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Short storability of *Caesalpinia echinata* Lam. seeds as a consequence of oxidative processes[1]

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ABSTRACT - (Short storability of *Caesalpinia echinata* Lam. seeds as a consequence of oxidative processes). The seed bank is one of the strategies for the preservation of endangered species, such as *Caesalpinia echinata* Lam. In this work we studied the changes in O_2 consumption and CO_2 release by seeds incubated at different temperatures and water contents, evaluating the deterioration of seeds through germination and tetrazolium tests. Our results demonstrated that the deterioration processes occurring in *C. echinata* seeds are related to respiration and possibly other oxidative processes, causing the death of embryonic tissues in short periods and loss of seed viability. This characteristic means that seed bank is an alternative for *Caesalpinia echinata* conservation, however it depends on the control of these oxidative processes.
Key words: brazilwood, deterioration, seed respiration, storage

RESUMO - (Baixa capacidade de armazenamento de sementes de *Caesalpinia echinata* Lam. como consequência de processos oxidativos). O banco de sementes é uma das estratégias para a preservação de espécies ameaçadas de extinção, como *Caesalpinia echinata* Lam. Neste trabalho foram estudadas mudanças no consumo de O_2 e na liberação de CO_2 por sementes incubadas em diferentes temperaturas e teores de água, avaliando-se também a deterioração das sementes por meio dos testes de germinação e de tetrazólio. Os resultados demonstraram que os processos de deterioração que ocorrem nas sementes de *C. echinata* estão relacionados com a respiração e possivelmente com outros processos oxidativos, causando em curtos períodos a morte de tecidos embrionários e a perda da viabilidade das sementes. Essas características significam que a utilização do banco de sementes como alternativa para a conservação de *Caesalpinia echinata* depende do controle desses processos oxidativos.
Palavras-chave: armazenamento, deterioração, pau-brasil, respiração de sementes

Introduction

Caesalpinia echinata Lam. (brazilwood or pernambuco) is one of the most important plant species in Brazil and is confined to the Brazilian Atlantic rainforest due to its over-exploitation (Rocha *et al.* 2007, Rocha 2010). The species is now extinct in significant part of its range (Abensperg-Traun 2009) and was included in 2008 in the official list of the Brazilian native species in danger of extinction (Pilatti *et al.* 2011).

In situ conservation should be the highest priority to conserve species but the *ex situ* conservation, for example in seed banks, of threatened or endangered plant species has become a necessary and suitable strategy (Godefroid *et al.* 2010, Khoury *et al.* 2010, Enβlin *et al.* 2011), including *C. echinata*. However, this conservation strategy depends on the longevity of the seeds as well as the knowledge of the physiology of seed deterioration, including biochemical, physiological or genetic degenerative processes.

The period and speed of seed deterioration may be determined primarily by genetic inheritance, initial quality, moisture content and storage conditions (Rajjou & Debeaujon 2008). Temperature and water content are among the most important storage conditions since they can modify the energetic status of water, affecting the respiratory metabolism, accelerating the deterioration process (Leopold & Vertucci 1989, Vertucci & Roos 1990, Kibinza *et al.* 2006).

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The metabolic reactions differ in their kinetics for each level of hydration of the seed during storage, practically ceasing when almost all water is removed from the cells, for example in the anhydrobiotic organisms (Murdoch & Ellis 2000, Alpert 2005, Kibinza *et al.* 2006, Pagnotta & Bruni 2006). Water, either associated or not with solutes as sugars and proteins, is directly involved with the formation of an intracellular glass matrix, which is essential to reduce or even stop metabolic reactions (Pagnotta & Bruni 2006). In this so-called glassy state, at water potential below - 150 MPa, the oxidative metabolism is limited; however, the protections against free radicals are reduced when the cells hydrate, starting oxidative activity, respiration and the synthesis of both protein and nucleic acids (Vertucci & Farrant 1995). Water starts producing free radicals and other reactive oxygen species (Voeikov 2006) which, in tissues lacking enzymatic antioxidant agents, can modify cellular components and cause damage (Bernal-Lugo *et al.* 2000, Møller *et al.* 2007).

As a matter of fact, it is clear the potential accelerating effect of respiration and other oxidative processes on seed deterioration. However, there is insufficient information in the literature to establish the relationship between respiratory and deterioration rates as well as the interference of the hydration degree and temperature on these processes. The establishment of relations among the energetic state of water, the intensity of temperature and respiration rate as well as seed deterioration can bring substantial contribution to seed conservation and hence, germplasm conservation.

