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Anti-oxidant activity of seedlings from rice seeds stored in different temperatures over 10 years

Fabíola de Oliveira Krüger[1], Dario Munt de Moraes[2], Daniel Fernandez Franco[3], Caroline Jácome Costa[3], Juliana de Magalhães Bandeira[4], Patrícia Marini[5]

[1] Universidade Federal de Lavras Departamento de Biologia, Programa de Pós-Graduação em Fisiologia Vegetal, CEP 37200-000, Lavras- MG, Brasil. Caixa Postal 3037. E-mail: fabiolaoliveirakruger@gmail.com
[2] Universidade Federal de Pelotas, Instituto de Biologia, Departamento de Botânica, Campus Capão do Leão, CEP 96010-900, Pelotas-RS, Brasil. Caixa Postal 354. E-mail: dariommoraes@gmail.com
[3] Embrapa Clima Temperado, Rodovia BR-392, km 78, CEP 96010-971, Pelotas-RS, Brasil. E-mail: daniel.franco@embrapa.br; caroline.costa@embrapa.br
[4] Instituto Federal Farroupilha, Campus São Borja, Rua Otaviano Castilho Mendes, 355, Betim, CEP 97670-000, São Borja-RS, Brasil. E-mail: bandeira_jm@hotmail.com
[5] Instituto Federal de Educação, Ciência e Tecnologia Farroupilha, Campus Alegrete, RS-377, km 27, Passo Novo, CEP 97555-000, Alegrete-RS, Brasil. E-mail: marinipati@gmail.com

ABSTRACT

Seed deterioration can occur in premature maturation and bad storage conditions. In these two cases, the excessive production of the reactive oxygen species (ROS) has contributed for losses in physiological potential seeds. Thus, the objective of this study was to evaluate the physiological potential and antioxidant enzymes in rice seedlings against 10 years storage conditions at different temperatures. The cultivars BRS Pelota, BRS Atalanta and BRS Firmeza were kept in waterproof bag at -15, 1 and 18 ºC until evaluation. Then, the physiological potential and antioxidant enzymes (SOD, CAT and APX) were estimated. Storage at -15 and 1 ºC provided the best physiological potential conditions of cv. BRS Pelota, Atalanta and Firmeza. These results were compatible with the enzymatic activity of seedlings from seeds stored at different temperatures. Storage at 18 °C was detrimental to the maintenance of physiological potential of the cv. BRS Pelota, Atalanta and Firmesa over 10 years. The lower activity of antioxidant defense system enzymes the higher seed liveliness.

Key words: anti-oxidant enzymes; *Oryza sativa* L.; physiological potential; viability

Atividade antioxidante de plântulas provenientes de sementes de arroz armazenadas em diferentes temperaturas por 10 anos

RESUMO

A deterioração de sementes pode ocorrer durante a maturação precoce e armazenamento inadequado. Nos dois casos, a produção excessiva de espécies reativas de oxigênio tem contribuído com a perda do potencial fisiológico das sementes. Objetivou-se avaliar o potencial fisiológico de sementes e a atividade de enzimas antioxidantes em plântulas oriundas de sementes de arroz armazenadas por dez anos em diferentes temperaturas. Foram utilizadas sementes de arroz das cv. BRS Pelota, BRS Atalanta e BRS Firmeza acondicionadas em embalagem impermeável e armazenadas por dez anos a -15; 1 e 18 °C. Após esse período avaliou-se o potencial fisiológico e a determinação da atividade de enzimas do sistema antioxidativo (SOD, CAT, APX). O armazenamento a -15 e 1 °C proporcionaram melhores condições para a manutenção da viabilidade e do vigor das sementes das cv. BRS Pelota, Atalanta e Firmeza. Corroborando com a atividade enzimática das plântulas provenientes de sementes armazenadas sob temperaturas -15 e 1 °C. O armazenamento a 18 °C foi prejudicial à manutenção do potencial fisiológico das cv. BRS Pelota, Atalanta e Firmeza ao longo de 10 anos. A menor atividade das enzimas do sistema de defesa antioxidante indica sementes de maior vigor.

