academicJournals

Vol. 14(31), pp. 2434-2441, 5 August, 2015
DOI: 10.5897/AJB2015.14568
Article Number: 9E2B0F554556
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Physiological and enzymatic changes in rice seeds stored at low temperatures

Raimundo Wagner de Sousa Aguiar*, Deyvid Rocha Brito, Magnólia de Mendonca Lopes, Gil Rodrigues dos Santos, Clovis Maurilio Sousa, Ezequiel Marcelino Marcelino da Silva and Julcemar Didonet

Federal University of Tocantins, Department of Plant Production, University Campus Gurupi, Tocantins 77410-530, Brazil.

Received 14 March, 2015; Accepted 15 July, 2015

This study aimed to evaluate the effect of low temperatures on the physiological and enzymatic changes of rice seeds. The seeds were packed in airtight chambers and maintained at temperatures of 8 and -50°C for periods of 15, 30 , 45, 60, 75 and 90 days. The same procedure was adopted for the control treatment with the seeds kept at a temperature of 25°C. The seeds were evaluated regarding germination test; seedling emergency; emergency speed index; length and dry weight of radicle; and seedling of shoot. The activity of amylase and total protein content were also evaluated. The temperatures of 8 and -50°C significantly influenced the physiological quality and the enzyme amylase activity of rice seeds, resulting in higher germination, seedling emergence and enzyme activity. The temperature is a promising alternative for the maintenance of physiological quality and enzymatic activity of rice seeds during storage.

Key words: *Oryza sativa* L., enzymatic activity, physiological quality, storage.

INTRODUCTION

The conservation of seed quality during the storage period is a key factor to be considered in the complex system of seed production, since the establishment in the field is directly related to the physical, physiological and sanitary conditions of the seed. Among the factors that determine the maintainability of the quality of seeds during storage are natural or modified conditions that favor their conservation (Carvalho and Villela, 2006), such as the storage temperature (Caldwell et al., 2005; Toledo et al., 2009). Temperature plays a main role in influencing the rates of biochemical processes and indirectly affecting the water content of the seeds. Therefore, the period of seed viability can be increased by reducing the humidity and storage temperature. This reduces the respiratory rate of the seeds and, therefore, minimizes the degradation of its reserve tissue (Bewley

*Corresponding author. E-mail: rwsa@uft.edu.br.

Abbreviations: ESI, Emergence speed index; **PVP,** polyvinylpyrrolidone.

and Black, 1994; Takaki, 2004). Low storage temperatures favor the maintenance of biochemical processes in the embryo, subsequently allowing normal seedling development and uniform germination. High temperatures, according to MacDonald (2000), may denature proteins and alter membrane permeability, thereby, accelerating deterioration.

The initial maintenance of physiological processes of seed depends on structural enzymes which have specific requirements for temperature (Bewley and Black, 1994). During seed germination, the accumulated material in starchy endosperm is mobilized by enzymes that are synthesized and secreted (Devi et al., 2007). Among the enzymes, the amylases are predominantly synthesized during germination and they hydrolyze the starch granules to produce monosaccharides, which are energy source for the development of seedlings (Helland et al., 2002). Generally, the α-amylase hydrolyzes the starch granules; β-amylase and α-glucosidase have a minor role only in the hydrolysis of dextrin, an α-amylase product. In this sense, for the quality control program of seed production, monitoring the amylase activity in rice seeds is extremely important, especially when the objective is to produce viable and of high quality seeds. Thus, the test of α-amylase may be of fundamental importance to evaluate the physiological potential of seeds after storage period (Panobianco et al., 2007).

This study aimed to evaluate the physiological changes that occur in rice seeds stored at low temperatures.

MATERIALS AND METHODS

The cultivars of the rice seeds used were IRGA 423 and 424, obtained from seed producers in the municipalities of Formoso do Araguaia (11°47'48" S latitude, 49°31'44 "W longitude) and Lagoa da Confusão (10°47'37" S latitude, 49°37 '25" W longitude), at the state of Tocantins - Brazil. After the standardization of the humidity at 11%, the seeds were placed in airtight chambers with volume of 2 L and stored at the temperatures of 8 ± 1°C and -50 ± 1°C, the control kept in paper bags at room temperature around 25°C. The seeds remained stored during periods of 15, 30, 45, 60, 75 and 90 days.

Germination test

Initially, the dormancy and initial germination was determined with the seeds that were obtained after the moisture uniformity. Four samples of 50 seeds, for each repetition, were placed on germitest paper moistened with sterile distilled water at a ratio of 2.5 times the weight of paper and placed in a climatic chamber at 25°C. At 14 days after seeding, there was the final count of germination, by determining the percentage of normal seedlings (Brasil, 2009).

