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

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2017-05-16

# Abstract

*Jatropha curcas* L. is a plant that can be used in the production of biofuel with 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, were studied the effect of the addition of five different concentrations of NaCl in the irrigation (0, 50, 75, 100 and 150 mM) under the 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. 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, 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.

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 study the mechanims of the tolerance of *J. curcas* to salinity between the accesses of 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 germination 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 (TSC), total soluble amino acids (TSA) and starch (STR), the samples were solubilized in 50% (v/v) ethanol [[35](#ref-trethewey1998combined)], whereas for analysis of total soluble proteins (TSP), 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 (OIL) 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 (RWC) of seeds was calculated as described in detail in Vertucci [[42](#ref-vertucci1993predicting)] and Moncaleano-Escandon [[20](#ref-moncaleano2013germination)]. The water potential (WTP) 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 (RPR), 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 germination boxes containing two sheets of germination test 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 (GRP), mean germination time (MGT), germination uncertainty (GRU) and germination synchrony (GRS). 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 (SDL) and mean stem diameter (STD) 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 (LA) was evaluated by plant and for experimental unit (TLA) [[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 (LDW), dry stem weight (STDW) and root dry weight (RDW) were used to calculate several biomass parameters, such as: total dry weight (TDW); aerial part dry weight (BDW); Leaf weight ratio (LWR; leaf dry weight divided by total dry weight); Dry stem weight ratio (DSR; stem dry weight divided by total dry weight); (RPA, aerial part dry weight divided by total dry weight) and root dry weight ratio (RTR, 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)].

# Results

**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).

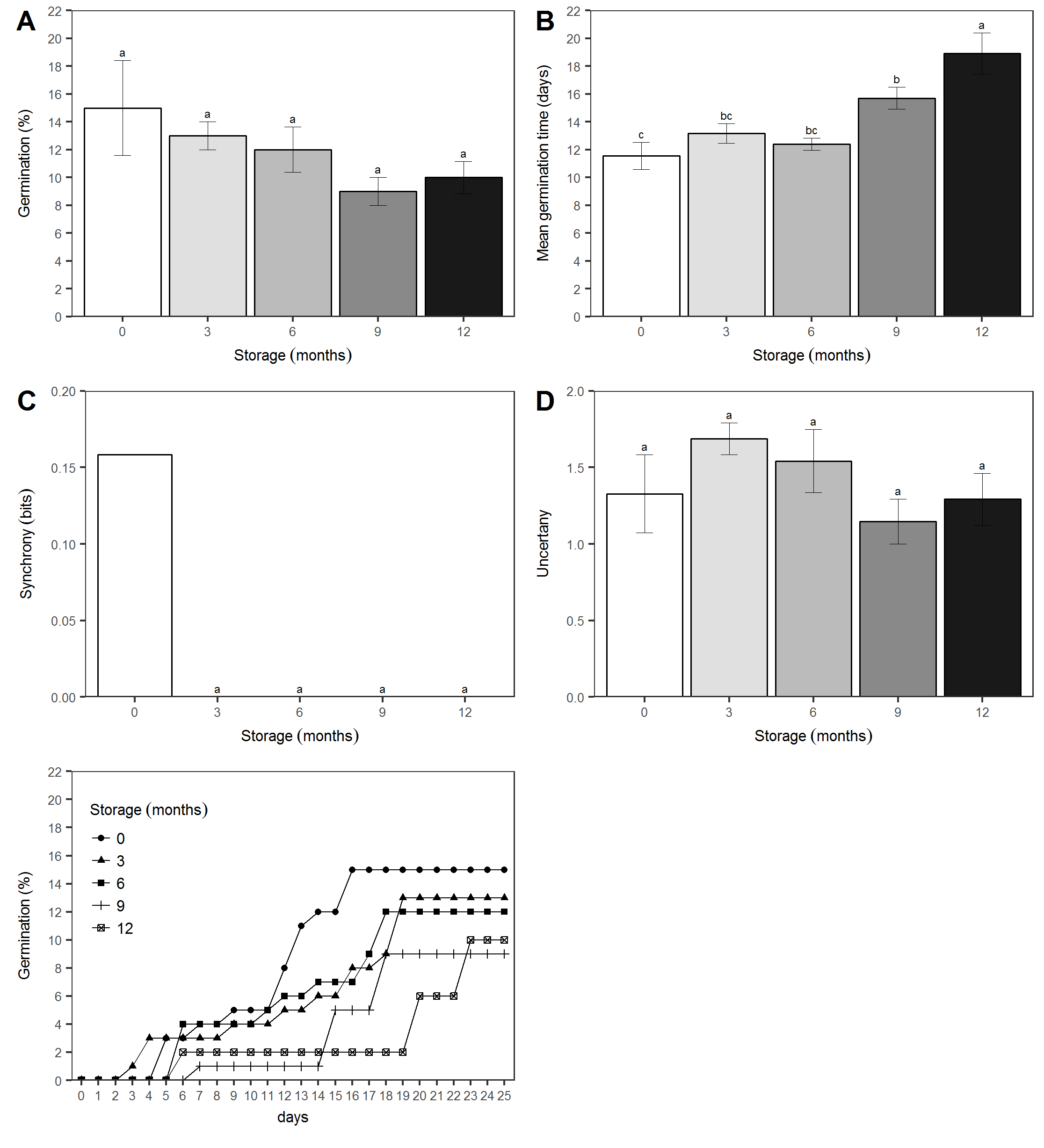


Figure 1 Germinability (A), mean germination time (B), synchrony index (C), germination uncertainty (D) and temporal germination (E) evaluated in *Jatropha curcas* seeds in genotype 171 stored at 0, 3, 6, 9 And 12 months. The bars represent the mean (± SE). The mean differences between the storage months 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)