Palavras-chave: enzimas antioxidantes; *Oryza sativa* L.; potencial fisiológico; viabilidade

Introduction

Brazil is one of the 10 countries with higher rice production (*Oryza sativa* L.), and 66.9% of national production is based in Rio Grande do Sul state (Conab, 2014). For this reason, the use of seeds with high physiological potential is extremely important for successful crops, which aim uniformity coming from attributes such as high genetic quality, physical and physiological sanitary (Marcos Filho, 2005).

Thus, storage comprises an essential step to preserve physiological potential of seeds without losses in the period from harvesting to sowing. As plant breeding programs improves, it has been observed the need for storage of small seed banks for longer periods due to their importance for future breeding programs. Therefore, germplasm banks were created aiming the conservation of genetic material of actual or potential use for as long as possible, requiring constant monitoring of the major effects which may interfere in seed conservation such as temperature, moisture level of seeds and relative humidity (Carvalho & Nakagawa, 2012).

However, it is known that the deterioration process is continuous and starts just after physiological maturity in a progressive way determining a decrease in quality and culminating with seed death (Marcos Filho, 2005). Even though this process is irreversible, controlling environmental conditions throughout storage in an effective way makes it possible to drop its speed/intensity.

It is also known, therefore, that the deterioration of seeds may occur faster when they exhibit early maturation or even due to inadequate storage. In the last cases, excessive production of reactive oxygen species (ROs) takes place, such as superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$), which may limit the growth and development of the seedling vegetal (Carvalho et al., 2009; Forman et al., 2010). Looking forward to contain deleterious effects of ROs, plants developed a complex anti-oxidant system that comprises primary defenses against the radical generated under limiting conditions, such as superoxide dismutase enzyme which catalyzes dismutation of superoxide radical H_2O_2 and O_2, peroxidase ascorbate and catalase which may break H_2O_2 molecule into H_2O and O_2. Thus, the balance between ROs production and the capacity of quickly activating anti-oxidant defense system will likely impact the capacity of adaptation and survival of seedlings to the injuries caused by feasible early maturation or inadequate storage and consequent seed deterioration (Carvalho et al., 2009; El-Shabrawi et al., 2010; Deuner et al., 2011).

It has been observed that maize seeds kept down in different temperatures exhibited higher activity of anti-oxidant defense system enzymes for seeds kept down in the highest temperature (Bandeira et al., 2013). Although, cowpea seeds laid in 150 mM NaCl obtained high SOD activity whereas APX has not played an efficient role, evidencing the treatment with 200 mM NaCl with a lower activity observed in control treatment (Deuner et al., 2011). In another work with cowpea beans subjected to saline stress, a reduction in CAT and APX activities was observed (Maia et al., 2012). The results indicate variability in the response from different species regarding seed deterioration. But there is little information about anti-oxidant enzymes activity in the deterioration process of irrigated rice seeds stored for a long time.

In face of the above mentioned, the aim was to analyze the physiological quality of seeds as well as anti-oxidant enzymes activity in seedlings from rice seeds stored over 10 years in different temperatures.

Material and Methods

Three cultivars of irrigated rice were used: BRS Pelota, BRS Atalanta and BRS Firmeza harvested in the area of seed production of experimental station lowlands in the crop 2001/2002. After harvesting, seeds exhibited 22% moisture and afterwards they were dried until reaching 9.9% moisture for cultivars BRS Pelota and BRS Atalanta and 11.3% for Firmeza cultivar. After drying, seeds were packed in aluminum cans hermetically sealed with capacity for 1.0 Kg but filled with 0.5 Kg seeds. After packing, seeds were sent for storage in seed conservation chamber, refrigerator and freezer over 10 years with average temperature 18, 1.0 and -15 ºC, respectively.

To determine physiological potential of seeds after storage, it was assessed:

Water content (WC) – samples of seeds from each cultivar were taken out of their respective storage environments and put in room temperature for 24 h before determining moisture level. The oven drying method at 105 ºC for 24 hours was adopted, 200 seeds were used (Brasil, 2009) and results were expressed in percentage.

