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Germination responses of *Jatropha curcas* L. seeds to storage and aging

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ABSTRACT

The present study investigated the effects of storage and aging on seed germination and seedling vigor in *Jatropha curcas* seeds, an oilseed plant with great potential for biodiesel production. Seeds were collected in 2009 and 2010 and stored under either room temperature or refrigerator conditions for 3, 6, 9 or 12 months. Analyses of seed germination and vigor, seed reserves and several biochemical factors were conducted in the stored seeds. We show that *Jatropha* seeds have a short viability period (less than 6 months) and that the increase of storage temperature accelerates the loss of seed germination potential. The loss of seed viability is due to metabolism of the seed itself, which remains active even under low levels of water and consumes the reserves of the seeds. Therefore, seeds stored for long periods demonstrated a marked decrease in their levels of starch and soluble proteins. Moreover, the presence of a high concentration of reducing sugars leads to the glycosylation of proteins and then lipid peroxidation, which increases the electrolyte leakage and subsequently causes extensive embryo damage or deterioration. These data are of great importance for decision making regarding the allocation of a particular seed lot, as they will directly influence the possibility of seed storage.

1. Introduction

Biodiesel, an alternative diesel fuel has attracted considerable attention during the past decade as a renewable, biodegradable, and non-toxic fuel. It is becoming increasingly important due to diminishing petroleum reserves and lower environmental impact when compared to petroleum diesel fuel (Berchmans and Hirata, 2008). Fortunately, inedible vegetable oils, mostly produced by seed-bearing trees and shrubs can provide an alternative to petrodiesel. With no competing food uses, this characteristic turns attention to *Jatropha curcas* L., which grows in tropical and subtropical climates across the world (Achten et al., 2008; Berchmans and Hirata, 2008; Pompelli et al., 2011; Santos et al., 2013). This species require little water and fertilizer, can survive on infertile soils, and is not browsed by cattle (Sarin et al., 2007) and making then suitable for cultivation on degraded soils (Achten et al., 2010). Oil contents, physicochemical properties, fatty acid composition and energy values of *J. curcas* were investigated (Achten et al., 2010; Banerji et al., 1985; Kandpal and Madan, 1995; Pramanik, 2003). The seeds contain between 25–40% (w/w) oil (Kumar and Sharma, 2008; Pompelli et al., 2010), with highest amount of unsaturated fatty acids (~73%) (Kumar and Sharma, 2008), which makes it ideal for biodiesel industries (Pramanik, 2003). Furthermore, seed production of *J. curcas* range from approximately 0.4 to more than 12 t ha^{-1} y^{-1} after 5 years of growth (Achten et al., 2010).

Much of the world's biodiesel comes from oil seeds (like soybean, sunflower, peanut), thus oilseeds species currently being widely studied. However, little attention has been given to the processes of seed storage, which constitutes a major problem for agriculture (Tekrony, 2006). The process is responsible for serious losses worldwide, especially in the tropics, where high temperatures and relative humidity prevail during the maturation and storage of seeds (Bilia et al., 1994). While deterioration is both irreversible and inevitable, the speed of the process can be controlled with appropriate harvesting, drying and storage techniques. There are several factors that are known to influence the progress of deterioration during seed storage. Both high temperatures and humidity during storage increase the deterioration speed of seeds (McDonald, 1999; Pukacka et al., 2009), and decreasing either of these factors significantly increases the storage life of seeds (Castellión et al., 2010).

For many years, the germination test was the only method for evaluating the physiological quality of seeds (Maeda et al., 1986). However, biochemical tests (*e.g.*, electrical conductivity, water content), stress tests (*e.g.*, cold, accelerated aging, controlled deterioration), and seedling growth tests have been studied in a

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wide range of crop species. Electrical conductivity (EC) test estimate the degree of cellular membrane damage resulting from seed deterioration by determining the quantity of lixiviated ions in a solution with a fixed volume of deionized water. The accelerated aging is induced by exposure to high temperature and humidity. This technique has been studied in several crop species, such as sunflower (Maeda et al., 1986), onion (Rao et al., 2006), rape (Takayanagi and Harrington, 1971), corn (Fessel et al., 2006), lettuce (Peñaloza et al., 2005), cotton (Mendonça et al., 2008) and others. In small seeds, however, the AA test is not accurate because of rapid water absorption. Because of these difficulties, the use of a saturated salt accelerated aging (SSAA) test has received much attention. The main purpose of the SSAA test is to promote slower aging of the seeds, ensuring that the observed deterioration effects are due to temperature and exposure period, but not by the increase of water content in the seeds (Mendonça et al., 2008). SSAA thereby provides more precise and repeatable measurements of seed vigor (McDonald, 1999).

