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PHYSIOLOGICAL QUALITY OF *Inga laurina* (*Sw.*) Willd. SEEDS UNDER DIFFERENT STORAGE METHODS

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ABSTRACT

The species *Inga laurina* (Sw.) Willd. belongs to the Fabaceae, popularly known as ingá, is widely distributed in Brazil, has wood of good quality, fruit with sweetened pulp, and can be used in the reforestation of riparian forests and recovery of degraded areas. Considering the importance of this species, the objective of this study was to evaluate the physiological quality of *Iinga laurina* seeds stored under different conditions. The experiment was carried out at Laboratório de Análise de Sementes, with the treatments distributed in a factorial scheme 3 x 6 (seeds inside the pods, seeds outside the pods with sarcotesta and seeds outside the pods without sarcotesta) and storage periods (0, 7, 14, 21, 28 and 35 days). Water content, percentage, first counting of germination, germination speed index, root and shoot length, dry matter of roots and shoot were evaluated. The storage of *I. laurina* seeds inside the pods provides the maintenance of the viability for a longer period of time, whereas seeds stored outside the pods with and without sarcotesta have their germinative potential and vigor reduced in the first days of storage.

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INTRODUCTION

The genus Inga Mill. belongs to the Fabaceae family and includes about 300 woody species (Pennington, 1997). *Inga laurina* (Sw.) Willd., with center of origin is the South of Central America (PENNINGTON and FERNANDES, 1998), is exclusively Neotropical, what means that it can be found in the southern part of the North America, Central and South America (FERRÃO, 2001). This tree is popularly known as ingá mirim, ingá branco or ingá do brejo (LORENZI, 2008), with occurrence in many states of Brazil such as Pará, Amazonas, Acre, Maranhão, Ceará, Paraíba, Pernambuco, Bahia, Goiás, Mato Grosso do Sul, Minas Gerais, Espírito Santo, São Paulo and Rio de Janeiro (SILVA *et al*., 1989). Similarly to the other species of the genus Inga, the fruits are appreciated by birds, fish and man, due to its fleshiness, high palatability and sugar content (CUNHA *et al*., 2011). In addition, the tree has environmental and ornamental importance and has potential use for reforestation of riparian forests and recovery of degraded areas (BILIA *et al*., 2003) and can also be planted in urban environments since it has dense crowns and is perennial through out the year (BRANDÃO *et al*., 2002).

In some regions, *I. laurina* is used exclusively for shade of cacao in Venezuela and tea plantations in the eastern slopes of the Andes in Peru (LEON, 1998). The seeds, of Iinga laurina have polyembryony (PENNINGTON and FERNANDES, 1998) and high percentage of germination (70-80%) which is obtained between ten to fifteen days after sowing. However, they have short longevity and should be sown soon after the extraction of the pulp that surrounds the seeds (LORENZI, 2008). They are characterized by not suffering desiccation in the mother plant during its maturation, since they are dispersed with high water content, and if reduced to a critical level causes a rapid loss of its viability (ROBERTS, 1973). These seeds are among those of greater intolerance to desiccation, due to their recalcitrance and, because of the absence of adequate storage techniques, they can not be stored for long periods (BARBEDO and MARCOS FILHO, 1998). In recalcitrant seeds the water content reaches levels of 50-70% when they reach physiological maturity (CARVALHO and NAKAGAWA, 2012), and normally the metabolic activity is high throughout its formation and after harvest, because they are maintained with significant levels of water content (BARBEDO and MARCOS FILHO, 1998; CASTRO *et al*., 2004; MARCOS FILHO, 2005). In the development of the seeds there is no dehydration phase (FARRANT *et al*., 1988) but a decline in their water content not significant when compared to the drying phase of orthodox seeds (KIKUTI, 2000). Recalcitrant seeds are more sensitive to storage conditions (BARBEDO and MARCOS FILHO, 1998), this is why it is difficult to maintain their vigor and viability, since factors such as temperature and relative humidity should be considered during the storage, aiming to prolong longevity and viability (OLADRIAN and AGUNBIADE, 2000). However, the storage of recalcitrant seeds is a challenge for conservation, since it is only possible for short periods of time, usually weeks or a few months. The objective of the storage is to preserve and maintain the physiological, physical and sanitary quality of the seeds for later sowing and obtain healthy plants after germination, aiming their use for the formation of plantations for the purpose of reforestation, commercialization, gene banks of forests and seedling banks (FLORIANO, 2004). The objective of this study was to evaluate the physiological quality of seeds of *Inga laurina* (Sw.) Willd. stored under different conditions.

