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# Conditioning in the promotion and uniformization of Umbu seed germination

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**Abstract -** Seed dormancy may decrease during storage and some environmental conditions may accelerate this process. The aim of this work was to determine efficient techniques to condition umbu seeds in order to promote and standardize their germination. Seeds were stored for 180 days in paper bags kept in five ambient conditions: laboratory (25 ºC and 55% RH); warm oven (40 °C and 53% RH); hot oven (50 °C and 49% RH); dry chamber (18 °C and 65% RH) and cold chamber (10 °C and 65% RH). Seed quality was evaluated every 60 days by means of the following tests and determinations: water content; germination test (25 °C and 55% RH, weekly evaluated up to 91 days after sowing); first count test (14 days); germination rate index; mean germination time and electrical conductivity. The conditioning of umbu seeds in laboratory, or in warm oven (40 ºC) used efficient techniques to promote and standardize germination; under these conditions, after six months of storage, germination increased from 31% to 84 and 74%, respectively.
**Index terms:** storage, dormancy, germination, fruit trees, *Spondias tuberosa*.

# Condicionamento na promoção e uniformização da germinação de sementes de Umbuzeiro

**Resumo** – A dormência das sementes pode diminuir durante o armazenamento, e algumas condições do ambiente podem acelerar este processo. O objetivo deste trabalho foi determinar técnicas eficientes para condicionar as sementes de umbu de modo a promover e a uniformizar a germinação. As sementes foram armazenadas por 180 dias em sacos de papel mantidos em cinco condições de ambiente: laboratório (25 ºC e 55% UR); estufa morna (40 ºC e 53% UR); estufa quente (50 ºC e 49% UR); câmara seca (18 ºC e 65% UR) e câmara fria (10 ºC e 65%UR). A qualidade das sementes foi avaliada a cada 60 dias, por meio dos seguintes testes e determinações: teor de água; teste de germinação (25 ºC e 55% UR, avaliado semanalmente até 91 dias após a semeadura); teste da primeira contagem (14 dias); índice de velocidade de germinação; tempo médio de germinação e condutividade elétrica. O condicionamento das sementes de umbu em laboratório, ou em estufa morna (40 ºC), foi técnica eficiente para promover e uniformizar a germinação; nestas condições, após seis meses de armazenamento, a germinação aumentou de 31% para 84 e 74%, respectivamente.
**Termos para indexação:** armazenamento, dormência, germinação, frutíferas, *Spondias tuberosa*.

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## Introduction

The demand for exotic fruits has increased significantly in recent years and the trend is for growth. For some fruits typical of northeastern Brazil, such as umbu, consumption is related to consumers' eating habits and traditionalism in relation to their region of origin (WATANABE; OLIVEIRA, 2014).

Umbu tree (*Spondias tuberosa*, Arr. Câmara) is a plant native to the semiarid region of Brazil and its fruits are consumed fresh or transformed into juices, pulps, jellies or ice creams (SANTOS et al., 2005). Fruits of species of the genus Spondias are rich in bioactive compounds, presenting antioxidant potential, which increases their value to the consumer as functional food (NEVES et al., 2015).

The commercial plantation of umbu trees presents as an obstacle in the production of seedlings due to the low and uneven seed germination, which occurs between 12 and 90 days after sowing. At the end of the period, maximum germination varies between 30 and 40% of seeds (NEVES; CARVALHO, 2005). This phenomenon can be caused by dormancy, which hinders the formation of seedlings on commercial scale, as verified by some authors (SOUZA et al., 2005; MELO et al., 2012).

Thus, there are studies that have investigated the dormancy of umbu seeds, and the best result found was mechanical scarification (NASCIMENTO et al., 2000; ARAÚJO et al., 2001; LOPES et al., 2009). However, this is a laborious and costly method, since it requires the individual treatment of each seed using tools such as lathe and saws or pruning shears with resistant and sharp steel (LOPES et al., 2009).