Quality control and the potential storage of seeds involve, among other activities, the evaluation of germination and seed vigor. The benefits of high-quality vegetable seed include (i) rapid and uniform germination; (ii) the production of seedlings and plantlets that are better to able to withstand environmental stresses; (iii) the establishment of desired plant population targets and (iv) more uniform crop maturity and increased harvest efficiency. To our knowledge, the loss of physiological capacity in aged *Jatropha* seed has rarely been discussed. Therefore, the aim of the present study was to test the germination and vigor of *Jatropha* seeds that had been subjected to traditional storage over 12 months and to evaluate their vigor after planting and food mobilization during storage.

2. Materials and methods

2.1. Seed collection

The experiment was carried out in a commercial plantation of *J. curcas* L. in the Atlantic rain forest region (09°28′S; 35°51′W, 39 m a.s.l.). Each *Jatropha* plantation consisted of plants that were at least 8 years of age, and the spacing between plants was 2 m × 2 m. Fruits of *J. curcas* were randomly collected during the rainy season from May to June 2009–2010 and represented the entire genetic diversity of the population. The fruits were transported immediately to the laboratory, where the seeds were manually separated from the fruits. The seeds were air-dried for 2–3 days and stored (Pompelli et al., 2010) until use.

2.2. Aging tests

Fruits of *J. curcas* were collected and transported to the laboratory, as previously described in Section 2.1. Seeds were separated from the fruits and divided into two groups. The first group was stored in paper bags at room temperature (25 °C) over 12 months and subsequently be referred to as "2009 seeds". The other seed group was collected in 2010 and referred to as "2010 seeds or non-aged seeds". Both seed groups were treated by accelerated aging (AA), saturated salt accelerated aging (SSAA) or controlled deterioration (CD) tests. However, SSAA tests were not performed in "2009 seeds", because many seeds were contaminated by fungi and completely lost their viability after 12 months of storage in paper bags. *Accelerated aging* tests were conducted with 20 g of seeds placed on a wire mesh screen and suspended over 40 mL of water inside a plastic box (110 mm × 110 mm × 35 mm). The boxes were placed in a growth chamber, which was maintained at 42 °C and approximately 100% relative humidity for either 48 h or 72 h (Maeda et al., 1986). *Saturated salt accelerated aging* tests were conducted by placing the seeds on a screen inside a plastic box. Forty milliliters of a saturated NaCl solution (40%) was then added into each of the plastic boxes. The boxes were placed in a growth chamber, which was maintained at 42 °C and 76% relative humidity for either 48 or 72 h, as described by Peñaloza et al. (2005). The *controlled deterioration* test was conducted using seeds whose water content had been was adjusted to 18% (Rosseto and Marcos-Filho, 1995). These seeds were placed in aluminum foil bags and kept in a water bath at 41 °C for either 12 h or 24 h.

2.3. Seed storage

Fruits of *J. curcas* were collected during the rainy season from August 2010 and transported to the laboratory, as previously described in Section 2.1. Seeds were separated from the fruits and divided into two groups. The first group was germinated within 7 days of harvest; this is subsequently referred to as the control group. The other seed groups were dry-stored in paper bags at either room temperature (25 ± 2 °C) or refrigerator (4 ± 2 °C) conditions for 3, 6, 9 or 12 months; the seeds were refereed to as "aged seeds". The relative humidity at seed level during the experiments was 86 ± 5% and 42.5 ± 1.5% for the room temperature and refrigerator treatments, respectively.

2.4. Seed germination

Four replicates of 25 seeds per treatment were allowed to germinate in germination boxes (110 mm × 110 mm × 35 mm) with three sheets of Whatman No. 1 filter paper (Whatman Paper, Whatman International, Maidstone, UK) that had been moistened with 10 mL of distilled water plus 500 U of Mycostatin solution (100 mg L^{-1}) (Bristol-Myers Squibb Pharmaceutics, New York, NY, USA) to prevent fungal growth. More water was added each day as necessary. The germination boxes were sealed and then placed in a NT 708 growth chamber (New Technical Instruments, Piracicaba, SP, Brazil). Incubators were provided with four 20 W Sylvania cool-white fluorescent lamps, performing 40 μmol photons m^{-2} s^{-1} at the level of the germination boxes. The photoperiod and temperature conditions were 12 h at 25 ± 0.5 °C. Seed germination was evaluated daily, and seeds were considered to have germinated when its radicle extended at least 0.5 mm out of the seed. When no germination was observed in all treatments at least in five consecutive days, the germination was considered completed, as recommended by Ranal and Santana (2006). After 25 days, the germinability (%), germination rate ($\bar{t}$), uncertainty (U) and germination synchrony (Z) were recorded (Ranal and Santana, 2006). To ensure that the seeds used for the experiments were viable and maintained their viability after treatments, seed viability was determined by the ability to reduce 2,3,5-triphenyltetrazolium chloride to red colored formation (Brewer, 1949). Thus, seeds were reserved before and after the treatments, which were tested by the ability to reduce 2,3,5-triphenyltetrazolium chloride to red colored formation.