Length and dry weight of seedlings

The evaluation was performed according to the method described by Vanzolini et al. (2007), with seedlings that originated during the germination test, randomly constituting four replications of 10 seedlings. The shoot and root length (cm.plântula^{-1}) was determined through a millimeter ruler, with the mean obtained by the sum of each repetition divided by the number of normal seedlings. To determine the dry mass, four replications of ten normal seedlings were placed in paper bags and taken to the greenhouse at 70°C until constant weight. The weigh-in was held in 0,001 g precision scale and the data were expressed in grams seedling^{-1}.

Seedling emergence

It was performed according to the method used by Fleck et al. (2003) using plastic trays with dimensions of 50 × 25 × 15 cm; the seeds were sown in 10 cm thick at properly sterilized sand. At the end of each storage period (15, 30, 45, 60, 75 and 90 days), four replications of 100 seeds of each cultivar were performed. The emerged seedlings were performed at five periods (6, 8, 10, 12 and 14 DAE) and the results expressed as a percentage of normal seedlings. For the emergence speed index (ESI), we used the formula Maguire (1962), in which ESI = N1 / N2 + D1 / D2 + ... + Nn / Dn where: ESI = seedling emergence speed index; N = number of emerged seedlings, computed from first to last count; D = number of days from sowing of first to last count.

Specific activity of amylase

The evaluation of the specific activity of amylase was performed with seeds after 90 days storage. The germination of seeds was induced by incubating them in Petri dishes lined with filter paper moistened with distilled water and kept at room temperature. After induction of germination at periods of 0, 12, 24, 48, 72 and 96 h, the amylase extraction was performed according to the methodology used by Jose et al. (2004). The extractions were performed through maceramento - seeds in the presence of liquid nitrogen (N_2) in porcelain mortar. For each treatment, 4 extractions were performed, each extraction constituted in a repetition. Then, 200 mg of seed powder were suspended in 600 µL of extraction buffer (Tris – HCl 0.2 M (pH = 8.0) with 0.4% of PVP (Polyvinylpyrrolidone) where they remained under agitation in table at 6°C for 12 h. After this period, the solution was transferred to Eppendorf tubes and centrifuged at 4000 rpm for 1 h at 4°C. After, a process for achievement of the enzyme extract was held in order to review the specific amylase activity using the methodology described by Miller (1959), which were used 40 µL for each treatment of the extracts in 60 µL of 50 mM sodium acetate buffer, pH = 5.5 with 100 uL of 0.5% starch solution and maintained for 3 h of incubation at 50°C. After that, the reaction was quenched by the addition of 1000 µL DNS reagent and boiled again for 10 min. Reducing sugars present in the enzyme extract were deducted from the calculation of enzyme activity. For this, 40 µL of the enzyme extract were added to 160 µL of the same buffer and the mixture was incubated under the same test conditions prior to the addition of reagent DNS. Reducing sugars were determined by the DNS method compared to a calibration curve prepared with glucose solution at different concentrations within the same test conditions. The absorbance was read with a spectrophotometer at a wavelength of 540 ηm zeroed with white consisting of 40 uL of distilled water, 160 µL of the same buffer and 1 mL of DNS. Reading for dilutions were performed when necessary. The activity was expressed as µmol of reducing sugar released per minute of reaction per mg of total protein.

Determination of total proteins

The determination of the total protein present in the extracts of the seeds was carried out according to the methodology described by

Table 1. Percentage of germination (G), seedling emergence (SE) and the emergence speed index (ESI) in seeds of rice (cultivars IRGA 423 and 424) depending on room temperature (25, 8 and -50°C); and periods of storage (15, 30, 45, 60, 75 and 90 days).

