Effect of light, temperature, and salinity and drought stresses on seed germination of *Hypericum ericoides*, a wild plant with ornamental potential

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Abstract

Hypericum ericoides is a rock plant of potential ornamental use in sustainable gardening in Mediterranean urban environment. This study was carried out to investigate the effects of temperature, light, salinity and soil moisture at the stage of plant life most sensitive to abiotic stresses (seed germination). The results indicate that light is not a germination requirement, while temperature the main factor that regulates the germination process of this species. Seed germination was inhibited by warm temperatures of 25 and 30 ºC. By contrast, intermediate temperatures of 10, 15 and 20 ºC induced high germination percentages both in alternating light/darkness and continuous darkness. The alternating temperature of 12/20 ºC led to germination percentages close to 100% and the highest germination speed, making this the suggested optimal for germination. The germination values attained demonstrated the absence of dormancy in *H. ericoides* seeds. In general, salinity and drought stress (induced by NaCl and PEG solutes, respectively) caused similar effects, reducing and delaying seed germination as the osmotic potential decreased (from 0 to -1.68 MPa). However, while most non-germinated seeds remained viable during exposure to all the osmotic potentials induced by PEG and germinated when drought stress was alleviated, the highest levels of salt stress permanently inhibited

germination, although not in all seeds. These results indicated that germination inhibition under both NaCl and PEG stress is mainly due to the low water potential caused by osmotic stress, while salt stress had the added toxic effect of specific ions at the highest concentrations of NaCl. So, *H. ericoides* seeds can germinate well under conditions of moderate salinity and high drought stress, making it a promising species for use in sustainable urban gardening, with a low input of irrigation water of low quality.

Keywords: abiotic stresses, seed ecology, urban gardening, osmotic potential, germination recovery, ornamental species

Introduction

The genus *Hypericum* L (Guttiferae) includes about 488 species of shrubs, perennial and annual herbs, and more infrequently, trees with a worldwide distribution (Robson, 2012). Many *Hypericum* species are medicinal plants used by traditional medicine systems in many countries around the world, or are sold as ornamentals (Crockett and Robson, 2011). Among the wild species, *Hypericum ericoides* is a rock plant that can be considered of potential use as an ornamental species for sustainable gardening in Mediterranean urban environments, as we will justify later. It is an evergreen dwarf shrub (2-40 cm) that grows in a cracks in the calcareous rock or scree on calcareous soils in the east and south-east of the Iberian Peninsula (Castroviejo et al., 1993). Its stems are articulated and very branched, with 4 longitudinal lines at the internodes. The leaves range in size between 1-4.5 and 0.5-0.8 mm (length/width ratio: 3-7), are whorled in groups of four, densely imbricated, from linear to lanceolate and recurved in shape, papillose, with translucent glands. Its flowers, which start to bloom in May and continue

to bloom throughout summer, are often yellow in colour, and have five petals 4-7 mm in length. The seeds are brown and 0.9-1 mm long.

Mediterranean species of wild flora are of increasing interest for use in semi-arid climate garden designs because of their capacity to adapt to adverse environmental conditions: hot day and low night temperatures, drought and salinity (Franco et al., 2006). In addition, due to their endemic origin, Mediterranean species represent the most suitable vegetation for tolerating the stresses of urban environments (Benvenuti et al., 2016). In this type of species, succulent leaves, compact twiggy growth, small evergreen leaves with a thick cuticle are all adaptations to water loss, necessary characteristics in plants exposed to the climatic conditions of semi-arid gardens (Dunnett and Kingsbury, 2004). Many of these characteristics are present in the rock plant *H. ericoides*, which makes it a good candidate for use in urban landscaping, including coverage applications in vertical gardens and green roofs. In the last few years, new urban greening concepts have been developed to mitigate the multiple contaminants resulting from human activities within the built-up environment (Hunter et al., 2014). Although vertical gardens were initially inspired by the epiphytic plant growth of tropical forests, a wide range of plant types could adapt to and grow well in a vertical garden (Pérez-Urrestarazu et al., 2016).

In semi-arid Mediterranean regions, such as SE Spain, with structural water deficits, the use of treated municipal wastewater in gardening is widespread because it leads to a more effective water resources management (Pedrero et al., 2010). However, in general, as salinity increases in the treated wastewater used for irrigation, so do the problems associated with the establishment of ornamental plants. In such situations, an appropriate selection of plants with high salt tolerance, combined with good irrigation management, can minimize the potential impact of the salts or specific ions of concern.