Germination (G) and First germination count (FGC) – evaluated in four replicates of 100 seeds for each treatment, sowed in a paper specific for germination dampened with distilled water in quantity equivalent to 2.5-fold their dry matter and kept in germinator at 25 ºC. Evaluations were carried out five and 14 days after sowing, results expressed in percentage of normal seedlings (Normal seedlings are those that show potential to continue its development and give rise to normal plants, when grown under favorable conditions) (Brasil, 2009).

Tetrazolium test – seeds underwent pre-dampening in germination paper dampened at temperature 25 ºC for 18 hours. Following this period, seeds were sectioned longitudinally with a scalpel, in the lateral extremity along embryo. For staining, seeds were kept in 2, 3, 5-triphenyl tetrazolium chloride at 0.05% for 4 hours at 25 ºC in darkness. Afterwards, seeds were rinsed in tap water and submerged in distilled water up to evaluation (Brasil, 2009). The test was conducted only in seeds which did not present germination, so that it was possible to analyze embryo viability.

Length of seedlings shoots (SL) and roots (RL) – determined concomitantly with germination test at 14 days (Nakagawa, 1999). Mean length of seedling shoots and roots obtained dividing the sum of measurements taken from sub-samples by the number of measured seedlings, results were expressed in cm seedling^{-1}.

Dry matter of seedling shoots (DMS) and roots (DMR) – obtained from seedlings of the germination test. Each sample was packed in paper bags and kept in an oven with forced air circulation at 70 ºC until reaching constant mass. Seedling

dry matter was determined in precision scale of ±0.001g (Nakagawa, 1999) and results expressed in g seedlings^{-1}.

Cold test – four replicates of 100 seeds from each treatment were given out in germination paper, dampened with water in quantity equivalent to 2.5-fold their dry matter. Seeds were covered with sieved soil from a rice crop and covered with another sheet of germination paper. Rolls of germination paper were packed in plastic bags, sealed and kept in germination chamber type BOD chambers at 10 ºC temperature during seven days. After this period, rolls were transferred into a germinator at 25 ºC temperature where they stayed for seven more days (Cícero & Vieira, 1994). Results were expressed in percentage of normal seedlings.

At five and 14 days after sowing (DAS), seedlings from seeds submitted to different storage conditions were collected to evaluate anti-oxidant enzyme activity superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT). For this, 300 mg of fresh material were used, macerated in 10% polyvinylpolypyrrolidone (PVPP) and homogenized in 1.5 mL of extraction buffer, pH 7.8 with potassium phosphate 100 mM, EDTA 0.1 mM and ascorbic acid 10 mM. Following centrifugation at 12.000 g for 20 minutes at 4 ºC, supernatant was collected and used for enzymatic activity determination and protein quantification by Bradford (1976) method.

SOD activity was evaluated according to the capacity of the enzyme to inhibit photo reduction of nitrotetrazolium blue (NBT) (Giannopolitis & Ries, 1977) in medium containing potassium phosphate (50 mM, pH 7.8), methionine (14 mM), EDTA (0.1 µM), NBT (75 µM) and riboflavin (2 µM) with 50 µL of enzymatic extract, completing a final volume of 2 mL with distilled water. Readings were performed at 560 nm, taking into account that one SOD unit corresponds to the quantity of enzymes able to inhibit 50% NBT photo reduction in essay conditions.

APX activity was determined according to Nakano & Asada (1981), evaluating the oxidation rate of ascorbate for 2 minutes at 290 nm. For the analysis, reaction media composed by potassium phosphate (100 mM, pH 7.0) and ascorbic acid (0.5 mM) incubated at 37 ºC was used. Following incubation, H_2O_2 (0.1 mM) and 25 µL of enzymatic extract were added, completing a final volume of reaction of 2.0 mL immediately proceeding the reading.

CAT activity was determined according to Azevedo et al. (1998) with modifications, estimated by the decrease in the absorbance at 240 nm during the same reaction period for APX. Reaction media composed by potassium phosphate buffer (100 mM, pH 7.0) was also incubated at 37 ºC and before performing the reading, added with H_2O_2 (12.5 mM) and 15 µL of enzymatic extract, completing a final volume of 2.0 mL.