2.5. Seedling growth

The resulting seedlings ($n \geq 20$) from the aging tests were transferred to polyethylene bags (80 cm^3) filled with soil and sand (3:1), then grown in a greenhouse (28 ± 2 °C and 78.3 ± 9% RH). After 40 days, the shoot height, diameter at 1 cm from substrate level, total shoot and root biomass, shoot:root ratio and leaf area of each seedling were measured. Plant heights were measured from the substrate level to the top of the apical meristem. To measure the leaf areas, leaves were scanned using a scanner (Genius

1200 × 1200 dpi), and the images were analyzed using Image-Pro® Plus software (Pompelli et al., 2012).

2.6. Biochemical analysis of seeds

Seeds stored at 25 °C and 4 °C were collected at 0, 3, 6, 9 and 12 months of storage and used for subsequently analysis. All seed samples were thoroughly ground with a cold mortar and pestle in an ice bath until no fibrous residue could be observed. The ground seeds were placed in a 50% (v/v) ethanol solution (Trethewey et al., 1998) for starch, soluble-sugar and amino acids analyses, and in Stitt buffer (Armengaud et al., 2009) for soluble proteins analysis. Starch (Trethewey et al., 1998), soluble sugars (Dubois et al., 1956), soluble proteins (Bradford, 1976) and amino acids (Moore and Stein, 1954) were analyzed colorimetrically. All analyses were performed in triplicate.

The seed water content (SWC) was calculated using the formula: $[(W_f - W_i)/W_i]$, where W_i is the weight of fresh seeds and W_f is the weight after n months of storage. For this analysis were used four replicates of 25 seeds each per treatment. The solute leakage of stored seeds was estimated by placing three replicates of 1 g of seeds each into 50 mL deionized water for 24 h at 25 ± 0.5 °C. The electrical conductivity of the medium was measured with a conductivity meter (Conductivity meter model CD-4306, Lutron Electronic Enterprise Co., Ltd., Taipei, Taiwan).

3. Results

3.1. Germination of aging seeds

The germinability of the seeds after treatments with the aging tests is shown in Table 1. The storage of seeds affected all of the evaluated characteristics. We verified that seeds collected in 2009 had a lower germinability than seeds collected in 2010. The 72 h AA treatment promoted a significant increase in the germination of the 2009 seeds (*T*2) (29.33%) in comparison with the control seeds (*T*1; 7%). Moreover, *T*2 seeds showed a lower $\bar{t}$ and greater synchronization, which leads us to believe that the acceleration of metabolism is beneficial for the stored seed. In 2010 seeds (*T*4), the germinability was 65.33% and did not differ significantly from seeds subjected to either AA or SSAA. However, SSAA increases the $\bar{t}$ from 5.54 days in the control to 7.93 or 8.63 days after 48 or 72 h of incubation, respectively. These data reinforce the concept that aging seeds lose the ability to develop into uniform and vigorous seedlings more quickly than they lose the ability to germinate.

The maximum germinability was obtained in seeds collected in 2010 without seed aging treatments (control or *T*4 seeds) (Table 1). The AA increased the germinability of the 2009 seeds by 4.2-fold, although the germinability was still very low (29.33%; Table 1); however, this effect was not observed in the 2010 seeds. Independent of year, controlled deterioration negatively affected the germinability, and this effect was more evident in seeds exposed for 24 h (*T*3 and *T*10; Table 1) than in those exposed for 12 h (*T*9; Table 1). Independently of exposition time, all seeds submitted to controlled deterioration lose their seed viability inducing embryo dead, fact confirmed with yellowish embryo color after tetrazolium test (Table 2). Another treatment shows this pattern; however, the color of the embryos in seeds submitted to saturate salt accelerated aging were light red, while control seeds show a light and bright red color, in 2009 and 2010 seeds respectively (Table 2 and Supplementary Figure).

As shown in Table 3, all aging treatments significantly influenced seedling growth in both 2009 and 2010. Independent of aging treatments, 2010 seeds (*T*4) grew faster and accumulated more biomass (shoot and root) in comparison with other treatments.