In this context, a study of the germination behaviour of plants must be the first step toward the introduction of a new ornamental crops, since seed germination is the initial stage in the life cycle of plants (Ungar, 1995), and the most sensitive stage to abiotic stress (Patade et al., 2011). Temperature and water potential are the most important external factors that influence germination (Alvarado and Bradford, 2002), the environmental stresses of salinity and drought being the most common problems affecting seed germination in arid and semi-arid regions (Mohammadizad et al., 2013). An increase in salinity leads to a reduction and/or delay in germination of both halophyte and glycophyte seeds (Albregts and Howard, 1973). Salinity may inhibit seed germination due to osmotic effects that prevent water uptake by the seed, or as a result of toxic ionic effects on germinating seeds (Dodd and Donovan, 1999). If inhibition is due to osmotic potential, seed germination recovery will occur once the osmotic effect is removed. But if it is due to ionic toxicity, the recovery is not to be expected. Many authors have used sodium chloride solutions to study salinity tolerance in the germination of both types of seed (Herranz et al., 2004). Moreover, to know whether the saline inhibition of germination in NaCl treatments is osmotic and/or toxic and irreversible, polyethylene glycol (PEG) has been used in many of these germination studies (Mohammadizad et al., 2013). The same water potential of NaCl treatments can be simulated using PEG solutions, although the inhibition of seed germination under PEG treatments is mainly due to osmotic effects (Dodd and Donovan, 1999) because PEG is chemically inert and non-toxic, and seems not to penetrate the seminal cover (Thill et al., 1979). Indeed, to assess plant drought tolerance in the germination and seedling stages, drought stress can be induced using polyethylene glycol as described by Michel and Kauffman (1973) (Lin et al., 2015).

When it is possible to carry out direct sowing in a garden of semi-arid climate with low inputs of low quality irrigation water, information about salinity and drought

tolerance during seed germination would be of great practical interest. In the case of *H. ericoides*, its optimal germination conditions and its response to salinity and water deficit during germination are still unknown. Seeds of several *Hypericum* species require light and an alternating temperature regime for germination (Sánchez-Coronado et al., 2015), which is a common requirement of small seeds (Thompson et al., 2001). Although the germination of several *Hypericum* species has been studied, their salt and drought tolerance at germination have not been characterised. Therefore, in the present study, the effect of light, temperature and salinity and drought stresses on seed germination of *H. ericoides*, a wild plant of ornamental potential was evaluated.

2. Materials and methods

2.1. Seed collection

The study was carried out using seeds of *H. ericoides* collected from the population at Roldán, a mountain flanking the coast of Cartagena (Murcia; 37º 35´ 21´´N; 1º 2´ 25´´W), with a semi-arid Mediterranean climate characterized by irregular rainfall and a severe, dry summer period. Annual mean precipitation is around 300 mm, most of which falls in autumn, and the mean annual temperature is 17 ºC. August is the warmest month, with an average temperature of 24.9 ºC and a maximum of 42 ºC. The coldest month is January, with an average temperature of 10.6 ºC and a minimum always > 0 ºC. All seeds were collected from mature fruits in October 2015 and taken to the laboratory, where they were cleaned and divided into two lots. They were kept in paper bags and stored at room temperature (20 ºC) for 3 weeks and for two months, respectively, until the germination tests began. Before starting the experiments, seeds were sterilized with a solution of 3% sodium hypochlorite for two minutes.

2.2. Germination experiments

2.2.1. Experiment 1: effect of light and temperature regime on seed germination

For this experiment, the lot of seeds stored at room temperature for 3 weeks was tested at five constant (10, 15, 20, 25 and 30 ºC) and two alternating (12/20 and 21/30 ºC) temperature regimes. At each temperature, seeds were incubated in continuous darkness and with a 12 h/12 h light/dark regime (hereafter alternating light/darkness). In seeds incubated at alternating temperature with alternating light/darkness, the light hours coincided with the 12 h of highest temperature. The light source was a white fluorescent tube with a mean photon flux density of 35 μmol m^{-2} s^{-1} (400–700 nm). Each treatment contained four replicates of fifty seeds placed on Petri dishes with filter paper moistened with 5 ml distilled water. The Petri dishes were hermetically sealed with parafilm to prevent evaporation. For the continuous darkness treatments, the Petri dishes were wrapped in two layers of aluminium foil until the end of the experiment. Seeds were counted every 2 d in the treatments with alternating light/darkness, removing the germinating seeds. Seeds were considered to have germinated when the radicle emerged with at least 1 mm visible. In order not to interrupt seed exposure to darkness, germination in the continuous darkness treatments was only recorded at the end of the experiment. Both the alternating light/darkness and continuous darkness experiments finished after 30 d. At the end of the germination period, the germination percentage and the mean time to germination (MTG) were calculated. The latter was determined according to the following formula (Brenchley and Probert, 1998): $MTG = (\sum_i n_i \times d_i)/N$, where n is the number of seeds germinated at day i, d the incubation period in days and N the total number of seeds germinated in the treatment. MTG was not calculated in the seeds exposed to continuous darkness.