Cultivar		Storage (days)																	
		15			30			45			60			75			90		
		Temperature (°C)																	
		25	8	-50	25	8	-50	25	8	-50	25	8	-50	25	8	-50	25	8	-50
Irga423	G	83^{a}	92.5^{a}	91^{a}	82.7^{a}	93.5a	90^{a}	83^{a}	94^{a}	93^{a}	79.2^{b}	93.2^{a}	92.5^{a}	76.7^{b}	93^{a}	91.7^{a}	68.7^{b}	93.7^{a}	90^{a}
	SE	83.6^{a}	92^{a}	92.3^{a}	83.3^{a}	94^{a}	93^{a}	78.6^{b}	93^{a}	92^{a}	76^{b}	94^{a}	94.2^{a}	73^{b}	91^{a}	92.2^{a}	66.6^{b}	91^{a}	93^{a}
	ESI	51.5^{b}	64.2^{a}	64.7^{a}	50.8^{b}	62.9^{a}	64.6^{a}	54.4^{b}	71.7^{a}	69.6^{a}	52.9^{b}	62^{a}	61a	60,6^{b}	66.7^{a}	67.7^{a}	54.2^{b}	71.3^{a}	71.1^{a}
	VC(%)	9.0	7.8	10.2	9.3	8.7	8.0	7.4	12.4	10.6	6.4	9.7	8.4	9.7	13.8	14.9	9.4	11.8	13.9
Irga424	G	91.2^{a}	91.7^{a}	92.2^{a}	90.5^{a}	92^{a}	93.2^{a}	90.5^{a}	92.5^{a}	92^{a}	83.5^{a}	93^{a}	92.7^{a}	83.5^{b}	92.7^{a}	92.5^{a}	80.5^{ab}	93^{a}	91.2^{a}
	SE	94.3^{a}	92^{a}	93^{a}	91^{a}	92^{a}	92.3^{a}	90.3^{a}	92.3^{a}	92.3^{a}	83^{a}	93^{a}	92.3^{a}	82^{a}	92.3^{a}	92.6^{a}	81^{b}	93^{a}	92.8^{a}
	ESI	57.8^{a}	63.2^{a}	71.1^{a}	63^{a}	70.9^{a}	62.6^{a}	62.3^{a}	71.2^{a}	75.2^{a}	58^{b}	69^{a}	61.8^{a}	61.5^{a}	68.2^{a}	72.1^{a}	60.5^{ab}	73.1^{a}	76.2^{a}
	VC(%)	11.8	8.9	8.7	9.0	14.8	12.3	10.9	8.7	6.9	7.9	9.8	8.6	11.9	15.9	7.9	8.0	7.2	12.9

Means followed by the same lower case letter in the line do not differ by Tukey test (P ≤ 0.05); CV: Coefficient of variation.

Bradford (1976), being used at a rate of 100 uL of sample extract with 1000 uL of Bradford reagent (Kit BIORAD®), with four replications per treatment. The quantitation of total protein was calculated through comparison with a calibration curve prepared with different concentrations of bovine serum albumin under the same test conditions. Readings were taken in a spectrophotometer at a wavelength of 595 ηm and the values expressed in mg/mL.

Amylase activity gel SDS-PAGE

The polyacrylamide gel was made 4.5% (concentrating gel) and 7.5% (separating gel containing 5% of soluble starch). The analysis of amylase activity was performed through the use of electrophoresis system under non-denaturing conditions as described by Jose et al. (2004) using a vertical electrophoresis Loccus Biotecnologia® as apparatus. The samples were suspended another time in 1X sample buffer (50 µL of Tris-HCl 0.5 mmol L^{-1} buffer pH 6.8, 100 µL glycerol, 0.005 mg of bromophenol blue and enough distilled water for 1.0 mL), boiled for 5 min in water bath and was applied in the gel. The race was made using a voltage of 100 V and 80 mA. After the run, the gels were stained by placing them on resublimed iodine for visualization of starch degradation bands.

Experimental design

The experimental design was completely randomized in a factorial arrangement 6 × 3 × 2, consisting of storage periods (15, 30, 45, 60, 75 and 90 days), three temperatures (room temperature: 25, -50 and 8°C) and two cultivars (IRGA 423 and 424). The comparison of means was performed through Tukey test (P≤0.05) using the statistical program SISVAR 5.0 (Ferreira, 2003).

RESULTS AND DISCUSSION

Germination

The control seeds were kept at a temperature of 25°C (Table 1). Temperatures above 25°C are considered harmful to maintain the physiological quality of rice seeds, depending on the storage period, due to the physiological and biochemical changes that occur gradually (Marini et al., 2012). Germination of IRGA 423 was lower when stored at room temperature (average 25°C) after 15 days storage (Table 1). For IRGA 424, the percentage of germination was affected negatively and significantly by the temperature after 70 days of storage, at 25°C. Storage temperature directly influences the activity of gas exchange in seeds. Mild temperatures decrease the rate of gas exchange (Patane et al., 2006), favoring the maintenance of physiological quality of stored seeds, verified by the highest percentage of germination. The results of the emergency speed index were similar to the results of the germination test (Table 1). For both varieties stored at 8°C and -50°C, significant differences became most pronounced in 90 days in relation to seeds stored at room temperature, resulting in a reduction of the emergence speed. Reducing the emergency speed of seeds stored at room temperature may be related to the consequent deterioration, which is probably associated to temperature fluctuations and varying humidity, occurring on site during storage. Tunes (2014) showed that seeds stored under conditions of temperature fluctuation and

Table 2. Length and dry weight of shoot radicle and rice seedlings (cv. IRGA 423) depending on room temperature (25 ° C), -50 ° C and 8°C, storage periods (15, 30, 45, 60, 75 and 90 days).

