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Germination of mucilaginous seeds of *Plantago albicans* (Plantaginaceae): effects of temperature, light, pre-sowing treatments, osmotic stress and salinity

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Abstract. *Plantago albicans* L. (Plantaginaceae) is a perennial herbaceous plant widely distributed throughout the Mediterranean region. The germination requirements (under different controlled conditions of light and temperature, and after two pre-sowing treatments) and tolerance to osmotic stress (polyethylene glycol, PEG 6000) and salinity (NaCl) of *P. albicans* seeds were studied. Seeds were germinated under constant temperatures (5°C, 10°C, 15°C, 20°C, 25°C and 30°C) and alternating temperature regimes of 20/10°C and 25/15°C with a 16 h/8 h light/dark photoperiod. The outer layer of seeds become mucilaginous when wetted and the presence of mucilage on seeds significantly increased germination percentages at all temperatures tested. *P. albicans* seeds were non-dormant and temperature significantly affected germination percentages and germination rate (germination velocity expressed as mean germination time, MGT). The final germination percentages ranged from 34% to 89% for intact seeds (seeds with mucilage) and from 9% to 62% for demucilaged seeds, depending on the temperature. Temperatures of 25°C and 25/15°C gave the highest germination percentages. Light did not affect seed germination at both temperature regimes assayed (25°C and 25/15°C). Germination percentages of seeds soaked for 24 h in distilled water or in a gibberellic acid (GA_3) solution were not significantly higher than that of untreated seeds. In general, both the final germination percentage and germination rate were reduced by increasing salinity and PEG concentration. Seeds germinated in up to 35% PEG and 300 mmol·L^{-1} NaCl. Recovery of germination for seeds when transferred to distilled water after being in PEG or salinity treatments for 15 days was quite high, suggesting that *P. albicans* seeds are tolerant to osmotic and salt stresses.

Additional keywords: gibberellic acid, *Plantago*, polyethylene glycol, recovery germination, seed germination.

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Introduction

Seeds of many species produce mucilage (myxospermy) on imbibition of water, which can play a significant role in seed germination and dispersal (Yang *et al*. 2010; Western 2012). Development of mucilage by seeds after wetting can aid seed germination in heterogeneous environments (Kigel 1995; Gutterman and Shem-Tov 1997; Yang *et al*. 2010). Thus, the mucilage can ensure seed germination by protecting the seed against drying during germination, by retaining moisture and increasing the area of contact of seed with soil (Gutterman 2002; Western 2012). Seed mucilage can play an ecologically important role in seed germination under conditions of salt stress (Mott 1974; Yang *et al*. 2010; Sun *et al*. 2012; Western 2012). Besides, the mucilage can prevent further dispersal of the seeds by seed predators (ants) by adhering strongly to soil once the mucilage is dry (Gutterman and Shem-Tov 1997; Huang *et al*. 2000; Engelbrecht and García-Fayos 2012; Sun *et al*. 2012). The precise role of mucilage appears to be dependent on species and their environmental context (Thapliyal *et al*. 2008; Western 2012).

The ability of seeds to germinate fast at low water potentials is an advantageous adaptation for species colonising water-stressed environments (Bochet *et al*. 2007). It has been shown in a large number of species that an increase in salt concentration usually delays and reduces seed germination (Ungar 1978; Khan and Ungar 1984; Khan *et al*. 2001; Zia and Khan 2004; El-Keblawy *et al*. 2007; El-Keblawy and Al-Shamsi 2008; Tlig *et al*. 2008). However, there is a wide range of variability in salt tolerance among species (Khan and Gulzar 2003; Khan and Gul 2006). Moreover, many seeds that are unable to germinate at high saline concentrations might recover the ability to germinate when salinity decreases (Pujol *et al*. 2000; Baskin and Baskin 2001; Zia and Khan 2008).

Plantago albicans L. (Plantaginaceae) is a perennial herbaceous plant widely distributed throughout the Mediterranean region and south-western Asia to Iran, along a

wide altitudinal gradient of 0–1100 m asl. Because of its wide distribution, the habitat of *P. albicans* can be very heterogeneous (Puech *et al*. 1998). Thereby, it grows in wastelands, slopes and stony pastures, on dry and sun-exposed soils (Pedrol 2009). *P. albicans* colonises open, arid and semiarid environments of the Mediterranean region where saline soils are common (Puech *et al*. 1998).

As in other *Plantago* species (Western 2012), *P. albicans* seeds contain mucilage that can imbibe a large amount of water when wetted. Salt stress and low water availability are two frequent environmental stress factors that *P. albicans* encounters during seed germination. This species is considered a successful coloniser plant on road embankments, and it could be used in plant restoration programs and in revegetation projects in its natural range (Tormo *et al*. 2006). Therefore, it is of great interest to study the effects of osmotic and salt stresses on the seed germination of *P. albicans*.

According to Puech *et al*. (1998), germination performance of *P. albicans* seeds is correlated with its biogeographical distribution. Seeds of the populations of *P. albicans* from arid and desert environments germinate in successive waves, whereas seeds from the populations of moist regions germinate immediately (Puech *et al*. 1998). Bochet *et al*. (2007) found that *P. albicans* seeds reached a high germination percentage (close to 90%) at a water potential of –0.05 MPa, and no germination occurred at –1.50 MPa. However, little is known of the temperature and light requirements for germination of this species and about the role of mucilage in seed germination. To our knowledge, nothing is known about its tolerance to salinity.

We hypothesised that mucilage might affect the germination of *P. albicans* seeds. The specific aims of the present study were to determine (1) the effect of different temperature and light regimes on germination of *P. albicans* seeds with and without mucilage, (2) the effect of osmotic potential and salinity on germination of intact seeds (seeds with mucilage), and (3) the effect of soaking of seeds in distilled water or in a gibberellic acid (GA_3) solution on germination.

Materials and methods

Seed collection

Ripe seeds of *P. albicans* were collected in July and August 2012, from wild plants growing in semiarid environments of the Navarra region, north-eastern Spain. After collection, seeds were manually cleaned, kept in paper bags, and then stored dry under laboratory conditions (at ~23°C under darkness, 35% relative humidity, RH) until the start of the trials in November 2012. Visibly damaged seeds were excluded from the experiments.

Seed mass

To quantify mean seed mass, two replicates of 100 seeds each were randomly selected and weighed using an analytical balance with an accuracy of 0.0001 g. The same two lots of 100 seeds were imbibed with distilled water for 10 min and then reweighed. The increase in mass was calculated by subtracting the mass of imbibed seeds from that of the original seeds, and then the relative increase in mass was compared with the mass of the original seeds.

