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Salinity and temperature influencing seed germination of Mediterranean Aleppo pine (*Pinus halepensis* Mill.): an ecological adaptation to saline environments

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Abstract

In Mediterranean basin, *Pinus halepensis* Mill. is the dominant conifer used in afforestation and landscape projects. The ecological, aesthetic and commercial benefits make this conifer a precious tree for both woody production and land rehabilitation. Nevertheless, detailed studies on the adaptation of this tree to abiotic environmental constraints in the Mediterranean areas remain limited up until now. Therefore, laboratory experiments were conducted to assess the effects of the interactive impact of temperature and salinity on the germinability potential of *P. halepensis* seeds. The cones of *P. halepensis* used in this experiment were harvested from the Gotaïa forest at Djelfa province, Algeria. The seeds were removed from their cones and surface-sterilized by soaking for five min in 10% NaClO. Seeds were germinated at three temperature regimes (10–20°C, 15–25°C and 20–30°C) and four levels of salinity (0, 50, 100, and 150 mM NaCl) at photoperiodic lighting (16 h of light : 8 h of dark). For each treatment 04 replicates of 25 seeds were placed in Petri dish with 5 ml of test solutions. A completely randomized design (CRD) was used for the experiment. Results revealed that the uppermost germinability was recorded in distilled H_2O and the progressive enhancement of NaCl significantly ($P < 0.001$) suppressed germination. At 15–25°C (dark : light thermoperiod), *P. halepensis* seeds showed approximately 90% of germination at 0 mM NaCl. However, less germination percentage (GP) was obtained at 10–20°C and 20–30°C. Values of *Timson's* index were also suppressed significantly ($P < 0.001$) with an increase under salt stress at all thermoperiods but at least at 15–25°C. These funding suggest that seeding of *P. halepensis* may be an effective path for rehabilitation of degraded lands where salinity and drought are major features of the arid ecosystems. Yet, this conclusion still needs verification by field experiments.

Keywords: *Pinus halepensis*, arid zones, sowing dates, afforestation, salt tolerance

Introduction

The Aleppo pine (*Pinus halepensis* Mill.) is a Mediterranean conifer of high ecologic and economic importance. However, harsh environmental conditions and illicit exploitation of wood reduce *P. halepensis* forests and lead to restraint its natural propagation (Guit et al. 2015). The ecological adaptation and land rehabilitation use of *P. halepensis* makes this species essential for land restoration and multi-purpose forestry (Maestre and Cortina 2004).

Soil salinization is one of the most negative stressors perturbing seed germination and woody species regeneration in arid and semi-arid Mediterranean lands. Salt stress affects seed germinability by reducing osmotic potential (ψ_0), limiting seed hydration, and/or through ion toxicity, decreasing seed viability (Sidari et al. 2008). Soil salinity can interact with thermoperiods to affect seed germination behaviour of many plant species, with the highest suppression due to salt stress habitually found at the minimal or maximal thresholds of temperature tolerance (Delgado Fernández et al. 2016).

Pinus halepensis produces abundant cones and seeds at the end of summer. After dissemination, the seeds persist in the seed bank until the next rainwater season which not only reduce the high temperature and salinity but also offer much required moisture for seed imbibition (Kadik 1986).

Previous studies have shown that many species of genus *Pinus*, such as *P. banksiana* (Croser et al. 2001), *P. nigra* (Yücel et al. 2008), *P. brutia* (Al-Hawija et al. 2014) and

P. elliottii (Zhang and Yu 2019) could survive under saline conditions (> 200 mM NaCl); and low salinity (50 mM) did not effects seed germinability of these species. In Algeria, *P. halepensis* is the most widespread and common woody conifer of the natural forests, where it occupies 852,000 ha and predominantly colonizes the calcareous soils (Guit et al. 2015). Nevertheless, despite the importance of this species, there is little information documenting its tolerance to salinity (Nedjimi 2017, Zouidi et al. 2019). Therefore, selection of coniferous tree species that could be used in restoration of degraded lands necessitates information about their seed germination requirements for its establishment in the field environment. Under field conditions, success or failure of natural propagation of species depends essentially on seed germination responses to various external stressors as heat, salinity and drought (Bhatt et al. 2019). The knowledge of the relationship between seed germination, drought and salinity is essential to determine the occupation capability of species in harsh biotopes (Nedjimi et al. 2014, Nedjimi and Zemmiri 2019).