Other works attributed the dormancy of umbu seeds to phytohormonal factors due to the balance between substances that promote and inhibit germination. However, the treatment of umbu seeds with phytohormones still presents inconclusive data regarding the improvement of final germination (MARCOS FILHO, 2016; LOPES et al., 2009).

Seed dormancy is a phenomenon naturally overcome with time, both for seeds stored in soil banks and those stored in man-made stores (CARVALHO; NAKAGAWA, 2012). Therefore, storage is a procedure that can also be used by growers to obtain seeds without dormancy. According to Marcos Filho (2016), seed storage overcomes all causes of dormancy, such as: control of the balance between substances that promote and inhibit germination, mechanical coverage resistance, embryo dormancy, coverage impermeability to gases and water, and by the combination of causes.

For umbu seeds, storage at low temperatures seems to delay the overcoming of dormancy and promotion of germination. Araújo et al. (2001) and Cavalcanti et al. (2006) verified that umbu seeds take two to three years to overcome dormancy when stored in cold and dry chamber (10 °C and 40% RH), increasing germination from 23 to 74% in 24 months (ARAÚJO et al., 2001).

However, it is still not possible to establish reliable comparisons on the subject, since all studies on the storage of umbu seeds evaluated the quality of seeds in a single conservation environment condition (ARAÚJO et al., 2001; AZEVEDO et al., 2004; CARVALHO et al., 2001; CAVALCANTI et al., 2006; SANTOS et al., 2005; SOUZA et al., 2005).

However, there is evidence that seed maintenance in environments with relatively high temperatures or the application of oxidizing agents may promote the physical or chemical removal of short-chain saturated fatty acids that primarily control seed dormancy in strongly dormant plants from tropical regions (SESHU; DADLANI, 1991).

The application of temperatures between 40 and 50 ºC in greenhouses has shown good results for seeds of some plants native to hot climate regions (ALMEIDA; SILVA, 2004; BRASIL, 2009), including fruit trees such as tucumã (BRASIL, 2013). This occurs due to the adaptation of species to the environmental conditions of the place of origin, and the application of these conditions in an artificial way could overcome dormancy and promote seed germination (ALMEIDA; SILVA, 2004; MENDONÇA et al., 2015).

Although several studies have sought to clarify the problem of overcoming dormancy in umbu seeds, other aspects such as longevity and methodologies for storage / conditioning of seeds still need to be better clarified. In this way, the aim of this research was to define efficient techniques to condition umbu seeds in order to promote and standardize germination.

## Materials and Methods

The experiment was carried out at the Laboratory of Seed Analysis, Department of Plant Production, Faculty of Agrarian and Veterinary Sciences (FCAV), "Júlio de Mesquita Filho" State University (UNESP), Campus of Jaboticabal - SP.

Umbu fruits were harvested from 10 trees located in the orchard and in several places of FCAV-UNESP campus and farms of the municipality of Jaboticabal-SP. Fruits were pulped by washing and rubbing on sieve under running water and placed to dry on laboratory bench for two days. Visibly healthy and well formed endocarps (nuculans) were selected and used to conduct the research.

Nuculans containing the true seeds were called seeds, according to definition of the Rules for Seed Analysis (BRASIL, 2009), since they are the structure used for seeding and propagation of the species.

Seeds were then stored in paper bags under the following conditions: laboratory environment at 25 ºC

and 55% RH; forced ventilation oven at 40 °C and 53% RH; forced ventilation oven at 50 °C and 49% RH; dry chamber at 18 °C and 45% RH and cold chamber at 10 °C and 65% RH. The quality of seeds was evaluated at 0, 60, 120 and 180 days, by means of the following tests and determinations:

**Water content -** determined by the oven method at 105 ± 3 °C for 24 hours (BRASIL, 2009) using two subsamples of seven seeds.