2.2.2. Experiment 2: effect of salinity and drought stresses on seed germination

149 The second lot of seeds were used to study species response to salinity and drought
150 stresses, which were induced using NaCl and PEG 8000, respectively. Distilled water was
151 used in both experiments as the control treatment. Seven levels of salinity (50, 100, 150,
152 200, 250, 300 and 350 mM NaCl) were used, which corresponded to a water potential of
153 -0.24, -0.48, -0.72, -0.96, -1.20, -1.44 and -1.68 MPa, respectively, as determined by a
154 dew point microvolter. Polyethylene glycol solutions at the same NaCl potentials were
155 prepared by dissolving different concentrations of PEG 8000 in deionized water
156 according to the values of the water potentials, as described by Michel and Kaufmann
157 (1973). Four replicates of 50 seeds per treatment were placed in tightly sealed Petri dishes
158 with filter paper moistened with 5 ml of a NaCl solution or PEG 8000 at zero (distilled
159 water control), -0.24, -0.48, -0.72, -0.96, -1.2, -1.44 and -1.68 MPa. The light and
160 temperature germination conditions were those that induced the highest percentage of
161 germination in the previous experiment (12 h/12 h light/dark regime and 12/20 ºC).
162 Germination parameters were recorded every 2 d, removing the germinating seeds from
163 the plates. Germination percentage in the osmotic treatments of NaCl and PEG solutes
164 and the mean time to germination (MTG) were calculated as described in experiment 1.
165 After 30 d, ungerminated seeds from the NaCl and PEG treatments were
166 transferred to new Petri dishes containing distilled water and maintained in the same
167 temperature/light regime for another 30 d to study germination recovery. The recovery
168 test was made from the osmotic potential -0.72 MPa for both salt and drought stresses
169 due to the high germination rate reached in the treatments with the lower potentials (-0.24
170 and -0.48 MPa). The recovery percentage was calculated by using the equation: [(a - b)
171 / (c - b)] × 100, where a = total number of seeds germinated after recovery (in osmotic
172 treatments for the NaCl and PEG solutes plus recovery in distilled water), b = total
173 number of seeds germinated in osmotic treatments of NaCl and PEG solutes, and c = total

number of seeds (Gulzar and Khan, 2001). Also, MTG was calculated as described in experiment 1. Lastly, the final germination percentage was analysed (in osmotic treatments for the NaCl and PEG solutes plus recovery in distilled water).

2.3. Statistical analysis

For experiment 1, a two-way ANOVA was used to evaluate the effect of light (two levels) and temperature (five levels) on seed germination, and a one-way ANOVA to test the effect of temperature on MTG (light was excluded as a factor since there were no data on MTG for the continuous darkness treatments). When different osmotic treatments of NaCl and PEG solutes were used in experiment 2, a two-way ANOVA was used to evaluate the effects of solutes on both seed germination and MTG. The germination percentage data were arcsine transformed before statistical analysis to ensure homogeneity of variance. When significant main effects existed, differences were tested by Tukey's multiple comparison test at 95% confidence level. All statistical tests were performed using the software Statgraphics Plus version 2.1.

3. Results

3.1. Experiment 1: effect of light and temperature regime on seed germination

The results of the analysis of variance demonstrated that the effect of temperature on seed germination in *H. ericoides* was significant (Table 1). By contrast, light did not affect germination, although the interaction between both factors did. Analysis of variance comparing the temperatures showed that the highest germination percentage was obtained at alternating temperature of 12/20 ºC (Table 2), both in seeds incubated in alternating light/darkness (97%) and in continuous darkness (95%), while higher temperatures of 25, 30 and 21/30 ºC completely inhibited germination. Seeds exposed at

intermediate temperatures of 10, 15 and 20 ºC reached high percentage of germination in the two light conditions tested, which varied between 81 and 91%. In the same way to the germination percentage, temperature affected the speed of germination (Table 1). The shortest mean time to germination (6.9 ± 0.20 d) was achieved at 12/20 ºC (Table 2) and the longest mean time to germination (15.2 ± 2.04 d) was in seeds incubated at 10ºC.