3.2. Germination of storage seeds

The germinability of stored seeds was significantly reduced ($R_a^2 = -0.966$; $P \leq 0.001$) with a consequent elevation of germination rate ($R_a^2 = 0.435$; $P \leq 0.01$) (Fig. 1). The highest percentages of seed germination were obtained in control (non-stored) seeds, and the germinability decreased as age increased (Fig. 1). Storage duration, combined with temperature, had strong effects on the germination of *Jatropha* seeds. Only 7% and 2% of the seeds germinated after 12 months in storage at 4 °C and 25 °C, respectively (Fig. 1). Seeds stored under 25 °C showed more asynchronous (*i.e.*, high uncertainty) germination than their 4 °C counterparts (Fig. 1). These seeds also required longer germination times, *i.e.*, higher $\bar{t}$ (Fig. 1), and had lower germinability rates (Fig. 1). This reduction in seed germination can be attributed to the more stressful conditions of storage at high temperatures, such as 25 °C. The high synchrony (*i.e.*, low uncertainty) found in the seeds submitted to 6–12 months of storage was influenced by the low number of seeds that germinated on the same day (germinability <50% in 6 and 9 months treatments and <10% in seeds submitted to 12 months of storage). The coefficient of variation of uncertainty was greater, a result that confounds the significant differences between seeds germinated at 25 °C and 4 °C.

Likewise described in aging tests, the storage time affected the seed viability, with seeds stored of more than 6 months showed a decrease in the intensity of red after tetrazolium test (data not shown).

The *Jatropha* seeds demonstrated a reduction of starch ($R_a^2 = -0.878$; $P \leq 0.001$), without effects of sugars ($R_a^2 = 0.023$; $P = 0.838$) levels as their age increased (Fig. 2). Independently of temperature

Table 1
Germinability (%), germination rate ($\bar{t}$), uncertainty (U) and germination synchrony (Z) of *Jatropha curcas* seeds after control, accelerated aging (AA), saturated salt accelerated aging (SSAA) and controlled deterioration (CD). The values represent the media (±SE) of four replicates of 25 seeds. Means followed by different letters within columns are significantly different (Newman–Keuls test; $P < 0.05$).

Treatment	Germinability (%)	$\bar{t}$ (days)	Uncertainty (U)	Synchrony (Z)
Seeds collected in 2009				
Control = *T*1	7.00 ± 3.42c	5.17 ± 0.14ab	0.75 ± 0.48b	0.00 ± 0.00b
AA72h = *T*2	29.33 ± 1.33b	5.04 ± 0.11ab	1.11 ± 0.50b	0.50 ± 0.18a
CD24h = *T*3	0.0 ± 0.0c	nd	nd	nd
Seeds collected in 2010				
Control = *T*4	65.33 ± 0.94a	5.54 ± 0.16ab	2.30 ± 0.10a	0.24 ± 0.01ab
AA48h = *T*5	64.00 ± 5.16a	4.26 ± 0.22b	1.97 ± 0.26a	0.25 ± 0.05ab
SSAA48h = *T*6	41.00 ± 9.98ab	7.93 ± 0.76a	2.15 ± 0.13a	0.11 ± 0.05b
AA72h = *T*7	64.00 ± 4.62a	6.17 ± 0.28ab	2.64 ± 0.10a	0.14 ± 0.01b
SSAA72h = *T*8	41.00 ± 7.72ab	8.63 ± 0.28a	2.21 ± 0.22a	0.19 ± 0.02b
CD12h = *T*9	2.67 ± 1.33c	7.50 ± 2.25ab	0.38 ± 0.37c	0.00 ± 0.00b
CD24h = *T*10	0.0 ± 0.0c			

nd: not determined.

Table 2
Viability (%) determined by the tetrazolium test performed on *Jatropha curcas* staining with a 2,3,5-triphenyltetrazolium chloride solution and by immersion in that solution. For more details of the treatments, see Table 1.

Treatment	*n*	Viable seeds (%)	Non-viable seeds (%)	Predominant color
Seeds collected in 2009				
Control	50	9.5	90.5	Light red
AA72h	50	32.0	68.0	Light red
CD24h	50	0.0	100.0	Yellowish
Seeds collected in 2010				
Control	30	92.4	7.6	Bright red
AA48h	30	68.0	32.0	Bright red
SSAA48h	40	36.0	62.8	Light red
AA72h	25	42.0	58.0	Bright red
SSAA72h	50	17.0	83.0	Light red
CD12h	50	7.67	92.3	Yellowish
CD24h	50	0.0	100.0	Yellowish

of storage, the level of soluble proteins, measured by the Bradford method, decreased ($R^2_a = -0.935$; $P \leq 0.001$) after 12 months, with a drop of 90% and 87% in seeds stored at 25 °C and 4 °C, respectively (Fig. 2). The pattern of soluble sugars and amino acids during storage was curvilinear (Fig. 2). This curvilinear pattern indicates an increase in the soluble sugars and amino acid concentration through 6 months of storage, then a sharp decline.

