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

Flavio Lozano-Isla, Mariana L.O. Campos, Lauricio Endres, Agnaldo R. Chaves, Egidio B. Neto, Marcelo F. Pompelli

2017-05-12

# 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

**Germination of aged seeds.** The germination of the seeds of *J. curcas* submitted to storage ranged from 9% to 15%, with values that are statistically similar to each other (Figure 1A). Although twinning was not affected by storage, the mean germination time was significantly increased with time (Figure 1B). The germination was completely asynchronous for all storage times, with a mean of 0.16 being recorded before storage and close to zero at other times (Figure 1C). As it was observed, the germination in the time was significantly affected by the storage time, however, the germination uncertainty was not affected by the storage (Figure 1D). It is verified that the seeds before storage and stored for 3 months began their germination on the 4th and the 3rd day after sowing respectively, while in other times the germination of the first seed was only computed from the 6th day the time for stabilization Of the germination was increased as the storage time was increased, being completed at 15 days without storage and at 23 days at 12 months of storage (Figure 1E).

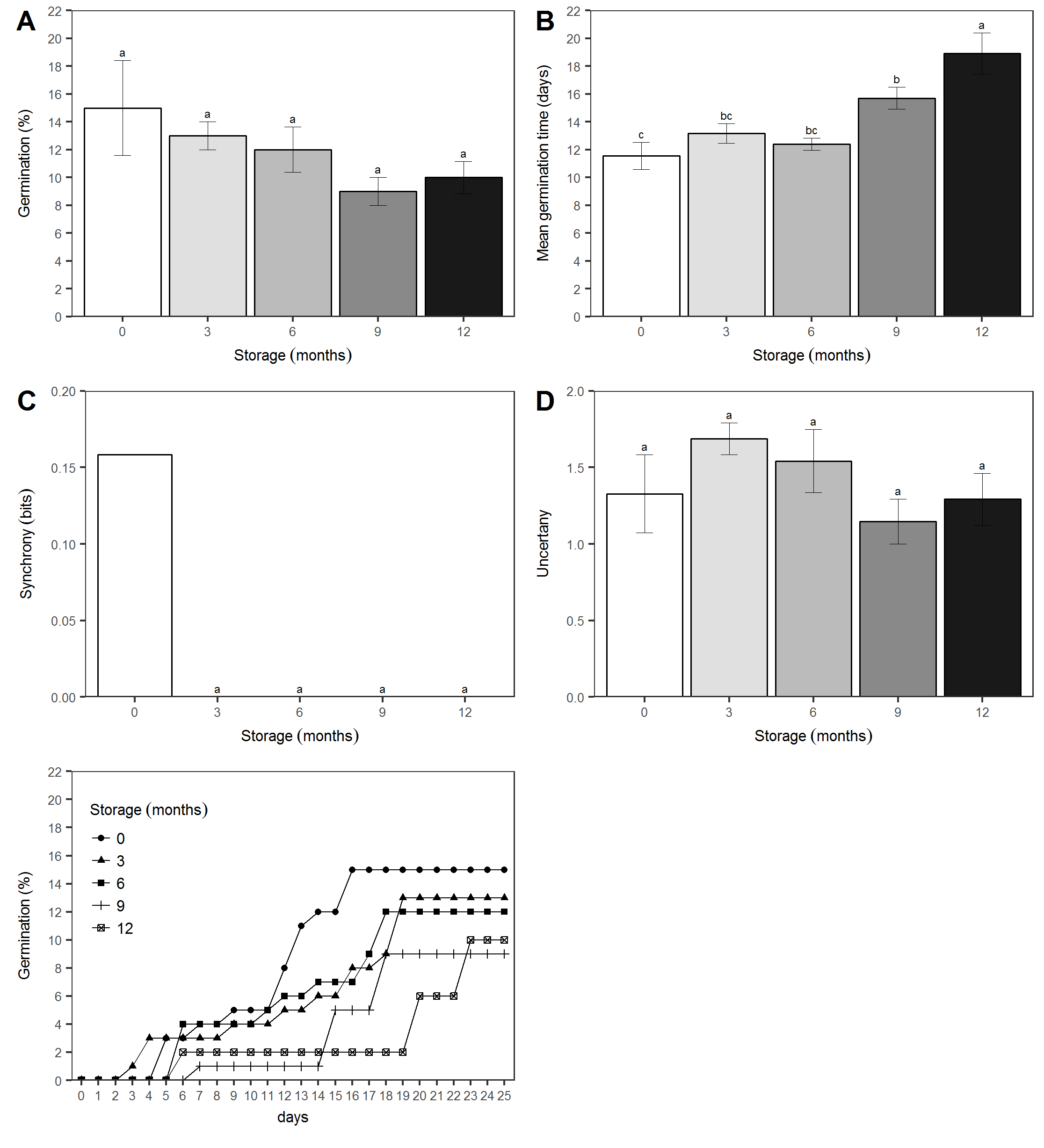


Figure 1 Germinability (A), mean germination time (B), synchrony index (C), germination uncertainty (D) and temporal germination (E) evaluated in *Jatropha curcas* seeds in genotype 171 stored at 0, 3, 6, 9 And 12 months. The bars represent the mean (± SE). The mean differences between the storage months are represented by the lower case letters (SNK, p = 0.05). N = 4

**Biochemical responses of seeds submitted to aging.** It was verified that the oil content in the seeds remained practically stable until the sixth month of storage at the rate of 35%, with significant reductions from that date until the 12th month, when the oil content was approximately 29% (Figure 2A ), And the oil content in the seeds showed a reduction in time (r = -0.91, p ≤ 0.05, Figure 5). On the other hand, the starch was rapidly metabolized with approximate reduction of 33% with time 12 months of storage (Figure 2B). Total soluble protein and total soluble amino acid content was increased by 160% and approximately 67% (Figure 2C-D) during storage. A high correlation (r = 0.92, p ≤ 0.05) is shown between total soluble proteins and amino acid syntheses.