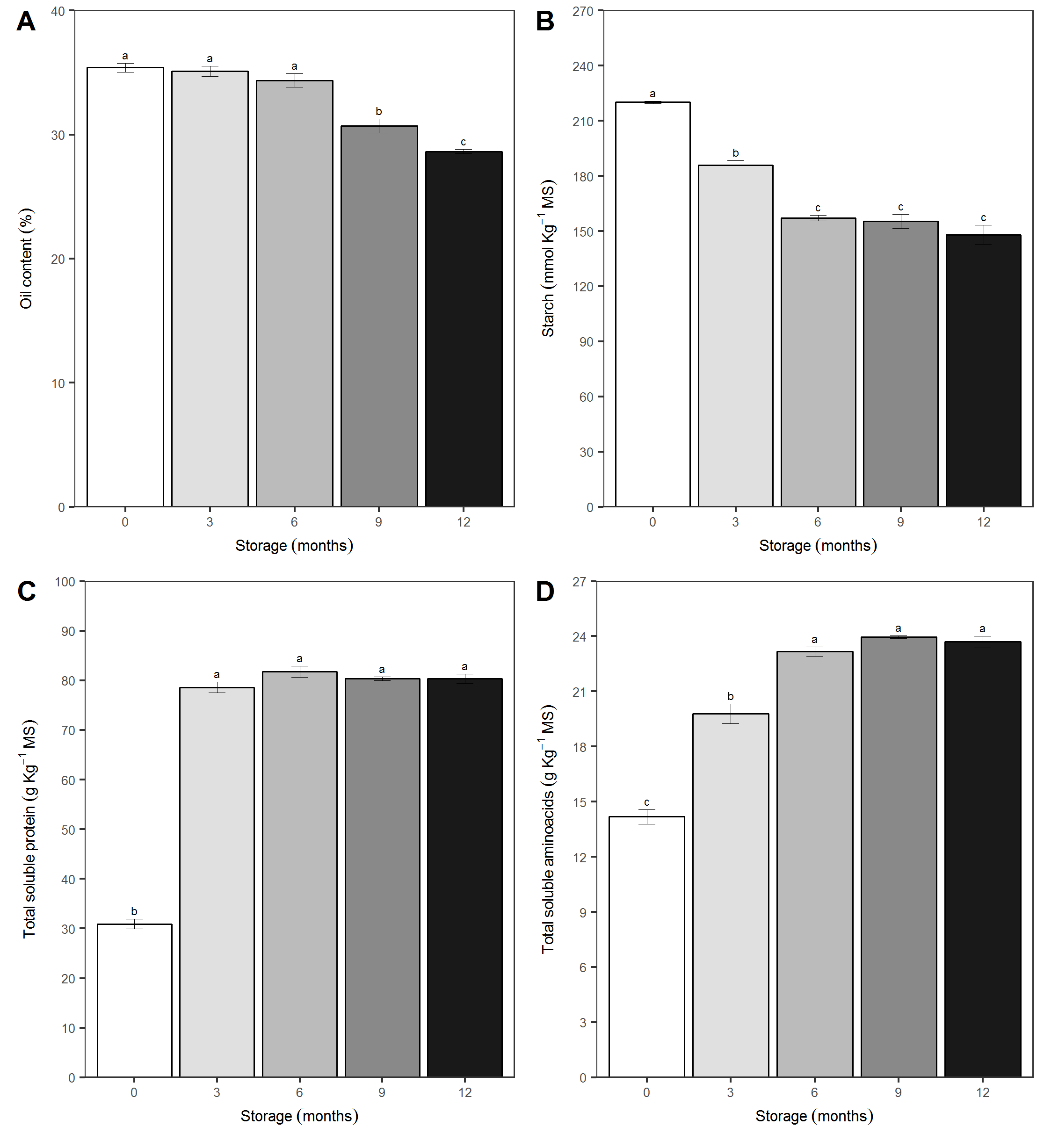


Figure 2 Oil content (A), amino acids B), total soluble protein (C), total soluble amino acids (D) evaluated in *Jatropha curcas* seeds in genotype 171 stored for 0, 3, 6, 9 and 12 months. The bars represent the mean (± SE). The mean differences between the storage months are represented by the lower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

