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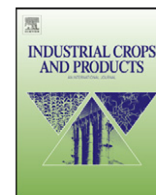

Germination responses of *Jatropha curcas* L. seeds to storage and aging

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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.

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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

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