There was a gradual reduction in the total soluble carbohydrate content at the third month of storage compared to the control, without showing differences until the 12th month of storage (Figure 3A). Sucrose levels had a reduction of approximately 49% between 3 and 12 months of storage (Figure 3B), but glucose levels remained stable until the 3rd month of storage. From the 6th month the glucose was rapidly elevated, reaching 71% in the 12th month in relation to the non-stored seeds (Figure 3C). On the other hand, fructose levels did not show a trend during the months evaluated (Figure 3D), while sucrose levels decreased (r = -0.57, p ≤ 0.05) throughout the storage period. Glucose (r = 0.67, p ≤ 0.05) had an opposite behavior, ie increase with storage (Figure 5)

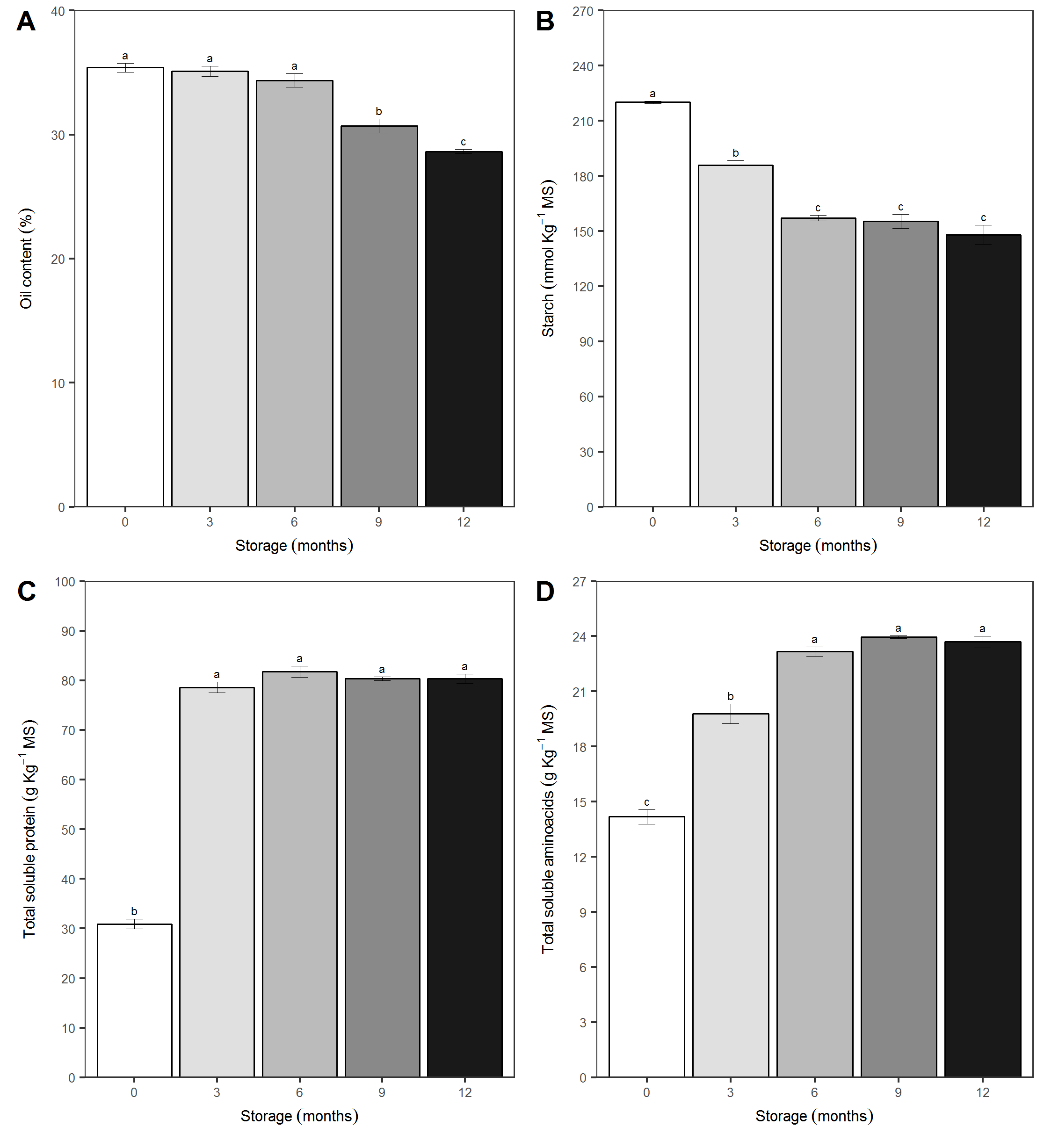


Figure 2 Oil content (A), amino acids B), total soluble protein (C), total soluble amino acids (D) evaluated in *Jatropha curcas* seeds in genotype 171 stored for 0, 3, 6, 9 and 12 months. The bars represent the mean (± SE). The mean differences between the storage months are represented by the lower case letters (SNK, p = 0.05). N = 4.

