

# *Jatropha curcas* L. and *J. macrocarpa* Griseb: Seed Morphology, Viability, Dormancy, Germination and Growth of Seedlings

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## Abstract

To achieve a good production of a crop, it is essential to know the ability of the species to successfully complete two critical stages in the life cycle such as germination and seedling establishment. In this paper we study in comparative form structure of the seed, the importance of tegument in dormancy, the effect of accelerated aging on seed germination and viability, and the early and late growth in *J. curcas* and *J. macrocarpa*. External morphology of the seeds allow difference and internally also the embryos show evident differences. *J. macrocarpa* germination is around 0% - 4%. The total removal of tegument showed a 50% increase and the other treatments between 0% - 10%. Aging accelerated by Tetrazolium test allowed a comparative analysis of VP and GP. *J. curcas* maintains both to 96 h, while *J. macrocarpa* the seed viability is registered along the all treatment. *J. macrocarpa* seeds have less synchronicity than those of *J. curcas*. ABA and JAs were detected in tegument of *J. macrocarpa* and *J. curcas* seeds. JA could have a roll in inhibition of germination of *J. macrocarpa* seeds. Early and late growth, FW and DW of root, hypocotyl, epicotyl and leaf of *J. curcas* were significantly different in both species.

## Keywords

Dormancy, Germination, Phytohormones, Growth, Seeds, Viability

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## 1. Introduction

The genus *Jatropha* (Euphorbiaceae) includes 172 species native to Central America and is also widely distributed in Africa, Asia and South America. In Argentina, it is reported that 11 native species of *Jatropha* include *J. curcas* L. and *J. macrocarpa* Griseb. These plants are perennial deciduous shrub, with the greatest importance mainly from its biofuel potential [1]. The interest in the cultivation of some species of genus *Jatropha*, suitable for production of biodiesel oil from their seeds [2], is due to their adaptation to marginal conditions for agriculture production. *J. curcas* and *J. macrocarpa* growing in semi-arid and arid soils, and their non-edible seeds have high oil content [3]. Some years ago it has been known that biodiesel obtains from *J. curcas* and *J. macrocarpa* meets international standards [2] [4] and is reported to exhibit better performance than conventional petroleum diesel [5]. However, the potential of *J. curcas* as a biofuel plant is limited by its low seed production [6]. Despite the clear evidence of the abundant aboveground biomass generated by *J. curcas*, these data are not indicative of high seed productivity [7].

While that *J. curcas* has aroused much interest worldwide as a new oleaginous crop for biodiesel, it is not a suitable crop for arid zones and plants are sensitive to frost [8] and need annual rainfall is greater than 700 mm for good fruit production [3]. In arid areas with winter frost, the species *J. macrocarpa* could be an interesting alternative because its natural distribution area presents in such climatic conditions [2].

To achieve a good production of a crop, it is essential to know the ability of the species to successfully complete two critical stages in the life cycle such as germination and seedling establishment. The information that exists on *J. macrocarpa* is very scarce and is especial referring to botanical aspects, and does not yet have studies on the seed morphology or requirements for germination and establishment of seedling. While it is true that *J. curcas* grows readily from seeds or cuttings; trees propagated by cuttings show a lower longevity and possess a lower drought and disease resistance than those propagated from seeds [9]. Spatial distribution of germination is generally controlled through seed and fruit morphology, which enhances dispersal of the offspring from the maternal habitat. In contrast, temporal distribution of germination is controlled mainly by the physiological status of seeds. A variation among individual seeds in a population, in terms of physiological status, allows each seed to germinate at a different time, which is an important strategy for seeds to avoid competition with their siblings or extinction of all individuals due to a disastrous condition [10]. On the other hand, the plants have evolved seed dormancy, a temporal suppression of germination under the conditions favorable to germination; which ensures that seeds germinate at the appropriate time. Dormancy is a complex trait because it is influenced by both environmental and endogenous factors. Moreover, the final level of dormancy is determined by the contributions of the different tissues that comprise a seed; between them the seed coat [11] [12]. Induction of seed