Caesalpinia echinata seeds can be considered as anhydrobiotic organisms as they are desiccation-tolerant. However, their germinability remains viable no longer than 3 months at natural environment (ca. 25 °C), around 18 months when dried and stored at 7 °C and for 18 months at -18 °C (Barbedo *et al.* 2002, Hellmann *et al.* 2006). In order to multiply an endangered species, seed technology shall be developed to provide long term storage in seed banks. The physiological processes involved in rapid loss of viability of *C. echinata* seeds still remain unclear, but they may be related to seed respiration or oxidative reactions such as lipid peroxidation. Therefore, the development of seed technology to allow long term storage in germplasm banks is crucial to the formulation of sound strategies for the conservation of the species.

Material and methods

Two studies were carried out so as to corroborate our hypothesis that oxidative processes (respiratory or others) are the main cause of rapid deterioration of *Caesalpinia echinata* Lam. seeds in storage. First, we studied the changes in O_2 consumption and CO_2 release by seeds incubated at different temperatures and different water contents by analysing both the deterioration of the whole seeds via germination tests as well as cut seeds via tetrazolium test. Secondly, we analysed the changes in the kinetic of the gases consumed/released by seeds with different deterioration rates while incubated at different temperatures and moisture contents, as shown on the results of the first study.

Plant material - Seeds of *Caesalpinia echinata* were obtained from approximately 20 trees within the Reserva Biológica e Estação Experimental de Mogi-Guaçu, (22°15-16'S, 47°8-12'W), Brazil. Mature fruits were harvested at pre-dehiscence point (Borges *et al.* 2005), then each seed was manually removed and immediately dried at laboratory conditions up to ca. 0.10 g H_2O g^{-1} dry weight. Then seeds were stored at 7 °C, but no longer than seven days until the beginning of the experiments (Barbedo *et al.* 2002). These seeds were called high vigour seeds (HVS). Part of HVS were submitted to accelerated aging (42 °C, 100% RH, according to Marcos Filho 1994 and Lamarca *et al.* 2009) for 12 hours, and then they were called low vigour seeds (LVS). Recent (less than 24 hours) dispersed seeds (RDS) were also collected from the ground. Part of RDS were submittted to the same artificial aging of the HVS, they were then called high deteriorated seeds (HDS).

Seed analyses - The seeds were analysed for water content, water potential (only embryos), germination, respiration rate (O_2 consumption and CO_2 release) and vigour (tetrazolium test). Water content was determined gravimetrically at 103 °C for 17 hours (Ista 1985) and the results were expressed on g H_2O g^{-1} dry weight. The water potential of the embryo was measured in WP4 potentiometer (Decagon Devices, Pullman, USA) based on the dew point temperature of the air after equilibrium with the sample. The assessment of the potential was performed by sorption isotherms in solutions of polyethylene glycol (PEG 6000) at different osmotic potential, based on Michel & Kaufmann (1973). Germination tests were carried out on germination chambers MA400 (Marconi Ltda,

Piracicaba, Brazil) at 25 °C in continuous light (Mello & Barbedo 2007). Seed germination (primary root protrusion) as well as normal seedling development were recorded daily.

Tetrazolium test was based upon the methodology and the eight categories described by Lamarca *et al.* (2009). We also estimated the frequency of viable and unviable tissue distribution, corresponding to the percentage of the area occupied by living, dead or damaged tissues for each seed. Thus, we considered the sum of the percentages of each type of tissue identified in all seed treatment. The frequency of tissue distribution was based on the quantification of damaged areas described for soybean and barley (Chauhan 1985).

Incubation of seeds to evaluate respiratory rates - Respiratory rates were estimated by packaging the seeds in sealed glass flasks (600 mL). The caps were punched and the hole (2 mm) was coated by a rubber septum in which the needles were inserted to collect air samples. The total air volume of the flask was determined hydrostatically. Seed volume was previously determined and then seeds were introduced into the flasks. The closure of the flask determined the beginning of the experiment. Based upon previous experiments of the average daily consumption of O_2 and of CO_2 release by *C. echinata* seeds, two air samples were collected at 15 and 30 days after the beginning of the experiment. The air samples collected from the flasks were analysed by Illinois analyser 6600 (Illinois Instruments, Inc., Johnsburg, USA), based on a zirconium oxide (ZrO_2) potentiometric sensor in order to measure the difference of oxygen partial pressure between two electrodes for O_2 analysis. For CO_2 analysis, an infrared sensor measures the red radiation absorption by CO_2. The percentages of the total volume were converted to µmol of O_2 or CO_2 g dry mass^{-1} day^{-1} (µmol g DM^{-1} d^{-1}) taking into account the local atmospheric pressure (0.9 atm), the volume of the air into the flasks, the volume of the seeds, the partial pressure of each gas, the temperature at the time of the readings, the dry mass of seeds (as described above) as well as the total number of days throughout seed incubation.

