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ACKNOWLEDGEMENTS. This work was carried out under the National Surveillance Programme for Aquatic Animal Diseases (NSPAAD), coordinated by the ICAR-National Bureau of Fish Genetic Resources (NBFGR), Lucknow. The authors thank the Indian Council of Agricultural Research (ICAR) and National Fisheries Development Board (NFDB), Govt. of India, for financial support to carry out this work. The authors are grateful to the Referral Laboratory at ICAR-CIBA, Chennai for validating the IHHNV positive samples.

Received 13 June 2016; revised accepted 12 May 2017

doi: 10.18520/cs/v113/i10/2027-2031

How NaCl, Na_2SO_4, $MgCl_2$ and $CaCl_2$ salts affect the germinability of *Pinus halepensis* Mill.

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In the Mediterranean forests, *Pinus halepensis* Mill. (Aleppo pine) plays an important role against desertification, reforestation of degraded lands and soil rehabilitation. Therefore, knowledge of its seed germinability requirements is necessary for its propagation in field conditions to colonize new territories habitually not conventional for other species. The study was carried out to assess the effects of different soluble salts (NaCl, Na_2SO_4, $MgCl_2$ and $CaCl_2$) on seed germination characteristics [germination percentage (GP) and rate of germination (RG)] of this conifer. Data show that all soluble salts decreased both parameters GP and RG. The highest GP was obtained in conditions without salinity. The maximum values of germination were obtained by low concentrations of $MgCl_2$. Comparatively, NaCl was generally the most toxic salt followed by $CaCl_2$ and Na_2SO_4. The present findings could be useful in the design of future projects for reforestation of degraded arid lands.

Keywords: Aleppo pine, rate of germination, reforestation, saline soils.

ECO-PHYSIOLOGICAL studies about regeneration of endemic conifers species grown in arid and semi-arid areas and the factors influencing them are important for the

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protection and propagation of tree species in projects of restoration of degraded lands facing erosion[1,2].

Algerian saline soils are formed from the accumulation of various chloride and sulphate salts dominated by NaCl (>50%). The main salt components of these soils are Na^+, Ca^{2+} and Mg^{2+} cations, and SO_4^{2-} is the second major anion after Cl^- (ref. 3).

In many studies, sodium chloride (NaCl) is the principal soluble salt examined to evaluate salt tolerance during germination of pine species[4,5]. However, rare information exists about other soluble salts such as sodium sulphate (Na_2SO_4), calcium chloride ($CaCl_2$) and magnesium chloride ($MgCl_2$) that were present at higher levels in the saline soils of arid regions[3].

The amount of soluble salts in arid soils can be greater than the tolerable limits for seed germinability of most conventional species, and therefore the emergence and propagation of plant species in these areas can be limited[6].

Mediterranean species use many strategies to cope with the harsh-ecological conditions in their local biotope, such as salinity and drought[7]. Saline soils in the arid regions of Algeria contain multiple types of soluble salts, which have various influences on germination and the first growth stage of species[3].

Aleppo pine (*Pinus halepensis* Mill.) is one of the most abundant endemic conifer species growing throughout the Mediterranean basin. However, in the recent decades, the main threat to its conservation is the devastation of natural forests in addition to lack of knowledge among the local population on the importance of *P. halepensis* forest ecosystems. Therefore, the possibilities of rehabilitation and restoration of this valuable tree need to be explored. In this context, information about seed germination requirements is of relevance to reforestation or replanting of degraded forests, and to colonize new territories habitually not conventional for other species.

In Algeria, *P. halepensis* constitutes the dominant coniferous tree of the natural and artificial forests, where it covers 852,000 ha area[8]. Notwithstanding its ecological and economic importance, exhaustive studies about seed germinability and seedling establishment of *P. halepensis* are required for propagation in field conditions. Therefore the present study was conducted in order to determine the effects of different types of soluble salts on germination of this species. Information from this study offers new knowledge about germination requirements of *P. halepensis* in saline conditions that can be used to enhance the chance of successful propagation under these conditions.