dormancy during the maturation stage and its release at a dry state after a certain period of time, which is called “after-ripening”, is widespread phenomena observed in diverse species of seed plants [13]. In fact, in various species the mechanisms related to dormancy imposed by the seed head are related to restrictions the permeability of water and/or oxygen, with the existence of a mechanical resistance to the protrusion of the radicle, with the presence of inhibitors and/or the inability to leach inhibitors from the embryo [14] [15] [16]. Studies previous showed high variation in *J. curcas* seeds germination [17] [18]. This variation is influenced by genotype, age, storage conditions of the seed and environmental conditions of the crop [19] [20] [21] [22] [23]. The germination rate decreases with age and with the storage of seed, this strongly affects the content of reserve substances in seeds and low germination rate [23]. Also, it has been reported that the mechanical rupture of the tegument as a pre-planting treatment significantly increased seed germination and slightly stimulated the growth of *J. curcas* seedlings [24]. *J. curcas* and *J. macrocarpa* present a hard seminal covering that encloses the endosperm and the embryo. Several authors consider that this tegument is one of the factors that induce dormancy in *J. curcas* [21] [25] [26]; however, in the *J. macrocarpa* the effects of the tegument in the low germinative power, is not yet studied. Several plant hormones play a role in dormancy and germination control [27] [28]. Abscisic acid (ABA) is one of such hormone; that plays a prominent role in dormancy and germination control in coordinated interaction with various others [10] [29] [30]. Recently, evidences have been provided for an interaction between ABA and jasmonates (JAs) in the regulation of these processes [31] [32]. In particular, in ABA-JA interaction, Dave *et al.* [31] confirmed that *Arabidopsis thaliana* seed dormancy is correlated with the accumulation level of oxo-phytodienoic-acid (OPDA), which acts synergistically with ABA, ABI5 transcription factor, DELLA RGL2 protein and MFT dormancy promoting factor in regulation of this process. On the contrary, Xu *et al.* [32] reported that JAs and ABA have opposing roles in the regulation of dormancy release by stratification in wheat.

We hypothesize that seed morphological, anatomical and physiological characteristics determine the differences in the establishment of crops of *J. curcas* and *J. macrocarpa*.

The aim of the present research was to study in comparative form structure of the seed, the effect of accelerated aging on seed germination and viability, and the importance of tegument in dormancy and the early growth in *J. curcas* and *J. macrocarpa*.

## 2. Material and Methods

Seeds of *J. macrocarpa* were collected in a wild population located 30 km south of La Rioja city, Argentina (29.3°S; 66.8°W, 438 m above sea level) while the seeds of *J. curcas* were obtained from experimental plots located in Siete Palmas, Formosa, Argentina (58°17'59.67"W - 25°13'21.04"S).

### **Seed morphology**

Ten *J. macrocarpa* and *J. curcas* seeds were imbibed in distilled water for 24 h to facilitate removal of tegument to observe the embryo and nutritive tissues and ten endosperm of each species.

### **Seed treatments to break dormancy in *J. macrocarpa***

The seeds of *J. macrocarpa* were subjected to different scarification and stratification treatments: T1) Control (not ripping); T2) Scarification with sandpaper; T3) Total elimination of the tegument; T4) Immersion in boiling water for 1 min and then immersed in cold water for 24 h; T5) Alternating hot and cold water for 5 min each one T6) Immersion in concentrated H<sub>2</sub>SO<sub>4</sub> for 15 min; T7) Immersion in concentrated H<sub>2</sub>SO<sub>4</sub> for 30 min; T8) Stratification in wet and cold paper (4°C) for 90 days; T9) Stratification in moist sand and cold (4°C) for 90 days. After each treatment the seeds were immediately placed on filter paper in Petri dishes containing 3 ml of distilled water at 30°C temperature. The test was conducted under dark condition. Germination percentages (GP) were determined during 30 days. We used 20 seeds by treatment, with three replications each one.

The seeds of *J. curcas* don't were subjected to different treatments scarification and stratification because they haven't dormancy.

### **Accelerated aging treatment**

Seed aging aims to study the effect of environmental stress conditions on the germination of seeds. Seeds from both species (*J. macrocarpa* without tegument) were introduced in fine mesh bags and then placed in glass jars with water half way, so that the seeds are held without contact with water. They were placed in an oven at 40°C and darkness for 0 (control), 24, 48, 72, 96 and 120 h [33]. The experiment was performed in triplicate and each glass jar contained 20 seeds. After the aging treatment, the seeds were tested for Tetrazolium viability and germination test.

