Original Article

POTENCIAL FISIOLÓGICO DE SEMENTES DE JENIPAPO ARMAZENADAS EM DIFERENTES EMBALAGENS

PHYSIOLOGICAL POTENTIAL OF JENIPAPO SEEDS STORED IN DIFFERENT PACKAGES

Sebastião Alves ARRUDA[1]; Gisele Herbst VAZQUEZ[2]; Adílio de SÁ JÚNIOR[3]; Luiz Sérgio VANZELA[4]

1. Mestrando do curso em Ciências Ambientais da Universidade Brasil, Fernandópolis/SP, sebastiao.arruda@ufu.br; 2. Professora, Doutora, Universidade Brasil, Fernandópolis/SP, gisele-agro@uol.com.br; 3. Doutorando em Fitotecnia da Universidade Federal de Uberlândia, Uberlândia/MG, adilio.junior@yahoo.com.br; 4. Professor, Doutor, Universidade Brasil, Fernandópolis/SP, lsvanzela@yahoo.com.br

ABSTRACT: Seed storage through the reduction of its water content is an important strategy for the conservation of many plant species, but some, such as the jenipapo, are susceptible to desiccation, which may lead to loss of viability and even death, in case its water content is reduced to a critical level. The aim of this work was to evaluate the interference of different packaging conditions in the conservation of the physiological potential of jenipapo seeds stored for 105 days. Specifically, this research sought to analyze the feasibility of the use of vacuum conditions and the coating of the seed with biofilm. The evaluated treatments were: storage in paper bag, plastic bag, plastic with vacuum, plastic with seeds coated with biofilm and plastic with seeds with biofilm and vacuum condition for 15, 45, 75 and 105 days, besides the control. Seeds with biofilm were treated with cassava starch at 3% of the mass to volume ratio. The following tests were performed: water content, germination, germination speed, aerial part and root lengths, wet and dry mass of the aerial part and root. It was concluded that plastic packaging, vacuum condition and biofilm coating are not recommended for storage of jenipapo seeds. Storage in paper bags, when at 24 ºC and with 65% relative humidity for 46 days, is capable of conserving the seeds of jenipapo with germination of 60%.

KEYWORDS: Recalcitrant seeds. Germination. Native trees. *Genipa americana* L.

INTRODUCTION

There is no consensus on which region the jenipapo (*Genipa americana* L.) originated from. For some researchers, it originated in the northwestern region of South America and, for others, in Brazil, where the species is widely distributed throughout a coastal strip that begins in the state of Maranhão and ends in the state of São Paulo (PRUDENTE, 2002; LORENZI, 2008).

The word jenipapo comes from the tupi-guarani language and means "fruit that is used to paint" (MOURA; SOUSA; CONDE JUNIOR, 2016). The jenipapo tree has commercial value for both its wood and fruits, being used to make urban areas greener, in folk medicine, in dyeing, as a forage and apiculture plant, and in the recovery of degraded areas in riparian forest environments (LORENZI, 2008; SOUZA et al., 2013)

In general, in what concerns desiccation tolerance and storage behavior seeds can be classified into three categories: recalcitrant, orthodox and intermediate. The recalcitrant seeds present low germinative power when stored for long periods and cannot be dehydrated to a water content below 12-31%. The orthodox seeds can be dehydrated to rates between 2% and 5% and stored in low-temperature environments (ROBERTS, 1973), while the intermediates (ELLIS; HONG; ROBERTS, 1990) as the jenipapo, can stand reductions to around 7% and 10% of water content, having a loss of viability in values inferior to these (CARVALHO; NASCIMENTO, 2000; SALOMÃO, 2004; MAGISTRALLI et al., 2013).