**Germination test -** conducted with four subsamples of 25 seeds per treatment at 25 °C with eight hours of light in sterilized vermiculite (BRASIL, 2013) and moistened with one and a half times the weight of the substrate in water, packaged in clear plastic boxes (11.0 x 11.0 x 3.5 cm). Seedling counts were weekly performed from the 14th to the 91st day after sowing (NEVES; CARVALHO, 2005; CAVALCANTI et al., 2006), when the germination percentage (normal seedlings), abnormal seedlings and non-germinated seeds were calculated. Normal seedlings were those with well developed root system and aerial part.

**First germination count test -** performed in conjunction with the germination test, calculating the percentage of normal seedlings present on the 14th day after sowing (NEVES; CARVALHO, 2005).

**Germination rate index (GRI) -** determined by weekly counting of the number of seedlings emerged from the 14th to the 91st day by adapting the criterion and formula proposed by Maguire (1962) with results presented in number of seedlings.

**Mean germination time -** determined by weekly counting of the number of seedlings emerged from the 14th to the 91st day according to formula described by Santana and Ranal (2004). The results were presented in days after sowing.

**Electrical conductivity -** evaluated by measuring seed soak solution using four replicates of 20 seeds per treatment, which were weighed and placed in disposable plastic cups containing 75 ml of distilled water and kept to soak at 25 °C for 24 hours; after this period, the electrical conductivity of the solution was determined by means of a conductivity meter and the results were expressed as µS $cm^{-1}$ $g^{-1}$ of seed (MARCOS FILHO, 2016).

The experimental design was completely randomized in a 4 x 5 factorial scheme, with four replicates. Data were submitted to analysis of variance and, afterwards, polynomial regression analysis ($p < 0.05$) was performed, in which the significant model of higher order ($R^2$) was selected using the equation that best fit to data.

## *Results and discussion*

The permeable paper bag packaging allowed the water exchange between the conditioning environments and the seeds, since initially they had 12.7% of water content, and during the conditioning period, fluctuations of these values were verified ( Figure 1). At 180 days, seeds had water contents of 8.1; 4.4; 3.0; 10.7 and 12.8%, respectively, under laboratory environments, drying oven at temperatures of 40 and 50 °C, dry and cold chamber. These results can be attributed to the hygroscopic equilibrium between umbu seeds and the temperature and relative humidity conditions of each environment (CARVALHO; NAKAGAWA, 2012).

There was a significant effect of the interaction between environments and conditioning periods for all evaluated parameters (germination, germination rate index, mean germination time and electrical conductivity). The evaluation periods influenced the seed quality of all storage conditions, and the best fit of data was verified for the linear model equation (Figure 2).

It was verified, by the tendency of the germination lines of umbu seeds that the longevity of seeds was superior to 180 days in all environmental conditions. However, the conditioning of umbu seeds in oven at 40 ºC and laboratory at 25 ºC presented the best results as a method to promote germination in all evaluated periods.

Seeds had 53% of initial germination and after the period of 180 days in laboratory environment and oven at 40 ºC, germination increased to 84 and 74%, respectively. For the same period, seed germination had a small increased in the dry chamber, only 59%. The dormancy of umbu seeds may be more intense than that verified in the present study, resulting in lower germination values, since Neves and Carvalho (2005) reported that the initial germination of the species is between 30 and 40%. The greater the dormancy, the more important it is to overcome it for the production of seedlings and this is a characteristic that depends on the species genotype and the environment at the place of origin (MARTINS; NAKAGAWA, 2008).

Similar results were found by Lopes et al., 2009, who evaluated storage of *S. tuberosa* endocarps under 22.5 °C and obtained time from 120 to 180 days, with approximately 83% of germination.

Temperature plays an important role in the maintenance of seed viability and deterioration during storage, being directly related to biochemical processes (MARCOS FILHO, 2016). Some storage environment conditions may be detrimental to seed vigor and viability (CARVALHO; NAKAGAWA, 2012). Probably, this must have occurred in seeds kept in oven at 50 ºC and cold chamber (10 ºC), which caused significant reductions in germination.