Salt stress is predominantly detrimental in arid areas due to the interactive impacts of rainfall scarcity and the high diurnal temperatures (An et al. 2011). Generally, at the cardinal temperatures of germinability, the effect of salt stress is less harmful (Gul et al. 2013). In brackish lands, successful woody species establishment was related to salinity tolerance during the first stage of development (Sidari et al. 2008, Nasri and Benmahioul 2015). A critical phenomenon leading to seed germination failure in these areas is the existence of excessive salt concentrations in topsoil horizons due to high evapotranspiration (ETP) (Khadhri et al. 2011). However, seed germinability under hypersaline conditions improves after washing of salts by rainwater (Khan and Ungar 1996).

Previous research by Tsitsoni (2009) showed that *P. halepensis* seeds are not latent and accomplish better germination at humid season in natural forests of Greece. The maximum seed germination of *P. halepensis* was recorded at 20°C (Thanos 2000). To examine the tolerance of Spanish *P. halepensis* to heat temperatures, Martínez-Sánchez et al. (1995) demonstrated that heating of cones for 1 minute at 90, 110, 150 and 200°C did not affect seed viability. In this way, Calvo et al. (2013) indicated that seed viability and post-fire regeneration could give *P. halepensis* an advantage to cope with wildfires compared to *P. canariensis* seeds.

The germination inhibition by salinity may be accentuated by extreme temperatures, and the highest germination is usually found at optimal temperature (Gul et al., 2013). Our previous study revealed that *P. halepensis* seeds can germinate and tolerate a wide range of salinities (Nedjimi 2017). But the effect of interaction between salinity and temperature on germination of *P. halepensis* was not investigated.

Given the above information, the present work was undertaken to: (i) comprehend the seed germinability requirements of *P. halepensis,* and (ii) assess the effects of salt stress (S), temperature (T), and their combined action ($S \times T$) on germination percentage (GP) and the rate of germination (RG) of this species. Information obtained from this investigation will lead to better understanding seed germination requirements in saline conditions that can be applied for future reforestation projects.

Materials and methods

Mature cones of *P. halepensis* were harvested from trees grown in Gotaïa forest at Djelfa province, Algeria, and stored at 4°C. Prior to test germination, seeds were taken out their cones and sterilized with NaClO (10%) for five minutes to fungal decontamination and washed thoroughly with distilled water. NaCl solutions with 0, 50, 100, and 150 mM concentrations were prepared. For each treatment, 25 seeds/replicate, i.e. 100 seeds/treatment, were placed uniformly in Petri dishes with double sheets of filter paper and completely randomized pattern (CRD) were applied with four repetitions. In total, experiment was carried out using 1,200 seeds of *P. halipensis*. Germinability test was conducted in a programmed Phytotron at the temperatures of 10–20°C (low), 15–25°C (moderate) and 20–30°C (high), with a photoperiod of 16 h light : 8 h dark. Germinability was counted when seed radicle reached elongation of 2 mm.

Timson's index (% day^{-1}) was used to assess the rate of germination (RG) using the formula:

$$RG = \frac{\sum G_{2d}}{T} ,$$

where G_{2d} is the SG % after a 2-day interval and T is the total duration of the germinability (Hadi et al. 2018).

Speed of germinability was assessed by the time taken to reach 50% (T50) of germination (Soltani et al. 2015).

Data analysis

A two-way analysis of variance was applied using the SPSS 9.0 software package (SPSS 1999) to test the impact of NaCl, thermoperiod and their combined action (NaCl × thermoperiod) on GP and RG. Germinability results were arcsine converted to establish the variance homogeneity. *Tukey*'s test ($P < 0.001$) was used to determine if significant change between treatments. A linear regression between the SG and NaCl treatments was applied for different thermoperiods.

Results

A two-way analysis of variance showed significant impact of NaCl (F values = 99.61, $P < 0.001$), thermoperiod (F values = 117.81, $P < 0.001$) and their combination (NaCl × thermoperiod) (F values = 2.92, $P < 0.001$) on GP of *P. halepensis* seeds (Table 1).

Seed germinability decreased with increases salinity and the maximum germination occurred for seeds treated without salt stress (distilled water) (Figure 1). *Pinus halepensis* seeds were capable to emerge at all thermoperiods and the cardinal thermoperiod corresponds to 15–25°C (Figure 1). The closest regression relationships were found between GP and salinity with R^2 extending between 0.82 and 0.95 across the temperature regimes (Figure 2).

