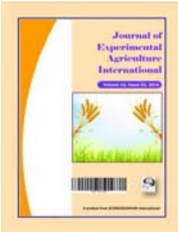

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Physiological Quality of Rice Seeds Stored in Different Environments and Packages

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Authors' contributions

This work was carried out in collaboration between all authors. Author ASS designed the study, managed the analyses of the study and wrote the protocol. Authors LPS and FM auxilied on performance of the study and managed the literature searches. Author BEP performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Evaluate the physiological quality of rice seeds during storage in different packages and environments, identifying the best condition to conserve the seeds.
Study Design: Completely randomized with four replicates.
Place and Duration of Study: Plant Science Laboratory and Genetics Laboratory of the Federal University of Piauí, Bom Jesus-PI, from April to October 2016.
Methodology: Seeds of rice were stored for 180 days in plastic bottles, kraft paper packaging, trifoliate paper packaging and polyethylene bags in cold and humid chamber (10°C and 85% RH), in a temperature controlled environment (25°C) and in an uncontrolled environment (21-36.4°C and 30-67% RH). The water content and vigor characteristics (first counting germination and germination speed index and electrical conductivity) were evaluated before (control), and at 90 and 180 days of storage, additional tests were also evaluated during these periods the Absolute growth rate of the

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root (AGRR), Absolute growth rate of the aerial part (AGRAP), Relative growth rate of the root (RGRR) and Relative growth rate of the aerial part (RGRAP).

Results: The values of AGRAP and RGRAP were reduced, differing from the others, for the trifoliate paper packages (0.168 cm day^{-1}) and polyethylene bag (0.168 cm dia^{-1}) in environment 1 at 180 days of storage. The plastic bottles presented the highest values for the characters 1st count (%) and germination (%) in all environments and storage periods. At the end of the storage (180 days) the environment with temperature control was the one that presented the best results of 1st counting of germination (%) and germination (%). At 90 and 180 days, the plastic bottles presented higher Germination speed index. The environment with temperature control presented better results for electrical conductivity (μS cm^{-1} g^{-1})

Conclusion: The environment with temperature control is efficient in storing rice seeds for 180 days using a plastic bottle package. During storage, the greatest changes in the physiological quality of rice seeds are verified in the cold and humid chamber environment and in the polyethylene bag package.

Keywords: Deterioration; viability; vigor; Oryza sativa L.

1. INTRODUCTION

Rice, *Oryza sativa* (L.), is the third largest cereal crop in the world, being surpassed by corn and wheat [1]. Rice is considered to be the most socioeconomically important product in many developing countries, serving as a staple food for about 2.4 billion people, especially for low-income population [2].

Rice grains harvest occurs seasonally during the year, which makes post-harvest stages important and determinant to maintain seed quality. During storage, there are several factors that interfere with seed quality, starting with the control of physical-chemical, biochemical and metabolic reactions [3,4].

Temperature and relative humidity are the main factors that affect seed quality during storage [5], having a direct relationship in the process of seed viability loss during storage and changes in the product quality, in contrast, by-products [6,7]. According to Cardoso et al. [8], the deterioration process is inevitable but can be delayed depending on the storage conditions and the seed characteristics.

In addition to the environment, the packaging used in storage should help to reduce the rate of the deterioration process, keeping the initial seed moisture content stored in order to reduce respiration [9]. Seed deterioration is related to the characteristics of the packaging containers, depending on the greater or lesser ease of water vapor exchange between the seeds and the atmosphere [10].

Therefore, the objective of this study was to evaluate the physiological quality of rice seeds during storage in different packages and environments, identifying the best condition to conserve the seeds.

2. MATERIALS AND METHODS

The experiment was conducted at the Plant Science Laboratory and Genetics Laboratory of the Federal University of Piauí, Bom Jesus-PI, from April to October 2016. Rice seeds, provided by Embrapa, of cultivar BRS *Sertaneja* were used for being a cultivar widely used in dry rice cultivation in Brazil.

The seeds were packed in plastic bottles (104 mm at the base and 351 mm in height), kraft paper packaging (150 x 300 mm), trifoliate paper packaging (150 x 300 mm) and polyethylene bags (500 x 1000 mm, and 2 mm thick), and then stored for a period of six months in a cold and humid chamber (10°C and 85% RH), in a temperature controlled environment (25°C) and in an uncontrolled environment (21-36.4°C and 30-67% RH). In each environment 4 packages of each type were stored. Each package had, in average 0.2 kg of seeds. All packaging was properly sealed.