Mucilage removal

For removing the mucilage, seeds were first imbibed with distilled water for 10 min and then the mucilage formed was rubbed on filter paper according to Huang *et al*. (2008) and Yang *et al*. (2010). This treatment was continued after five further imbibition periods of 10 min each, until little more mucilage was formed on rewetting. Hereafter, seeds with and without mucilage are termed ‘intact seeds’ and ‘demucilaged seeds’, respectively.

Effect of temperature and light regimes on seed germination

The aim of these trials was to determine the optimal temperature and light requirements for radicle emergence. Intact and demucilaged seeds were tested for germination at different constant temperatures (5°C, 10°C, 15°C, 20°C, 25°C, 30°C), with a 16-h light photoperiod (provided by cool white fluorescent tubes with an irradiance of 35 $\mu mol \cdot m^2 \cdot s^{-1}$), and the alternate temperature regimes of 20/10°C and 25/15°C (the highest temperature for 16 h in light and the lowest one for 8 h in dark). In each trial, four replicates of 25 seeds each were tested for germination on top of two sheets of filter paper (previously moistened with 3.5 mL of 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 and germinated seeds were counted and removed. For the lowest incubation temperatures assayed (5°C, 10°C and 20/10°C), the germination period was extended up to 30 days. Seeds were considered germinated on emergence of the radicle from the seed coat. In all trials, seeds that had not germinated at the end of the incubation period were opened to determine whether the seed was empty. If so, they were excluded from calculation of final germination percentages (Baskin and Baskin 2001). The number of empty seeds was always equal or less than 5% of the total seeds.

Non-germinated intact seeds from the 15-day temperature trials (30 days for 5°C, 10°C and 20/10°C) were transferred to 25°C and then incubated for another 15-day period. Recovery percentage (RP) was calculated by the following formula: $RP = (a - b/c - b) \times 100$, where a is the number of seeds germinated at the different temperature regimes assayed (except 25°C) after a 15-day period plus those that recovered to germination at 25°C after another 15-day period, b is the number of seeds germinated at the different temperature regimes and c is the total number of seeds tested (Gul and Webber 1999). Initial germination was recorded as $(b/c) \times 100$ and final germination as $(a/c) \times 100$ (Gul and Webber 1999; Yang *et al*. 2010).

For constant temperature of 25°C and alternating temperatures of 25/15°C, intact seeds were subjected to light conditions of constant darkness and 16-h light photoperiod. For incubation in total darkness, Petri dishes were wrapped in a double layer of aluminium foil. A green safe light was used to examine the dark-incubated seeds.

Effect of pre-sowing treatments on seed germination

Two treatments were applied to enhance seed germination.

- *Soaking in distilled water*, where intact seeds were soaked in distilled water (three volumes of water for each volume of seeds) at room temperature for 24 h.
- *Gibberellic acid*, where intact seeds were soaked in a GA_3 solution (1000 mg·L^{-1}) for 24 h (three volumes of GA_3 for each volume of seeds) at room temperature.

After these treatments, *P. albicans* seeds were set to germinate at 25/15°C under a 16-h light photoperiod. Untreated seeds were sown in the same conditions and they were used as a control.

Effect of osmotic stress on seed germination

Polyethylene glycol (PEG) 6000 was used to check the effect of osmotic stress on seed germination of *P. albicans*. Intact seeds were germinated in 2.5%, 5%, 10%, 15%, 20%, 25% and 35% (w/v) PEG 6000 solutions. According to Michel and Kaufmann (1973), water potentials of these PEG solutions were –0.02, –0.05, –0.16, –0.31, –0.51, –0.76 and –1.41 MPa, respectively. Distilled water was used as a control. Four replicates of 25 seeds each were tested for germination in Petri dishes on top of two sheets of filter paper moistened with 3.5 mL of either distilled water or PEG solution. Petri dishes were sealed with Parafilm to minimise evaporation of water from the solutions. Seeds were germinated for a period of 15 days at 25/15°C, and under a 16-h light photoperiod.

Ungerminated seeds from the 15-day PEG incubation tests were rinsed five times with distilled water, and then incubated for additional 15 days in Petri dishes on top of two sheets of filter paper moistened with 3.5 mL distilled water at the same incubation temperature. RP, initial germination and final germination were calculated as previously detailed.

Effect of salinity on seed germination

To evaluate the effect of salt stress on seed germination of *P. albicans*, intact seeds were germinated in 1, 5, 10, 25, 50, 75, 100, 150, 200, 300, 400, 600, 800, 1000, and 1500 mmol·L^{-1} NaCl solutions. Distilled water served as a control. Four replicates of 25 seeds each were germinated in Petri dishes on top of two sheets of filter paper moistened with 3.5 mL of either distilled water or NaCl solution. Petri dishes were sealed with Parafilm to reduce loss of water. Seeds were germinated for 15 days at 25/15°C, under a 16-h light photoperiod.

Ungerminated seeds from the 15-day NaCl incubation tests were rinsed five times with distilled water, and then incubated for additional 15 days in Petri dishes on top of two sheets of filter paper moistened with 3.5 mL distilled water at the same incubation temperature. RP, initial germination and final germination were calculated as previously detailed.

Effect of wetting and drying on seed germination

The effects of wetting and drying of seeds were investigated to ascertain the effects of rain showers on the germination behaviour of *P. albicans* seeds. Intact seeds were soaked for 24 h in distilled water before being dried for 4 days or 7 days under ambient conditions (~23°C, 35% RH). The seeds were then tested for germination at 25/15°C under a 16-h light photoperiod. Seeds being soaked for 24 h before being dried for 7 days were considered as single cycle of wetting and drying seeds. Two, three and four repeated cycles of wetting and drying seeds were tested. After each cycle, seeds were tested for germination at 25/15°C under a 16-h light photoperiod.