Periods (days)	Radicle length (cm)			Aerial part length (cm)		
	25°C	08°C	-50°C	25°C	08°C	-50°C
15	12.8[a]	13.4[a]	13.2[a]	16.1[a]	16.0[a]	15.9[a]
30	12.0[a]	12.8[a]	13.0[a]	15.5[a]	16.1[a]	16.0[a]
45	11.8[a]	12.5[a]	13.1[a]	13.6[b]	15.9[a]	16.2[a]
60	10.7[b]	11.7[a]	12.9[a]	12.4[b]	16.5[a]	16.1[a]
75	8.1[c]	11.2[b]	13.5[a]	12.3[b]	16.8[a]	16.5[a]
90	6.5[c]	11.5[b]	13.3[a]	9.8[b]	15.6[a]	15.8[a]
VC%	8.31			8.16		
Periods (days)	**Weight of radicle (g)**			**Weight of Aerial Part (g)**		
	25°C	08°C	-50°C	25°C	08°C	- 50°C
15	0.0126[a]	0.0128[a]	0.0131[a]	0.0180[a]	0.0181[a]	0.0182[a]
30	0.0127[a]	0.0132[a]	0.0132[a]	0.0172[ab]	0.0184[a]	0.0184[a]
45	0.0125[b]	0.0133[ab]	0.0137[a]	0.0175[a]	0.0176[a]	0.0180[a]
60	0.0107[a]	0.0129[a]	0.0129[a]	0.0145[a]	0.0172[a]	0.0178[a]
75	0.0090[b]	0.0131[a]	0.0132[a]	0.0131[b]	0.0171[a]	0.0176[a]
90	0.0091[b]	0.0133[a]	0.0130[a]	0.0127[b]	0.0170[a]	0.0172[a]
VC%	6.11			6.42		

Means followed by the same lower case letter in the line do not differ by Tukey test (P≤0.05).

varying humidity may have reduced viability compared with those stored under constant temperature and humidity.

In general, the results obtained also show that the storage temperature is an important factor for the preservation of rice seeds, directly influencing the physiological seed quality. Storage at low temperatures (-50 and 8°C) promoted the conservation of seeds of both cultivars (IRGA 423 and 424). This process may be associated to the decrease of respiration and, consequently, reduction of the metabolism and maintenance of enzymes, reserve tissues and membrane permeability, as evidenced by higher germination during storage up to 90 days compared to the control (Table 1). According to Filho (2005), low temperature conditions allow for the reduction of seed metabolism, contributing to a longer life, while higher temperature conditions produce a more rapid loss of seed viability and the accelerated metabolic reactions often result in increased water content.

Length and dry weight of seedling

The results for radicle (Fc = 31.750; Pr > Fc = 0.004) (P ≤ 0.05) and for shoot (Fc = 42.313; Pr > Fc = 0.001) (P ≤ 0.05) of seedling for both cultivars (IRGA 423 and 424) showed superiority of seedlings originated from seeds stored at low temperatures (Table 2). Seeds that were stored at 25°C originate seedlings with shorter length of the primary root and shoot, after 60 days of storage for IRGA 423 and 30 days for IRGA 424 (Tables 2 and 3). Seedlings originated from seeds stored at -50 and 8°C that remained with similar sizes from beginning until the end of the experiments, especially the ones -50°C, which showed higher values than at the end of 90 days of storage (Tables 2 and 3).

With the dry weight of shoot and radicle, it was observed that the seeds of both cultivars (IRGA 424 and 423), kept at room temperature conditions, had their dry matter reduced, especially for IRGA 424, with a significant difference at 90 days storage; while at low temperature treatment (-50 and 8°C), maintained constant mass at storage periods (Tables 2 and 3). This may be due to the inhibition of respiration and, thus, the preservation of the reserves of seeds (Zuchi and Bevilaqua, 2012).

Specific amylase activity

The specific activities of amylase were more significant for cultivar IRGA 423 than for cultivar IRGA 424 among all established treatments (Figure 1A and 1B). Seeds stored at room temperature did not show a significant increase in amylase activity (Figure 1A). However, the seeds of IRGA 423 stored under refrigeration at 8 and -50°C for 90 days showed a significant increase in amylase activity in the first 12 h of germination, reaching approximately 0.5 up to 0.8 IU / mg, respectively. There was a decline of activity within 24 h up to 96 h and consequent increase in germination. The same effect was observed in the specific activity of amylase for seed

Table 3. Dry mass and length of the primary root and shoot of rice seedlings (cv. IRGA 424) depending on room temperature (25, 8 and -50°C) and storage periods (15, 30, 45, 60, 75 and 90 days).