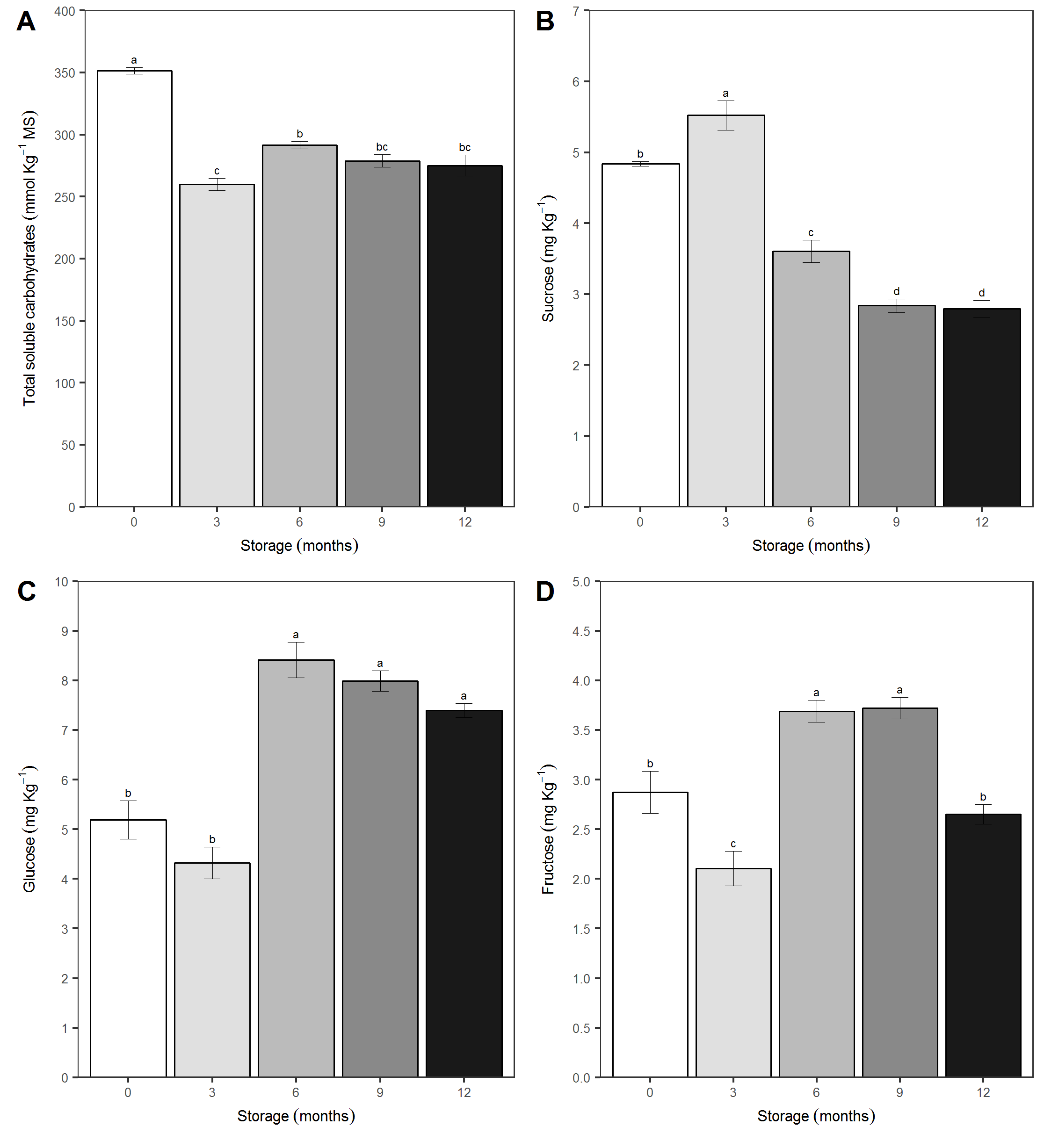


Figure 3 Total soluble carbohydrates (A), sucrose B), glucose (C) and fructose (D) evaluated in *Jatropha curcas* seeds in genotype 171 were stored for 0, 3, 6, 9 and 12 months. The bars represent the mean (± SE). The mean differences between the storage months are represented by the lower case letters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

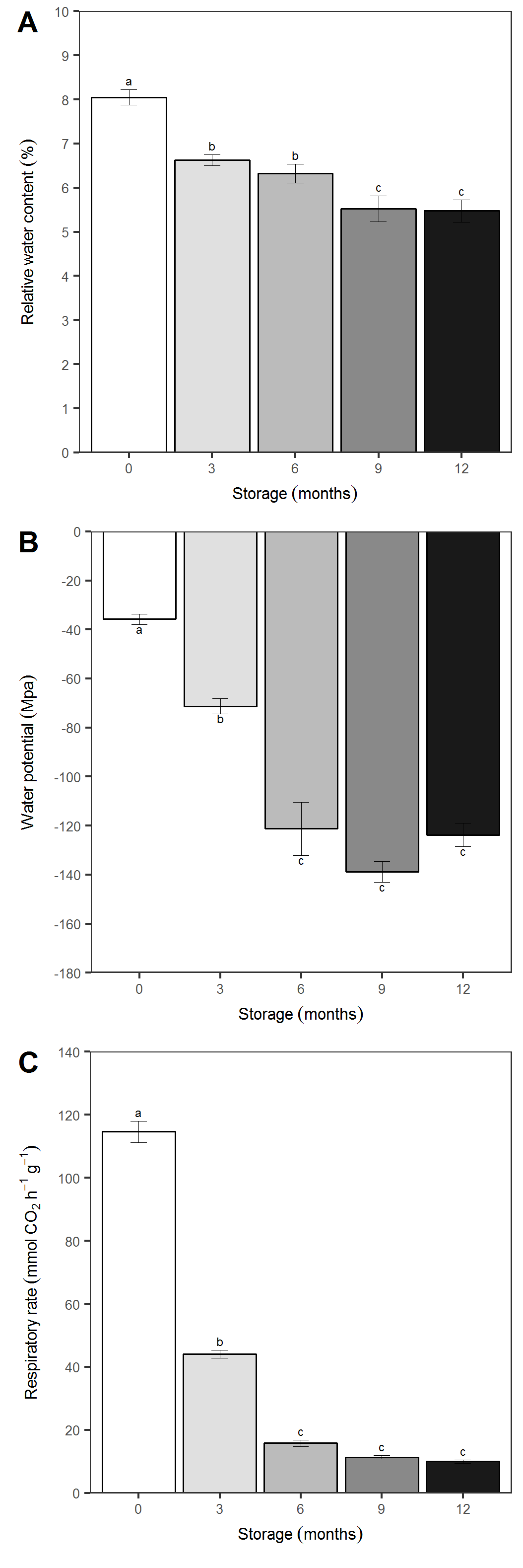


Figure 4 Relative water content (A), water potential (B), respiratory rate (C), evaluated in *Jatropha curcas* seeds in genotype 171 stored at 0, 3, 6, 9 and 12 months. The bars represent the mean (± SE). The mean differences between the storage months are represented by the lower case letters (SNK,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