### **Viability test after aging treatment**

The viability from two species was measurement from seed under 0, 24, 48, 72, 96, 120 h of aging treatment by Tetrazolium method [34] [35]. Twenty seeds were cut and placed in a 1% solution of 2,3,5-triphenyl tetrazolium chloride for 24 h. at 40°C - 45°C and were classified as viable and non-viable seeds [36]. All experiment was made by triplicate. Images were captured using Nikon Stereoscopic Zoom Microscope SMZ1000/SMZ800 and digital camera.

### **Germination test after aging treatment**

Seeds of two species from aging treatment for 0, 24, 48, 72, 96 and 120 h were performed to germination test. We used 20 seeds by treatment, with three replications each one. The seeds were placed on filter paper in Petri dishes containing 3 ml of distilled water at 30°C temperature. The test was conducted under dark condition. Germination percentages (GP) were determined during 30 days.

### **Extraction and purification of endogenous hormones**

ABA and JA were extracted from both *Jatropha* species tegument using a modification of the protocol of Durgbanshi *et al.* [37]. 200 mg of tegument were

homogenized in a mortar with liquid nitrogen and 5 ml ultra-pure water. D<sub>6</sub>-ABA (NRC-Plant Biotechnology Institute, Saskatoon, Canada) and D<sub>6</sub>-JA (Leibniz-Institute of Plant Biochemistry, in Halle, Germany) were used as internal standards. Extracts were transferred to 50-ml tubes, centrifuged at 1500× g for 15 min, pH of the supernatant was adjusted to 2.8 with 15% acetic acid, and supernatant was partitioned twice against an equal volume of diethyl ether. The aqueous phase was discarded, and the organic fraction was evaporated under vacuum. Dried extracts were dissolved in 1 ml methanol. Samples were filtered through a syringe filter tip on a vacuum manifold at flow rate < 1 ml min<sup>-1</sup>, and the eluate was evaporated at 35°C under vacuum in a SpeedVac SC110 (Savant Instruments Inc. NY, USA). The assay employed four biological replicates.

#### **Hormone identification and quantification with liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI MS-MS)**

ABA and JAs were separated from tissue using reverse-phase high-performance liquid chromatography (HPLC). An Alliance 2695 separation module (Waters, Milford, MA, USA) equipped with a RestekC<sub>18</sub> column (100 m × 2.1 mm, 3 μm) was used to maintain performance of the analytical column. Fractions were separated using a gradient of increasing methanol concentration, constant glacial acetic acid concentration (0.2% in water) and initial flow rate 0.2 ml·min<sup>-1</sup>. The gradient was increased linearly from 40% methanol/60% water—acetic acid at 25 min, to 80% methanol/20% water—acetic acid. After 1 min, the initial conditions were restored, and the system was allowed to equilibrate for 7 min. The identification and quantification of all hormones was performed with a quadruple tandem mass spectrometer (Quattro Ultima, Micromass, Manchester, UK) fitted with an electrospray ion (ESI) source, in multiple reactions monitoring mode (MRM) using precursor ions and their transitions ABA (m/z 263/153) and D<sub>6</sub>-ABA (m/z 269/159) and JAs (m/z 209/59), D<sub>6</sub>-JA (m/z 215/59), with retention times of 8.25 and 14.30 min, respectively. The collision energies used were 20 eV for JAs and ABA, and the cone voltage was 35 V. The spec spectrometry software used was Mass Lynx version 4.1 (Micromass).

#### **Early and late growth parameters**

Seeds without tegument of *J. curcas* and *J. macrocarpa*, were sowed in terrines with soil:perlite 50:50 and kept at field capacity. They were placed at 30°C of temperature and photoperiod 16 h light/8 h dark and 60% of relative humidity. Fresh weight (FW) and dry weight (DW) of root, hypocotyls and epicotyls and true leaves were measured. Samples were dried in an oven at 60°C until constant DW was obtained. FW and DW were expressed in g plant<sup>-1</sup>. FW and DW were recorded at once a week, for 40 days (early growth) and then every 20 days (late growth) for 8 months old plants. The experiment took place in five replicates with 20 seeds per each replication.