Cones of *P. halepensis* were collected in August 2014 from the natural forest of Gotaïa in the province of Djelfa, Algeria (2°47′E long., 34°37′N lat.; 1198 m asl). Seeds were detached from the cones and surface-sterilized with 60% alcohol (ethanol) for 10 min, followed by a treatment with 10% NaClO solution for 1 min and then washed abundantly with distilled H_2O.

Seeds were germinated in different concentrations (0, 50, 100 and 150 mM) of four soluble salts (NaCl, Na_2SO_4, $MgCl_2$ and $CaCl_2$); these concentrations reflect the range of salt content in the Algerian salt steppe[9]. The experiment was carried out in an incubator with a 12 h photoperiod under 15–25°C dark light. Radicle elongation of 2 mm was used as the criterion for germinability. A completely randomized design was applied in the germination tests. For each treatment, 100 seeds (in four replicates each) were placed in 90 mm petri dishes with 5 ml of test solution.

Modified Timson's index was applied to calculate the rate of germination (RG) as follows: $RG = \sum g/t$, where g is the percentage of seeds germinated after two-days interval and t is the total period of germination[10].

A two-way ANOVA was carried out to test the effects of salts (S), concentration (C) and their interactions ($S \times C$) on the germination percentage (GP) and RG using the SPSS 9.0 software package. To ensure homogeneity of variance, data were arcsine converted before statistical analysis. Newman–Keuls test was applied ($P < 0.001$) for a comparison between treatments.

A two-way ANOVA revealed significant effect of S and C ($P < 0.001$) on final GP of *P. halepensis* seeds, but their interactions ($S \times C$) did not significantly affect GP (Table 1). GP decreased with increasing salinity, the highest GP was found in non-saline (control) treatment (Figures 1 and 2). In field conditions this might occur because soluble salts reduce osmotic potential (ψ_0) of the soil and prevent seed hydration[11].

A strong inhibition of GP was observed in NaCl solution and maximum GP was recorded at all concentration of $MgCl_2$. The level of toxicity for different soluble salts in decreasing order was as follows: $NaCl > CaCl_2 > Na_2SO_4 > MgCl_2$. Strongest regressions were recorded between germination (GP) and salt concentration (S) with a coefficient of determination (R^2) ranging from 0.57 to 0.89 (Figure 3).

RG values calculated using a modified Timson's index, decreased in response to increasing salinity (Figure 4). A two-way ANOVA of RG showed significant ($P < 0.001$) effect of the two variants (S and C) but not of their interactions ($S \times C$) (Table 1). The inhibition action of salt stress on RG was highest for NaCl treatment than compared to other soluble salts. However, the harmful effect of salt stress was generally less with $MgCl_2$.

Table 1. A two-way ANOVA of the effects of salts (S), concentration (C), and their interaction ($S \times C$) on germination characteristics of *Pinus halepensis* seeds

Independent variable	S	C	$S \times C$
Germination %	1.52*	41.56**	0.56^{ns}
Rate of germination	0.72*	52.52**	0.28^{ns}

Numbers represent F-values.
ns, Not significant; *$P < 0.05$; **$P < 0.001$.