The experiment was conducted in a completely randomized design with four replicates, the data obtained subjected to analysis of variance, and means compared by Tukey test at 5% probability. The statistical software WinStat version 2.0 (Machado & Conceição, 2007) was used.

Results and Discussion

The water content of seeds from three rice cultivars after storage period at -15; 1 and 18 ºC temperatures decreased when compared to the values presented at the moment of storage (Table 1). However, even with this small reduction in moisture, these levels are considered suitable for safe storage of rice seeds, as they narrow the respiratory rate of seeds and, therefore, narrowing the uptake of reserves during respiration and deterioration speed (Machado & Biava, 2006).

The germination percentage before storage of seeds in different temperatures was 97 for cv. BRS Pelota and 96% for BRS Atalanta and BRS Firmeza cultivars. After storage period, seeds presented 91, 92 and 94% of germination for BRS Pelota, BRS Atalanta and BRS Firmeza cultivars, respectively, stored at -15 ºC. Seeds stored at 1 ºC obtained 87, 93 and 96% germination and the ones stored at 18 ºC presented 66, 0 and 0% germination for cv. BRS Pelota, BRS Atalanta and BRS Firmeza (Table 1). There was no difference among cultivars when stored at – 15 ºC, BRS Pelota, BRS Atalanta and BRS Firmeza cultivars obtained 91, 92 and 94% germinated seeds, respectively. But this was not observed when the seeds of the three cultivars were stored at 1 ºC, since in this temperature BRS Pelota cultivar presented significant difference for germination percentage (87%) when compared to cultivars BRS Atalanta and BRS Firmeza. The storage at 18 ºC allowed germination only for cv. BRS Pelota with a rate of 66% germination. Seeds of cultivars BRS Atalanta and BRS Firmeza did not show viability, which was ensured by tetrazolium test demonstrating that the embryo of the seeds when stored at 18 ºC temperature have lost viability after 10 years of storage (Figure 1).

Researches with sunflower seeds have shown that storage at low temperatures (10 °C) occasioned the maintenance of seed quality compared with those stored at 25 °C, but the reduction in germination occurred independent of temperature after three months of storage (Abreu et al., 2011). *Passiflora setacea* seed maintains viability when stored at subzero temperatures for three months, but after the eighth month of storage the seeds not treated with gibberellic acid do not germinate and those treated with GA3 showed very low germination (3-15%) (Pádua et al., 2011).

When comparing the percentage of germinated seeds before and after storage, it was possible to verify that, in general, there was a decrease in germination after the period of storage. However, seeds of cv. BRS Firmeza stored at 1 ºC kept

Table 1. Moisture level (M), germination, first germination count (FGC) and cold test (Cold) in seeds of three irrigated rice cultivars stored over 10 years in different temperatures.

Variables (%)	Cultivar	-15	1	18	CV (%)
		°C			
Moisture	BRS Pelota	6.69	7.41	6.82	-
	BRS Atalanta	7.89	7.73	8.02	
	BRS Firmeza	8.06	7.98	7.96	
Germination	BRS Pelota	91 Aa	87 Bb	66 Ab	3.02
	BRS Atalanta	92 Aa	93 Aa	0 Bb	
	BRS Firmeza	94 Aa	96 Aa	0 Bb	
FGC	BRS Pelota	91 Aa	86 Ba	56 Ab	4.84
	BRS Atalanta	83 Bb	93 Aa	0 Bc	
	BRS Firmeza	85 Bb	93 Aa	0 Bc	
Cold	BRS Pelota	65 Ca	56 Cb	16 Ac	6.32
	BRS Atalanta	78 Ba	80 Ba	0 Bb	
	BRS Firmeza	90 Aa	89 Aa	0 Bb	

Upper case both in the column and lower case in line are not different between them according to the Tukey test (p≤0,05).

Figure 1. Tetrazolium test in seeds of cultivars BRS Atalanta (a) and BRS Firmeza (b) stored over ten years at 18 ºC.

the germination standards required for marketing of irrigated rice seeds, which is above 80% (Rio Grande do Sul, 2000).