The water content and vigor characteristics (first counting, germination and germination speed index and electrical conductivity) were evaluated at 0 day, and at 90 and 180 days of storage, except for electrical conductivity and humidity test at 90 days. The following evaluations were performed:

Determination of the degree of relative humidity - the greenhouse method was used at 105 ± 3°C for 24 h, using two replicates for each sample, according to the Rules for Seed Analysis [11].

Germination test - 200 seeds of each treatment using "Germitest" paper, moistened with distilled water, in a ratio of 2.5 times the weight of the paper. The paper with 50 seeds each were placed in a BOD-type germination chamber at 25°C and photoperiod programmed for 16 hours, until the end of the test. The evaluation was performed 10 days after, and the normal seedlings were evaluated according to Brasil [11].

First germination count - Performed together with the germination test, recording the number of normal seedlings 5 days after the test implementation according to Brasil [11].

Germination speed index (GSI) - Carried out together with the germination test. The evaluations of the normal seedlings were performed daily, at the same time, from the 1st day until the 10th day. After obtaining the number of normal seedlings, the germination speed was calculated, using the formula indicated by Edmond & Drapala [12]:

$$GSI = \frac{\sum_{i=1}^{k} NiGi}{\sum_{i=1}^{k} Gi}$$ being:

GSI: germination speed index; Ni: number of days counted from sowing to day of observation; Gi: number of seeds germinated on the day of observation.

Electrical conductivity (EC): Four subsamples of 50 seeds were weighed (0.001 g precision) and placed in plastic cups containing 75 ml of deionized water in a BOD-type germination chamber at 25°C for 24 hours. After this period, the electrical conductivity of the soaking solution was determined and the results expressed in µS cm^{-1} g^{-1} of seed [13].

Absolute growth rate of the root (AGRR) and Absolute growth rate of the aerial part (AGRAP) - was held with the germination test, measuring the root and shoot length of the seedlings on the 1st and 10th day, obtained from the equation: TCA = (W2-W1) / (T2-T1). Where, M2 = final length; M1 = initial length; T2 - T1 = time interval, with results expressed in cm seedling day^{-1} [14].

Relative growth rate of the root (RGRR) and Relative growth rate of the aerial part (RGRAP) - Performed together with the germination test, measuring the root and shoot length of the seedlings on the 1st and 10th day, obtained from the equation: TCR = (lnW2 - lnW1) / (T2 -T1), where: ln is the logarithm neperian; Wl final length; M1 = initial length; T2 - T1 = time interval; with results expressed in cm cm^{-1} day^{-1} [11].

The experimental design was completely randomized with four replicates, the experiment had different factorial schemes, and the characteristics AGRR, AGRAP, RGRR and RGRAP had 2x4x3 scheme being three storage periods, four packages and three environments following the mathematical model:

$$y_{izkj} = \mu + p_i + a_z + r_k + p_i a_z + p_i r_k + a_z r_k + p_i a_z r_k + e_{izkj}$$

being:

$y_{izkj} =$ observed in the plot corresponding to *i-th* storage period of *z-th* environment inside of *k-th* storage container *j-th* repetition; $\mu =$ mean effect of the experiment $p_i =$ effect of *i-th* storage period; $a_z =$ effect of *z-th* environment; $r_k =$ *effect of k-th* storage container; $p_i a_z =$ effect of the interaction between *i-th* storage period with the *z-th* environment; $p_i r_k =$ effect of the interaction between *i-th* storage period with *k-th* storage container ; $a_z r_k =$ effect of the interaction between *z-th* environment with *k-th* storage container; $p_i a_z r_k =$ effect of the triple interaction between *i-th* storage period with *z-th* environment and *k-th* storage container; $e_{izkj} =$ effect of the error associated with observation of the plot containing *i-th* storage period of *z-th* environment inside of *k-th* storage container of *j-ésima* repetition.