MATERIAL AND METHODS

The experiment was carried out at the no Laboratório de Análise de Sementes (LAS), of the Departamento de Fitotecnia e Ciências Ambientais. Ripe fruits of *Inga laurina*, characterized by the yellowish coloration of the pods, were manually harvested in a mother tree. After being harvested they were taken to the laboratory for selection of pods without any apparent damage and, then obtained the seeds that were stored separately, with the presence and absence of sarcotesta. The seeds kept inside the pods (IP), outside the pods with sarcotesta (OPWS) and outside the pods with nosarcotesta (OPWNS) were conditioned in transparent polyethylene 0.3 mm thick plastic bags and stored in a refrigerator with temperature oscillating between 3.9 to 5.8 °C and relative humidity of 30 to 40% for different periods: 0, 7, 14, 21, 28 and 35 days. At the end of each storage period, the seeds were submitted to the following tests and determinations:

Water Content - The water content of the seeds was determined by the greenhouse method at 105 ± 3 °C for 24 hours (BRASIL, 2009), with four replications of ten seeds, and the results were expressed as percentage.

Germination test - Four replicates of 25 seeds treated with the fungicide Captan® at the dose of 240 g 100 kg^{1} were distributed on two "germitest" paper sheets, covered with a third layer and organized in the form of rolls, which were distributed in Biological Oxygen Demand (BOD) with photoperiod of 8 hours and regulated at a constant temperature of 25 °C. The paper was previously moistened with an amount of distilled water equivalent to 2.5 times its dry weight. The evaluations were performed daily after the installation of the experiment. The number of germinated seeds were counted from the seventh to the twelfth day, when the germination was stabilized. The germinated seeds were considered those that had emitted the primary root and the epicotyl (normal seedlings), with the data expressed as percentage. The seeds that emitted only root were considered abnormal and those that did not germinate, were considered as not germinated.

First Counting of Germination - Performed concurrently with the germination test, by counting the number of seeds germinated on the seventh day after sowing, and the data were expressed as percentage.

Germination Speed Index - This evaluation was performed concurrently with the germination test, by daily counts of the number of germinated seeds, from the seventh to the twelfth day after sowing, calculated according to the formula proposed by Maguire (1962).

Root and shoot length - After the final counting of the germination test, roots of normal seedlings were measured, both with the aid of ruler graduated in centimeters, the results were expressed in centimeters.
Dry matter of roots and shoots - After measuring, the roots and shoots of the seedlings were placed separately in paper bags, taken to the regulated lab stove at 80 °C/24h (NAKAGAWA, 1999). The samples were weighed in an analytical scale with an accuracy of 0.001 g, and the results expressed in milligrams.

Experimental Design and Statistical Analysis - The experiment was carried out in a completely randomized design, with treatments distributed in a factorial scheme 3 x 6 (storage ways x storage period). The data obtained were submitted to generalized linear models, applying the beta distribution for percentage of germination, first germination counting, non-germinated seeds and abnormal seedlings, and gamma distribution for germination speed index, length and seedlings dry matter. The statistical program SAS 9.3 (SAS INSTITUTE, 2011) was used.

RESULTS AND DISCUSSION

In the period of zero days of storage, the water content of *Inga laurina* seeds was around 52% and at seven days it increased its content from 56.4, 57.4 and 58.5% in the seeds stored outside the pods with sarcotesta (OPWS), outside the pods without sarcotesta (OPWNS) and inside the pods (IP), respectively (Figure 1). Among the evaluated ways of storage, the lowest oscillations in the water content of the seeds were observed in those kept outside the pods without sarcotesta (OPWNS), which minimum and maximum values were recorded at 0 and at 21 days of storage, respectively. For the seeds stored outside the pods with sarcotesta (OPWS), the water content increased until 21 days reaching 59%, with decreases in the subsequent periods, reaching 56.5% at the end of the storage period. In the seeds kept inside the pods (IP), the water content presented higher values than those obtained for the seeds stored outside the pods without sarcotesta (OPWNS) and outside the pods with sarcotesta (OPWS) in all storage periods. From the seventh to the fourteenth day, there was a slight oscillation between the values registered with a subsequent increase up to 28 days, reaching 61.5% and at the end of storage period (35 days) the value decreased to 60.3%.