The first germination count test may be considered an indicative of liveliness, once the results demonstrated that storage at – 15 and 1 ºC resulted in higher liveliness of seeds for cv. BRS Pelota seeds with a percentage of 91 and 86% for the first germination count (FGC), respectively. But in the storage at 18 ºC FGC was lower for this cultivar (Table 1). For cultivar BRS Atalanta and BRS Firmeza FGC was higher for seeds stored at 1 ºC temperature. In researches made in *Tabebuia serratifolia* seed stored in a cold room (8 °C) and in ambient conditions (22 °C) was observed that in the PCG test, the seeds stored in a cold room had 90% normal seedlings after twelve months of storage. Otherwise, the seeds stored in ambient conditions, the reduction in the percentage in the PCG was continuous, becoming zero after nine months of storage (Silva et al., 2011). Although, this test is not enough to assure the performance of seeds in field conditions even with elevated percentage, since its potential also depends on external environmental conditions (Nascimento & Pereira, 2007).

In face of this, the cold test with soil is an important parameter to be analyzed because it evaluates the physiological quality of seeds with a combination of low temperature, high moisture level of substrate and pathogenic agents (Marcos Filho, 2005). The results observed for the cold test showed that cv. BRS Pelotas showed the lowest force values obtained 65, 56, 16% at temperatures of -15, 1 and 18 °C respectively. For BRS Atalanta and BRS Firmeza, the seeds coming from storage at -15 and 1 °C had higher vigor. The cv. BRS Atalanta and BRS Firmeza stored at -15 °C obtained 78 and 90%, however the seeds stored at 1 °C showed 80 and 89% of germinated seeds in the cold test, respectively. It detaches that values observed for these two cultivars and storage temperatures were significantly higher to those observed for seeds of cv. BRS Pelota (Table 1).

Seeds with high physiological potential must have, at least, 70 and 85% normal seedlings in the cold test (Grabe, 1976), and the data obtained were 78, 90, 80 and 89% normal seedlings for seeds of cultivars BRS Atalanta and BRS Firmeza stored at -15 and 1 ºC, respectively demonstrating that these cultivars preserve the physiological potential for these two storage temperatures.

Regarding the parameters of seedling growth, there was an alteration among cultivars (Table 2), for cv. BRS Pelota, shoots (SL) and dry matter (DML) length were significantly higher when seeds were stored at 18 ºC. For cv. BRS Atalanta there was a significantly increase in SL for seeds stored at -15 ºC and for cv. BRS Firmeza, the highest seedling shoot and dry matter lengths were observed after storage of seeds at 1 ºC. In these two cultivars, shoot dry matter has not differed between storage temperatures of -15 and 1 ºC.

The root length (RL) in cv. BRS Pelota demonstrated better response for seedlings from seeds stored at -15 and 18 ºC, which differed significantly from the storage at 1 ºC (Table 2). Length evaluation takes into account the measurement of physical quantities, which is justified by the fact that seed lots with high germination percentage not always result in seedlings with higher growth, since these characteristics depend on the size of seeds, initial stage of cell division as well as constitution of reserve tissue of seeds (Vanzolini et al., 2007; Guedes et al., 2009; Socolowski et al., 2011)

The dry matter of root (DMR) of the cultivar BRS Pelota was significantly higher only in the storage at -15 ºC. According to Patané et al. (2006), depending on the storage conditions, seeds may undergo severe stress, leading to a fast uptake of their reserves just as the beginning of the germination process and a fast growth, fact that may explain the higher shoot length and root dry matter of seedlings of cv. BRS Pelota from seeds stored at 18 ºC (Table 2). This process may also have led to a decreased advance in synthesis processes, causing decreased germination and liveliness (Table 1). This response may be related to the deterioration process of seeds stored at 18 ºC, which occurs gradually, emerging in seeds with a sequence of biochemical or physiological events (Marini et al., 2012).

In cultivars BRS Atalanta and BRS Firmeza, results obtained for Length of roots and Dry matter of roots did not differ between storage temperatures -15 and 1 ºC, emphasizing that growth parameters were not computed for these two cultivars when stored in conservation chamber, since there were no germinated seeds in this condition (Table 1).

