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GERMINATION RESPONSES OF *LIMONIUM INSIGNE* (COSS.) KUNTZE TO SALINITY AND TEMPERATURE

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Abstract

Limonium insigne (Plumbaginaceae) is a perennial halophyte endemic to the SE of the Iberian Peninsula. Experiments were conducted to determine the effects of different salinities (0, 100, 200 and 400 mM NaCl) on the seed germination of *L. insigne* under different temperature regimes (20/10, 25/15, 30/20 and 35/25 °C), both in a 14 h light and 10 h dark photoperiod. Seed germination of *L. insigne* was affected significantly by salinity levels, temperature and their interaction. Maximum germination was observed in the least saline media (100 mM NaCl) and distilled water (0 mM NaCl) at 20/10 °C temperature. No seeds germinated at concentrations higher than 200 mM NaCl at the highest temperature (35/25 °C). The increase in salinity delayed the beginning and ending of germination, reduced final germination percentage and increased mean time to germination. The rate of germination decreased with an increase in salinity and temperature.

Key words: Germination, Halophyte, Limonium insigne, Salinity, Temperature.

Introduction

Limonium insigne (Coss.) Kuntze (known in Spanish as *siempreviva de saladar* or *siempreviva rosa*) is an evergreen plant endemic to the SE of the Iberian Peninsula (provinces of Almería, Granada, Murcia & Alicante). The plant belongs to coastal and continental halophilous vegetation. According to Salazar & Lendínez (2009), the species can be found on coastal cliffs, littoral steppes and dry inland areas (0-400 m). The plant grows on soils which are temporarily wet (but not waterlogged) due to superficial run-off saline water. These soils are very dry in the summer giving rise to saline efflorescence. Fields of *L. insigne* can also be found in the driest part of the land strip of the coastal marshlands and saltmarshes (De la Cruz, 2009). The plant communities belong to the *Limonietalia* order, a priority habitat for the "Habitats Directive" (1510 Mediterranean salt steppes, Directive 92/43/EEC). This plant is of interest for the restoration of natural saltmarshes and areas degraded by different kinds of infrastructure, in particular, road or railway embankments in saline and semiarid environments, where plants unfit for these conditions are not viable. This plant is also very beautiful and it is not surprising that it is used as an ornamental plant and in xerogardening.

Germination is the most critical period in the life cycle of halophyte plants. Salinity becomes a decisive factor at this point (Waisel, 1972; Taleisnik *et al*., 1998; Khan & Gulzar, 2003; Abbas *et al*., 2013a,b). Under saline conditions, temperature fluctuations can also have a variable impact on halophyte germination. In some species seeds delay germination until salt stress becomes moderate and temperatures are optimal (Khan & Ungar, 1997). There is no doubt that the interaction between temperature and salinity during germination plays a crucial role in the fitness and successful colonization of plant species in saline habitats (Ungar, 1995). There are no published data on the performance of *Limonium insigne*. This paper aims at analysing the impact of temperature and salinity, together with their possible interaction, on seed germination under laboratory conditions. These results will show us the best conditions for the germination of the seeds of this species so that the plant can reproduce successfully for use in the restoration of wild populations and in the production and commercialization of ornamental plants.

Materials and Methods

The *L. insigne* seeds were collected in July and August 2010 in Cerro de los Lobos (Vícar, Almería, 30SWF3575), an enclave located at the foot of the Sierra de Gádor, in the SE of the Iberian Peninsula. Once dried, the seeds were cleaned and selected manually. The seeds were then stored in glass jars in a chamber at 8°C until later use. The experiment began with the sterilization of the seeds by means of a sodium hypochlorite 5% solution for 5 minutes. The seeds were then washed with abundant distilled water. Four replications of 25 seeds in Petri dishes (90 mm in diameter) were put on filter paper and submerged in 5 ml saline solution. Four different saline solutions (0, 100, 200, 400 mM NaCl) were used. Germination took place in a monitored growth chamber under a 14/10 h light/darkness photoperiod (25 μmol photons $m^{-2}S^{-1}$, 400-700 nm with fluorescent lamps) and at different temperature regimes 20/10, 25/15, 30/20, 35/25°C. These temperature regimes were selected to reflect temperature fluctuations in spring and summer in the study area. The emergence of the radicle was considered as evidence of germination. Germination was recorded every other day during the 24 days of the experiment.

