Acta Scientiarum

http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v40i1.39347

CROP PRODUCTION

Isoenzyme activity in monitoring deterioration of *Balfourodendron riedelianum* seeds

Cristina Fernanda Schneider[1*], Fabiane Cristina Gusatto[2], João Alexandre Lopes Dranski[3], Marlene de Matos Malavasi[2] and Ubirajara Contro Malavasi[2]

*[1]Pontifícia Universidade Católica do Paraná, Avenida da União, 500, Jardim Coopagro, 85902-532, Toledo, Paraná, Brazil. [2]Centro de Ciências Agrárias, Universidade Estadual do Oeste do Paraná, Marechal Cândido Rondon, Paraná, Brazil. [3]Centro Universitário Dinâmica das Cataratas, Medianeira, Paraná, Brazil. *Author for correspondence. E-mail: cristina.schneider@pucpr.br*

ABSTRACT. In this study, we sought to identify efficient enzymes for use in monitoring the deterioration and loss of germination of *Balfourodendron riedelianum* (Engler) Engler seeds. The research was conducted at the Laboratório de Tecnologia de Sementes e Mudas of the Universidade Estadual do Oeste do Paraná, *Campus* Marechal Cândido Rondon, Paraná State, Brazil. Seeds were harvested at three collection sites and later submitted for processing, drying, initial characterization and storage in airtight packaging under controlled and uncontrolled storage conditions and uncontrolled. Tests were conducted at 0, 120, 240, and 360 days using a germination test, germination speed index, accelerated ageing test and the activity of peroxidase, phenylalanine ammonia-lyase and β-1,3-glucanase. The experiment was a completely randomized design. The results were subjected to regression analysis at 5% probability. Ageing was different among sample sites and between different storage conditions. The decrease in viability of seeds of *Balfourodendron riedelianum* (Engler) Engler was detected based on the activity of peroxidase enzymes in seeds from the Diamante do Oeste and Missal sites and the enzyme β-1,3-glucanase in seeds from the Missal site after storage periods of 0, 120, and 240 days.

Keywords: ivory wood; ageing; physiological quality; enzymatic activity.

Isoenzimas no monitoramento da deterioração de sementes de *Balfourodendron riedelianum*

RESUMO. Neste trabalho buscou-se identificar enzimas eficientes no monitoramento da deterioração e perda da capacidade germinativa de sementes de *Balfourodendron riedelianum*. Adotou-se delineamento inteiramente casualisado em esquema fatorial constituído pela combinação de quatro períodos (0, 120, 240 e 360 dias) e dois ambientes (controlado e não controlado) de armazenamento perfazendo oito tratamentos. As sementes foram obtidas de três locais de coleta (Missal, Mercedes e Diamante do Oeste, Estado do Paraná). Foram realizados os seguintes testes: germinação; índice de velocidade de germinação; envelhecimento acelerado e atividade das enzimas peroxidase, fenilalanina amônia-liase e β-1,3-glucanase. Os dados foram avaliados pela análise de regressão a 5% de probabilidade de erro. O padrão de envelhecimento das sementes de *B. riedelianum* foi variável para diferentes locais de coleta e condições de armazenamento. Por meio da atividade da enzima peroxidase nos locais Diamante do Oeste e Missal e da enzima β-1,3-glucanase para o local Missal, nos períodos de armazenamento de 0, 120 e 240 dias foi possível ser detectado o decréscimo na viabilidade de sementes da espécie.

Palavras-chave: pau marfim; envelhecimento; qualidade fisiológica; atividade enzimática.

Introduction

Balfourodendron riedelianum (Engler) Engler is in the family Rutaceae and is commonly known as pau-marfim, guatambu, and pequiá-mamona, among other names (Lorenzi, 2008). The fruit is ellipsoid according to Lorenzi (2008) and a samara, indehiscent, woody, dry fruit, with two or more locules. The seeds are ellipsoids, black, anatropous, endotestal with reduced inner integument, exarillate and albuminous, and the endosperm has a protein grain and lipid storage (Silva & Paoli, 2006). The seeds are classified as orthodox according to storage behaviour.

Because decay cannot be avoided, the seed storage of forest species is a practice that aims to control the speed of decay. However, seeds can be maintained with the minimal deterioration possible through proper storage. According to Bewley, Bradford, Hilhorst, and Nonogaki (2013), of the factors that influence the longevity of seeds, the most important are the amount of moisture in the seeds and temperature during storage. When these

factors are not ideal, the process of seed deterioration begins and/or accelerates.

Many physiological and biochemical manifestations in damaged seeds are widely reported (Marcos Filho, 2015; Bewley et al., 2013; Shaban, 2013). The most obvious are the physiological manifestations, which are observed during germination and early seedling development. However, long before the identification of these manifestations, other ultrastructural and biochemical changes must have occurred previously (Marcos Filho, 2015). These changes are related to membrane systems (Shaban, 2013) with respiratory and enzymatic activities (Bewley et al., 2013). Among the most common enzymatic changes related to the deterioration process are lipid peroxidation, removal of free radicals and seed respiratory metabolism.

Peroxidases are responsible for most oxidation-reduction reactions, stimulated by environmental changes, injuries and infectious reactions. According to Marcos Filho (2015), the increased activity of this enzyme indicates the evolution of deterioration due to the requirement for more intense action by the enzyme in the antioxidant complex.