Any stored seed undergoes deterioration, which may be faster or slower depending on the characteristics of the environment or the species itself. In the case of recalcitrant and intermediate seeds, the water content must be kept high by packing them in airtight containers, which often favors the growth of microorganisms and subsequent deterioration (CHIN; ROBERTS, 1980). In addition to that, in order to increase the viability time of these seeds, there are techniques such as biofilm coating, as it reduces the metabolic activity of the seeds by limiting the gas exchange with the external environment, keeping the water content high for a longer period (ALEGRETTI et al., 2015) and the conservation in vacuum packaging, which has the advantages of reducing the respiration rate of seeds in the absence of oxygen, variations of relative air humidity and seed colonization by fungi and insects (CAMARGO; CARVALHO, 2008).

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These techniques in the conservation of recalcitrant seeds have been proven efficient for various species. Some examples are the use of biofilm in *Poncirus trifoliata* (PIROLA et al., 2016) and vacuum-wrapping in carambola (*Averrhoa carambola* L.) (OLIVEIRA et al., 2009) and urucum (*Bixa orellana* L.) (CORLETT; BARROS; VILLELA, 2007), or both in jabuticaba (*Plinia trunciflora*) (HOSSEL et al., 2013) and the cherry of the rio grande (*Eugenia involucrata*) (ALEGRETTI et al., 2015; HOSSEL et al., 2016), yet little it is known about the behavior of jenipapo seeds, during and after storage.

Due to the great loss of biodiversity in Brazil, studies aimed at the conservation and storage of seeds of species susceptible to desiccation, such as recalcitrant and intermediate, are necessary. This work intended to evaluate the interference of different packaging conditions on the conservation of the physiological potential of jenipapo seeds stored for 105 days. Specifically, we sought to analyze the feasibility of the use of vacuum conditions and the coating of the seed with biofilm in the storage of these seeds.

MATERIAL AND METHODS

The experiment was conducted in the seed laboratory of the Agricultural Sciences Institute of the Agronomy program of the Federal University of Uberlândia/MG from December/2015 to June/2016. In December of 2015, 200 ripe fruits still in the plant – a jenipapo matrix in Uberlândia/MG – were selected. They presented brown coloration and the tegument with a wrinkled appearance. Next to this tree, the matrix, there were other specimens of jenipapo, guaranteeing the exchange of pollen and cross-fertilization.

For the removal of the mucilage, the fruits were cut in half and the seeds removed and immersed in a plastic container filled with water for eight days. The seeds were then rubbed onto a sieve along with sand, and then washed and laid to dry in the shade for seven days.

The experimental design was the randomized blocks distributed in a 5 x 5 factorial scheme, the first factor consisted of the packages and the second factor, the storage period, that is, 15, 45, 75 and 105 days, besides the control in the Season 0, with eight replicates of 25 seeds.

The packages tested were as follows: seeds stored in a paper bag (SP), plastic bag (P), plastic bag with biofilm-coated seeds (PB), vacuum plastic bag (PV), vacuum plastic bag with seeds coated with biofilm (PVB).

We used 11 x 17 cm brown paper bags and 0.16 micron clear plastic bags, measuring 16 x 25 cm, sealed with adhesive tape. To carry out the treatment of the seeds packed in a vacuum, the air was removed with the aid of a pump and rotary compressors, which were also sealed with adhesive tape in order to avoid the exchange of gases.

For biofilm treatment, a solution of hot water (80 °C) containing cassava starch dissolved at a concentration of 3% was prepared according to Henrique & Cereda (1999). The seeds were submerged in this solution under agitation for two minutes, and then were distributed in sheets of germitest paper for two hours to dry.

All packages were kept in ambient conditions for 105 days and the conditions of temperature and relative humidity were monitored daily with the use of a thermo-hygrometer.

The following evaluations were carried out:

- Ascertainment of water content: conducted in a drying oven at 103 ± 3 ºC for 24 h with two replications, according to the Rules for Seed Analysis - RAS (BRASIL, 2009) and evaluated at 0, 45, 75 and 105 days after storage.