Rapid rates of water uptake are observed in *Jatropha* seeds submitted to aging, probably due to the elevated concentration of osmotic solutes (Fig. 3). Independent of storage time, the seeds stored at 25 °C showed higher SWC values than seeds stored at 4 °C, likely because of the increased permeability of the seed coat. This response may affect membrane integrity during storage and elevate the electric conductivity of the seeds (Fig. 3). Conductivity values, corrected for seed size, were significantly greater as seed storage duration increased (Fig. 3) and were higher in seeds stored for 12 months at 25 °C (509 mS m^{-1} g^{-1} FW) than in those stored at 4 °C (424 mS m^{-1} g^{-1} FW). Both SWC ($R^2_a = -0.829$; $P \leq 0.001$) and EC ($R^2_a = -0.827$; $P \leq 0.001$) were significantly and negatively correlated with germinability. The increase in electrical conductivity during seed storage (Fig. 3) suggests that seeds lost significant vigor during storage. In this sense, the loss of vigor in seeds stored at 25 °C was higher in comparison with their 4 °C counterparts.

4. Discussion

The agricultural literature describes that seed storage significantly affects the viability of the seeds (Rao et al., 2006; Scalon et al., 2012), which great loss of their reserves as age increased. These conditions significantly affect seed germination (Bilia et al., 1994; Rice and Dyer, 2001). Thus, the goal of using seeds collected in 2009 was to test the effects of seed age on seed vigor. We germinated *J. curcas* seed stored for 12 months in paper bags to determine the germination response of seed of different age, as well as the efficiency of accelerated aging in the detection of seed vigor. In this sense, in 2009 seeds, the germinability was commonly low, with elevation of the $\bar{t}$ and the loss of synchronization in germination, *i.e.*, high uncertainty and low synchrony (Table 1). When uncertainty is applied to seed germination, the conventional interpretation is that low values indicate more synchronized germination (Ranal and Santana, 2006). It has been reported that dry storage increases the germinability of stored seeds over that of fresh seeds in many species, including *Prosopis juliflora* (El-Keblawy and Al-Rawai, 2006), *Lasia spinosa* (Tang and Long, 2008) and *Onopordum acanthium* (Qaderi et al., 2003). In *Jatropha* seeds, however, dry storage decreased germinability and increased $\bar{t}$ (Table 1; Fig. 1). The period between imbibition and emergence partly reflects the time necessary for the repair of damaged tissues upon hydration, because tissue repairs are energetically costly (Rice and Dyer, 2001) and time-consuming, the negative effects of delayed germination may be compounded by the reduction of stored reserves.

As shown in Table 1, the germination of aged seeds was 10% of that observed in freshly seeds. As discussed above, we believe the lower germination of aged seeds was due to the natural aging process, with consequent loss of organic solutes throughout the storage period, caused by continued respiratory activity of the seeds (Booth and Sowa, 2001; Srivastava, 2002), even when stored in temperature and humidity controlled.

Lin (1990) observed a decrease in the germination and vigor of bean seeds subjected to 1, 2, 3 and 4 days of aging that was related to an increase in solute leakage from seed cells, suggesting a close relationship between the deterioration of biological membranes and the loss of vigor and germination. This decay in the viability of aged seeds would normally be attributed to the loss of seed vigor

Table 3
Growth and vigor of *Jatropha curcas* seedlings after seed treatments. Means followed by different letters within columns are significantly different (Newman–Keuls test; $P \leq 0.05$). Values represent means ± SE of 10 replicates. For more details of the treatments, see Table 1.

Treatment	Shoot height (cm)	Diameter (cm)	Shoot biomass (g)	Root biomass (g)	Shoot:root	Leaf area (cm^2)
Seeds collected in 2009						
Control	11.20 ± 0.56c	1.09 ± 0.04e	2.01 ± 0.24c	0.51 ± 0.12d	4.33 ± 0.45a	179.70 ± 18.47c
AA72h	15.00 ± 0.45ab	1.32 ± 0.02b	3.78 ± 0.14b	1.06 ± 0.10b	3.83 ± 0.28abc	244.21 ± 8.04b
CD24h	nd	nd	nd	nd	nd	nd
Seeds collected in 2010						
Control	16.56 ± 0.88a	1.46 ± 0.05a	5.25 ± 0.52a	1.79 ± 0.08a	2.90 ± 0.16d	285.96 ± 4.02a
AA48h	13.75 ± 0.59b	1.26 ± 0.02bc	3.50 ± 0.14b	0.85 ± 0.03bc	4.12 ± 0.11ab	212.12 ± 13.97bc
SSAA48h	11.54 ± 0.12c	1.16 ± 0.03de	2.39 ± 0.17c	0.65 ± 0.04cd	3.36 ± 0.10cd	183.54 ± 6.98c
AA72h	14.18 ± 0.58b	1.30 ± 0.01b	3.44 ± 0.16b	0.96 ± 0.04b	3.54 ± 0.02bcd	215.65 ± 8.54bc
SSAA72h	12.88 ± 0.60bc	1.19 ± 0.04cd	3.16 ± 0.22b	0.71 ± 0.04cd	4.46 ± 0.15a	241.62 ± 16.18b
CD12h	nd	nd	nd	nd	nd	nd
CD24h						

nd: not determined.

