

Full Length Research Paper

Dormancy-breaking and salinity/water stress effects on seed germination of Atlas cypress, an endemic and threatened coniferous species in Morocco

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Atlas cypress (*Cupressus atlantica* Gaussen) is an endemic coniferous medicinal species geographically restricted to the N'Fis valley river in the High Atlas Mountains in South-Western Morocco. Eight dormancy-breaking treatments, 5 NaCl concentrations, and 5 water potentials were tested on the germination of Atlas cypress seeds that had been stored in a cold room for 5 years after collecting from Aghbar population. Hand scarification, gibberellic acid and hot water increased the seed germination percentage (up to 75%), and mechanical scarification and gibberellic acid (1000, 2000 ppm) induced a faster speed germination. Soaking in sulfuric acid (10%) did not improve the seed germination of *C. atlantica*. In addition, salinity higher than 160 mM NaCl and water potential below -0.53 MPa drastically reduced seed germination.

Key words: *Cupressus atlantica*, seeds dormancy, scarified seeds, NaCl, water potential.

INTRODUCTION

Cupressus atlantica Gaussen, commonly known as Atlas cypress or "sarw al Atlas" in Arabic, is an endemic coniferous tree restricted to the valley of the Oued n'Fis river in the High Atlas mountains in South-Western Morocco. The species is drought and frost-resistant and tolerates a variety of soils. It grows at 1100 to 2200 m elevation in a Mediterranean arid climate with rainfall of 350 to 700 mm/year (Alifriqui et al., 1996). The species have attracted considerable interest due to its high socio-economic (production of timber, fodder and firewood) and ecologic importance (protection against erosion) and for its valuable medicinal properties. The plant was classified by Food and Agriculture Organization (FAO) among the 17 world forest species whose genetic inheritance is

impoverished (FAO, 1976). This situation is largely due to its low natural regeneration coupled with high anthropozoic pressure and habitat degradation. The species and its natural geographical distribution is represented by several stands and a number of isolated trees distributed over a total of 200 km² (Bechir, 2004). The estimate area of species occupancy in Aghbar station considered as one of the most important Atlas cypress forest, have drastically declined from 5500 ha to 1460 ha over a period of 50 years (Barbero et al., 1990). To maintain and improve these Atlas cypress forest, many nurseries have been managed to produce seedlings under controlled condition. But, even under these conditions, it has been shown that seeds germinated poorly and the mass production of *C. atlantica* seedlings was lower under nursery conditions. It appeared evident that for the successful propagation of such endemic and threatened species and consequently reduce the cost of its restoration operation, it's therefore essential to have a good understanding of its specific

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germination requirements. Germination was reported as a critical stage in the life cycle and establishment of many forest species (Bani and Michmerhuizen, 2001). It's well known that germination percentage may be influenced by several uncontrolled factors. Among these, seed dormancy, soil salinity and/or water deficit contribute in part to germination failure of many forest species under arid environmental conditions (Kolowski et al., 1991; Bewley, 1997; Baskin and Baskin, 1998; Dirik, 2000; El-Keblawy and Al-Rawai, 2005). Dormancy is the inability of a seed to germinate, even under conditions that are normally considered favorable for germination (Guner and Tilki, 2009). Two categories of dormancy were reported: Coat-enhanced dormancy where the embryos isolated from these seeds are not dormant and embryo-dormancy where the embryos themselves are dormant (Bewley, 1997; Ellery and Chapman, 2000). Many pretreatments have been recommended to break seed dormancy and enhance germination of many forest species such as plant growth regulators (gibberellic acid, GA) (Bonner et al., 1994; Bewley and Black, 1994), chemicals such as sulfuric acid (Rincón-Rosales et al., 2003, Likoswe et al., 2008) and physical treatments such as mechanical scarification and hot water (Ren and Tao, 2004). It's evident that the mechanism for breaking seed dormancy varies from species to species. For each forest species, finding the successful germination treatments is a very important step for its conservation and for its reintroduction program especially for those that are in danger of extinction.

For Atlas Cypress, there has been little experimental research dealing with the seed germination requirement based on the effect of seed breaking treatments on germination. Thus, the main objective of the present study was to find an effective and simple procedure for Atlas cypress seed germination using physical, mechanical and chemical pre-sowing treatments that would be adaptable to various nursery situations. In addition, water deficit and salt stress were recognized as the main environmental constraints for seed germination, plant growth and survival in Mediterranean-type ecosystems (Galmès et al., 2007), and thus the tolerance level of scarified seeds to salinity and water potential were also evaluated.

