



Physiological quality of castor bean seed genotypes stored at two temperatures

Anailda Angélica Lana Drumond^{1*}, Juliana de Fátima Sales¹, Jacson Zuchi², Osvaldo Resende¹, Gessimar Nunes Camelo³ and Moara Mariely Vinhais Souza⁴

¹Instituto Federal de Educação, Ciência e Tecnologia Goiano, Rodovia Sul Goiana, km 01, 75901-970, Rio Verde, Goiás, Brazil. ²Pólo de Inovação Tecnológica, Instituto Federal de Educação, Ciência e Tecnologia Goiano, Rio Verde, Goiás, Brazil. ³Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso, Campo Novo dos Parecis, Mato Grosso, Brazil. ⁴Programa de Pós-Graduação em Ciências Agrárias, Instituto Federal de Educação, Ciência e Tecnologia Goiano, Rio Verde, Goiás, Brazil. *Author for correspondence. E-mail: anailda14@yahoo.com.br

ABSTRACT. Environment and storage time influence stored seed quality, especially for oilseeds such as castor beans because they have a high lipid content and are more prone to deterioration. This study evaluated the physiological quality of seeds from three castor bean genotypes stored at 10 and 20°C for 300 days. The experimental design was completely randomized, with 4 replications, in a 2 x 6 (2 temperatures x 6 storage periods) split-plot design. The seeds were placed in low-density polypropylene bags and stored in climatic chambers at 10 and 20°C, in which the temperature and relative humidity were registered. Physiological quality (germination, germination rate index, emergence, rate of emergence, electrical conductivity and accelerated aging) were evaluated at baseline and after 60, 120, 180, 240, and 300 days of storage. Castor seeds of genotype EVF 106 showed better physiological quality when stored at 10°C. Castor seeds of genotype EVF 701 could be stored at 10 and 20°C for 300 days. Castor seeds of genotype EVF 712 presented reduced physiological quality during their 300 days in storage, regardless of temperature.

Keywords: *Ricinus communis* L.; seed conservation; dormancy; temperature.

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Introduction

Castor beans (*Ricinus communis* L.) are plants that produce inedible oilseeds with 40-60% oil (David et al. 2013), which are widely used in the chemical and bioenergy industries (Merkouropoulos et al. 2016).

However, the high content of oil in the seeds makes it difficult to store, since the oil seeds have less storage potential than the starches, due to the lower chemical stability of the lipids, in relation to the starch, since a moderate temperature increase, as a consequence of the respiratory process, is sufficient for the decomposition of lipids and increase of the deterioration rate (Fanan, Medina, Camargo, & Ramos, 2009; Marcos Filho, 2015a).

Castor bean production in Brazil is currently restricted to certain regions with great traditions of castor bean cultivation, such as Bahia state, the largest national producer. However, yield has been very low due to the minimal use of technologies such as improved seeds, fertilization, phytosanitary management and irrigation (*Empresa Brasileira de Pesquisa Agropecuária* [Embrapa], 2018). For the 2017/18 harvest, a production area increase is estimated to reach 33,900 hectares, representing a 21.1% increase compared with the previous harvest. This trend is attributed to the climate and market prices, especially for the last three years (*Companhia Nacional de Abastecimento* [Conab], 2018).

In Brazil, agroclimatic zoning, which presents ideal conditions for castor bean cultivation, comprises the Central region, more specifically, the Brazilian Cerrado. In addition to this fact, castor bean cultivation is an economically viable alternative, which can be used in the second crop of the year, in a crop rotation system, due to the low rainfall presented in this area.

In this sense, Torres et al. (2015a; 2015b) observed the phenotypic adaptability of castor bean cultivars to the Cerrado-Pantanal ecotone region in Brazil, demonstrating the crop's viability in this area. However, the production of high quality castor bean seeds is essential for increasing productivity and establishing the crop.

Storage is an excellent alternative to meet the logistics of seed production and marketing. In this way, information related to the behavior of the seeds in the face of the probable climatic conditions, which occur during the storage, can help in the decision making on the safe storage of the product (Smaniotto, Resende, Marçal, Oliveira, & Simon, 2014).

