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.

# Material and methods

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Table 1 Main functions in the GerminaR R package for seed germination variables and graphical analysis.

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| --- | --- |
| Function | Description |
| ger\_summary | Calculate ten germination indices maintaining the factors levels for analysis of variance |
| ger\_intime | Calculates and displays cumulative germination data. |
| fplot | Function that allows to graphic the results in bar or line plot. |
| GerminaQuant | Runs the interactive application in offline mode for use on a personal computer. |
| prosopis | Dataset with germination experiment in *Prosopis juliflor* seeds under under different osmotic potentials and temperatures. |

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# 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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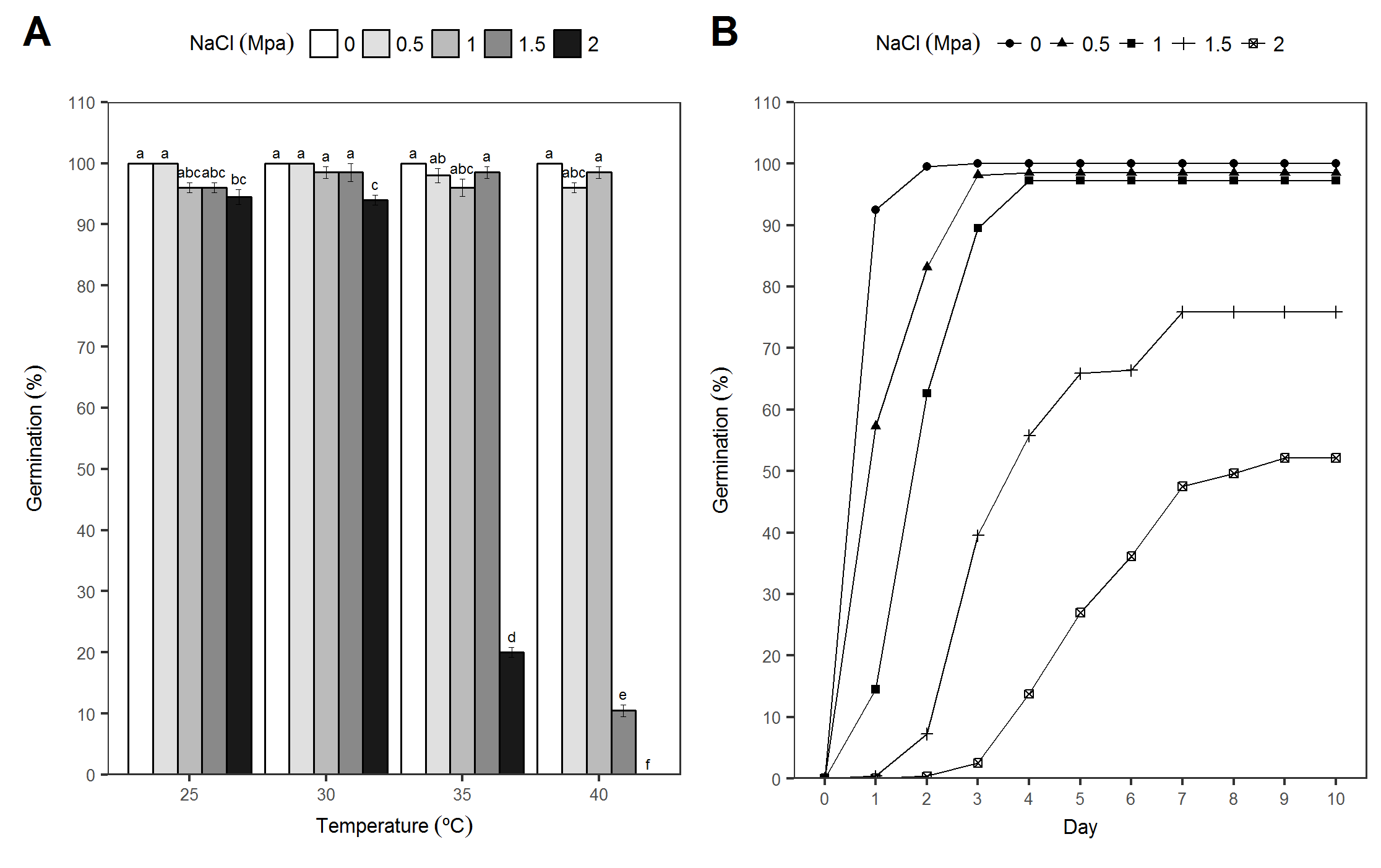


Figure 1 Germination experiment with *Prosopis juliflor* under different osmotic potentials and temperatures. A) Bar graph with germination percentage in a factorial analisys. B) Line graph from cumulative germination under different osmotic potentials.