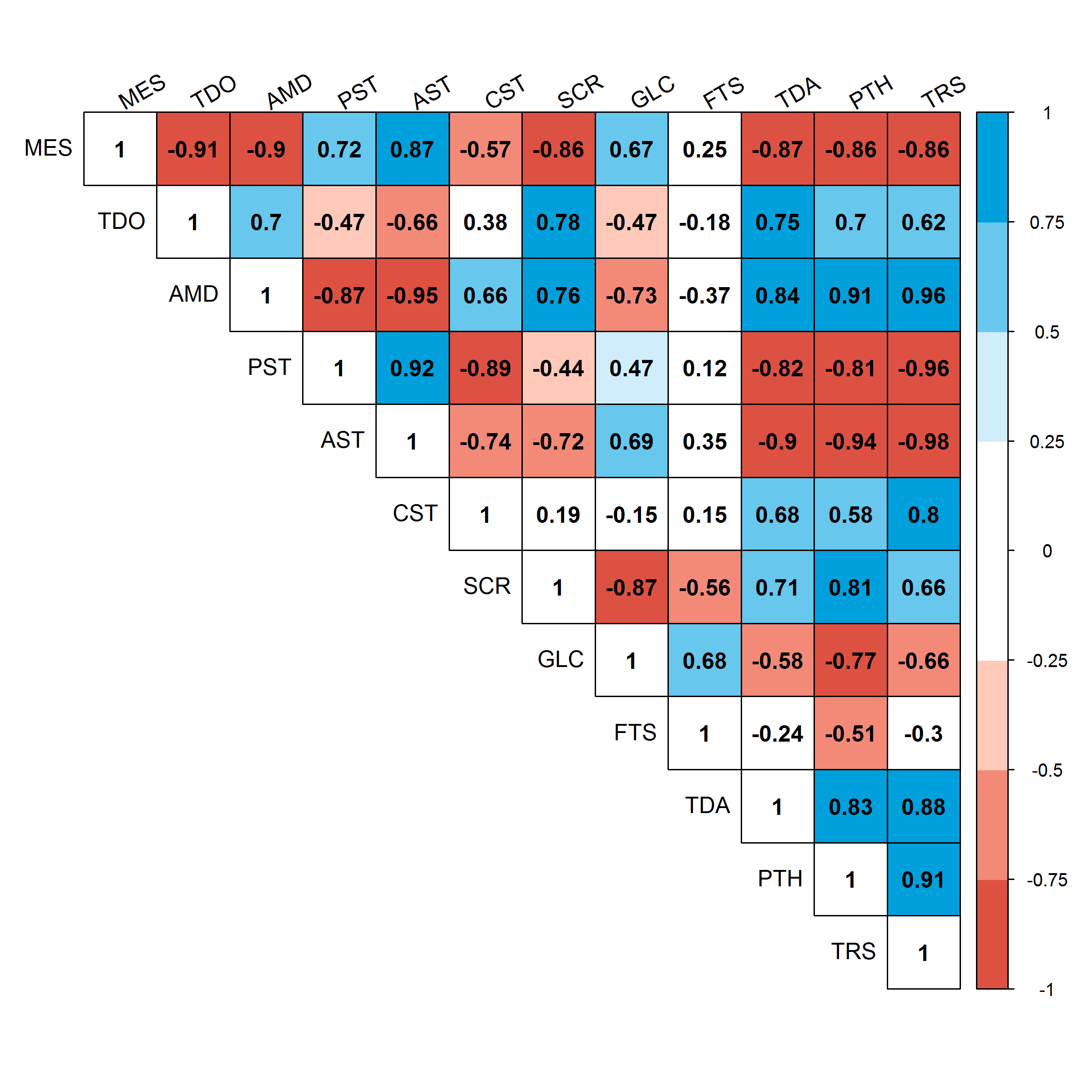


Figure 5 Pearson correlation (P = 0.05) of the variables evaluated in Jatropha curcas seeds in genotype 171 stored for 0, 3, 6, 9 and 12 months. TDO, oil content; AMD, starch; PST, total soluble protein; AST, total soluble amino acids; CST, total soluble carbohydrates; SCR, sucrose; GLC, glucose; FTS,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

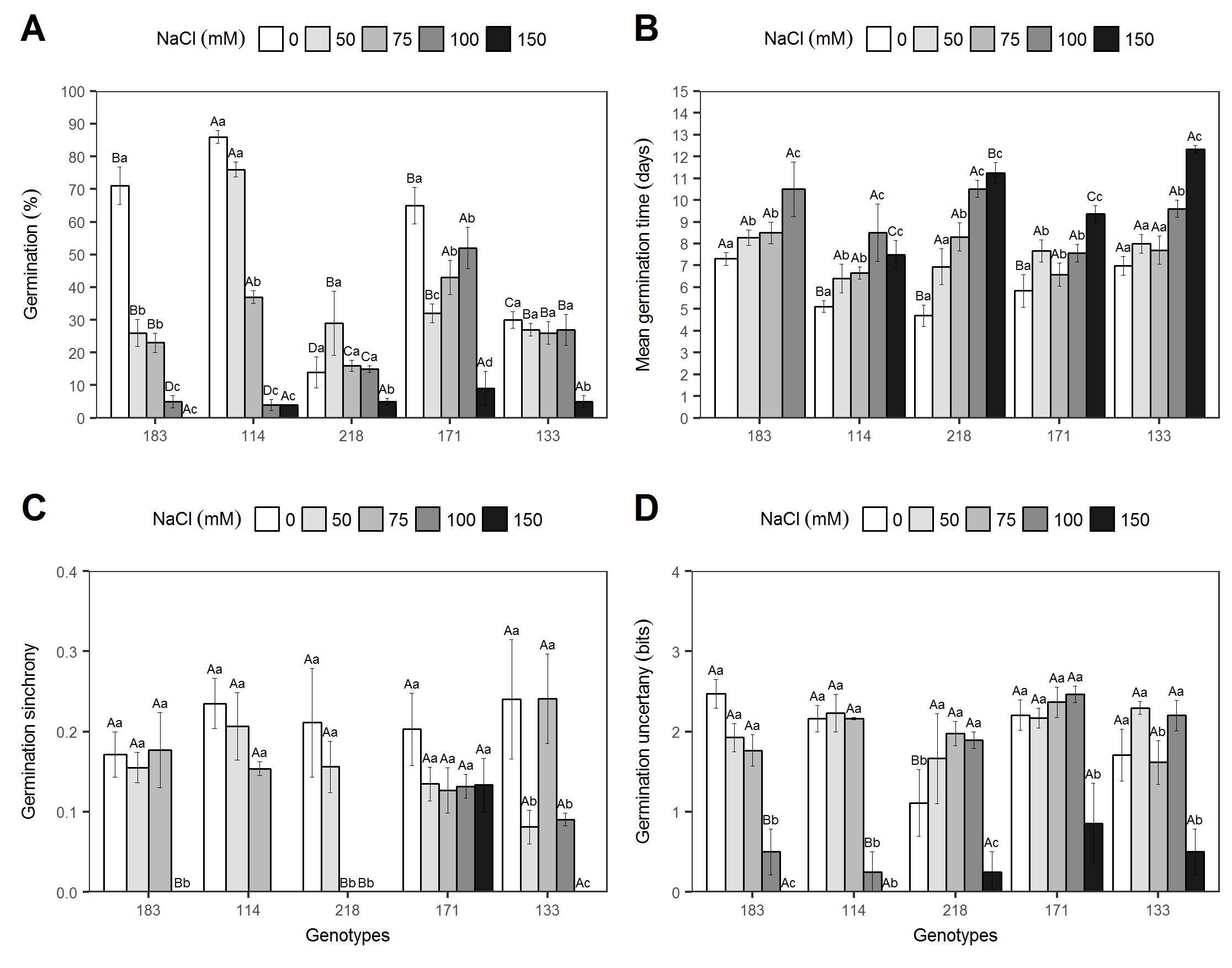


Figure 6 Percentage of germination (A), mean germination time (B), germination synchrony (C) and germination uncertainty (D) evaluated in five genotypes of *Jatropha curcas* L. under different NaCl concentrations (0, 50, 75, 100 And 150 mM). The vertical bars represent the mean (± SE). The mean differences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