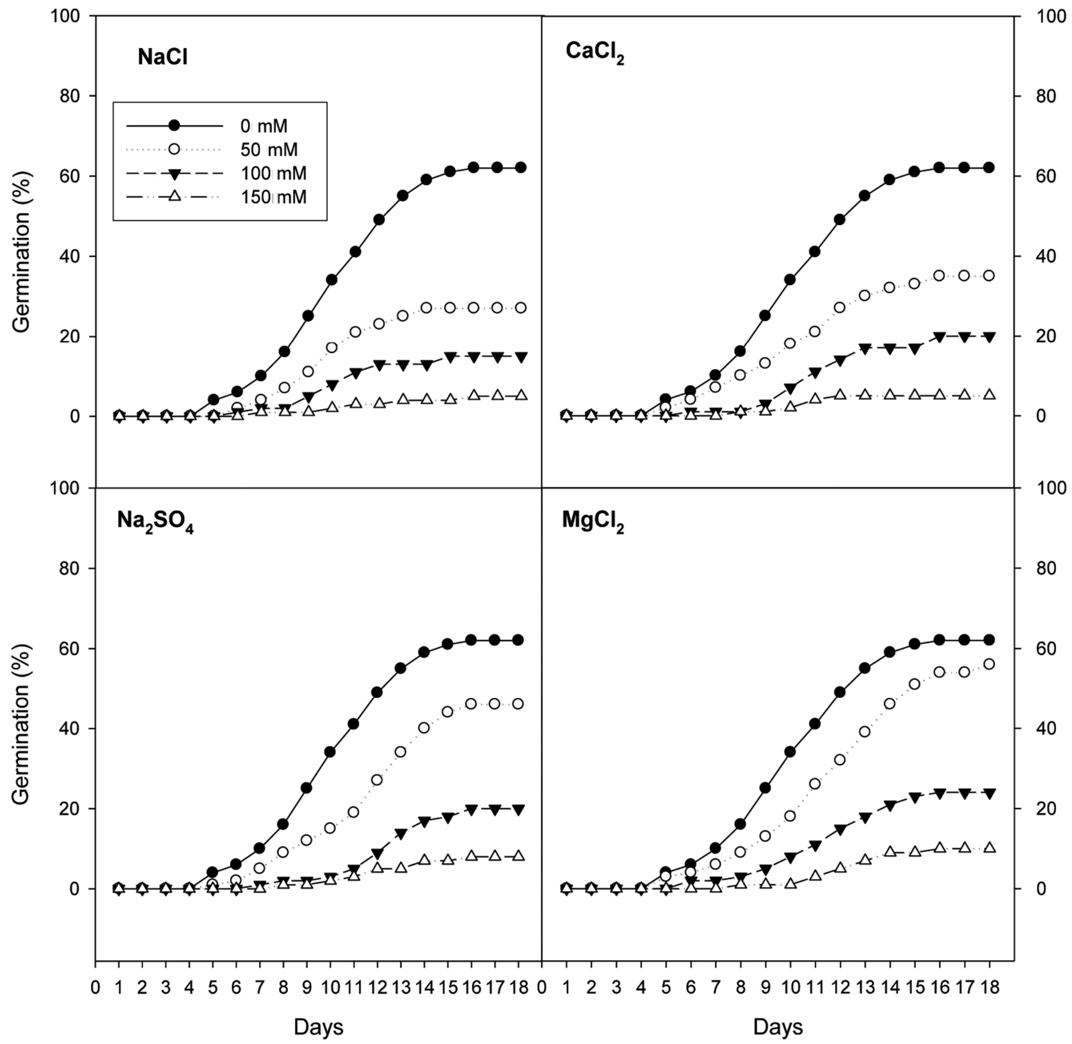

Figure 1. Mean germination percentage as a function of time of *P. halepensis* seeds treated with different soluble salts.

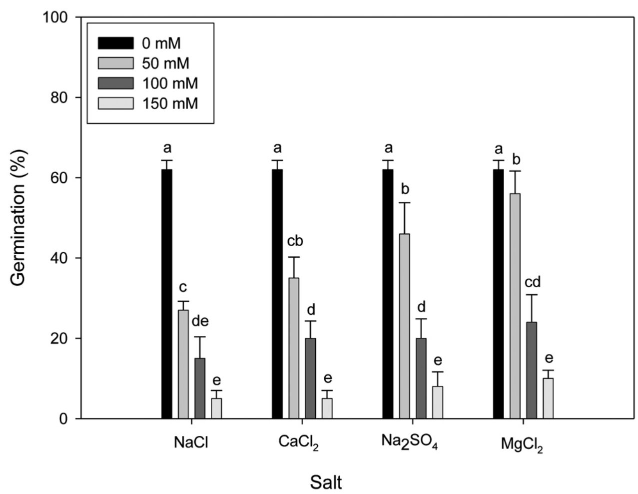

Figure 2. Final germination percentages of *P. halepensis* seeds treated with different soluble salts. Bars represent mean ± SE ($n = 4$). Different letters indicate significant difference between treatments (Newman–Keuls test, $P < 0.001$).