3.2. Experiment 2: effect of salinity and drought stresses on seed germination

Significant differences were obtained for the two factors considered (osmotic potential and solute) and their interaction regarding both germination percentage and mean time to germination in *H. ericoides* seeds subjected to increasing levels of both salinity and drought stresses (Table 3). The results showed that this species was able to germinate up to -1.44 MPa osmotic potential (5.5%) induced by NaCl solution (Fig. 1.A). Analysis of variance comparing the decreasing osmotic potential levels of salinity stress showed that seed germination at -0.24 MPa NaCl (90%) was similar to that of the distilled water control (0.0 MPa) and at -0.48 MPa it was slightly lower (85%) than the control (Fig. 1.A). Moreover, at -0.72 and -0.96 MPa of salinity stress seed germination remained above 60%, and only at -1.68 MPa NaCl that was it almost completely inhibited. When decreasing osmotic potentials induced by PEG were compared, germination under drought stress showed a similar trend to that of seeds subjected to NaCl stress for the iso-osmotic potentials, except above the potential -0.72 MPa that drastically reduced seed germination under PEG stress (Fig. 1.A). On the other hand, decreased osmotic potentials induced by NaCl led to a gradual increase in mean time to germination compared to the control (Table 4). However, the mean time to germination of seeds under PEG stress was not affected up to -0.72 MPa, when the germination speed was significantly reduced compared with the control (Table 4).

When ungerminated seeds from osmotic treatments above -0.48 MPa induced by NaCl and PEG were transferred to distilled water, only the germination recovery was affected by the solute, while the mean time to germination was also affected by the osmotic potential (Table 3). Both under salinity and drought stresses, the recovery in seed germination was similar at all osmotic potentials induced by either solute (Fig. 1.B), the fact that the differences were not significant probably being due to a very high standard deviation in the germination recovery data (data not shown). Generally, germination recovery in seeds that were subjected to drought stress was greater (51-81%) than that of seeds under salt stress (33-53%), differences being significant at the iso-osmotic potentials above -0.96 MPa (Fig. 1.B). The mean time to germination in the recovery of seeds both under salinity and drought stress was similar in all osmotic treatments, except for -1.68 MPa induced by PEG, which significantly reduced the speed of germination recovery (Table 4).

Lastly, the final germination percentage after the recovery experiments in seeds subjected to salinity and drought stresses was calculated. Analysis of variance showed that the final germination of *H. ericoides* seeds was significantly affected for the two factors considered (osmotic potential and solute) and their interaction (Table 3). Under salinity stress induced by NaCl, the two lower levels of salinity (-0.24 and -0.48 MPa NaCl) did not cause a reduction in the final germination percentage compared with the non-saline conditions of the control (Fig. 1.C). Moreover, increasing levels of salinity from -0.72 to -1.20 MPa did not affect the final percentage of germination compared with that obtained at -0.48 MPa, only higher salinity levels of -1.44 and -1.68 MPa NaCl significantly reducing the seed germination (to below 35%). As regards drought stress, the final germination percentage was very high for all osmotic potentials, 73-88% of seeds germinating (Fig. 1.C), but without reaching in any case the germination percentage

obtained in the corresponding control treatment (96%). Comparing both stresses, only for the higher levels of stress (-1.22, -1.44 and -1.68 MPa), seeds that were subject to drought stress showed higher final germination percentages than seeds under salinity stress (Fig. 1.C).

4. Discussion

Germination of the *H. ericoides* seeds was not affected by the light regime, and took place both in light and darkness with a slight improvement in light at a constant temperature of 20 ºC. These results disagree with those obtained in other *Hypericum* species, where their small seeds demonstrated a near absolute requirement for light to germinate (Sánchez-Coronado et al., 2015), while temperature was not a significant factor for the germination of some of those species (Pérez-García et al., 2006). In those small-seeded species, only seeds that are near to the soil surface will be able to germinate, since small seeds have much lower food reserves and their seedlings could have difficulties in emerging if they germinate too deep in the soil (Fenner and Thompson, 2005), as light is used by these seeds to detect whether they are close to the soil surface to germinate. In the case of *H. ericoides*, a rock plant, is clearly adapted to the scarcity and practically absence of soil to establish roots and survive, suggesting that light is not a requirement its small seeds to germinate. That the germination of this species is not light-dependent allows their seeds to germinate even when they are buried in the crevices of the rock or in accumulations of soil in its natural habitat, where sunlight cannot penetrate. If this species is used in urban gardening by direct seeding methods, this germination feature leads us to suppose that its seeds will have no problem germinating whether they are on the surface of the growing medium or buried by alterations in the sowing depth due to subsequent cultivation practices. The fact that light is not relevant for the germination of

small seeds is a characteristic of plants of arid zones, like *H. ericoides*, where vegetation cover is scarce and, therefore, competition for space is not a major factor that affects seedling establishment (Khurana and Singh, 2001). Photosensitivity also seems to be associated with perenniality, seeds of annual species showing higher light dependence for germination (De Villiers et al., 2003).