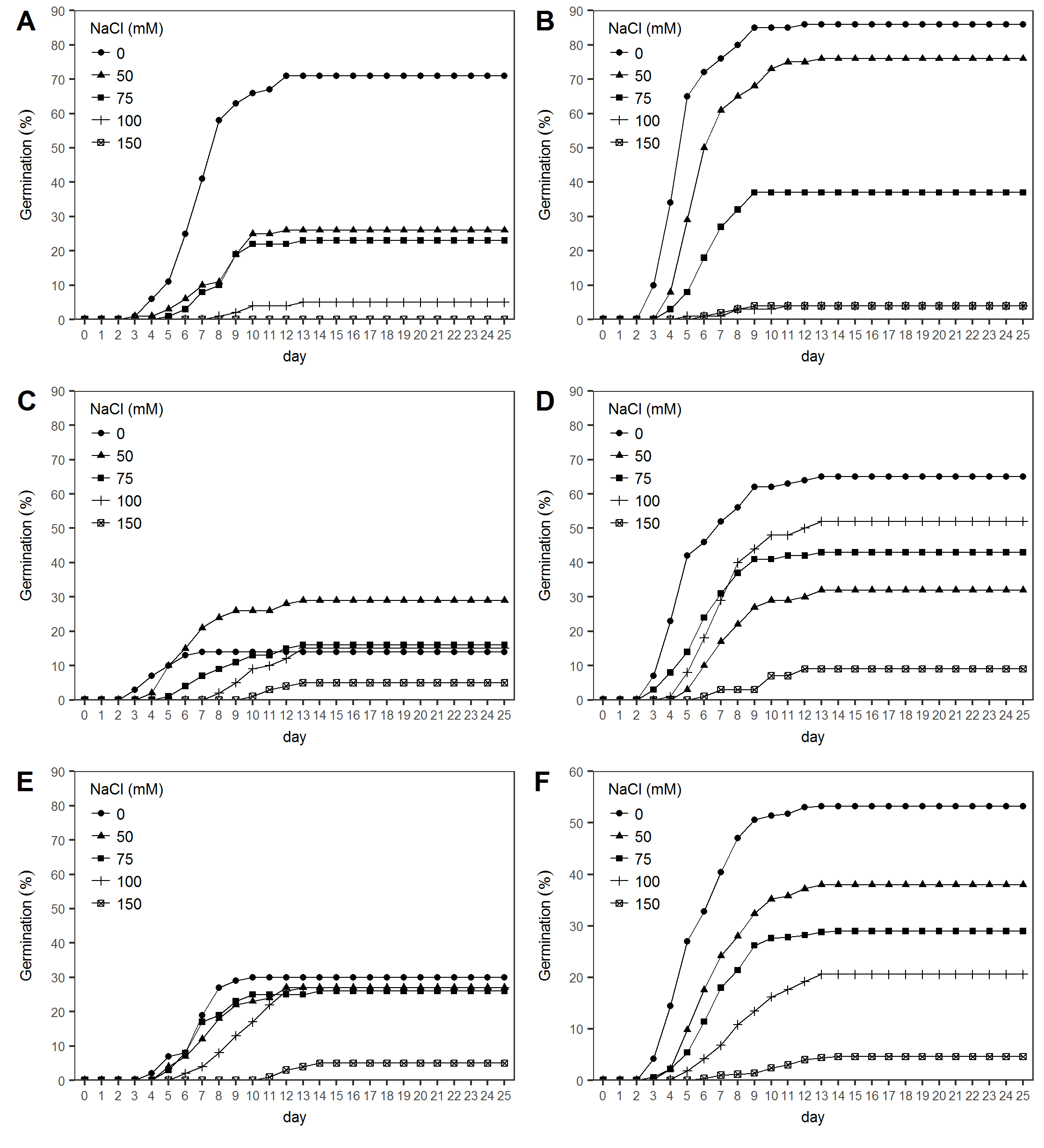


Figure 7 Cumulative germination in the time evaluated in five genotypes of *Jatropha curcas* L. under different concentrations of NaCl (0, 50, 75, 100 and 150 mM). (A) Genotype 183. (B) Genotype 114. (C) Genotype 218. (D) Genotype 171. (E) Genotype 133. (F) Average germination of five genotypes per NaCl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

Table 2 Parameters of biometric and biomass components evaluated in five accessions of *Jatropha curcas* L. under salinity stress at 25 days after sowing. Where: PSF, leaf dry weight. PSR, root dry weight. PSC, dry stem weight. PST, total dry weight. AF, leaf area. AFT, total leaf area. CMP, plant length. DMC, stem diameter. The values are represented by the mean (± SE) for four replicates of 25 seeds in each experimental unit. The differences between the means between the accessions are represented by the different ones by capital letters and between the different levels of salt by lowercase letters (SNK, p = 0.05)