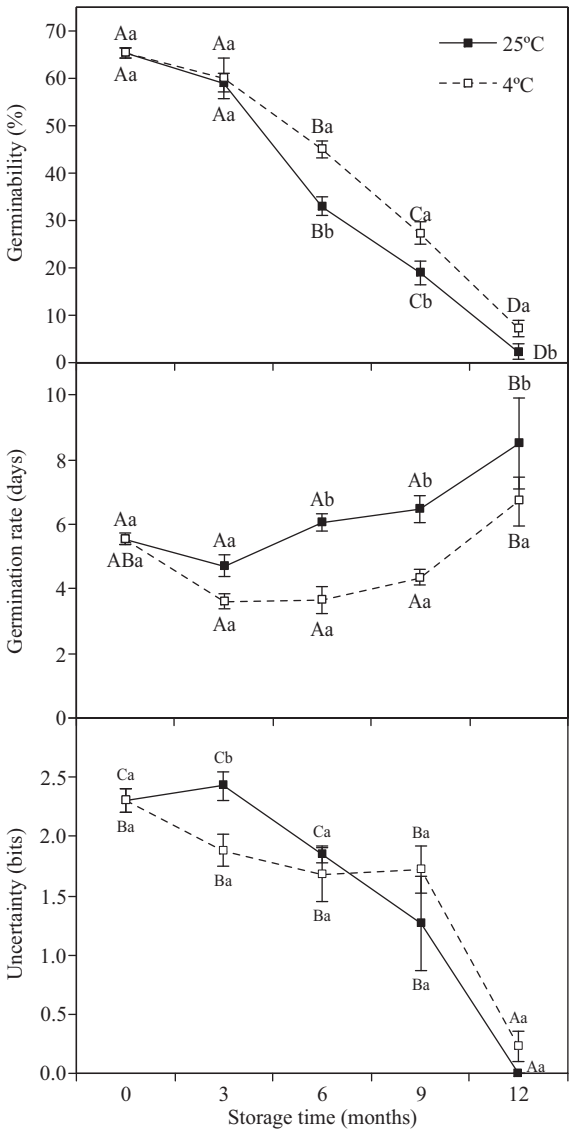

Fig. 1. Germinability, germination rate and uncertainty of *Jatropha curcas* seeds after 0, 3, 6, 9 or 12 months of storage at 25 °C (black symbols) or 4 °C (open symbols). Means followed by different upper case letters represent statistically significant differences between the means for each period of storage, and different lower case letters represent statistically significant differences among the means of each storage temperature ($P \leq 0.05$, Newman–Keuls test). The values represent the media (±SE) of four replicates of 50 seeds.

due to ultracellular changes as temperatures increase. In fact, seed germination of *Jatropha* was strongly and negatively affected by the treatments of aging and storage, which was also reported in soybean (Torres et al., 2004), corn (Fessel et al., 2006), beech (Pukacka et al., 2009), onion (Rao et al., 2006) and other herbaceous (Cruz et al., 2012) seeds.

In distinct form of soybean (Rosseto and Marcos-Filho, 1995), the *Jatropha* seeds cannot resist to CD treatment and completely lost their germination capacity (Table 1). As this result did not occur with seeds subjected to AA and SSAA, it is believed that the more drastic acceleration of metabolic activities in the CD tests caused the high temperatures and high RH to affect the physiological quality of seeds more significantly. Consequently, germinability of seeds in the CD treatment was significantly reduced in comparison with both control seeds and those submitted to the accelerated aging test (Table 1). A probable cause may be the originally high moisture content of *Jatropha* seeds, which can occasionally reach 20% of fresh weight (Pompelli et al., 2010).

Seed aging causes a decrease of seedling growth (Table 3), with 2010 seeds producing taller (*i.e.*, higher stalk diameter, shoot