The main objective of the storage of seeds is to store the production, maintaining the quality, mainly of the physiological and sanitary attributes. The chemical constitution, tegument characteristics, number of reserves, physiological changes, the internal cellular arrangement of the seed and the conditions of temperature and relative humidity are factors that will influence the intensity of deterioration during the storage period (Zuchi, 2018).

The storage conditions are determinant to guarantee the physiological quality of the seeds and the control of the environment, temperature and relative humidity of the air, contributes to decrease the deterioration process (Almeida, Jerônimo, Alves, Gomes, & Silva, 2010; Bewley, Bradford, Hilhorst & Nonogaki, 2013; Neves, Serigatto, Dalchiavon, & Silva, 2014). In tropical regions, such as Brazil, storage is more complex due to high temperatures and relative humidity in several regions of the country, especially in castor bean cultivars.

Understanding the seeds' behavior during storage is essential for determining their handling. Therefore, the physiological quality of castor bean seeds with three genotypes (EVF 106, EVF 701 and EVF 712) stored at two temperatures, 10 and 20°C, for 300 days was evaluated.

Material and methods

Seeds from three castor bean genotypes (EVF 106, EVF 701 and EVF 712) were obtained from crops in production fields located at the 2P Farm (18.00' 28.90" S/50.57' 59.52" O), belonging to the Sementes Goiás LTDA company, 30 km from the city of Rio Verde, Goiás State, Brazil.

Planting was carried out in early March and harvesting in the last week of September 2015, totaling a cycle of 210 days. The mechanical harvest of the fruits (PLM 08L Platform) was performed after physiological maturity when the plants had 100% senescence (natural drying), with water content between 6 and 7% wet basis.

The fruits were pre-treated in a peeling machine and the seeds were followed by an air machine and sieves to remove the impurities, after being stored in big bags for three months in a store at 12°C in the Goiás Seed Processing Unit LTDA.

The seeds were sent to the Seeds Laboratory of the Goiano Federal Institute - Rio Verde Campus, where manual removal of the plant residues, broken seeds and without tegument was carried out. The seeds of the castor bean genotypes evaluated did not go through the process of overcoming dormancy, since they are not registered in the country and do not present scientific reports to analyze this characteristic.

Samples of 1,600 seeds from the genotypes EVF 106, EVF 701, and EVF 712 were packed in low-density polypropylene bags (semipermeable packaging) for each storage time, which was considered a subplot consisting 6 levels: zero, 60, 120, 180, 240, and 300 days. The temperatures were considered plots, consisting of two separate BOD (Biochemical Oxygen Demand) incubators, regulated at 10 and 20°C, in which dataloggers were placed to record the temperature ($10 \pm 0.5^\circ\text{C}$, $20 \pm 0.5^\circ\text{C}$) and relative humidity ($45 \pm 3\%$, $53 \pm 2\%$).

Seed storage began in January 2016, which was considered time zero (baseline). The seeds' water content was determined by the gravimetric method, using a forced-air oven at $105 \pm 3^\circ\text{C}$ for 24 hours, with two replicates (Brasil, 2009).

The experimental design was completely randomized in a 2 x 6 (2 temperatures x 6 storage periods) split-plot design, with four replicates, and the genotypes were evaluated separately.

In each period, the physiological seed quality was evaluated: Germination test and germination rate index - sowing was performed on germitest paper sheets, moistened with distilled water in an amount equivalent to 2.5 times the dry substrate mass with four replicates of 50 seeds. The paper rolls were maintained in a BOD incubator regulated at an alternating temperature of 20-30°C, with a photoperiod of 12/12 hours (Brasil, 2009). The germination percentage was evaluated at 7 and 14 days to determine the number of normal seedlings (well-developed essential structures with no damage), abnormal seedlings (with lesions or missing parts of essential structures), hard seeds (no signs of imbibition and remain hard when pressed with the index finger) and dead seeds (rotten appearance). The result was expressed as the percentage of normal seedlings (Brasil, 2009). The germination rate index (GRI) was calculated by the sum of the number of germinated seeds, with radicles larger than or equal to 1 cm, recorded daily, divided by the number of days elapsed between sowing and daily counts (Maguire, 1962).

