Scientia Agraria Paranaensis

Scientia Agraria Paranaensis – Sci. Agrar. Parana.
ISSN: 1983-1471 – Online

Jatropha curcas SEED TOLERANCE TO DESICCATION AND STORAGE AT LOW TEMPERATURES

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SAP 22682 Received: 25/06/2019 Acepted: 03/09/2019
Sci. Agrar. Parana., Marechal Cândido Rondon, v. 18, n. 4, oct./dec., p. 396-400, 2019

ABSTRACT - Jatropha seeds were classified according to tolerance to desiccation and storage, as a way to subsidize future propagation programs, as jatropha seed shows to be a promising oilseed for biodiesel production. The objective of this work was to study the physiological classification regarding the tolerance of physic nut seeds to desiccation and storage at low temperatures and to verify if their behavior was closer to that of seeds classified as orthodox, recalcitrant or intermediate. The seeds were harvested in the morning and sent to the Laboratory, being processed and submitted to drying, storage and emergency assessment. For seed quality analysis, the germination test and the emergence speed index were used, using a completely randomized design, with four treatments (no drying, 12% humidity, 5% humidity and 12% humidity a -20ºC for 90 days) and five repetitions. After physiological classification, it was found that seeds of physic nut are orthodox, thus can be stored at low temperature (-20ºC) and with low water content (<5%), without compromising their viability.
Keywords: *Jatropha curcas* L., oilseed, biodiesel, drying, orthodox.

TOLERÂNCIA DA SEMENTE DE PINHÃO MANSO À DESSECAÇÃO E AO ARMAZENAMENTO EM BAIXAS TEMPERATURAS

RESUMO - Sementes de pinhão manso foram classificadas de acordo com a tolerância à dessecação e ao armazenamento, como forma de subsidiar futuros programas de propagação, pois a semente de pinhão manso mostra ser uma oleaginosa promissora para produção de biodiesel. Objetivou-se com este trabalho estudar a classificação fisiológica quanto à tolerância de sementes de pinhão manso à dessecação e armazenamento em baixas temperaturas e verificar se seu comportamento foi mais próximo ao de sementes classificadas como ortodoxas, recalcitrantes ou intermediárias. As sementes foram colhidas pela manhã e encaminhadas ao Laboratório, sendo beneficiadas e submetidas à secagem, armazenamento e à avaliação da emergência. Para análise da qualidade das sementes foram utilizados o teste de germinação e o Índice de velocidade de emergência, sendo utilizado o delineamento inteiramente casualizado, com quatro tratamentos (sem secagem, 12% de umidade, 5% de umidade e 12% de umidade a -20ºC por 90 dias) e cinco repetições. Após a classificação fisiológica, verificou-se que as sementes de pinhão manso são ortodoxas, assim, podem ser armazenadas à baixa temperatura (-20ºC) e com baixo teor de água (<5%), sem comprometer sua viabilidade.
Palavras-chave: *Jatropha curcas* L., oleaginosas, biodiesel, secagem, ortodoxa.

INTRODUCTION

Jatropha is a perennial, monoecious plant, that belongs to the family Euphorbiaceae. It resists well to drought, presents faster growth in hot climate regions and is popularly known in Brazil as *pinhão manso* and *pinheiro de purga* (purging nut), among other names (CAB INTERNATIONAL, 2017). In Brazil, jatropha has been cultivated by producers from the Northeast, Midwest and Southeast regions (NUNES, 2009). The center of origin of the species is undetermined but it is believed that it may have been disseminated by Portuguese navigators from Central America and Mexico through the Island of Cape Verde and the Island of Guinea Bissau to other countries in Africa and Asia (BRITTAINE and LUTALADIO, 2010, VIRGENS et al., 2017).

It is easy to cultivate and its oil has little significant variation of acidity, besides having better stability to oxidation than soybean and more adequate viscosity when compared to castor oil (NUNES et al., 2008). The jatropha flowers are small, yellow-greenish, and the fruit is a capsule with three dark seeds. The seed is oval, endospermic with smooth, lightly striated coat, and

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caruncle attached to the ventral part. The raphe is longitudinally marked and not very evident. The integument is hard and brittle. Under the seed coat there is a white film covering the nut; the albumens are abundant, white, oily, and contain the embryo with two flattened cotyledons (NUNES et al., 2009). Jatropha seeds are among the main oleaginous seeds considered to be promising in the biodiesel production program. According to Câmara and Heiffig (2006) their seeds have 52% of oil.