MATERIALS AND METHODS

In September 2004, cones were collected from Aghbar population, a site of biological and ecological interest and an important natural seeds bank (MCEFCS, 1997). To obtain the representative sample of population, cones were collected from several parent plants. Then, the cones were exposed to sun heat for 24 h and were collected after natural opening. Seeds were stored until 2009 in a cold room with controlled temperature (4°C) and hygrometry (about 30%). Therefore, when the germination test was carried out, the seeds had been in storage for five years. In autumn 2009, the seeds were surface sterilized for 20 min in 3% sodium hypochlorite, rinsed in distilled water and dried before the experiment.

Dormancy-breaking treatments

Seeds were submitted to mechanical, physical and chemical treatments. The mechanical treatments consisted of vigorous rubbing of the seed with 100 grit sandpaper to abrade the seed coat. Physical treatments were performed through soaking the seeds in hot distilled water at 60 and 80°C for 15 min. Two different chemical treatments were applied. The first one consisted of soaking and agitating seeds in 50 ml of 10% concentrated sulphuric acid for 15 min. After the treatment, the seeds were thoroughly rinsed with tap water and distilled water after. In the second treatment, the seeds were soaked for 48 h in a gibberellic acid (GA3) solution at 1000 or 2000 ppm. After treatments, seeds were placed on a single Whatman (N° 3) filter paper in 9 cm Petri dishes moistened with 5 ml of distilled water. Four replicates of 30 randomly selected seeds each were used for each treatment. For each experiment, a batch of untreated seeds was used as the control. The dishes were randomized in a thermostatically controlled incubator ($\pm 1^\circ\text{C}$) in the dark at 20°C (Bechir, 2004). After the beginning of germination, every 2 days and for a period of 28 days (incubation period), the germinated seeds (young radicles over 1 mm in length) were counted and removed from each Petri dish. Final germination percentage and mean germination time (MGT) were calculated, the last according to the following formula (Scott et al., 1984):

$$\text{MGT (days)} = \sum (ni \times di) / N$$

Where, ni is the number of germinated seeds on day i ; d is the incubation time (day); N is the total number of seeds germinated.

Salinity and water stress treatments

Seeds were scarified mechanically and placed to 9 cm Petri dishes on filter paper moistened with 5 ml of test solution. Four replicates of 30 randomly selected seeds each were used for each treatment. A seed was considered to have germinated at the emergence of the radicle (> 1 mm). Five NaCl concentration (0.0, 40, 80, and 120, 160 and 200 mM) and six water potential solutions (0.0, -0.07, -0.14, -0.22, -0.32 and -0.53 MPa) were tested. The latest were prepared by calculating the amount of polyethylene glycol (PEG 6000) using the equation of Michel and Kaufmann (1973). PEG 6000 and NaCl solutions were renewed every 48 h under sterile conditions to ensure relatively constant water potential and NaCl concentration in the treatment. Assays were performed in the dark at 20°C ($\pm 1^\circ\text{C}$) in a thermostatically controlled incubator. Germination was noted on alternate days for 28 days. Seeds which did not germinate after 28 days at higher PEG (6000) and NaCl concentrations were placed in new Petri dishes with filter paper moistened with distilled water and incubated under the same conditions for additional 28 days to study the recovery of germination. The cumulative germination rate expressed as the percentage of seeds germinated, recovery germination and total germination were evaluated.

Statistical analysis

Data were subjected to the statistical analyses. $P < 0.05$ was used to define statistical significance using SPSS for windows 10.0 version (SPSS, 1999). If a significant difference was determined among means, a Student-Neuman-Keuls (SNK) test was used to determine significant difference between pairwise comparisons among individual treatments. Germination data were arcsine