Emergence test and emergence rate index - 200 seeds divided into four replicates of 50 seeds were used. The seeds were sown in a 3-cm deep sand bed in a greenhouse with a sprinkler irrigation system, which operated for 15 min. every 4 hours. Seedling emergence was evaluated until stabilized, and only normal seedlings were considered, with the result expressed as the final emergence percentage. The seedling emergence rate index (ERI) was evaluated by daily observations after the emergence test was set up, counting the number of seedlings emerged per day (with two cotyledons above sand level) and dividing it by the number of days elapsed since sowing (Maguire, 1962).

Accelerated aging - The gerbox method (Marcos Filho, 2015b), with four replicates of 50 seeds, was used. In each gerbox, 50 seeds were placed on the screen, and 40 mL of distilled water was placed in the bottom. Subsequently, the gerboxes were placed in a BOD incubator at 42°C, where they remained for 72 hours. After this period, each replicate of 50 seeds was sown, as described for the germination test, in which normal and abnormal seedlings and hard and dead seeds were evaluated at 7 and 14 days (Brasil, 2009).

Electrical conductivity - To evaluate the electric conductivity of the seed imbibition solution, the 'bulk conductivity' test was performed with four samples of 25 seeds each, which were weighed on an analytical balance, with a precision of two decimal places. The seeds were then soaked in a plastic container with 75 mL of deionized water and kept in a BOD incubator at 25°C for 24 hours. Next, the electrical conductivity in the imbibition solution was measured using a Tecnal TEC-4MP conductivity meter. The results are expressed in $\mu\text{S cm}^{-1} \text{ g}^{-1}$ of seeds (Vieira & Krzyzanowski, 1999).

Data were subjected to analysis of variance, and when significant ($p < 0.05$), regression models were analyzed. The models were selected based on a coefficient of determination (R^2) $\geq 70\%$ or the goodness of fit to the biological phenomenon. Tukey's test was applied to the means, based on the experimental factor characteristic. The Analysis of Variance System - Sisvar (Ferreira, 2011) was used to analyze the data.

Results and discussion

The initial water content of the seeds of genotypes EVF 106, EVF 701 and EVF 712 were 6.92, 6.39, and 6.55% (wet basis), respectively. During storage there were no significant variations in moisture contents, which were between the values of 4.71 to 6.97% (EVF 106); 5.64 to 6.86% (EVF 701) and 5.13 to 6.56% (EVF 712). These values are adequate before the tests, since the variations were within the tolerable limits, that is, 2 to 3 percentage points (Marcos Filho, 1999).

Germination of the genotype EVF 106 seeds stored at 10°C increased after 154 days of storage, while the opposite was observed for the seeds stored at 20°C, which showed decreased germination after 71 days of storage (Figure 1A). The difference between the mean germinations at 10 and 20°C occurred from 120 days of storage, being significant in the evaluations of seeds stored for 300 days (Figure 1A).

Temperature and relative air humidity of the storage site are the main factors affecting the physiological quality of the seed. The relative humidity of the air controls the moisture content of the seed, while temperature affects the speed of biochemical processes (Goldfarb & Queiroga, 2013).

Moderate temperature elevation because of the respiratory process is already sufficient for lipid decomposition and elevation of deterioration rate (Marcos Filho, 2015a), which may have led to a reduction in the germination of seeds stored at 20°C.

The germination rate index (GRI) of the genotype EVF 106 seeds stored at 10°C decreased until day 144 of storage (Figure 1B). After this, the GRI increased concomitantly with the percentage of normal seedlings (Figure 1A), and seeds stored at 10°C also had higher GRIs when stored for 180, 240 and 300 days (Figure 1B).

Storage may have enabled the seeds to break their physiological dormancy over time because some castor bean genotypes exhibit this dormancy. Machado, Martins, Cruz, Nakagawa, and Pereira (2010) observed that 9.3% of castor bean seeds were dormant after harvest. This decreased to 5.5% when the seeds were stored for 12 months at room temperature.