Three characteristics of germination were determined: *Final Germination Percentage* (FGP), *Rate of Germination* (RG) and *Mean Time to Germination* (MTG). MTG was calculated using the equation:

$$MTG= \sum_i(n_i \times d_i)/N$$

where *n* is the number of seeds germinated up to day *i*; *d* is the incubation period in days, and *N* is the total number of seeds germinated in the treatment (Brenchley & Probert 1998), which means that the lower the value, the faster the germination.

RG was estimated by using a modified Timson's index of germination velocity: germination velocity = $\Sigma G/t$, where G is the percentage of seed germination at 2-d intervals and t is the total germination period (Khan & Ungar, 1997). The maximum value possible for our data using this index was 50 (i.e., 1200/24), so the higher the value, the more rapid the germination.

Data were subjected to a two-way analysis of variance (ANOVA) after arcsine transformation to evaluate the effect of temperature and salinity and their interaction on FGP, RG and MTG. Untransformed data are presented in figures and tables. Bonferroni's test was carried out to perform all-pairwise comparisons between individual treatments ($P < 0.05$). All statistical analyses were performed using IBM SPSS Statistics 20 (IBM Corp., Armonk, NY, USA).

Results

Final Germination Percentage (FGP): The ANOVA reveals that the germination of *L. insigne* is highly dependent on salinity ($F=124.544$, $p<0.0001$), temperature ($F=60.596$, $p<0.0001$) and the interaction of both factors ($F=6.254$, $p<0.03$) (Fig. 1 and 2, Table 1).

In the control test (0 mM) the FGP of the *Limonium insigne* seeds decreased as temperature increased. There is also a progressive decrease in FGP as temperature increases with saline solutions (100, 200 and 400 mM). The best results for all saline concentrations were recorded under a 20/10 °C temperature regime: 95 and 72% on the fourth day after sowing for 0 and 100 mM treatments respectively. The highest germination percentage was recorded on the 14th day (97% at 0 mM and 81% at 100 mM).

The highest FGP was recorded with no salinity at all (0 mM) under any temperature regime. Exposure to different saline concentrations reduces FGP significantly. FGP reduction is even greater as the temperature of the culture medium increases.

The lowest FGP values are recorded with the highest temperature regimes. At 35/25°C, FGP is 0 with a 400 mM concentration treatment. Germination of cultivated seeds under conditions of no salinity (0 mM) even decreases significantly at these temperatures, when compared with other temperature regimes.

At lower temperature regimes (30/20 and 25/15°C) results improve progressively and reach their maximum at 20/10°C. FGP records also decrease as salinity increases, regardless of temperature.

The best FGP results for *L. insigne* seeds (97%) were obtained under conditions of no salinity and at the lowest temperature (20/10°C). An increase in temperature causes these values to decrease to 93%, 48% and 26% under the other temperature regimes monitored: 25/15 °C, 30/20°C and 35/25°C, respectively.

Good FGP results are also obtained at 20/10°C with low salinity levels (100 mM). Under such conditions germination was 81% as compared to 46%, 15% and 13% under other temperature regimes (25/15°C, 30/20°C and 35/25°C).

A high salinity level (from 200 to under 400 mM) has a significant impact on FGP records and FGP even becomes null at 35/25°C with 400 mM salinity.

Our data clearly indicate that temperature fluctuations significantly affect FGP.

Mean Time to Germination (MTG): MTG is highly dependent on salinity ($F=3.173$, $p<0.0001$), on temperature ($F=3.547$, $p<0.0001$) and on the interactions of both factors ($F=6.058$, $p<0.0001$) (Table 1 and Table 2).

At 20/10°C an increase in salinity causes the MTG to rise, whereas the opposite response was observed in the other temperature ranges (25/15°C, 30/20°C and 35/25°C). This negative impact is more apparent as salinity values increase, and MTG becomes null at 400 mM.

The lowest MTG was recorded at 20/10°C and no salinity (0 mM). Higher temperature ranges induce significant changes in the MTG at all the salinity levels under study.

Rate of germination (RG): The ANOVA indicates that the RG of *L. insigne* seeds is influenced by salinity ($F=170.582$, $p<0.0001$), temperature ($F=72.262$, $p<0.0001$) and the interaction between these two factors ($F=8.501$, $p<0.0001$) (Table 1 and 2 and Fig. 3).

RG decreases as salinity and temperature increase. The highest RG values for all saline concentrations were recorded in a temperature range of 20/10°C. The increase in temperature causes significant RG decreases and RG becomes null at temperature regimes above 35/25°C and 400 mM salinity. Surprisingly, under this temperature regime (35/25°C), the RG for medium salinity levels (100 and 200 mM NaCl) is higher than the records obtained for lower temperatures (30/20°C).