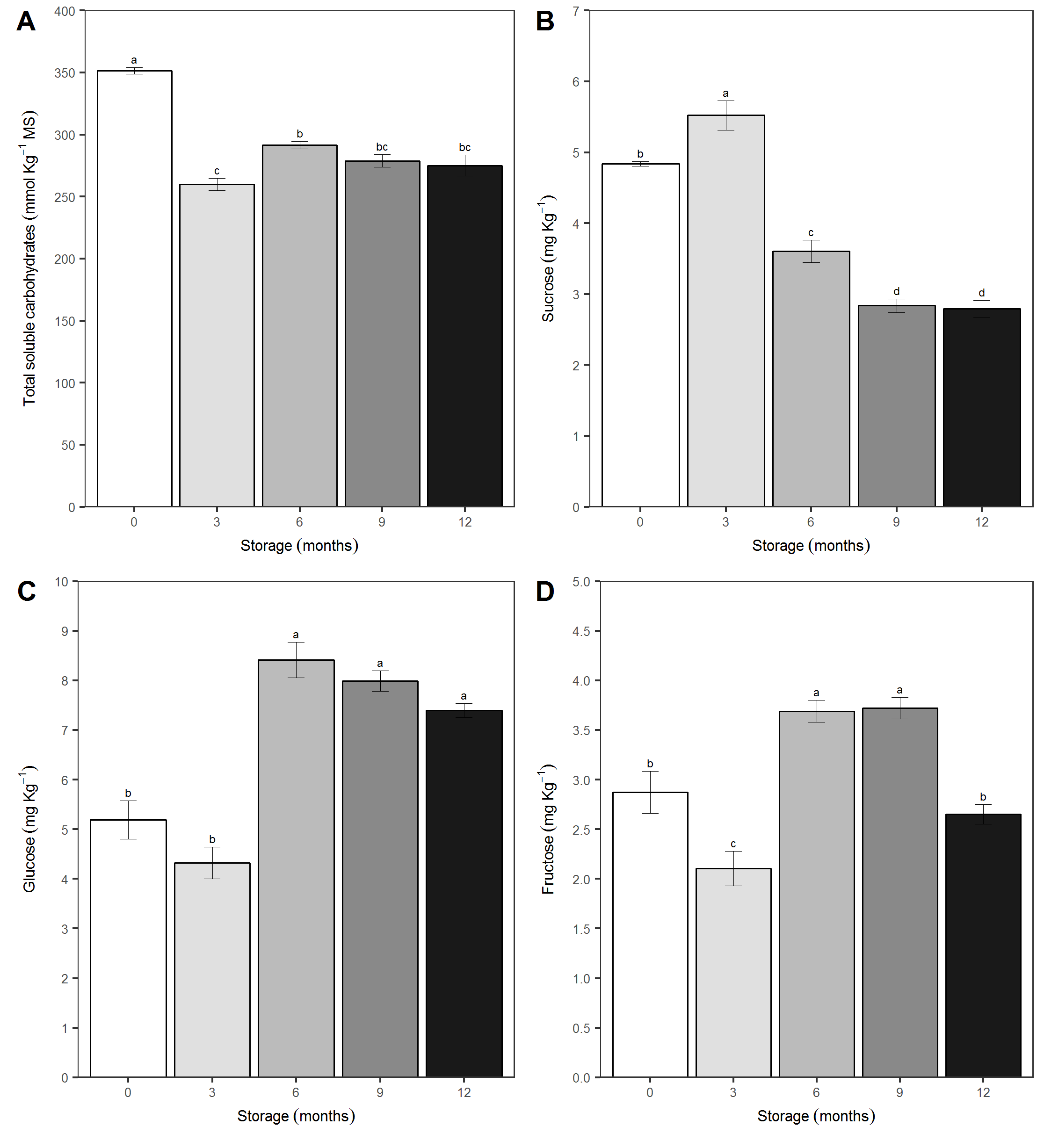


Figure 3 Total soluble carbohydrates (A), sucrose B), glucose (C) and fructose (D) evaluated in *Jatropha curcas* seeds in genotype 171 were stored for 0, 3, 6, 9 and 12 months. The bars represent the mean (± SE). The mean differences between the storage months are represented by the lower case letters (SNK, p = 0.05). N = 4.

**Physiological responses of seeds submitted to aging.** With the storage time in desiccant agent, it was verified that the water content in the seeds was greatly reduced. Seeds prior to storage had water content of 8%, but reduced to 5.5% at 12 months of storage (Figure 4A). The water potential showed a reduction parallel to the water content, presenting a strong correlation (r = 0.83, p ≤ 0.05) between these two characteristics (Figure 5). It is verified that the water potential of the seeds was decreased from -35 Mpa, without storage, to -124 MPa at 12 months of storage (Figure 4B). With the reduction of the relative water content and the water potential there was a drastic reduction in the respiratory rate of the seeds (r = 0.88, p ≤ 0.05), from 115 mmol CO2 h-1 g-1 MF to 10 mmol CO2 h-1 g-1 MF at time zero (Figure 5), with a 91% reduction in respiratory rate in the seeds over the 12 months of storage (Figure 4C).