- Germination test: conducted in a gerbox box containing substrate based on pine tree bark and maintained at 60% retention field capacity in a Mangelsdorf model germinator at 25 °C. The counting was carried out at 30 days after sowing (DAS) at 0, 15 and 45 days, at 40 DAS at 75 days and at 50 DAS at the time of 105 days, using normal and abnormal seedlings according to RAS (BRASIL, 2009).

- Germination Speed Index (IVG): determined during germination test conduction by daily counts of normal seedlings and calculated according to the formula proposed by Maguire (1962), the seedlings are considered normal after the expansion of the cotyledons.

- Aerial and root length: after determining the percentage of germination, the seedlings were separated from the substrate with the help of water jets and each normal seedling was evaluated for aerial and root length with the aid of a graduated ruler. The results being expressed as a percentage of the average (cm seedling^{-1}).

- Weight of the fresh and dry mass of the aerial part and root: after ascertaining the length, the cotyledons of the normal seedlings were removed and the aerial parts were then weighed, as well as the radicular, obtaining the weight of the fresh mass of each fraction. Then, the material was taken to oven drying at 65 °C until constant weight, when weighed again in a 0.001 g precision scale and the results expressed in mg seedling^{-1}.

The collected data were submitted to analysis of the variance using the F test, which when significant was analyzed by the Tukey test at 5% of probability and by regression. The percentage data were initially transformed into arcsen $\sqrt{x} / 100$ and the averages presented in the tables refer to the original values. The statistical program SISVAR version 4.2 was used to analyze the data as described by Ferreira (2011).

RESULTS AND DISCUSSION

There was a significant interaction of packing factors and storage period for all variables analyzed, i.e., water content, germination, IVG, shoot length and seedling root length, and wet and dry mass of shoot and shoot seedlings of the jenipapo.

At the time of installation of the experiment (season 0), the water content of the jenipapo seeds was 14.7% in the paper and plastic packaging treatments and 21.6% in those that used the biofilm coating, surpassing the others, a fact that was repeated at 45 days. At 75 days, the PB treatment exceeded the others, and at 105 days, the PVB presented the highest water content (15.1%), while the SP was lower, with only 5.0% of humidity, probably from the loss of water to the environment until it reaches the hygroscopic equilibrium with air (Table 1).

Table 1. Averages of the water content of jenipapo seeds as a function of the packaging factor unfolding in each storage period evaluated.

Packages	0	45	75	105
	days			
Paper bag (SP)	14.7b	15.5d	10.2e	5.0e
Plastic (P)	14.7b	19.7b	12.3d	6.2d
Plastic + Vacuum (PV)	14.7b	18.4c	14.3c	11.4b
Plastic + Biofilm (PB)	21.6a	22.2a	17.6a	8.5c
Plastic + Vacuum + Biofilm (PVB)	21.6a	22.3a	16.2b	15.1a
CV %	6.71			

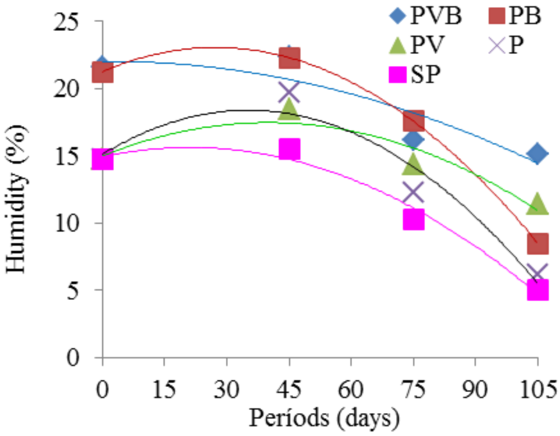
Averages followed by the same letter in the column at each time do not differ among themselves by the Tukey test at 0.05 probability. CV (coefficient of variation).

The average time for the seed of a particular species to reach the hygroscopic equilibrium depends on the temperature and the relative humidity of the air (BORGES et al., 2009), in this case, during the storage period the averages were 23.5 °C and 64.8%, respectively. Magistrali et al. (2013), evaluating the effect of slow drying with the use of saline solutions on jenipapo seeds, showed that the water content of fresh seeds (47%) decreased progressively, reaching 10% and 5% after 42 and 45 days, respectively. In this experiment, with the environment at 20 ºC, the seeds in SP reached 10% at 75 days and 5% at 105 days.