The germination rate showed behavior similar to that of germination during conditioning (Figure 3). The germination rate index curve assumed the linear model for laboratory, dry chamber and oven at 40 °C. For the cold chamber, there was no curve adjustment, and in oven at 50 °C, the curve followed a quadratic trend. The index increased from 0.24 at the beginning of conditioning to 1.03 in the last period.

Similar results were found by Lopes et al. (2009)

in a study in which the storage of umbu seeds for seven months provided better germination rate index. Cavalcanti et al. (2006) verified that the dormancy of umbu seed is overcome by storage for 24 to 36 months. Seed storage overcomes all causes of dormancy, such as control of the balance between substances that promote and inhibit germination, mechanical coverage resistance, embryo dormancy, coverage impermeability to gases and water, and by the combination of causes (MARCOS FILHO, 2016).

The mean germination time was also influenced by storage environment conditions, but mainly by the conditioning period (Figure 4). There was a reduction in the mean germination time of umbu seeds due to the increase of the germination rate (Figures 4 and 3). In general, without considering the particularities of storage environments, newly harvested seeds required time between 41 and 51 days to germinate and at the end of 180 days, the time required for this phenomenon was reduced to a period between 14 and 28 days (Figure 4).

This reduction in the mean germination time represents a significant decrease in the production costs of a seedling production nursery, as seedlings would require less time under controlled production conditions. The rapid and uniform germination of seeds, followed by the early emergence of seedlings, are highly desirable characteristics in the formation of seedlings of fruit species (RAMOS et al., 2011).

Regarding the effect of the different storage environments over the mean germination time, a greater reduction of the germination time was observed when seeds were kept in oven at 40 ºC and laboratory, verifying that these seeds demanded, respectively, 16 and 18 days to germinate after 180 days of conditioning. For seeds kept in cold chamber, dry chamber and oven at 50 ºC, the mean germination time was higher in most conditioning periods. The gradual overcoming of seed dormancy that naturally occurs during storage promotes greater germination rate and uniformity (LOPES et al., 2009). There are species whose seeds are released from the mother plant with immature embryo, which would be one of the types of dormancy, verified in tucumã (BRASIL, 2013), peach (FISCHER et al., 2013) and persimmon (PECHE et al., 2016) seeds. These seeds are able to complete maturation if kept in stratification environment, with temperature and humidity specific to each species.

Over the conditioning period, progressive and linear increase of the electrical conductivity of seeds was verified (Figure 5). The electrical conductivity test is based on the disintegration of cell membranes, which occurs with the seed deterioration process, which may be more or less intense depending on the environment conditions (MARCOS FILHO, 2016).

Thus, from the 60 days, the differentiation of this seed quality parameter was intensified according to the storage environments and at the end of the 180 days, seeds conditioned in oven at 50 ºC, 40 ºC, laboratory (25 ºC ), dry chamber (18 °C) and cold chamber (10 °C) were classified in decreasing order of electrical conductivity (Figure 5). These results can be attributed to the advancement of the deterioration process caused by high storage temperatures (PANOBIANCO et al., 2007).

In the case of umbu seeds, deterioration due to storage would degrade the spongy endocarp of the fruit that contains the true seeds. This structure provides a physical barrier that hinders germination and has been reported as one of the possible causes of dormancy of this species (NASCIMENTO et al., 2000; ARAÚJO et al., 2001; LOPES et al., 2009). The electrical conductivity results obtained in the present study corroborated this hypothesis. Therefore, the electrical conductivity test can be used as an indication of dormancy in umbu seeds.

Considering the conditioning of umbu seeds as a methodology to be used to overcome dormancy, it could be inferred that the best storage environments were oven at 40 ºC and laboratory (25 ºC). These storage conditions promoted the deterioration of the spongy endocarp that causes dormancy and promoted more quick and uniform germination (Figures 2, 3 and 4 and 5). Storage at 50 ºC, although being the environment that caused the highest endocarp deterioration, was not able to promote germination, probably because it caused the death of seeds due to the excessive temperature.