For the conversion of percentage of O_2 and CO_2 for µmol initially values obtained in percentage were converted to partial pressure of gas, through equation $p_1/P = v_1\%/V\%$ (Feltre 1985), where: p_1: partial pressure of gas (atm), P: local atmospheric pressure (= 0.9 atm), v_1%: gas volume (%) and V%: total volume (= 100%). These values were then converted to µmol de O_2 e de CO_2 through the equation of "Clapeyron", $p_1V = nRT$, where: V: total volume of air of flasks (L), n: number of moles of gas, R: universal constant of gas (0.082 atm L mol^{-1} K^{-1}), T: temperature (in Kelvin).

The respiratory quotient (RQ = CO_2 released/O_2 consumed) was also calculated as described by Kader & Saltveit (2002).

Changes in respiration rates as a result of seed moisture and incubation temperature - Samples of HVS were oven-dried at 40 °C up to 0.06 g H_2O g^{-1} dry weight and hydrated (plastic sandwich boxes, with 100% RH at 25 °C) in order to obtain seeds with 0.06, 0.12, 0.18, 0.25 and 0.32 g H_2O g^{-1} dry weight. Immediately after reaching each level the seeds were incubated into the flasks at 25 °C, for respiratory analysis, as described above. For the analyses at different temperatures, HVS were hydrated up to 0.18 g H_2O g^{-1} dry weight, and then incubated into the flasks at 3, 7, 10, 13, 16, 19 and 22 °C. In this experiment, an additional evaluation was performed at 45-day incubation. After the latest assessment of O_2 consumption and CO_2 release, seeds were evaluated for both water content and water potential. They were also submitted to tetrazolium test.

Changes in respiratory rates by seeds of different deterioration levels - Samples of HVS, LVS, RDS and HDS were hydrated up to 0.11, 0.16 and 0.19 g H_2O g^{-1} dry weight and incubated in the flasks at 13 °C for 30 days so as to assess O_2 consumption and CO_2 release. At the end of the incubation period, the seeds were submitted to tetrazolium test and analysed for germination and normal seedling development.

Data analysis - All experiments were based on a complete randomized design, by using three replications. The data were submitted to ANOVA and the means were compared by Tukey test at 5% probability, using SISVAR 5.3 (Ferreira 2008). All statistical analyses of water potential values (negative values) were performed based upon module data (e.g., with data transformed to positive values).

Results

Changes in respiratory rates as a result of incubation temperature and moisture content of seeds - Nearly all target water contents were reached, except for 0.32 g H_2O g^{-1} dry weight, which were 0.37 g H_2O g^{-1} dry weight (figure 1a). The increase in water content from 0.06 to 0.37 g H_2O g^{-1} dry weight was accompanied by

an increase in water potential (figure 1b). However, the initial increases in water content (up to 0.12 g H_2O g^{-1} dry weight) promoted proportionally higher increases in water potential (from -180 MPa to - 40 MPa) than other hydration levels, indicating a greater change in the energy of water at low hydration levels. At the end of the 30-day incubation, the higher the initial water content of the seeds, the higher the reduction on final water content (figure 1a), probably due to the greater amount of water needed to achieve the hygroscopic equilibrium between the air inside the flasks and the seeds. However, the water potential showed an opposite behaviour, with low decreases among seeds with higher initial water content (figure 1b), except for the lowest moisture content ones (0.06 g H_2O g^{-1} dry weight).