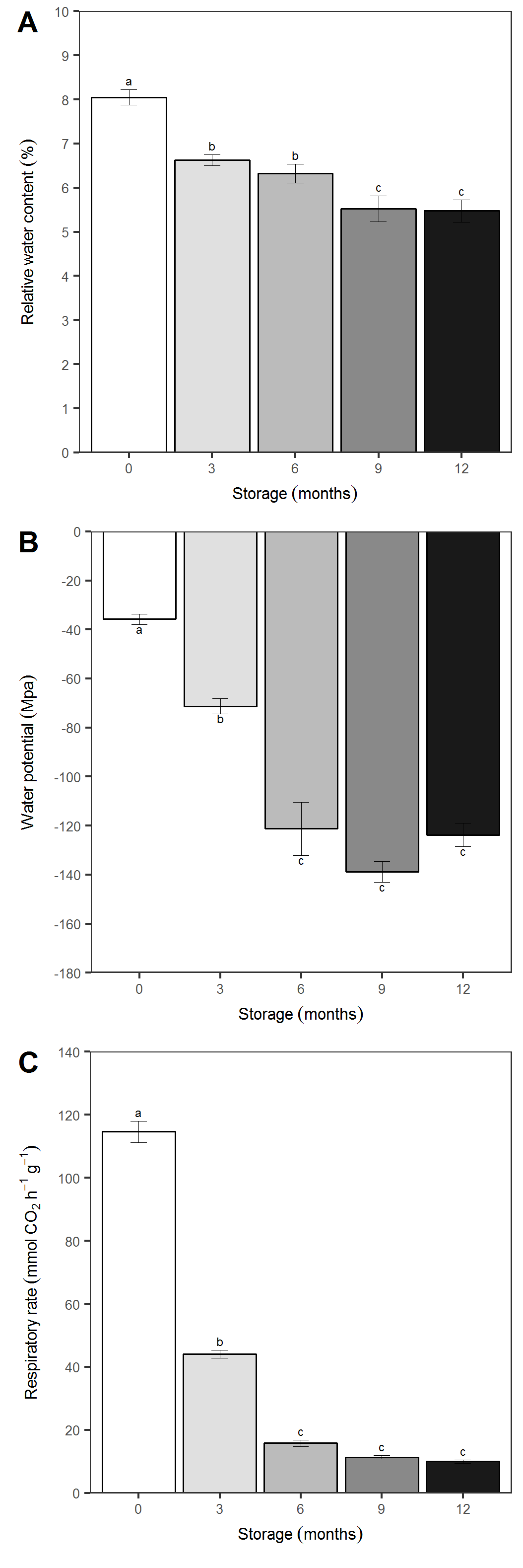


Figure 4 Relative water content (A), water potential (B), respiratory rate (C), evaluated in *Jatropha curcas* seeds in genotype 171 stored at 0, 3, 6, 9 and 12 months. The bars represent the mean (± SE). The mean differences between the storage months are represented by the lower case letters (SNK, p = 0.05). N = 4.