Período	Radicle length (cm)			Aerial part length (cm)		
(days)	25°C	08°C	-50°C	25°C	08°C	-50°C
15	13.7[b]	13.7[b]	13.2[ab].	16.5[a]	16.0ab	16.5[a]
30	12.2[b]	13.5[a]	13.6[a]	13.9[c]	16.6a	16.3[ab]
45	11.3[b]	13.4[a]	13.5[a]	13.1[b]	15.8ab	16.5[a]
60	11.1[c]	13.7[ab]	13.3[a]	13.3[b]	15.5a	15.9[a]
75	8.3[c]	12.9[ab]	13.6[a]	12.6[b]	15.3a	16.0[a]
90	6.9[b]	13.0[a]	13.2[a]	10.2[b]	14.9ab	15.7[a]
CV%		9.16			7.43	

Período	Weight of radicle (g)			Weight of Aerial Part (g)		
(days)	25°C	08°C	-50°C	25°C	08°C	- 50°C
15	0.0140[ab]	0.0135[a]	0.0136[a]	0.0180[a]	0.0185[a]	0.0187[a]
30	0.0130[b]	0.0137[ab]	0.0135[a]	0.0170[a]	0.0180[a]	0.0188[a]
45	0.0111[a]	0.0131[a]	0.0133[a]	0.0172[a]	0.0183[a]	0.0186[a]
60	0.0112[a]	0.0130[a]	0.0136[a]	0.0161[a]	0.0179[a]	0.0188[ab]
75	0.0104[a]	0.0129[a]	0.0133[a]	0.0158[a]	0.0174[a]	0.0184[a]
90	0.0097[c]	0.0126[b]	0.0131[ab]	0.0128[b]	0.0176[a]	0.0183[a]
CV%		7.51			7.13	

Means followed by the same lower case letter in the line do not differ by Tukey test (P≤0.05).

IRGA 424 stored at low temperatures for 24 up to 48 h of germination (Figure 1B). The amylase activity tends to increase significantly as the germination process is intensified, which may also be linked to the fact that low temperatures induce the biosynthesis of gibberellin - the precursor of biosynthesis of the amylase hormone (José et al., 2004; Neves and Moraes, 2005). Veluppillai et al. (2009) observed, from the first day, a significant increase in rice seed germination through the reduction of sugars and endogenous amylase activity; followed by a linear sharp rise on the third day of germination. Amylase expression during grain germination of cultivation can vary from one to another (Nandi et al., 1995), which was also observed in this study. Excluding only the amylase expression as an important factor, it is possible to affirm that the practice of seed germination has been used to improve their nutritional value. Germination has an important effect on the chemical composition, nutritional value and acceptability of the product for human consumption (Bailly, 2004).

Amylase activity gel SDS-PAGE

Through the analysis of the amylase activity in non-denaturing SDS-PAGE containing 5% starch, it is clearly observed that amylase activity was seen in the starch and it was possible to see starch degradation bands in the gels (Figure 2). Regarding both cultivars, it is possible to see that the amylase activity was more pronounced during germination, and that low temperature (-50 and 8°C) treatments stand out in comparison to the control treatment, especially within 96 hours of germination. These results corroborate with those found in the specific activity of amylase (Figure 1), in which the seeds stored under low temperatures (-50 and 8°C), for 90 days, presented higher specific activity of amylase during germination, in comparison to the control.

Total protein

The statistical analysis of total protein content recovered from all treatments during the germination period showed no significant differences by Tukey test at 95% confidence (Figure 3A and 3B). The total protein content and constant during the process of germination of rice seeds for 5 days, was also observed by Veluppillai et al. (2009). According to these results and considering that the specific activity is the amount of amylase activity per mg protein (UI/mg), it is possible to infer that the amylase expression occurred with greater intensity during germination of seeds that were stored at low temperatures (Figures 1A and 1B). For Petruzzelli and Taranto (2010) the development of amylase activity is an important event, which can be detected at the beginning of seed germination, and its primary role is to provide substrates for seedling use until they become photosynthetically independent. Therefore, the high specific activities of amylase observed in treatments performed with seeds stored at low temperatures for 90 days confirm the results of germination, seedling emergence