In the first 15 days of incubation, seeds consumed O_2 at all hydration levels (figure 1c), but in lower amounts up to 0.25 g H_2O g^{-1} dry weight (below 10 µmol g DM^{-1} d^{-1}) and substantially higher among seeds with 0.37 g H_2O g^{-1} dry weight (ca. 40 µmol g DM^{-1} d^{-1}). Throughout the following 15 days of incubation, the O_2 consumption remained almost the same for the least hydrated seeds; however, this consumption increased at least 2 times more in seeds hydrated up to 0.37 g H_2O g^{-1} dry weight, reaching values close to 90 µmol g DM^{-1} d^{-1}. Conversely, the production of CO_2 in the first 15 days of incubation (figure 1d), practically non-existent among seeds at lower hydration levels, increased substantially in seeds hydrated up to 0.25 and 0.37 g H_2O g^{-1} dry weight, reaching values close to 30 µmol g DM^{-1} d^{-1}. Within the following 15 days of incubation, seeds hydrated up to 0.18 g H_2O g^{-1} dry weight levels continued to release negligible amounts of CO_2, however, seeds hydrated up to 0.37 g H_2O g^{-1} dry weight released values above 60 µmol g DM^{-1} d^{-1} (figure 1d). By comparing the values in figure 1 (c, d), it is clear that O_2 consumption was always higher than CO_2 release at both 15 or 30 days of incubation, resulting in respiratory quotient much lower than 1.0. Such values suggest that there were other oxidative reactions other than respiration.

Prior to the incubations, seeds showed 73% germination in which 40% of them were able to produce normal seedlings. At the end of the 30-day incubation, only the seeds initially with 0.06 and 0.12 g H_2O g^{-1} dry weight maintained some germination (respectively, 53 and 10%) and only the former retained their ability to produce normal seedlings (20%). According to Barbedo *et al.* (2002), this would be actually expected since the incubation resembled the storage at 25 °C which promotes the total loss of seed viability in less than 90 days, even when seeds have less than 0.10 g H_2O g^{-1} dry weight. However, the reduction to 0.06 g H_2O g^{-1} dry weight preserved some capacity of these seeds to germinate and develop into normal seedlings. Interestingly, the seeds with 0.06 g H_2O g^{-1} dry weight were the ones with the lowest consumption of O_2 and CO_2 release. However, even these seeds, which are considered suitable for storage due to their supposedly very low metabolic activity, consumed about 3 times more O_2 than CO_2 release (respectively, 1.0 and 0.3 µmol g DM^{-1} d^{-1}). This corroborates the hypothesis that oxidation processes are responsible for the rapid deterioration of these seeds.

O_2 consumption and CO_2 production also changed throughout incubation temperature changes (figure 2a), but no differences were detected among 15, 30 and 45 days of incubation. Changes in seed water content during the period were less than 0.04 g H_2O g^{-1} dry weight. Both O_2 consumption and CO_2 release increased with rise of temperature, however the rate of this increase was more pronounced in the consumption of O_2 (figure 2a). As this was always superior to the release of CO_2, once again, the presence of oxidation reactions other than respiration takes place. Furthermore, considering the difference between the rates of increased O_2 consumption and CO_2 release, these results show that the oxidative reactions are much more intense at higher temperatures, leading to even faster deterioration, whereas under refrigerated conditions, seeds would not be conserved at long-term storage as oxidative processes would still be severe. As a matter of fact, seeds stored at 7 °C consumed over 4 times more O_2 (2.2 µmol g DM^{-1} d^{-1}) than the CO_2 released (0.5 µmol g DM^{-1} d^{-1}) (figure 2a). Even when the temperature approached the freezing point there was still a large consumption of O_2 (ca. 0.5 µmol g DM^{-1} d^{-1}) and practically no release of CO_2 (figure 2a). This consumption of O_2 for respiration or other oxidation processes caused damages to the seeds and consequently to germination and normal seedling development, as severe as higher the temperature (figure 2b). Just 45 days of incubation at 7 °C were enough to decrease seed viability to approximately one half of the initial one. Tetrazolium test showed that the higher the temperature, the greater the amount of non-viable tissues in the embryos of *C. echinata*. (figure 2c). Images of the tetrazolium test showed that the increase of respiration affected

the integrity of meristematic regions which are essential to maintain seed vigour and viability. The main changes were noted in the radicle, hypocotyl, plumule, at the insertion of the cotyledons and all areas of the cotyledons (figure 2d). Such damages to tissues resulted in progressive damages to the development of normal seedlings (figures 2b, e) and even for germination (figures 2b, f) according to the increasing in temperature of incubation, leading to more and more abnormal seedlings (figure 2g) and ending up in almost complete lost of germinability (figure 2b), this one was measured by primary radicle protrusion (figure 2f).