Data analysis

For all experiments, final germination percentage (mean ± s.e.) and mean germination time (MGT, mean ± s.e., in days) were calculated. The latter was determined according to the following formula: $MGT = \Sigma DN / \Sigma N$, where D is the number of days counted from the date of sowing and N is the number of seeds germinated on Day D (Ellis and Roberts 1981). 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 (untransformed data appear in tables). The effect of temperature regime (eight levels) and seed type (seeds with and without mucilage; two levels) on the final germination percentage was analysed with a two-way factorial ANOVA. The statistical analysis of MGT values was also carried out with a two-way factorial ANOVA. A two-way factorial ANOVA was also used to test the effect of light conditions (photoperiod and total darkness) and the two temperature regimes (25°C and 25/15°C) on seed germination and MGT. One-way factorial ANOVA was applied to test the effect of different concentrations of PEG and NaCl on the final germination percentage. Similarly, RP and MGT values were analysed with a one-way factorial ANOVA. The relationship between PEG or NaCl concentration and seed germination was also analysed following a linear regression (SPSS, Chicago, Illinois, USA). One-way ANOVA was applied to determine the statistical significance of differences among pre-sowing treatments, cycles of wetting and drying, and their correspondent MGT values. Where ANOVA indicated a significant effect, a comparison of mean values was carried out through the least significant difference (l.s.d.) test at 0.05 level of probability.

Results

Seed mass

Mean mass (±s.e.) per 100 seeds was 0.114 ± 0.003 g for original seeds and 1.410 ± 0.023 g for seeds imbibed with distilled water for 10 min. Therefore, the relative increase in mass for imbibed seeds compared with original seeds was 11.37.

Effect of temperature and light regimes

The effect of incubation temperature on the final germination percentage and germination rate (as expressed by MGT) of intact and demucilaged seeds and the ANOVA results are shown in Table 1. Temperature and seed type (intact and demucilaged seeds) had significant ($P < 0.001$) effects on seed germination; however, the two-way interaction between both factors was not significant ($P = 0.225$). A one-way ANOVA was used to compare final germination percentages at the

Table 1. Final germination percentage (mean ± s.e.) and mean germination time (MGT, mean ± s.e., in days) of *Plantago albicans* seeds at different temperature regimes

For each temperature and parameter (germination and MGT), the significance level between the results from intact and demucilaged seeds is shown. Means within a column followed by the same letter are not significantly different according to the l.s.d. test at 0.05 level. ***$P < 0.001$; **$P < 0.01$; n.s., not significant

Incubation temperature (°C)	Germination (%)		MGT (days)	
	Intact seeds	Demucilaged seeds	Intact seeds	Demucilaged seeds
5	34 ± 1.39a	9 ± 1.52a***	16.85 ± 0.41d	19.82 ± 0.28d**
10	42 ± 3.89ab	15 ± 1.82ab**	10.12 ± 0.88c	10.45 ± 1.40c n.s.
15	50 ± 4.70abc	24 ± 2.90bc**	5.55 ± 0.17b	6.37 ± 0.47b n.s.
20	68 ± 3.05bcd	29 ± 3.25c***	2.95 ± 0.31a	3.30 ± 0.38a n.s.
25	89 ± 2.97d	62 ± 2.56e**	2.55 ± 0.36a	3.10 ± 0.28a n.s.
30	72 ± 2.08cd	49 ± 3.26d**	2.92 ± 0.24a	3.17 ± 0.33a n.s.
20/10	63 ± 5.90bc	21 ± 4.62bc**	3.90 ± 0.39a	4.50 ± 0.62ab n.s.
25/15	86 ± 4.42d	57 ± 3.29de***	2.70 ± 0.15a	3.20 ± 0.65a n.s.
ANOVA table				
Source of variation				
Temperature (T)	d.f. = 7, MS = 1236.02	$F = 51.37$, $P < 0.000$	d.f. = 7, MS = 239.15	$F = 148.25$, $P < 0.000$
Seed type (ST)	d.f. = 1, MS = 5680.64	$F = 236.11$, $P < 0.000$	d.f. = 1, MS = 10.16	$F = 6.30$, $P = 0.016$
T × ST	d.f. = 7, MS = 33.81	$F = 1.40$, $P = 0.225$	d.f. = 7, MS = 1.62	$F = 1.00$, $P = 0.442$
Error	d.f. = 48, MS = 24.06		d.f. = 48, MS = 1.61	

different temperatures. For all temperatures assayed, the germination percentage of intact seeds was significantly ($P < 0.01$ and $P < 0.001$, depending on the temperature) higher than that of demucilaged seeds. Germination of intact and demucilaged seeds ranged from 34% to 89% and from 9% to 62%, respectively. The highest germination percentages were obtained at 25°C and 25/15°C (89% and 86% for intact seeds, 62% and 57% for demucilaged seeds) and the lowest at 5°C and 10°C (34% and 42% for intact seeds, 9% and 15% for demucilaged seeds).

Germination velocity was significantly affected by temperature and seed type, but their interaction (temperature × seed type) was not significant ($P = 0.442$; Table 1). For each temperature regime tested, no significant differences were found between the MGT values reached by intact and demucilaged seeds (except for 5°C). However, overall germination speed was higher for intact seeds. The highest germination rate (i.e. the lowest MGT value) of intact and demucilaged seeds was recorded at 25°C (2.55 and 3.10 days, respectively).

When intact seeds that did not germinate 15 days after being sown at the different temperatures assayed were transferred to 25°C, the germination percentage was increased in all cases, except for 25/15°C (Table 2). The RPs ranged from 2.1% to 68.1%, depending on the initial temperature.

Final germination percentage reached by intact seeds incubated at 25°C and 25/15°C was not significantly ($P > 0.05$) affected by either light regime tested (Table 3). Similarly, the two-way interaction between both factors (light and temperature) was not significant ($P = 0.409$). For alternating temperatures of 25/15°C, seeds incubated under complete darkness germinated significantly ($P < 0.001$) slower than did seeds incubated under a 16-h light photoperiod (Table 3). However, when seeds were germinated at 25°C, germination rate was not significantly ($P > 0.05$) affected by light. The highest germination percentage and germination rate were found at 25°C under light or darkness (Table 3). The two-way interaction between both factors (light and temperature) was not significant ($P = 0.082$).

Table 2. Germination percentages (mean ± s.e.) recorded for *Plantago albicans* seeds incubated for 15 days at several temperature regimes (initial germination) and germination percentages when non-germinated seeds from these temperature regimes were transferred to 25°C and then incubated for another 15 days (final germination)

Means within a column followed by the same letter are not significantly different according to the l.s.d. test at 0.05 level

Incubation temperature (°C)	Initial germination (%)	Final germination (%)	Recovery percentage (%)
5	34 ± 1.39a	79 ± 2.25ab	68.15 ± 3.02b
10	42 ± 3.89ab	51 ± 4.00a	21.65 ± 12.99a
15	50 ± 4.70abc	61 ± 7.13ab	33.27 ± 9.65ab
20	68 ± 3.05bcd	74 ± 3.35ab	22.11 ± 6.79a
25	89 ± 2.97d	–	–
30	72 ± 2.08cd	79 ± 3.68ab	25.72 ± 8.64ab
20/10	63 ± 5.90bc	64 ± 4.96ab	2.07 ± 1.80a
25/15	86 ± 4.42d	86 ± 4.42b	0a

Effect of pre-sowing treatments

Soaking for 24 h in distilled water or in a GA_3 solution (1000 mg L^{-1}) did not significantly ($P > 0.05$) increase the final germination percentage of *P. albicans* seeds when compared with control seeds (untreated seeds) (Table 4). Germination velocity was significantly ($P < 0.001$) higher for seeds soaked for 24 h in a GA_3 solution than that of control seeds (Table 4).

