Original Article

GROWTH AND DEVELOPMENT OF HALOPHYTE *Funaria hygrometrica* HEDW. (Funariaceae) UNDER SALT STRESS

CRESCIMENTO E DESENVOLVIMENTO DA HALOFITA Funaria hygrometrica *HEDW. (Funariaceae) SOB ESTRESSE SALINO*

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ABSTRACT: The ability of bryophytes to tolerate salt is determined by a number of biochemical routes, whereas the salt ends up driving the activation of adaptive responses to tolerate this adverse condition. Salinity is the main limiting environmental factor under plant development, and is caused by excess salt ions in the environment, mainly Na^+ and Cl^-. The optimal growth of plants in saline environment is obtained in concentrations of 50% of NaCl. Due to these findings, the importance of the study of the effect of salinity on the germination of plants, in this case in *Funaria hygrometrica* Hedw., is noticed.

KEYWORDS: Mosses. Salt stress. Axenic tissue culture. I*n vitro* development.

INTRODUCTION

Various environmental situations can cause stress in plants (BOGDANOVIC et al., 2012). Many physiological processes such as photosynthesis are affected by abiotic stresses, such as salt stress (GAO et al., 2016). Salinity is the main limiting environmental factor in the development of plants, and is caused by excess salt ions in the environment, mainly Na^+ and Cl^- (PARIDA; DAS, 2005). However, the environment for plants that develop the ability to adapt to salt-rich environment, these are called halophytes. In contrast to flowering plants, relatively few bryophytes occupy saline environments (BATES, 2000). The Bryopsida class is unique among mosses, which have certain adjustments against salt tolerance (CHOUCKR-ALLAH et al., 1996). Bryopsida belonging to class, and the family of the most influential among bryophytes moss, *Physcomitrella patens* (Hedw.) Bruch & Schimp, the briófita *Funaria hygrometrica* Hedw. it served as an organism for the study, since mosses are organisms that serve as good model for study of plant (COVE et al., 2006).

Birds can disperse plant units (diaspores) via ingestion (endozoochory) or externally by the attachment of diaspores to the body (ectozoochory). Endozoochory is known to be the primary means of bird-mediated dispersal at local, regional, and continental scales (LEWIS et al., 2014). Spores of bryophytes traveling in the body of various migratory birds and end up being exposed to sea water, since birds require feed. This time in contact with salt water, can cause various consequences to the plant, even desiccation, but some mosses have the ability to tolerate salt, which is determined by various biochemical pathways, since this salinity ends up driving the activation of adaptive responses to tolerate this adverse condition (WANG et al., 2008), as it occurs in halophytes as *Tortella flavovirens* (Bruch.) Broth and *Schistidium maritimum* (Sm. ex R. Scott) Bruch & Schimp, which in biochemical studies showed only small increases intracellular sodium when incubated in sea water (BATES et al., 2009).

The plants have a depression of 50% in their growth when in contact with salt (PARIDA; DAS, 2005). *P. patens* is chosen to study differentiation processes in plants, as it has been in the haploid phase protonemal moss seen this, the first analysis of *Physcomitrella* tolerance to salt indicated that the plants are able to tolerate NaCl concentrations to 600 mM . This high degree of tolerance has been attributed to the presence of a Na + ATPase pump, which is usually not found in flowering plants, and therefore may have been lost during the development of terrestrial plants (FRANK et al., 2005; BENITO; RODRIGUEZ NAVARRO, 2003).

This study aimed to measure the growth and development of halophyte plant tests on different submersion times in saline (NaCl, 100%), similar to seawater, were conducted and the data computed. It

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is expected that longer in seawater decrease the the sporophytic regeneration as well as developing *F. hygrometrica.*

