Industrial Crops & Products 118 (2018) 214–224

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Effects of seed storage time and salt stress on the germination of *Jatropha curcas* L.

Flavio Lozano-Isla[a], Mariana L.O. Campos[a], Laurício Endres[b], Egídio Bezerra-Neto[c], Marcelo F. Pompelli[a,*]

[a] Plant Physiology Laboratory, Federal University of Pernambuco, Department of Botany, Recife, PE 50670901, Brazil
[b] Plant Physiology Laboratory, Federal University of Alagoas, Maceió, AL, Brazil
[c] Department of Chemistry, Federal Rural University of Pernambuco, Recife, PE, Brazil

ARTICLE INFO

Keywords:
Salt tolerance
Seed storage
Seed germination
Biofuel
Carbon credit
NaCl

ABSTRACT

Jatropha curcas L. has been proposed to be a potential tropical plant for augmenting renewable energy sources. Due to its several merits, *J. curcas* deserves to be considered as candidate for biofuel production instead of for food seed sources. Its seeds contain 27–40% oil that can be processed to produce a high quality biodiesel fuel that is usable in a standard diesel engine. While *J. curcas* grows well under low-rainfall conditions, the salt tolerance capacity of this species has received meager attention. Furthermore, another major problem faced by this species is the lack of a safe system capable of guaranteeing seed germination when stored for medium or long periods of time. Thus, *J. curcas* seeds show two important problems: (*i*) rapid loss of viability, resulting from the high respiratory rate of the seeds during storage, and (*ii*) seed sensitivity when germinated under salt conditions. Therefore, the main objectives were (*i*) to verify if storage in very low humidity boxes can improve germination without affecting the oil content of the seeds and (*ii*) to compare different genotypes to prove their salt tolerance capacities. The results indicate that from the use of desiccants, all the germination parameters were kept reasonably constant, and germination was essentially unchanged during the entire storage period. Biochemical activity, supported by the drastic reduction in respiratory activity of the seeds, was also decreased during the storage in order to keep the seed reserves intact; therefore, *J. curcas* seeds may be resistant to storage when stored in a controlled process. On the other hand, it was shown that *J. curcas* presents a moderate tolerance to salinity. This species is able to germinate in the presence of up to 150 mM of Sodium chloride (NaCl), although a drastic reduction in the biomass accumulation was observed with the increase in salt concentration in the irrigation water. It was shown that the germination was reduced to values close to 4% at 150 mM NaCl; whereas biometric and biomass characteristics were strongly affected by the increase in salt content. However, it was shown that genotypes 114, 171 and 183 were shown to be potentially tolerant, whereas genotypes 218 and 133 were sensitive.

1. Introduction

There is a growing consensus that the use of the dirtiest fossil fuels may have leveled off (Horan, 2017). At the same time, renewables, particularly wind and solar, are booming. For example, demand for oil and gas is growing, and most electricity still comes from fossil fuels. Aviation and shipping have no viable alternative to hydrocarbons. However, several reports have shown that the replacement of kerosene used in aviation can be, at least, in part replaced by biofuels (Airbus, 2016; Baroutian et al., 2013; Gutiérrez-Antonio et al., 2016; Jim, 2009). In addition, the impending shortage of fossil fuels has caused humanity to rationally use the primary energy sources, consequently, new technologically developed versions of power plants have been designed in order to increase not only energy efficiency but also ecological efficiency. From these concepts, the idea of carbon credits arose. Carbon credits create a market for reducing greenhouse gases emission by giving a monetary value to the cost of polluting the air. In this sense, *Jatropha curcas* (purging nut) comes into play, because this species requires little water and fertilizer (Achten et al., 2010; Pompelli et al., 2010a; Sapeta et al., 2013), can survive on infertile soils and is not grazed by cattle (Sarin et al., 2007), making this species suitable for cultivation on degraded soils (Achten et al., 2010). Furthermore, *J. curcas* should be the opportunity to gain future project financing through carbon credits (Horan, 2017; Torres et al., 2011; van Rooijen,

* Corresponding author.
E-mail address: marcelo.pompelli@ufpe.br (M.F. Pompelli).

https://doi.org/10.1016/j.indcrop.2018.03.052
Received 22 July 2017; Received in revised form 21 March 2018; Accepted 23 March 2018

2014; Wani et al., 2012). The majority of the present projects combine plantations and small-scale production (Divakara et al., 2010; Pandey et al., 2012).

J. curcas is a species belonging to the Euphorbiaceae family and has multiple uses; this species is widely distributed in many tropical and subtropical regions in the Americas, Africa and Asia (Achten et al., 2010). Over the past 20 years, this species has gained much attention as a potential crop for bioenergy production, since its seed oil can easily be converted to high-quality biodiesel. In addition, this species is not edible and therefore does not compete with the other oilseeds used as food sources, such as maize and soy (Pompelli et al., 2011). *J. curcas* is easily propagated and has a growth rate, short period until the first fruit harvest, low seed cost (Heller, 1996), high oil content (40–58%) (Pandey et al., 2012; Pompelli et al., 2010b), and good adaptation to different agroclimatic conditions (Divakara et al., 2010; Fini et al., 2013).