The germination test, first germination count and GIS had a 2x4x3 + 1 factorial experimental design, two storage periods, four storage containers and three environments with one control (characteristics evaluation before the application of the treatments) following the mathematical model:

$$y_{izk(t)j} = \mu + T_t + p_{i(t)} + a_{z(t)} + r_{k(t)} + p_i a_{z(t)} + p_i r_{k(t)} + a_z r_{k(t)} + p_i a_z r_{k(t)} + e_{izk(t)j}$$

being:

$y_{izk(t)j} =$ observed in the plot corresponding to *i-th* storage period of *z-th* enviornment inside of *k-th* storage container inside of *t-ésimo* type (control or treatment) of *j-th* repetition; $\mu =$ mean effect of the experiment; $p_{i(t)} =$ effect of *i-th* storage period within the type; $a_{z(t)} =$ effect of *z-th* environment inside the type; $r_{k(t)} =$ *effect of k-th* storage container within the type; $p_i a_{z(T)} =$ effect of the interaction between *i- th* storage period with *z-th* environment; $p_i r_k =$ effect of the interaction between *i-th* storage period with *k-th* storage container within type; $a_z r_{k(T)} =$ effect of the interaction between *z-th* environment with *k-th* storage container within the type; $p_i a_z r_{k(t)} =$ effect of triple interaction between *i-th* storage period with *z-th* environment and *k-th* storage container within the type; $e_{izk(T)j} =$ effect of the error associated with observation of the plot containing *i- th* storage period in *z- th* environment inside of *k-th* storage container of *j-th* repetition inside of the type.

The electrical conductivity character followed the experimental design of factorial 4x3 + 1, with four storage containers and three environments with a control (characteristics evaluation before the application of the treatments) following the mathematical model:

$$y_{zk(t)j} = \mu + T_t + a_{z(t)} + r_{k(t)} + a_z r_{k(t)} + e_{zk(t)j}$$

being:

$y_{izk(t)j} =$ observed in the plot corresponding to *z-th* environment inside of *k-th* storage container inside of *t-th* type (control or treatment) of *j-th* repetition; $\mu =$ mean effect of the experiment; $a_{z(t)} =$ effect of *z-th* enviornment insde of the type; $r_{k(t)} =$ *effect of k-th* storage container inside of the type; $a_z r_{k(T)} =$ interaction effect inside of *z-th* environment with *k-th* storage container within type; $e_{izk(T)j} =$ effect of the error associated with the observation of the plot *z-th* environment inside of *k- th* storage container of *j-th* repetition within type.

The comparison between means was done using the Tukey test at 5% probability. To verify the need to transform the percentage data, the analysis of homogeneity of variance between treatments was done using the Cochran test (p = 0.05). All statistical analyzes were performed with SAS® software (1999).

3. RESULTS AND DISCUSSION

The AGRR, AGRAP characteristics of the rice were highly significant for all the studied factors, with triple interaction significance. Proving the effect on these characters of the period, the container and storage environment of rice seeds (Table 2). The interaction between the factors causes different behaviors of the periods according to the container and the environment; therefore, there is no pattern that causes better systematic conditions.

The values of AGRR and AGRAP decreased, differing from the others, for the trifoliate and polyethylene bags in cold and humid chamber at 180 days of storage (Table 4). These results agree with statement that the longevity of seeds that are stored in different types of packaging may vary due to water vapor exchange [15].

Trifoliate paper and polyethylene bag have characteristics that provide little or no resistance to the water vapor exchanges of the seeds with the medium [15], and because seeds are hygroscopic, they either absorb or loose moisture depending upon the air moisture within the container in which they are stored until they reach a stable moisture content with the air enclosed in the container [16]. This factor causes an increase in deterioration, which is proven in the seed moisture content values stored in these two types of packaging (Table 1), considering that Smaniotto et al. [5] observed decreased seed vigor related to deterioration with increasing humidity, and according to Corte et al. [17] this deterioration slows seedling growth. It was also possible to observe that in the other packages and environments, the means did not differ.