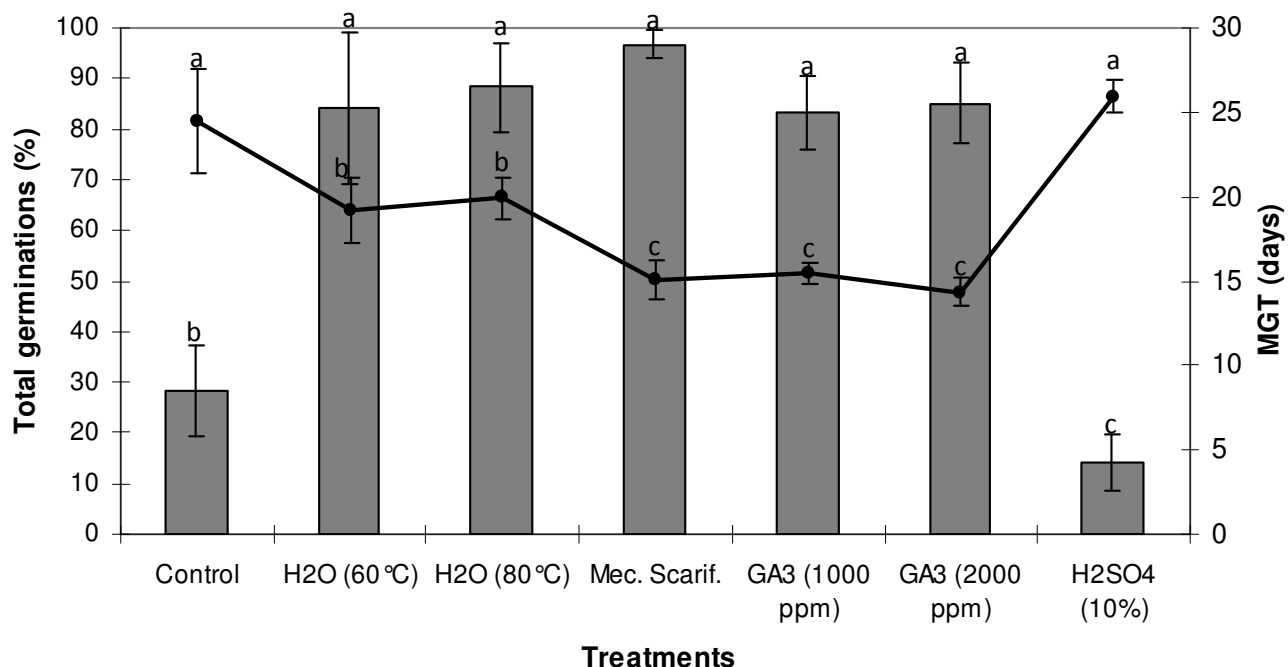


Figure 1. Variation of the final germination percentage (%) and the mean germination time (MGT, days) of *Cupressus atlantica* seeds submitted to different pre-treatment. For each parameter, different letters note significant difference at 5% (SNK test).

transformed before statistical analysis to ensure homogeneity of variance.

RESULTS AND DISCUSSION

Effects of dormancy breaking on seed germination

Non-treated seeds of *C. atlantica* germinated to a maximum of 28% (Figure 1). Dormancy breaking treatments significantly affected the germination of *C. atlantica* seeds. The highest germination percentage (96.7%) was observed for seeds that were hand scarified with sand paper, indicating that it was the most efficient treatment. *C. atlantica* seeds possess exogenous dormancy (physical dormancy) due to the hard seed coat (hardseededness). Indeed, removing the thick cellular layer beneath the seed coat by a hand scarification with abrasive paper, almost full germination was attained. Hand scarification was reported as the best treatment to overcome seed coat impermeability of many forest species (Mapongmetsem et al., 1999; Valbuena and Tarrega, 1998).

Exogenous application of gibberellic acid (GA3) at 1000 or 2000 ppm and soaking in hot and boiled water were also effective in increasing the germination percentage of *C. atlantica* seeds (Figure 1). Gibberellic acids appear to be responsible in metabolism activation and consequently in induction of certain enzymes that are involved in the cell walls weakness of seed coat of many

species (Nakajima et al., 2004). The effectiveness of hot water in promoting seed germination are also reported on many species (Uzum and Aydin, 2004; Patanè and Gresta, 2006). These treatments induced a reduction in the time to the start of germination expressed by low values of mean germination times (MGT) as compared to the other treatments (Figure 1).

In contrast to what had been reported by Bechir (2004) on the species and on others forest species such as *Ceratonia siliqua* (Goor and Barney, 1968), *Colutea armena* (Dirr, 1990) and *Acacia angustissima* (Rincón-Rosales et al., 2003), soaking in sulfuric acid (10%) did not improve the seed germination of *C. atlantica*. It appears that sulfuric acid was lethal for *C. atlantica* seeds. Infact, many seeds died indicating that acid had probably made contact with the embryos. Similar results with sulfuric acid have been reported for *Casuarina equisetifolia* (Eze and Ahonsi, 1993), *Alstonia boonei*, *Cordia platythyrsa*, *Terminalia superb* and *Triplochiton scleroxylon* (Mapongmetsem et al., 1999).