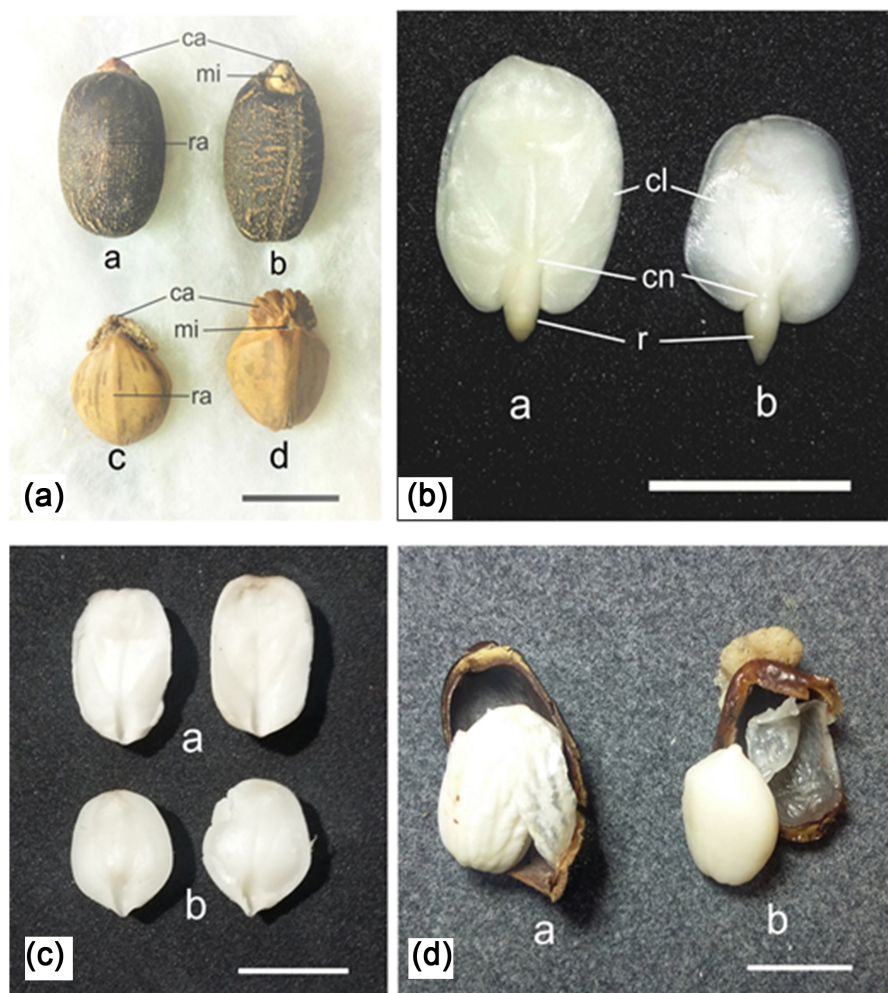
#### **Statistical analysis**

Analysis of variance (ANOVA) was applied and data were subjected to Multiple Range the Duncan. Test using the software INFostat-UNC.

### 3. Results

#### External and internal seed morphology

Seeds of *J. curcas* are oblong in shape with a convex dorsal area along which the raphe is visualized and in the hilar region a small conical caruncle of ivory color is observed (Figure 1(a), a). The average measurements of these seeds are: length  $1.8 \pm 0.03$  cm, width  $1.0 \pm 0.01$  cm and thickness  $0.8 \pm 0.02$  cm. The tegument is very dark brown, smooth with porous texture and with small cracks that are more evident in the ventral zone. In this area, in the center of the caruncle the micropyle is observed (Figure 1(a), b). The tegument of *J. macrocarpa* is also smooth but light brown, mottled with dark brown. The seeds are sub-spherical, on average they are  $1.5 \pm 0.05$  cm long,  $1.3 \pm 0.04$  cm wide and  $0.9 \pm 0.02$  cm thick. The dorsal area is slightly convex and is traversed by an evident



**Figure 1.** (a) Visual external aspect of seeds of *J. curcas* (a and b) and *J. macrocarpa* (c and d). (b) Embryo of *J. curcas* (a) and *J. macrocarpa* (b). (c) Internal surfaces of the endosperm in contact with the embryo of *J. curcas* (a and b) and *J. macrocarpa* (c and d). (d) Seeds with broken tegument showing the outer surface of the endosperm and the protective membrane, *J. curcas* (a) and *J. macrocarpa* (b). Abbreviations: ca caruncle; cl, cotyledonal leaf; cn, cotyledonal node; r, radical; mi, micropyle; ra, raphe. Scale bar = 1 cm.