The mean water content of jenipapo seeds in all packages evaluated was adjusted to quadratic equation regression equations of order 2 in the different periods evaluated (Figure 1). The seeds packaged in SP presented lower values of seed moisture at all times, as the paper was porous and favored the changes with the environment. At 105 days, PVB presented the highest content, followed by PV, which was expected, since there were no changes of humidity with air due to the vacuum condition in these packages.

At the beginning of the experiment, the germination percentage (G) of the seeds was 96% and, at 15 days, only in the SP, P and PV packages did the seeds maintain the high G (91%, 90% and 84% respectively). In the other storage periods (45, 75 and 105 days), SP was the most efficient packaging in the conservation of the physiological potential, surpassing the others that made the seeds unfeasible, reaching 48%, 54% and 29% germination, respectively (Table 2). According to Silva et al. (2001), the seeds harvested from jenipapo can expect an average germination of 75% at 25-30 days after sowing.

According to the equations obtained, for G it is expected that the jenipapo seeds completely lose viability in the PVB, PB, PV and P packages after 64.6; 65.8; 67.7 and 89.6 days of storage, respectively. The seeds conserved in SP are expected to have a germination percentage of 32.7 ($SP = 0.0034x^2 - 0.9777x + 97.871$) after 105 days of storage (Figure 2).



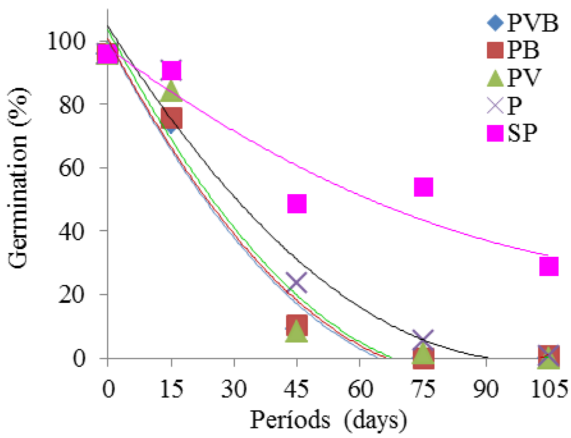
$PVB = -0,0007x^2 + 0,002x + 22,004$ $R^2 = 0,82^{**}$
$PB = -0,0024x^2 + 0,1315x + 21,242$ $R^2 = 1^{**}$
$PV = -0,0015x^2 + 0,1239x + 14,972$ $R^2 = 0,89^{**}$
$P = -0,0026x^2 + 0,1859x + 15,101$ $R^2 = 0,93^{**}$
$SP = -0,0016x^2 + 0,066x + 14,905$ $R^2 = 0,98^{**}$

Figure 1. Water content of jenipapo seeds submitted to different packages and storage periods.

Table 2. Averages of the germination percentage of genotype seeds as a function of the packaging factor unfolding in each storage period evaluated.

Package	0	15	45	75	105
			days		
Paper Bag (SP)	96a	91a	48a	54a	29a
Plastic (P)	96a	90a	24b	5b	0b
Plastic + Vacuum (PV)	96a	84a	8c	1b	0b
Plastic + Biofilm (PB)	96a	76b	10c	0b	0b
Plastic + Vacuum + Biofilm (PVB)	96a	74b	9c	0b	0b
CV %	18,3				

Averages followed by the same letter in the column at each time do not differ among themselves by the Tukey test at 0.05 probability. CV (coefficient of variation).