Effect of PEG concentration

Germination percentages of *P. albicans* seeds significantly ($P < 0.001$) decreased for 20% and 25% PEG when compared with the germination reached by control seeds (seeds germinated in distilled water) and no seed germinated at 35% PEG (Table 5). The PEG concentration was significantly related to a decrease in seed germination capacity ($r^2 = 0.716$, $P = 0.008$). The lowest MGT value (2.7 days) was reached in distilled water. Germination speed declined with increasing PEG concentrations, and these decreases were significant

Table 3. Final germination percentage (mean ± s.e.) and mean germination time (MGT, mean ± s.e., in days) under the combination of two temperatures and two light regimes (16-h light photoperiod and total darkness) for *Plantago albicans* seeds

For each temperature, the significance level between the MGT values from photoperiod and darkness is shown. MGT values within a column followed by the same letter are not significantly different according to the l.s.d. test at 0.05 level. ***$P < 0.001$; n.s., not significant

Incubation temperature (°C)	Germination (%)		MGT (days)	
	Photoperiod	Darkness	Photoperiod	Darkness
25	89 ± 2.97	87 ± 2.77	2.55 ± 0.36a	3.35 ± 0.20a n.s.
25/15	86 ± 4.42	76 ± 4.52	2.70 ± 0.15a	4.50 ± 0.12b***
ANOVA table				
Source of variation				
Light	d.f. = 1, MS = 98.46	$F = 2.28$, $P = 0.157$	d.f. = 1, MS = 6.76	$F = 24.43$, $P < 0.000$
Temperature	d.f. = 1, MS = 98.85	$F = 2.29$, $P = 0.156$	d.f. = 1, MS = 1.69	$F = 6.11$, $P = 0.029$
Light × Temperature	d.f. = 1, MS = 31.61	$F = 0.73$, $P = 0.409$	d.f. = 1, MS = 1.00	$F = 3.61$, $P = 0.082$
Error	d.f. = 12, MS = 43.24		d.f. = 12, MS = 0.28	

Table 4. Final germination percentage (mean ± s.e.) and mean germination time (MGT, mean ± s.e., in days) of *Plantago albicans* seeds after two pre-sowing treatments

Results after 15 days of incubation at 25/15°C under a 16-h light photoperiod. Means within a column followed by the same letter are not significantly different according to the l.s.d. test at 0.05 level

Pre-sowing treatment	Germination (%)	MGT (days)
Control (untreated seeds)	84 ± 2.91a	2.40 ± 0.13b
Soaking in distilled water for 24 h	87 ± 2.19a	1.95 ± 0.10ab
Soaking in GA_3 for 24 h (1000 mg·L^{-1})	95 ± 2.60a	1.40 ± 0.03a

Table 5. Germination percentages (mean ± s.e.) and mean germination time (MGT, mean ± s.e., in days) of *Plantago albicans* seeds after incubation in different polyethylene glycol (PEG 6000) concentrations for 15 days (initial germination), and germination percentages when ungerminated seeds were incubated for other 15 days in distilled water (final germination)

Means within a column followed by the same letters are not significantly different according to the l.s.d. test at 0.05 level

PEG concentration (% w/v)	Initial germination (%)	MGT (days)	Final germination (%)	Recovery percentage (%)
0	82 ± 1.43c	2.70 ± 0.15a	–	–
2.5	77 ± 1.92bc	3.05 ± 0.19a	81 ± 1.77a	5.00 ± 4.33a
5	83 ± 2.65c	3.02 ± 0.21a	84 ± 1.92a	19.57 ± 6.13a
10	78 ± 2.60bc	3.42 ± 0.14a	83 ± 1.34a	14.57 ± 7.43a
15	76 ± 2.25bc	3.90 ± 0.21ab	82 ± 1.63a	22.90 ± 6.82a
20	65 ± 2.72b	5.07 ± 0.15bc	77 ± 2.61a	34.70 ± 7.36a
25	63 ± 2.57b	5.95 ± 0.28c	72 ± 3.34a	33.60 ± 5.97a
35	0a	–	79 ± 4.22a	79.37 ± 4.13b

($P < 0.001$) for 20% and 25% PEG solutions (Table 5). A positive significant correlation was observed between MGT value and PEG concentration ($r^2 = 0.937$, $P = 0.0003$).

Seeds that did not germinate after 15 days of incubation in PEG solution were able to germinate when they were incubated for another 15 days in distilled water (Table 5). There were no significant ($P > 0.05$) differences among recovery germinations. The RP value reached at 35% PEG (79.4%) was significantly ($P < 0.05$) different from all other RP values. No significant differences ($P > 0.05$) were observed among RP values from 2.5% to 25% PEG solutions, ranging from 5.0% to 34.7% (Table 5).

Effect of NaCl concentration

The highest germination (82%) was obtained in distilled water (control seeds). The response to increasing NaCl concentrations was a reduction in the final germination percentage (Table 6). No significant ($P > 0.05$) differences were detected among final germination percentages from 0 (control) to 75 mmol·L^{-1} NaCl. However, these decreases were significant ($P < 0.001$) for 100, 150 and 200 mmol·L^{-1} NaCl when compared with the control. No germination occurred for NaCl concentrations equal to or higher than 300 mmol·L^{-1} (Table 6). The saline concentration was significantly related to a decrease in seed germination ($r^2 = 0.913$, $P = 0.0000$). Similarly, germination speed declined with an increasing NaCl concentration (Table 6), and these decreases were significant ($P < 0.001$) for 100, 150 and 200 mmol·L^{-1} NaCl. A significant positive correlation was observed between MGT value and saline concentration ($r^2 = 0.841$, $P = 0.0002$).

A proportion of ungerminated, salt-treated seeds were able to germinate when they were transferred to distilled water (Table 5). Recovery germination significantly ($P < 0.001$) decreased with an increasing NaCl concentration (Table 6). The highest RP values were reached for 300 and 400 mmol·L^{-1} NaCl (79.9% and 75.8%, respectively; Table 6).