Conditioning in oven at 40 ºC or laboratory would be a more practical and low-cost method compared to mechanical scarification, in which each seed must be individually worn with tools such as lathe, saws and sharp pruning shears and resistant steel (LOPES et al., 2009).

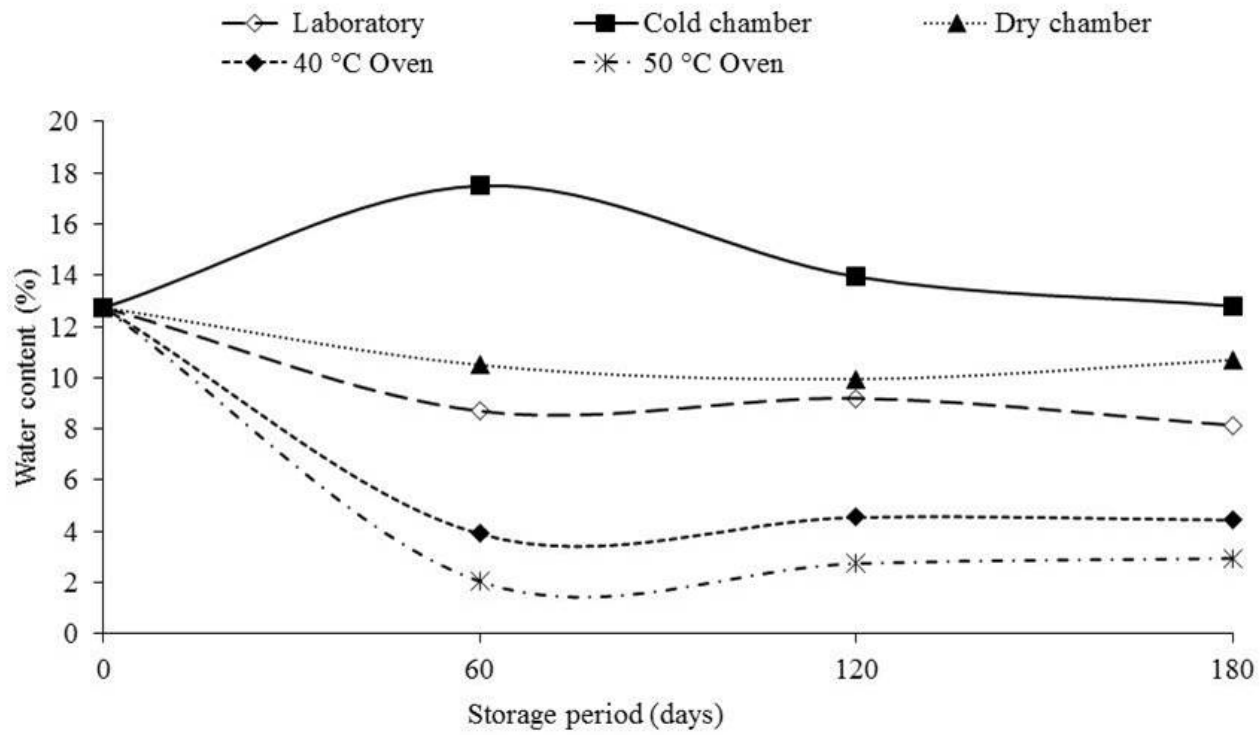