Our results show that the seeds of *H. ericoides* were not dormant, taking into account the 97% of seed germination obtained at an alternating temperature of 12/20 ºC. It seems that the characteristic thick seed coat of members of Hypericaceae (Baskin and Baskin, 2007) is not an important obstacle for germination. By contrast, other species of this genus present a complex dormancy, involving physiologically immature embryo and a hard seed coat that mechanically constraints embryo growth, requiring different conditions to maximise germination and overcome the seed dormancy (Camas and Caliskan, 2011; Sánchez-Coronado et al., 2015). Among the tested temperature conditions, intermediate temperatures between 10 and 20º C led to high germination percentages (81-90%), but only the alternating temperature of 12/20 ºC permitted almost 100% germination in both light and darkness. Moreover, this alternating temperature also promoted the highest germination speed (6.9 days), so it could be qualified as the "optimal temperature of germination". By contrast, at higher temperatures (> 20 ºC) seed germination was completely inhibited. Thermo-inhibition of germination has been found in other plant species (Belmedhi et al., 2018) and, according to the authors, it may be caused by high levels of endogenous abscisic acid (ABA). As occurs in other species in Mediterranean climates (Shütz et al., 2002), the germinative behaviour of this species is an adaptive response to the environmental conditions in its natural habitat - characterized by mild winters and dry hot summers. This may confer an ecological advantage, preventing germination in the hot temperatures of summer to avoid having to endure

299 extreme drought conditions as a seedling, but allowing extensive and rapid germination
300 during the autumn and winter with the corresponding greater availability of moisture in
301 the soil.

302 When moisture conditions are favourable, not only temperature but also salinity
303 represents a major factor affecting seed germination and seedling establishment in arid
304 and saline regions (Maraghni et al., 2010). In the present study, salinity stress resulted in
305 a reduction in the germination and a delay in the speed of seed germination. *H. ericoides*
306 is not described in the bibliography as a halophytic species since it does not grow naturally
307 in saline environments. However, if we compare it with most glycophytes, *H. ericoides*
308 can be considered a highly salt tolerant species. Results from other studies on glycophyte
309 species have shown greater sensitivity to salt stress, as in the case of *Salvia* species where
310 an increase in salinity above 50 mM quickly decreased seed germination (Al-Gharaibeh
311 et al., 2017), while *Diplotaxis harra* germination was completely inhibited at 200 mM
312 (Tlig et al., 2008). Flowers et al. (2010) proposed a salinity threshold of 200 mM NaCl to
313 separate glycophytes from halophytes. By contrast, although halophyte seeds germinate
314 best under non-saline conditions, they can germinate at higher salinities than glycophytes
315 (Ungar, 1995), and the threshold of salinity for a significant reduction in seed germination
316 ranges from 100-500 mM NaCl (Khan and Ungar, 2001). In the present study, seed
317 germination in *H. ericoides* was 5.5% at 300 mM NaCl (-1.44 MPa), confirming the
318 germination ability of this species in high salinity conditions. Compared with some
319 halophyte species such as *Chloris virgata* (Lin et al., 2015), *H. ericoides* seeds showed
320 tolerance to higher salinity concentrations and slower decreases in germination
321 percentages as salt stress increased. On the other hand, although the seeds of halophytes
322 may be unable to germinate under hypersaline conditions, they may germinate when the
323 salinity decreases. The resistance to salinity is very well studied in halophytes and is used