Effects of salinity and water potential on seed germination and recovery

The salt concentration and water potential effects on seed generation were highly significant ($P < 0.001$). Increasing salt concentration and decreasing water potential delayed the beginning of germination, decreased the germination rate and reduced the final

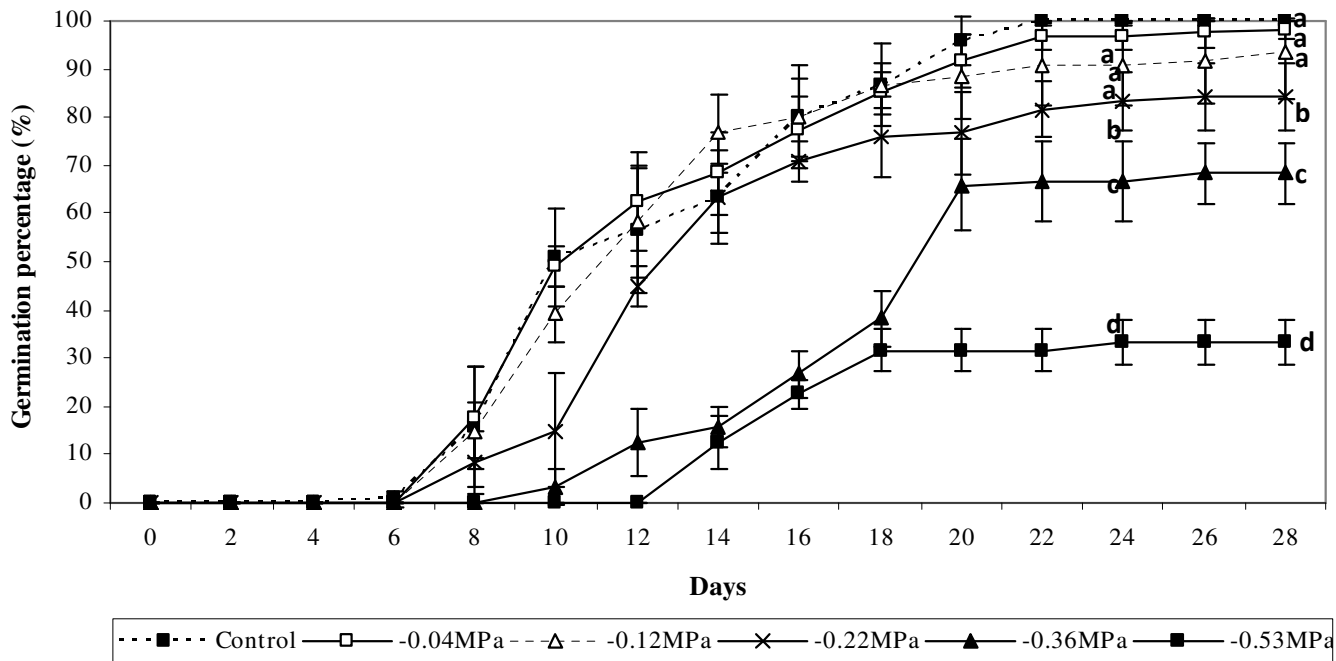


Figure 2. Mean cumulative germination percentage of scarified *C. atlantica* at six water potentials. Different letters note significant difference at 5% (SNK test).

