

# Seed germination requirements and responses to salinity and water stress of *Centaurea eriophora*



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## Abstract

*Centaurea eriophora* L. (Asteraceae) is a rare annual plant, found in some Mediterranean regions and requiring conservational measures. The present study was carried out to highlight the species germination behavior under two controlled environmental constraints: salt and water stress. Achenes collected on Mount Tessala (North-West Algeria) were germinated under a water potential gradient (0 to –2.45 MPa) induced by different concentrations of polyethylene glycol and under different concentrations of NaCl (0 to 102 mM). The experiments were conducted at constant temperature. The obtained results showed a high significant effect of salinity and water stress, especially on final germination percentage, while the highest germination (85%) was obtained at control conditions in both of the tests. Under salinity and water stress conditions, there was a significant deterioration in most germination parameters, particularly a lower final germination percentage. The tolerance thresholds for salinity and water deficit were 68 mM and –1.2 MPa respectively. Beyond these values, seed germination of *C. eriophora* was completely inhibited. The sensitivity of *C. eriophora* achenes to environmental pressures should be considered in the long-term effective conservation plans.

**Key words:** achenes, *Centaurea eriophora* L., conservation, germination, salinity, water stress.

**Abbreviations:** FGD, final germination day; FGP, final germination percentage; IGD, initial germination day; MGT, mean germination time; PEG, polyethylene glycol.

## Introduction

The rapid global change and the increasing anthropogenic pressure on the Mediterranean region have led the global conservation assessments to consider the biodiversity of the Mediterranean biome as a priority (Médail, Quézel 1997; Médail, Quézel 1999; Syphard et al. 2009; Underwood et al. 2009). The reinforcement of plant populations through the introduction of *ex situ* conserved species is considered as a valid method of reducing the risk of extinction of threatened species and populations (Cerabolini et al. 2003).

Regeneration of natural populations includes the production and dispersal of seeds. It depends on germination and successful establishment of seedlings (Fangqing et al. 2012). Germination is considered to be the most critical stage and vulnerable step in the plant life cycle (Loic et al. 2012) and in the colonization process, which are essential in understanding the ecosystem dynamics (Baskin, Baskin 2014). In fact, seed germination has been advocated as one of the most viable biotechnological tools for the *ex situ* conservation of species (Kameshwararao 2004). However, each species has particular requirements for their

seed germination in a patchy and changing environment (Simons, Johnston 2006; Vandeloek et al. 2008). The knowledge of these requirements is very important in the conservation of rare and threatened species (Heywood, Iriondo 2003; Padmalatha, Prasad 2007), such as *Centaurea eriophora* L.

Propagation from seed is inexpensive and usually effective, but germination requirements for native species are often unknown, particularly for rare and/or endemic species from which material is more difficult to obtain (Cochrane et al. 2002; Cerabolini et al. 2004). On the other hand, seed germination is one of stages that is most sensitive to environmental stress and it is characterized by extremely high mortality rate and intense natural selection acting on the entire life cycle (Kolb, Barsch 2010). Hence, each species responds to abiotic factors in a particular way. In fact, water deficit and water/soil contamination with salt are two major environmental worldwide issues (Geissler et al. 2009) that influence seed germination. Drought causes severe limitation of plant growth, development and productivity as well, particularly in arid and semi-arid regions where it could delay germination and induce

decreased germination percentage, rate and growth (Gallé et al. 2007). Despite this, seeds tolerant to water stress may have an ecological advantage in these conditions and can establish plants in areas in which drought dominates (Bewley, Black 1994).

Soil salinization is one of the main abiotic factors that affects seed germination regeneration in arid and semi-arid Mediterranean lands (Nedjimi, Guit 2021). Salt stress affects seed germination by reducing the osmotic potential, limiting seed hydration, and/or through ion toxicity that decreases seed viability (Sidari et al. 2008; Alatar 2011). Also, increased salinity has detrimental effects on germination and can reduce the percentage of germinating seeds (Shila et al. 2016). Soil salinity can interact with the thermoperiod to affect seed germination of many plant species, with higher suppression due to salt stress habitually found at the minimal or maximal thresholds of temperature tolerance (Delgado Fernández et al. 2016). Seed tolerance to salinity during germination is critical for the establishment of plants growing in saline soil of arid regions (Khan, Gulzar 2003).