However, according to Pereira et al. (2013), seeds of oleaginous plants are difficult to preserve during storage since they are very prone to deteriorate. Generally, the intensity and speed of the deterioration process are linked to the chemical composition of the seeds. The longevity of the seed during storage depends, besides the chemical composition, on factors such as water content, environmental conditions, packaging, microorganism's activity, among others (MARCOS FILHO, 2015).

Thus, storage conditions are decisive to ensure the physiological quality of the seeds and, although the quality cannot be improved, good storage conditions can help to maintain seed quality for longer, slowing down the process of deterioration (BEWLEY et al., 2013; NEVES et al., 2014). This is due to the fact that uncontrolled conditions of temperature and the relative humidity during the storage period result in a rapid reduction of germination (CARDOSO et al., 2012). Thus, there is a great importance in studying the different types of conditions and time of seed storage.

The classification of seeds according to their behavior during desiccation and storage is currently divided into three categories: orthodox seeds, which tolerate desiccation to low water contents (2-5%) and can be stored at low temperatures (-0°C). Conditions that maximize the storage time; intermediate seeds, which do not tolerate desiccation to low water contents (10-12%), but can be stored at low temperatures (generally above 0°C); and recalcitrant seeds, which do not tolerate desiccation to low water contents (<12%), nor storage at low temperatures (BRASIL, 2009).

Currently, there are no concise works on the classification of jatropha seeds in terms of tolerance to dehydration and storage under low temperatures. However, for effective seed conservation, it is important to know previously its physiological behavior during drying and storage, since not all seeds tolerate desiccation and may require special storage conditions.

In this context, the objective of this work was to study the physiological classification as for to the tolerance of jatropha seed to desiccation and storage at low temperatures, and to verify if its behavior was closer to that of seeds classified as orthodox, recalcitrant or intermediate.

MATERIAL AND METHODS

It was used jatropha (*Jatropha curcas* L.) seeds of mature fruits from the experimental field of PESAGRO (Agricultural Research Company of the State of Rio de Janeiro) in Campos dos Goytacazes County, Rio de Janeiro, Brazil. They were harvested in the morning and sent to the Agricultural Engineering Laboratory (LEAG) of the Center for Agricultural Sciences and Technologies (CCTA), from the State University of North Fluminense Darcy Ribeiro (UENF), where they were benefited and submitted to drying, storage and emergence evaluation. The initial water content of the seeds was determined before and after the drying tests, according to the recommendations proposed by the Rules for Seed Analysis (BRASIL, 2009).

In order to reduce the initial water content, the seeds were dried in a fixed bed dryer, capable of supplying the drying air under controlled conditions of flow and temperature in the drying room. The dryer was built with galvanized double-sheet walls, filled with glass wool throughout its air heating section. The drying chamber was composed of three circular trays with internal diameter of 23 cm and 5 cm high, with perforated stainless-steel sheet bottom. The dryer has a 1.0 cv centrifugal fan, a set of electric heating elements for air heating, a frequency inverter for changing and controlling the fan motor rotation, a temperature controller with an N 480 microprocessor, a plenum chamber and a set of glass beads to reduce turbulence and to uniform the air velocity before it enters the drying chamber.

The drying tests were performed using only one level of drying air velocity (1.0 m s^{-1}) and one temperature level (38°C). The reduction in the water content of the samples was monitored by gravimetry, by weighing the sample-tray set at regular intervals of 5 min. in the first 30 min., of 10 min. until 120 min., of 15 min. until 180 min. and of 30 min. until 180 min., with a digital scale of 0.01 g precision, until the material reached the desired final water content.

When the desired water content (10-12% wet basis - w.b.) was reached, the seeds in the trays were mixed and the batch was divided into two parts. The first part was used to perform the tests to determine the water content and to evaluate the physiological quality of the seeds, by means of an emergence test performed right after drying (ASHRAE, 1992; CARLESSO et al., 2009).

The second part of the batch remained in the dryer until reaching a water content of approximately 5% w.b. When this water content was reached, the physiological quality evaluation test was performed. The rest of the seeds were duly stored in a glass bottle and then in a freezer at -20ºC, where it remained stored for 90 days. After this storage time, new determinations of water content and emergence tests were performed to determine the physiological quality of the seeds.