Table 1. Moisture content (%) of rice seeds before and after storage of 180 days in different packages and environments

Packing / storage environment	Moisture content (%)
Moisture (Before Storage)	8.0
Plastic bottles / Cold and humid chamber	9.5
Plastic bottles / Temperature controlled	8.6
Plastic bottles / Uncontrolled environment	8.1
Kraft paper / Cold and humid chamber	9.5
Kraft paper / Temperature controlled	8.9
Kraft paper / Uncontrolled environment	8.9
Trifoliate paper / Cold and humid chamber	9.8
Trifoliate paper / Temperature controlled	8.6
Trifoliate paper / Uncontrolled environment	8.5
Polyethylene bags / Cold and humid chamber	10.1
Polyethylene bags / Temperature controlled	9.6
Polyethylene bags / Uncontrolled environment	9.1

Depending on the storage conditions of the seeds, deterioration can be delayed or accelerated. The seeds are subjected to the greatest respiratory activity; increase the wear and tear on the seeds' reserves, thus accelerating the deterioration process. It may be associated with the increase in moisture levels. [18].

It is likely that the temperature and RH of the storage environment contributed to the seed deterioration when associated to these types of packages, since the cold and humid chamber environment presented lower mean values of AGRR and AGRAP (Table 4), as Smaniotto [5] states that although the most important factors in seed conservation are not considered, temperature and RH contribute significantly to their deterioration, affecting the speed of the biological processes. The conditions of relative humidity and temperature during storage in which the seeds will obtain their specific hygroscopic equilibrium, will determine the maintenance of their physiological quality, for a greater or lesser time [19].

At 180 days of storage, AGRR values were higher than those observed at 90 days (Table 4), indicating that the storage period influenced the growth of rice seedlings.

The AGRR character was not affected by any of the factors tested in rice seeds (Table 2) showing that storage period, container and environment do not directly interfere with this character.

In general, the plastic bottle package presented the highest values for the 1st germination count (%) and germination (%) characters in all environments and storage periods. On the other hand, trifoliate paper and polyethylene bag stored in the cold and humid chamber at 180 days had the lowest values (Table 4), as shown

Table 2. Summary of variance analysis of Absolute growth rate of the root (AGRR) and Absolute growth rate of the aerial part (AGRAP), Relative growth rate of the root (RGRR) and Relative growth rate of the aerial part (RGRAP) in different forms of seed storage rice

VF	DF	Mean square			
		AGRR	AGRAP	RGRR	RGRAP
Storage period (SP)	1	0.241**	0.084**	0.001^{ns}	0.0095**
Packaging (P)	3	0.031^{ns}	0.019**	0.002^{ns}	0.0025**
Environment (E)	2	0.034^{ns}	0.013*	0.004^{ns}	0.0037**
SP x P (T)	3	0.003^{ns}	0.012*	0.0005^{ns}	0.0020**
SP x E (T)	2	0.047^{ns}	0.063**	0.004^{ns}	0.0078**
P x E (T)	6	0.040^{ns}	0.045**	0.002^{ns}	0.0042**
SP x P x E (T)	6	0.045^{ns}	0.016**	0.003^{ns}	0.0023**
Error	95	0.028	0.004	0.002	0.0005
CV%		26.22	16.52	18.74	13.63
Mean		0.642	0.384	0.207	0.162

*ns, *, ** not significant, significant at 5 and 1%, respectively, of probability by the test F* in the AGRAP and RGRAP variables. Da Silva et al. [20] working with the storage of rice, maize and beans seeds, with different packages, concluded that, regardless of the species, the plastic bottle package presented better values of 1st count (%) and germination (%), this was due to the seed moisture content that were stored in the permeable packages, in which they suffered a greater influence of the atmospheric conditions of the storage site, since this type of packaging does not offer any resistance to the water vapor exchanges of the seeds with the medium in which they are stored different than the impermeable, and this is shown in the values of moisture degree of the seeds in Table 1.

Therefore, the use of packaging that allows or not a certain water vapor exchange between the seed and the outside environment is important to avoid fluctuations in the degree of seed moisture. The reduction of metabolic activity, with a reduced deterioration rate, allows the maintenance of the physiological quality for a longer period [8].

The 1st germination count (%) at 180 days of storage, the lowest averages were observed in the uncontrolled environment (Table 4), proving the increase in the respiratory rate in the seeds, and according to Marini et al. [21], this increase allows degenerative transformations, which can lead to the rapid consumption of their reserves, at the beginning of the germination process, reducing respiratory efficiency and, consequently, leading to less use in the synthesis processes, causing a decrease in germination and seed vigor. According to Mano [22] the seed deterioration under high temperature conditions occurs from the interference of the metabolic activities of the cell, due to the oxidation of lipids, proteins and nucleic acids.