*Centaurea* L. (Asteraceae) is represented by about 700 species distributed in the Mediterranean region and the Middle East, with few species in northern Eurasia, north and east Africa, North America, and Australia (Bancheva et al. 2014). The Mediterranean region is considered as a refugium for many of these species (Greuter 1979), and several endemic taxa of *Centaurea* have narrow distributions in this region (Pisanu et al. 2011). The Algerian flora includes 45 species of *Centaurea* and seven of them are localized in the Sahara (Quézel, Santa 1963). *Centaurea eriophora* L. is an annual plant, native to the western part of the Mediterranean region, growing on calcareous soils, on roadsides and in open arid spaces (De Clavijo 2002). *C. eriophora* is considered as a relatively rare plant species in northern Algeria (Quézel, Santa 1963). Our work constitutes a part of the conservation efforts of the *C. eriophora* population growing on Tessala Mount (western Algeria). The population is exposed to anthropo- and zoogenic pressure and drought, which remain the major causes of degradation of the flora (Cherifi et al. 2017).

Because of lack of information about *C. eriophora* salt or drought tolerance, the aim of the present study was to determine the suitable germination requirements under water and salinity and to evaluate its tolerance. Thus, the outputs of this study would be included in the elaboration of *ex situ* conservation protocol for this rare species.

## Materials and methods

### Achenes collection site

Achenes of *Centaurea eriophora* were collected during the period of maturation at the end of July, 2018, from local populations growing in Tessala Mount (north western Algeria) (35°16'08.56"N; 0°46'48.26"W; altitude 889 m).

This area is arid to semi-arid with a typical Mediterranean climate characterized by an irregular rain events and a harsh dry summer period with annual precipitation between 290 and 420 mm and mean monthly temperatures in the range 9.4 to 26.6 °C (Dadach et al. 2016).

### Germination test conditions

Considering that pathogen and non-pathogen organisms from the soil may affect achenes, their surface was sterilized for 5 min in 5% sodium hypochlorite solution (NaOCl), and then thoroughly rinsed two times with deionized water for 5 min to eliminate the effect of chlorine. Germination tests were carried out in quadruplicate on 20 achenes placed inside closed glass Petri dishes (90 mm of diameter) on two certified nontoxic No. 1 Whatman paper disks. The experiments were conducted in a Memmert incubator in darkness at constant optimum temperature (20 °C) for 30 days according to De Clavijo (2002) and Bouker et al. (2016).

### Effect of salt and water stress on germination

For salt and water stress tests, germination was conducted using different solutions of NaCl (0, 2, 4, 6, 8 g L<sup>-1</sup>) corresponding to the molar concentrations 0, 34, 68, 102, 136 mM, and polyethylene glycol (PEG6000) (0, 5, 10, 20, 40, 80, 120 g L<sup>-1</sup>) corresponding to hydric potentials 0, -0.03, -0.08, -0.19, -0.47, -1.2 and -2.45 MPa, evaluated according the equation of Michel and Kaufman (1973). The moisture of the filter paper was kept constant by adding distilled water for the controls and by solutions of NaCl and PEG6000 for the other tests. We used the integument breakthrough by the radicle as a germination criterion (Baskin, Baskin 1998). The counting of germinated achenes was carried out every 24 h.

The cumulative germination curve was obtained by daily counting of the germinated achenes of *C. eriophora*. Furthermore, three parameters of germination were then determined: initial germination day (IGD), final germination percentage (FGP) and mean time of germination (MGT). MGT was calculated using the equation:

$$MGT = \sum (n_i \times d / N),$$

where  $n_i$  is the number of achenes germinated until day  $i$ ;  $d$  is the incubation period in days, and  $N$  is the total number of achenes germinated in the treatment (Brenchley, Probert. 1998). Therefore, the MGT value is inversely proportional to the germination rate (Delgado Fernández et al. 2016).