The germination test and the emergence speed index were used to analyze the seed quality, by using a completely randomized design with four treatments (no drying, 12% water content, 5% water content and 12% water content at -20°C for 90 days) and 10 repetitions. The seeds (50 per repetition) were seeded in perforated plastic trays, with dimensions of 39 x 25 x 7 cm in length, width and depth, respectively, with washed sand as substrate. They were placed at approximately 3 cm depth and kept in a germination room at 35°C, in a photoperiod of 8-16 h light/dark for 10 days.

After four days of emergence test, the first count was realized, constituting the percentage of normal seedlings (MARTINS et al., 2008, BRASIL, 2009). It was considered germinated the seedlings that presented the hypocotyl above the soil. The germination speed index (GSI) and the percentage of germination were calculated according to the methodology recommended by Maguire (1962). The results were submitted to variance analysis and mean test (Tukey at 5%) for comparison of means. All values were transformed, for variance analysis, in arcsin√x/100.

RESULTS AND DISCUSSION

Figure 1 displays a drying curve for jatropha seeds, in which is shown their RU% behavior response in a period of approximately 8 h. Seeds had an initial water content of 38.9% w.b. When the drying time reached 3.5 h, the seeds had a water content of 11.4% w.b.; after four more hours of drying, the seeds had a water content of 4.9% w.b. Sinniah et al. (2009) analyzed the impact of desiccation on the germination and storage of jatropha seeds under three storage conditions (freezer, refrigerator and at room temperature) and found that seeds tolerated desiccation at low RH% (10), thus not being sensitive to cold. This indicates that the seeds can be classified as orthodox. However, if seeds water content exceeds 10%, they freeze. These authors did not observe a significant decrease in seed germination for water content below 10%, regardless the conditions or time of storage, and suggested that additional studies should be realized, in order to understand the long-term storage of *Jatropha curcas* seeds.

When studying the storage of fruits and seeds of Iaracatiá (*Vasconcellea quercifolia*) in different temperatures (10 or 25ºC) and durations (30, 60, 90, 120 and 150 days), Pissatto (2015) observed that seeds obtained from fresh fruits and soaked in water for 24 h and seeds obtained from fruits stored in refrigerator at 10±1ºC and treated with gibberellic acid (GA_3) showed the highest germination rates. The storage of the seeds in this refrigerator for one week did not favor germination, and the storage for different periods of time and temperatures caused reduction in their germinative potential.

In this work, at the end of the drying process, the seeds were stored at 12% water content for 90 days at -20°C. They kept their germination capacity, with 92% emergence (Table 1), but differed significantly from the other treatments.

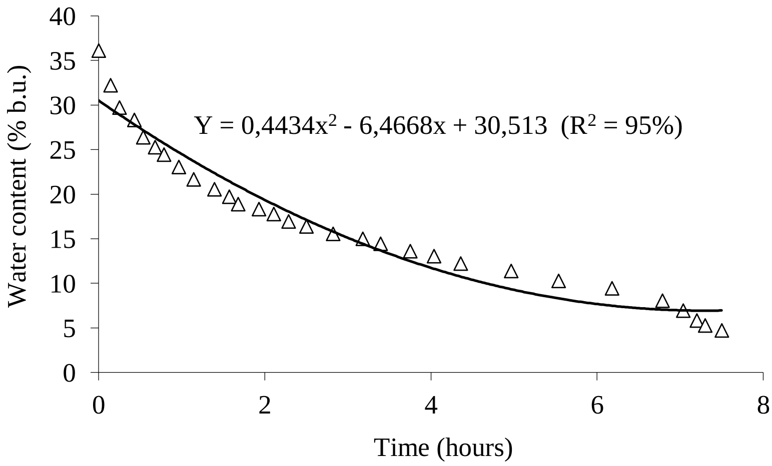

FIGURE 1 - Drying curve of whole physic nut seeds (*Jatropha curcas* L.) dried in a fixed bed canvas dryer.

TABLE 1 - Germination percentage of physic nut seeds (*Jatropha curcas* L.) before and after drying and after storage at -20ºC, for 90 days.

Seeds	Undried	12%	5%	-20ºC (90 days)
Germination (%)	100 a*	100 a	100 a	92 b
GSI**	3.23 a	3.12 a	3.15 a	3.23 a

*Means followed by the same lowercase letter in the line do not differ among themselves according to Tukey test (5%). **Germination speed index (GSI).