raphe (**Figure 1(a)**, c) in these seeds the ventral zone is the most convex and to the hilar zone end is inserted. With a prominent caruncle that forms a ridge with irregular edges, the micropyle can be seen at the base of the crest (**Figure 1(a)**, d). The embryo of *J. curcas* has a cylindrical radicle of approximately 6 mm in length and two whitish ovoid, foliate, cotyledons that are inserted into the embryonic axis knot at its base. The blade of these leaves are thin and show a trinervia venation as it presents three main nerves that are born from the base of the blade foliar, two of them open laterally. The ribs are very marked on both the abaxial and adaxial sides (**Figure 1(b)**). In *J. macrocarpa* the embryo is smaller than in *J. curcas*, the radicle reaches 3 mm in length and its apex is markedly conical, its cotyledons are broad-bodied with apex rounded. The blade is fleshy and also trinervia although the ribs are less evident than in *J. curcas* (**Figure 1(b)**). In both species the cotyledons are faced for their adaxial face protecting the sheepish, and are externally surrounded by the nutrient tissue that in these seeds is the endosperm. This nutrient tissue is strongly attached to the embryo and has on its outer surface the impression of the radicle and the veins running along the abaxial surface of each cotyledon blade (**Figure 1(c)**). On the outside the endosperm is protected by a whitish membrane that is in contact with the tegument. The protective membrane of the seed of *J. curcas* is thicker and is furrowed by a set of important veins that leave their imprint on the inner side of the endosperm to which it covers firmly (**Figure 1(d)**). This membrane in *J. macrocarpa* is very tenuous and although this innervate does not form remarkable grooves in the surface of the same (**Figure 1(d)**).

#### **Break dormancy**

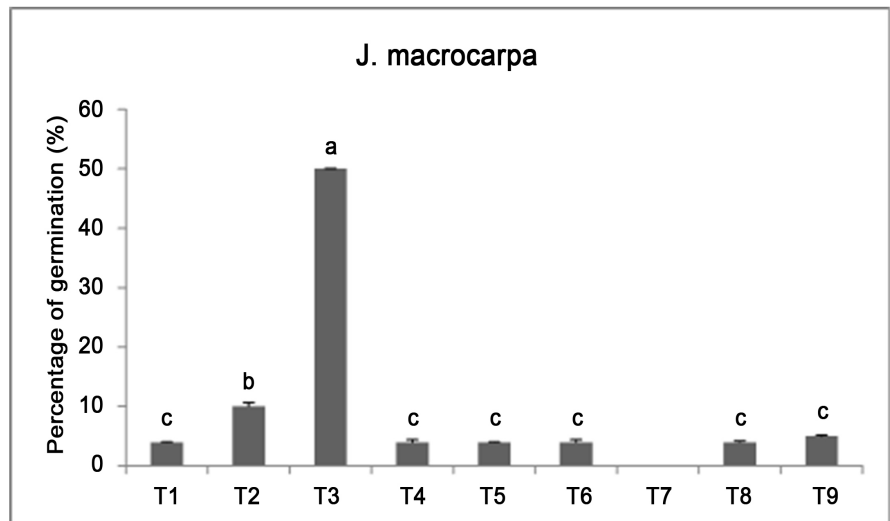
*J. macrocarpa* present dormancy since it does not germinate by traditional methods. The effect of the different treatments to break seed dormancy of *J. macrocarpa* is showed in **Figure 2**. The total removal of tegument showed a 50% increase in germination percentage, with the other treatments achieved between 0% - 10%. The seeds of *J. curcas* germinate without treatments for that reason the treatment was done only in the seeds of *J. macrocarpa*.