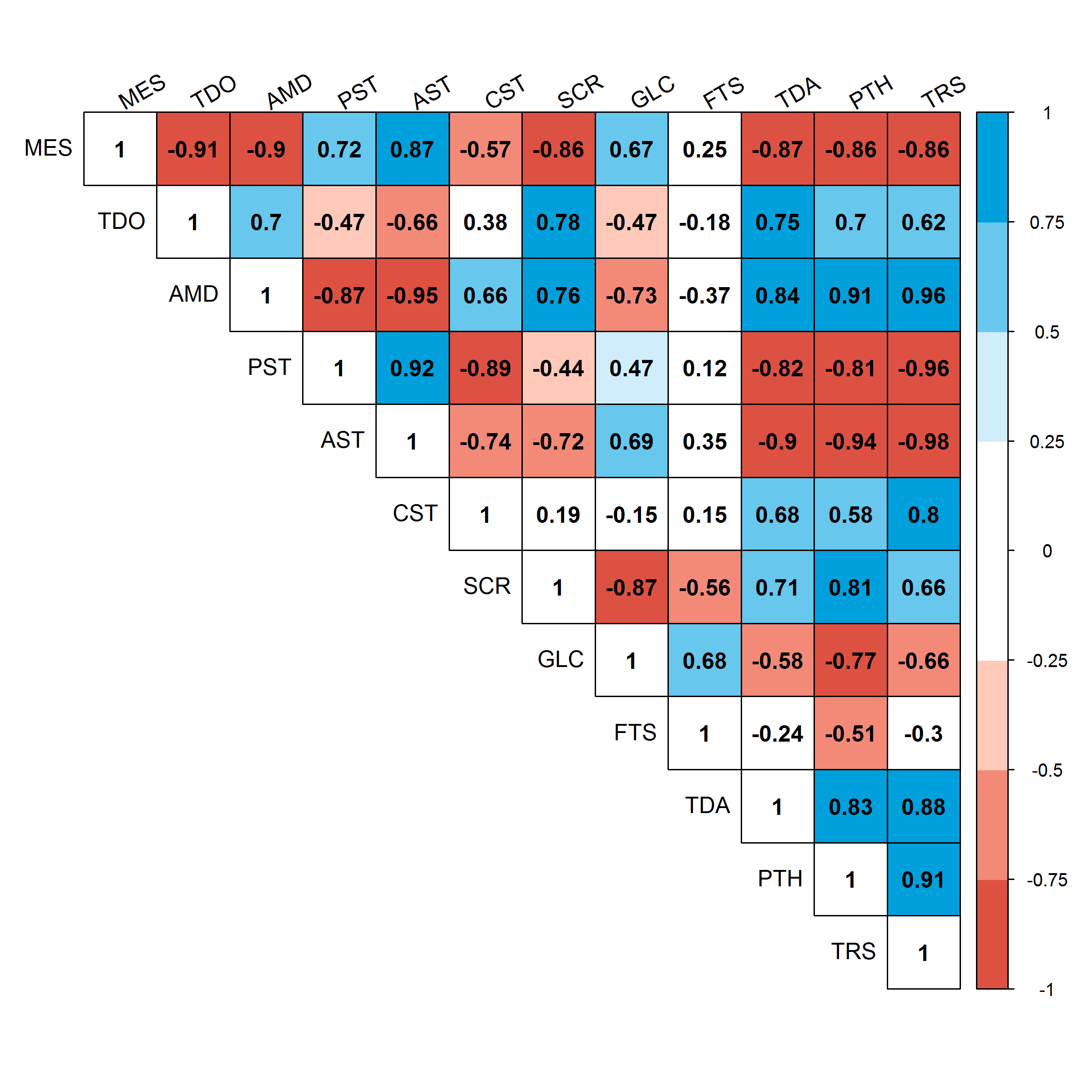


Figure 5 Pearson correlation (P = 0.05) of the variables evaluated in Jatropha curcas seeds in genotype 171 stored for 0, 3, 6, 9 and 12 months. TDO, oil content; AMD, starch; PST, total soluble protein; AST, total soluble amino acids; CST, total soluble carbohydrates; SCR, sucrose; GLC, glucose; FTS, fructose; TDA, water content in seeds; PTH, water potential; TRS, seed respiration rate.

**Seed germination treated with NaCl.** Germination was almost naked at the concentration of 150 mM NaCl for all genotypes. For the control concentration (0 mM), seeds of accessions 183 and 114 had 71% and 86% of germination, respectively, with gradual decrease with the increase in NaCl concentration, from 0% to 150 mM and 4% to 100 mM . Genotypes 218 and 133 did not differ by up to 100 mM NaCl, although germination was reduced to approximately 5% at the concentration of 150 mM. Seeds of the accession 171 showed 65% germination in the control, reducing to 32% and 9% in 50 mM and 150 mM NaCl respectively (Figure 6A). The mean germination time for the control was 5 to 7 days in general for all accessions, while for the 150 mM NaCl concentration the interval was longer, ranging from 7.2 days to 12.3 days. No significant differences were observed in mean germination time from 50 mM to 75 mM, with a general average of 7.5 days for all genotypes (Figure 6B). There was no difference in the germination synchrony between the control accessions up to 75 mM NaCl; However, the synchronization at 100 mM was null at accesses 183, 114 and 218, as well as for access 133 in the concentration at 150 mM NaCl. It was observed that the synchrony values were always lower than 0.25 for all concentrations (Figure 6C), denoting a very asynchronous germinative profile. The maximum value for the uncertainty in germination in this experiment was 4.64 bits. Genotypes 183 and 114 showed a tendency to reduce uncertainty with the increase in NaCl concentration. The germination uncertainty was stable up to 75 mM NaCl, but with a significant increase in the concentration of 100 mM, where the uncertainty was 0.5 and 0.3 for the accessions 183 and 114, respectively. Accesses 218, 171 and 133 showed a trend in increasing uncertainty from 0 mM to 100 mM without showing significant differences. At 150mm, the uncertainty was 0.3, 0.7 and 0.5 for genotypes 218, 171 and 133 respectively (Figure 6D).

The cumulative germination presented differences for each of the genotypes. It was observed that, irrespective of access, germination in the treatments without stress began between the 3rd and 4th day after sowing; Whereas for the treatments with NaCl addition the maximum values of germination were observed between the 9th and 12th day (Figure 7A-E). Regardless of the evaluated genotype, germination was observed on the 3rd day in the control, while in the 50 mM and 75 mM treatments the germination was generally initiated on the 4th day, values that were elevated with 100 mM (5th day) and 150 mM NaCl (7th day). However, regardless of treatments and salinity levels, germination became stable from day 13 (Figure 7F).