### Statistical data processing

Data were subjected to one-way analysis of variance (ANOVA), to evaluate the effect of salt and water stress on the individual parameters (IGD, FGP and MGT), followed by the Duncan's multiple range post hoc test. Data were analyzed using IBM statistics SPSS (23 version). The relationships between the germination parameters under

the effect of different salt concentrations and different water potentials were analyzed by the evaluation of the coefficient of determination  $R^2$  and using linear regression analysis. The graphs were produced using Excel software.

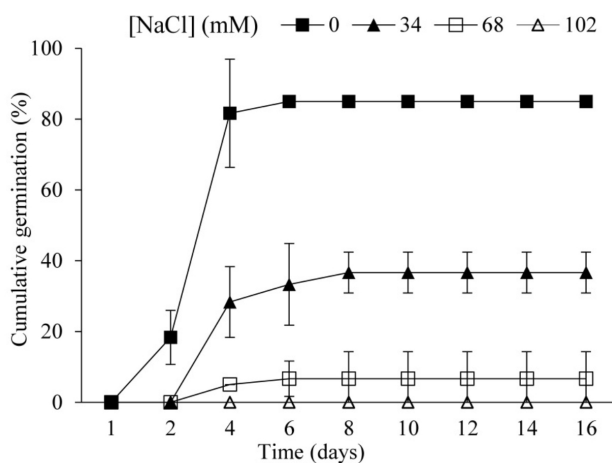
## Results

### Effect of salinity

The cumulative germination (Fig. 1) was modelled using the daily records of emerged radicles on *C. eriophora* achenes under saline stress conditions. For all of the tested NaCl concentrations, the cumulative germination curve can be divided into three phases: a latency phase, an exponential acceleration phase, and a stationary phase corresponding to a level reflecting the end germination result.

Germination was significantly affected by different levels of salinity ( $p < 0.05$ ) (Table 1). Control achenes of *C. eriophora* were non-dormant and started germinating within the second day of incubation (Day 1) reaching FGP = 85% in Day 6 (Fig. 1). The lowest IGD (2 days) was obtained for control seeds. A positive and moderate linear relationship was evident between IGD and salinity ( $R^2 = 0.42$ ) (Fig. 2A), which means that the achenes of *C. eriophora* germinated abundantly and faster in distilled water.

According to Table 1, the effect of NaCl was significant particularly on FGP ( $p < 0.001$ ;  $F = 196.21$ ). In addition, a non-significant effect was observed on MGT. Also, a strong and inverse relationship was noted between the concentration of NaCl and FGP ( $R^2 = 0.90$ ) (Fig. 2C). However, the weakest correlation was noted between MGT and salinity ( $R^2 = 0.001$ ; Fig. 2B). Regarding the effect of the salinity on germination parameters shown in Table 1, the lowest MGT was observed for the control (3.12 days), and the highest FGP (85%) was obtained also in control conditions. In addition, FGP followed a steady decrease

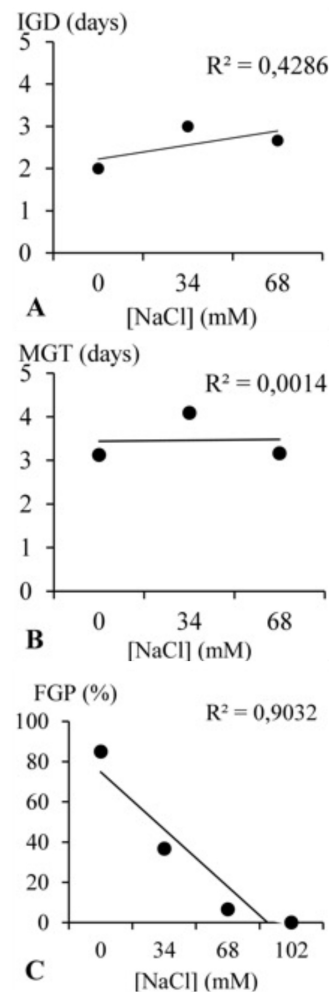


**Fig. 1.** Cumulative germination percentages in response to different molar concentrations of NaCl. Bars represent  $\pm$  SE, ( $n = 4$ ). Confidence interval were calculated at the threshold of 5%.