Table 1. Analysis of variance (ANOVA) indicating effects of salinity (*S*), temperature (*T*), and their combination (*S* × *T*) on germination of *P. halepensis* seeds

Independent variables	Salinity (*S*)		Temperature (*T*)		Interaction (*S* × *T*)	
	df	*F* values	df	*F* values	df	*F* values
Germination percentage (*GP*)	3	99.61***	2	117.81***	6	2.92***
Rate of germination (*RG*)	3	130.28***	2	132.95***	6	10.23***
T50	3	35.22***	2	1385.76***	6	29.15***

Note: Data represent degree of freedom (df) and *F* values significant at $P < 0.001$(***).

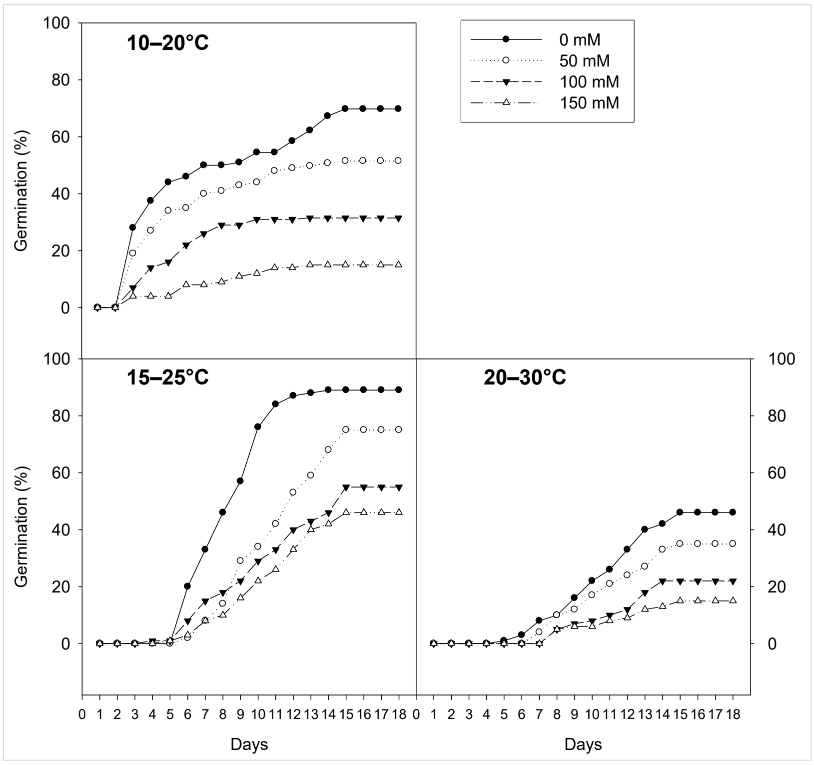

Figure 1. Mean final germination of *P. halepensis* seeds at different NaCl concentrations and thermoperiods