Phenylalanine ammonia-lyase is an enzyme widely studied by physiologists and although important to normal plant development, is also a key enzyme indicating plant stress; the enzyme activity increases in response to stressors, increasing synthesis of protectors (Schwan-Estrada, Stangarlin, & Pascholati, 2008; Taiz & Zeiger, 2009).

β-1,3-glucanase is an enzyme that accumulates in plants after contact with pathogens (Martins, 2008) but also acts on cell division, pollen grain germination, pollen tube growth, and seed germination (Leubner-Metzger & Meins, 1999). The enzyme is also relevant to dormant seeds, because the activity decreases in dormant seeds (Farashah, Sharifzadeh, & Chavoshinasab, 2011).

Thus, as the subject of this study, we sought to identify efficient enzymes for use in monitoring the deterioration and loss of germination of *Balfourodendron riedelianum* (Engler) Engler seeds.

Material and method

The research was conducted at the Laboratório de Sementes e Mudas of the Universidade Estadual do Oeste do Paraná (UNIOESTE), Campus Marechal Cândido Rondon, Paraná State, Brazil.

Balfourodendron riedelianum fruits (RNC: 23531) were collected manually with help of a trimmer in June 2013, with four matrixes from each collection site, previously marked within the agreement Unioeste/Itaipu Binational in Paraná basin III in forest fragments.

Three batches of ripe fruits (brownish) of pau marfim were used, and these fruits were collected in Diamante do Oeste, Mercedes, and Missal, Paraná State, Brazil.

The Diamante do Oeste region has an average altitude of 266 m and the geographical coordinates 24°57'532" S, 54°09'556" W. The Mercedes region is at 24°24'503" S, 54°07'896" W, at an altitude of 281 m. The region of Missal is at 25°01'661" S, 54°12'411" W, at an altitude of 358 m.

After collection, the fruits were dried in covered wooden structures with air circulation at the Experimental Station Professor Dr. Antônio Carlos dos Santos Pessoa at Universidade Estadual do Oeste do Paraná (UNIOESTE), Campus Marechal Cândido Rondon, Paraná State, Brazil. The fruits were dried for 12 days, until reaching a degree of humidity (Bu) of 9%, which is ideal for airtight storage.

After drying, manual processing was conducted to remove the winged part. Fruits were packed inside a polypropylene bag and strongly treated mechanically with the help of a piece of wood. Then, fruits were removed and rubbed on a metal sieve for the complete withdrawal of the winged parts. A part of these fruits was used for preliminary assessments, and the other part was stored.

The fruits were stored in airtight packaging, transparent glass jars with screw cap and 500 mL capacity sealed with duct tape. Each package contained 175 fruits, and for each assessment period, two packages per treatment were used because they contained sufficient fruits required for tests.

The fruit packages were stored in two environments: controlled and uncontrolled. The controlled condition had air conditioning and a dehumidifier to control environmental conditions, and temperatures ranged between 13 and 17°C, monitored by a thermo-hygrometer, model Gehaka (São Paulo, São Paulo State, Brazil). In the uncontrolled environment, the fruit packages were stored on a shelf in an airy room, and the temperature varied as shown in Figure 1, monitored by a thermo-hygrometer, model Incoterm.

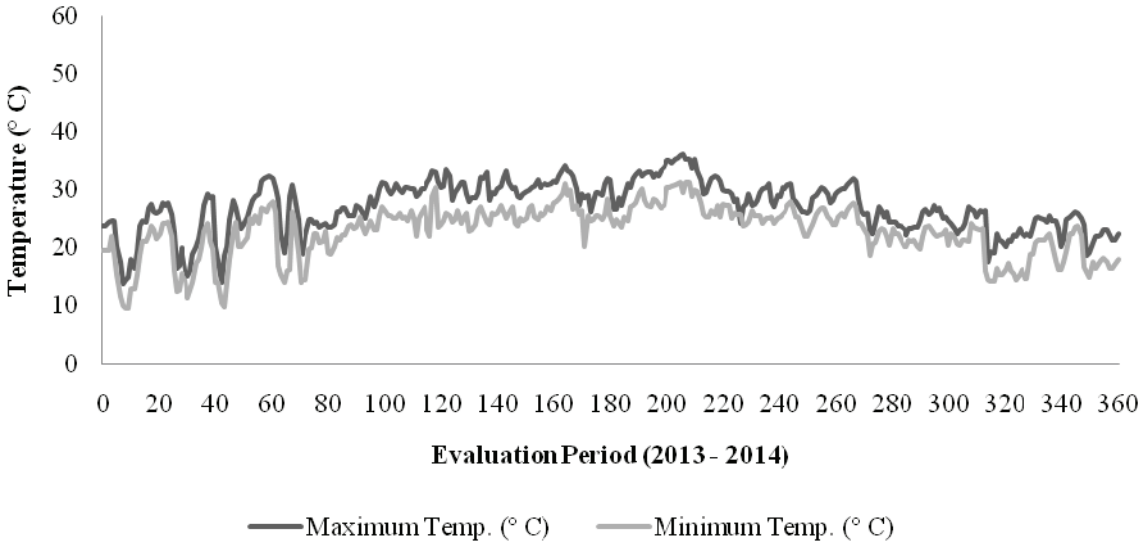

Figure 1. Maximum and minimum temperature data (°C) of the uncontrolled storage environment in Marechal Cândido Rondon, Paraná State, Brazil, between July 2013 and June 2014.