In taking into account that *J. curcas* is a potential species for the large-scale generation of biodiesel, it is easy to think that it will be necessary to plant hundreds or thousands of hectares of trees to produce a satisfactory amounts for commercial exploitation (Contran et al., 2013). In this sense, it is also important to remember that in times of harvest, market prices usually fall sharply (Summer and Mueller, 1989), which is where the storage of seeds comes into play. Seed storage is one the most important factors that negatively affect seed viability, which includes the time elapsed between harvest and utilization (Marcos-Filho, 1998; Marcos-Filho et al., 1984). Marcos-Filho (1998) reported that seed storage is a major problem for agriculture, as seed storage is responsible for considerable losses throughout the world, especially in the tropics (Tekrony, 2006), where high temperatures and high relative humidity prevail during seed maturation and storage (Marcos-Filho, 1998). *J. curcas* does not escape this pattern, as it presents high metabolism, causing its seeds to rapidly lose their viability during storage (Moncaleano-Escandon et al., 2013). Although deterioration is irreversible and unavoidable, the speed of the process can be controlled by appropriate harvest, drying and storage techniques (Summer and Mueller, 1989). In this sense, the use of a drier atmosphere can protect seeds (Hay et al., 2012; Rao et al., 2006), and slow down the respiratory process, which is responsible for the loss of organic compounds that will be used as energy sources by the embryo during germination (Chidananda et al., 2014).

Another factor that negatively influences agriculture, mainly in irrigated crops, is salinity (Kumar and Sharma, 2008), with NaCl being the predominant salt. At the world level, approximately 33% of agricultural lands irrigated by saline water are facing the problem of salt stress (Shrivastava and Kumar, 2015). Seed germination begins with imbibition of the quiescent seed and ends with the elongation of the embryonic axis, at which point the reserves begin to be mobilised to provide energy to the developing embryo. Salinity can also affect germination by limiting the absorption of water in the seeds (osmotic effect) (Almansouri et al., 2001; Hegarty, 1977), increasing the toxicity of ions or the combination of both (Apse et al., 1999). In addition, NaCl can affect the mobilization of reserves (Bouaziz and Hicks, 1990), structural organization and protein synthesis in embryos (Alencar et al., 2015). In saline environments, plant adaptation during germination involves decisive stages for species establishment, and such factors can negatively influence the germination process (Ungar, 1995). Furthermore, salinity affects plant growth and development (Munns and Tester, 2008), negatively influencing the stages of its development (Almansouri et al., 2001; Kumar and Sharma, 2008). However, throughout their evolution, plants have developed mechanisms for regulation and tolerance to salts. Some studies have described the ecophysiological aspects of the tolerance of *J. curcas* to NaCl (Campos et al., 2012; Díaz-López et al., 2012; Rajaona et al., 2012); however, these studies focused on only one genotype, and there is no known research that has examined the effect of NaCl on different genotypes during germination and early seedling growth. In this study, five distinct genotypes of *J. curcas* exposed to different NaCl treatments were studied to determine their tolerance and to understand the morphological and physiological responses of these species under conditions of salinity with respect to germination and initial development.

Thus, the main hypotheses of this work were (*i*) to verify if the use of a desiccant agent could help maintain the viability and germinability of the seeds of *J. curcas* when stored for long periods of time and (*ii*) to study the mechanism of the tolerance of *J. curcas* to salinity among the some genotypes cultivated in Brazil.

2. Materials and methods

2.1. Aging tests

To test the effect of storage on seed viability, an artificial aging test was used to reduce the water content in the interstices of the seeds with a desiccant material composed of silica gel (Sigma-Aldrish, part number 10087, Sigma-Aldrich Chemie, Schnelldorf, Germany). The genotype used in this experiment was 171 from Maceió, AL, Brazil. In each experimental unit, 50 seeds of *J. curcas* were arranged in germination boxes (110 mm × 110 mm × 35 mm) were added under a stainless steel mesh suspended 2 cm from the desiccant. In each germination box, 1.5 g of silica gel were added per gram of seed (as previously tested where it was shown to be the best in preventing vigor, and seed germination; Supplementary Fig. 1). Four storage times (*i.e.*, 0, 3, 6, 9, and 12 months, with zero representing non-stored seeds) were tested and stored in a refrigerator at 4 ± 2 °C. After each storage time, the seeds were removed from their storage boxes and then allowed to germinate.

2.2. Germination tests with aged seeds

After removal from the germination boxes that contained the desiccant, the seeds were allowed to germinate in other germination boxes (110 × 110 × 35 mm) that contained two sheets of germination test paper soaked with twice the weight of the paper in water; the boxes were sealed with Parafilm® M (Sigma-Aldrish, part number P7793-1EA, Sigma-Aldrich Chemie, Schnelldorf, Germany) and placed in a growth chamber (mod. NT 708, New Technical Instruments, Piracicaba, SP, Brazil). The incubator was equipped with four cold white fluorescent lamps of 20 W that produced 40 μmoles $m^{-2} s^{-1}$ at the level of the germination boxes. The photoperiod was 12 h, and the temperature conditions were

25 ± 0.5 °C. Germination was evaluated daily for a period of 25 days. The seeds whose radicles had emerged from the integuments were considered germinated.

2.3. Biochemical analysis of the seeds used in the aging test

A portion of 30% of the aged seeds was carefully ground in liquid nitrogen and stored at −20 °C until use. For extraction of soluble carbohydrates (TSC), soluble amino acids (TSA) and starch (STR), samples were solubilized in 50% (v/v) ethanol (Trethewey et al., 1998), whereas for the analysis of total soluble proteins (TSP), the samples were extracted in Stitt buffer (Armengaud et al., 2009). To measure soluble carbohydrates and starch, soluble proteins and soluble amino acids, the methods described by Dubois et al. (1956), Bradford (1976) and Moore and Stein (1954), respectively, were used. For the quantification of the oil content (OIL), the method described in detail by Ahmad et al. (1981) was used. To quantify the glucose (GLC), fructose (FTS) and sucrose (SCR), coupled to the production of 6-phosphogluconate, in the sequential presence of hexokinase, phosphoglucoisomerase, glucose-6-phosphate dehydrogenase and invertase enzymes was used, as described by Stiit et al. (1989). All these analyzes were performed in triplicate.