# Conclusions

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# Acknowledgments

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# References

[1] J. Heller, Physic nut, jatropha curcas l., Bioversity international, 1996.

[2] Y. Takeda, others, Development study on jatropha curcas (sabu dum) oil as a substitute for diesel engine oil in thailand., Journal of the Agricultural Association of China. (1982) 1–8.

[3] M.F. Pompelli, A.D. Jesús, J. Orozco, M.T.D. Oliviera, B. Rafael, M. Rodrigues, M.O. Barbosa, M.G. Santos, Crise energética mundial e o papel do Brasil na problemática de biocombustíveis., Agronomía Colombiana. 29 (2011) 361–371.

[4] V.C. Pandey, K. Singh, J.S. Singh, A. Kumar, B. Singh, R.P. Singh, Jatropha curcas: A potential biofuel plant for sustainable environmental development, Renewable and Sustainable Energy Reviews. 16 (2012) 2870–2883.

[5] M.F. Pompelli, D.T. da R.G. Ferreira, P.G. da Silva Cavalcante, T. de Lima Salvador, B.S. de Hsie, L. Endres, Environmental influence on the physico-chemical and physiological properties of jatropha curcas seeds, Australian Journal of Botany. 58 (2010) 421–427.

[6] B. Divakara, H. Upadhyaya, S. Wani, C.L. Gowda, Biology and genetic improvement of Jatropha curcas L.: A review, Applied Energy. 87 (2010) 732–742. doi:[10.1016/j.apenergy.2009.07.013](https://doi.org/10.1016/j.apenergy.2009.07.013).

[7] A. Fini, C. Bellasio, S. Pollastri, M. Tattini, F. Ferrini, Water relations, growth, and leaf gas exchange as affected by water stress in jatropha curcas, Journal of Arid Environments. 89 (2013) 21–29.

[8] S. Gao, C. Ouyang, S. Wang, Y. Xu, L. Tang, F. Chen, others, Effects of salt stress on growth, antioxidant enzyme and phenylalanine ammonia-lyase activities in jatropha curcas l. seedlings, Plant Soil Environ. 54 (2008) 374–381.

[9] A. Günster, Seed bank dynamics—longevity, viability and predation of seeds of serotinous plants in the central namib desert, Journal of Arid Environments. 28 (1994) 195–205.

[10] J.D. Bewley, K.J. Bradford, H.W. Hilhorst, H. Nonogaki, Mobilization of stored reserves, Springer, 2013.

[11] L. Sánchez-Linares, M. Gavilanes-Ruíz, D. Díaz-Pontones, F. Guzmán-Chávez, V. Calzada-Alejo, V. Zurita-Villegas, V. Luna-Loaiza, R. Moreno-Sánchez, I. Bernal-Lugo, S. Sánchez-Nieto, Early carbon mobilization and radicle protrusion in maize germination, Journal of Experimental Botany. (2012) ers130.

[12] M. Almansouri, J.-M. Kinet, S. Lutts, Effect of salt and osmotic stresses on germination in durum wheat (triticum durum desf.), Plant and Soil. 231 (2001) 243–254.

[13] T. Hegarty, Seed and seedling susceptibility to phased moisture stress in soil, Journal of Experimental Botany. 28 (1977) 659–668.

[14] M.P. Apse, G.S. Aharon, W.A. Snedden, E. Blumwald, Salt tolerance conferred by overexpression of a vacuolar na+/h+ antiport in arabidopsis, Science. 285 (1999) 1256–1258.

[15] A. Bouaziz, D. Hicks, Consumption of wheat seed reserves during germination and early growth as affected by soil water potential, Plant and Soil. 128 (1990) 161–165.

[16] N.L. Alencar, C.G. Gadelha, M.I. Gallão, M.A. Dolder, J.T. Prisco, E. Gomes-Filho, Ultrastructural and biochemical changes induced by salt stress in jatropha curcas seeds during germination and seedling development, Functional Plant Biology. 42 (2015) 865–874.