The laboratory tests were performed at 0, 120, 240, and 360 days after the fruit processing, to feature the effect of storage period. The following parameters were evaluated: degree of moisture, seed dry mass, seed size, germination, germination speed index (GSI), accelerated ageing and the activity of peroxidase, phenylalanine ammonia-lyase and β-1,3-glucanase enzymes.

Pau marfim has a samara-type fruit, and the extraction of the fruit seed is very difficult. Therefore, the fruit was used as the reproductive unit. All analyses in this study, except for enzyme determinations, were conducted with the fruit. Seeds will be used as the fruit denomination to facilitate the understanding of this work.

The degree of moisture was determined gravimetrically after the seeds were subjected to drying at the temperature of 105 ± 2°C for 24 hours (Brasil, 2009); we used four replicates of 25 seeds. The results are expressed as percentage on a wet basis.

Seed dry mass was measured simultaneously with the degree of moisture, with four repetitions of 25 seeds used for each treatment, and the results are expressed in grams.

With the aid of digital pachymeter, the diameter and length of pau marfim fruits were measured for a composite sample of 4 repetitions with 25 fruits. The length was the measure of the distal part of the fruit between the ends, and the average diameter was the measure between the two transversal measures perpendicular to the length. The results are expressed in millimetres.

The germination test was conducted with 25 seeds for each repetition in a germitest paper roll moistened with water at the ratio of 2.5-fold the weight of the substrate (Brasil, 2009). The paper rolls were maintained in a germination chamber (BOD) at an alternating temperature of 20-30°C with 8 hours of photoperiod light, associated with the high temperature, and 16 hours of darkness. Counts were performed daily to evaluate the germinated seeds for a period of 90 days. A seed was considered germinated when the seedling issued cotyledons and presented a developed radicle system. Because more than one seedling can be found in each fruit, only the first seedling was counted. The results are expressed as percentage of normal seedlings.

The germination speed index (GSI) was obtained simultaneously with the germination test using four replicates of 25 seeds for each treatment.

In the accelerated ageing test, 25 seeds were used for treatment repetitions. The seeds were distributed in a single layer on plastic canvas fastened inside a plastic box (gerbox type), with dimensions of 11.0 x 11.0 x 3.5 cm, containing 40 mL of water. The capped boxes were kept in a BOD at 41°C for 48 hours. After this period, the seeds were subjected to a germination test under the same conditions as above, with daily assessment for a period of 90 days, counting the percentage of normal seedlings.

Three repetitions were used for biochemical analyses. An initial enzymatic extraction was prepared. Samples were composed of 0.1 g of pau marfim seeds, which was homogenized in 4 mL of sodium phosphate buffer solution 0.01 MoL L^{-1} (pH 6.0) (extraction buffer) in a previously cold porcelain mortar. The

homogenate was centrifuged for 2 hours at 20,000 g. The supernatant, considered the fraction containing soluble proteins, was stored at 4°C (Lusso & Pascholati, 1999) for subsequent determinations of total proteins, peroxidase, phenylalanine ammonia-lyase and β-1,3 glucanase.

Peroxidase activity was determined at 30°C using a direct spectrophotometric method that measured guaiacol in tetraguaiacol at 470 nm (Lusso & Pascholati, 1999). The reaction mixture was composed of 0.10 mL of protein extract and 2.9 mL of solution with 250 μL of guaiacol and 306 μL of hydrogen peroxide in 100 mL of phosphate buffer solution 0.01 MoL L^{-1} (pH 6.0) (Lusso & Pascholati, 1999). The reference cuvette contained 3 mL of solution with 250 μL of guaiacol and 306 μL of hydrogen peroxide in 100 mL of phosphate buffer solution 0.01 MoL L^{-1} (pH 6.0). Peroxidase activity is expressed as specific activity (seed units of absorbance $min.^{-1} g^{-1}$).

The activity of phenylalanine ammonia-lyase was determined by colourimetric quantification of trans-cinnamic acid released from phenylalanine substrate (Umesha, 2006). The reaction mixture, incubated for 2 hours at 40°C, contained 100 μL of protein extract, 400 μL of Tris HCl 25 mMoL L^{-1} (pH 8.8) and 500 μL of L-phenylalanine (50 mM Tris HCl 25 mMoL L^{-1}, pH 8.8). The sample absorbance was determined at 290 nm, against extraction buffer, and the control value was subtracted from each sample (the control corresponded to 100 μL of protein extract and 900 μL of Tris-HCl 25 mMoL L^{-1}, pH 8.8). The absorbance readings were plotted on standard curve for the trans-cinnamic acid, and enzymatic activity is expressed in μg of seed trans-cinnamic $min.^{-1} g^{-1}$.

For spectrophotometric determination of β-1,3-glucanase activity, a solution of remazol brilliant blue-carboximetilcurdlan (CM-Curdlan-RBB, 4.0 mg mL^{-1}; Loewe Biochemia GmbH) was used as substrate, in accordance with the methodology described by Guzzo and Martins (1996). Protein extract, 200 μL, was mixed with 600 μL of the same extraction buffer and 200 μL of CM-Curdlan-RBB (4.0 mg mL^{-1}). After incubation for 20 minutes at 40°C, the reaction was stopped with the addition of 200 μL of HCl solution 1 MoL L^{-1}, followed by ice cooling for 10 minutes and centrifugation for 5 minutes at 10,000 g. The absorbance at 600 nm of supernatant was determined with extraction buffer in the reference cuvette. The results are expressed in units of protein absorbance $min.^{-1} g^{-1}$, discounting absorbance values of the control (800 μL of extraction buffer and 200 μL of CM-Curdlan-RBB).