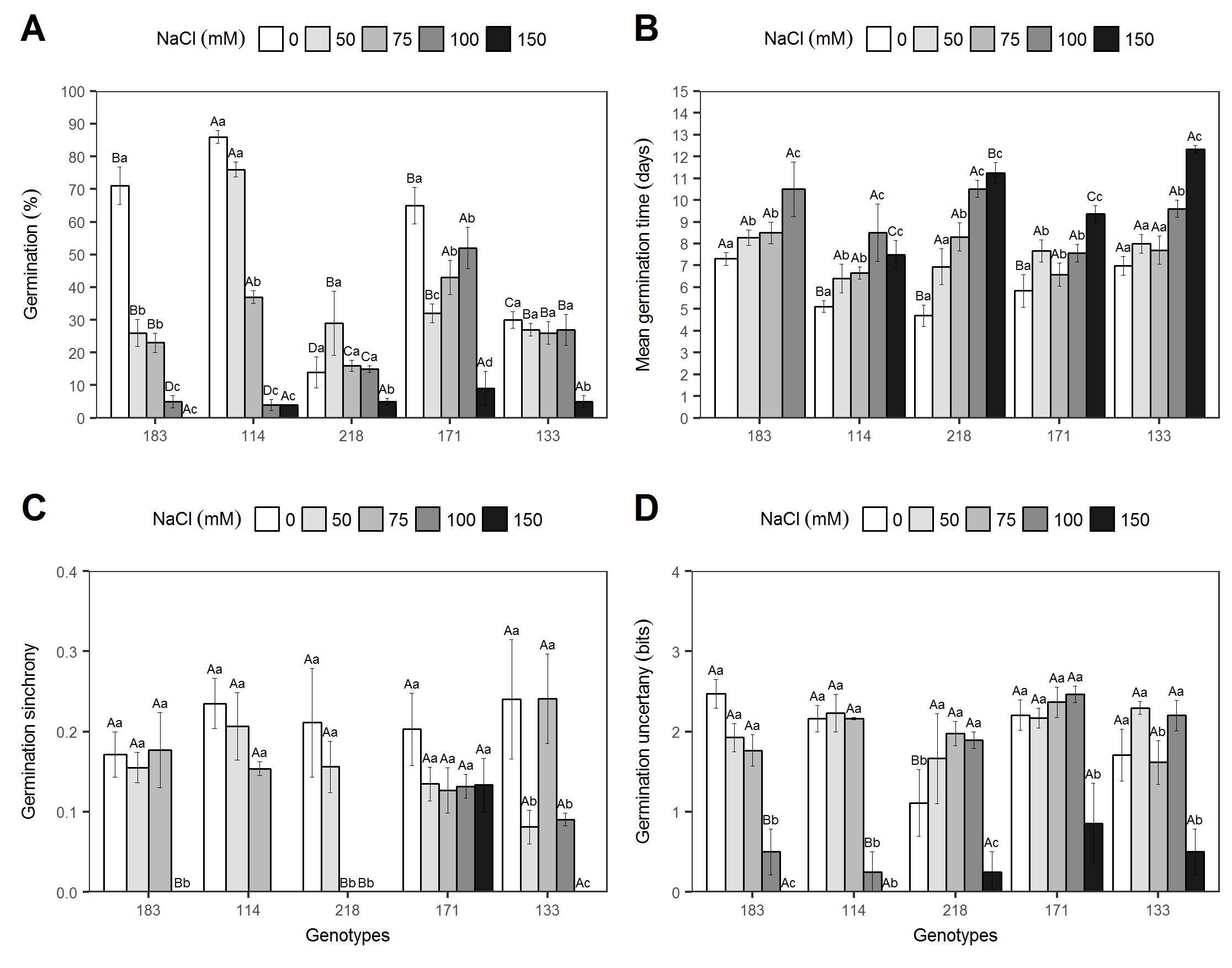


Figure 6 Percentage of germination (A), mean germination time (B), germination synchrony (C) and germination uncertainty (D) evaluated in five genotypes of *Jatropha curcas* L. under different NaCl concentrations (0, 50, 75, 100 And 150 mM). The vertical bars represent the mean (± SE). The mean differences between the accessions are represented by different capital letters and between salt levels by different lowercase letters (SNK, p = 0.05). N = 4

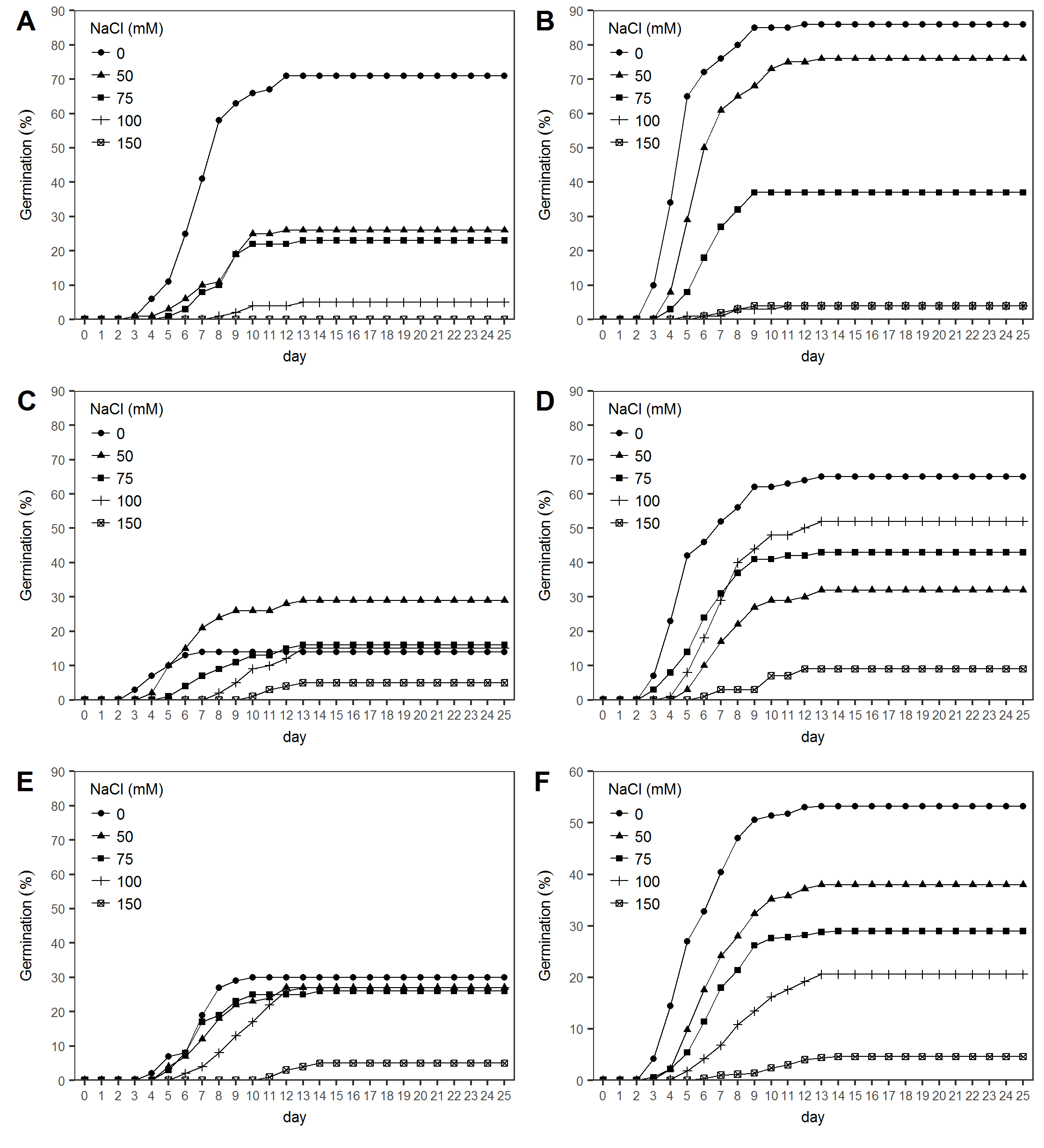


Figure 7 Cumulative germination in the time evaluated in five genotypes of *Jatropha curcas* L. under different concentrations of NaCl (0, 50, 75, 100 and 150 mM). (A) Genotype 183. (B) Genotype 114. (C) Genotype 218. (D) Genotype 171. (E) Genotype 133. (F) Average germination of five genotypes per NaCl concentration in irrigation water.