$PVB = 0,015x^2 - 2,5206x + 100,07$ $R^2 = 0,98^{**}$
$PB = 0,0147x^2 - 2,4958x + 100,6$ $R^2 = 0,98^{**}$
$PV = 0,0147x^2 - 2,526x + 103,66$ $R^2 = 0,95^{**}$
$P = 0,0105x^2 - 2,1088x + 104,67$ $R^2 = 0,96^{**}$
$SP = 0,0034x^2 - 0,9777x + 97,871$ $R^2 = 0,90^{**}$

Figure 2. Percentage of germination of jenipapo seeds submitted to different packages and storage periods.

Still according to the equations obtained (Figures 1 and 2), it is expected that, after 15 days of storage, seeds packed in SP present values of water content and percentage of germination of 15.5% and 82%, respectively, of 14.6% and 60.8% at 45 days and of 13.1% and 51.4% at 60 days. This is in agreement with the results obtained by Salla, José and Faria (2016), who showed, in a study on the behavior of genotype jenipapo seeds with 56% humidity and 96% germination, that drying to 30 and 20% humidity did not interfere. However, when the water content is reduced to 15%, 10% and 5%, reductions in seed viability are observed for 58, 41 and 1%, respectively. According to the same authors, seeds of *G. americana* form a transient seed bank, with maintenance of its viability only until the fourth month after being arranged in a natural environment.

In the same way, Oliveira et al. (2011), evaluating the drying of genotype jenipapo seeds in a screened environment (averages of 33 °C and 70% relative humidity) or in the laboratory (28 °C and 75% relative humidity), concluded that drying the seeds up to 25.1% of humidity (48 h in the laboratory) and 39.5% of humidity (24 h in screen) does not compromise the physiological potential.

However, this goes against Carvalho and Nascimento (2000) and Salomão (2004), who observed the reduction in longevity only when jenipapo seeds were dried below 10% of water content. Magistrali et al. (2013), studying drying techniques, reported that genotype seeds dried at 10% and 5% of water content had a reduction in viability after 30 days of storage, but there was no total loss of germination after this period. In another study, Magistrali (2013) reports that fast drying negatively influences the germination of genotype seeds below 20% of water content and that 10% of water content can be considered the deadly point for this species.

Therefore, according to the equations obtained and considering 60% of germination as the minimum value for commercialization, jenipapo seeds could be stored in paper bags and in environmental conditions for up to 46 days, with a water content of 14.5%.

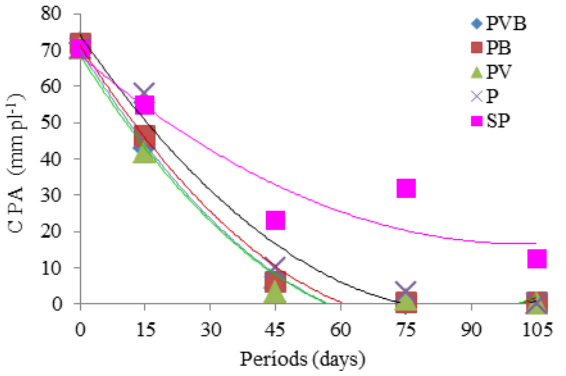
On the other hand, at 15 days, in all other packages evaluated, even with the humidity being above 15%, the G was drastically reduced, which shows that jenipapo seeds do not stand the restriction of O_2 (plastic and vacuum use) or biofilm coating, i.e., techniques that limit gas exchange with the external environment and reduce respiratory rate are not recommended for this species. This is also in agreement with Zanela et al. (2011), who evaluated the effect of the application of cassava starch biofilm on the germination of 'Ya-Cy' (*Psidium cattleyanum* Sabine) cultivar seeds, and Freitas, Santana and Camargo (2011), who tested the vacuum storage of ipê-verde seeds under different pressure conditions (200; 400 and 600 mm Hg) for 2, 4 and 6 months, observed that pressures between 200 and 400 mm Hg significantly increased the percentage of abnormal plants, as well as that of damaged plants. According to Schimidt (2007), in waterproof packages, the water content recommended for the storage of seeds should be equal to or lower than 10%, since values above this can accelerate seed respiration, increasing the temperature, humidity and reducing the rate of germination.

