Echophysiological study of germination and initial development in *Jatropha curcas* during storage and salinity stress

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# Abstract

*Jatropha curcas* L. is a plant that can be used in the production of biofuel, whose species presents strong resistance to drought. However, the crop presents two important problems: i) rapid loss of viability, resulting from the high respiratory rate of the seeds during the storage period; ii) seed sensitivity when germinated under salinity conditions. To achieve these objectives, two experiments were developed. In the first experiment were verified how the storage of seeds in a drier environment can influence germination, respiration rate and the main biochemical and physiological parameters. In the second experiment, we studied the effect of the addition of five different concentrations of NaCl (0, 50, 75, 100 and 150 mM) under the parameters of germination and initial growth of five accessions of *J. curcas* originating from different producing regions of Brazil. The results of the first experiment show that the use of desiccant, can stabilized the germinability of the seeds stored, a fact corroborated by the reduction of the water potential of the seeds and the strong reduction of the respiratory rates. On the other hand, we showed that *Jatropha curcas* presents a moderate tolerance to salinity, being able to germinate up to 150 mM NaCl, even though a drastic reduction in the biomass accumulation was observed with the increase of the salt concentration in the irrigation water. The results show that the germination was reduced to values close to 4% in the treatment of 150 mM, while the average time of germination increased with the increase in the concentration of salts. In the biometric and biomass variables, the dry weight of the leaf, leaf area, plant length and total biomass were strongly affected by the increase of the salts, while in the biomass allocation parameters, accumulation was observed in the stem of the seedlings. In this sense, genotypes 114, 171 and 183 were shown to be potentially tolerant while genotypes 218 and 133 were sensitive.

**Key words:** salinity tolerance, seed germination, biomass, biofuel, NaCl

# Introduction

*Jatropha curcas* (pinhão-manso) is a species belonging to the family Euphorbiaceae with multiple uses, abundantly distributed in many tropical and subtropical regions in the Americas, Africa and Asia [[1](#ref-heller1996physic),[2](#ref-takeda1982development)]. Over the last 20 years they have gained a lot of attention as a potential crop for bioenergy production, since their seed oil can easily be converted to good quality biodiesel. In addition, the species does not present as edible and therefore does not compete with the other oilseeds [[3](#ref-Pompelli2011)]. It has a high growth rate, easy propagation, short period until the first fruit harvest, low seed cost [[1](#ref-heller1996physic)], high oil content (40-58%) [[4](#ref-pandey2012jatropha),[5](#ref-pompelli2010environmental)], and good adaptation to different agroclimatic conditions [[6](#ref-Divakara2010)–[8](#ref-gao2008effects)]

Germination is the process that determines when and where the seeds will initiate their growth [[9](#ref-gunster1994seed)], allowing the embryo to germinate and develop as a photosynthetically active organism. It begins with the imbibition of the quiescent seed and ends as the elongation of the embryonic axis, which can be visualized by the emergence of the radicle under the surface of the soil. At the moment, the reserves contained in the seeds begin to be mobilized by yielding energy to the developing embryo [[10](#ref-bewley2013mobilization),[11](#ref-sanchez2012early)]. In this sense, salinity can affect germination, limiting the absorption of water in the seeds (osmotic effect) [[12](#ref-almansouri2001effect),[13](#ref-hegarty1977seed)] increases the toxicity by ions or the combination of both, Na+ ion affect the biochemical processes in plants [[14](#ref-apse1999salt)]. In addition, it may affect the mobilization of reserves [[15](#ref-bouaziz1990consumption)], structural organization and protein synthesis in embryos [[16](#ref-alencar2015ultrastructural)]. In saline environments, plant adaptation during germination and early development are decisive stages for species establishment, and such factors may negatively influence this process [[17](#ref-ungar1995seed)].