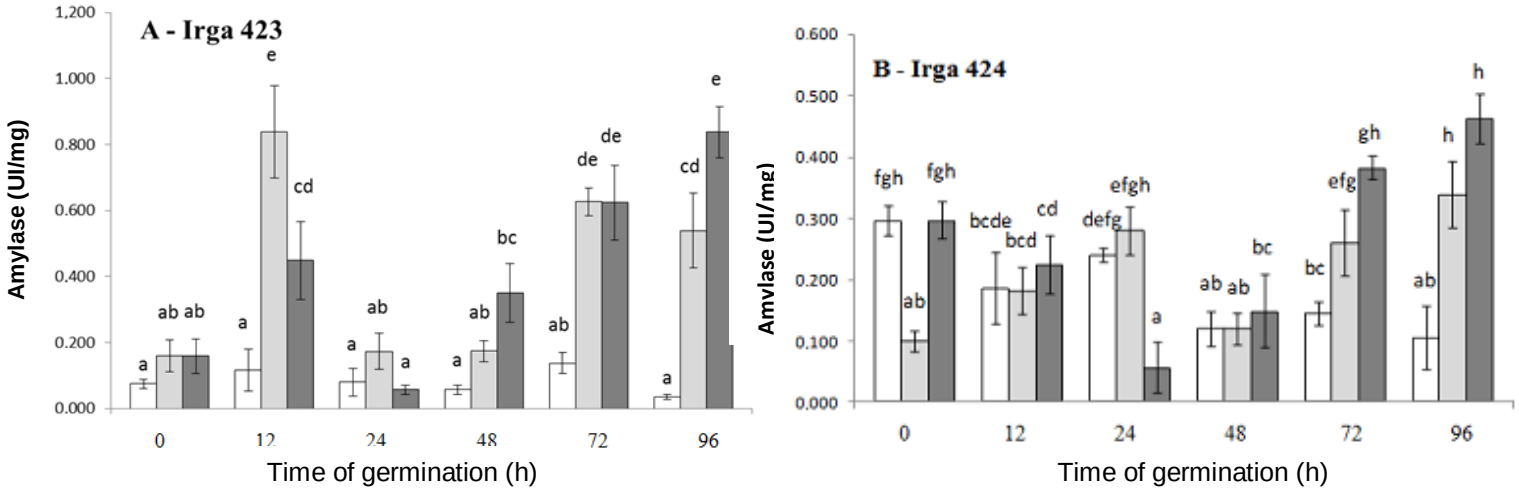

Figure 1. Activity of amylase extracted from rice seeds (*Oryza sativa* L.) after 90 days of storage under treatments. ☐ room temperature; ▒ 8°C and ■ -50°C. **A =** Cultivar IRGA 423 and **B =** Cultivar IRGA 424. Means followed by the same lower case letter in the columns do not differ by Tukey test at 95% confidence. The bars represent the standard error.

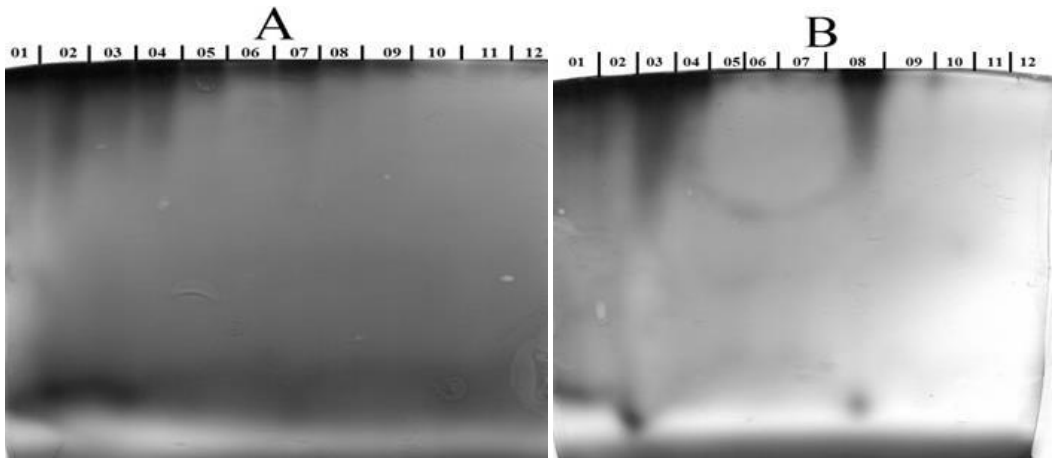

Figure 2. Evaluation of amylase activity extracted from rice seeds (*Oryza sativa* L.) polyacrylamide gel (5% starch) after 90 days of storage at room temperature (25°C), -50 and 8°C. **A**- Cultivar IRGA 423 and **B-** Cultivar IRGA 424. Sequence: **1**) control: 12 h of germination; **2**) 08°C: 12 h of germination; **3**) -50°C: 12 h of germination; **4)** control: 48 h of germination; **5)** 8°C: 48 h germination; **6**) -50°C: 48 h germination; **7**) control: 72 h of germination; **8**) 08°C: 72 h germination; **9)** -50°C: 72 h germination; **10**) control: 96 h of germination; **11**) 08°C: 96 h of germination; and **12**) -50°C: 96 h of germination.