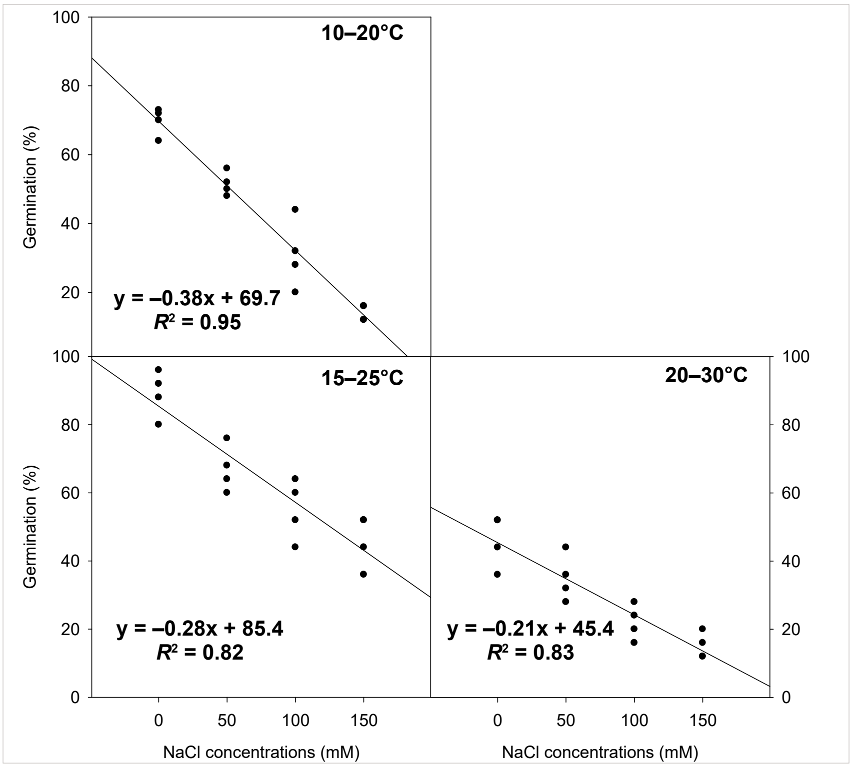

Figure 2. Regression plots of *P. halepensis* seed germination percentage (*GP*) at different NaCl concentrations and thermoperiods

Figure 3 showed that addition of NaCl significantly decreases the *RG* (*Timson*'s index) at all temperature regimes. A two-way analysis of variance indicated a significant effect of the two variants salinity (F values = 130.28, $P < 0.001$) and temperature (F values = 132.95, $P < 0.001$) and their combination (F values = 10.23, $P < 0.001$) on rate of germination (Table 1). *RG* at high thermoperiod (20–30°C) was lower than at other temperature regimes.

Although *RG* decreased with NaCl increments, the harmful effect of salt stress was less strong at 10–20°C and 15–25°C temperatures. There was no significant change in *RG* between the 10–20°C and 15–25°C treatments, except at the highest salt concentration where the 15–25°C treatment gave a significantly higher rate of germination.

A two-way analysis of variance showed significant effects of NaCl (F values = 35.22, $P < 0.001$), temperature (F values = 1385.76, $P < 0.001$) and their interactions (F values = 29.15, $P < 0.001$) on T50 of *P. halepensis* seeds (Table 1). For all thermoperiods, the T50 substantially increased with increasing NaCl increment, in which the maximum values were obtained by the higher levels of NaCl (Table 2).

Woody species selected for land afforestation programmes have to be resistant to considerable abiotic constraints such as heat/cold conditions, drought and salinity, particularly if they are established outside their natural geographic biotopes (Al-Hawija et al. 2014). Therefore, the propagation success or failure of these tree species needs a good acknowledgement of its germination requirements and behaviour towards these harsh conditions.

Table 2. Time to 50% germination (T50) of *P. halepensis* seeds at different NaCl concentrations and thermoperiods. Data are mean ± S.E. ($n = 4$). Different letters in the same column indicate significant difference (*Tukey's* test, $P < 0.001$)

NaCl, mM	Time to 50% germination (T50)		
	10–20°C	15–25°C	20–30°C
0	2.50 ^{c}	6.00 ^{c}	10.23 ^{b}
50	3.05 ^{b}	8.28 ^{b}	11.57 ^{b}
100	6.03 ^{a}	9.01 ^{b}	14.07 ^{a}
150	-	10.03 ^{a}	-

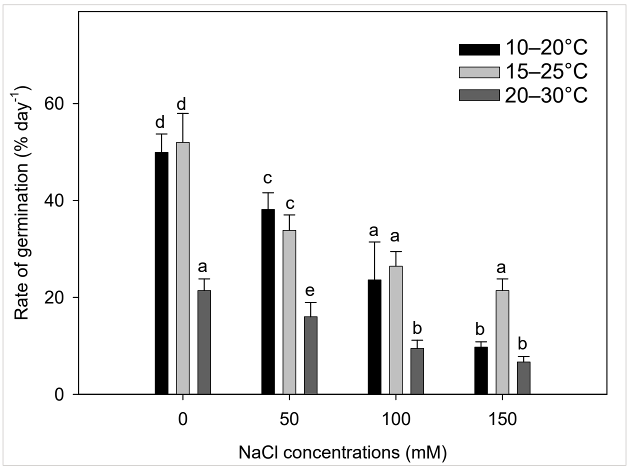

Figure 3. Rate of *P. halepensis* seed germination (*Timson's* index) at different NaCl concentrations and thermoperiods. Vertical bars are mean ± S.E. ($n = 4$). Different letters over identical bars indicate significant differences (*Tukey's* test $P < 0.001$)

