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EFFECT OF WATER AND SALT STRESS ON SEEDS GERMINATION AND VIGOR OF DIFFERENT *EUCALYPTUS* SPECIES

de Sá-Martins R*, Cleiton-José A, Rocha-Faria JM & de Melo LA

Department of Forest Sciences, Federal University of Lavras (UFLA), Lavras, MG, 37200-000, Brazil

**rayanamartins@gmail.com*

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Seed plays a key role in the propagation of *Eucalyptus*, allowing greater distribution and adaptation of the material under different soil and weather conditions. Since water and salt stress are the main factors that affect plant development, studying their effect on germination and initial growth of seedlings of these species is relevant. In order to determine the species with the most contrasting behavior, to tolerate water and salt stress, germination tests at different osmotic potentials, obtained by aqueous solutions of polyethylene glycol and NaCl, were performed to simulate water and salt stress. Radicle protrusion was counted daily while normal seedlings were counted at the end of the tests (45 days). Generalised linear model analysis was used and the data were analysed by the LSD test at 5% probability. It was verified that the maximum limit of tolerance to water stress ranged from -0.6 to -0.8 MPa for the six studied species. *Eucalyptus urophylla* and *E. tereticornis* were more tolerant to drought while *E. camaldulensis* and *E. tereticornis* were the most tolerant to salt stress during germination.

Keywords: Abiotic stress, tree seeds, *Eucalyptus*, tolerance

INTRODUCTION

The vegetative propagation of *Eucalyptus* is an extremely sophisticated reproduction method, mainly because of its greater effectiveness in capturing the genetic gains obtained from breeding programs. However, in this breeding technique there is no genetic variability, and therefore, no chance of an evolution in the genetic material of the species (Hoppe et al. 2004).

In sexual reproduction, it is possible that genetic characteristics commute among individuals. *Eucalyptus* plants propagation by seeds allows certain phenotypic characteristics of interest to be inherited to the next generation simultaneously as genetic variability is present, allowing new genetic gains when crossing from one generation to another (Hoppe et al. 2004). Thus, the establishment of plantations via seedlings obtained by seeds becomes an extremely important procedure until new clones of proven value are available (dos Santos et al. 2007). It is known that the abiotic stress can limit the growth and development of plants with, emphasis on water and salt stresses that are the main factors affecting crop yield, especially in arid and semiarid regions (Harfouche et al. 2014, Gholami et al. 2015) .

Water deficit usually reduces the germination speed index (GSI) and germination rate of seeds, and can reduce the probability for establishment of seedlings, since the water availability is considered extremely important for the progress of several metabolic reactions (Yang et al. 2010). Salinity effect on germination does not only restrict seed imbibition and causes water stress, but also causes toxicity due to the high content of soluble salts in the soil, thus increasing the concentration of ions in the cytoplasm of cells (Betoni et al. 2011).

Thus, the reduction of water potential, associated to the toxic effect of salts, interferes initially in the water absorption process by seeds, and may affect the germination, vigor of seedlings and hence the normal development of plants (Ghaderi-Far et al. 2010).

The period between sowing and establishment of seedlings is crucial for the survival of seedlings, since a shorter emergence time confers a possible ecological advantage in establishing the plant in areas with suboptimal conditions, whereas it results in a shorter exposure period of plants to adverse environmental factors, such as low soil moisture and salinity (Bewley et al. 2013).

One way to simulate water/salt stress conditions in the laboratory is to use aqueous solutions at different osmotic potentials, which may delay the germination process and/or decrease the final germination rate. Among the different solutions used to simulate water stress conditions, the polyethylene glycol (PEG) is highlighted, being chemically inert, non-toxic and non-penetrating osmotic agent, due to its high molecular weight (Landjeva et al. 2008). The solute widely used in the simulation of salt stress is NaCl, since the soil salinisation occurs with the accumulation of certain ionic species, being Na^+ and Cl^- the most frequent ions, considered the main ones to hinder the plant metabolism (Nobre et al. 2010).