as a criterion to distinguish them from most glycophytes (Ungar, 1995). In the case of *H. ericoides*, a recovery of its germination response has been demonstrated when seeds are transferred to distilled water after being exposed to saline solutions. Even the seeds exposed to high salinity concentrations (300-350 mM) showed a 24-33 % recovery of germination in distilled water. These results suggest that *H. ericoides* seeds could remain viable in the soil under natural conditions when the salinity stress exceeds its tolerance limit, and germinate when the salinity decreases. This ability suggests the existence of resistance to salinity in this species, as in most halophytes (Ungar, 1995). Some studies have demonstrated that similar strategies are used by most halophytes and glycophytes in response to salt stress (Hasegawa et al., 2000). As regards the final percentage of germination after 30 days of a recovery period, *H ericoides* seeds exposed to increasing levels of salinity from 150 to 250 mM reached final germination percentages slightly lower than those attained in non-saline conditions. So, this range of salinity only delayed their germination but did not lead the seeds to lose viability. These results indicate that seeds exposed to moderate salinity levels were not damaged by a specific ionic toxicity and that the osmotic effect prevented their germination. However, the higher salinity levels (300-350 mM) led to much lower final germination (< 35%) than the control, demonstrating that only exposure to higher concentrations of sodium chloride permanently inhibit germination, but not in all seeds. High concentration of sodium chloride probably resulted in the accumulation of Na^+ and Cl^- toxic ions in the embryo, which would have compromised metabolic processes of seed germination and killed more than half of the seeds (Bajji et al., 2011). However, a significant fraction of seeds was still tolerant to the toxic ionic effect induced by the highest concentrations of NaCl. All this strongly suggests that salinity stress affects seed germination of *H. ericoides* through an osmotic effect that reduces seed imbibition under a moderate salt stress, and through

an ion toxic effect on some of the seed embryos exposed to high salt stress, which can make them lose their viability (Guan et al., 2009). Caliskan et al. (2017) also demonstrated that *H. pruinatum* is a salt tolerant species and that salinity stress has a marked influence on the accumulation of phenolic constituents, which play an important physiological role in such tolerance.

In general, the effect of drought stress had a similar trend to that of salinity stress, reducing and delaying seed germination with increasing values of osmotic potential. However, the source of osmotic potential influenced the response of *H. ericoides* to the stresses, and germination was more inhibited under PEG stress compared with NaCl stress at the equivalent osmotic potentials below -0.72 MPa. This result indicates a stronger effect of stress by drought. Similar results have been reported in species of other plant families (Patané et al., 2013). However, most *H. ericoides* seeds remained viable during exposure to all osmotic potentials induced by PEG and germinated when the drought stress was alleviated, reaching almost the same final percentage of germination after recovery as in non-stress conditions. Thus, all the studied levels of drought stress simply reduced seed germination but did not cause a loss of viability. This demonstrates that seed germination inhibition under PEG stress is mainly due to the low water potential caused by osmosis stress, the same factor that inhibits seed germination under salt stress (Debez et al., 2004). By contrast, the highest levels of salt stress also caused loss of seed viability. We can conclude that the low osmotic potential inhibited the germination of *H. ericoides* seeds in both stress situations, but that salt stress added the toxic effect of specific ions at the highest concentrations of NaCl. Although both salinity and drought are important factors that can inhibit seed germination, *H. ericoides* seeds still germinates well under conditions of moderate salinity and high drought stress, and most non-germinated seeds will remain viable and germinate when both stresses are alleviated.

Conclusions

Of the abiotic factors that may have an effect on germination, such as temperature, light, salinity and soil moisture, this study has demonstrated that temperature is the main factor that regulates the germination process of this species. Our results suggest that light sensitivity does not play an important role in *H. ericoides* germination. By contrast, the inhibition of germination by warm temperatures was clear. However, while moderate temperatures favor high germination percentages, alternate temperatures of 12/20 ºC produced almost 100% germination. This indicates that *H. ericoides* seeds are not dormant Moreover, a germinating potential of seeds under salt and drought stress conditions has been demonstrated. Therefore, we suggest that *H. ericoides* is a promising species for use in sustainable urban gardening, where it can be established using direct seeding methods, with a low input of irrigation of low quality water. Whatever the case, good irrigation management can minimize the potential impacts of both salt and drought stresses, although further field trials will be needed to study the development of *H. ericoides* plants under these abiotic stress conditions.

Conflicts of interest: none

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Table 1. Analysis of variance for germination percentage (two factors) and mean time to germination (MTG, one factor) of the *Hypericum ericoides* seeds exposed to alternating light/darkness and continuous darkness at different conditions of temperature.

Factor	Germination percentage			MTG		
	df	F	*P*	df	F	*P*
Light (A)	1	0.08	0.776			
Temperature (B)	6	345.57	< 0.001	3	28.40	< 0.001
A x B	6	2.34	0.048			
Residual	42			12		

Degrees of freedom (df). F- Ratio and *P* values. Effects are considered significant for $P < 0.05$ and very significant for $P < 0.01$, except for light for germination percentage.