In order to survive under harsh conditions (high soil salinity and water-deficit), salt-tolerant plants have adapted many strategies, such as delaying germinability to prevent seedling mortality due to lowest water potentials (ψ_w)[12]. The ability of seeds to preserve their longevity under saline conditions for prolonged periods constitutes an imperative characteristic of salt-tolerant plants, permitting them to colonize the saline soils with low osmotic potential (ψ_0)[13].

P. halepensis seeds showed a highest GP under non-saline conditions for all salinities. This result has been reported by several other studies, which indicates that optimal GP of different Mediterranean coniferous trees occurs under non-saline conditions[14].

The probable reason for limited germination in the control treatments (reaching maximum of 62%) may be the dormancy of seeds. The enforced dormancy response in arid conditions is a selective strategy of plants growing in harsh habitats (drought and salinity). In these situations,

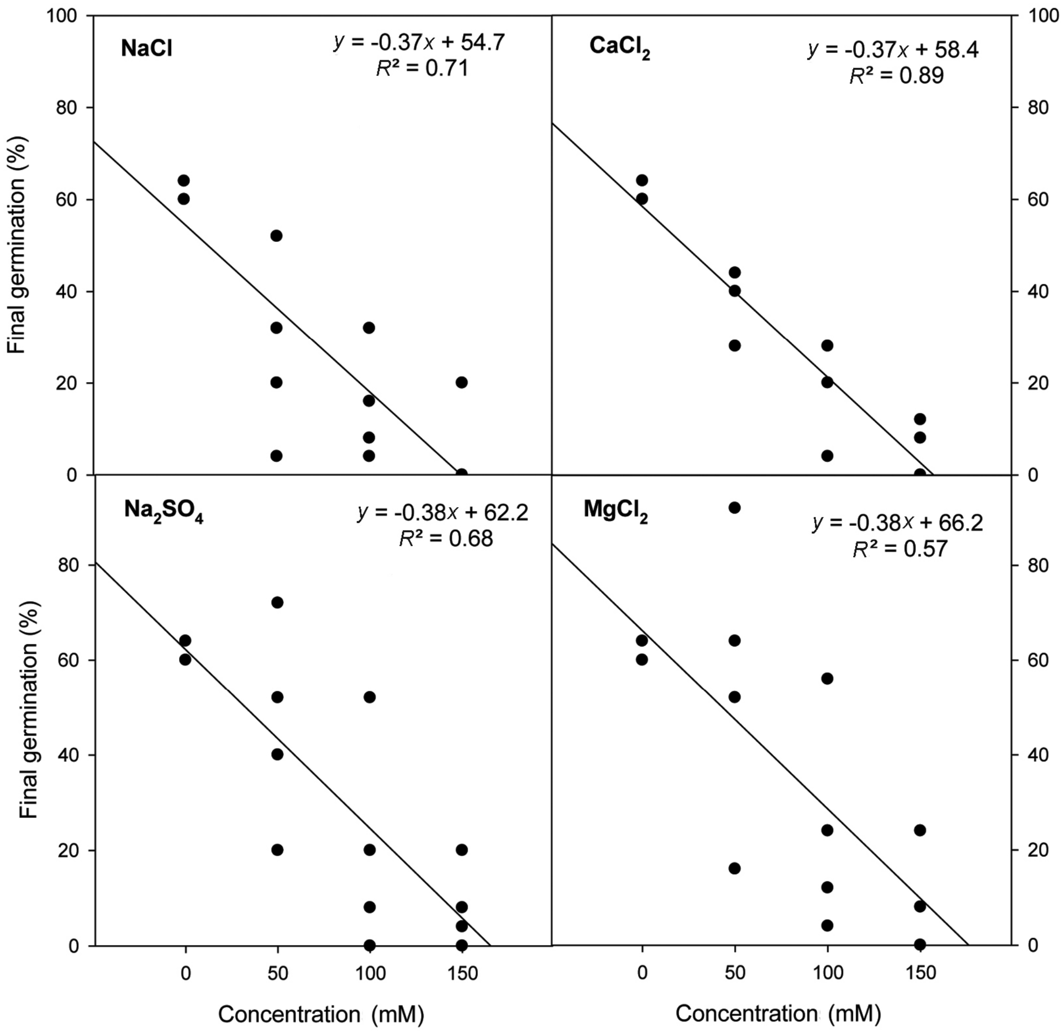

Figure 3. Regression plots of final germination percentage of *P. halepensis* seeds treated with different soluble salts.