To assist in the development of new clones tolerant to abiotic stresses, such as water deficit and salinity, it is important to select species that tolerate these stresses in the germination phase, leading to the identification of individuals with a mechanism of effective drought/salinity tolerance in commercial plantations (Echer et al. 2010). One of the most commonly used methods for studying plant tolerance to abiotic stresses is the assessment of the germination capacity of seeds in such conditions (Larcher 2006).

Despite the importance of eucalypt, research on seed germination under stress conditions has received little attention.

The hypothesis of this study is that seeds of different *Eucalyptus* species behave distinctly when exposed to different osmotic potentials in PEG and NaCl. Thus, the present study aims to evaluate the tolerance to water and salt stress of seeds from different *Eucalyptus* species during germination.

MATERIAL AND METHODS

Plant selection and acquisition of seeds

Eucalyptus species were selected from contrasting environments, especially in relation to water availability. Seeds were acquired from the Institute of Forest Research and Studies (IPEF) and stored in plastic containers in a cold chamber (5 ± 2 °C) until the time for their use. To perform the tests, sieves were used to separate seeds from the husk.

Germination under water and salt stress conditions

In order to determine the responses of species to water and salt stress, germination tests were performed at different osmotic potentials with seeds of six *Eucalyptus* species.

To simulate water stress, seeds were subjected to different osmotic potentials in PEG 6000 solutions at potentials of -0.4, -0.6 and -0.8 MPa. Salt stress was performed in aqueous solutions of NaCl at potentials of -0.5, -1.0 and -1.5 MPa. The PEG 6000 and NaCl solutions were prepared according to Sun (2002). As a control, seeds were placed to germinate in distilled water.

Plastic boxes containing blotting paper moistened with water (control) and PEG 6000 or NaCl solution equivalent to 2.5 times the mass of non-hydrated paper were used, following recommendations of Brasil (2009). The boxes were incubated in germination chambers maintained at 25 °C (*E. brassiana*, *E. urophylla*, *E. saligna* and *E. grandis*) or 30 °C (*E. tereticornis* and *E. camaldulensis*), according to the rules for seed analysis (Brasil 2009, 2013).

The tests were performed in a completely randomised design (CRD), in a 6 × 4 factorial design (six *Eucalyptus* species x four osmotic potentials of PEG and NaCl), using five replicates of 20 seeds per treatment.

Daily counts were performed to determine the germination speed index (GSI), being the 45th day after sowing computed the germination percentage (Brasil 2009). The formula proposed by Maguire (1962) was used to calculate GSI:

$$GSI = (G1/N1) + (G2/N2) + ... + (Gn/Nn)$$

where GSI = germination speed index, G1, G2, ..., Gn = number of germinated seeds in first, second, to the last count and N1, N2, ..., Nn = number of days from sowing to the first, second and last count.

Statistical analysis

All data were submitted to normality (Shapiro–Wilk) and homoscedasticity (Bartlett) tests. When data distribution was abnormal and/or homoscedasticity, where none transformation normalised the data, the analysis was performed using Generalised Linear Models (GLM). Observation data on effect of treatments on germination and GSI by Chi-squared test were analysed by Fisher's Least Significant Difference (LSD) test at 5% probability. All analyses were carried out using the software R for Windows 3.4.1 (R Core Team 2017).

RESULTS

Effect of water stress on germination

In general, seeds showed better performance, in relation to the final germination rate and GSI, when germinated in water, i.e., without water stress induction.

Table 1 shows the average germination rates of seeds from six *Eucalyptus* species at different osmotic potentials (0, -0.4, -0.6 and -0.8 MPa) in PEG. All tested species germinated above 80% in water, whereby *E. tereticornis* and *E. brassiana* showed the lowest germination rate. At water potential of -0.4 MPa, *E. urophylla*, *E. grandis* and *E. camaldulensis* showed the best performance. When water potential was reduced to -0.6 MPa, *E. urophylla* and *E. tereticornis* showed the highest germination rate, while *E. grandis*, *E. saligna* and *E. brassiana* showed the lowest values for both water potentials (-0.6 and -0.8 MPa). At the most severe stress (-0.8 MPa), germination was zero or reduced to values close to zero for all species.