No significant difference was observed (P>0.05) in the GSI and seeds germination evaluation. When analyzing the storage of *Mastique* seeds (*Pistacia lentiscus*) in different packaging and environments, Guedes et al. (2012) noticed a sharp reduction in the GSI of seeds stored in the laboratory, where the climate conditions were not controlled. Similar results were noticed by Joker and Jepsen (2003), in which seeds of jatropha were dried to low water contents (5-7%) and stored at low temperature. They kept a high viability for at least one year, what indicates that seeds of this oleaginous plant can be stored dehydrated to a low water content for a long time, as a possible alternative to preserve their viability.

Oleaginous seeds have a high oil content; therefore, they are not supposed to be preserved for a long time as most orthodox plants. An increase in temperature, as an consequence of the respiratory process, is enough to break down lipids and to increase the deterioration rate related to ezymatic hydrolysis, peroxidation and oxidation (BRACCINI et al., 2001). For that reason, Marcos-Filho (2015) recommends that the oleaginous seeds should be stored at an inferior water content than the one indicated for starchy seeds (11-12%).

Studies indicate that the storage time of orthodox seeds is influenced by their water content and by storage temperature, and there is a rapid transition from the phase of intolerance to tolerance to desiccation, whose viability period can be increased by reducing the water content to 2-5%. According to Marcos Filho (2015), an environment with low relative humidity and lower temperature has proven Zaidman et al (2010) to be more adequate to a better conservation of orthodox seeds, since these conditions allow the maintenance of a low level of chemical reactions and the preservation of germinative capacity and seeds vigor.

Differently from the results obtained by Zaidman et al. (2010), in this study, the mass of seeds had no influence on their germinative power, since 100% of germination was obtained in most tests performed, even with batches little homogenous in mass. Chaves et al. (2012) studied the physiological quality of jatropha seeds during storage in different conditions and noticed that the reduction in the percentage of germination was more evident in seeds stored in natural environment, with a reduction of 23.3 percent, while those kept in refrigerated and climatized rooms decreased 6.0 and 4.0 percentual points, respectively.

Silva et al. (2016) examined the physiological and phytosanitary behavior of *E. dysenterica* seeds after drying and storage in cold and dry rooms and concluded that storage in a cold and dry chamber at 7±1ºC, 45±7% of relative humidity (RH) for up to 120 days maintains the physiological and phytosanitary quality of *Cagaita (Eugenia dysenterica)* seeds with about 35% water content. Similar results were observed by Balbinot et al. (2006) and Aroucha et al. (2007) for papaya (*Carica papaya*), what evidences the need to store seeds at low temperature to improve seed vigor and increase germination potential.

In storage studies with jatropha seeds with water content in the range of 4 to 5% w.b., Gusman and Aquino (2009) found only a slight decrease in germination when the seeds were kept in impermeable packages. However, Figueiredo et al. (2006) analyzed the storage of castor beans, oleaginous plant that behaves similarly to jatropha, kept in laboratory conditions for six months in different types of packaging. In this case, the authors found that there was a decrease in germination and vigor of the seeds stored in both permeable and semipermeable packages.

Dias et al. (2016) evaluated different environment and packaging for conservation of jatropha seeds along 12 months of storage. They realized that there was a decrease in the physiologic quality of the seeds kept in laboratory, regardless the packaging used, and that the use of plastic bags in cold rooms (10±2°C; 55±5% RH), in other words at low RH%, was the most suitable condition for seeds storage. According to Marcos Filho (2015), the combined action of high humidity and temperature speeds up the deterioration process of orthodox seeds as castor beans and decreases their longevity. Zonta et al. (2011) also noticed a decrease in jatropha seed germination during storage when they were submitted to 33°C and final humidity content of 8,5% w.b.

However, Guzman and Aquino (2009) consider that the storage temperature is not the most important factor to maintain the physiologic quality of jatropha seeds, but the water content is what most influences the reduction of germination and vigor of the seeds. According to these authors, seeds with water content between 4 and 5% stored in impermeable packages may be stored for one year with little reduction in the germination percentage. They also found that a water content of 9.5% was harmful for seed quality.

Pinto Junior et al. (2012) observed that seeds stored in glass packaging and under refrigeration kept their physiological quality and could be stored for a period of 180 days. For the germination test, no significant effect was observed for the different environments and storage times (90 days, 87% and 180 days, 89%) and packaging (90 days, 87% and 180 days, 90%).

CONCLUSION

Jatropha (*Jatropha curcas* L.) seeds tolerate desiccation and storage at -20ºC being classified as orthodox.

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