Changes in respiratory rates among seeds of different deterioration levels - All seeds (HVS, LVS, RDS and HDS) showed similar behaviour in the kinetics of gases, increasing the respiratory rate as water content increased (figures 3a-d). Also, it can be seen that the oxygen consumption was higher in seeds at 0.19 g H_2O g^{-1} dry weight hydration level and, as occurred at different moisture levels and temperatures described above, CO_2 production was always lower than O_2 consumption independent of seed deterioration level. The seed respiratory rates into each hydration level, as well as both respiration and other oxidation processes, were higher in vigorous (figure 3a) than in deteriorated (figures 3b-d) seeds. This triggered higher deterioration rates taking into account the initial viability of the tissues in each seed group (figures 3e-h). This loss of embryonic tissue viability reflected directly in the reduction of germination and decline in normal seedling development (table 1). The reduction in respiration rates resulting from seed deterioration might be understood as a result of mitochondrial membranes deterioration and, consequently, the reduction in the energy supply for germination (McDonald 1999 and references therein). It might also represent a decline in the cytochrome pathway activity, as observed among soybean seeds by Amable & Obendorf (1986). Conversely, these data somewhat contradict the expectation that deteriorated seeds, which were supposed to have lower efficiency of mitochondria activity, should have higher oxidative rates than vigorous seeds. The difference between O_2 consumption and CO_2 release from vigorous to deteriorated seeds may also result from changes in the detoxification potential present in the seeds that, due to damage accumulation during the incubation period may lose the ability to control the reactive oxygen species (ROS) (Rajjou & Debeaujon 2008). In this case, the rise of temperature would increase the metabolic activity so that vigorous seeds would depend on the detoxification systems.

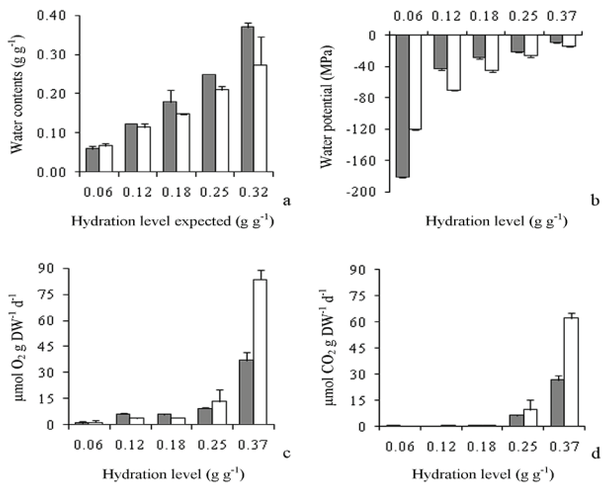

Figure 1. a. Water content in g H_2O g^{-1} dry weight (g g^{-1}), b. Water potential, c. O_2 consumption, d. CO_2 release by seeds of *Caesalpinia echinata* Lam. at seven levels of hydration (in g H_2O g^{-1} dry weight, g g^{-1}), incubated by 30 days at 25 °C. Grey columns, in (a) and (b), represent the values before incubation; white columns, after 30 days of incubation. Grey columns, in (c) and (d), represent the values after 15 days of incubation, white columns after 30 days of incubation. Values are means plus standard deviation.

Discussion

Our study clearly demonstrated that the deterioration processes occuring at *Caesalpinia echinata* seeds are complex and therefore the development of seed storage technology aimed at this species conservation will probably not take place from short - to medium -term. It becomes evident that *C. echinata* seeds have short life-span under natural conditions. There is also a strong influence of both respiration and other oxidative processes (e.g. ROS activity) which damage seed tissues, affecting seed vigour or even causing viability loss.

Caesalpinia echinata seeds have no dormancy and germinate up to 3 days after shedding (Borges *et al.* 2005, Mello & Barbedo 2007). Therefore, this species propagation is likely to happen through nursery grown seedlings rather than seeds. However, even under controlled storage conditions, *C. echinata* dry seeds at refrigeration or even freezing temperatures would be exposed to oxidative processes as well as deterioration in short - to medium - term, as shown in this work. As a matter of fact, Hellmann *et al.* (2006) demonstrated that it is possible to store *C. echinata* seeds for at least

2 years, at freezing temperatures. However, the authors described that even when seeds were stored at -5 or -18 °C they started showing signs of deterioration, as shown by some percentage of abnormal seedling development.