For *E. grandis*, *E. camaldulensis* and *E. brassiana*, the higher the water stress, the lower the germination rate, whereas for *E. urophylla* and *E. tereticornis*, germination rate was reduced only at -0.6 and -0.8 MPa. For *E. saligna*, the lowest germination rate was already achieved at -0.6 MPa.

GSI values from the six *Eucalyptus* species at different osmotic potentials (0, -0.4, -0.6 and -0.8 MPa) in PEG are shown in Table 2. Under normal conditions of germination, seeds of *E. grandis*, *E. camaldulensis*, *E. saligna* and *E. urophylla* showed the highest GSI values. When water stress simulated by PEG (-0.4, -0.6 and -0.8 MPa) was applied, the GSI did not differ statistically among the species, however it was lower than control (0 MPa).

Table 1 Average germination rates of seeds from six *Eucalyptus* species at different osmotic potentials (0, -0.4, -0.6, and -0.8 MPa) in PEG

Species	Osmotic potential (MPa)							
	0		-0.4		-0.6		-0.8	
Eucalyptus grandis	97	Aa	82	ABb	9	Cc	0	Bd
Eucalyptus camaldulensis	91	ABa	82	ABb	39	Bc	5	ABd
Eucalyptus saligna	90	Ba	75	Cb	4	Cc	0	Bc
Eucalyptus urophylla	90	Ba	87	Aa	45	ABb	9	Ac
Eucalyptus tereticornis	81	Ca	77	BCa	46	Ab	5	ABc
Eucalyptus brassiana	80	Ca	66	Db	8	Cc	0	Bd

Averages followed by the same capital letter on the column and lowercase on the line do not differ statistically among themselves by LSD test at 5% probability ($p \leq 0.05$)

Table 2 Averages of germination speed index (GSI) of seeds from six *Eucalyptus* species at different osmotic potentials (0; -0.4; -0.6 and -0.8 MPa) in PEG

Species	Osmotic potential (MPa)							
	0		-0.4		-0.6		-0.8	
Eucalyptus grandis	6.0	Aa	1.4	Ab	0	Ab	0	Ab
Eucalyptus camaldulensis	6.4	Aa	1.7	Ab	0.2	Abc	0	Ac
Eucalyptus saligna	6.7	Aa	1.3	Ab	0	Ab	0	Ab
Eucalyptus urophylla	6.2	Aa	2.2	Ab	0.1	Ac	0	Ac
Eucalyptus tereticornis	3.5	Ba	1.0	Ab	0.2	Ab	0	Ab
Eucalyptus brassiana	3.5	Ba	1.0	Ab	0.1	Ab	0	Ab

Averages followed by the same capital letter on the column and lowercase on the line do not differ statistically among themselves by LSD test at 5% probability ($p \leq 0.05$)

Effect of salt stress on germination

Table 3 shows the average germination rates of seeds from the six *Eucalyptus* species at different NaCl osmotic potentials (0, -0.5, -1.0 and -1.5 MPa). Under normal conditions of germination (water), all tested species germinated above 80% wherein *E. grandis*, *E. camaldulensis*, *E. saligna* and *E. urophylla* showed the highest germination rate. At -0.5 MPa, germination rate was significantly reduced only for *E. brassiana*. By decreasing the potential to -1.0 and -1.5 MPa, all species showed reduced germination, however, *E. camaldulensis* and *E. tereticornis* were more tolerant to salt stress. At the most severe stress treatment (-1.5 MPa), germination was below 10% for *E. grandis*, *E. saligna*, *E. urophylla* and *E. brassiana*. The species *E. brassiana* progressively reduced the germination with the increase of salt stress. GSI values of the six *Eucalyptus* species at different osmotic potentials (0, -0.5, -1.0 and -1.5 MPa) are shown in Table 4. Under normal conditions of germination (water) and at -0.5 MPa, *E. tereticornis* and *E. brassiana* showed the lowest GSI compared to other species. In the lowest tested osmotic potentials (-1.0 and -1.5 MPa), the GSI did not differ among species. All species showed reduced GSI when exposed to salt stress simulated by NaCl at -0.5 MPa. With the exception of *E. brassiana*, the germination of other species was not affected at this potential, however, germination speed was reduced for these species.