At the end of the storage (180 days), the temperature control environment showed the best results for the 1st germination count (%) and germination (%) (Table 4), due to the uniformity of the values, regardless of the type of packaging , different than the control, but absolute values presented a smaller decrease when compared to the other environments. A similar result was verified by Almeida et al. [23], who emphasized that the physiological quality of stored seeds is better when kept in an environment where the temperature is controlled.

Seed packaging influenced the results of the germination speed index (Table 4). At 90 and 180 days, the plastic bottle package presented higher values, since this type of packaging can preserve or alter the minimum physiological characteristics of the seeds during storage, reducing the deterioration and according to Marini et al. [21] provides a slower growth in the seedlings.

These results show that the type of packaging used while storing the seeds is important to preserve the physiological quality of the seeds, which agrees with Bessa [24] and Carvalho et al. [25].

Table 3. Summary of analysis of variance of first germination count, germination, germination speed index (GSI) and electrical conductivity in different forms of storage of rice seeds

VF	DF	Mean square			
		1st ger. Count	Germination	GSI	Electrical conductivity
Treatment	24 (12)	213.9**	2097.4**	223.5**	17,65*
Type (T)	1	1120.7**	3432.0**	847.5**	2,158^{ns}
Storage period (SP)	1	253.5**	1998.4**	248.4**	-
Packaging (P)	3	232.6**	3466.7**	385.7**	12,15^{ns}
Environment (E)	2	20.04^{ns}	2923.0**	141.7**	5,355^{ns}
SP x P (T)	3	163.4**	1522.0**	138.0**	-
SP x E (T)	2	195.9**	1577.6**	232.6**	-
P x E (T)	6	227.8**	1685.0**	168.9**	27,09**
SP x P x E (T)	6	128.9**	1805.0**	156.0**	-
Error	75 (39)	21.15	70.16	5.474	6,967
CV%		19.64	13.78	14.88	12,20
Mean		11.60	60.80	15.73	21,62

*ns, *, ** not significant, significant at 5 and 1%, respectively, of probability by the test F*

Table 4. Deployment of means of absolute growth rate of the root, absolute growth rate of the aerial part, relative growth rate of the root, 1st germination count, germination, germination speed index and electrical conductivity of rice seeds stored for a period of six months in a cold and humid chamber (1), in a temperature controlled environment (2) and in an uncontrolled environment (3)