MATERIAL AND METHODS

Plants materials and growth conditions

Sporophytes of *F. hygrometrica* were obteined in urban area of the city of São Gabriel-RS (30º20'37.8"S, 54º19'36.9"W) Capsules were surface sterilised for 3 minutes in solution of 70% NaOCl comercial (2,5%). After disinfestation, the capsules were treated with salt solution and ruptured in petri dishes contained medium KNOP adapted from Reski e Abel (1985) that includes 250g.L^{-1} of KH_2PO_4, KNO_3 and $MgSO_4$ x $7H_2O$, 1g.L^{-1} of Ca and 12,5mg.L^{-1} of $FeSO_4$ x $7H_2O$. The medium was suplemented with agar-agar (Vetec) 12g.L^{-1} and the pH was adjusted for 5,8 with NaOH and HCl. The petri dishes are held in chambers photoperiod at 25±1 ºC under long day conditions (16h light / 8h dark). The explants were monitored for 30 days

Experimental design

Capsules *F. hygrometrica* after sterilization were immersed in 3.26% NaCl solution equal to 100% sea water, according to the standard described by Pangua et al. (2009). Five capsules were selected for each treatment consisting in control without influence of sea water and treatment with capsules immersed in sea water for 6, 12, 18 and 24min.

Statistical analysis

The data were submitted to nonparametric test (Kruskal-Wallis). The number of regenerated spores and the diameter of each protenemal regenerated spore growth were analyzed. Data analysis was performed using the software Statistix 10.0.

RESULTS AND DISCUSSION

Regenerated spores

F. hygrometrica tolerated all immersion times in saline (α=0.05), without significant differences between the salt exposition (Figure 1). Treatment control shows the highest number of protonemal regeneration (18.40 average), regeneration treatment 2 (18 min in saline soluction) shows the lowest protonemal regenerated (average 8.10) (Figure 2A).

Halophytes have the ability to survive in salt developing a new state of metabolic equilibrium, thus allowing their survival and development. When different salt concentrations were available for *Bryum argenteum* Hedw., *Atrichum undulatum* (Hedw.) P. Beuv. cultured in vitro the average survival and other characteristics showed a decrease in culture media with the highest salt concentrations (BOGDANOVIC et al., 2012). Comparing these results to that found in *F. hygrometrica* and *P. patens*, one realizes that these mosses have greater ability to survive the salty conditions, it can be adapted to the high time of exposure to salt.

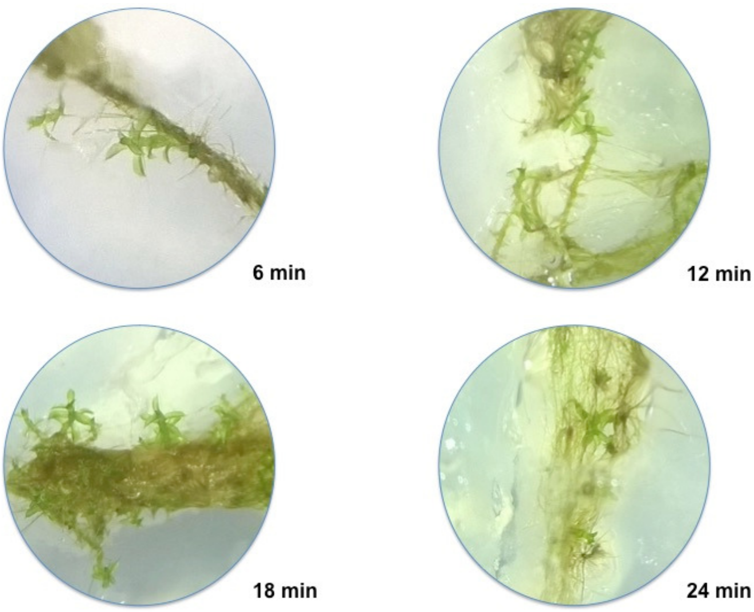


Figure 1. Development of *Funaria hygrometrica* Hedw. in each exposition time in salt solution.