**Table 1.** Effect of different molar concentrations of NaCl on the germination parameters of *Centaurea eriophora* seeds (mean  $\pm$  SE,  $n = 4$ ). IGD, initial germination day; FGP, final germination percentage; MGT, mean germination time. Different lowercase letters (column) show significant differences between the averages. According to Duncan multiple comparisons test  $F$ -probabilities are indicated by symbols: \*\*\*, significant differences at  $p < 0.001$ ; ns, non-significant

NaCl (mM)	IGD (days)	FGP (%)	MGT (days)
0	2 b	85 c	3.12 $\pm$ 0.67 a
34	3 b	36.66 $\pm$ 5.77 b	4.1 $\pm$ 0.94 a
68	2.66 $\pm$ 2.3 b	6.66 $\pm$ 7.63 a	3.16 $\pm$ 1.18 a
102	0 a	0 a	–
$F$ value	–	196.21***	1.04 ns

with increasing NaCl concentration (Fig. 2C). There was a strong linear relationship ( $R^2 = 0.90$ ) between FGP and salinity (Fig. 2C). At 102 mM NaCl, the germination of *C. eriophora* achenes was entirely inhibited (Fig. 2).



**Fig. 2.** Effect of different molar NaCl concentrations on germination parameters. Bars represent  $\pm$  SE, ( $n = 4$ ). The confidence intervals were calculated at the threshold of 5%.

**Effects of water stress**

As presented in Table 2, a significant effect was observed between water potential and the germination parameters: IGD ( $p < 0.001$ ,  $F = 8.4$ ) and FGP ( $p < 0.001$ ,  $F = 18.73$ ). Water potential induced by PEG6000 did not show any significant effect on MGT. In the water stress test, the highest germination percentage was found for distilled water treatment with a value 85% on Day 6 of incubation. The increase in PEG6000 concentration decreased water potential, which resulted in a steady decrease in FGP to reach a minimum (6.66 %) at water potential of -1.2 MPa (Fig. 3). The lowest IGD time (2 days; Fig. 4A) and mean germination time (MGT; 3.13 days; Fig. 4B) occurred in the control condition (0 MPa). Germination percentage was completely inhibited at water potential -2.45 MPa (Fig. 4C). Linear regression analysis showed a highly pronounced relationship between FGP and water potential ( $R^2 = 0,87$ ), while a weak relationship occurred between water stress and IGD ( $R^2 = 0.26$ ) (Fig. 4A) and MGT ( $R^2 = 0.018$ ; Fig 4C).

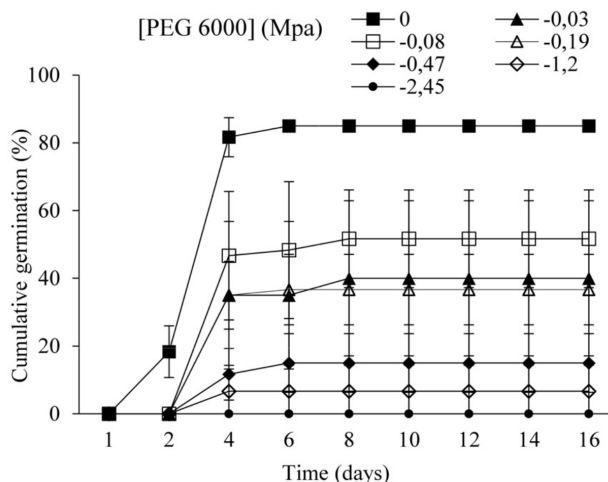
**Discussion**

This study investigated germination requirements of *C. eriophora* achenes, which were collected from field, under different salinity and water stress conditions using a constant optimum temperature in order to understand effect of salinity and water stress on seed germination. These are the main abiotic factors all around the world. In fact, they constitute the main reasons of limited propagation and regeneration of some annual plant species (Heard, Ancheta 2011; Medjebeur et al. 2018; Zare, Moosavi 2020).