Seed storage is one of the factors that most negatively affects seed viability, which includes the time elapsed between harvesting and utilization [[18](#ref-marcos1984testes),[19](#ref-marcos1998new)]. *J. curcas* does not escape this pattern, since it presents high metabolism, causing its seeds to rapidly lose their viability with storage [[20](#ref-moncaleano2013germination)]. Marcos-Filho [[19](#ref-marcos1998new)] describes that seed storage is a major problem for agriculture [[21](#ref-tekrony2006seeds)], since it is responsible for large losses worldwide, especially in the tropics, where High temperatures and high relative humidity prevail during seed maturation and storage [[22](#ref-bilia1994comportamento)]. Although deterioration is irreversible and unavoidable, the speed of the process can be controlled by appropriate harvesting, drying and storage techniques [[20](#ref-moncaleano2013germination)]. In this sense, the use of drier atmosphere environments could protect seeds [[23](#ref-hay2012evaluation)–[25](#ref-rao2006storability)].

Another factor that negatively influences agriculture is salinity, mainly in irrigated crops [[26](#ref-kumar2008effects)], with NaCl being the predominant salt. Soil salinization can occur naturally by deposition or contact with sea water, and approximately 20% of the world's cultivated land is affected by salts [[27](#ref-sun2009nacl)]. This problem is more relevant in arid and semi-arid regions of the globe, where the lack of rainfall and the high evaporative demand caused by high temperatures and low relative humidity contribute to soil salinity intensification. In addition, salinity affects plant growth and development [[28](#ref-munns2008mechanisms)], negatively influencing different stages of its development [[12](#ref-almansouri2001effect),[29](#ref-khajeh2003interaction),[30](#ref-khan2003light)]. However, throughout their evolution, plants have developed mechanisms for regulation and tolerance to salts. Some studies have studied the ecophysiological aspects of the tolerance of *J. curcas* to NaCl [[31](#ref-diaz2012tolerance)–[34](#ref-rajaona2012effect)]; However, these studies focused on only one batch of seeds. Although interest in the growth response of *J. curcas* is increasing, there is no known research that has examined the effect of NaCl on different genotypes during germination and early development of seedlings. In this work five distinct genotypes of *J. curcas* exposed to different NaCl treatments were studied to determine tolerance and to understand the morphological and physiological responses of this species under conditions of salinity in their germination and initial development, Of plants with good characteristics in their early stages of development.

Thus, the main hypotheses of this work were (i) to verify if the use of a desiccant agent could help to maintain the viability and germinability of the seeds of *J. curcas* when stored for long periods of time, and (ii) to identify genotypes tolerant to salinity between the accesses of *J. curcas* marketed in Brazil.

# Materials and methods

**Aging tests.** To test the effect of storage on seed viability, an artificial aging test was used to reduce the water content in the interstices of the seeds with a desiccant material composed of silica gel (Sigma-Aldrish, No. 10087). The genotype used in this experiment was 171 from Maceió, Alagoas. In each experimental unit 50 seeds of *J. curcas*, arranged in germbox type boxes (110 x 110 x 35 mm) were added under a stainless steel mesh suspended 2 cm from the desiccant. Five storage times (i.e., 0, 3, 6, 9, 12 months) were tested and stored under refrigerator condition at a temperature set at 4 ± 2 °C. After each storage period, the seeds were placed to germinate.

**Biochemical analysis of the seeds used in the aging test.** A portion of 10% of the samples in each storage period was carefully ground in liquid nitrogen and stored at -20 °C until use. For the analysis of total soluble carbohydrates (CST), total soluble amino acids (AST) and starch (AMD), the samples were solubilized in 50% (v/v) ethanol [[35](#ref-trethewey1998combined)], whereas for analysis of total soluble proteins (PST), the samples were extracted in Stitt buffer [[36](#ref-armengaud2009multilevel)]. The methodologies described in Dubois [[37](#ref-dubois1956colorimetric)], Bradford [[38](#ref-bradford1976rapid)] and Moore and Stein [[39](#ref-moore1954modified)] were used for the analysis of starch, total soluble carbohydrates, total soluble proteins and soluble amino acids, respectively. For the quantification of the oil content (TDO) the methodology described in detail by Ahmad [[40](#ref-ahmad1981ricinoleic)] and for the determination of glucose (GLC), fructose (FTS) and sucrose (SCR) [[41](#ref-stitt198932)], coupled to the production of 6-phosphogluconate, in the sequential presence of hexokinase, phosphoglucoisomerase, glucose-6-phosphate dehydrogranase and invertase enzymes. All these analyzes were performed in triplicate.