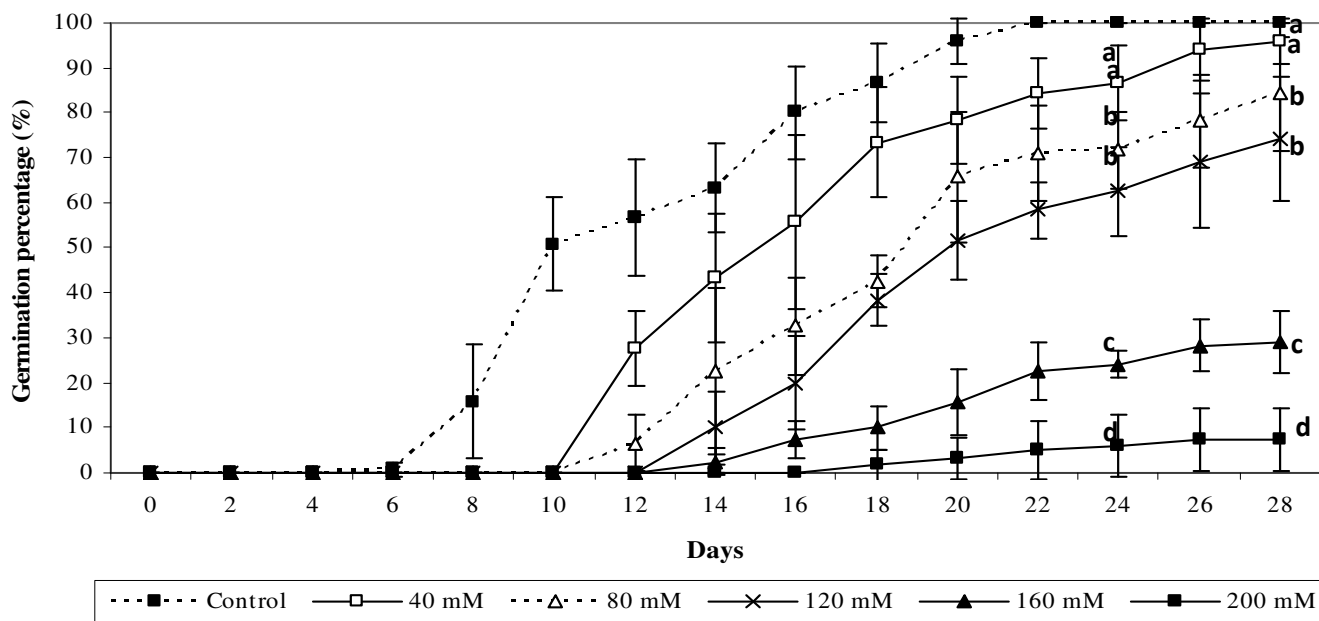


Figure 3. Mean cumulative germination percentage of scarified *C. atlantica* at five salinity treatments. Different letters note significant difference at 5% (SNK test).

germination percentage (Figures 2 and 3). Germination was drastically reduced at 200 mM NaCl (<10%) and -0.53 MPa (<35%), indicating that the species resistance limits is below -0.53 MPa for water stress and between 160 and 200 mM NaCl for salt stress (Figures 2 and 3). Regarding this result, it appears that *C. atlantica* reacted

to salinity like a glycophyte, showing a steady decrease in germination with increasing salinity. Its behavior differed markedly from halophytes such as *Haloxylon recurvum* (Khan and Ungar, 1996) or *Sporobolus ioclados* (Khan and Gulzar, 2003) in which germination occur with high percentage at 200 mM NaCl. Similar

Table 1. Mean germination (%) of *C. atlantica* seeds indicating the recovery after 28 days of transfer to distilled water and total germination (mean \pm SE, n=4).

Solution	Concentration	Germination	
		Recovery (%)	Total (%)
PEG (-MPa)	0.36	7.2 \pm 2.6	68.5 \pm 3.9
	0.53	2.7 \pm 1.4	35.8 \pm 8.33
NaCl (mM)	160	2.5 \pm 3.19	31.4 \pm 11.1
	200	1.66 \pm 3.33	8.3 \pm 10.6

results were reported on some forest species such as *Argania spinosa* (Bani Aameur and Michmerhuizen, 2001) and *Picea asperata* (Yang et al., 2010). Considering the water stress, the species showed a moderate tolerance since the germination persist (up to 30%) at water potential of -0.53 MPa. However, it differed significantly from high tolerant xerophytic species such as *Zizyphus lotus* in which germination percentage was higher (up to 60%) under water potential below -0.53 MPa (Maraghni et al., 2010).

The recovery germination test showed a significant depressive effect of salt and water stress marked by low recovery germination percentages (Table 1). This situation implies that the exposure of scarified seeds to high NaCl concentrations and low water potential may probably affect the viability of embryo and confirmed the glycophytic (salt-sensitive) and non-xerophytic behavior of *C. atlantica* at least at germination stage.

Conclusion

C. atlantica possess a physical dormancy attributed to hard seed coat and that may reduce its natural regeneration. Hand scarification, gibberellic acid (1000 to 2000 ppm) and hot water appear more effective to improve seed germination of this species. The germinative capacity of *C. atlantica* seeds was higher even after five years of storage, suggesting that seed storage may be a valuable tool for conserving such endemic and threatened species where the production of seeds is irregular over the years. In addition, *C. atlantica* did not behave as a xero-halophyte species but rather as moderate tolerant species, and salinity higher than 160 mM NaCl and water potential below -0.53 MPa may drastically reduce seed germination.

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