The experiment followed a completely randomized design (DIC) in factorial scheme 4 x 2 (4 storage periods and 2 storage environments), with four repetitions, except for enzymatic activity, with three repetitions. The collection sites were evaluated separately.

Lilliefors and Chi-square tests were used to check the normality of the distribution of the data, followed by analysis of variance. The significance was determined by an F-test, and data were analysed by polynomial regression at 5% probability with the aid of Sisvar software 5.3 (Ferreira, 2011).

Result and discussion

Based on the characterization of fruits (Table 1), the average length of fruit varied from 14.61 to 16.42 mm, and the diameter ranged from 11.80 to 14.64 mm. Silva and Paoli (2006) found the length of pau marfim fruits ranged from 20 to 26 mm, longer than the fruits measured in this work. This variation occurs because of the interference of environmental factors in matrices, influencing the formation of fruits with different sizes.

Fruits from the Missal collection site had highest averages of length and mass, those from Mercedes had the greatest diameter, whereas the values for all variables of fruits from Diamante do Oeste were lower than those from other sites. Proportional to the size of the fruit was dry mass, as shown clearly in fruits from the Missal collection site.

Table 1. Characterization of fruits of *Balfourodendron riedelianum* (Engler) Engler from three collection sites.

Collection Site	Length (mm)	Diameter (mm)	Dry Mass (g)	Degree of Moisture (%)
Diamante d'Oeste	14.61 ± 1.20*	11.80 ± 1.01	0.1808 ± 0.01	9.20 ± 0.08
Mercedes	15.56 ± 1.54	14.64 ± 1.23	0.2698 ± 0.02	9.64 ± 0.09
Missal	16.42 ± 1.74	13.67 ± 1.43	0.2962 ± 0.01	9.68 ± 0.23

*Standard deviation.

The degree of humidity varied little among fruits from different collection sites (9.20 to 9.68%; Table 1). Moreover, the data are consistent with the conclusion of Marcos Filho (2015), which states that seeds stored in waterproof packages must have a degree of humidity below 10%. The data also illustrated that fruits from different collection sites were subjected to similar conditions during the storage period. According to Marcos Filho (2015), the aim of drying of seeds at post-harvest is to reduce the degree of humidity of seeds to safe levels. This process is extremely important, because when done correctly, the destructive metabolism tends to slow without promoting seed disturbances.

The storage period interfered with all evaluated attributes; only from some collection sites, no significant changes were observed. The storage conditions showed significance for seeds from the

Missal site only for activities of peroxidase, phenylalanine ammonia-lyase and β-1,3-glucanase. The interaction was significant in seeds in from the Diamante do Oeste collection site for peroxidase activity and in those from the Missal site for phenylalanine ammonia-lyase.

The germination percentage of pau marfim seeds presented a significant difference ($p \leq 0.05$) for the three collection sites during the period of storage (Figure 2a). The percentage declined until 240 days of storage, reaching the minimum, and later increased at 360 days, although during this period, the germination did not differ statistically from 0. Thus, quadratic behaviour described the germination percentage for all collection sites.

Corroborating the results obtained in this work, Pinto Junior, Guimarães, Dranski, Malavasi, and Malavasi (2012) and Zonta et al. (2014) discovered that seed germination of *Jatropha curcas* did not suffer from different influences in controlled and uncontrolled environments during storage in waterproof packages.

The germination speed index (GSI) was significant ($p \leq 0.05$) for seeds from all three sources in different storage periods (Figure 2b), following similar behaviour to that of germination percentage.

The best vigour expression of seeds from the sources Mercedes and Missal was observed at 0 and 360 days of storage. For the source Diamante do Oeste, at 360 days, the seed vigour was superior to that in the other periods.

The pericarp of mature fruit from *B. riedelianum* has an endocarp composed of several rows of parallel fibres oriented in relation to the fruit axis. In the mesocarp, the parenchyma cells of the subepidermal layers have more elongated and thicker walls, and in the exocarp, the epidermal cells have thick cuticles and walls. No reports are available related to germination with the formation of fruit similar to the species studied in this work. Most likely, the similar behaviours observed for seeds from the three collection sites for germination and GSI were related to certain fruit characteristics, such as moisture retention or inducing a chemical deterrent, because the storage was airtight, and no exchanges occurred with the environment.

Additionally, the high percentage of germination and high GSI at the initial storage period (0 days) in comparison with periods 120 and 240 days could be due to ethylene synthesis from fruit processing because synthesis is induced by mechanical stresses, such as bending, rubbing or agitation (Abeles, Morgan, & Saltveit Jr., 1992). Uchida and Yamamoto (2002) reported a similar observation working with Arabidopsis thaliana seeds that had increases in germination rates after being subjected to mechanical vibrations.

