Ecological Engineering 102 (2017) 636–640

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Short communication

Germination of *Atriplex halimus* seeds under salinity and water stress

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ARTICLE INFO

Article history:
Received 10 December 2015
Received in revised form 17 February 2017
Accepted 18 February 2017
Available online 11 March 2017

Keywords:
Germinability
Halophyte
Hydrotime model
Mean germination time
Osmotic potential

ABSTRACT

Direct seeding is an effective approach for plant re-establishment and revegetation of post-mining areas. However, germination of seeds is influenced by environmental stressors such as soil water conditions and salinity, eventually affecting revegetation success. In this study, we conducted laboratory experiments to evaluate the effect of water stress and salinity on the germination of *Atriplex halimus* seeds. We exposed seeds to various solutions of NaCl and Polyethylen Glycol (PEG) ranging from soil saturation (0 MPa) to permanent wilting point (−1.5 MPa). We measured the germinability and mean germination time to quantify seed germination. Based on the measured germination data, we estimated the parameters of the hydrotime model, which is a critical tool to predict timing and success of seed emergence. At an osmotic potential of −1.5 MPa, 31% or 3% of the seeds germinated when exposed to PEG or NaCl solution, respectively. This indicates that *Atriplex halimus* seeds are highly tolerant to water stress, but less tolerant to salinity stress. The results of this study suggest that direct seeding of *Atriplex halimus* may be an effective path for revegetation of post-mining landscapes where salinity and water stress are predominant features of the environment.

1. Introduction

Land revegetation can occur either through passive regeneration (Musselman et al., 2012), the transplanting of seedlings (Bouzid and Papanastasis 1994), or direct seeding (Brofas and Karetsos 2002; Doust et al., 2006; Doust et al., 2008; Millsom 2002). The latter may be an effective and economic path for land revegetation on a large spatial scale (Abbad et al., 2004; Engel and Parrotta 2001), but it is potentially limited by environmental factors such as water availability. In this regard, seed germination is a significant indicator of revegetation success since it initially drives plant establishment and plant community dynamics (Abbad et al., 2004; Arnold et al., 2014a). Seed germination is influenced by environmental stressors such as salinity and periods of water deficit (Abbad et al., 2004; Arnold et al., 2014a; Cavallaro et al., 2014), particularly in arid and semi-arid environments. Under rare and erratic rainfall conditions, seed germination may be successful only once in several years (Breckle 1995). Hence, estimating the time and extent of seed germination under water and salinity stress is an essential integral part of any land rehabilitation planning, which is commonly supported by numerical modelling (Bradford 2002; Bullied et al., 2012; Cavallaro et al., 2016; Köchy and Tielbörger, 2007).

In arid and semi-arid environments, post-mining lands can be revegetated using halophytes (Al-Nasir 2009; Chaudhri et al., 1964; Devi et al., 2008; Keiffer and Ungar 2001; Keiffer and Ungar 2002) that can survive, live and complete their life cycle in high concentrations of salt (∼200 mM NaCl) (Flowers et al., 1986; Khan and Gul 2006). Particularly *Atriplex halimus* is considered desirable due to its high fodder quality and being a perennial plant species (Abbad et al., 2004). However, while several authors (Abbad et al., 2004; Bajji et al., 2002; Katembe et al., 1998; Khan and Rizvi 1994; Ungar 1996) reported the tolerance of *Atriplex* species to high levels of salinity and water stress, literature still lacks of any quantitative information regarding the effects of osmotic pressure (water stress) and ion toxicity (salinity stress) on the germination of *Atriplex halimus* seeds. Therefore, the primary aim of this study was to evaluate the germination response of *Atriplex halimus* seeds to salinity and water stress. Based on a germination study on *Acacia harpophylla* (Arnold et al., 2014a,b), we hypothesized that, under low to moderate levels of salinity, germination is primarily controlled by the osmotic effect rather than ion toxicity. We also aimed to estimate the parameters of the hydrotime model for *Atriplex halimus* for the purpose of evaluating the extent and timing of germination in relation to soil water potential and salinity.

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http://dx.doi.org/10.1016/j.ecoleng.2017.02.050
0925-8574/

2. Materials and methods

2.1. Germination tests

We used seeds of *Atriplex halimus*, collected in 2010 from Tunisia and stored at 4 °C, for germination testing in relation to water potential and salinity. We established seed germination tests in 2013. We sterilized *Atriplex halimus* seeds (seeds with enclosing bracts) in 0.01% $NaClO^-$ (sodium hypochlorite) for 10 s and rinsed three times with deionised water. Then, we removed the seeds manually from the bracts by tweezers. We placed 4 replicates of 25 seeds within petri dishes (9 cm, sealed with Parafilm® to minimize water loss) for each treatment and incubated them in a germination chamber with 25 °C and a 16 h/8 h light and dark cycle (Nedjimi and Daoud 2009). We monitored seed germination once per day and removed the germinated seeds from dishes after each counting. The experiments ceased after 15 days.

To evaluate the germination response in relation to water deficit, we exposed the seeds to 15 mL solutions of polyethylene glycol (PEG 6000) on absorbent substrates (Wettex®) within the petri dishes. The osmotic water potentials used for this study included −0.01, −0.03, −0.07, −0.14, −0.28, −0.56, −1.12 and −1.5 MPa. We used distilled triple deionized water (TDI-water) to simulate soil water saturation (0 MPa). The required water potential (Ψ in kPa) was calculated as (Wood et al., 1993):

$$\Psi = \left(6.3 \times 10^{-5} T - 0.021 \times 96\right) O^{2.2357} \quad (1)$$

where T is the temperature (298.15 K) and O is the osmolality (g 1000 g^{-1} of water). The use of PEG 6000 and PEG 10000 is equally appropriate (Wood et al., 1993).