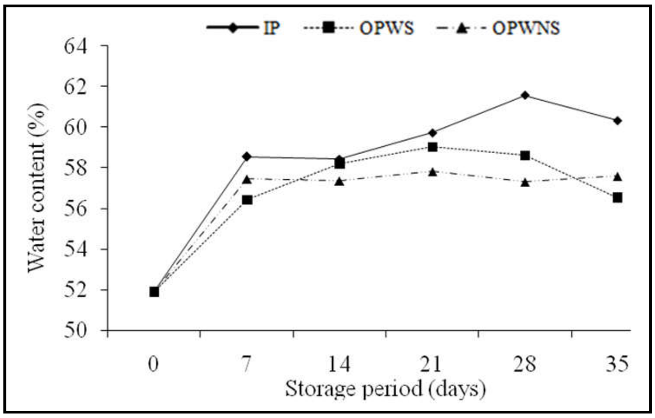
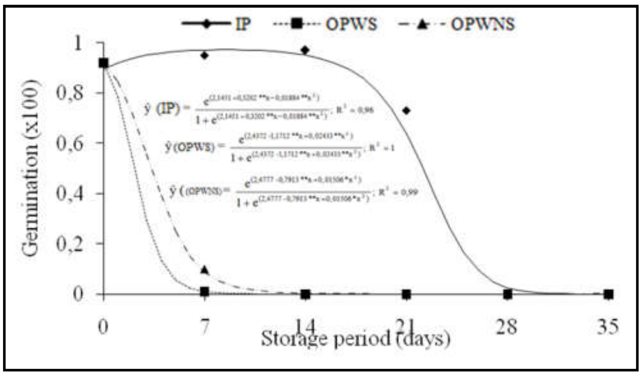


Figure 1. Water content of *Inga laurina* seeds stored in a refrigerator environment inside the pods (IP), outside the pods with sarcotesta (OPWS) and outside the pods without sarcotesta (OPWNS)

The seeds of *Inga laurina* were stored in impermeable plastic containers, which do not promote a moisture exchange of the seeds with the external environment. This way, these packages favored an intense respiration of the seeds, releasing water, consequently increasing the moisture content of the seeds (MACEDO *et al.,* 1998). Based on these observations, the periods of 21 and 28 days of storage were enough to cause water accumulation and loss of viability of the seeds stored outside the pods (OPWS and OPWNS) and inside the pods (IP), respectively. Seeds stored inside the pods (IP), where the water content remained higher, presented a greater viability and vigor, probably due to its natural storage way, inside thepods. Oscillations in water content were also observed in seeds of *Piper marginatum* Jacq. and *Piper tuberculatum* Jacq. when stored in a cold chamber in impermeable packages, because the hygroscopic balance of the seed varies according to the relative humidity of the environment in which these seeds are stored, in a way that, if the vapor pressure of water in the seed is less than what is found in the air, humid absorption occurs, on the other hand, if the vapor pressure of water in the seed is higher than the air, the seed loses water to the air (BATISTA, 2015). The impermeable packages, does not allow moisture exchange, retaining the initial moisture of the seeds during the entire storage period, favoring their deterioration if it is high. This internal humidity causes an increase in respiratory rate, microorganisms activity and even their germination. The use of low temperatures, which could inhibit these problems, is detrimental because these seeds are damaged by temperatures close to or below zero, where ice crystals are formed, leading to the rupture of important membranes present in the seed (BATISTA, 2015).