Effect of wetting and drying of seeds

The final germination percentage of *P. albicans* seeds significantly ($P < 0.01$) decreased after three and four cycles of wetting and drying of seeds (Table 7). Seeds imbibed for 24 h in distilled water before being dried for 4 days germinated significantly faster than did control seeds as expressed by MGT values (1.30 vs 2.70 days, respectively; Table 7).

Discussion

Intact seeds of *P. albicans* showed higher germination percentages and germination speed than did demucilaged seeds for all temperature regimes tested. Germination percentages ranged from 34% to 89% for intact seeds and from 9% to 57% for demucilaged seeds, depending on the temperature. Therefore, the mucilage plays an important role in seed germination of *P. albicans*. As occurs in several species

Table 6. Germination percentages (mean ± s.e.) and mean germination time (MGT, mean ± s.e., in days) of *Plantago albicans* intact seeds after incubation in different NaCl concentrations for 15 days (initial germination), and germination percentages when non-germinated seeds were incubated for another 15 days in distilled water (final germination)

Means within a column followed by the same letter are not significantly different according to the l.s.d. test at 0.05 level

NaCl concentration ($mmol \cdot L^{-1}$)	Initial germination (%)	MGT (days)	Final germination (%)	Recovery percentage (%)
0	82 ± 1.43d	2.70 ± 0.15a	82 ± 1.43d	–
1	71 ± 3.77cd	2.62 ± 0.09a	75 ± 4.68cd	16.67 ± 5.10ab
5	72 ± 4.24cd	3.02 ± 0.21a	75 ± 2.90cd	9.37 ± 5.18ab
10	72 ± 2.48cd	3.22 ± 0.25ab	73 ± 1.67cd	2.77 ± 2.40a
25	72 ± 2.02cd	3.10 ± 0.31ab	76 ± 2.32cd	10.72 ± 9.29ab
50	70 ± 6.23cd	2.70 ± 0.20a	75 ± 3.33cd	13.12 ± 8.17ab
75	67 ± 3.07cd	3.27 ± 0.28abc	78 ± 5.40cd	38.25 ± 12.74abcd
100	57 ± 3.27bc	3.80 ± 0.24bcd	71 ± 1.08cd	28.77 ± 8.60abc
150	54 ± 1.60bc	3.97 ± 0.10cd	74 ± 2.51cd	43.32 ± 6.13bcde
200	42 ± 2.88b	4.40 ± 0.27d	77 ± 1.77cd	58.92 ± 5.20cde
300	0a	–	80 ± 3.66cd	79.92 ± 3.62e
400	0a	–	76 ± 4.55cd	75.80 ± 4.49de
600	0a	–	67 ± 8.36c	66.85 ± 8.34cde
800	0a	–	47 ± 6.50b	47.20 ± 6.51bcde
1000	0a	–	42 ± 3.88b	41.85 ± 3.99bcde
1500	0a	–	16 ± 3.03a	16.35 ± 2.96ab

Table 7. Germination percentages (mean ± s.e.) and mean germination time (MGT, mean ± s.e., in days) recorded in *Plantago albicans* seeds submitted to different cycles of wetting and drying

Means within a column followed by the same letter are not significantly different according to the l.s.d. test at 0.05 level

Cycles of wetting and drying	Germination (%)	MGT (days)
Control (untreated seeds)	82 ± 1.43b	2.70 ± 0.15bcd
Imbibition for 24 h + drying seeds for 4 days	83 ± 1.84b	1.30 ± 0.12a
Imbibition for 24 h + drying seeds for 7 days		
One cycle	84 ± 4.92b	1.72 ± 0.10ab
Two cycles	76 ± 5.24b	2.37 ± 0.48bc
Three cycles	50 ± 8.84a	2.80 ± 0.42cd
Four cycles	55 ± 9.26a	3.40 ± 0.18d

(Harper and Benton 1966; Western 2012), removal of the mucilage layer in *P. albicans* seeds had a significant negative effect on seed germination, suggesting that the mucilage of intact seeds could increase their capacity for water uptake. Demucilaged seeds imbibed water slower than did intact seeds. Therefore, demucilaged seeds of *P. albicans* would be less adapted to drought-stress environment, where water shortage is an important limitation for successful habitation.

The range of temperatures within which germination occurs is highly variable among species (Baskin and Baskin 2001; Luna and Moreno 2010; Chamorro *et al*. 2013). As occurs in several Mediterranean species (Galmés *et al*. 2006; Zaidi *et al*. 2010; Martínez-García *et al*. 2012), *P. albicans* seeds are able to germinate over a wide range of temperatures (5−30°C). It appears logical that seeds having a wide range of temperatures for germination germinate whenever the chance of sufficient rain occurs. This germination behaviour may suggest that *P. albicans* seeds could germinate during most of the year. Therefore, this species appears to show an opportunistic strategy for germination. That is, after rainfall, and depending on the temperature, a certain portion of the total number of seeds in the population may germinate. However, the highest germination percentage was obtained at 25°C, followed by 25/15°C. This suggests that most *P. albicans* seeds will germinate in early autumn and throughout spring, if rain water is available. In the natural habitats in which the species grows in Spain, soil moisture conditions would be the most decisive factor for germination of *P. albicans* seeds.

Intact seeds of *P. albicans* reached germination percentages higher than 85% without any pre-treatment and therefore they can be considered non-dormant (according to Baskin and Baskin 2004). It has been shown that the optimal germination temperatures for several *Plantago* species range from 15°C to 25°C (Arnold 1973; Blom 1992; Fons *et al*. 2008). In *P. ovata* (Hammouda and Bakr 1969), and in *P. algarbiensis* and *P. almogravensis* (Martins *et al*. 2012), two endemic plantains from south of Portugal, the best germination results were obtained at 15°C. However, the highest germination of *P. albicans* seeds was reached at 25°C and 25/15°C. These results are in agreement with the reports of Puech *et al*. (1998) in this same species (several populations from Tunisia and Morocco) and Pons and Van Der Toorn (1988) concerning *P. major*. A positive effect of alternating temperatures of 25/15°C on seed germination has been reported for several species from arid and semiarid habitats (Hammouda and Bakr 1969; Baskin and Baskin 2001; Albert *et al*. 2002). This temperature regime corresponds to mid-spring mean temperatures in the natural habitats where *P. albicans* grows in Spain. However, the alternating temperatures of 25/15°C did not significantly increase the germination of *P. albicans* seeds compared with the constant temperature of 25°C.