Table 1. ANOVA results for final germination percentage (FGP), mean time to germination (MTG) and rate of germination (RG) according to salinity (S), temperature (T), and S/T interaction (SxT). Numbers indicate F-values significant at p<0.0001 for T an S; p<0.03 for SxT.

Factor	FGP	MTG	RG
S	124.544	3.173	170.582
T	60.596	3.547	72.262
SxT	6.254	6.058	8.501

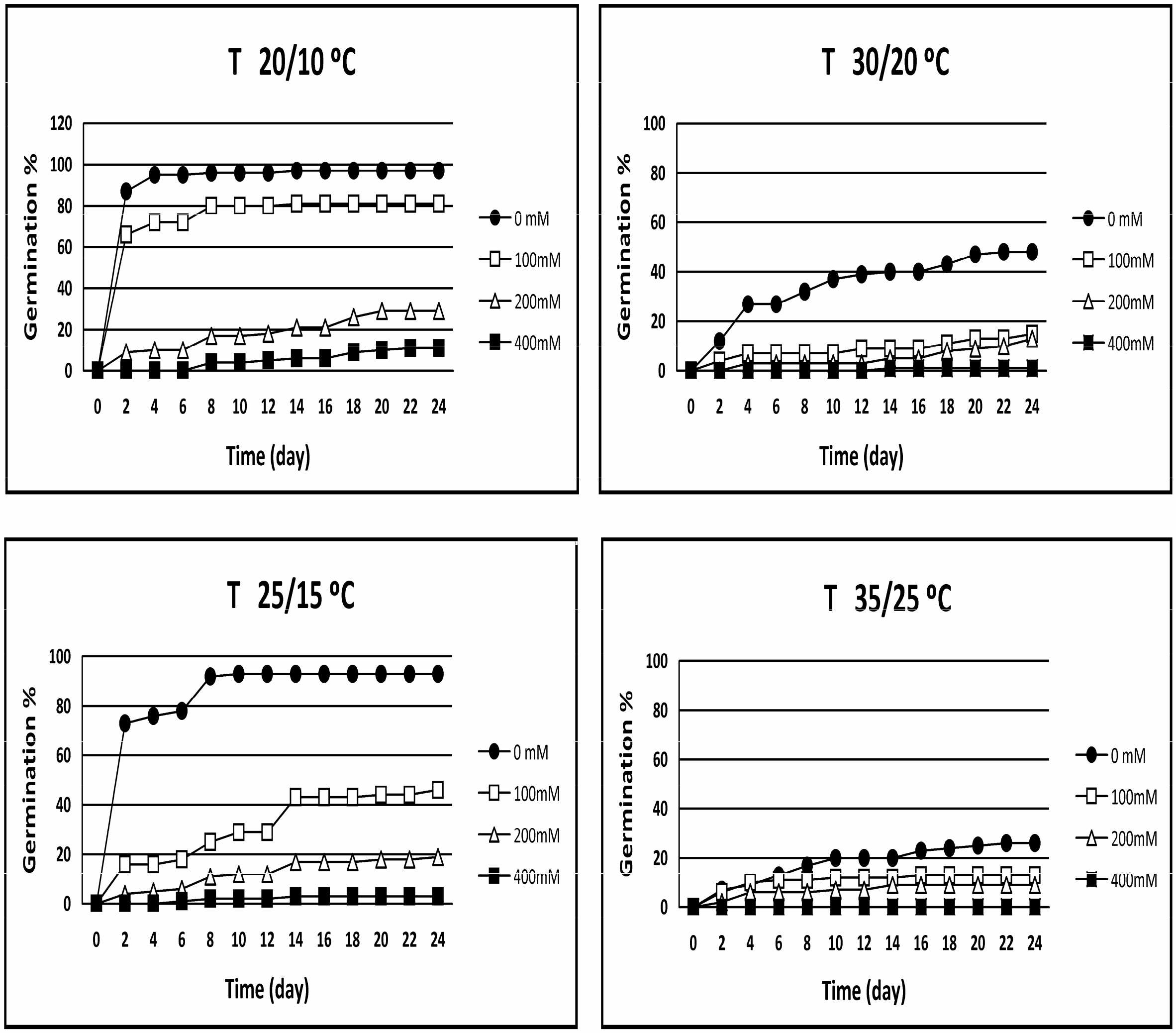

Fig. 1. Cumulative germination percentage of *Limonium insigne* seeds during 24 days under different temperature regimes (20/10, 25/15, 30/20, and 35/25 °C) and different salinity concentrations (0, 100, 200, and 400 mM).