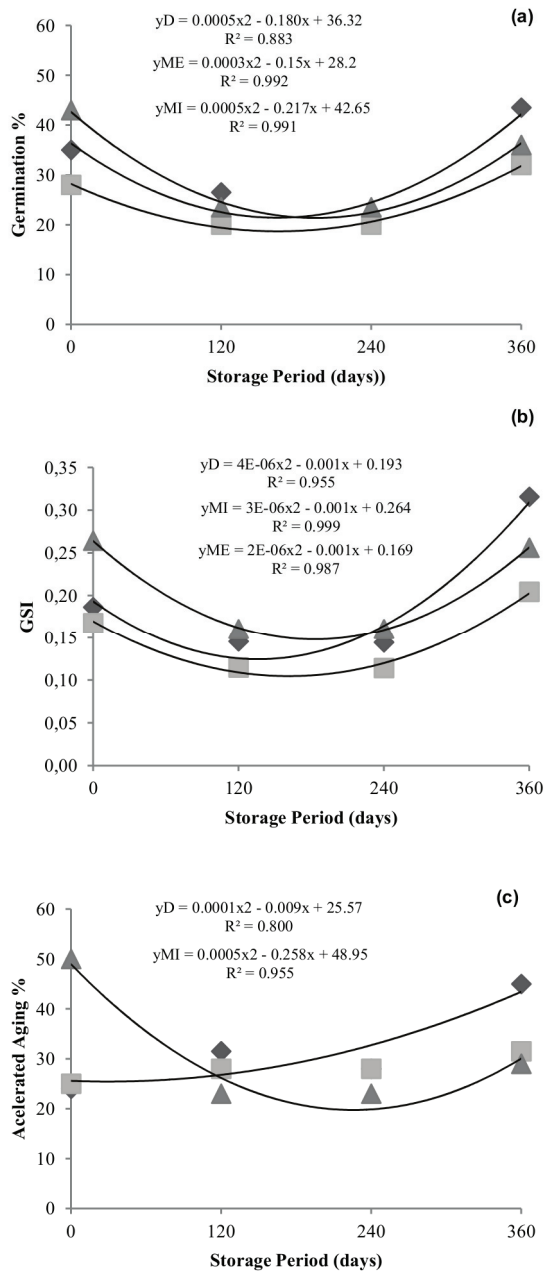

Figure 2. Germination (a), Germination Speed Index (GSI) (b) and Accelerated Ageing (c) of *Balfourodendron riedelianum* (Engler) Engler seeds from the collection sites of Diamante do Oeste (D) (♦), Mercedes (ME) (■), and Missal (MI) (▲) during the storage period.

The accelerated ageing test identified primary differences among seeds from the three collection sites in terms of resistance to stress imposed by extreme humidity and temperature, and significant differences were found in seeds from Diamante do Oeste and Missal during the storage period. For seeds from Mercedes, the imposed conditions of temperature and humidity were not sufficient to identify differences in seed vigour ($p \geq 0.05$; Figure 2c).

With the increase in storage period, a percentage linear increase of seed germinations was observed for those from Diamante do Oeste subjected to accelerated ageing with the minimum point of 24% at 0 days and the peak of 45% at 360 days. By contrast, the seeds from the collection site at Missal began with high performance (50%); whereas over the period of storage, a decrease in vigour was identified, reaching the germination minimum of 23% at 240 days. Linear and quadratic polynomial equations explained these results for seeds from Diamante do Oeste and Missal, respectively.

Several studies, in addition to this study, identified differences among sources using an accelerated ageing test, including for aroeira (Araújo, Andrade, Rêgo, Gonçalves, & Araújo, 2013), *Corymbia citriodora* (Gonzales, Valeri, & De Paula, 2011), *Sebastiania commersoniana* (Santos & Paula, 2009) and cedar (Cherobini, Muniz, & Blume, 2008).

These results can be explained by morphological and physiological differences among seeds collected at different sites, as clearly noted earlier during fruit characterization and based on the results of the accelerated ageing test. These phenotypic variations may occur because of population genetic properties, in addition to environmental influences on genotype expression (Moraes, Zanatto, Moraes, Freitas, & Sebbenn, 2011).

Peroxidase enzyme activity showed significant interaction between storage time and different storage conditions in seeds from the Diamante do Oeste collection site. Peroxidase activity in the controlled condition of storage at 120 days was lower than that at other periods In the uncontrolled condition, the activity was lower than that in other periods at 360 days.

The development of the interaction is shown in Figure 3a; a quadratic equation described the storage effect in the controlled environment and a linear equation for the effect in the uncontrolled environment. The lowest activity was observed after 120 days in the controlled environment; however, in the uncontrolled environment, activity decreased over storage time, with lowest activity being observed at 360 days.

In seeds from the Missal collection site in the uncontrolled environment, peroxidase activity increased. During the storage period (Figure 3b), enzyme activity increased linearly, with the highest activity at 360 days.

Diamante do Oeste seeds stored in an uncontrolled environment showed a more advanced degree of deterioration than those in the controlled environment, given that the peroxidase activity was reduced. Shaban (2013) explains that as the progress of deterioration occurs beyond germination, enzymatic activity also decreases. However, in seeds from the Missal site, the peroxidase activity was higher than that in seeds from the Diamante do Oeste site, showing that Missal seeds had not yet deteriorated to the advanced degree observed in Diamante do Oeste seeds. Although the germination percentage of these sources increased at the end of the storage period, peroxidase activity showed different behaviours in seeds from different collection sites, which are due to genetic variation within a single species that may be related to their geographical distribution or influenced by environmental variations of each site (Biernaski, Higa, & Silva, 2012).

Martins, Nakagawa, and Ramos (2011) described peroxidase activity and discovered that only the activity of this enzyme was effective in monitoring the deterioration of *Euterpe espiritosantensis* Fernandes seeds. However, in seeds of *Mimusops elengia* L., the removal of free radicals by peroxidase activity and other enzymes was not sufficient to prevent lipid peroxidation of membranes, caused by extreme loss of water (Tang, 2012).