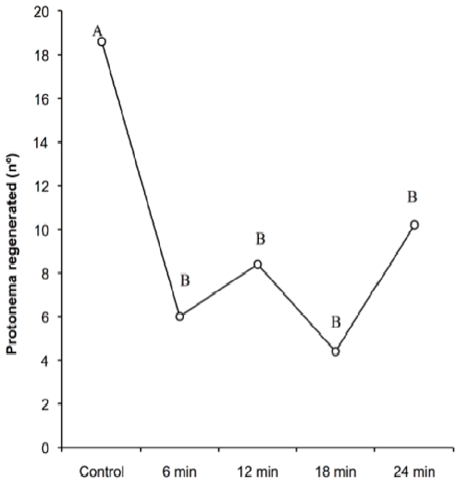
Diameter of each protenemal growth

There was a decline in diameter with increase in salt immersion time, except for the treatment 5 where it increased diameter. The mean of the control treatment were grouped with treatment 5 (24min) with α=0.05. Treatments 2 and 3 (12min-18min) showed no significant difference between them, with an average diameter of 7.70 and 7.60 respectively (Table 1). Since the control treatment and treatment 4 (24min) did not show significant differences among themselves with averages of 16.10 and 12.40 respectively. Treatment 1 (6min) was different from all other treatments with an average of 21.20 (Figure 2B).

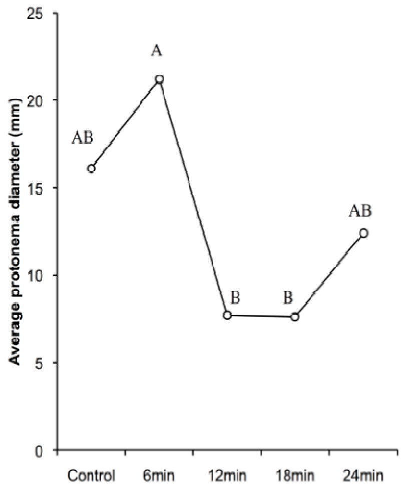
Table 1. Average diameter protonemal growth of *Funnaria hygrometrica* under distinct exposures in salt solution.

Treatments	Protonemal growth* (mm)
Control	16.10^{AB}
6min	21.20^{A}
12min	7.70^{B}
18min	7.60^{B}
24min	12.40^{AB}

* Means followed by the same lowercase letter in the line do not differ by Tukey test (α = 0.05).



A



B

Figure 2. A. Average of protonemal regenerated in each treatment. B. Average diameter protonemal growth.
* Means followed by the same lowercase letter in the line do not differ by Tukey test (α = 0.05).

The saline state of change leads to initial reductions in growth. These reductions depend on the concentration and duration of exposure to salt provided to the plant. (HASEGAWA et al 2000;. PARIDA; DAS, 2005;). As quoted by Munns and Tester (2008) salt causes morphological and anatomical changes in plants. High salt concentrations favoring the development of protonemal in *P. patens* as found in *F .hygrometrica*. In all salt exposure time, the only way was found vegetative protonemal (BENITO; RODRIGUEZ-NAVARRO; 2003). The results suggest that protonemal growth *F .hygrometrica* is not affected by in saline immersion time, although there was a decline in protonemal diameter after treatment 1, the largest salt exposure time, said normal conditions moss development were - established.

The salt ends up providing many effects on different plants, and the response to this condition depends on your physiology. In leaves of rice (*Oryza sativa* L.), salt eventually causes of senescence due to high intracellular concentrations of Na^+ ions and Cl^- (LUTTS et al., 1996). Salinity also affects the intracellular concentration of metals in aquatic plants, which increase with decreased salinity (FRITIOFF et al., 2005).

CONCLUSION

The exposure *F. hygrometrica* from saline solution, at different times, does not cause significant changes in their development. Being a halophyte plant, with metabolic, morphological and anatomical modifications , which causes a greater survival rate this plant to salt immersion, whether caused shaped soaking diasporas or environmental exposure.

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RESUMO: A capacidade das briófitas para tolerar meios salinos é determinada por uma série de vias bioquímicas, uma vez que o sal acaba por conduzir a ativação de respostas adaptativas para tolerar esta condição adversa. A salinidade é o principal fator ambiental limitante no desenvolvimento da planta e é causada pelo excesso de íons salinos no ambiente, principalmente Na^+ e Cl^-. O crescimento ótimo de plantas em ambiente salino foi obtido em concentrações de 50% de NaCl. Devido a essas descobertas esses achados, observa-se a importância do estudo do efeito da salinidade sobre a germinação de plantas, neste caso em *Funaria hygrometrica* Hedw**.**

PALAVRAS-CHAVE: Musgos. Estresse salino. Cultivo axênico. Desenvolvimento *in vitro.*

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