**Biometric and biomass components** Although the germination was evaluated up to the concentration of 150 mM NaCl, the biomass production was only computed up to 100 mM NaCl, and the biomass parameters were strongly affected by the increase in salinity. Regardless of the genotype, dry leaf biomass (PSF) accumulation was reduced to zero in 100 mM. For the control, the PSF did not show difference between the accessions 114, 171 and 183 with 5.19 g, 4.62 g and 5.11 g respectively. On the other hand, for genotypes 133 and 218 the PSF were 2.37 and 2.43, respectively. At the concentration of 50 mM, genotype 114 obtained the highest PSF at 1.39 g, followed by genotypes 171, 133, 183. Genotype 218 was the most sensitive to salinity, since it had PSF of 0.23 g. For the treatment of 75 mM, the PSF was reduced to values close to zero for all accessions (Table 2). For the dry weight of the root (PSR), genotype 114 presented the best development with 0, 50 and 75 mM NaCl in comparison to the other accessions, whereas for 100 mM the genotype 218 had greater accumulation of biomass in relation to the other accessions. Genotype 171 did not show differences in the accumulation of biomass for irrigation of 50, 75 and 100 mM with values of 0.31 g, 0.22 g and 0.26 g respectively. For stem dry weight (PSC), access 114 showed the best development at 0 mM and 50 mM, while accession 183 did not show significant differences between 50 mM and 75 mM NaCl treatments. At the concentration of 100 mM the genotypes 218, 133 and 171 showed an increase in the biomass accumulation of the stem with values of 0.98 g, 1.59 g and 2.40 g respectively. For the total dry weight (PST), genotype 114 was higher than the others, at least at concentrations of 0 and 50 mM, presenting values of 20.63 g and 4.31 g, respectively. Genotypes 171 and 183 did not show statistically significant differences in PST. Genotypes 133, 218 and 171 showed an increase in biomass at 100 mM, with values of 1.78 g, 1.85 g and 2.26 g, respectively, while the same genotypes at the 75 mM concentration showed no significant differences.

The parameters of unitary leaf area (AF) and total leaf area (AFT) were only possible to be evaluated until the concentration of 75 mM NaCl, since these were the parameters most affected by the increase of salts in the irrigation solution. The genotypes 218, 171 and133 presented the highest values in the control concentration, while for 50 mM the genotypes with the highest performance were 133, 114 and 183. The genotypes 133, 114, 171 and 183 for 75 mM presented values of 0, 4, 0.58, 0.73 and 1.97 cm 2, respectively. For the AFT variable, access 114 presented the highest value in the control treatment, followed by accessions 183 and 171 that did not show significant differences between them. At the concentration of 50 mM, genotype 114 continued to present larger leaf area, followed by access 133, while at the 75 mM concentration there was no difference between the accessions. There was a tendency of plant height reduction (CMP) with increasing salts (Table 2). The CMP reached approximately 13 cm in the control, but was strongly reduced until values smaller than 3 cm to 100 Mm of NaCl, fact clearly recorded in genotype 183 (Table 2). The diameter of the stem (DMC) presented reduction with the increase of the concentration of the salts, even without statistical differences. At concentrations of 50 mM and 75 mM, the accessions 183 and 114 obtained the highest values of stem diameter, while at the concentration of 100 mM they had the lowest values (Table 2).

For the leaf weight ratio (RPF), it is possible to observe a tendency in the reduction with the salinity increase, being genotype 218 presenting a ratio of 0.42, followed by genotype 183 with a ratio of 0.39. These two genotypes were the ones that best behaved in this allometric parameter. Genotype 171 showed no significant difference in RPF up to 50 mM, while genotype 114 increased its biomass accumulation to 50 mM. All genotypes showed drastic reduction from 75 mM NaCl, with a reduction of biomass accumulation in the leaf with increasing NaCl concentration in the irrigation water (Figure 8A). A distinct profile is verified in the stem dry weight ratio (RPC), which was increased with elevation of salinity, even though no significant effect was observed up to the 50 mM concentration. For the 75 mM concentration, genotype 183 was better than the others, although similar to genotypes 171 and 133 at 100 mM concentration (Figure 8B). The root weight ratio (RPR) showed a distinct behavior for each genotype, with genotypes 171, 133 and 218, increasing biomass of the roots up to 75 mM, and the latter showed a continuous increment up to 100 mM. On the other hand, the genotype 183 showed increment up to 50 mM and from there had its values reduced. Genotype 114 was apparently unaffected by the increase in NaCl concentration (Figure 8C). In the aerial weight characteristic (APR), it was not possible to observe a trend with increasing NaCl concentration; However, genotypes 183, 114, 171 and 133 in the treatment of 100 mM obtained the highest values of RPA in this respect of the other salt treatments, while genotype 218 showed reduced RPA when salinity increased (Figure 8D).