The increased germination and GRI may also be related to the castor bean seed caruncle deteriorating while in storage because this structure hinders water from entering imbibition and delays germination. Mendes, Dias, Pereira, and Berger (2009) also stated that removing the caruncle contributes to accelerating freshly harvested seed germination, which positively affects the seedlings' initial growth. Fogaça et al. (2017) found that removing the caruncle positively affected the characteristics evaluated in the seed physiological quality tests.

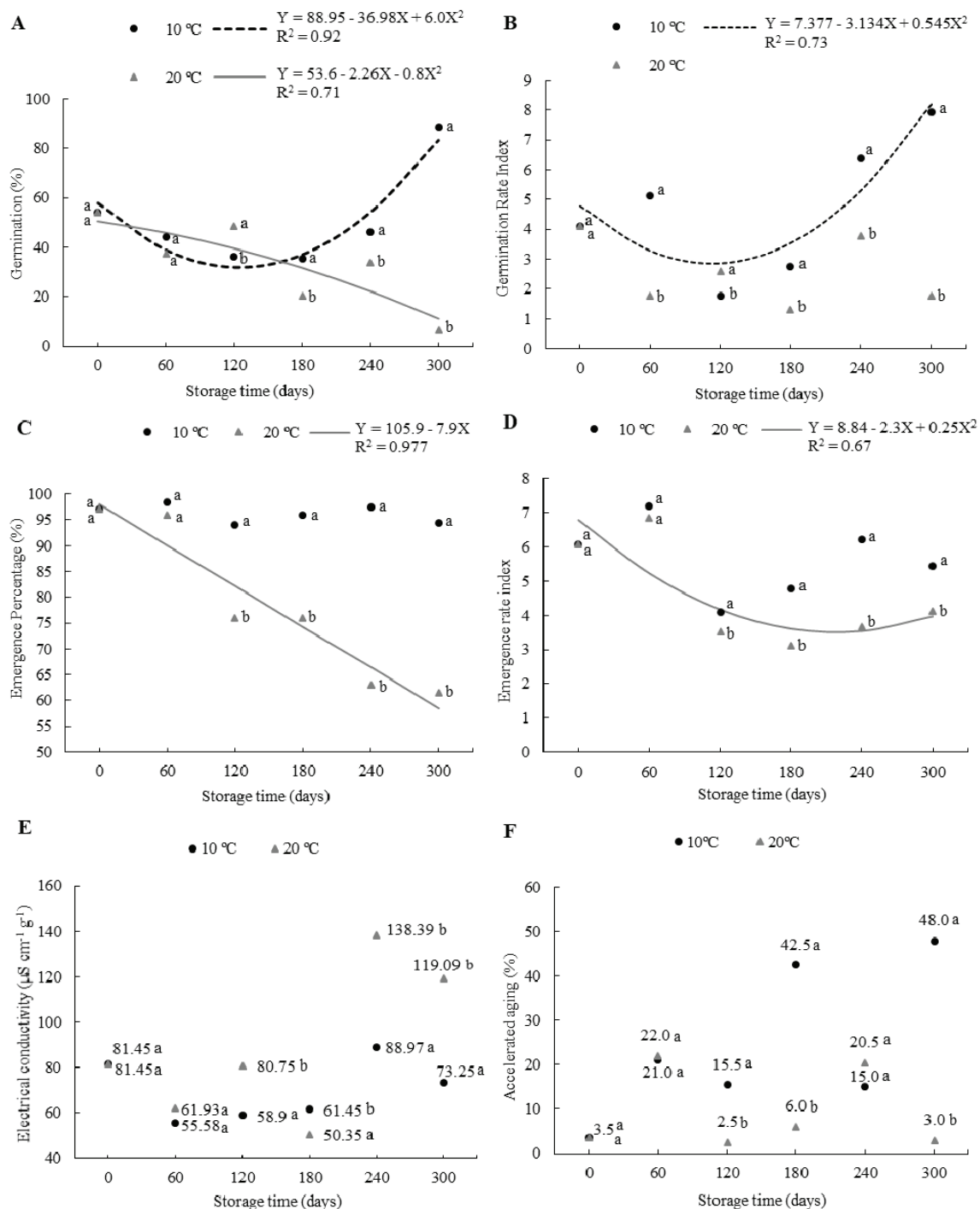


Figure 1. Germination percentage (A), germination rate index (B), emergence percentage (C), emergence rate index (D), electrical conductivity (E), and accelerated aging (F) of genotype EVF 106 castor bean seeds stored for 300 days at 10 and 20°C. Means followed by lower case letters at the same storage time did not differ by Tukey's test ($p \leq 0.05$).