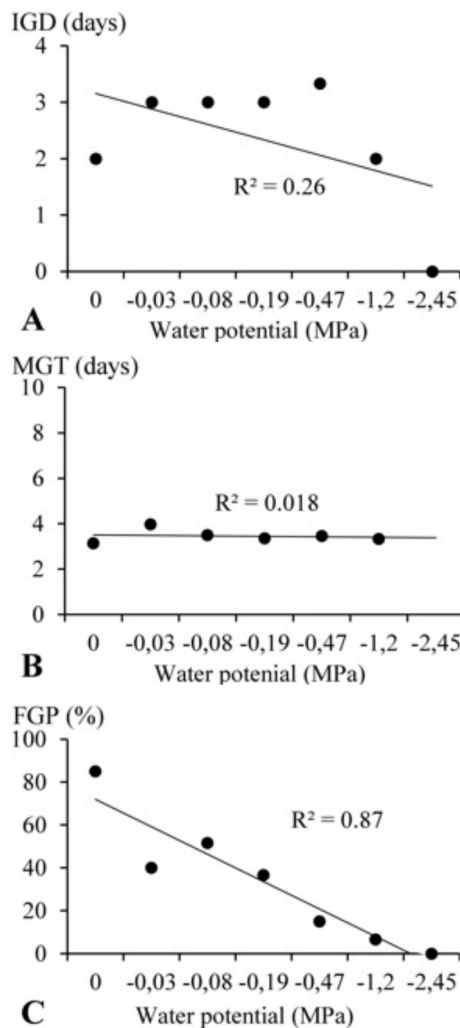
The obtained results showed that *C. eriophora* achenes are variable in their germination under salinity and water stress. At the optimum germination temperature (20

**Table 2.** Effect of different water potentials on the germination parameters of *Centaurea eriophora* seeds (mean  $\pm$  SE,  $n = 4$ ). IGD, initial germination day; FGP, final germination percentage; MGT, mean germination time. Different lowercase letters (column) show significant differences between the averages. According to Duncan multiple comparisons test  $F$ -probabilities are indicated by symbols: \*\*\*, significant differences at  $p < 0.001$ ; ns, non-significant

Water potential (MPa)	IGD (days)	FGP (%)	MGT (days)
0	2 b	85c	3.13 $\pm$ 0.68 a
-0.03	3 b	40 $\pm$ 22.91 b	3.96 $\pm$ 0.66 a
-0.08	3 b	51.66 $\pm$ 14.43 b	3.49 $\pm$ 0.58 a
-0.19	3 b	36.66 $\pm$ 10.40 b	3.53 $\pm$ 0.34 a
-0.47	3.33 $\pm$ 0.57 b	15 $\pm$ 8.66 a	3.45 $\pm$ 1.63 a
-1.20	2 $\pm$ 1.73 b	6.66 $\pm$ 7.63 a	3.33 $\pm$ 0.46 a
-2.45	0 a	0 a	-
$F$ value	8.40***	18.73***	0.27 ns



**Fig. 3.** Cumulative germination percentages in response to different water potentials. Bars represent  $\pm$  SE, ( $n = 4$ ). Confidence interval were calculated at the threshold of 5%.



**Fig. 4.** Effect of different hydric potentials on germination parameters. Bars represent  $\pm$  SE ( $n = 4$ ). The confidence intervals were calculated at the threshold of 5%.