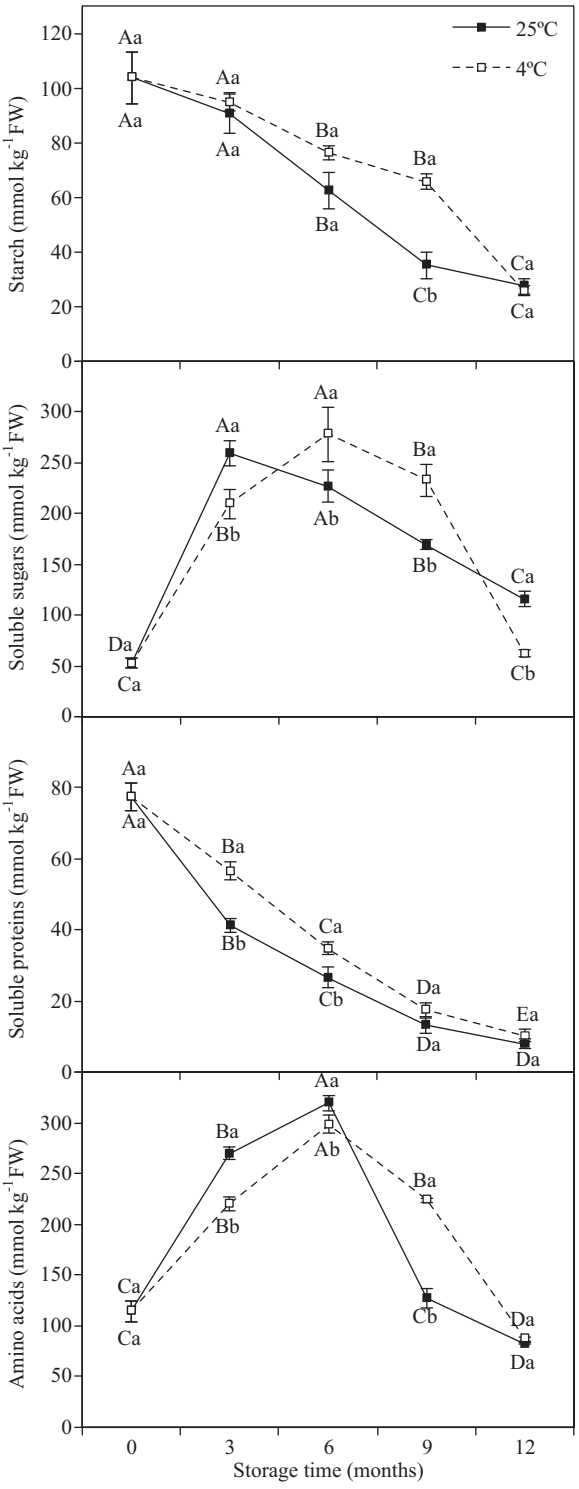

Fig. 2. Starch, soluble sugars, soluble proteins and amino acids contents of *Jatropha curcas* seeds after 0, 3, 6, 9 or 12 months of storage at 25 °C (black symbols) or 4 °C (open symbols). Means followed by different upper case letters represent statistically significant differences between the means for each period of storage, and different lower case letters represent statistically significant differences among the means of each storage temperature ($P \leq 0.05$, Newman–Keuls test). The values represent the media (±SE) of four replicates.

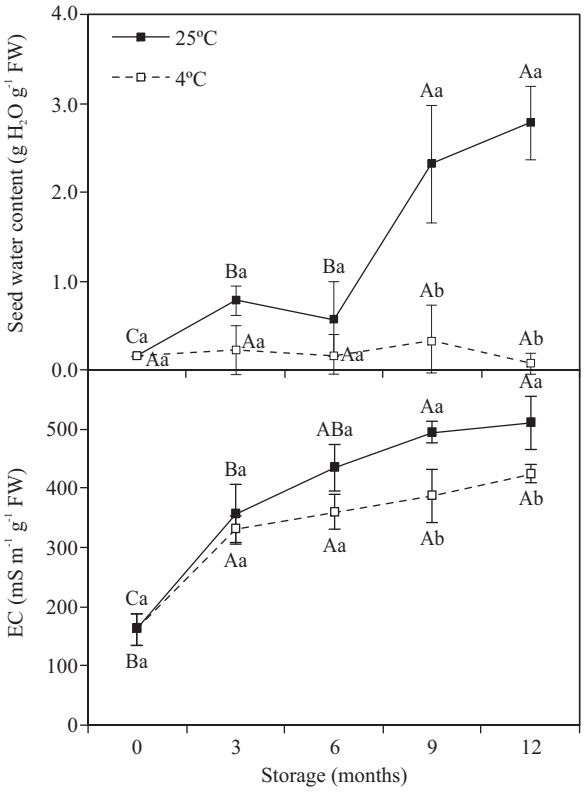

Fig. 3. Relative water content (RWC) and electrical conductivity (EC) of *Jatropha curcas* seeds after 0, 3, 6, 9 or 12 months of storage at 25 °C (black symbols) or 4 °C (open symbols). Means followed by different upper case letters represent statistically significant differences between the means for each period of storage, and different lower case letters represent statistically significant differences among the means of each storage temperature ($P \leq 0.05$, Newman–Keuls test). The values represent the media (±SE) of four replicates.

and root biomass) and more vigorous plantlets (*i.e.*, higher leaf area). In some species, the AA and SSAA test caused a decrease in percent germination and a consequent increase in abnormal seedlings (Maeda et al., 1986; Peñaloza et al., 2005) seemingly due to the high ethylene production of aged seeds (Takayanagi and Harrington, 1971). In this study, however, all *Jatropha* seedlings were normal through 40 days of growth, and no morphological alterations were noted in seedlings as a result of the seed aging treatments (Table 3).