#### **Seed viability test after aging treatment**

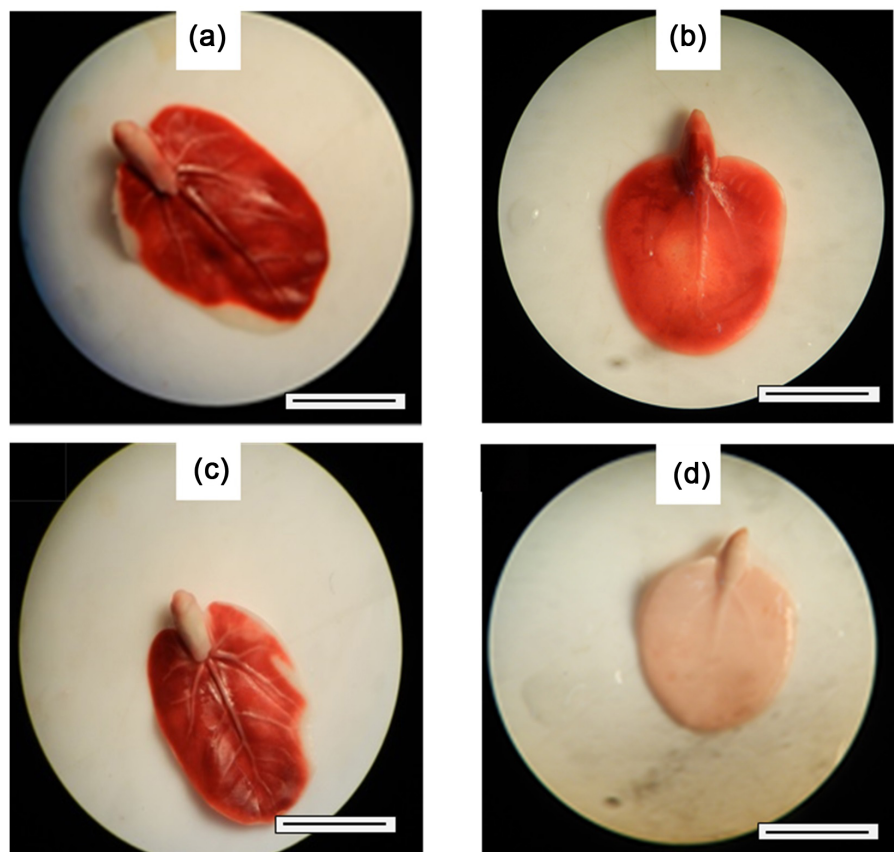
Seed viability is showed in **Figure 3**. In *J. curcas* (**Figure 3(a)**) and *J. macrocarpa* (**Figure 3(b)**) embryo of viable seeds showed a red colour. Moreover, in non-viable seed unstained embryo was observed in both *J. curcas* (**Figure 3(c)**) and *J. macrocarpa* (**Figure 3(d)**). In *J. curcas*, seed viability was maintained until 96 h of aging accelerated, from 86.67%  $\pm$  0.33% at T0 to 50% at T96, not registering viable seeds at 120 h. However, in *J. macrocarpa* the percentage of viability (PV) in seeds decreased slowly along the experiment (93.33%  $\pm$  0.33% at T0; 76.67%  $\pm$  0.33% at T24; 63.33%  $\pm$  0.33% at T48; 56.67%  $\pm$  0.33% at T72; 36.67%  $\pm$  0.33% at T96 and 33.33%  $\pm$  0.33% at T120) (**Table 1**).

#### **Seed germination test after aging treatment**

*J. curcas* seeds showed a GP between 80% and 90% at T0, T24, T48 and T72 h and decreased after T96 of treatment. Seeds with 120 h of aging with viability of



**Figure 2.** Scarification and stratification treatments of *J. macrocarpa* seeds. T1) Control (not ripping); T2) Scarification with sandpaper; T3) Total elimination of the tegument; T4) Immersion in boiling water 1 minute and cold water 24 h; T5) Alternating hot and cold water; T6) Immersion in H<sub>2</sub>SO<sub>4</sub> for 15 min; T7) Immersion in H<sub>2</sub>SO<sub>4</sub> for 30 min; T8) Stratification in paper and cold (4°C); T9) Stratification in moist sand and cold (4°C). Values are mean ± SE from triplicate experiments. Different letters indicate significant differences (p ≤ 0.05).



**Figure 3.** *J. curcas*: Visual aspect of viable seeds (a) and non-viable seeds (c). *J. macrocarpa*: Visual aspect of viable seeds (b) and non-viable seeds (d). Scale bar = 1 cm.



0% did not germinate (Table 2). In *J. macrocarpa* the highest GP was found at T0 (66.66%), then, GP decreased to 10 and 3.33% at T24 and T48, respectively. After 48 h of aging, GP was 0% (Table 2).