According to some researchers, jenipapo seeds are classified as intermediate behavior in storage (CARVALHO; NASCIMENTO, 2000; SALOMÃO, 2004; FERREIRA et al., 2007; MAGISTRALI et al., 2013), with a short longevity, and that they would be able to tolerate storage humidity between 7% and 10%, which is not in accordance with this experiment, where the seeds were not able to tolerate desiccation at 15% of water content and below. However, the results obtained from this study agree with Oliveira et al. (2011) and Salla, José and Faria (2016), that also showed viability reduction when seeds were dried to around 15%.

Regarding the vigor of jenipapo seeds evaluated by the length of the aerial part and root, green and dry mass of the aerial part and green and dry mass of the root of the seedlings (Figure 3) and IVG (Figure 4), in the same way as observed for G, there were significant decreases after 15 days of storage in all evaluated packaging, but the SP is the less harmful to the seeds.

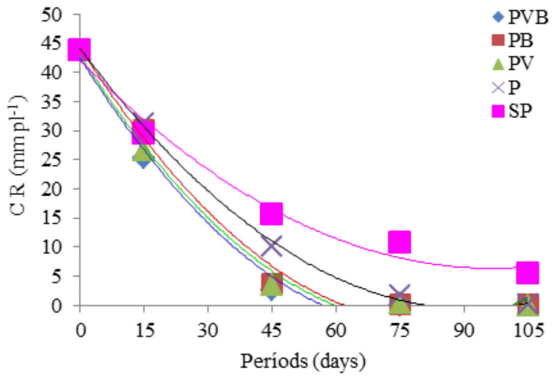
On the other hand, the reduction in the water content of the seeds observed during the storage resulted in an increase in the number of days for the beginning of germination, since the count of the number of normal emerged seedlings was performed at 30 DAS in the 0, 15 and 45 days, at 40 DAS in the 75 days and at 50 DAS in the 105 period, which can be proven by reducing the IVG value during storage for each package evaluated (Figure 4).

A



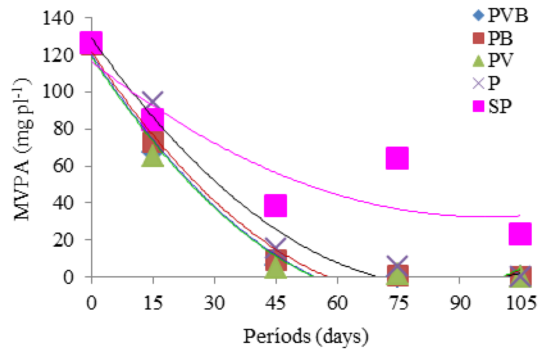
$PVB = 0,0121x^2 - 1,9145x + 70,072 \quad R^2 = 0,99^{**}$
$PB = 0,0115x^2 - 1,8723x + 71,291 \quad R^2 = 0,99^{**}$
$PV = 0,0119x^2 - 1,8812x + 68,531 \quad R^2 = 0,98^{**}$
$P = 0,0097x^2 - 1,7145x + 74,095 \quad R^2 = 0,97^{**}$
$SP = 0,005x^2 - 1,0149x + 68,493 \quad R^2 = 0,89^{**}$

B



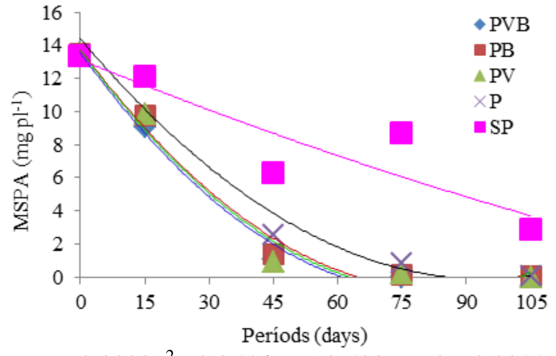
$PVB = 0,0074x^2 - 1,1648x + 42,401 \quad R^2 = 0,98^{**}$
$PB = 0,007x^2 - 1,1484x + 44,181 \quad R^2 = 0,99^{**}$
$PV = 0,0071x^2 - 1,1406x + 42,827 \quad R^2 = 0,99^{**}$
$P = 0,0053x^2 - 0,9725x + 44,008 \quad R^2 = 1,0^{*}$
$SP = 0,0038x^2 - 0,7389x + 42,02 \quad R^2 = 0,98^{**}$

