsi, 2008; Zia & Khan, 2008; Guma & *al.*, 2010), and a proportion of them recovered their capacity germinate when transferred to distilled water after being incubated under high NaCl concentrations (200 and 300 mmol·L^{-1}).

In summary, seeds from both populations of *P. lanceolata* were non-dormant and temperature significantly affected the final germination percentage and germination rate. The highest germination percentages were reached at 20ºC. Inter-population variability on seed germination was found at alternate temperature regimes. Final germination percentage was significantly affected by the treatment factor (PEG and NaCl) but not by the population factor and neither of them by the interaction between both factors. For both tested populations, germination percentage decreased by increasing PEG and NaCl concentration. However, inter-population variability was found when recovery germination after a treatment of osmotic or salt stress was assessed. The production of seeds with different germination response is the mechanism by which many plant species adapt to changing environmental conditions, and is a common strategy in Mediterranean wild species (Pérez-García, 1993, 2009; Cochrane & *al.*, 2014). Our results indicate that the degree of salt tolerance shown by *P. lanceolata* seeds may be sufficient to allow germination at low levels of salinity. Finally, the origin of the plant material used in revegetation in Mediterranean environments must be strongly considered, while standardization of plant propagation protocols should take into account the intraspecific variation of Mediterranean species.

Acknowledgements

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