Table 2. Shoots (SL) and root (RL) length and dry matter of shoot (DMS) and root (DMR) of seedlings of the germination test of seeds from three rice cultivars stored in different temperatures.

Variables	Cultivar	-15	1	18	CV (%)
		°C			
SL (cm)	BRS Pelota	9.29 Bb	9.16 Bb	11.80 Aa	3.16
	BRS Atalanta	10.19 Aa	9.56 Bb	0 Bc	
	BRS Firmeza	9.6 Bb	10.04 Aa	0 Bc	
RL (cm)	BRS Pelota	11.81 Ab	8.21 Bb	10.67 Aa	10.06
	BRS Atalanta	9.92 Ba	10.75 Aa	0 Bb	
	BRS Firmeza	8.63 Ba	11.20 Aa	0 Bb	
DMS (mg)	BRS Pelota	43.43 Bb	47.25 Aab	50.6 Aa	10.19
	BRS Atalanta	51.93 Aa	53.81 Aa	0 Bb	
	BRS Firmeza	48.18 Aa	47.87 Aa	0 Bb	
DMR (mg)	BRS Pelota	52.43 Aa	29.87 Bb	31.50 Ab	11.47
	BRS Atalanta	32.93 Ca	33.56 Ba	0 Bb	
	BRS Firmeza	41.25 Ba	40.75 Aa	0 Bb	

Upper case both in the column and lower case in line are not different between them according to the Tukey test (p≤0,05).

The activation of anti-oxidant defense mechanism in rice seedlings from seeds stored at -15, 1 and 18 ºC after five and 14 days following sowing was quantified through the activity of SOD, CAT and APX enzymes. For cv. BRS Atalanta and BRS Firmeza, these enzymes were not evaluated in storage condition at 18 ºC due to the lack of seed germination after storage.

Amongst enzymes involved in removing reactive oxygen species (ROS) made up in stress conditions, SOD is the first in the defense line against oxidative stress (Pompeu et al., 2008; Pereira & Martins Filho, 2012). However, the activity of this enzyme did not exhibit significant difference at five DAS among analyzed seedlings of three rice cultivars from seeds stored at -15 and 1 ºC (Table 3). The same behavior was observed for CAT and APX enzymes indicating that in these storage conditions there was no induction of stress in seedlings, not intensifying anti-oxidant enzymes activity. Although, APX activity in rice seedlings of cv. BRS Pelota at five days after sowing presented interactions between different storage temperatures, and values significantly higher in comparison to those analyzed in seedlings from seeds stored at -15 ºC were observed for storage at 1 and 18 ºC (Table 4). APX enzyme has high affinity for H_2O_2 since it can eliminate efficiently this radical during oxidative stress (Hasanuzzaman et al., 2012).

The results indicate high ROS production in seedlings from seeds stored at 1 and 18 ºC in comparison to the storage at -15 ºC, this fact may be related to the responses obtained in tests of physiological quality evaluation of seeds from this cultivar at three storage temperatures, where the best response was obtained for storage at -15 ºC. These results have already been observed in other studies, evidencing seeds which exhibited lower activity of this enzyme presenting higher physiological activity and, therefore, lower ROS production characterizing lower de-structuration of the membrane system and lower seed deterioration level (Borba et al., 2014).

The evaluation of SOD and APX enzymes activity in rice seedlings of three cultivars at 14 DAS in storage conditions of -15 and 1 ºC did not exhibit significant variance among cultivars (Table 5). On the other hand, CAT activity presented interactions for storage temperature and cultivar factors, observing activity significantly higher in cv. BRS Atalanta in storage conditions at -15 ºC when compared to the storage at 1 ºC (Table 6). The lowest CAT activity was observed in seedlings of cv. BRS Atalanta 14 DAS from seeds stored at -15 ºC, possibly related to their higher liveliness (Table 1), since seedlings with higher liveliness tend to lower generation of free-radical, such as the radical H_2O_2, for instance, which is the substrate for CAT enzyme acting.