Phenylalanine ammonia-lyase activity was significant during the storage period in seeds from the Diamante do Oeste collection site (Figure 3c). However, at 360 days of storage, the method used to detect the activity of this enzyme failed to detect activity, and therefore, the equation describing the data was quadratic.

A similar trend was observed in seeds from Missal, which showed a significant interaction, and phenylalanine ammonia-lyase activity could not be detected at 240 and 360 days in controlled and uncontrolled conditions, with activity detected only at day 0 (Figure 3d).

In seeds from both Diamante do Oeste and Missal during the period of storage, no significant difference was detected in the activity of this enzyme, which might indicate that the entire route of phenylpropanoids was not affected during the storage period. Thus, mechanisms such as synthesis of lignin, phenolic compounds, and quinones, among others, might not have been enhanced during the storage period, although activity was detected.

The low activity of enzymes should not have influenced the identification of phenylalanine ammonia-lyase activity in certain periods. The results observed in seeds of Diamante do Oeste were related to the enzyme peroxidase in the uncontrolled condition in which activity decreased. In seeds from Missal, this relation was not identified.

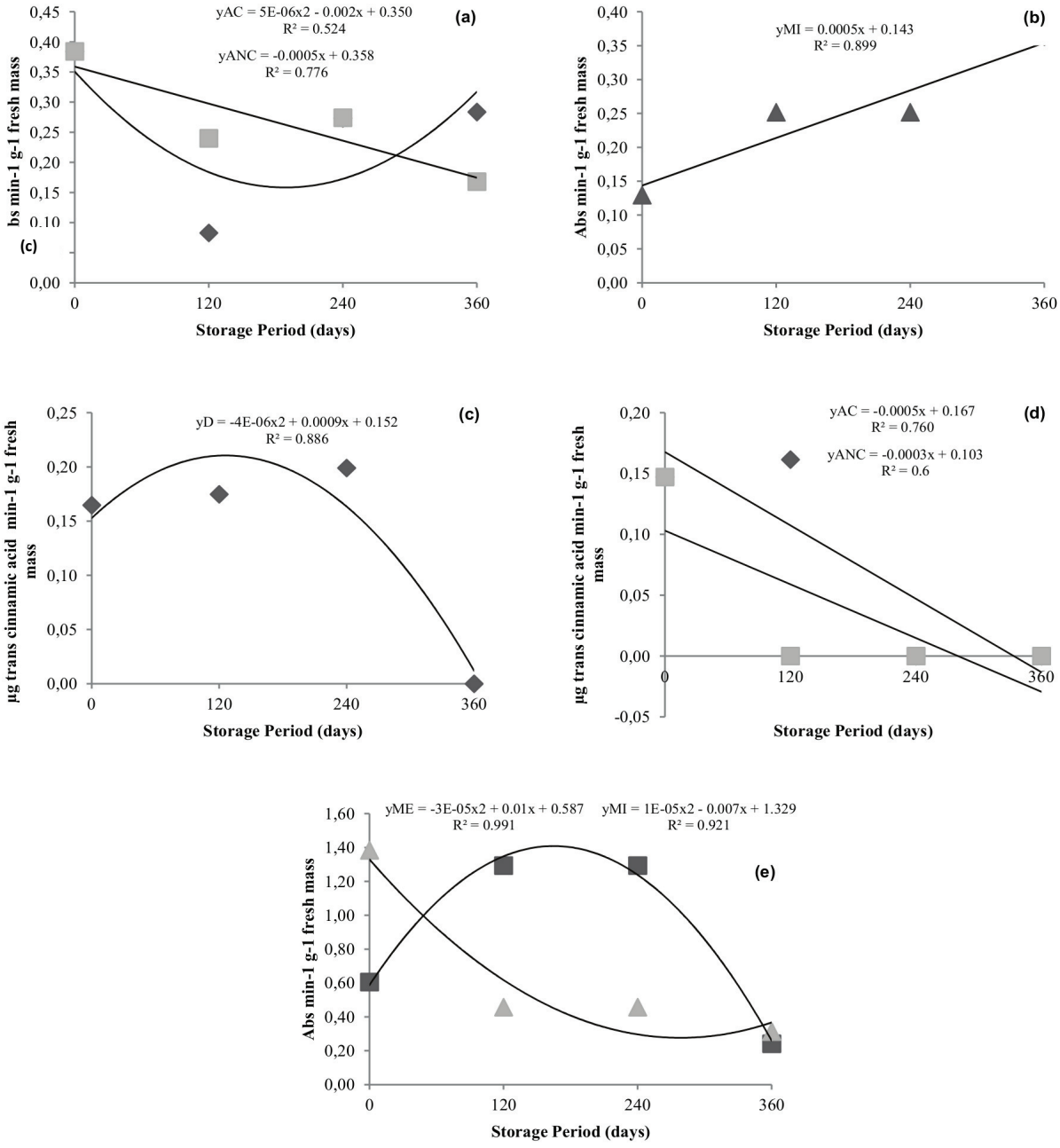

Figure 3. Seed peroxidase activity of *Balfourodendron riedelianum* from the collection site of Diamante do Oeste and the unfolding of the interaction between different storage environments: controlled environment (AC) (♦) and non-controlled environment (ANC) (■) (a); Peroxidase activity of seeds from the Missal collection site (b); Phenylalanine ammonia-lyase activity in seeds from the Diamante do Oeste collection site (c); Phenylalanine ammonia-lyase in seeds from the Missal collection site and the unfolding of the interaction between different storage environments: controlled environment (CA) (♦) and non-controlled environment (ANC) (■) (d); and β-1,3-glucanase in seeds from the Mercedes (ME) (■) and Missal (MI) (▲) collection sites (e), as a function of storage period.