The emergence of castor bean seeds from the EVF 106 genotype decreased linearly while in storage at 20°C (Figure 1C), decreasing by 13.4% every 60 days of storage. Storing seeds at 10°C allowed greater seedling emergence for 300 days (Figure 1C). Per Bewley et al. (2013), the reduced seedling emergence percentage reflects the seeds' natural aging process, which is related to damages in the biomembrane system composed of phospholipids.

Lipid peroxidation is one of the most frequent causes of deterioration and loss of viability of the seeds. Often, this is due to the activity of oxygen in polyunsaturated fatty acids present in the seed membranes, which affects their quality (Abreu, Carvalho, Pinto, Kataoka, & Silva, 2013).

The seed germination and emergence rate is influenced by the storage environment as well as by the seeds' initial quality (Marcos Filho, 2015a). In addition, the ERI of EVF 106 genotype seedlings stored at

20°C decreased until 230 days of storage, and the 10°C temperature produced higher mean values after 120 days (Figure 1D).

EVF 106 genotype seeds, when stored at 10°C, presented lower EC values in the initial storage period between 60 and 120 days (Figure 1E). The 20°C temperature provided higher EC values at 240 and 300 days, suggesting decreased seed vigor.

Several authors (Fessel, Vieira, Cruz, Paula & Panobianco, 2006; Panobianco, Vieira, & Perecin, 2007) state that electrical conductivity test results can be influenced by storage temperature, suggesting that the seed deterioration at low temperatures is likely unrelated to the loss of cell membrane integrity, since, at 10°C, membrane damage does not occur at the same intensity as when seeds are stored between 20 and 25°C.

Germination and the germination rate index of genotype EVF 701 seeds showed no significant interaction. Emergence, electrical conductivity and accelerated aging were only significant for the storage time factor in the EVF 701 genotype.

The 20°C temperature provided greater germination at 240 and 300 days of storage for the genotype EVF 701 seeds (Figure 2A). These storage times also differed in their GRIs based the storage temperatures. In addition, after 176 days, seeds stored at 20°C germinated faster (Figure 2B). The GRI of the seeds stored at 10°C decreased until day 293 of storage. Emergence of cultivar EVF 701 seedlings was greatest at day 157 of storage, then decreased (Figure 2C).

Jatropha seeds stored in different packaging and environments were evaluated by Zonta et al. (2014), who observed reduced physiological quality and recommended storage in a refrigerated environment, with temperatures between 18-20°C, regardless of packaging. The seed quality of castor bean cultivar IAC-226 stored for 12 months was evaluated by Santos et al. (2016), who identified higher emergence rates for seeds stored in liquid nitrogen for 12 months. Seeds stored in a cold chamber and vacuum packed also showed lower emergence.

The electrical conductivity of the seeds of the cultivar EVF 701 increased after 180 days regardless of the storage temperature (Figure 2E). The increase of the electrical conductivity during storage may be related to the seed reumidation during the test, which reflects greater damage in the membrane system (Smaniotto et al., 2014).

In addition, the degradation process is characterized by the disruption of the cellular membranes system, which results in damage to the solutes retention capacity, being considered as one of the first events of the deterioration process of the seeds (Santos et al., 2016; Fessel et al., 2006).

The temperature factor showed that a 20°C storage effectively provided higher EC values than 10°C, with values of 111.65 and 108.38 $\mu\text{S cm}^{-1} \text{g}^{-1}$, respectively. Under field conditions, the exudation of exudates after sowing, reflecting the loss of cell membrane organization and selective permeability, may stimulate the growth of pathogenic microorganisms and impair the emergence of seedlings (Marcos Filho, 2015a).