Biochemical parameters are increasingly used as indicators of seed viability and vigor (Ramiro et al., 1995). During storage, *Jatropha* seeds show a decrease in starch levels and an increase in sugars (Fig. 2). Prolonged storage generally results in considerable nutrient loss, mainly of sugars and proteins (Sandoval et al., 2002; Shah et al., 2002). Seed deterioration could result from a gradual hydrolysis of the soluble sugars (Fig. 2). The hydrolysis of sugars in the seeds would lead to an accumulation of reducing sugars, which would eventually threaten the integrity of a proteins due to the formation of Maillard products (Sun and Leopold, 1995). The Maillard reaction refers to a series of complex reactions that cause proteins to become aggregated and lose solubility. These modifications can take place by non-enzymatic glycosylation with a reducing sugar or by reaction with the aldehydes produced from free radical-mediated lipid peroxidation. The Maillard reaction may contribute to seed aging through the chemical alteration of functional proteins, thereby depressing metabolic capability and reducing the ability of the metabolic system to limit free radical damage and repair this damage during germination (see more details in Castellión et al., 2010). Furthermore, although these glycosylated proteins may remain as in the embryo as a food source (Sandoval et al., 2002), the way in which this process would occur is not clear. It is known, however, that glycosylated proteins are not detected by the standard methods of protein detection (Castellión et al., 2010). The electrophoretic pattern obtained by SDS-PAGE denoted the presence of storage proteins, further supporting the hypothesis of disaggregation of high-molecular-weight protein aggregates created during storage. These aggregates are most likely destroyed upon activation of the repairing mechanisms during pre-germinative humidification (Castellión et al., 2010). Protein insolubilization could be reverted during water uptake in the first stages of germination. However, the ability to overcome protein insolubilization did not necessarily lead to higher germination rate, as evidenced by the low germination percentage (Castellión et al., 2010). It is possible that the Maillard reaction found in the *Jatropha* seeds an environment for its occurrence. This hypothesis can be confirmed by the high relative water content (~18%) on *J. curcas* seed (Pompelli et al., 2010).

Seed storage may influence seed viability and reduce seed vigor depending on the time span and conditions of storage (Panobianco et al., 2007). Stress due to high temperature and humidity aggravates the deleterious lipid peroxidation reactions (Bilia et al., 1994; Panobianco et al., 2007) of aging seeds (Sung and Jeng, 1994). Water content has an important effect on the rate of seed deteriorative reactions and aging during storage (Rosseto and Marcos-Filho, 1995). During storage, physical and chemical changes occur that alter the tensile strength of seed coats and increase their permeability to water and gases (Qaderi et al., 2003), thereby reducing the hard-seededness of the coats and causing the leakage of solutes, such as organic and inorganic ions, sugars, amino acids and even proteins, into the surrounding medium (Govender et al., 2008). The physiological basis for seed-coat impermeability is not fully understood, but morphological (Egley and Paul, 1981), enzymatic (Egley et al., 1983), and phenolic (Marbach and Mayer, 1975) differences have been implicated in the phenomenon. Depending of the conditions of seed storage, this loss can lead to a severe loss of intracellular constituents and often results in extensive embryo damage or deterioration (McDonald, 1999). In deteriorated seeds, the repair mechanism is either absent or inefficient (Sung and Jeng, 1994) or the membranes are completely damaged, thus permitting the leaching of greater electrolyte amounts (Fessel et al., 2006; Rao et al., 2006) and causing the loss of vigor (Panobianco et al., 2007).

5. Conclusion

We were able to demonstrate, with a high degree of consistency, that *Jatropha* seeds have generally high vigor. However, the high moisture content of the seeds can interfere with this vigor, which can quickly be lost depending on the conditions of seed storage; this loss was confirmed by the accelerated aging tests, which did not reduce the germination of *Jatropha* seeds. The results of the present study provide useful information regarding the use of electrolyte leakage as an indicator of *Jatropha* seed vigor. Along with salt saturated accelerated aging and controlled deterioration tests, this indicator may offer an alternative method of seed vigor measurement for *Jatropha* seed producers and technologists. Although there are numerous reports in the literature regarding the effects of storage on the viability of various seed species, this is the first study to report this effect in seeds of *J. curcas*. It should be noted that with the advent and expansion of the use of biodiesel from *Jatropha* seeds, new knowledge must be added of this species, either at the plant (*i.e.*, growing conditions, agronomic characteristics) and the level of seeds, with better techniques of storage, and faster methods for evaluating and detecting compounds force stored in the seeds. These data are of great importance for decision making regarding

the allocation of a particular seed lot, as they will directly influence the possibility of seed storage.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.indcrop.2012.08.035.

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