CAT has low affinity for H_2O_2 and it is more active only when this compound accumulates (Jaleel et al., 2009). Therefore, more liveliness seeds such as the seeds of cv. BRS Atalanta which has been stored at 1ºC exhibited lower production of this radical, evidencing lower reason to activate the enzymatic defense system.

Most works analyzing the activity of anti-oxidant enzymes (SOD, APX and CAT) relate the influence of abiotic and biotic stress in different crops (Cakmak et al., 2010; Mei & Song, 2010; Cai et al., 2011). In these cases, the lower activity of the enzymes refers to seeds and seedlings with low viability and liveliness (Demirkaya et al., 2010; Chauan et al., 2011; Prodanović et al., 2012). However, studies exploring the relation between the liveliness of the seeds and the activity of these enzymes were not found in literature.

Table 4. Activity of ascorbate peroxidase (APX) enzyme in rice seedlings of cv. BRS Pelota five days after sowing from seeds stored over ten years in different temperatures.

APX (μmol ASA min^{-1} mg^{-1} Prot.)	
Environment	**BRS Pelota**
-15 °C	0.8764 b
1 °C	1.2723 a
18 °C	1.4041 a
CV (%)	16.39

Means followed by the same letter do not differentiate between them by Tukey test (p≤0,05).

Table 6. Activity of catalase (CAT) enzyme of seedlings from the 14th day after sowing of seeds from cultivars BRS Pelota, BRS Atalanta and BRS Firmeza stored in -15 and 1 ºC over ten years.

CAT (μmol H_2O_2 min^{-1} mg^{-1} Prot.)		
Cultivars/environment	**-15 °C**	**1 °C**
BRS Pelota	0.0688 Aa	0.0764 Aa
BRS Atalanta	0.0978 Aa	0.0596 Ab
BRS Firmeza	0.0740 Aa	0.0791 Aa
CV (%) 21.72		

Upper case both in the column and lower case in line are not different between them according to the Tukey test (p≤0,05).

Table 3. Activity of superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) enzymes of seedlings from three rice cultivars, five days after sowing (DAS) of seeds stored over ten years at -15 and 1 ºC.

Cultivar	SOD (U mg^{-1} Prot.)	CAT (μmol H_2O_2 min^{-1} mg^{-1} Prot.)	APX (μmol ASA min^{-1} mg^{-1} Prot.)
BRS Pelota	0.6527^{ns}	0.0960^{ns}	1.0743^{ns}
BRS Atalanta	0.7186^{ns}	0.0894^{ns}	1.3301^{ns}
BRS Firmeza	0.6693^{ns}	0.0981^{ns}	1.2467^{ns}
CV (%)	18.14	20.88	19.95

Not significant, at 5% probability, Tukey test.

Table 5. Activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX) enzymes of rice seedlings of cultivars BRS Pelota, BRS Atalanta and BRS Firmeza 14 days after sowing from seeds stored in -15 and 1 ºC over ten years.

Cultivar	SOD (U mg^{-1} Prot.)	APX (μmol ASA min^{-1} mg^{-1} Prot.)
BRS Pelota	0.5713^{ns}	0.9835^{ns}
BRS Atalanta	0.6962^{ns}	1.0617^{ns}
BRS Firmeza	0.7321^{ns}	1.2961^{ns}
CV (%)	25.91	17.08

Not significant, at 5% probability, Tukey test.

Thereby, the increase observed in the activity of APX and CAT enzymes in different storage conditions indicate higher ROS production and therefore emphasize the beginning of the deterioration process settling a reduction in seeds liveliness (El-Shabrawi et al., 2010). Thus, when evaluating the enzymatic activity of seedlings from seeds stored in different temperatures, it was possible to notice that the lowest activities of the anti-oxidant defense system were detected in seeds stored in temperatures which allow better conservation of their physiological potential.

Conclusion

Rice seeds of cultivars BRS Pelota, BRS Atalanta and BRS Firmeza do not keep their viability and liveliness after 10 years of storage at 18 ºC, but they do it when stored at -15 and 1 ºC.

The lowest enzyme activity of the anti-oxidant defense system indicates seed with higher liveliness.

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