Table 2. Germination percentage of the *Hypericum ericoides* seeds exposed to alternating light/darkness and continuous darkness at different conditions of temperature and mean time to germination (MTG) in alternating light/darkness at the same temperatures. Means within a column that have a different uppercase letter are significantly different from each other, and means within a row that have a different lowercase letter are significantly different from each other (Tukey test, $P < 0.05$).

Temperature (ºC)	Germination percentage			MTG (days)
	Alternating light/darkness	Continuous darkness	Statistics	
10	89 ± 8.25 Ca	84 ± 4.62 Ba	F = 1.40, df = 1/6, *P* = 0.282	15.2 ± 2.04 C
15	90 ± 5.16 Ca	88 ± 0.00 BCa	F = 0.81, df = 1/6, *P* = 0.402	9.8 ± 0.54 B
20	81 ± 3.83 Ba	91 ± 2.00 Cb	F = 25.18, df = 1/6, *P* = 0.002	10.2 ± 1.49 B
25	1 ± 2.00 Aa	0 ± 0.00 Aa	F = 1.00, df = 1/6, *P* = 0.356	*
30	0 ± 0.00 A	0 ± 0.00 A	-	*
12/20	97 ± 2.00 Da	95 ± 3.83 Da	F = 0.25, df = 1/6, *P* = 0.634	6.9 ± 0.20 A
21/30	0 ± 0.00 A	0 ± 0.00 A	-	*
Statistics	F = 140.95 df = 6/21 *P* < 0.001	F = 229.12 df = 6/21 *P* < 0.001		F = 28.40 df = 3/12 *P* < 0.001

Mean time to germination values could not be estimated because germination percentage was ≤1%. Therefore, these treatments were excluded from ANOVA

Table 3. Analysis of variance for germination percentage (two factors) and mean time to germination (MTG, two factors) of the *Hypericum ericoides* seeds exposed to stresses induced by NaCl and PEG solutes and their recovery in distilled water after stresses.

Factor	Germination (%) under stresses			MTG (days) under stresses		
	df	F	*P*	df	F	*P*
Osmotic potential (A)	7	93.87	< 0.001	5	141.69	< 0.001
Solute (B)	1	6.40	0.015	1	7.39	0.010
A x B	7	2.23	0.048	5	5.28	0.001
Residual	48			36		
	Recovery germination (%)			MTG (days) in the recovery		
	df	F	*P*	df	F	*P*
Osmotic potential (A)	4	1.43	0.2478	4	2.93	0.038
Solute (B)	1	22.80	< 0.001	1	5.89	0.022
A x B	4	1.64	0.191	4	1.73	0.169
Residual	30			30		
	Total germination (%)					
	df	F	*P*			
Osmotic potential (A)	7	13.66	< 0.001			
Solute (B)	1	17.11	< 0.001			
A x B	7	7.63	< 0.001			
Residual	48					

Degrees of freedom from the numerator (df). F- Ratio and *P* values. Effects are considered significant for $P < 0.05$ and very significant for $P < 0.01$.