The highest percentages of germination (Figure 2) were obtained at 14 days for the *Inga aurina* seeds stored inside the pods (IP), when they had 58% of water content. At 21 days, with water content of 60%, the germination of the seeds began to decrease, reaching 0% at 28 days. In the seeds stored outside the pods with sarcotesta (OPWS) and outside the pods without sarcotesta (OPWNS) there was anaccentuated decrease in germination at 7 days of storage, with a rapid loss of viability for the seeds with sarcotesta. A better physiological quality for seeds of *Inga subnuda* Salzm. ex Benth. and *Inga cylindrica* (Vel.) Mart. was obtained when the seeds were stored outside the pods in polyethylene bags at 12 °C, with initial water content around 49.04 and 52.42%, respectively, probably because the lower humidity of the environment that allowed its aeration, when compared to those stored inside the pods, which had reduced germination potential and vigor (MATA, 2009). The results showed that the physiological quality is directly related to the water content of the seeds, but mainly to the manner that the seeds are stored. At 14 days of storage, the germination of the seeds kept inside the pods was higher when compared to the other ways of storage. After 14 days of storage, there was an accentuated decrease in germination, resulting in the total loss of viability at 35 days. In partially dehydrated *Inga uruguensis* Hook. et Arn. seeds with up to 50% water, stored in polyethylene bags inside the pods in a cold storage chamber at 10 ºC for 60 days, the physiological quality of the seeds was maintained (BILIA, 1998).



**; * Significant at 1 and 5% of probability by the chi-square test

Figure 2. Germination of *Inga laurina* seeds stored in a refrigerator environment inside the pods (IP), outside the pods with sarcotesta (OPWS) and outside the pods without sarcotesta (OPWNS)

The occurrence of fungi during the experiment was observed in the seeds stored outside the pods with and without sarcotesta but was not measured. In the seeds outside the pods with sarcotesta, the development of microorganisms was probably favored by the mucilaginous pulp, which contains a large quantity of sugars and also due to the accumulation of water in the plastic bags. This fact may have negatively influenced the germination of the seeds, together with the high water content of the seeds. Changes in the color of the seeds, followed by a very strong odor were also observed, which characterize signs of deterioration, which according to Villela and Peres (2004) involve biochemical, physical and physiological changes, being determined by genetic, biotic and abiotic factors, harvesting ways and storage. For the first counting of germination of *Inga laurina* (Figure 3), the highest values were obtained from seeds stored inside the pods (IP), but with decreases over the storage period. For the seeds that were stored outside the pods with sarcotesta (OPWS) and outside the pods without sarcotesta (OPWNS), the lowest values were observed, with an accentuated decrease at 7 days of storage.

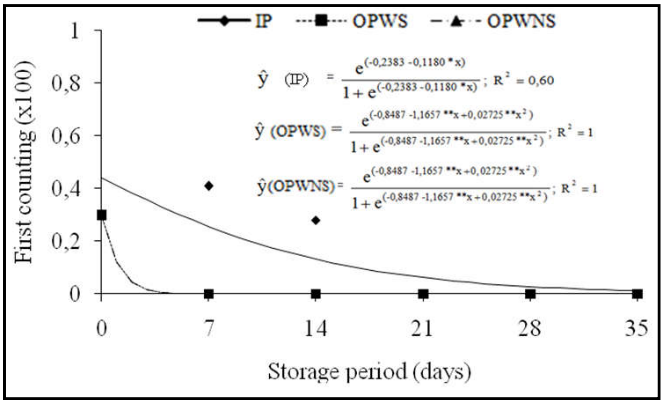


Figure 3. First counting of germination of *Inga laurina* seeds stored in a refrigerator environment inside the pods (IP), outside the pods with sarcotesta (OPWS) and outside the pods without sarcotesta (OPWNS) **; * Significant at 1 and 5% of probability by the chi-square test

The low values for the seeds stored outside the pods with sarcotesta (OPWS) may be related to the presence of inhibitory substances of germination, which according to Carvalho and Nakagawa (2012), are mostly composed of phenolic compounds. For the seeds stored outside the pods without sarcotesta (OPWNS) the values were similar to the seeds stored outside the pods with sarcotesta (OPWS) (Figure 3). The oscillations in seed moisture may have started the process of deterioration and favored the colonization of fungi, as observed in *Piper marginatum* and *Piper tuberculatum* seeds stored in glass containers (BATISTA, 2015). Throughout the storage period, there was an accentuated increase in the number of non-germinated seeds stored outside the pods with and without sarcotesta (OPWS and OPWNS). For these storage conditions, at 7 days of storage, the percentage of non-germinated seeds was greater than 50% and was intensified until the end of the storage period. For seeds stored inside the pods (IP) the mean values increased over time, however the regression curve estimated a more expressive increase after 14 days of storage (Figure 4).