[17] I. Ungar, Seed germination and seed-bank ecology in halophytes, Seed Development and Germination. (1995) 599–628.

[18] J. Marcos Filho, H.M. Pescarin, Y.H. Komatsu, C.G. Demétrio, A.L. Fancelli, Testes para avaliação do vigor de sementes de soja e suas relações com a emergência das plântulas em campo, Pesquisa Agropecuária Brasileira. 19 (1984) 605–613.

[19] J. Marcos-Filho, New approaches to seed vigor testing, Scientia Agricola. 55 (1998) 27–33.

[20] J. Moncaleano-Escandon, B.C. Silva, S.R. Silva, J.A. Granja, M.C.J. Alves, M.F. Pompelli, Germination responses of jatropha curcas l. seeds to storage and aging, Industrial Crops and Products. 44 (2013) 684–690.

[21] D.M. TeKrony, Seeds, Crop Science. 46 (2006) 2263–2269.

[22] D. Bilia, A. Fancelli, J. Marcos Filho, J. Machado, Comportamento de sementes de milho híbrido durante o armazenamento sob condições variáveis de temperatura e umidade relativa do ar, Scientia Agrícola. 51 (1994).

[23] F. Hay, P. Thavong, P. Taridno, S. Timple, Evaluation of zeolite seed’Drying beads’for drying rice seeds to low moisture content prior to long-term storage, Seed Science and Technology. 40 (2012) 374–395.

[24] F.R. Hay, R.J. Probert, Advances in seed conservation of wild plant species: A review of recent research, Conservation Physiology. 1 (2013) cot030.

[25] R. Rao, P. Singh, M. Rai, Storability of onion seeds and effects of packaging and storage conditions on viability and vigour, Scientia Horticulturae. 110 (2006) 1–6.

[26] N. Kumar, S. Pamidimarri, M. Kaur, G. Boricha, M. Reddy, Effects of nacl on growth, ion accumulation, protein, proline contents and antioxidant enzymes activity in callus cultures of jatropha curcas, Biologia. 63 (2008) 378–382.

[27] J. Sun, S. Chen, S. Dai, R. Wang, N. Li, X. Shen, X. Zhou, C. Lu, X. Zheng, Z. Hu, others, NaCl-induced alternations of cellular and tissue ion fluxes in roots of salt-resistant and salt-sensitive poplar species, Plant Physiology. 149 (2009) 1141–1153.

[28] R. Munns, M. Tester, Mechanisms of salinity tolerance, Annu. Rev. Plant Biol. 59 (2008) 651–681.

[29] M. Khajeh-Hosseini, A. Powell, I. Bingham, The interaction between salinity stress and seed vigour during germination of soyabean seeds, Seed Science and Technology. 31 (2003) 715–725.

[30] M.A. Khan, S. Gulzar, Light, salinity, and temperature effects on the seed germination of perennial grasses, American Journal of Botany. 90 (2003) 131–134.

[31] L. Díaz-López, V. Gimeno, V. Lidón, I. Simón, V. Martínez, F. García-Sánchez, The tolerance of jatropha curcas seedlings to nacl: An ecophysiological analysis, Plant Physiology and Biochemistry. 54 (2012) 34–42.

[32] A.Z. Elhag, M.O. Gafar, Effect of sodium chloride on growth of jatropha (jatropha curcas l.) young transplants, Universal Journal of Plant Science. 2 (2014) 19–22.

[33] M.F. Pompelli, R. Barata-Luís, H.S. Vitorino, E.R. Gonçalves, E.V. Rolim, M.G. Santos, J.S. Almeida-Cortez, V.M. Ferreira, E.E. Lemos, L. Endres, Photosynthesis, photoprotection and antioxidant activity of purging nut under drought deficit and recovery, Biomass and Bioenergy. 34 (2010) 1207–1215.

[34] A.M. Rajaona, H. Brueck, C. Seckinger, F. Asch, Effect of salinity on canopy water vapor conductance of young and 3-year old jatropha curcas l., Journal of Arid Environments. 87 (2012) 35–41.

[35] R Core Team, R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria, 2017. <https://www.R-project.org/>.

[36] Y. Xie, Knitr: A general-purpose package for dynamic report generation in r, 2016. <https://CRAN.R-project.org/package=knitr>.