°C), achenes were non-dormant and reached maximum germination under control conditions in both tests. Such a response has been shown also for other species of *Centaurea*, such as *Centaurea calcitrapa* L. (Micheal et al. 2002) and *Centaurea solstitialis* L. (Young et al. 2005). Our results are in agreement with the study of Dadach et al. (2018), which found that the best germination of *Marrubium vulgare* from the same study area occurred under control conditions. Germination of *C. eriophora* achenes was negatively affected by salinity. Significant decrease in FGP and an extension in IGD was observed when salt concentration increased. A low concentration of NaCl (34 mM) had little effect, and the tolerance threshold was 68 mM NaCl. Higher concentration (102 mM) caused a lower germination of *C. eriophora* achenes. The decrease in germination rate with increasing NaCl in the environment can be explained by low water potential generated by salt stress, causing the alteration of enzymes and hormones in the seeds (Botia et al. 1998). Similar to results of our study, high germination sensitivity to salt stress was also observed for the Turkish endemic species *Centaurea tuzgoluensis* (Yildiztugay et al. 2011). These findings are in accordance with the reports of Nosratti et al. (2017) and Zare, Moosavi (2020), where *Centaurea virgata* Lam. and *Centaurea balsamita* Lam. germination was affected by salt stress conditions. Furthermore, *Lygeum spartum* L., a species of the arid steppes of Algeria, is considered as moderately salt-tolerant in the germination stage, with a tolerance threshold concentration of 150 mM (Nedjimi 2013).

Hence, it can be concluded that the ion disproportion is the main cause of lower salinity tolerance in *C. eriophora*. Moreover, Panuccio et al. (2014) noted that the negative effects of high salinity on seed germination may be due to ion toxicity as a consequence of a coincident increase in anion and cation concentration. In addition, it was noted that salinity stress led to disruption of hormonal balance in seeds, which may reduce consumption of seed reserves (Shokohifard et al. 1989; Albacete et al. 2014).

Drought tolerance during germination is considered as an important criterion for distinguishing the types of species that can sustain water deficit. Germination of *C. eriophora* achenes at different osmotic potentials was significantly affected by the severity of the water deficit constraint. The maximum FGP and shortest IGD and MGT occurred in the control (0 MPa). The germination percentage was intensely decreased with decrease of water potential. The tolerance threshold of *C. eriophora* was at -1.2 MPa and germination was halted -2.45 MPa. Decreasing water potential results in higher osmotic potential, impeding seed hydration and growth, which hinder radicle emergence and delay germination (Gill et al. 2003). These mechanisms are related to reduction of enzymatic activities due to the low availability, thus obstructing seed metabolism (Bewley, Black 1994).

Our results are similar to these of previous studies on

germination of other species of *Centaurea*. A decrease in germination percentage of *Centaurea solstitialis* L. was found to occur at -1.5 MPa (Lary, Gary 1997), and of *Centaurea iberica* achenes at -1.2 MPa (Nosratti et al. 2017). Also, *Glebionis segetum* (L.) Fourr., an endangered Asteraceae annual species, showed similar germination during water stress (Ruhl et al. 2015). Hamdini et al. (2021) reported that the germination of *Ruta chalepensis*, found in the same study area as *C. eriophora*, was also moderately tolerant to a range of water potential from 0 to -1.16 MPa. Thus, each species germinates under its specific critical osmotic potential.

It can be suggested that the moderate tolerance of *C. eriophora* achenes to water stress is due to their ability to absorb a sufficient amount of water during seed germination, even with its low availability. However, the global climate change affecting the Mediterranean region may provide more frequent and longer harsh periods, with higher temperature and lower precipitation (Rey et al. 2011), which potentially threatens the populations of *C. eriophora*. Despite its tolerance, the undesirable impact of climatic changes on the Mediterranean region will consequently threaten the long-term sustainability of this species. Regarding its ecological status, *C. eriophora* is considered as a rare plant requiring serious interest. Water insufficiency and salt stress, even at relatively low intensity can inhibit germination of *C. eriophora*.

Therefore, germination of this species of concern is sensitivity to salinity and water stress, which restricts its distribution in natural habitats. As the consequences of climate change are not avoidable, further conservation efforts and measures must be undertaken to compensate for these negative effects e.g. *ex situ* propagation. The salinity and drought effect in the germination stage requires further investigation to better understand the physiological mechanisms of this species that enable it to survive in limiting natural environmental conditions of these semi-arid and arid regions. The results of this study provide information about germination requirements that are needed to elaborate conservation and restoration plans.

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