We used a similar experimental set up to investigate the germination in relation to salinity stress. We established this experiment in parallel with PEG study. We placed the seeds on filter paper soaked with 5 mL sodium chloride (NaCl) solutions (Abbad et al., 2004) corresponding to osmotic potentials of −0.07,−0.14, −0.28, −0.56, −1.12 and −1.5 MPa (0.015, 0.030, 0.061, 0.122, 0.245, 0.328 M, respectively) (Lang 1967). We also used the distilled TDI-water to simulate non-saline condition.

We measured germinability (%) (Arnold et al., 2014a; Ranal and De Santana 2006) and mean germination time (days) (Arnold et al., 2014a; Czabator 1962) in relation to salinity levels and water potentials. Germinability is defined as:

$$G = \left(\frac{g}{n}\right) \times 100 \quad (2)$$

where g is the number of germinated seeds and n is the number of seeds per replicate.

We calculated the mean time required for maximum germination of a seed lot as follows (Demir et al., 2008):

$$\bar{t} = \frac{\Sigma_{i=1}^{k} g_i t_i}{\Sigma_{i=1}^{k} g_i} \quad (3)$$

where $\bar{t}$ is the mean germination time required for maximum germination, t_i is the time elapsed since the beginning of the germination, g_i is the number of seeds germinated at time t_i, and $\sum_{i=1}^{k} g_i$ is the final germination. Finally, we compared the effect of the two treatments (PEG and NaCl) on the mean values of germinability and germination time using the one-way ANOVA (Khan and Rizvi 1994; Montgomery 2013) followed by Duncan's multiple comparison test ($p < 0.05$).

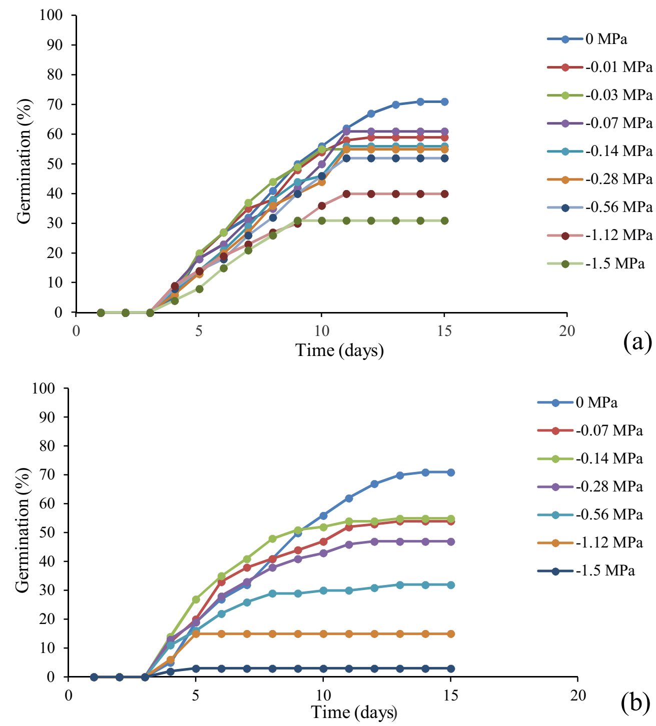

Fig. 1. Cumulative germination of *Atriplex halimus* seeds exposed to (a) PEG (polyethylene glycol) solutions and (b) NaCl solutions.

2.2. Parameters of the hydrotime model

The hydrotime model (Bradford 1990; Gummerson 1986) can be used to estimate "*the extent of germination and timing of germination for the specific seed population in relation to the soil water potential of environment*" (Bradford 2002). It is defined as:

$$\theta_H = (\Psi - \Psi_b(P))\, t_P \quad (4)$$

where θ_H (MPa h) is the hydrotime constant, which "*quantifies the inherent speed of germination and varies among species and physiological states*" (Bradford 2002), t_P (h) is the time for the germination of percentage (P) of the seed population, and $\Psi_b(P)$ (MPa) is the base water potential required to complete the germination of P. The hydrotime model can be used to predict the germination time courses such as the rate and extent of germination of the seed population under any conditions of water potential at a constant temperature (Arnold et al., 2014a; Bradford 2002). We estimated the parameters of the hydrotime model (θ_H and Ψ_b) based on the observed values of t_P at each level of Ψ (Arnold et al., 2014a; Bradford 2002).

3. Results

3.1. Seed germination

Germination commenced after 3 days, and the osmotic potentials of the PEG solutions and salinity level of the solutions did not affect the starting point of germination (Fig. 1). The germination was highest (71 ± 5.2%) at 0 MPa corresponding to saturated soil water conditions and/or non-saline conditions (Fig. 2a). Germinability reduced when the seeds were exposed to PEG or NaCl solutions (Fig. 2a). The mean germination time also decreased ($p < 0.001$) with decreasing osmotic potentials when the seeds were exposed to NaCl solutions (Fig. 2b). Furthermore, the mean germination time reduced significantly when the seeds were exposed to