The activity of β-1,3-glucanase in seeds from Mercedes showed significant differences during the storage period (Figure 3e), described by a quadratic equation, and activity increased at 167 days and decreased at 360 days. In seeds from Missal, both period and storage condition were significant, and in uncontrolled conditions, activity increased. During the storage period, the peak of activity was at day 0 with a subsequent decrease and stabilization of activity (Figure 3e).

β-1,3-glucanase plays an important role during tobacco seed germination (Leubner-Mentzger Fründt, Vögeli-Lange, & Jr, 1995) and catalyses the hydrolysis of cell wall components, resulting in a weakening of the micropylar endosperm, leading to radicle protrusion. However, in tomato seeds

(Morohashi & Matsushima, 2000), the activity of β-1,3-glucanase was detected only after radicle protrusion.

According to Leubner-Mentzger (2003), β-1,3-glucanase acts as a key factor in the regulation of seed germination and dormancy in response to environmental and hormonal conditions, and because the expression of β-1,3-glucanase and the rupture of the embryo are promoted by gibberellin (GA) and ethylene, they can be inhibited by abscisic acid (ABA). Farashah et al. (2011) reported this relation in dormant seeds of *Origanum vulgare* that had low enzyme activity. However, enzymatic activity increased in germinated seeds, after the breaking of seed dormancy.

Activity of the enzyme β-1,3-glucanase showed different behaviour from seeds collected at various sites, which was also observed for peroxidase. Considering the role of β-1,3-glucanase in the germination of seeds, only in seeds from the collection site of Missal was a relationship observed between enzyme activity and germination and GSI at 0, 120, and 240 days of storage, However, at 360 days, the same relation was not observed. For the seeds from Mercedes, a contrasting behaviour was observed.

The results of this study reinforced that genetic differences among seeds from different sites, associated or not with climatic conditions, influenced morphological, physiological and biochemical characteristics of seeds in this species (Martins, Bovi, Mori, & Nakagawa, 2007; Martins et al., 2011).

Conclusion

The loss of germination capacity of *Balfourodendron riedelianum* (Engler) Engler seeds was detected by peroxidase enzyme activity in seeds from Diamante do Oeste and Missal and by β-1,3-glucanase enzyme activity in seeds from the Missal collection site during periods of storage for 0, 120 and 240 days. The ageing pattern of *B. riedelianum* seeds was different among collecting sites and for different storage conditions.

Acknowledgements

The Coordination for Higher Education Staff Development (CAPES) is acknowledged for granting a scholarship.

References

Abeles, F. B., Morgan, P. W., & Saltveit Jr., M. E. (1992). *Ethylene in plant biology* (2nd ed.). San Diego, US: Academic Press.

Araújo, E. R., Andrade, L. A., Rêgo, E. R., Gonçalves, E. P., & Araújo, E. (2013). Qualidade fisiológica e sanitária de sementes de aroeira produzidas no estado da Paraíba. *Revista Agropecuária Técnica*, *34*(1), 9-20. doi: 10.25066/agrotec.v34i1.20380

Bewley, J. D., Bradford, K. J., Hilhorst, H. W. M., & Nonogaki, H. (2013). *Seeds*: physiology of development, germination and dormancy (3rd ed.). New York, US: Springer.

Biernaski, F. A., Higa, A. R., & Silva, L. D. (2012). Variabilidade genética para caracteres juvenis de progênies de *Cedrela fissilis* Vell.: subsídio para definição de zonas de coleta e uso de sementes. *Revista Árvore*, *36*(1), 49-58. doi: 10.1590/S0100-67622012000100006

Brasil. (2009). Ministério da Agricultura Pecuária e Abastecimento. *Regras para análises de sementes*. Brasília, DF: Mapa/ACS.

Cherobini, E. A. I., Muniz, M. F. B., & Blume, E. (2008). Avaliação da qualidade de sementes e mudas de cedro. *Ciência Florestal*, *18*(1), 65-73. doi: 10.5902/19805098511

Farashah, H. D., Sharifzadeh, F., & Chavoshinasab, S. (2011). Germination improvement and α-amylase and β-1,3-glucanase activity in dormantand non-dormant seeds of Oregano (*Origanum* vulgare). *Australian Journal of Crop Science*, *5*(4), 421-427.

Ferreira, D. F. (2011). Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia*, *35*(6), 1039-1042. doi: 10.1590/S1413-70542011000600001

Gonzales, J. L. S., Valeri, S. V., & De Paula, R. C. (2011). Qualidade fisiológica de sementes de diferentes árvores matrizes de *Corymbia citriodora* (Hook.) K. D. Hill & L. A. S. Johnson. *Scientia Florestalis*, *39*(91), 171-181.

Guzzo, S. D., & Martins, E. M. F. (1996). Local and systemic induction of β-1,3-glucanase and chitinase in coffee leaves protected against *Hemileia vastatrix* by *Bacillus thuringiensis*. *Journal of Phytopathology*, *144*(9/10), 449-454. doi: 10.1111/j.1439-0434.1996.tb00322.x

Leubner-Mentzger, G. (2003). Functions and regulation of β-1,3-glucanase during seed germination, dormancy release after-ripening. *Seed Science Research*, *13* (1), 17-34. doi: https://doi.org/10.1079/SSR2002121

Leubner-Mentzger, G., & Meins, F. J. (1999). Function and regulation of plant β-1, 3-glucanases. In S. K. Datta, & S. Muthukrishnan (Ed.), *Pathogenesis-related proteins in plants* (p. 49-76.). Boca Raton, FL: CRC Press.