**Physiological analyzes coupled to the aging test.** The relative water content (TDA) of seeds was calculated as described in detail in Vertucci [[42](#ref-vertucci1993predicting)] and Moncaleano-Escandon [[20](#ref-moncaleano2013germination)]. The water potential (PTH) of the seeds was quantified with the dew point water potential meter (WP4C; Decagon Devices, Pullman, WA, USA), where the seeds were lightly cracked to allow water to pass through the seeds to the internal environment. The values were obtained in MPa. For respiratory rate estimation (TRS), 5% of the seeds used in each storage period were inserted whole in a CO2 flow chamber (6400-09; LiCOR, Lincoln, NE, USA). For each storage time, 10 different samples were used as replicates. In each measurement procedure of liquid respiration, three cycles of 102 seconds were performed, with a 2 second interval between the readings. During this time the increase in CO2 concentration inside the chamber was monitored. The reference CO2 was calibrated, at each measurement, according to the CO2 concentration of the environment (~ 400 μmol CO2 m-2 s-1). The net respiration rate was expressed, therefore in μmol CO2 h-1 g-1 seed.

**Germination tests with aged seeds.** At each evaluation time, 25 seeds of *J. curcas* were removed from the storage system and germinated in germbox boxes (110 x 110 x 35 mm) containing two sheets of germtest paper soaked with 2x the weight of the paper in water and were sealed and placed in a NT 708 growth chamber (New Technical Instruments, Piracicaba, SP, Brazil). The incubator was equipped with four cold white fluorescent lamps of 20 W with 40 μmoles m-2 s-1 at the level of the germination boxes. The photoperiod was 12 h and the temperature conditions were 25 ± 0.5 °C. Germination assessments were given daily for a period of 25 days. It was considered germinated, the seed whose radicle has emerged from the integument.

**Germination tests in the presence of NaCl.** The germination experiment was carried out in a greenhouse at the Federal University of Pernambuco, Department of Botany, Recife, PE (S 8°02"59.0' W 34°56"54.9', 4 masl), the average temperature being recorded during the experiments Of 30.6 ± 1.1 °C with a mean relative humidity of 70.4 ± 5.8%. Five different concentrations of NaCl (0, 50, 75, 100 and 150 mM) were tested in the irrigation water, with 0 mM NaCl free treatment in five genotypes of *J. curcas* (Table 1). Before seeding, the seeds were disinfected with NaOCl (2%) for 10 minutes and triply rinsed with distilled water. Next, the seeds were germinated in polypropylene trays (20 x 20 x 5 cm) containing 2500 g of river washed sand, air-dried; Each system containing 25 seeds was considered as an experimental unit. The pots were irrigated daily with 300 mL of water containing Hoagland nutrient solution [[43](#ref-epstein1972mineral)] at the final concentration of 25%, this being the volume of irrigation required (ie, previously tested) to leach excess salts and prevent their accumulation in soil. The experiment was evaluated daily for 25 days until the seedlings were collected.

**Plant material.** Seeds of *J. curcas* were supplied by EMBRAPA Agroenergia (Brasília, DF - Brazil), where they were kept at 4 °C until their use, Table 1.