DISCUSSION

Effect of water stress on germination

In water stress simulated by PEG, the high limit of tolerance ranged from -0.6 to -0.8 MPa for the six studied *Eucalyptus* species. These results corroborate those of Martins et al. (2014), who evaluated the effects of PEG-induced water

Table 3 Average germination rates of seeds from six *Eucalyptus* species at different NaCl osmotic potentials (0, -0.5, -1.0, and -1.5 MPa)

Species	Osmotic potential (MPa)							
	0		-0.5		-1.0		-1.5	
Eucalyptus grandis	97	Aa	94	Aa	21	Cb	4	Bc
Eucalyptus camaldulensis	91	ABa	87	BCa	61	Ab	18	Ac
Eucalyptus saligna	90	Ba	90	ABa	27	Cb	4	Bc
Eucalyptus urophylla	90	Ba	85	BCa	23	Cb	6	Bc
Eucalyptus tereticornis	81	Ca	83	Ca	62	Ab	18	Ac
Eucalyptus brassiana	80	Ca	59	Db	40	Bc	9	Bd

Averages followed by the same capital letter on the column and lowercase on the line do not differ statistically among themselves by LSD test at 5% probability ($p \leq 0.05$)

Table 4 Averages of germination speed index (GSI) of seeds from six *Eucalyptus* species at different NaCl osmotic potentials (0; -0.5; -1.0 and -1.5 MPa)

Species	Osmotic potential (MPa)							
	0		-0.5		-1.0		-1.5	
Eucalyptus grandis	6.0	Aa	2.8	ABb	0.2	Ac	0	Ac
Eucalyptus camaldulensis	6.4	Aa	3.3	ABb	0.8	Ac	0	Ac
Eucalyptus saligna	6.7	Aa	3.2	ABb	0.3	Ac	0	Ac
Eucalyptus urophylla	6.2	Aa	3.9	Ab	1.0	Ac	0.2	Ac
Eucalyptus tereticornis	3.5	Ba	1.7	Bb	0.5	Ab	0.1	Ab
Eucalyptus brassiana	3.5	Ba	1.7	Bb	0.4	Ab	0	Ab

Averages followed by the same capital letter on the column and lowercase on the line do not differ statistically among themselves by LSD test at 5% probability ($p \leq 0.05$)

stress on the germination of five *Eucalyptus* species, including *E. camaldulensis*, *E. grandis* and *E. urophylla*, and found that the osmotic potential limit for germination was -0.8 MPa. The knowledge about how stress affects seed germination is extremely important, since it can aid in the evaluation of tolerance limits and adaptive capacity of species, allowing a better distribution of the material in different soil and weather conditions (Larcher 2006).

Germination rates of *E. urophylla* and *E. tereticornis* were not affected at -0.4 MPa, showing their greater tolerance to water stress in comparison to others. The capacity of some species seed to germinate under water stress conditions confers ecological advantages over drought sensitive ones (Rosa et al. 2005). Based on these results, it can be inferred that *E. urophylla* and *E. tereticornis* are the most adapted to environments subjected to water stress.

E. saligna showed the lowest germination rate already at -0.6 MPa, thus demonstrating its greater sensitivity to water stress. According to Stefanello et al. (2006), reduced germination rate under water restriction can be a consequence of the inhibition of radicle protrusion or embryo death when seed starts to germinate and there is not enough water to keep the process.