**Multivariate analysis of the salinity experiment** The germination parameters were negatively affected by the increase in the salt concentration; There was a negative correlation between germination percentage (r = -0.55, p ≤ 0.05) and germination synchrony (r = -0.69, p value ≤ 0.001). On the other hand, the mean germination time was positively significant when correlated with the increase of salts (R = 0.66, p ≤ 0.001). However, there was no significant correlation between germination uncertainty and salt increase (r = -0.23, p = 0.321). The biomass parameters were significantly affected with increasing salts. The correlations between this parameter in relation to the leaf dry weight (r = -0.86, p ≤ 0.001), the leaf area (r = -0.89, p ≤ 0.001), and plant height ( R = -0.89, p ≤ 0.001) and stem diameter (r = -0.91, p ≤ 0.001). Other allometric parameters were also influenced by saline addition; (R = -0.90, p ≤ 0.001) and leaf area ratio (r = -0.94, p ≤ 0.001) were used to estimate the negative relationship between salinity and leaf weight ratio. Other allometric characteristics, on the other hand, were strongly increased with salinity; The increase in the ratio of stem weight (R = 0.74, p ≤0.001). The analysis of the main components shows that approximately 74% of the variation can be explained by the parameters studied in this work. In the first component the variables PSF, AFT, CMP and PST were the ones that presented the greatest contribution in the variance and are negatively correlated with the increase of NaCl, while the variables with positive correlation were the TMG and RPC. The genotypes that showed the best response were 114, 171 and 183 and the susceptible genotypes 133 and 218 (Figure 9).

# Conclusions

Class aptent taciti sociosqu ad litora torquent per conubia nostra, per inceptos himenaeos. Curabitur sodales ligula in libero. Sed dignissim lacinia nunc. Curabitur tortor. Pellentesque nibh. Aenean quam. In scelerisque sem at dolor. Maecenas mattis. Sed convallis tristique sem. Proin ut ligula vel nunc egestas porttitor. Morbi lectus risus, iaculis vel, suscipit quis, luctus non, massa. Fusce ac turpis quis ligula lacinia aliquet. Mauris ipsum. Nulla metus metus, ullamcorper vel, tincidunt sed, euismod in, nibh. Quisque volutpat condimentum velit. Class aptent taciti sociosqu ad litora torquent per conubia nostra, per inceptos himenaeos. Nam nec ante. Sed lacinia, urna non tincidunt mattis, tortor neque adipiscing diam, a cursus ipsum ante quis turpis. Nulla facilisi. Ut fringilla. Suspendisse potenti. Nunc feugiat mi a tellus consequat imperdiet.

# Acknowledgments

Donec lacus nunc, viverra nec, blandit vel, egestas et, augue. Vestibulum tincidunt malesuada tellus. Ut ultrices ultrices enim. Curabitur sit amet mauris. Morbi in dui quis est pulvinar ullamcorper. Nulla facilisi. Integer lacinia sollicitudin massa. Cras metus. Sed aliquet risus a tortor. Integer id quam. Morbi mi.

# 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.N. Trethewey, P. Geigenberger, K. Riedel, M.-R. Hajirezaei, U. Sonnewald, M. Stitt, J.W. Riesmeier, L. Willmitzer, Combined expression of glucokinase and invertase in potato tubers leads to a dramatic reduction in starch accumulation and a stimulation of glycolysis, The Plant Journal. 15 (1998) 109–118.

[36] P. Armengaud, R. Sulpice, A.J. Miller, M. Stitt, A. Amtmann, Y. Gibon, Multilevel analysis of primary metabolism provides new insights into the role of potassium nutrition for glycolysis and nitrogen assimilation in arabidopsis roots, Plant Physiology. 150 (2009) 772–785.

[37] M. DuBois, K.A. Gilles, J.K. Hamilton, P. t Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Analytical Chemistry. 28 (1956) 350–356.

[38] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Analytical Biochemistry. 72 (1976) 248–254.

[39] S. Moore, W.H. Stein, others, A modified ninhydrin reagent for the photometric determination of amino acids and related compounds., Journal of Biological Chemistry. 211 (1954) 907–913.

[40] M. Ahmad, S. Husain, S. Osman, Ricinoleic acid in phyllanthus niruri seed oil, Journal of the American Oil Chemists’ Society. 58 (1981) 673–674.

[41] M. Stitt, R.M. Lilley, R. Gerhardt, H.W. Heldt, [32] metabolite levels in specific cells and subcellular compartments of plant leaves, Methods in Enzymology. 174 (1989) 518–552.

[42] C.W. Vertucci, Predicting the optimum storage conditions for seeds using thermodynamic principles, Journal of Seed Technology. (1993) 41–53.

[43] E. Epstein, others, Mineral nutrition of plants: Principles and perspectives., 1972.

[44] F. Lozano Isla, O. Benites Alfaro, M.F. Pompelli, GerminaR: Germination indexes for seed germination variables for ecophysiological studies, 2017. <https://CRAN.R-project.org/package=GerminaR>.

[45] M. Pompelli, W. Antunes, D. Ferreira, P. Cavalcante, H. Wanderley-Filho, L. Endres, Allometric models for non-destructive leaf area estimation of jatropha curcas, Biomass and Bioenergy. 36 (2012) 77–85.

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

[47] F. de Mendiburu, Agricolae: Statistical procedures for agricultural research, 2016. <https://CRAN.R-project.org/package=agricolae>.

[48] T. Wei, V. Simko, Corrplot: Visualization of a correlation matrix, 2016. <https://CRAN.R-project.org/package=corrplot>.

[49] F. Husson, J. Josse, S. Le, J. Mazet, FactoMineR: Multivariate exploratory data analysis and data mining, 2017. <https://CRAN.R-project.org/package=FactoMineR>.