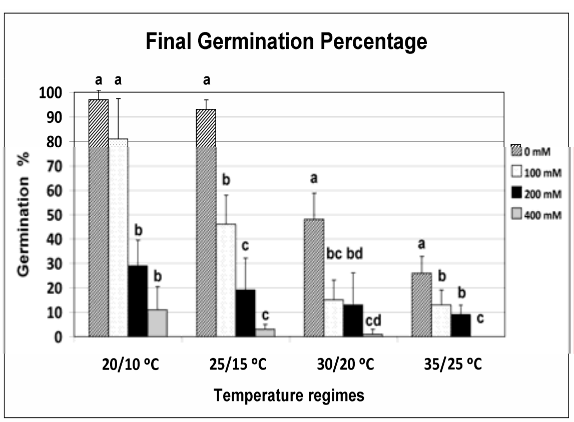

Fig. 2. Final germination percentage (FGP) of *Limonium insigne* seeds under different temperature regimes (20/10, 25/15, 30/20 and 35/25°C) and different salinity concentrations (0, 100, 200, and 400 mM). Values of FGP at each salinity level having the same letter are not significantly different (p<0.05); Bonferroni test.

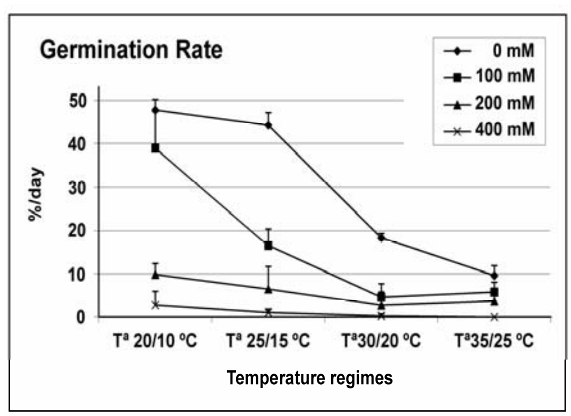

Fig. 3. Rate of germination (RG) of *Limonium insigne* seeds under different temperature regimes (20/10, 25/15, 30/20 and 35/25°C) and different salinity concentrations (0, 100, 200, and 400 mM).

Table 2. Final germination percentage (FGP), mean time to germination (MTG) and rate of germination (RG) in *Limonium insigne* taxa in four salinity treatments for 24 days.

Temperature regimes	Salinity treatment	FGP	MTG	RG
20/10 °C	0 mM	97 ± 3.83	2.36 ± 0.35	47.79 ± 2.44
	100 mM	81 ± 16.45	2.94 ± 0.50	39.00 ± 8.42
	200 mM	29 ± 10.52	9.20 ± 3.07	9.83 ± 2.53
	400 mM	11 ± 9.45	16.33 ± 3.71	2.75 ± 3.19
25/15 °C	0 mM	93 ± 3.83	3.15 ± 0.66	44.29 ± 2.89
	100 mM	46 ± 12.00	8.68 ± 1.59	16.50 ± 3.87
	200 mM	19 ± 13.22	10.01 ± 4.16	6.50 ± 5.18
	400 mM	3 ± 2.00	7.00 ± 5.77	1.04 ± 0.75
30/20 °C	0 mM	48 ± 10.83	7.19 ± 2.89	18.33 ± 1.01
	100 mM	15 ± 8.25	12.50 ± 4.92	4.63 ± 3.06
	200 mM	13 ± 13.22	12.57 ± 8.56	2.71 ± 3.10
	400 mM	1 ± 2.00	3.50 ± 7.00	0.25 ± 0.50
35/25 °C	0 mM	26 ± 6.93	8.13 ± 1.56	9.58 ± 2.28
	100 mM	13 ± 6.00	4.03 ± 1.81	5.79 ± 2.27
	200 mM	9 ± 3.83	6.00 ± 3.31	3.67 ± 1.58
	400 mM	0 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Values are mean ± S.D. (n = 4)

Discussion

The success of these halophyte populations depends on the germination performance of their seeds. Seed germination usually takes place in early spring, at a time when salt concentration in the soil decreases (Ungar, 1995). Research has shown that salinity prevents halophyte seed germination in two ways: it either inhibits germination processes when the salt tolerance of the species has been surpassed, or it delays germination as a result of the stress caused (Ungar, 1995; Gul & Weber, 1999).