GSI data decreased when seeds were subjected to -0.4 MPa in PEG. Due to the water restriction at this osmotic potential the water uptake becomes slower (Stefanello et al. 2006), decreasing GSI values. Coimbra et al. (2009) stated that germination test is only effective in determining the physiological quality of seeds when integrated with results from the vigor evaluation. The vigor tests allow estimating the physiological potential of seeds by identifying significant differences among species, which are generally not detected by germination test (ISTA 2012). Although the germination of *E. urophylla* and *E. tereticornis* was not affected at the potential of -0.4 MPa, their germination speed was reduced. In suboptimal conditions seeds are exposed to the environment for a longer period, which can be disadvantageous since it may lead to the formation of abnormal seedlings or even the absence of them. A similar result was observed in *Zeyheria montana* seeds, in which germination at 25 °C was not affected until a osmotic potential of -0.3 MPa in PEG, while GSI was reduced compared to the control (0 MPa) (Oliveira et al. 2017). Martins et al. (2014), who analysed the effects of PEG on the germination of different *Eucalyptus* species (*E. camaldulensis*, *E. grandis*, *E. robusta*, *E. citriodora* and *E. urophylla*) found that germination remained constant at -0.2 MPa for most of the species whereas the germination speed reduced for *E. grandis*, *E. robusta* and *E. urophylla*. These results highlight the importance of integrate germination test with vigor data, since germination per se, may not be able to identify which species is best suited to a given situation.

Effect of salt stress on germination

In the present study, *E. brassiana* was the most sensitive species to the environment with occurrence of slight salt stress, since it was the only species in which the potential of -0.5 MPa affected the germination. According to Taiz et al. (2017), the reduced germination capacity under salt stress, compared with control treatment, can be used as an indicator of salinity tolerance of the species.

Under a stress of -1,0 MPa, the species *E. camaldulensis* and *E. tereticornis* showed germination around 60% while others were below 40%. At -1,5 MPa, *E. grandis*, *E. urophylla*, *E. brassiana* and *E. saligna* showed a germination below 10%. These results demonstrated that *E. camaldulensis* and *E. tereticornis* were the most adapted species to environmental conditions subjected to salt stress.

Regarding the germination speed data, *E. tereticornis* and *E. brassiana* showed the lowest GSI, both in normal germination conditions and at -0.5 MPa. These results demonstrated that the mentioned species are less likely to be propagated in saline environments, due to a longer period in such conditions that limits the possibility of plant survival (Ditommaso 2004).

Dantas et al. (2014) verified that germination of *Aspidosperma pyrifolium* seeds was not affected by NaCl solutions above -0.36 MPa, however, GSI was reduced. Similar results were also observed in *Piptadenia moniliformis* seeds at 25 and 30 °C, where germination did not differ when subjected to an osmotic potential of up to -0.6 MPa, however, GSI decreased (Pereira et al. 2016). In the present study, the germination of *E. urophylla, E. tereticornis, E. grandis, E. saligna* and *E. camaldulensis* was not affected at -0.5 MPa, but their GSI was reduced. Factors that determine the physiological quality of seeds include germination (viability) and vigor. The decline in seed germination speed is an indicator that their vital functions have been harmed (Marcos Filho

2015). As observed, the water stress caused by the increase in salinity, acts on the seed by retarding the necessary water uptake to germinate, and as a consequence, delaying the germination process (Betoni et al. 2011). Thus, germination speed can be considered as the first variable affected by the reduction in water availability due to saline stress (Andréo-Souza et al. 2010, Oliveira et al. 2014).

Germination period is critical for survival, since the predominance of soluble salts in the soil can cause toxicity if accumulated in plant tissues, altering plant capacity to absorb, transport and use the necessary ions for its growth (Nobre et al. 2010). Thus, species in which germination is not affected in suboptimal environmental conditions, but decreases the GSI, may lead to a longer exposition to biotic and abiotic factors that can be potentially damaging.

CONCLUSIONS

In conclusion, seeds of different *Eucalyptus* species behave distinctly when exposed to different osmotic potential of water and salt. Considering the six *Eucalyptus* species studied, the high limit tolerance for water stress simulated by PEG ranged from -0.6 to -0.8 MPa. During germination processes, *E. urophylla* and *E. tereticornis* were the most tolerant species to water stress, while *E. camaldulensis* and *E. tereticornis* were more tolerant to salt stress.

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