The germination period of *P. albicans* seeds was very short. Except for the lowest temperatures (5°C and 10°C), seeds completed their germination within a period of 7 days. Rapid germination increases the likelihood of rapid establishment in a habitat with intermittent precipitation and could be an advantageous character for species (Verdú and Traveset 2005). In drought environments, a strategy of fast germination can ensure sufficient water availability to complete germination and achieve establishment (Montes-Recinas *et al*. 2012).

It is commonly accepted that light requirements for germination are more likely in small-seeded than in large-seeded species (Milberg *et al*. 2000), which is interpreted as a way to prevent small seeds with limited resources from germinating far from the soil surface (Fenner and Thompson 2005). However, the small seeds of *P. albicans* (2–3.5 mm long and 1–1.5 mm wide, and a mean mass per seed of 1.14 mg) did not need light for germination. Thereby, there were no significant differences in the final germination percentages reached by *P. albicans* seeds between light and dark regimes at both temperature regimes tested (25°C and 25/15°C). This fact indicated that *P. albicans* seeds can germinate at these temperatures without light, showing no photoblastic response. Similarly, several *Plantago* species can germinate under light or darkness (Blom 1992; Martins *et al*. 2012). However, other *Plantago* species require light to germinate (Hammouda and Bakr 1969; Arnold 1973; Gutterman and Shem-Tov 1997; Zaady *et al*. 1997). In our study, light had a positive effect on the germination speed of *P. albicans* seeds at the alternating temperatures of 25/15°C.

Pre-sowing treatments (soaking in GA_3 and distilled water) did not significantly enhance seed germination of *P. albicans*. These results are in accordance with the results obtained by Martins *et al*. (2012) for *P. algarbiensis* and *P. almogravensis*.

Different water potential conditions were simulated using PEG solutions. There was a significant negative correlation between PEG concentration and the final germination percentage, i.e. seed germination of *P. albicans* decreased as PEG concentration increased. However, these decreases were not significant until solutions of 20% and 25% PEG were used, ceasing germination at 35% PEG. Our results were similar to those obtained by Bochet *et al*. (2007) in this same species. Therefore, *P. albicans* seeds showed a high tolerance for osmotic stress; even when incubated for 15 days in 25% PEG, 63% seeds germinated. Besides, seeds showed a high recovery percentage (79%) when they were transferred from the highest PEG concentration (35% PEG) to distilled water. The ability of species to germinate under osmotic stress could be an indication of a species' potential for success under semiarid conditions (Bochet *et al*. 2007). In this respect, *P. albicans* could be considered a successful coloniser plant.

A significant negative correlation between germination percentage and NaCl concentration was found, i.e. seed germination of *P. albicans* decreased as saline concentration increased. The final germination percentage reached under saline solutions (from 1 to 200 $mmol \cdot L^{-1}$ NaCl) was always lower than for control seeds (seeds germinated in distilled water). Moreover, seed germination was totally inhibited at a concentration equal to or higher than 300 $mmol \cdot L^{-1}$ NaCl. Several studies have reported that salt stress negatively affects seed germination of several species (Khan *et al*. 2001; Khan and Gulzar 2003; Zia and Khan 2008; El-Keblawy *et al*. 2007; El-Keblawy and Al-Shamsi 2008; Mohammad Esmaeili *et al*. 2009; Vallejo *et al*. 2010). High saline concentrations can slow the rate of water uptake by seeds, and therefore inhibit germination and radicle elongation (Werner and Finkelstein 1995; Mohammad Esmaeili *et al*. 2009; Yang *et al*. 2010; Western 2012). *P. albicans* showed a pattern of increase in the saline concentration in delays and reduction of seed germination that is similar to those obtained in several halophyte species (Ungar 1978, 1995; Keiffer and Ungar 1997). Our results suggested that *P. albicans* seeds can germinate over a wide range of salinity. Even 42% of *P. albicans* seeds germinated at very high NaCl concentrations (200 $mmol\ L^{-1}$), representing a 50% decrease on the germination of control seeds. This result seems to indicate that this species is relatively tolerant to hypersaline conditions ($\geq$200 $mmol\ L^{-1}$), in which only halophyte plants complete their life cycle (Flowers and Colmer 2008). Furthermore, *P. albicans* seeds showed high recovery percentages (>75%) when subjected to high NaCl concentrations (300 and 400 $mmol \cdot L^{-1}$). Therefore, *P. albicans* seeds exposed to high salinity can germinate at high percentages once salinity decreases. Seeds of many species are capable of suspending germination under saline conditions and retaining the capacity to germinate when the saline conditions are alleviated (Ungar 1995; Barret 2003). Rainfall can dilute soil salinity and provide suitable conditions for seed germination (Ungar 2001). As occur in some halophyte species (Tobe *et al*. 2000; Qu *et al*. 2008; Yang *et al*. 2010), *P. albicans* seeds could maintain a high capacity for recovery germination in their natural habitats, and they will germinate when rainfall decreases soil salinity. Thereby, most *P. albicans* seeds could germinate early in the spring when it rains and soil salinity is reduced. Moreover, the presence of mucilage can aid germination in arid environments with both drought- and salt-stress (Yang *et al*. 2010; Western 2012).

Plantago albicans seeds are able to germinate after several cycles of wetting and drying and, therefore, they were desiccation tolerant under laboratory conditions. Under natural conditions, *P. albicans* seeds could withstand short drought periods without a marked decrease in germination capacity; however, too much wetting and drying was deleterious.

In summary, *P. albicans* seeds germinated readily at 25°C and 25/15°C under either light or darkness, reaching germination higher than 85%. Therefore, these two temperature regimes can be recommended for germination of this species. Presence of mucilage on *P. albicans* seeds significantly increased germination percentage and germination rate at all temperatures tested. According to the results obtained, seed mucilage appears to play an important role in seed germination of this species. Pre-sowing treatments did not significantly enhance seed germination. We found that increased osmotic stress and salinity caused a decrease in germination percentage and delayed germination rate, and seed germination was totally inhibited when seeds were exposed to the highest saline ($\geq$300 $mmol \cdot L^{-1}$ NaCl) and PEG 35%) concentrations. Moreover, seeds showed a high capacity for recovery germination, suggesting that *P. albicans* seeds are tolerant to osmotic and salt stresses. Recovery responses were related to

the intensity of exposure to saline solutions. Saline soils are common in semiarid environments of Spain where *P. albicans* grows. The results of the present study indicated that the degree of salt tolerance shown by this species may be sufficient to permit seed germination at the levels of salinity found in the soils of its natural habitat.