The values of the electric conductivity of the seeds of the casona variety, which originated from the primary racemus without caruncle, presented mean values of 45.29 $\mu\text{S cm}^{-1} \text{g}^{-1}$ (Fogaça et al., 2017). However, the electrical conductivity is affected by several factors, among them the genotype of the same species (Panobianco & Vieira, 1996).

Germination of the EVF 701 genotype seeds increased during storage regardless of temperature (Figure 2F). The increased germination could also be attributed to the break in physiological dormancy promoted by aging because similar behavior was observed in freshly harvested *Leucaena leucocephala* seeds, in which immature seeds were present (Araújo, Felix, Ferrari, Bruno, & Pacheco, 2017).

The vigor presented by the accelerated aging test (Figure 2F) increased, and may be attributed to imbibition caused by high temperature and relative humidity, since these conditions may function as a treatment to overcome seed dormancy (Vieira, Fraga, Vieira, Oliveira, & Almir, 2002). The castor bean seeds of the cultivar Al Guarany 2002, submitted to the accelerated aging test, decreased dormancy over the storage period (Machado et al., 2010).

Higher germination, after accelerated aging, was also observed in freshly harvested seeds from other Fabaceae species, such as *Anadenanthera falcata* (Benth.) Speg (Stallbaun, Souza, Martins, Matos, & Moura, 2015) and *B. forficata* (Guareschi, Lanzarini, Lazarotto, Gonzatto, & Barbieri, 2015). In addition, Nobre, Silva Neta, David, Gonçalves, and Amaro (2014) stated that castor bean seeds may be dormant while still in the plant, and the intensity and persistence of the dormancy depends on the cultivar and the maturation stage at harvest.