C



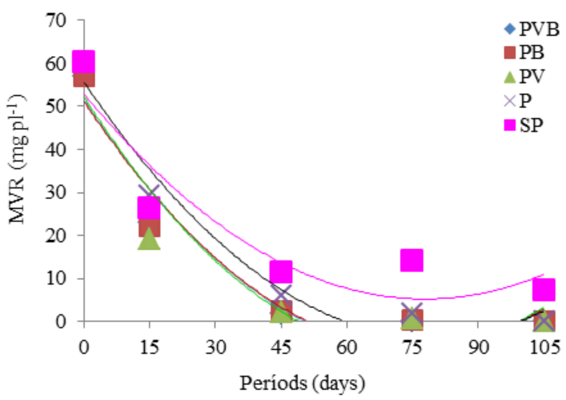
$PVB = 0,0215x^2 - 3,3594x + 119,62 \quad R^2 = 0,98^{**}$
$PB = 0,0207x^2 - 3,2925x + 121,33 \quad R^2 = 0,98^{**}$
$PV = 0,0216x^2 - 3,35x + 118,56 \quad R^2 = 0,97^{**}$
$P = 0,018x^2 - 3,0958x + 128,68 \quad R^2 = 0,98^{**}$
$SP = 0,009x^2 - 1,7309x + 116,14 \quad R^2 = 0,80^{**}$

D



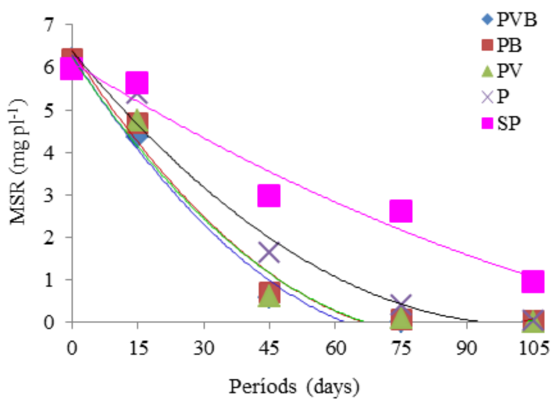
$PVB = 0,0022x^2 - 0,3546x + 13,538 \quad R^2 = 0,99^{**}$
$PB = 0,0021x^2 - 0,3477x + 13,765 \quad R^2 = 0,99^{**}$
$PV = 0,0022x^2 - 0,356x + 13,813 \quad R^2 = 0,98^{**}$
$P = 0,0016x^2 - 0,3075x + 14,399 \quad R^2 = 0,96^{**}$
$SP = 0,0002x^2 - 0,1057x + 13,136 \quad R^2 = 0,81^{**}$

E



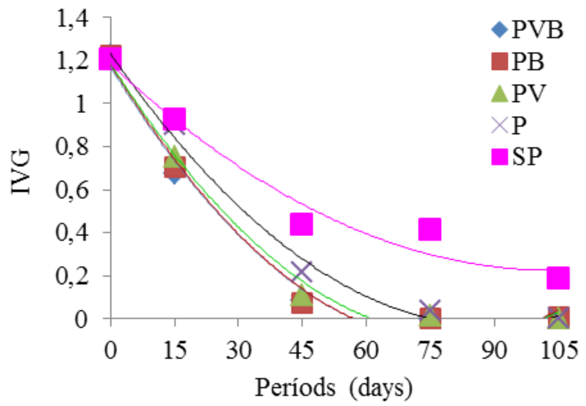
$PVB = 0,0102x^2 - 1,5321x + 51,422 \quad R^2 = 0,94^{**}$
$PB = 0,01x^2 - 1,5099x + 51,028 \quad R^2 = 0,94^{**}$
$PV = 0,0107x^2 - 1,5894x + 52,141 \quad R^2 = 0,90^{**}$
$P = 0,0094x^2 - 1,4952x + 55,607 \quad R^2 = 0,96^{**}$
$SP = 0,0079x^2 - 1,2248x + 52,854 \quad R^2 = 0,87^{**}$