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References

Albert MJ, Iriondo JM, Pérez-García F (2002) Effects of temperature and pretreatments on seed germination of nine semiarid species from NE Spain. *Israel Journal of Plant Sciences* **50**, 103–112. doi:10.1560/3HT7-P4UB-GA7N-PB3F

Arnold S (1973) Interactions of light and temperature on the germination of *Plantago maritima* L. *New Phytologist* **72**, 583–593. doi:10.1111/j.1469-8137.1973.tb04409.x

Barret GJ (2003) Seed dormancy and germinability in the halophyte *Halosarcia pergranulata* subsp. *pergranulata. Seed Science and Technology* **31**, 289–299.

Baskin CC, Baskin JM (2001) 'Seeds. Ecology, biogeography, and evolution of dormancy and germination.' (Academic Press: San Diego, CA)

Baskin JM, Baskin CC (2004) A classification system for seed dormancy. *Seed Science Research* **14**, 1–16. doi:10.1079/SSR2003150

Blom CW (1992) Germination and establishment. In '*Plantago*: a multidisciplinary study. Ecological studies 89'. (Eds PJC Kuiper, M Bos) pp. 88–98. (Springer-Verlag: Berlin)

Bochet E, García-Fayos P, Alborch B, Tormo J (2007) Soil water availability effects on seed germination account for species segregation in semiarid roadslopes. *Plant and Soil* **295**, 179–191. doi:10.1007/s11104-007-9274-9

Chamorro D, Luna B, Moreno JM (2013) Germination response to various temperature regimes of four Mediterranean seeder shrubs across a range of altitudes. *Plant Ecology* **214**, 1431–1441. doi:10.1007/s11258-013-0264-0

El-Keblawy A, Al-Shamsi N (2008) Effects of salinity, temperature and light on seed germination of *Haloxylon salicornium*, a common perennial shrub of the Arabian deserts. *Seed Science and Technology* **36**, 679–688.

El-Keblawy A, Al-Ansari F, Hassan N, Al-Shamsi N (2007) Salinity, temperature and light affect germination of *Salsola imbricada. Seed Science and Technology* **35**, 272–281.

Ellis RH, Roberts EH (1981) The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology* **9**, 373–409.

Engelbrecht M, García-Fayos P (2012) Mucilage secretion by seeds doubles the chance to escape removal by ants. *Plant Ecology* **213**, 1167–1175. doi:10.1007/s11258-012-0074-9

Fenner M, Thompson K (2005) 'The ecology of seeds.' (Cambridge University Press: Cambridge, UK)

Flowers TJ, Colmer TD (2008) Salinity tolerance in halophytes. *New Phytologist* **179**, 945–963. doi:10.1111/j.1469-8137.2008.02531.x

Fons F, Gargadennec A, Rapior S (2008) Culture of *Plantago* species as bioactive components resources: a 20-year review and recent applications. *Acta Botanica Gallica* **155**, 277–300. doi:10.1080/12538078.2008.10516109

Galmés J, Medrano H, Flexas J (2006) Germination capacity and temperature dependence in Mediterranean species of the Balearic Islands. *Investigación Agraria: Sistemas y Recursos Forestales* **15**, 88–95.

Gul B, Webber DJ (1999) Effect of salinity, light, and temperature on germination in *Allenrolfea occidentalis. Canadian Journal of Botany* **77**, 240–246. doi:10.1139/cjb-77-2-240

Gutterman Y (2002) 'Survival, strategies and annual desert plants. Adaptations of desert organisms.' (Springer: Berlin)

Gutterman Y, Shem-Tov S (1997) The efficiency of the strategy of mucilaginous seeds of some common annuals of the Negev adhering to the soil crust to delay collection by ants. *Israel Journal of Plant Sciences* **45**, 317–327. doi:10.1080/07929978.1997.10676695

Hammouda MA, Bakr ZY (1969) Some aspects of germination of desert seeds. *Phyton* **13**, 183–201.

Harper J, Benton R (1966) The behaviour of seeds in soil. II. The germination of seeds on the surface of a water supplying substrate. *Journal of Ecology* **54**, 151–166. doi:10.2307/2257664

Huang Z, Gutterman DJ, Hu Z (2000) Structure and function of mucilaginous achenes of *Artemisia monosperma* inhabiting the Negev desert of Israel. *Israel Journal of Plant Sciences* **48**, 255–266. doi:10.1560/49G5-CNE7-H6UD-PWVW

Huang Z, Boubriak I, Osborne DJ, Dong M, Gutterman Y (2008) Possible role of pectin-containing mucilage and dew in repairing embryo DNA of seeds adapted to desert conditions. *Annals of Botany* **101**, 277–283. doi:10.1093/aob/mcm089

Keiffer CH, Ungar IA (1997) The effect of extended exposure to hypersaline conditions on the germination of five inland halophytes species. *American Journal of Botany* **84**, 104–111. doi:10.2307/2445887

Khan MA, Gul B (2006) Halophyte seed germination. In 'Ecophysiology of high salinity tolerant plants'. (Eds MA Khan, DJ Weber) pp. 11–30. (Springer: Dordrecht, The Netherlands)

Khan MA, Gulzar S (2003) Light, salinity and temperature effects on the seed germination of perennial grasses. *American Journal of Botany* **90**, 131–134. doi:10.3732/ajb.90.1.131

Khan MA, Ungar IA (1984) The effect of salinity and temperature on the germination of polymorphic seeds and growth of *Atriplex triangularis* Willd. *American Journal of Botany* **71**, 481–489. doi:10.2307/2443323

Khan MA, Gul B, Weber D (2001) Germination of dimorphic seeds of *Suaeda moquinii* under high salinity stress. *Australian Journal of Botany* **49**, 185–192. doi:10.1071/BT00020

Kigel J (1995) Seed germination in arid and semiarid regions. In 'Seed development and germination'. (Eds J Kigel, G Galili) pp. 645–699. (Marcel Dekker: New York)

Luna B, Moreno JM (2010) Range-size, local abundance and germination niche-breadth in Mediterranean plants of two life-forms. *Plant Ecology* **210**, 85–95. doi:10.1007/s11258-010-9740-y

Martínez-García F, Guerrero-García S, Pérez-García F (2012) Evaluation of reproductive sucess and conservation strategies for *Senecio coincyi* (Asteraceae), a narrow and threatened species. *Australian Journal of Botany* **60**, 517–525. doi:10.1071/BT12109

Martins N, Gonçalves S, Palma T, Romano A (2012) Seed germination of two critically endangered plantain species, *Plantago algarbiensis* and *P. almogravensis* (Plantaginaceae). *Seed Science and Technology* **40**, 144–149.