The major difference between O_2 consumption and CO_2 production might be related to the use of fatty acids as initial substrate for respiration. As shown by Mello *et al.* (2010), lipids in cotyledons of *C. echinata* represent more than 17 percent of DM, whereas linoleic acid represents nearly half of them (more than 45 percent). Respiration using carbohydrates produces respiratory quotient (RQ) close to 1.0 since carbohydrate oxidation (highly oxygenated substrates) involves equal amounts of carbon dioxide and oxygen. On the other hand, the oxidation of fatty acids (poorly oxygenated substrates) produces less CO_2 per mole of oxygen and RQ values usually are close to 0.6-0.7 (Gnaiger & Kemp 1990, Tcherkez *et al.* 2003).

However, the low levels of CO_2 release in comparison to O_2 consumption (figure 2a and figures 3a-d) mostly resulted in RQ much lower than 0.5 and, in some cases even lower than 0.1, which indicates non-respiratory reactions. Furthermore, high intakes of O_2 could be related to lipid peroxidation and ROS formation, which also reduce O_2 (Vertucci & Leopold 1987, Vertucci 1989, Sacandé *et al.* 2000, Walters *et al.* 2002). These oxidative reactions could explain the low viability of *C. echinata* seeds while stored at temperatures higher than the freezing ones, even when they have low water content, as described by Barbedo *et al.* (2002) and Hellmann *et al.* (2006). Moreover, great amounts of linoleic acid were found in *C. echinata* cotyledons, (Mello *et al.* 2010), which is a polyunsaturated fatty acid (PUFA) and is particularly susceptible to oxidation by singlet oxygen ($^1O_2$) and hydroxyl radical (HO•) (Møller *et al.* 2007).