Table 1 Information and location of the five accessions of *Jatropha curcas* studied under salinity conditions with the objective of evaluating the germination parameters and initial development of the seedlings.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Genotype | City | State | Location | Altitude (masl) |
| 183 | Jaíba | Minas Gerais | 23°47'55.0'' S 53°18'48'' W | 478 |
| 114 | Umuarama | Paraná | 15°10'27.0'' S 43°53'18'' W | 430 |
| 218 | São Miguel do Araguaia | Goiás | 13°55'57.0'' S 50°09'17'' O | 350 |
| 171 | Maceió | Alagoas | 09°27'60.0'' S 35°49'41'' W | 131 |
| 133 | Santa Inês | Maranhão | 03°39'24.9'' S 45°22'36'' W | 31 |

**Evaluation of germination parameters.** For the two experiments the different germination parameters were calculated: germination percentage (PGR), mean germination time (TMG), germination uncertainty (ICG) and germination synchrony (SNG). All calculations, graphs and statistics were performed from the GerminaR package [[44](#ref-R-GerminaR)].

**Evaluation of biometric parameters and biomass.** The mean seedling length (CMP) and mean stem diameter (DMC) were evaluated at the end of the experiment [[20](#ref-moncaleano2013germination)]. For this, the seedlings were collected and separated into three components: leaves, stems and roots. The height of the plants was measured by means of a ruler calibrated in mm and the diameter of the stem measured by a digital caliper (Digital Caliper, ROHS, ZAAS Precision, Piracicaba, SP). Leaf area (AF) was evaluated by plant and experimental unit (AFT) [[45](#ref-pompelli2012allometric)]. The dry biomass was estimated from drying the fabrics in a forced ventilation oven at 70 °C; 72 hours. Leaf dry weight (PSF), dry stem weight (PSC) and root dry weight (PSR) were used to calculate several biomass parameters, such as: total dry weight (PST); Aerial part dry weight (PSA); Leaf weight ratio (RPF; leaf dry weight divided by total dry weight); Dry stem weight ratio (RPC; stem dry weight divided by total dry weight); (RPA, split dry weight divided by total dry weight) and root dry weight ratio (RPR, root dry weight divided by total dry weight).

**Experimental design and statistical analysis.** Both germination experiments were conducted in a completely randomized design. For the seed storage experiment, five storage times (0, 3, 6, 9 and 12 months) were used. The data for the 9 and 12 month plots of the aging experiment were submitted to the calculation of the lost plot. The salinity experiment was composed of a factorial, where five genotypes of *J. curcas* were tested, and five different concentrations of NaCl in irrigation water (0, 50, 75, 100 and 150 mM) were tested. Each treatment consisted of four replicates with 25 seeds. Statistical analysis and generation of graphs were performed in the statistical software R [[46](#ref-R-base)]. The analysis of variance (ANOVA) was performed to evaluate the differences between the factors and the comparison of the means with the Student-Newman-Keuls test (p <0.05) [[47](#ref-R-agricolae)]. For the multivariate analysis, correlation analysis was performed [[47](#ref-R-agricolae),[48](#ref-R-corrplot)] and principal components analysis [[49](#ref-R-FactoMineR)].

# Result and discusion

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# load data  
  
library(GerminaR)  
  
fb <- prosopis %>% dplyr::mutate( nacl = as.factor(nacl), temp = as.factor(temp), rep = as.factor(rep))  
  
# germination analysis  
  
gsm <- ger\_summary(SeedN = "seeds", evalName = "D", data = fb)  
str(gsm)  
  
# analisys of variance  
  
av <- aov(formula = GRP ~ nacl\*temp + rep, data = gsm)  
summary(av)  
  
# mean comparision test  
  
mc <- ger\_testcomp(aov = av, comp = c("temp", "nacl"), type = "snk")