**Figure 1**-Water content (%) of *Spondias tuberosa* seeds submitted to different environments and storage periods.

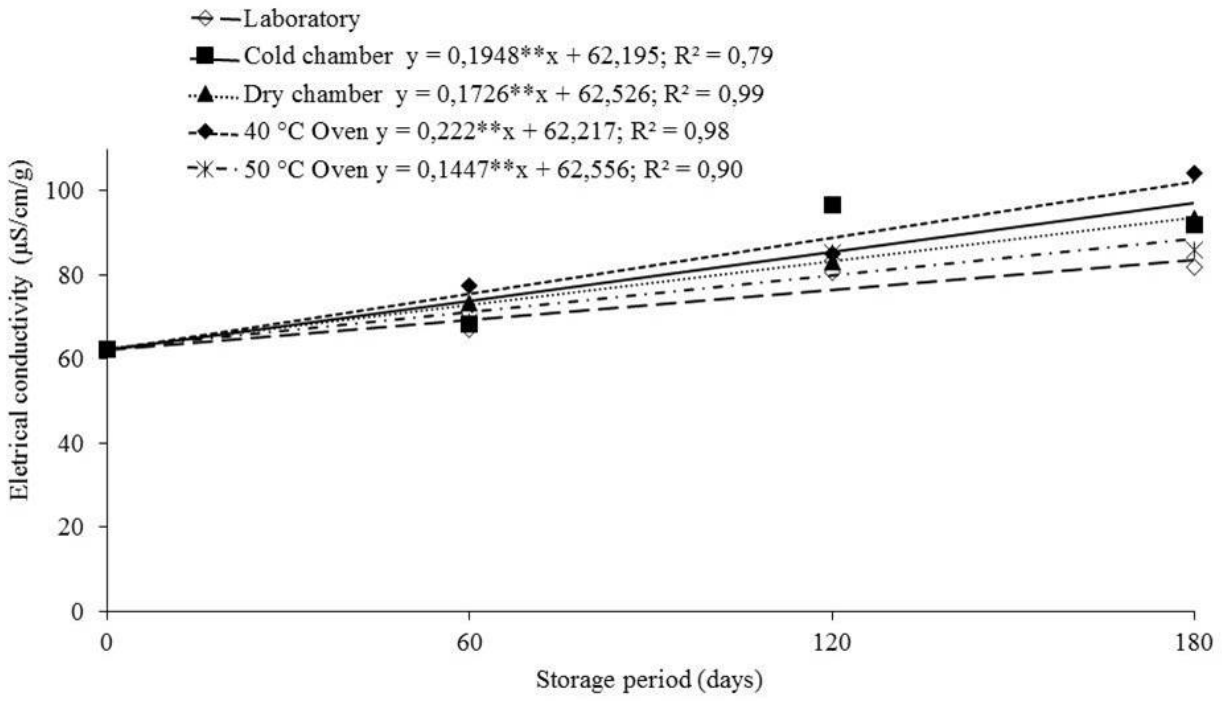


**Figure 2-** Germination (%) of Spondias tuberosa seeds submitted to different environments and storage periods.
** Significant at 1% probability