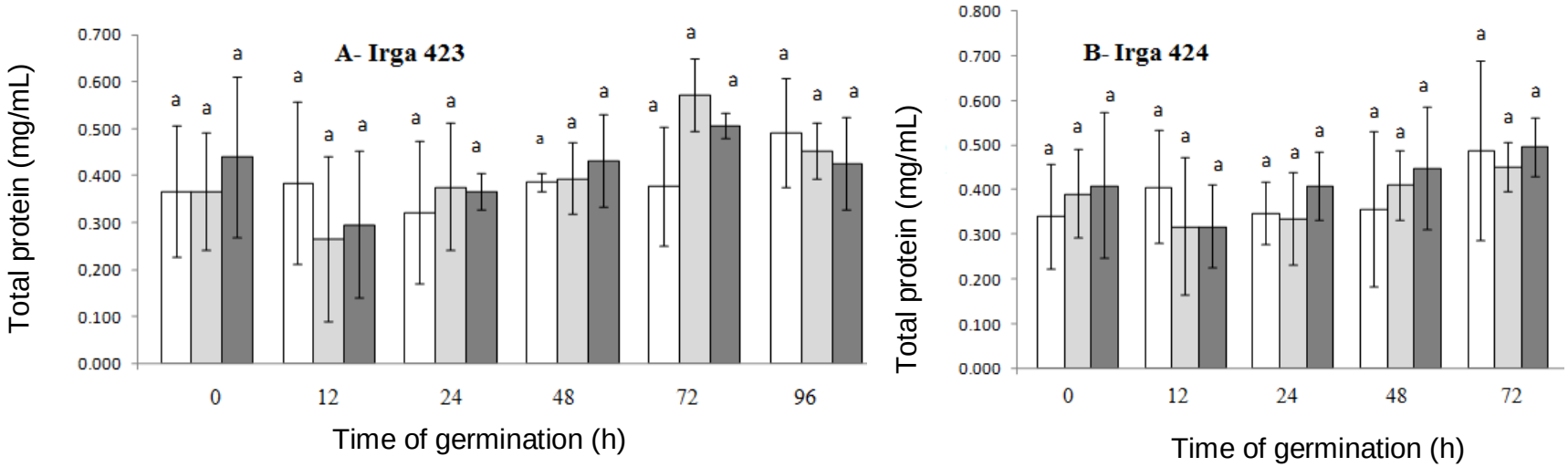

Figure 3. Total protein extracted of rice seeds (*Oryza sativa* L.), during germination after 90 days of storage under treatments: ☐ room temperature ▨ 8°C and ■ -50°C. The bars represent the standard error.

and emergence speed index of rice seeds (Table 1).

According to José et al. (2004), when seeds are exposed to high temperatures, the cellular membrane system ruptures and disintegrates, possibly due to changes in the constituent lipids. The solubility and protein binding capacity can also be reduced, causing injuries to the mitochondrial structure, thereby affecting the respiratory rate, and other subcellular systems. This results in metabolic and biochemical changes that are involved in the prevention of injuries caused by high temperatures, which directly interferes with amylase activity during germination.

Based on germination, emergence speed and seedling quality assessments, the reserve tissues of seeds stored under uncontrolled conditions may have been affected by the deterioration caused by high temperatures during storage. The starch in the seeds' reserves and, consequently, the expression of amylase activity may also have been affected by this deterioration. Spinola et al., (2000), concluded that monitoring enzyme change, rather than physiological assessment markers, may be more effective in detecting metabolic alterations indicating the beginning of the seed decay process during storage. However, the cultivars IRGA 423 and 424 stored at low temperatures remained protected from these deleterious effects, which allowed them to express their genetic potential.

In general, storage temperature influenced the physiological quality of rice seeds. Although inevitable and irreversible, the process of seed deterioration can be reduced by storage at appropriate temperatures. At low temperatures, the biochemical and physiological changes that cause seed deterioration are reduced, resulting in the maintenance of seed quality and, therefore, the quality of seedlings. Seeds stored at temperatures of −50°C and 8°C had higher germination rates compared with seeds stored at 25°C. Temperature is an important factor for the conservation of seeds, directly affecting the speed of biochemical processes and interfering with the increased amylase enzyme activity, making the activity favorable during the lag in rice seed germination.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

Barton LV (1961). Seed Preservation and Longevity. Interscience Publishers, London, UK. 216.