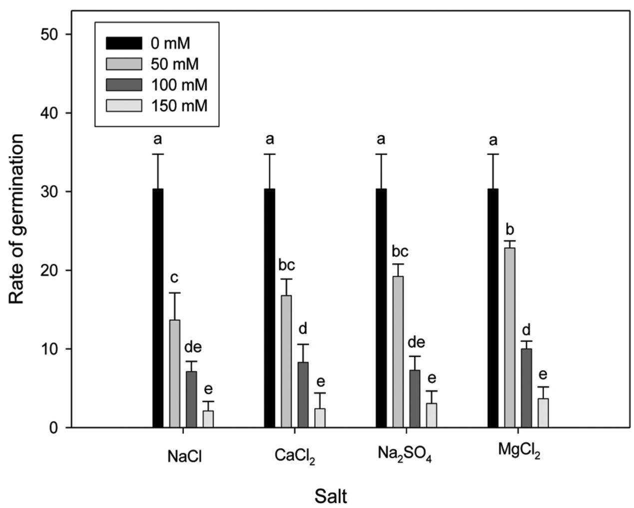

Figure 4. Rate of germination of *P. halepensis* seeds treated with different soluble salts. Bars represent mean ± SE ($n = 4$). Different letters indicate significant difference between treatments (Newman–Keuls test, $P < 0.001$).

seed germination would be limited to periods when soil humidity and salinity levels are within the species tolerance limits[15].

In the Mediterranean climate, rapid establishment of species after spring precipitation presents a successful key to plant colonization in saline environments. These species can propagate quickly and develop a deep root system which permits them to absorb water (H_2O) during drought from the profound soil horizons[16].

GP of *P. halepensis* seeds decreased when salinity increased in the medium. Similar results were found in other *Pinus* species as well[4,5,17]. This comportment may be due to high osmotic potential (ψ_0) that affects seed imbibition and delayed germination process.

In saline soils of arid regions, successful plant establishment depends on salt tolerance during seed emergence[18]. A critical phenomenon in these regions is high salt concentration at the superficial horizons of soils due to high evapo-transpiration causing germination failure by both osmotic and toxic effects[19]. However, seed germinability under hyper-saline conditions arises after leaching by abundant precipitations[20].

In field conditions, germination of *P. halepensis* seeds occurs during spring season when soil salinity is reduced by winter rainfall. Germination after wet season confers an optimal benefit to species growing in saline environments. The rain water dissolves soluble salts from the superficial soil layers and provides water for seed imbibition. In this situation, species propagate rapidly and develop high root density which permits them to absorb water and nutrients from deep horizons during harsh seasons (especially drought periods).

Thus it can be concluded that *P. halepensis* seeds germinate under a wide range of soluble salts and have the ability to tolerate salt stress. GP is less influenced by $MgCl_2$ followed by $CaCl_2$ and Na_2SO_4, whereas NaCl prevents germination more than the other soluble salts. Introduction of this coniferous tree has been proposed as a promising strategy to promote restoration in North African saline soils. Further studies are essential to explore these aspects under field conditions.

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ACKNOWLEDGEMENTS. This study was funded by the Ministry of Higher Education and Scientific Research of Algeria, agreement CNEPRU # D04N01UN170120140017. I thank the anonymous reviewer for constructive comments and Z. M. Souissi for technical assistance in the laboratory.

Received 12 January 2017; revised accepted 6 June 2017

doi: 10.18520/cs/v113/i10/2031-2035