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Genotype | Salt | LDW | RDW | SDW | TDW | LFA | TLFA | STD | HGT |
| G1 | 0 | 5.12 ± 0.37 Aa | 2.68 ± 0.13 Ba | 5.34 ± 0.51 Ba | 13.13 ± 0.99 Ba | 93.04 ± 6.69 Ba | 1624.49 ± 43.69 Ba | 6.61 ± 0.21 Aa | 13 ± 0.35 Aa |
| G1 | 50 | 0.55 ± 0.06 Bb | 0.68 ± 0.06 Bb | 0.93 ± 0.32 Ab | 2.15 ± 0.31 Bb | 5.26 ± 1.22 Bb | 31.45 ± 4.11 Ab | 5.65 ± 0.13 Ab | 6.24 ± 0.39 Ab |
| G1 | 75 | 0.06 ± 0.01 Ac | 0.12 ± 0.04 Cc | 0.99 ± 0.15 Ab | 1.32 ± 0.17 Ab | 1.97 ± 0.11 Ab | 11.83 ± 2.08 Ab | 5.16 ± 0.17 Ab | 5.12 ± 0.31 Ab |
| G1 | 100 | 0 ± 0 Ad | 0.01 ± 0.01 Cd | 0.12 ± 0.12 Bc | 0.13 ± 0.13 Bc | 0 ± 0 Ac | 0 ± 0 Ac | 1.8 ± 1.15 Bc | 0.88 ± 0.88 Bc |
| G2 | 0 | 5.19 ± 0.27 Aa | 7.48 ± 0.58 Aa | 7.97 ± 0.28 Aa | 20.64 ± 0.66 Aa | 87.33 ± 4.19 Ba | 1875.27 ± 82.33 Aa | 7.33 ± 0.2 Aa | 12.38 ± 0.34 Aa |
| G2 | 50 | 1.39 ± 0.15 Ab | 1.44 ± 0.19 Ab | 1.48 ± 0.24 Ab | 4.32 ± 0.56 Ab | 12.54 ± 2.56 Bb | 239.59 ± 52.25 Ab | 5.29 ± 0.1 Ab | 6.8 ± 0.35 Ab |
| G2 | 75 | 0.08 ± 0.01 Ac | 0.55 ± 0.09 Ac | 0.49 ± 0.11 Ac | 1.11 ± 0.19 Ac | 0.58 ± 0.11 Bc | 5.28 ± 0.83 Ab | 5.23 ± 0.11 Ab | 4.14 ± 0.23 Ac |
| G2 | 100 | 0 ± 0 Ad | 0.15 ± 0.03 Cd | 0.34 ± 0.02 Bc | 0.49 ± 0.04 Bc | 0 ± 0 Ad | 0 ± 0 Ac | 4.25 ± 0.26 Ac | 2.33 ± 0.33 Bd |
| G3 | 0 | 2.43 ± 0.34 Ba | 1.24 ± 0.08 Da | 1.99 ± 0.16 Ca | 5.65 ± 0.54 Ca | 178.15 ± 11.75 Aa | 839.17 ± 110.24 Ca | 7.27 ± 0.23 Aa | 10.81 ± 0.4 Aa |
| G3 | 50 | 0.23 ± 0.01 Bb | 0.35 ± 0.01 Cc | 0.55 ± 0.08 Ab | 1.12 ± 0.08 Bb | 2.69 ± 0.33 Bb | 25.86 ± 2.91 Ab | 5.11 ± 0.15 Ab | 4.96 ± 0.35 Cb |
| G3 | 75 | 0 ± 0 Ac | 0.28 ± 0.06 Bc | 0.47 ± 0.07 Ab | 0.74 ± 0.04 Ab | 0 ± 0 Bb | 0 ± 0 Ab | 4.19 ± 0.24 Ab | 2.33 ± 0.31 Bc |
| G3 | 100 | 0 ± 0 Bc | 0.87 ± 0.06 Ab | 0.98 ± 0.07 Ab | 1.85 ± 0.07 Ab | 0 ± 0 Ac | 0 ± 0 Ac | 4.45 ± 0.24 Ab | 2.57 ± 0.44 Ac |
| G4 | 0 | 4.62 ± 0.49 Aa | 2.64 ± 0.26 Ba | 5.13 ± 1.37 Ba | 12.4 ± 2.11 Ba | 97.45 ± 5.44 Ba | 1595.74 ± 199.32 Ba | 7.34 ± 0.25 Aa | 12 ± 0.48 Aa |
| G4 | 50 | 0.38 ± 0.09 Bb | 0.31 ± 0.03 Cb | 0.36 ± 0.11 Ac | 1.05 ± 0.11 Bc | 4.33 ± 0.35 Bb | 33.99 ± 1.39 Ab | 4.94 ± 0.14 Ab | 5.16 ± 0.3 Cb |
| G4 | 75 | 0.08 ± 0.01 Ac | 0.22 ± 0.06 Bb | 0.49 ± 0.08 Ac | 0.9 ± 0.25 Ac | 0.73 ± 0.08 Bb | 8.83 ± 1.67 Ab | 4.71 ± 0.2 Ab | 4.54 ± 0.3 Ab |
| G4 | 100 | 0 ± 0 Ad | 0.26 ± 0.08 Bb | 2.4 ± 0.38 Ac | 2.66 ± 0.45 Ab | 0 ± 0 Ac | 0 ± 0 Ac | 4.61 ± 0.15 Ab | 3.58 ± 0.29 Ab |
| G5 | 0 | 2.37 ± 0.2 Ba | 1.94 ± 0.1 Ca | 2.78 ± 0.35 Ca | 7.09 ± 0.62 Ca | 105.52 ± 5.19 Ba | 794.77 ± 84.01 Ca | 6.62 ± 0.17 Aa | 11.65 ± 0.44 Aa |
| G5 | 50 | 0.54 ± 0.21 Bb | 1.05 ± 0.15 Bb | 1.04 ± 0.13 Ab | 2.63 ± 0.47 Bb | 23.57 ± 4.41 Ab | 161.47 ± 37.86 Ab | 5.21 ± 0.15 Ab | 6.91 ± 0.42 Bb |
| G5 | 75 | 0.04 ± 0.01 Ac | 0.39 ± 0.09 Bc | 0.45 ± 0.05 Ab | 0.88 ± 0.13 Ab | 0.4 ± 0.05 Bc | 2.67 ± 0.63 Ab | 4.87 ± 0.19 Ab | 5.04 ± 0.23 Ac |
| G5 | 100 | 0 ± 0 Ad | 0.19 ± 0.02 Bc | 1.59 ± 0.23 Ab | 1.78 ± 0.25 Ab | 0 ± 0 Ad | 0 ± 0 Ac | 4.61 ± 0.22 Ab | 3.35 ± 0.33 Ad |