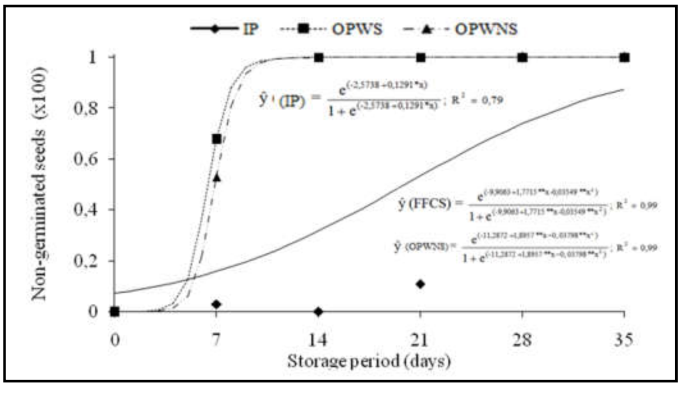


Figure 4. Percentage of non-germinated seeds of *Inga laurina* stored in a refrigerator environment inside the pods (IP), outside the pods with sarcotesta (OPWS) and outside the pods without sarcotesta (OPWNS) **; * Significant at 1 and 5% of probability by the chi-square test

For the abnormal seedling percentage (Figure 5), no adjustment to the regression models was observed, with mean values for the three storage ways being expressed. The higher percentage of abnormal seedlings were found in seeds stored outside of the pods without sarcotesta (OPWNS) and outside the pods with sarcotesta (OPWS). The lowest percentage of abnormal seedlings was obtained when the seeds were stored inside the pods (IP). The seed deterioration reduces the germinative capacity, followed by the smaller development of seedlings, being therefore one of the possible causes of the greater number of abnormal seedlings and seeds not germinated of the seeds stored outside the pods with and without sarcotesta (CARVALHO and NAKAGAWA, 2012).

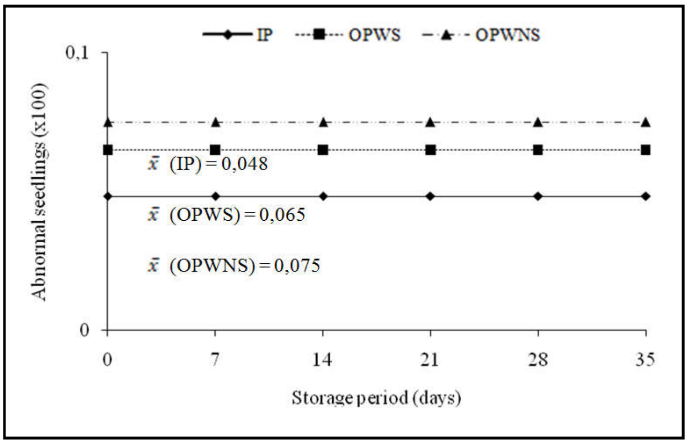


Figure 5. Percentage of abnormal seedlings of *Inga laurina*of seeds stored in a refrigerator environment inside the pods (IP), outside the pods with sarcotesta (OPWS) and outside the pods without sarcotesta (OPWNS) **; * Significant at 1 and 5% of probability by the chi-square test

The three storage ways resulted in differences in the germination speed index (GSI), specially on the seeds stored inside the pods (IP), which viability was better maintained (Figure 6). For this way of storage, the regression curve estimated an increase in the GSI, with a maximum value at 8 days of storage, with subsequent decrease along the storage period, while the seeds stored outside the pods with sarcotesta (OPWS) and outside the pods without sarcotesta (OPWNS) had a zero GSI at 7 days of storage.

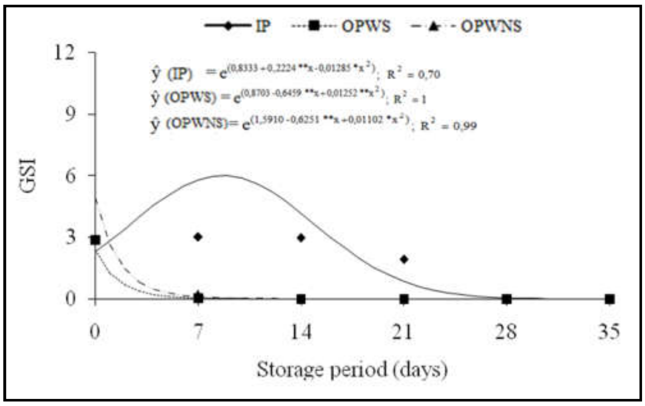


Figure 6. Germination speed index (GSI) of *Inga laurina* seeds stored in a refrigerator environment inside the pods (IP), outside the pods with sarcotesta (OPWS) and outside the pods without sarcotesta (OPWNS) **; * Significant at 1 and 5% of probability by the chi-square test