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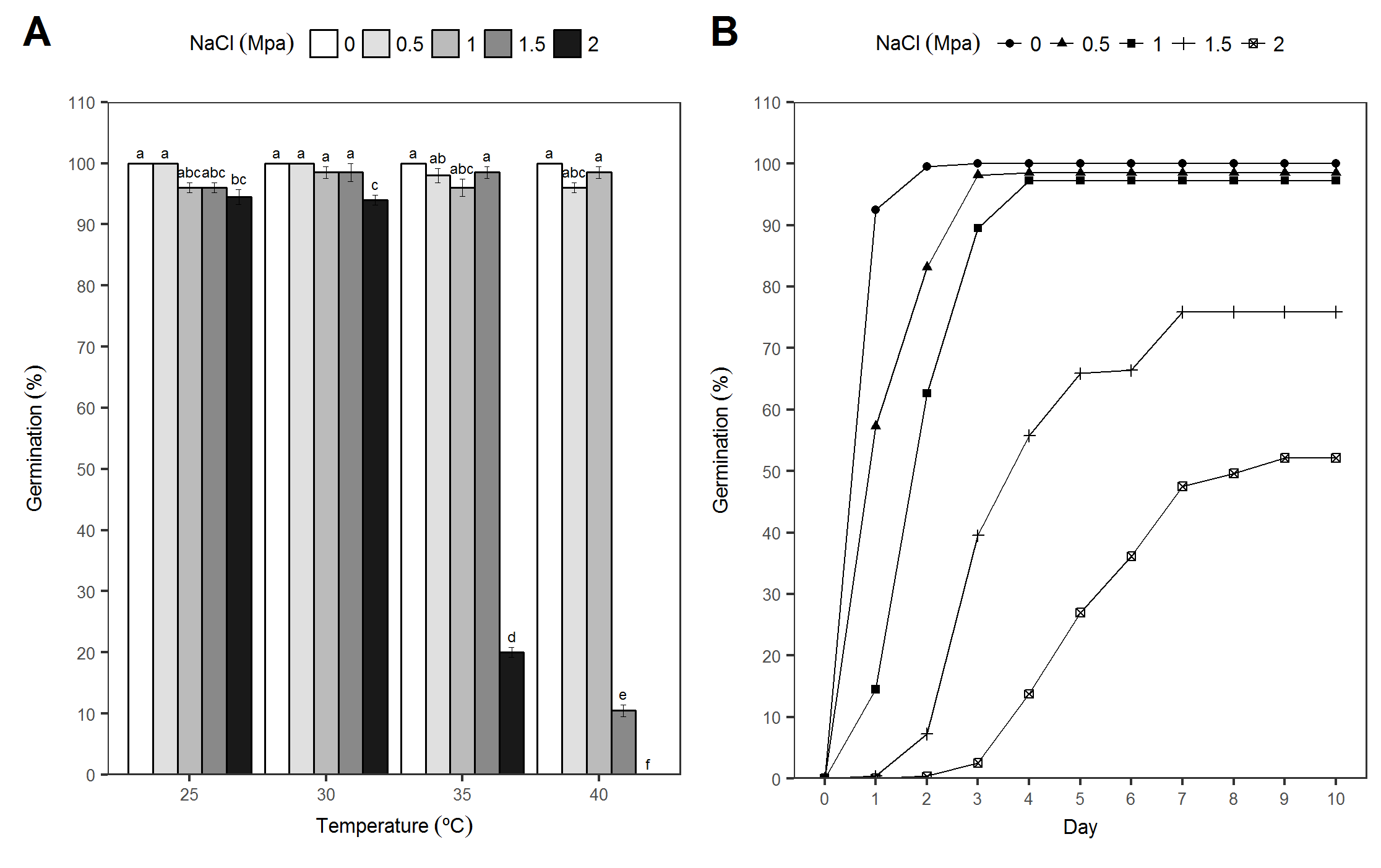


Figure 1 Germination experiment with *Prosopis juliflor* under different osmotic potentials and temperatures. A) Bar graph with germination percentage in a factorial analisys. B) Line graph from cumulative germination under different osmotic potentials.

# Conclusions

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# Acknowledgments

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