Leubner-Mentzger, G., Fründt, C., Vögeli-Lange, R., & Meins, F. (1995). Class I β-1,3-glucanases in the endosperm of Tobacco during germination. *Plant Physiology*, *109*(3), 751-759. doi: https://doi.org/10.1104/pp.109.3.751

Lorenzi, H. (2008). *Árvores brasileiras*: manual de identificação e cultivo de plantas arbóreas nativas do Brasil (5a ed.). Nova Odessa, SP: Instituto Plantarum.

Lusso, M. F. G., & Pascholati, S. F. (1999). Achtivty and isoenzymatic pattern of soluble peroxidases in maise

tissues after mechanical injury or fungal inoculation. *Summa Phytopathological*, *25*(3), 244-249.

Marcos Filho, J. (2015). *Fisiologia de sementes de plantas cultivadas* (2a ed.). Londrina, PR: Abrates.

Martins, C. C., Bovi, M. L. A., Mori, E. S., & Nakagawa, J. (2007). Isoenzimas na diferenciação de sementes de três espécies do gênero *Euterpe*. *Revista Árvore*, *31*(1), 51-57. doi: 10.1590/S0100-67622007000100007

Martins, C. C., Nakagawa, J., & Ramos, P. R. R. (2011). Isoenzimas no monitoramento da deterioração de sementes de *Euterpe espiritosantensis* Fernandes. *Revista Árvore*, *35*(1), 85-90. doi: 10.1590/S0100-67622011000100010

Martins, E. M. F. (2008). Proteínas relacionadas à patogênese. In S. F. Pascholati, L. Breno, J. R. Stangarlin, & P. Cia (Ed.), *Interação planta-patógeno*: *fisiologia, bioquímica e biologia molecular* (p. 387-410). Piracicaba, SP: Fealq.

Moraes, E., Zanatto, A. C. S., Moraes, M. L. T., Freitas, M. L. M., & Sebbenn, A. M. (2011). Comportamento e variação de procedências de *Corymbia citriodora* em diferentes tipos de solos. *Floresta*, *41*(2), 277-286. doi: 10.5380/rf.v41i2.21875

Morohashi, Y., & Matsushima, H. (2000). Development of β-1,3-glucanase activity in germinated tomato seeds. *Journal of Experimental Botany*, *51*(349), 1381-1387. doi: 10.1093/jexbot/51.349.1381

Pinto Junior, A., Guimaraes, V. F., Dranski, J. A. L., Malavasi, M. M., & Malavasi, U. C. (2012). Armazenamento de sementes de pinhão manso em diferentes embalagens e ambientes. *Revista Brasileira de Sementes*, *34*(4), 636-643. doi: 10.1590/S0101-31222012000400015

Santos, S. R. G., & Paula, R. C. (2009). Testes de vigor para avaliação da qualidade fisiológica de sementes de *Sebastiania commersoniana* (Baill.) Smith & Downs. *Scientia Florestalis*, *37*(81), 7-16. doi: 10.1590/S0101-31222005000200020

Schwan-Estrada, K. R. F., Stangarlin, J. R. & Pascholati, S. F. (2008). Mecanismos bioquímicos de defesa vegetal. In S. F. Pascholati, L. Breno, J. R. Stangarlin, & P. Cia (p. 227-248). *Interação planta patógeno*: fisiologia, bioquímica e biologia molecular. Piracicaba, SP: Fealq.

Shaban, M. (2013). Review on physiological aspects of seed deterioration. *International Journal of Agriculture and Crop Sciences*, *6*(11), 627-631.

Silva, L. L., & Paoli, A. A. S. (2006). Morfologia e anatomia da semente de *Balfourodendron riedelianum* (Engler) Engler – Rutaceae. *Revista Brasileira de Sementes*, *28*(1), 16-20.

Taiz, L., & Zeiger, E. (2009). *Fisiologia vegetal* (4a ed.). Porto Alegre, RS: Artmed. doi: 10.1590/S0101-31222006000100003

Tang, A. (2012). Desiccation-induced changes in viability, lipid peroxidation and antioxidant enzyme activity in *Mimusops elengi* seeds. *African Journal of Biotechnology*, *11*(44), 10255-10266. doi: 10.5897/ajb11.3736

Umesha, S. (2006). Phenylalanine ammonia lyase activity in tomato seedlings and its relationship to bacterial canker disease resistance. *Phytoparasitica*. *34*(1), 68-71. doi: 10.1007/BF02981341

Ushida, A., & Yamamoto, K. T. (2002). Effects of mechanical vibration of seed germination of *Arabidopsis thaliana* (L.) Heynh. *Plant Cell Physiology*. *46*(6), 647-651. doi: https://doi.org/10.1093/pcp/pcf079

Zonta, J. B., Araujo, E. F., Araujo, R. F., Jonta, J. H., Dias, L. A. S., & Ribeiro, P. H. (2014). Armazenamento de sementes de pinhão manso em diferentes embalagens e armazenamento. *Bioscience Journal*. *30*(2), 599-688.

Received on September 6, 2017.
Accepted on December 12, 2017.