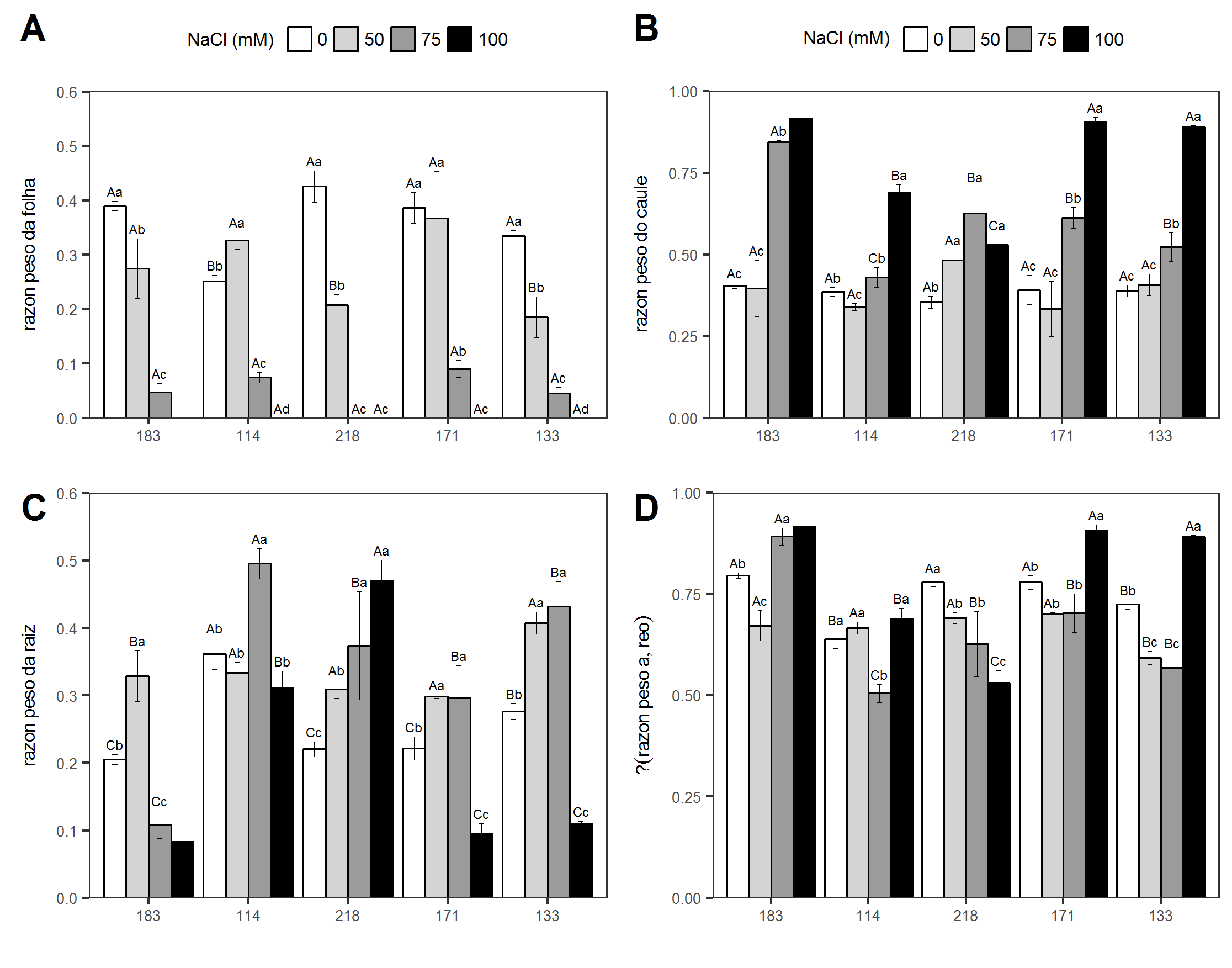


Figure 8 The dry weight ratio of leaf (A), root dry weight ratio (B), root weight ratio (C) and shoot weight ratio (D) evaluated in five genotypes of *Jatropha curcas* L. under different concentrations NaCl (0, 50, 75, 100 and 150 mM). The vertical bars represent the mean (± SE). The mean differences between the accessions are represented by different capital letters and between salt levels by different lowercase letters (SNK, p = 0.05). N = 4.

**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).

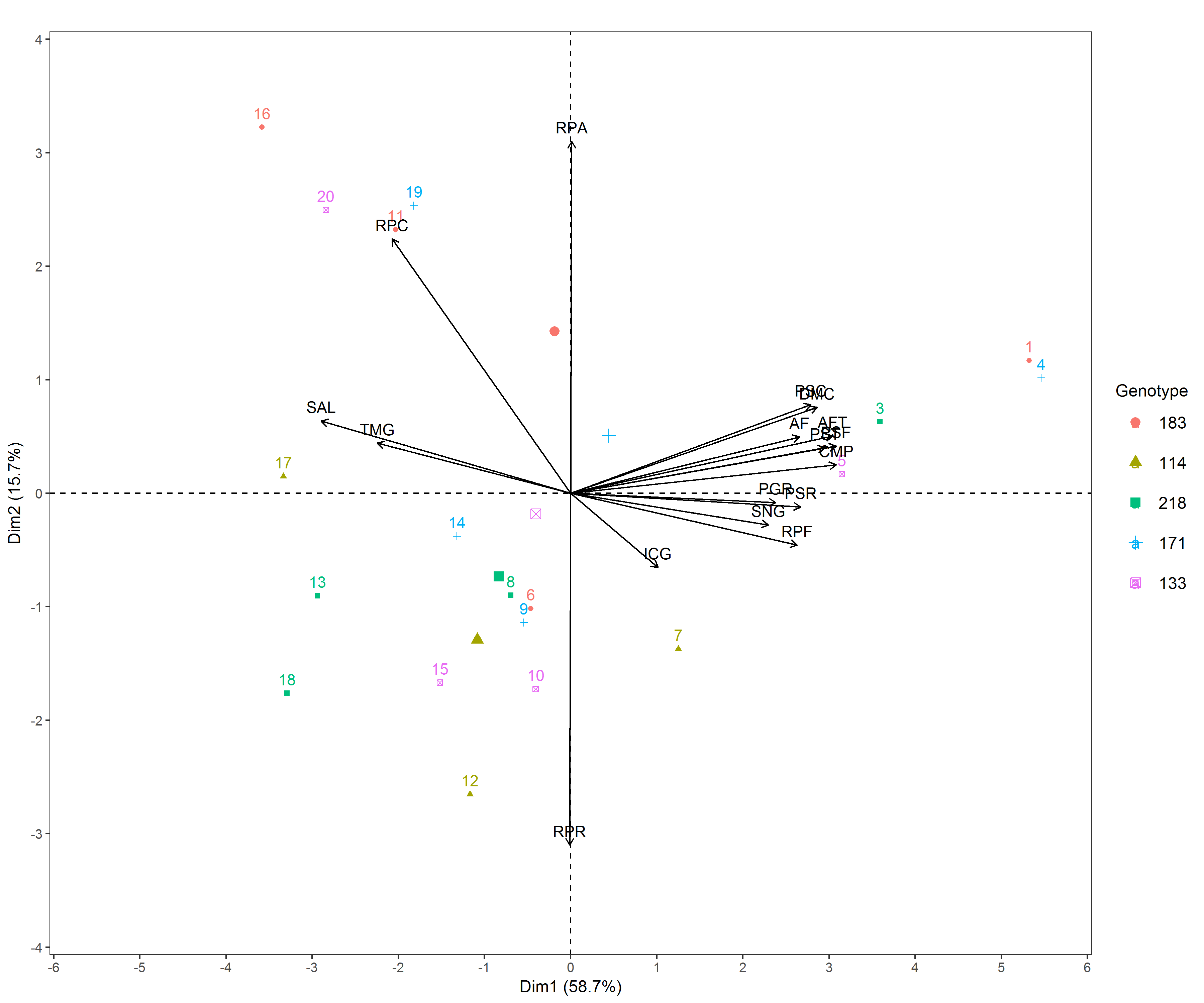


Figure 9 Principal components analysis (PCA) of the variables evaluated in five genotypes of *Jatropha curcas* L. under different concentrations of NaCl (0, 50, 75, 100 and 150 mM). PGR, Percentage of germination; TMG, Average germination time; ICG, Uncertainty of germination; SNG, Synchronism of germination; PSF, leaf dry weight; PSR, root dry weight; PSC, dry stem weight; PST, total dry weight; AF, total leaf area; AFT, total leaf area; RPF, leaf dry weight ratio; RPC, dry stem weight ratio; RPR, root weight ratio; RPA, shoot dry weight ratio; CMP, plant length; DMC, stem diameter.