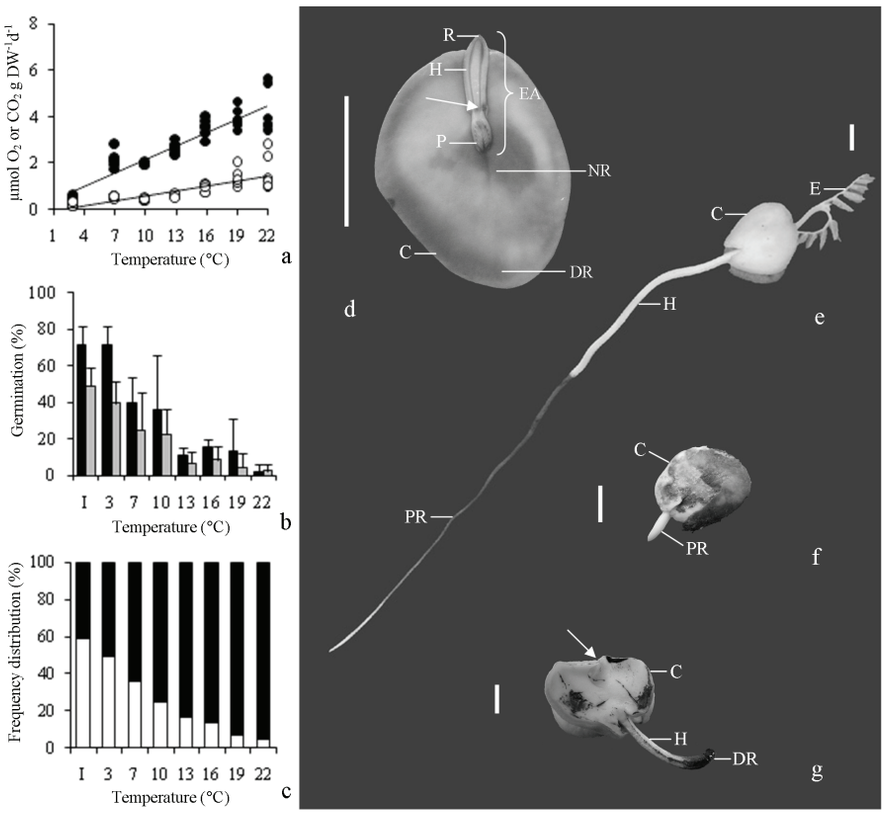

Figure 2. Respiration, germination and deterioration of *Caesalpinia echinata* Lam. seeds with water content of 0.19 g H_2O g^{-1} dry weight (g g^{-1}), after 45 days of incubation at different temperatures. a. O_2 consumption (closed circle) and CO_2 release (open circle): $y_{O2} = 0.196x + 0.196$, $R^2 = 0.84$; $y_{CO2} = 0.073x - 0.145$, $R^2 = 0.66$. b. Germination (black columns) and development of normal seedlings (grey columns). Values are means plus standard deviation. c. Frequency distribution of viable tissue (white columns) and not viable (black columns), obtained by the tetrazolium test. d. Longitudinal section of embryo. EA: embryogenic axis; R: radicle; H: hipocotyl; P: plumule; C: cotyledon; NR: the so-called nearest region; DR: the distal region; Arrow shows the cotyledon insertion region. e. Normal seedling. PR: primary root; H: hipocotyl; C: cotyledon; E: eophylls. f. Germination. PR: protruded primary root; C: cotyledon. g. Abnormal seedling development. DR: dead root; H: hipocotyl; C: cotyledon. Arrow shows the absence of eophylls. Scales: 1 cm.

Table 1. Germination (%) and normal seedling development (%) of *Caesalpinia echinata* Lam., from seeds at four deterioration levels and three hydration levels (in g H_2O g^{-1} dry weight, g g^{-1}), incubated for 45 days at 13 °C. HVS: high vigour seeds; LVS: low vigour seeds; RDS: recent dispersed seeds; HDS: high deteriorated seeds. Means followed by the same letter (small for columns, capital for lines) are not different by Tukey´s test ($p < 0.05$).

Deterioration Levels of levels	Before incubation	Hydration levels (g g^{-1})		
		0.11	0.16	0.19
		Germination (%)		
HVS	71	51 aA	13 aB	5 aB
LVS	29	20 bA	7 aB	0 aB
RDS	69	49 aA	13 aB	5 aB
HDS	20	7 bA	2 aB	0 aB
		Normal seedlings (%)		
HVS	58	24 aA	5 aB	2 aB
LVS	24	5 cA	0 aA	0 aA
RDS	62	15 bA	7 aB	0 aC
HDS	9	0 cA	0 aA	0 aA

Our results provide evidence on the damage caused by oxidative reactions as revealed by tetrazolium tests performed on seeds based upon different hydration treatments as well as different temperatures. In this work, the target levels of controlled seed hydration covered two energetic states of water (Vertucci & Farrant 1995). Seeds containing only Type I water released low amounts of CO_2; however, at this hydration level the protection system against free radicals decreased, as evidenced by the high consumption of O_2. A greater hydration level reached on Type II water increased oxidation, and probably resulted in degradation of cell components, extending the damage to the embryonic tissues. Instead, the temperature clearly interfered with the rapid deterioration of *C. echinata* seeds. Incubation of seeds at temperatures above freezing conditions generated the development of bright red by the tetrazolium test, indicating an intense process of tissue damage, especially in meristematic regions, such as radicle, plumule and cotyledons insertion (figure 2d) as these regions are considered essential for maintaining seed viability and vigour, which are aimed at seed conservation. Seed storage at its basal temperature for germination, as suggested by Pritchard *et al.* (1995) for the storage of *Araucaria hunteinii* K. Schum, could be appropriate to reduce the deterioration rate of *C. echinata* seeds, but not for conservation. As shown by Mello & Barbedo (2007), the basal temperature for the germination of these seeds would range between 10 and 14 °C which would cause severe damage to their physiological quality. Photooxidation process shall be considered in the deterioration of *C. echinata* seeds in the same way as *Salix nigra* March. has been accounted for (Roqueiro *et al.* 2010). Both species have desiccation tolerant seeds. However, both *S. nigra* and *C. echinata* differ from typical orthodox seeds because they lose viability in few weeks at room temperatures. Roqueiro *et al.* (2010) found that *S. nigra* seeds were very susceptible to photooxidation since there were large decreases in galactolipids which are contiguous in thylakoid membrane structure. Therefore, light and oxygen may promote strong photooxidation processes mediated by free radicals (FR) and ROS.