F



$PVB = 0,001x^2 - 0,1618x + 6,2793 \quad R^2 = 0,99^{**}$
$PB = 0,0009x^2 - 0,1585x + 6,3982 \quad R^2 = 0,98^{**}$
$PV = 0,0009x^2 - 0,1534x + 6,2372 \quad R^2 = 0,97^{**}$
$P = 0,0006x^2 - 0,125x + 6,3853 \quad R^2 = 0,97^{**}$
$SP = 0,0002x^2 - 0,0638x + 6,12 \quad R^2 = 0,96^{**}$

Figure 3. Aerial part length - CPA (A) and radicular - CR (B), shoot green mass - MVPA (C) and shoot dryness - MSPA (D) And root dryness - MSR (F) of jenipapo seedlings whose seeds were submitted to different packages and storage periods.



PVB = $0,0002x^2 - 0,0322x + 1,1712$ $R^2 = 0,98^{**}$
PB = $0,0002x^2 - 0,0325x + 1,1844$ $R^2 = 0,98^{**}$
PV = $0,0002x^2 - 0,0311x + 1,1868$ $R^2 = 0,99^{**}$
P = $0,0002x^2 - 0,0285x + 1,2329$ $R^2 = 0,99^{**}$
SP = $9E\text{-}05x^2 - 0,0185x + 1,1849$ $R^2 = 0,96^{**}$

Figure 4. Germination speed index (IVG) of the genotype seeds submitted to different packages and storage periods.

CONCLUSION

Plastic packaging, vacuum condition and biofilm coating are not recommended for storage of jenipapo seeds. The storage in a paper bag when carried out in an environment at 24 ºC and 65% relative humidity of the air for 46 days is able to conserve the seeds of jenipapo with germination of 60%.

RESUMO: O armazenamento de sementes por meio da redução do seu teor de água é uma importante estratégia para a conservação de muitas espécies vegetais, porém, algumas, como o jenipapeiro, são sensíveis à dessecação, podendo haver a perda da sua viabilidade, e até a morte, caso o seu teor de água seja reduzido a um nível considerado crítico. Neste trabalho, o objetivo foi avaliar a interferência de diferentes condições de embalagens na conservação do potencial fisiológico de sementes de jenipapo armazenadas por 105 dias. De forma específica, buscou-se analisar a viabilidade do uso de condições de vácuo e do revestimento da semente com biofilme. Os tratamentos avaliados foram: armazenamento em saco de papel, saco plástico, plástico com vácuo, plástico com sementes revestidas com biofilme e plástico com sementes com biofilme e condição de vácuo por 15, 45, 75 e 105 dias, além da testemunha. As sementes com biofilme receberam tratamento com fécula de mandioca a 3% da relação massa por volume. Os seguintes testes foram realizados: teor de água, germinação, velocidade de germinação, comprimento da parte aérea e radicular, massa úmida e seca da parte aérea e da raiz. Concluiu-se que a embalagem plástica, a condição de vácuo e o revestimento com biofilme não são recomendados para o armazenamento de sementes de jenipapo. O armazenamento em saco de papel quando realizado em ambiente com 24 ºC e 65% de umidade relativa do ar durante 46 dias é capaz de conservar as sementes de jenipapo com germinação de 60%.

PALAVRAS-CHAVE: Sementes recalcitrantes. Germinação. Árvores nativas. *Genipa americana* L.

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