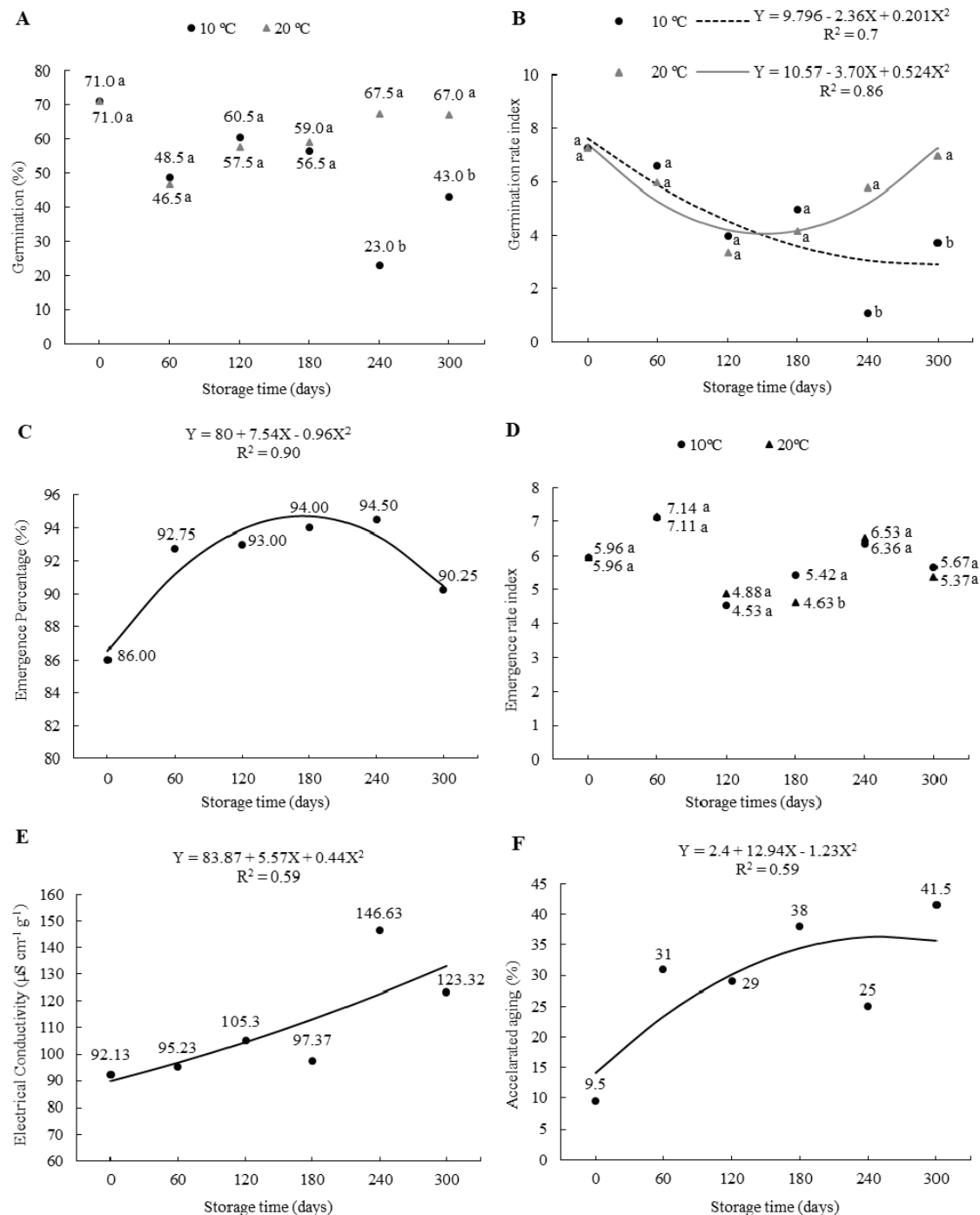


Figure 2. Germination percentage (A), germination rate index (B), emergence percentage (C), emergence rate index (D), electrical conductivity (E), and accelerated aging (F) of the EVF 701 genotype stored for 300 days at 10 and 20°C. Means followed by lower case letters at the same storage time do not differ by Tukey's test ($p \leq 0.05$).

The interaction among factors was significant for all analyzed variables of the EVF 712 genotype seeds, except for emergence, which was significant only for the time factor. Seed germination increased until day 167 at 10°C and day 173 at 20°C, followed by a reduction (Figure 3A). Higher germination and GRI were observed at 120 and 240 days in the seeds stored at 20°C, with a reverse in trend at day 300 of storage, with 10°C showing higher means (Figure 3A and B).

Emergence decreased until day 202 of storage, regardless of temperature (Figure 3C). The ERI also decreased until day 217 at 10°C, with a difference in the ERI between the temperatures at 240 days (Figure 3D). Storage at 20°C increased the electrical conductivity until day 300, with a difference between both temperatures at this storage time (Figure 3E). Germination of genotype 712 seeds also presented higher means when stored at 10°C, and germination differed between temperatures at 120 and 300 days (Figure 3F).

Chemical degradation of seed components during storage for Walters, Ballesteros, and Vertucci (2010) occurs through damage caused by oxidizing agents, but the speed of such reactions is defined by the properties of the seeds, which in turn are affected by temperature and humidity, and the intensity and speed of the degradation processes influence directly the germination and vigor of the seeds.

According to Marcos Filho (2015a) the conjugated action of high humidity and temperature accelerates the deterioration process of orthodox seeds, such as castor bean seeds, reducing their longevity. Zonta et al. (2014) also found a decrease in vigor of *Jatropha curcas* seeds during storage when subjected to a temperature of 33°C, with 8.5% b.u. of moisture content.