In seeds of *Inga edulis* Mart., removed from the pods and stored with and without the sarcotesta that involves them, Bacchi (1961) found total loss of viability within one or two weeks under natural conditions. Therefore, the seeds stored outside the pods are more sensitive to the deterioration process when compared to those maintained inside the pods. The decrease of seed vigor during storage is a result of the progressive deterioration process, associated to an intensification of the respiratory activity, which involves degeneration of the membranes (BATISTA, 2015). The higher maintenance of the physiological quality of *Inga laurina* seeds stored inside the pods was also verified by the evaluation of the root length of the seedlings (Figure 7). The regression curve estimated increasing values for the variable in question until 9 days of storage, with a subsequent decrease. The seeds stored outside the pods with sarcotesta (OPWS) and outside the pods without sarcotesta (OPWNS) had reduced vigor at 7 days, being estimated by the regression curve, at the period 0, a higher initial value for the root length from seeds stored outside the pods without sarcotesta.

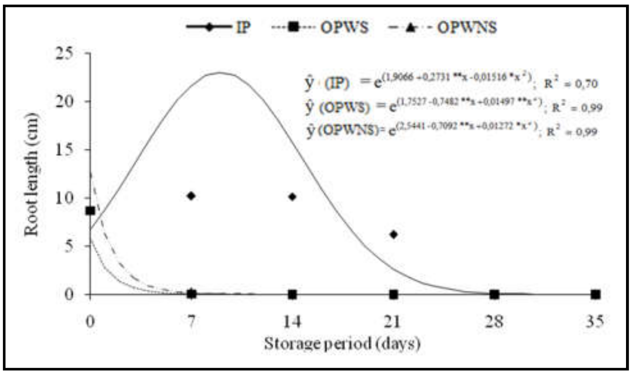


Figure 7. Root length of *Inga laurina* seedlings from seeds stored in a refrigerator environment inside the pods (IP), outside the pods with sarcotesta (OPWS) and outside the pods without sarcotesta (OPWNS) **; * Significant at 1 and 5% of probability by the chi-square test

Analyzing the physiological quality of *Inga laurina* seeds, considering the shoot length, it was observed that there was a decrease in the vigor of the seeds stored outside the podswhen compared to those stored inside the pods, which reflects a behavior similar to that observed for the others evaluated traits (Figure 8). When comparing to the results of root length it can be shown that there was a similarity between the periods of higher seed vigor for the different storage ways of *Inga laurina* seed. These results showed that the seeds whenstored inside the pods are more vigorous for a longer period, resulting in high values for the shoot length in the first storage periods. In a study made by MATA (2009) it was observed higher shoot and root lengths of seedlings originated of seeds stored outside the pods and low values for those originated of seeds stored inside the pods in *Inga. subnuda* and *Inga cylindrica*, stored in polyethylene bags at 12 °C.

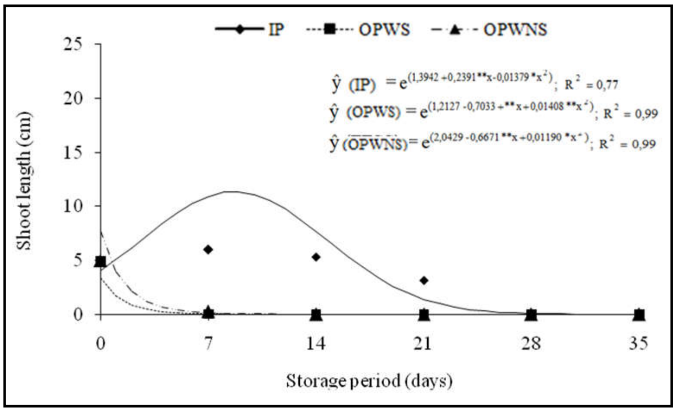


Figure 8. Shoot length of *Inga laurina* seedlings originated of seeds stored in a refrigerator environment inside the pods (IP), outside the pods with sarcotesta (OPWS) and outside the pods without sarcotesta (OPWNS) **; * Significant at 1 and 5% of probability by the chi-square test