In saline dry areas, seed emergence is affected by salinity stress which can reduce seed viability (Gul et al. 2013). In these environments, the detrimental impact of salt stress is often associated with temperature variations (Nedjimi et al. 2014). Our data indicate that germination and germination rate of *P. halepensis* were the highest in absence of salt stress (distilled H_2O) at all thermoperiods and reduced with increasing salinity. Comparable findings have been found in other pines species, i.e. *P. banksiana* (Croser et al. 2001), *P. nigra* (Yücel 2008) and *P. pinea* (Ganatsas and Tsakaldimi 2007, Sidari et al. 2008). Germination and germination rate of *P. halepensis* seeds were optimal at 15–25°C thermoperiod. The higher temperature regime of 20–30°C was more detrimental to germination than the lower one of 10–20°C.

In presence of salt stress, the decrease of the germinability of *P. halepensis* may be attributed to the incapacity of seeds to maintaining the water potential gradient, ψ_w, and overcome the high external osmotic potential (ψ_0) to absorb water from soil, and/or through the specific ion (Na^+ and Cl^-) toxicity to the embryo (Sidari et al. 2008).

The natural regeneration of *P. halepensis* stands depends exclusively upon seeds; their ecophysiological characteristics of germination were considered as of great importance in conservation of Mediterranean forest ecosystems. In unstressed circumstances, the seeds of *P. halepensis* are well recognized to emerge easily without specific environmental constraints (Tsitsoni 2009, Calvo et al. 2013). Temperature is considered to be the most important factors affecting the rate and speed of germinability, which directly affects the biochemical and enzymatic activities involved in the germination processes (reserve mobilization and radicle emergence) (Mazliak 1998). The dispersal of *P. halepensis* seeds occurs after natural opening of mature cones (in the age of 2–3 years) by high temperatures during summer (July–September) (Martínez-Sánchez et al. 1995). Under the field conditions, *P. halepensis* seeds follow a model very similar to the one when species are developing in the Mediterranean climate (Le Houérou 2004).

The rate of germination habitually increases linearly with temperature up to an optimal level, after which the germinability drops severely (Nedjimi 2014), but occasionally for some species, high or low temperatures induced secondary dormancy (named thermo-dormancy), in which germination will not arises at any thermoperiod, including the optimal temperature (Mazliak 1998). The optimal germination of Aleppo pine was recorded at 15–25°C. These particular thermoperiod requirements coincide with autumnal and spring temperatures, so the seed germinability is started early enough during wet season, and pine seedlings exploit the rainy period of winter and spring prior to unstressed period (salt and water stresses) founded in summer. Similar results have revealed that temperature of 20°C was cardinal for germination of *P. brutia* (Al-Hawija et al. 2014) and *P. bungeana* (Guo et al. 2020).

Our data show that the seeds were non-dormant and germinated highly (~90 %, Figure 1) in distilled H_2O without salt stress, and the decrease or increase of thermoperiod significantly affected germination percentage in presence or absence of salinity. High thermal regime and salinity concentrations affected considerably germination patterns. Despite the diminution of germination percentage by high NaCl treatments, the detrimental impact of salinity was generally less important at the optimal thermoperiod (Tlig et al. 2008).

In field conditions, the emergence of *P. halepensis* seeds starts during late autumn period. At this period of the year, germination occurs when temperatures are declining and after abundant rain which leaches salt accumulations from the upper soil horizons (El-Keblawy 2004). In arid environments, rainy water can rapidly wash salts decreasing salinity and provided water to seed imbibition. However, seedling mortality increases when there are only small quantities of precipitations and salinity increases due to strong soil evaporation (Shahbazi et al. 2014).

Conclusion

The present results show that maximum germination of *P. halepensis* seeds occurred in non-saline conditions, and an increase in NaCl concentration progressively impaired germination. The optimal germination percentage (~90 %) was recorded at 15–25°C. Rate of germination decreased with an increase in salinity at all thermoperiods but comparatively higher rates were obtained at 15–25°C. The highest values of T50 were obtained at higher levels of salinity. Therefore, to enhance the probability of seedling establishment and development in arid field conditions, late autumn sowings can be suggested when salinity and temperature are reduced. Further works need to be established in field conditions to confirm these findings.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

BN: Conceptualization, Methodology, Investigation and Writing – Review and Editing of the published work; BG: Methodology, Investigation and Analysis of Data.

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