Environment/	90 days			180 days		
Packaging	1	2	3	1	2	3
Absolute growth rate of the root (cm seedling day^{-1})						
Plastic bottles	0.487 Aa^{a}	0.401 Aa^{a}	0.401 Aa^{a}	0.435 Aa^{a}	0.369 Aa^{a}	0.380Aa^{a}
Kraft paper	0.465 Aa^{a}	0.378 Aa^{a}	0.379 Aa^{a}	0.438 Aa^{a}	0.378 Aa^{a}	0.379Aa^{a}
Trifoliate paper	0.414 Aa^{a}	0.394 Aa^{a}	0.417 Aa	0.168 Bb^{b}	0.377 Aa^{a}	0.370Aa^{a}
Polyethylene bags	0.399 Aa^{a}	0.420 Aa^{a}	0.405 Aa^{a}	0.081 Bb^{b}	0.453 Aa^{a}	0.424Aa^{a}
Absolute growth rate of the aerial part (cm seedling day^{-1})						
Plastic bottles	0.186 Aa^{a}	0.170 Aa^{a}	0.170 Aa^{a}	0.177 Aa^{a}	0.162 Aa^{a}	0.165 Aa^{a}
Kraft paper	0.182 Aa^{a}	0.165 Aa^{a}	0.165 Aa^{a}	0.177 Aa^{a}	0.165 Aa^{a}	0.165 Aa^{a}
Trifoliate paper	0.172 Aa^{a}	0.167 Aa^{a}	0.173 Aa^{a}	0.076 Bb^{b}	0.163 Aa^{a}	0.163 Aa^{a}
Polyethylene bags	0.169 Aa^{a}	0.174 Aa^{a}	0.170 Aa^{a}	0.056 Bb^{b}	0.180 Aa^{a}	0.175 Aa^{a}
Relative growth rate of the root (cm cm^{-1} day^{-1})						
	0.592^{b}			0.692^{a}		
1st germination count (%)						
Control	28.0 A	28.0 A	28.0 A	28.0 A	28.0 A	28.0 A
Plastic bottles	17.5 Ba^{a}	10.5 Bb^{a}	17.5 Ba^{a}	23.0 Aa^{a}	13.5 Bb^{a}	9.5 Bb^{b}
Kraft paper	4.0C Da^{b}	6.5B Ca^{b}	6.5 Da^{a}	24.0 Aa^{a}	13.0 Bb^{a}	8.0 Bb^{a}
Trifoliate paper	10.0 Ca^{a}	10.0 Ba^{b}	14.0B Ca^{a}	0.5 Bc^{b}	17.0 Ba^{a}	10.0 Bb^{a}
Polyethylene bags	1.5 Db^{a}	3.5 Cb^{b}	10.0 CDa^{a}	0.0 Bb^{a}	19.0 Ba^{a}	13.0 Ba^{a}
Germination (%)						
Control	89.5 A	89.5 A	89.5 A	89.5 A	89.5 A	89.5 A
Plastic bottles	86.0 ABa^{a}	76.0 Ba^{a}	86.5 Aa^{a}	68.0 Ba^{b}	69.0 Ba^{a}	71.0 Ba^{b}
Kraft paper	43.5 Cb^{b}	73.0 BCa^{a}	44.5 Cb^{a}	75.5 Ba^{a}	59.5 Bb^{b}	55.5 Cb^{a}
Trifoliate paper	75.0 Ba^{a}	62.0 Cb^{a}	74.5 Ba^{a}	3.0 Cc^{b}	69.5 Ba^{a}	54.5 Cb^{b}
Polyethylene bags	33.5 Cb^{a}	33.5 Db^{b}	82.0 ABa^{a}	4.5 Cb^{b}	70.5 Ba^{a}	60.0B Ca^{b}
Germination speed index (seeds day^{-1})						
Control	29.9 A	29.9 A	29.9 A	29.9 A	29.9 A	29.9 A
Plastic bottles	21.9 Ba^{a}	15.7 Bb^{b}	21.6 Ba^{a}	23.8 Ba^{b}	21.8 Bab^{a}	19.3 Bb^{a}
Kraft paper	7.4 Db^{b}	13.2 Ba^{b}	9.6 Db^{b}	24.5 Ba^{b}	18.2 Cb^{a}	13.6 Dc^{a}
Trifoliate paper	16.5 Ca^{a}	12.5 Bb^{b}	17.4 Ca^{a}	1.01 Cc^{b}	22.03 Ba^{a}	15.5 CDb^{a}
Polyethylene bags	5.3 Db^{a}	5.7 Cb^{b}	15.0 Ca^{a}	0.9 Cc^{b}	22.8 Ba^{a}	17.1 BCb^{a}
Electrical conductivity (μS cm^{-1} g^{-1}) at 180 days						
Control	20,91 B		20,91 A		20,91 AB	
Plastic bottles	25,17 Aa		20,07 Ab		23,24 ABab	
Kraft paper	21,66 Aba		21,51 Aa		20,03 Ba	
Trifoliate paper	16,59 Cb		21,61 Aa		23,70 Aa	
Polyethylene bags	21,24 Ba		23,08 Aa		22,26 Aba	

Means followed by the same uppercase letters in the columns, lowercase in the lines within the storage period and overwritten in the rows between periods within the same environment do not differ by the Tukey test at 5% probability; Means followed by the same uppercase letters in the columns, lowercase in the rows within the storage period and underwritten in rows between periods within the same environment do not differ among themselves by the Tukey test at 5% probability

Considering that the solute leakage from the seeds and, consequently, a higher electrical conductivity value, are associated with lower seed vigor [26,9], makes it possible to confirm that the environment that provided the best results was with temperature control, with no significant difference between the packages used, including the control (Table 4). This result confirms the results obtained in the previous test.

4. CONCLUSION

1. The environment with temperature control is efficient in storing rice seeds for 180 days using a plastic bottle package.
2. During storage, the greatest changes in the physiological quality of rice seeds are verified in the cold and humid chamber environment and in the polyethylene bag package.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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