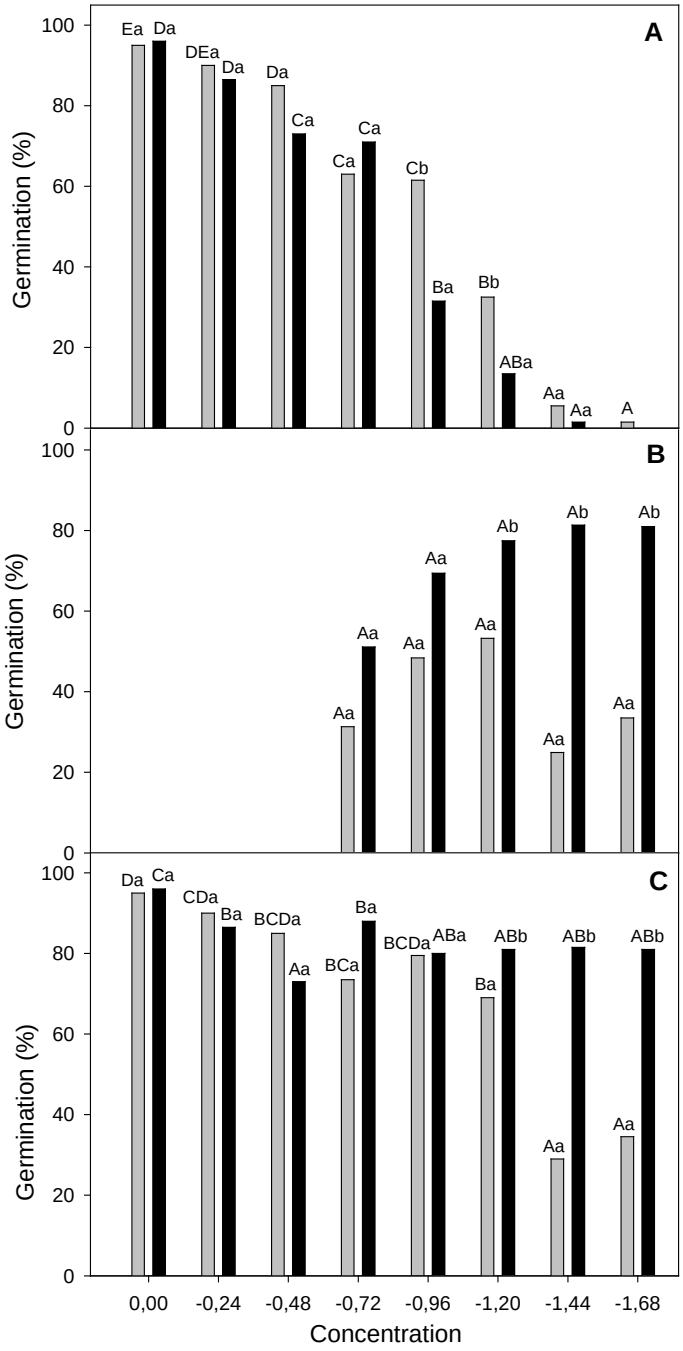

Figure 1: A) Germination percentage of *Hypericum ericoides* seeds in osmotic treatments of NaCl (clear bar) and PEG (dark bar) solutes at zero (distilled water control), -0.24, -0.48, -0.72, -0.96, -1.2, -1.44 and -1.68 MPa. B) Recovery percentage in distilled water of seeds from osmotic treatments of NaCl (clear bar) and PEG (dark bar) solutes at -0.72, -0.96, -1.2, -1.44 and -1.68 MPa. C) Final germination percentage of seeds after recovery experiments. For each subfigure the bars of the osmotic

potentials due to the same solute that have a different uppercase letter are significantly different from each other, and the bars of the same osmotic potential that have a different lowercase letter are significantly different from each other (Tukey test, $P < 0.05$).

Table 4. Mean time to germination (MTG) of the *Hypericum ericoides* seeds under salinity and drought stresses and in recovery experiments in distilled water after exposure to stresses. Means within a column that have a different uppercase letter are significantly different from each other, and means within a row that have a different lowercase letter are significantly different from each other (Tukey test, $P < 0.05$).

	MTG (days) under stress			MTG (days) in recovery experiments		
Osmotic potential (MPa)	Salinity	Drought	Statistics	Salinity	Drought	Statistics
0.0	6.8 ± 0.31 Aa	7.0 ± 0.34 Aa	F = 1.10, df = 1/6, *P* = 0.334			
-0.24	8.1 ± 0.76 Ba	7.2 ± 0.32 Aa	F = 5.10, df = 1/6, *P* = 0.064	-	-	
-0.48	8.9 ± 0.81 Ba	8.4 ± 0.41 Aa	F = 1.46, df = 1/6, *P* = 0.272	-	-	
-0.72	11.0 ± 0.91 Ca	11.5 ± 0.67 Ba	F = 0.70, df = 1/6, *P* = 0.434	4.9 ± 1.16 Aa	3.1 ± 2.52 Aa	F = 1.97, df = 1/6, *P* = 0.211
-0.96	12.8 ± 0.93 Da	15.3 ± 1.91 Ca	F = 5.64, df = 1/6, *P* = 0.055	6.8 ± 2.73 Aa	3.9 ± 1.19 Aa	F = 4.51, df = 1/6, *P* = 0.078
-1.20	15.6 ± 0.73 Da	18.3 ± 1.62 Db	F = 9.12, df = 1/6, *P* = 0.023	5.3 ± 0.94 Aa	4.0 ± 0.63 Aa	F = 5.37, df = 1/6, *P* = 0.059
-1.44	-	-		5.2 ± 0.82 Aa	4.4 ± 0.62 Aa	F = 2.66, df = 1/6, *P* = 0.154
-1.68	-	-		6.0 ± 2.02 Aa	7.0 ± 1.76 Ba	F = 0.52, df = 1/6, *P* = 0.500
Statistics	F = 72.80	F = 73.82		F = 0.80	F = 4.83	
	df = 5/18	df = 5/18		df = 4/15	df = 4/15	
	P < 0.001	*P* < 0.001		*P* = 0.543	*P* = 0.012	