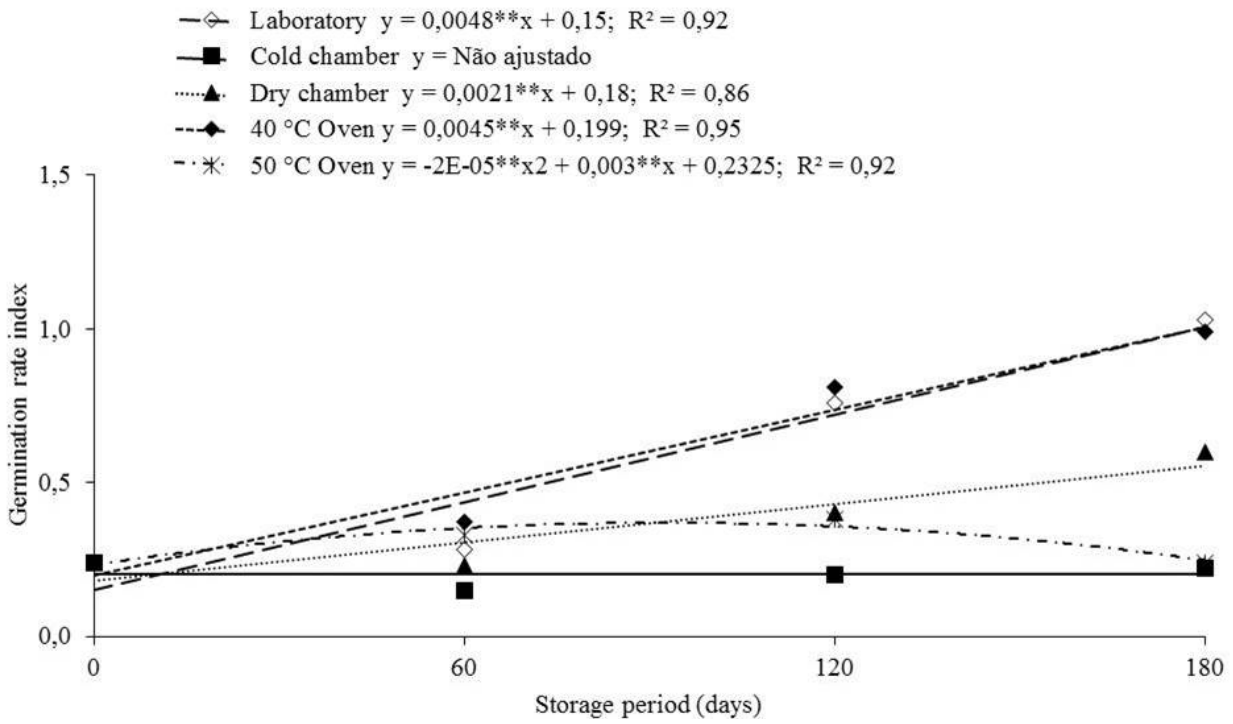


**Figure 3**- Germination rate index of Spondias tuberosa seeds submitted to different environments (A) and storage periods (P).
** Significant at 1% probability.