As regards seed germination, the responses of halophytes to salinity are highly variable and there are responses specific to each species. According to the studies conducted by certain authors (Badger & Ungar, 1989; Gutterman, 1992; Huang & Gutterman, 1999; Pujol *et al*., 2000), the germination of wild halophyte species is governed by various environmental factors, in particular, light, temperature and salinity. Salinity and temperature interact in the control of seed germination in such a way that tolerance limits to salinity can vary from one temperature to another (Gul & Weber, 1999). In our case, we confirmed that the performance of *L. insigne* seed germination under the saline conditions of each of our 24-day tests was governed by temperature.

Saline soils undergo moisture and salinity fluctuations throughout the year. In the summer months, the soil surface tends to have a higher level of salinity because of evaporation. This fact not only causes the germination rate to decrease but also delays the beginning of the whole process. Germination can be inhibited altogether when the salinity level exceeds the tolerance limits of the species (Pujol *et al*., 2000; Khan & Gulzar, 2003; Martins *et al*., 2012). According to Del Vecchio *et al*. (2012), environment salinity induces seed dormancy in *Crucianella maritima* and increases salt sensitivity at high temperatures. These results coincide with those of our experiment with *L insigne* seeds: increases in salinity at different temperatures, particularly above 30°C, reduce the germination rate and delay germination. According to Khan & Ungar (1996) and Rejili *et al*. (2009), the detrimental effect of NaCl at the highest temperatures can be explained by the toxicity of the Na^+ ion, which generally causes irreversible damage. However, not all halophytes behave in the same way. According to the studies conducted by Gulzar *et al*. (2001), *Urachondra setulosa* does not germinate well at low temperatures, in the 20/10°C range, nor at high temperatures, in the 35/25°C range. We conclude that each halophyte species presents an optimal germination temperature, below and above which its germination rate suffers. In the case of *L. insigne*, we can say that the optimal germination temperature is in the 20/10°C range, i.e., typical spring temperatures. The highest rainfall records are also in spring in their natural habitats. Higher temperatures reduce the FGP and RG. This indicates that the germination of *L. insigne* decreases in summer as a result of the increase in temperature. Similar results were observed by Ebrahimi & Eslami (2011) in seeds of *Ceratocarpus arenarius*. These ranges of temperature indicate that April and May are the most favourable months for germination in *L. insigne*.

Khan & Ungar (1998) have reported that the inhibiting effect of salinity at higher temperatures prevents seeds from germinating in saline habitats and therefore prevents high mortality rates in seedlings during severe water shortage. According to our results, exposure to high saline concentrations and a high temperature range not only inhibits FGP but also reduces MTG and RG. This same response has been observed by Debez *et al*. (2004) in *Cakile maritima*, by Li (2008) on the species *Limonium sinense*, *Glycine soja* and *Sorghum sudanese*, and by Rejili *et al*. (2009) on *Lotus creticus*. In their study on *Limonium emarginatum*, Redondo-Gómez *et al*. (2008) conclude that the two most important effects of salinity are a decrease in the germination rate and an increase in the germination time. We have observed both these effects in our experiments with *Limonium insigne* seeds.

Germination is one of the most critical processes in the life cycle of halophytes (Ungar, 1996). A number of studies (Greenwood & MacFarlane, 2006; Vicente *et al*., 2007; Engloner, 2009; Vicente *et al*., 2009) confirm that halophyte seeds behave in the same way in the face of salinity: the start of germination is delayed, the germination rate is reduced and some seeds remain dormant. In our first three tests (20/10 °C, 25/15°C and 30/20°C) the FPG exhibits a significant reduction as salinity increases. This leads us to conclude that the germination power of *L. insigne* decreases as room temperature and salt concentration in the soil increase.

In line with the studies conducted by Gulzar & Khan (2001), Wei *et al*. (2008), Ebrahimi & Eslami (2011) and Giménez *et al*. (2013), who observed that an increase in salinity causes a decrease in germination rate and germination speed, and even complete inhibition when the salt tolerance limit of the species is exceeded, we can confirm that the germination of *Limonium insigne* is affected by an increase in salinity.

With the results of our study we can say that the ideal conditions both for the germination rate and the germination speed of *L. insigne* seeds under laboratory conditions are 20/10°C and null salinity. These results could be helpful in the design of an action plan for the preservation and restoration of the wild habitats of this endemic plant. Further studies are needed for a proper understanding of the entire life cycle of the species in order to determine the most critical stages for the survival of wild populations.

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