For the seeds stored inside the pods, it was verified that the seedlings had a higher root dry matter content and the highest value at 8 days of storage, value estimated by the regression curve (Figure 9). Seedlings reduce the dry matter production as a longer storage period occurs and this tendency was more intense in seeds stored outside the pods with and without sarcotesta (OPWS and OPWNS), for these ways of storage the accentuated decrease recorded in the first period of evaluation (7 days), was probably due to the physiological changes of the seeds, such as the increase of water content or, due to the energy expenditure for its biological balance, reducing the reserve of energy used for the formation of the new seedling (BATISTA, 2015). For Carvalho and Nakagawa (2012), the translocation of dry matter of the reserve tissues from the vigorous seeds to the embryonic axis, during the germination process, gives rise to seedlings with greater mass, due to the greater accumulation of dry matter.

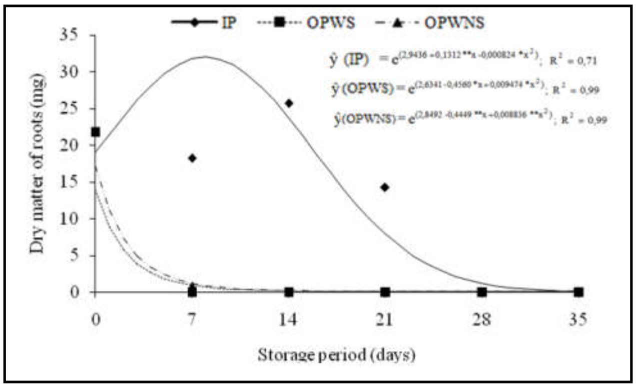


Figure 9. Dry matter of roots of *Inga laurina* seedlings originated of seeds stored in a refrigerator environment, inside the pods (IP), outside the pods with sarcotesta (OPWS) and outside the pods without sarcotesta (OPWNS) **; * Significant at 1 and 5% of probability by the chi-square test

The dry matter of *Inga laurina* seedlings of seeds stored inside the pods (Figure 10) shows that the values were higher than the seedlings of seeds stored outside the pods, whichreached higher values in the period of 0 days of storage, with a subsequent decrease. Thus, for seeds stored inside the pods (IP) it was observed a better quality when compared to the seeds stored outside the pods with sarcotesta and without sarcotesta (OPWS and OPWNS), obtaining the highest values for dry matter at 7 days of storage, differing from the dry matter of roots, which obtained the maximum vigor at 14 days of storage.

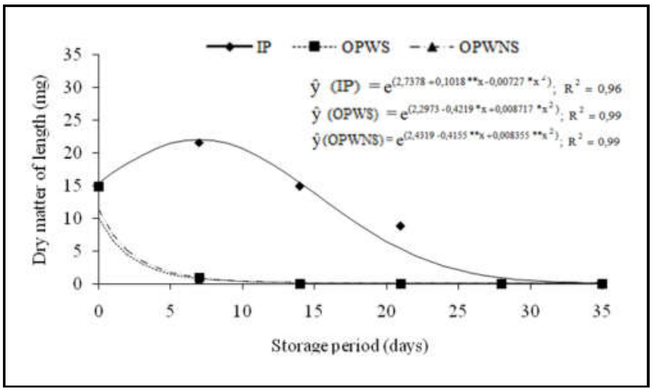


Figure 10. Dry matter of length of *Inga laurina* seedlings originated of seeds stored in a refrigerator environment, inside the pods (IP), outside the pods with sarcotesta (OPWS) and outside the pods without sarcotesta (OPWNS) **; * Significant at 1 and 5% of probability by the chi-square test

Studies focused on the germination of forest species enable the standardization of methods to analyze the viability of seeds, which contribute to the advances in research, as well as investments in the propagation of these species (CORREIA et al., 2020). In this context, the growth evaluation of the plants or the vigor classification using the test of germination, by the dry matter production, can be used to determine the vigor and characterize the seed lots, considering that the more vigorous seedlings potentially result in more vigorous plants (CARVALHO and NAKAGAWA, 2012).

CONCLUSIONS

The seeds of *Inga laurina* (Sw.) Willd. maintain their physiological quality when stored inside the pods, during 21 days of storage in a refrigerator; The storage of the seeds outside the pods, with and without sarcotesta, reduces their germinative performance and vigor.

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