Interestingly, *C. echinata* seeds, which were manually harvested directly from fruits and stored immediatelly after collection showed longer storability in comparison to seeds exposed to longer periods of light prior to their storage (Barbedo *et al.* 2002). Roqueiro *et al.* (2010) described that the embryonic tissues of *S. nigra* most damaged by FR were those of the abaxial side of the cotyledons and the most-external tissues of the axis and root tips. Therefore, the damage caused to seeds was evidenced by abnormal development in the roots and cotyledons, reducing the normal seedling development to zero level. This behaviour was also observed in our results, mainly at low temperatures. The low percentages of normal seedling development and the progressive germinability loss of *C. echinata* seeds, associated with the high amount of lipids (mainly linoleic acid)

as well as the great difference between O_2 consumption and CO_2 release, suggest that FR and ROS could be the most important factors leading to rapid seed deterioration. Furthermore, these seeds probably have insufficient amount of antioxidants which could reduce the damaging effects of ROS and FR, as tocopherols that are lipophilic antioxidants abundant in seeds of several species and are related to longevity (Sattler *et al.* 2004). Sucrosyl oligosaccharides (SOS) might detoxify ROS in chloroplasts and vacuoles and could act sinergistically with phenolic compounds as integrated redox system, quenching ROS and contributing to stress tolerance (Van den Ende & Valluru 2008). This could explain the reduction in stachyose levels of *C. echinata* seeds, greater for seeds stored at room temperature than seeds stored at cold temperatures (Garcia *et al.* 2006). However, the low initial levels of SOS in *C. echinata* seeds would not be enough for longer effects against FR and ROS. The conservation of *C. echinata* seeds remains a challenge and the effective conservation of this endangered species shall require incentive-driven preservation strategies. Therefore, *in situ* conservation is crucial for this species because apart from the difficulty in seed conservation, there are few remaining trees capable of producing seeds (therefore, the replacement of seed bank could be difficult), seed dispersal often coincides with less favorable periods to obtain high quality seeds (therefore, seed longevity could be very low) and the period in which the seeds are mature to be collected is summarized in a few days. Our results showed that free radical and reactive oxygen species production seem to be the main cause of tissue damage in *Caesalpinia echinata* seeds and, consequently, the main cause of its short storability.

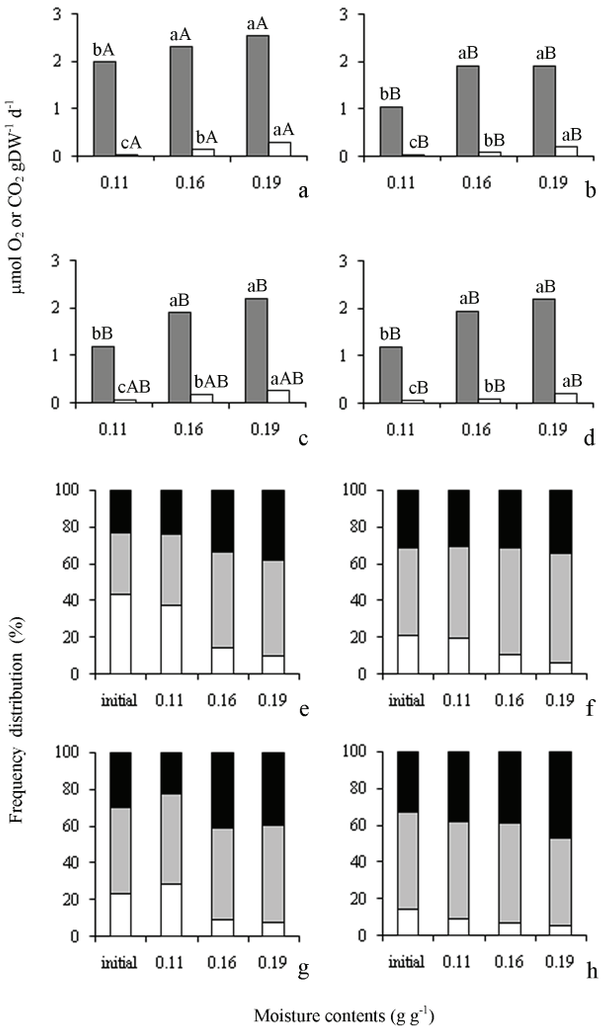

Figure 3. Seeds of *Caesalpinia echinata* Lam. with different initial levels of hydration (in g H_2O g^{-1} dry weight, g g^{-1}), incubated for 45 days at 13 °C. a-d. O_2 consumption (grey columns) and CO_2 release (white columns). e-h. Frequency distribution of viable (white), deteriorated (grey) and dead (black) tissues, obtained by the tetrazolium test. a and e: high-vigour seeds; b and f: low-vigour seeds; c and g: recent dispersed seeds; d and h: high deteriorated seeds. Means followed by the same letter (small, comparing water content, capital, comparing deterioration levels) are not different by Tukey's test ($p < 0.05$).

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