# Discussion

The seeds of *J. curcas*, because they are classified as orthodox [[24](#ref-hay2013advances)], resistant to desiccation, can often present water contents up to 18% [[5](#ref-pompelli2010environmental)] when freshly harvested. Moncaleano-Escandon [[20](#ref-moncaleano2013germination)], showed that the seeds of *J. curcas* can drastically reduce their germinability during storage at temperatures of 4 °C or 25 °C, accompanied by the reduction of some compounds such as starch and total soluble proteins. This author also showed that at 4ºC it was more interesting for the storage of seeds of this species. In this work, it was observed that the reduction of the relative humidity in the interstice of the seeds with the use of desiccant material reduced the water content of the seeds and decreased the water potential, helping to maintain the germinability of the seeds during the storage without significant reduction. In relation to the physiological characteristics, a reduction in the concentration of total soluble proteins and in total soluble amino acids, probably to provide energy to the embryo, could be observed that remained viable during the storage period. Similarly to that described previously, the total soluble carbohydrates content must have been reduced by the metabolism of sucrose, which acted as the carbon source for the embryo. Circumstantial evidence of this idea is based on the elevation of glucose and fructose contents while there has been a reduction in the levels of starch and sucrose. However, even under these conditions, the seeds remained viable, with expressive levels of respiration, which allowed the viability of the embryo at the expense of the solubilization of reserves. Such a hypothesis can be corroborated by the strong negative correlation between oil, starch and sucrose contents with degradation products (i.e. total soluble proteins, total soluble amino acids and glucose). Some of these responses are distinct from those previously described for species [[20](#ref-moncaleano2013germination)]. It should be noted that in the work of Moncaleano-Escandon [[20](#ref-moncaleano2013germination)] there was no change in moisture in the interstice of the seeds, a fact that was taken into account in this study. In addition, it was reported that seeds containing between 6% and 8% of moisture had a rapid reduction in seed oil content in the first 3 months of storage, together with an increase in the concentration of free fatty acids [[50](#ref-akowuah2012influence),[51](#ref-worang2008quality)]. Important parameters in this culture. Thus, it is verified that the moisture control in the interstice of the seeds should be taken into account to preserve both the viability and the oil content of the seeds of *J. curcas*.

The negative effects of saline stress were reflected in the delay in the germination period, from 3 days in the control and up to 13 days in 150 mM NaCl in the irrigation water, which is confirmed by the significant reduction in germination percentage and mean germination time [[16](#ref-alencar2015ultrastructural)]. Regardless of the storage time, germination was initiated between the third and fifth day, with complete finishing after 23 days of sowing, which occurred mainly with 12 months of storage. Both salinity and storage delayed germination; But among them, storage seems to be the factor that slows down germinability with greater magnitude. It was observed that the germination synchrony was reduced with the increase of the salts concentration, and the salinity stress promoted slow and more disorganized germination, a fact corroborated by the high values of germination uncertainty. Thus, it is verified that the synchrony and the uncertainty of the germination of the seeds can not be considered good parameters for judgment, at least in *J. curcas*. A possible explanation for this could arise from the fact that J. curcas is not yet a domesticated species [[52](#ref-achten2010towards)], which makes it present high levels of uncertainty in germination [[53](#ref-ranal2006and)]; A factor highly related to the survival of the species in its original habitat [[54](#ref-maes2009climatic)] and very unstable from the physiological point of view.

The salinity experiment reveals that *J. curcas* can tolerate up to 150 mM NaCl in the irrigation water for seed germination, although it has difficulties for the development of the seedlings in concentrations above 75 mM of NaCl, occurring a reduction in the accumulation of biomass , A fact that may even lead to the death of the plant. A possible explanation for this is presented by Alencar [[16](#ref-alencar2015ultrastructural)] as an increase of the Na + and Cl- contents in the embryonic axes and in the endosperm of the seeds of *J. curcas*; Or the loss of mobilization of cotyledon reserves on germination [[55](#ref-liu2010seed)] affecting seedling establishment [[56](#ref-marques2013increased)]. The delay of the germination accompanied with the decrease of the development of the leaves and the reduction of the root growth promotes delay of the autotrophic phase of the plants that, in extreme cases, leads to the death of the same one. Even if the cumulative germination is different for the different genotypes, a marked tendency can be observed between the salinity and the reduction in the germination and the accumulation of biomass. Among the parameters of biomass allocation, the PRC showed a strong increase with salinity. It is common for plants to increase their stem biomass to the detriment of other organs when subjected to salinity [[57](#ref-dantas2007germination)–[59](#ref-praxedes2010salt)]. Munns [[58](#ref-munns1986whole)] describes this as an indirect effect of decreasing water uptake by roots and lower leaf expansion, while Praxedes [[59](#ref-praxedes2010salt)] describes this effect as lower relative growth rate of the plant as a whole. On the other hand, Bayuelo-Jiménez [[60](#ref-bayuelo2002salinity)] and Debez [[61](#ref-debez2004salinity)] describe that all biometric components are reduced in plants submitted to salinity, whether this species is salinity sensitive [[60](#ref-bayuelo2002salinity)] or a halophyte [[61](#ref-debez2004salinity)]. In addition, Hasewaga [[62](#ref-hasegawa2000plant)] attributed this reduction and / or inhibition of plant growth, when subjected to salinity, to a number of factors such as changes in the water status of the plant caused by the osmotic effect of the salts, increase in the concentration of toxic ions, Producing physiological and biochemical variations and alteration in the absorption of essential nutrients. Principal component analysis showed that the biometric parameters better explain the salinity response than the germination parameters. Genotypes 114, 171 and 183 are possible candidates for salt stress tolerance, whereas genotypes 133 and 218 show sensitivity to NaCl addition in irrigation water.

# Conclusions

The viability of germination and oil content in *Jatropha curcas* seeds can be maintained, provided there is control of moisture in the interstices of the seed during storage. Salinity strongly affects the germination and initial development of *Jatropha curcas* L., and of the evaluated genotypes, 114, 171 and 183 present as potential candidates for future breeding programs.

# Acknowledgments

The authors thank the National Council for Scientific and Technological Development (CNPq Process nº 404357 / 2013-0) for the financial support. The first author thanks the Coordination of Improvement of Higher Education Personnel (CAPES) for the scholarship.

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