Bewley JD, Black M (1994). Seeds: physiology of development and germination. 2nd ed. Plenum Press, New York.

Bradford MMA (1976). Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein Dye Binding. Anal. Biochem. 72:248-254.

Brasil (2009). Ministério da Agricultura e Reforma Agrária. Regras para Análise de Sementes. Brasília: SNAD/CLAV, 398.

Caldwell CR, Britz SJ, Mirecki RM (2005). Effect of temperature, elevated carbon dioxide, and drought during seed development on the isoflavone content of dwarf soybean [*Glycine max* (L.) Merrill] grown in controlled environments. J. Agric. Food. Chem. 53(4):1125-1129.

Carvalho MLM, Vilella FA (2006). Armazenamento de sementes. Informe Agropecuário , Belo Horizonte 27(232):70-75.

Devi R, Munjral N, Gupta AK, Kaur N (2007). Cadmium induced changes in carbohydrate status and enzymes of carbohydrate metabolism, glycolysis and pentose phosphate pathway in pea. Environ. Exp. Bot. 61(02):167-174.

Ferreira DF (2003). Programa de análises estatísticas (statistical analysis sotware) e planejamento de experimentos – SISVAR 5.0 (Build 67). Lavras: DEX/UFLA.

Fleck NG, Junior AAB, Agostinetto D, Rizzardi MA (2003). Velocidade de estabelecimento em cultivares de arroz irrigado como característica para aumentar a habilidade competitiva com plantas concorrentes. Cienc. Rural 33(4): 635-640.

Helland MH, Wicklund T, Narvhus JA (2002). Effect of germination time on alpha-amylase production and viscosity of maize porridge. Food. Res. Int. 35:315-321.

Maguire JD (1962). Speed of germination aid in selection and evaluation for seedling emergence and vigor. Crop. Sci. 2(2):176-177.

Marini P, Moraes CL, Marini N, Moraes DM, Amarante L (2012). Alterações fisiológicas e bioquímicas em sementes de arroz submetidas ao estresse térmico. Rev. Ciência Agron. 43(4):722-730.

Matheus MT, Lopes JC (2009). Temperaturas cardinais para a germinação de sementes de *Erythrina variegata* L. Rev. Bras. Sementes 31(03):115-122.

Nandi S, Das G, Mandi SS (1995). β-amylase activity as an index for germination potential in Rice. Ann. Bot. 75: 463-467.

Neves LAS, Moraes DM (2005). Análise do vigor e da atividade da a-amilase em sementes de cultivares de arroz submetidas a diferentes tratamentos com ácido acético. Rev. Ciênc. Agrovet. 4(1):35-43.

Okamoto K, Akazawa T (1980). Enzymic mechanism of starch breakdown in germinating rice seeds. Plant physiol. 65: 81-84.

Panobianco M, Vieira RD, Perecin D (2007). Electrical conductivity as an indicator of pea seed aging of stored at different temperatures. Sci. Agric. 64:119-124.

Patane, C, Cavallaro V, Avola G, D"Agosta G (2006). Seed respiration of sorghum [*Sorghum bicolor* (L.) Moench] during germination as affected by temperature and osmoconditioning. Seed Sci. Res. 16(04):251-260.

Riley GJP (1981). Effects of high temperature on protein synthesis during germination of maize (*Zea mays* L.). Revista Planta 151:75-80.

Takaki M (1990). Effect of water stress on seed germination and seedling growth in *Oryza sativa* L. Biologia Plantarum 32:238-240.

Toledo MZ, Fonseca NR, Cesar ML, Soratto RP, Cavariani C, Crusciol CAC (2009). Qualidade fisiológica e armazenamento de sementes de feijão em função da aplicação tardia de nitrogênio em cobertura. Pesquisa Agropecuária Tropical 39(2):124-133.

Tunes LM (2009). Alterações fisiológicas e enzimáticas em sementes de cevada sob diferentes condições de armazenamento. Magistra, 21(3):154-164.

Vanzolini S, Araki CAS, Silva ACTM, Nakagawa J (2007). Teste de comprimento de plântula na avaliação da qualidade fisiológica de sementes de soja. Ver. Bras. Sementes. 29(2):90-96.

Veluppillai S, Nithyanantharajah k, Vasantharuba S, Balakumar S, Arasaratnam V (2009). Biochemical Changes Associated with Germinating Rice Grains and Germination Improvement. Rice Science 16(3):240-242.

Zuchi J, Bevilaqua GAP (2012). Qualidade fisiológica de sementes de arroz armazenadas em diferentes embalagens e temperaturas. Embrapa Clima Temperado – MAPA, Boletim de Pesquisa e Desenvolvimento 163:20.