Michel BE, Kaufmann R (1973) The osmotic potential of polyethylene glycol 6000. *Plant Physiology* **51**, 914–916. doi:10.1104/pp.51.5.914

Milberg P, Andersson L, Thompson K (2000) Large-seeded species are less dependent on light for germination than small-seeded ones. *Seed Science Research* **10**, 99–104. doi:10.1017/S0960258500000118

Mohammad Esmaeili M, Sattarian A, Bonis A, Bouzillé JB (2009) Ecology of seed dormancy and germination of *Carex divisa* Huds.: effects of stratification, temperature and salinity. *International Journal of Plant Production* **3**, 27–40.

Montes-Recinas S, Márquez-Guzmán J, Orozco-Segovia A (2012) Temperature and water requirements for germination and effects of

discontinuous hydration on germination seed survival in *Tillandsia recurvata* L. *Plant Ecology* **213**, 1069–1079. doi:10.1007/s11258-012-0066-9

Mott JJ (1974) Factors affecting seed germination in three annual species from an arid region of Western Australia. *Journal of Ecology* **62**, 699–709. doi:10.2307/2258950

Pedrol J (2009) *Plantago* L. In 'Flora Iberica. Vol. XIII'. (Eds C Benedi, E Rico, J Güemes, A Herrero) pp. 4–38. (Real Jardín Botánico, CSIC: Madrid)

Pons TL, Van Der Toorn J (1988) Establishment of *Plantago lanceolata* L. and *Plantago major* L. among grass. I. Significance of light for germination. *Oecologia* **75**, 394–399. doi:10.1007/BF00376942

Puech S, Rascol JP, Michel V, Andary C (1998) Cytogenetics and adaptation to increasingly arid environments: the example of *Plantago albicans* L. (Plantaginaceae). *Biochemical Systematics and Ecology* **26**, 267–283. doi:10.1016/S0305-1978(97)00111-7

Pujol JA, Calvo JF, Ramírez-Díaz L (2000) Recovery of germination from different osmotic conditions by four halophytes from southeastern Spain. *Annals of Botany* **85**, 279–286. doi:10.1006/anbo.1999.1028

Qu XX, Baskin JM, Wang L, Huang ZY (2008) Effects of cold stratification, temperature, light and salinity on seed germination and radicle growth of the desert halophyte shrub, *Kalidium capsicum* (Chenopodiaceae). *Plant Growth Regulation* **54**, 241–248. doi:10.1007/s10725-007-9246-3

Sun Y, Tan DY, Baskin CC, Baskin JM (2012) Role of mucilage in seed dispersal and germination of the annual ephemeral *Alyssum minus* (Brassicaceae). *Australian Journal of Botany* **60**, 439–449. doi:10.1071/BT11314

Thapliyal RC, Phartyal SS, Baskin JM, Baskin CC (2008) Role of mucilage in germination of *Dillenia indica* (Dilleniaceae) seeds. *Australian Journal of Botany* **56**, 583–589. doi:10.1071/BT08022

Tlig T, Gorai M, Neffati M (2008) Germination responses of *Diplotaxis harra* to temperature and salinity. *Flora* **203**, 421–428. doi:10.1016/j.flora.2007.07.002

Tobe K, Li X, Omasa K (2000) Seed germination and radicle growth of a halophyte, *Kalidium capsicum* (Chenopodiaceae). *Annals of Botany* **85**, 391–396. doi:10.1006/anbo.1999.1077

Tormo J, Bochet E, García-Fayos P (2006) Is seed availability enough to ensure colonization success? An experimental study in road embankments. *Ecological Engineering* **26**, 224–230. doi:10.1016/j.ecoleng.2005.10.003

Ungar IA (1978) Halophyte seed germination. *Botanical Review* **44**, 233–264. doi:10.1007/BF02919080

Ungar IA (1995) Seed germination and seed-bank ecology in halophytes. In 'Seed development and germination'. (Eds J Kigel, G Galili) pp. 599–628. (Marcel Dekker: New York)

Ungar IA (2001) Seed banks and seed population dynamics of halophytes. *Wetlands Ecology and Management* **9**, 499–510. doi:10.1023/A:1012236829474

Vallejo AJ, Yanovsky MJ, Botto JF (2010) Germination variation in *Arabidopsis thaliana* accessions under moderate osmotic and salt stress. *Annals of Botany* **106**, 833–842. doi:10.1093/aob/mcq179

Verdú M, Traveset A (2005) Early emergence enhances plant fitness: a phylogenetically controlled meta-analysis. *Ecology* **86**, 1385–1394. doi:10.1890/04-1647

Werner JE, Finkelstein RR (1995) *Arabidopsis* mutants with reduced response to NaCl and osmotic stress. *Physiologia Plantarum* **93**, 659–666. doi:10.1111/j.1399-3054.1995.tb05114.x

Western TL (2012) The sticky tale of seed coat mucilages: production, genetics, and role in seed germination and dispersal. *Seed Science Research* **22**, 1–25. doi:10.1017/S0960258511000249

Yang X, Dong M, Huang Z (2010) Role of mucilage in the germination of *Artemisia sphaerocephala* (Asteraceae) achenes exposed to osmotic stress and salinity. *Plant Physiology and Biochemistry* **48**, 131–135. doi:10.1016/j.plaphy.2009.12.006

Zaady E, Gutterman Y, Boeken B (1997) The germination of mucilaginous seeds of *Plantago coronopus, Reboudia pinnata*, and *Carrichtera annua* on cyanobacterial soil crust from the Negev Desert. *Plant and Soil* **190**, 247–252. doi:10.1023/A:1004269031844

Zaidi CA, González-Benito ME, Pérez-García F (2010) Morphological and physiological seed heterogeneity in the Mediterranean annual plant *Tuberaria macrosepala* (Cistaceae). *Plant Species Biology* **25**, 149–157. doi:10.1111/j.1442-1984.2010.00279.x

Zia S, Khan MA (2004) Effects of light, salinity, and temperature on seed germination of *Limonium stocksii*. *Canadian Journal of Botany* **82**, 151–157. doi:10.1139/b03-118

Zia S, Khan MA (2008) Seed germination of *Limonium stocksii* under saline conditions. *Pakistan Journal of Botany* **40**, 683–695.