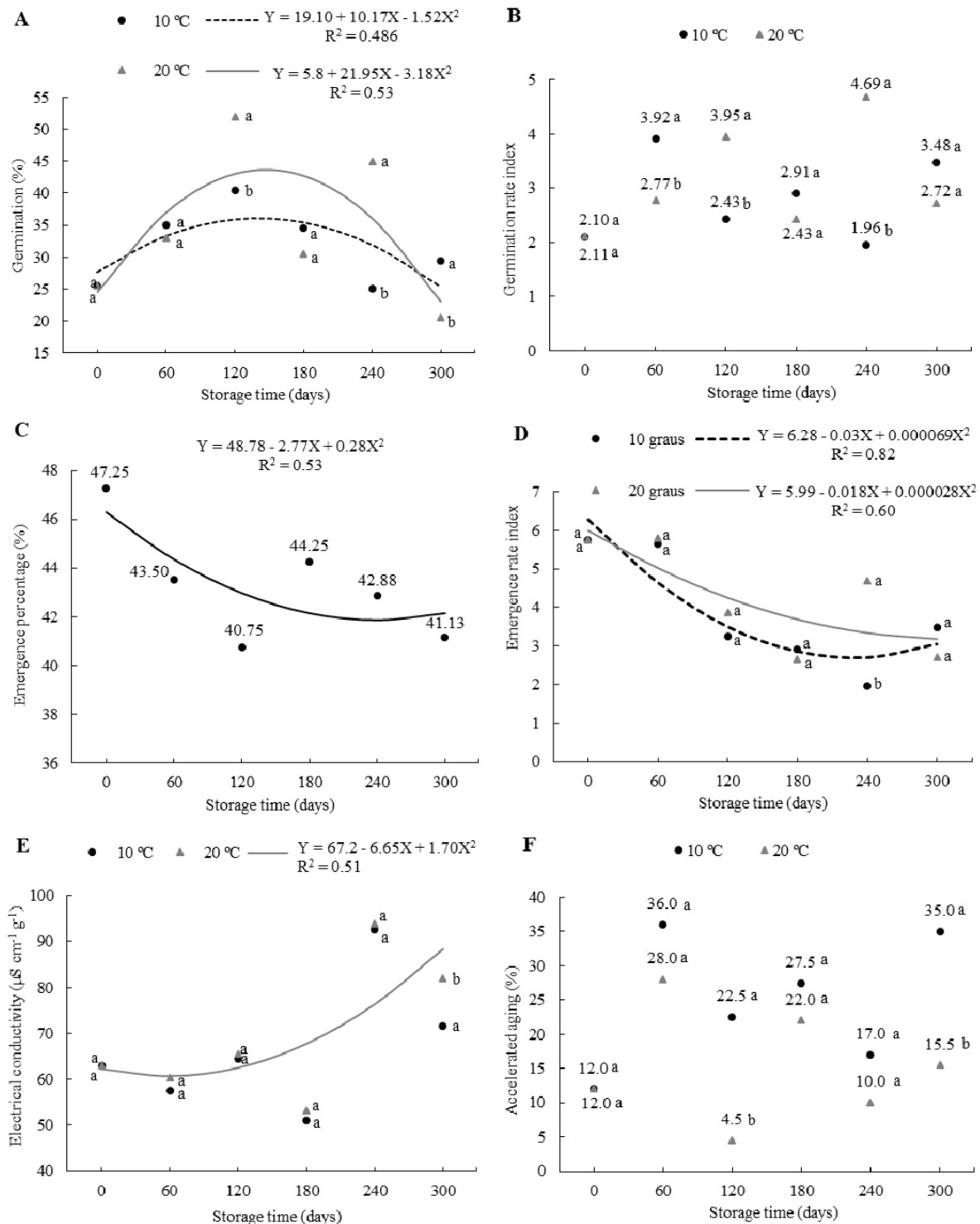


Figure 3. Germination percentage (A), germination rate index (B), emergence percentage (C), emergence rate index (D), electrical conductivity (E), and accelerated aging of castor bean genotype 712 seeds, stored for 300 days at 10 and 20°C. Means followed by lower case letters at the same storage time do not differ by Tukey's test ($p \leq 0.05$).

Vieira, Dardengo, Oliveira, Berbert, and Deminicis (2017) found that *Jatropha curcas* seeds, as well as other seeds with high oil content, showed a linear reduction over time in seed germination and vigor after storage at 15°C for 240 days. Coradi, Fernandes, Peralta, and Pereira (2015) found that 20°C and 60% relative humidity (RH) were more favorable for preserving sunflower seed quality during storage, which was adversely affected after three months of storage.

Conclusion

Seeds from castor bean genotype EVF 106 showed better physiological quality when stored at 10°C. Seeds from castor bean genotype EVF 701 can be stored at 10 and 20°C for 300 days. Seeds from castor bean genotype EVF 712 presented reduced physiological quality while in storage for 300 days, regardless of temperature.

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