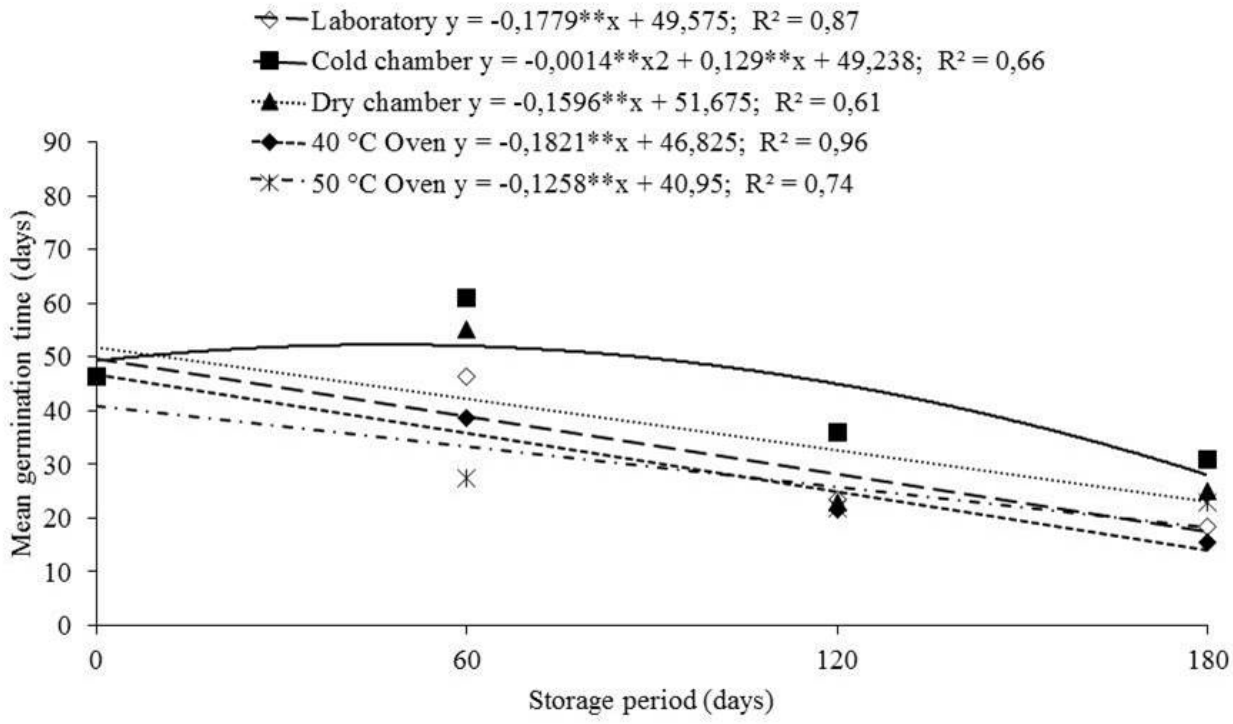


**Figure 4**- Mean germination time (days) of Spondias tuberosa seeds stored in different environments.
** Significant at 1% probability.

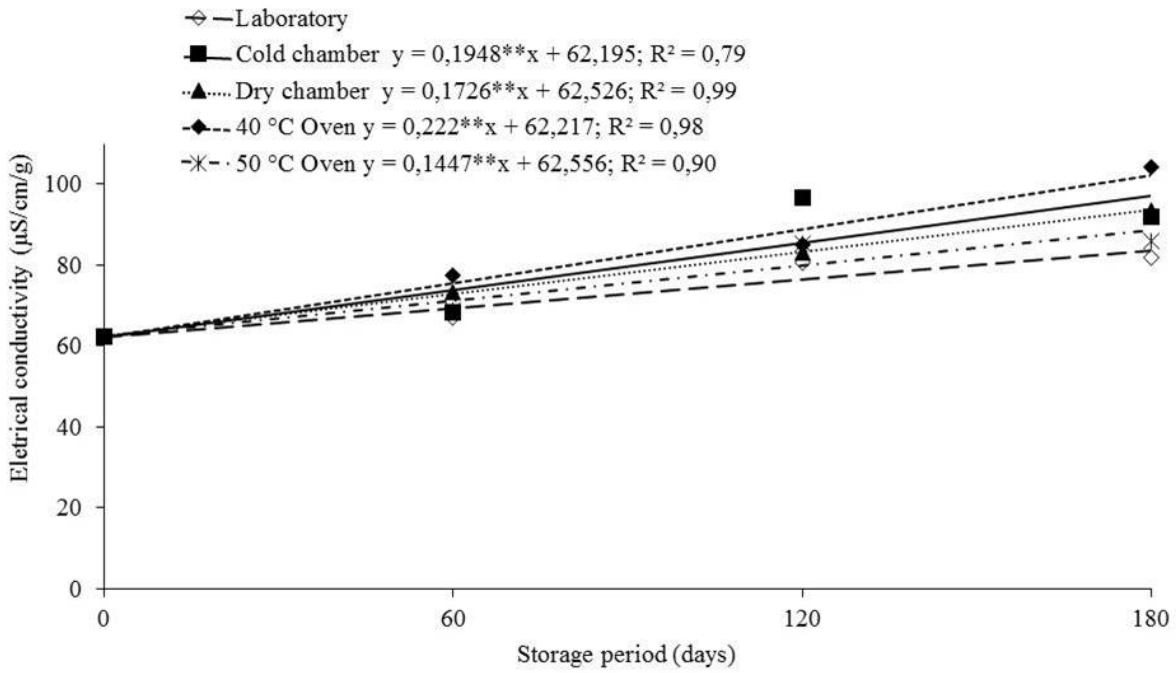


**FIGURE 5**- Electrical conductivity (mS/cm/g) of Spondias tuberosa seeds stored in different environments.
** Significant at 1% probability.

## *Conclusion*

The conditioning of umbu seeds in laboratory or in warm oven (40 ºC) was efficient to promote and standardize germination; under these conditions, after six months of storage, germination increased from 31% to 84 and 74%, respectively.

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