#### ABA and JAs level in *Jatropha* tegument

ABA and JAs were detected in tegument of *J. macrocarpa* and *J. curcas* seeds. JAs were the most abundant compound. Level of JAs was higher in *J. macrocarpa*

**Table 1.** Percentage of viability of *J. curcas* and *J. macrocarpa* using the tetrazolium technique at different times of aging. T0: Control, T24: 24 h, T48: 48 h, T72: 72 h, T96: 96 h; T120: 120 h. Values are mean  $\pm$  SE from triplicate experiments. Different letters indicate significant differences ( $p \leq 0.05$ ) in each time.

Different times of aging	Species	Viability (%)
T0	<i>J. curcas</i>	86.67 $\pm$ 0.33 b
	<i>J. macrocarpa</i>	93.33 $\pm$ 0.33 a
T24	<i>J. curcas</i>	76.67 $\pm$ 0.33 a
	<i>J. macrocarpa</i>	76.67 $\pm$ 0.33 a
T48	<i>J. curcas</i>	83.33 $\pm$ 0.33 a
	<i>J. macrocarpa</i>	63.33 $\pm$ 0.33 b
T72	<i>J. curcas</i>	76.67 $\pm$ 0.33 a
	<i>J. macrocarpa</i>	56.67 $\pm$ 0.33 b
T96	<i>J. curcas</i>	50 $\pm$ 0 a
	<i>J. macrocarpa</i>	36.67 $\pm$ 0.33 b
T120	<i>J. curcas</i>	0 $\pm$ 0 b
	<i>J. macrocarpa</i>	33.33 $\pm$ 0.33 a

**Table 2.** Percentage of germination of *J. curcas* and *J. macrocarpa*, after different times of aging. T0: Control, T24: 24 h, T48: 48 h, T72: 72 h, T96: 96 h; T120: 120 h of aging, evaluated at 7, 14, 21 and 30 days. Values are mean  $\pm$  SE from triplicate experiments. Different letters indicate significant differences ( $p \leq 0.05$ ) in each time.

Different time of aging	Species	Days			
		7	14	21	30
T0	<i>J. curcas</i>	83.33 $\pm$ 0.33 a	86.66 $\pm$ 0.33 a	86.67 $\pm$ 0.33 a	86.67 $\pm$ 0.33 a
	<i>J. macrocarpa</i>	50.00 $\pm$ 5.77 b	66.66 $\pm$ 0.33 b	66.66 $\pm$ 0.33 b	66.66 $\pm$ 0.33 b
T24	<i>J. curcas</i>	86.67 $\pm$ 0.33 a	86.67 $\pm$ 0.33 a	86.67 $\pm$ 0.33 a	86.67 $\pm$ 0.33 a
	<i>J. macrocarpa</i>	10.00 $\pm$ 0 b	10.00 $\pm$ 5.77 b	10.00 $\pm$ 5.77 b	10.00 $\pm$ 0 b
T48	<i>J. curcas</i>	80.00 $\pm$ 5.77 a	80.00 $\pm$ 0 a	80.00 $\pm$ 5.77 a	80.00 $\pm$ 0 a
	<i>J. macrocarpa</i>	3.33 $\pm$ 0.33 b	3.33 $\pm$ 0.33 b	3.33 $\pm$ 0.33 b	3.33 $\pm$ 0.33 b
T72	<i>J. curcas</i>	90.00 $\pm$ 0 a	90.00 $\pm$ 5.77 a	90.00 $\pm$ 5.77 a	90.00 $\pm$ 0 a
	<i>J. macrocarpa</i>	0 $\pm$ 0 b	0 $\pm$ 0 b	0 $\pm$ 0 b	0 $\pm$ 0 b
T96	<i>J. curcas</i>	33.33 $\pm$ 0.33 a	40.00 $\pm$ 5.77 a	40.00 $\pm$ 0 a	40.00 $\pm$ 5.7 a
	<i>J. macrocarpa</i>	0 $\pm$ 0 b	0 $\pm$ 0 b	0 $\pm$ 0 b	0 $\pm$ 0 b
T120	<i>J. curcas</i>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
	<i>J. macrocarpa</i>	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0

(101%) than in *J. curcas*. In contrast, ABA level was higher in *J. curcas* (628%) than in *J. macrocarpa* (Table 3).

**Early and late growth parameters**

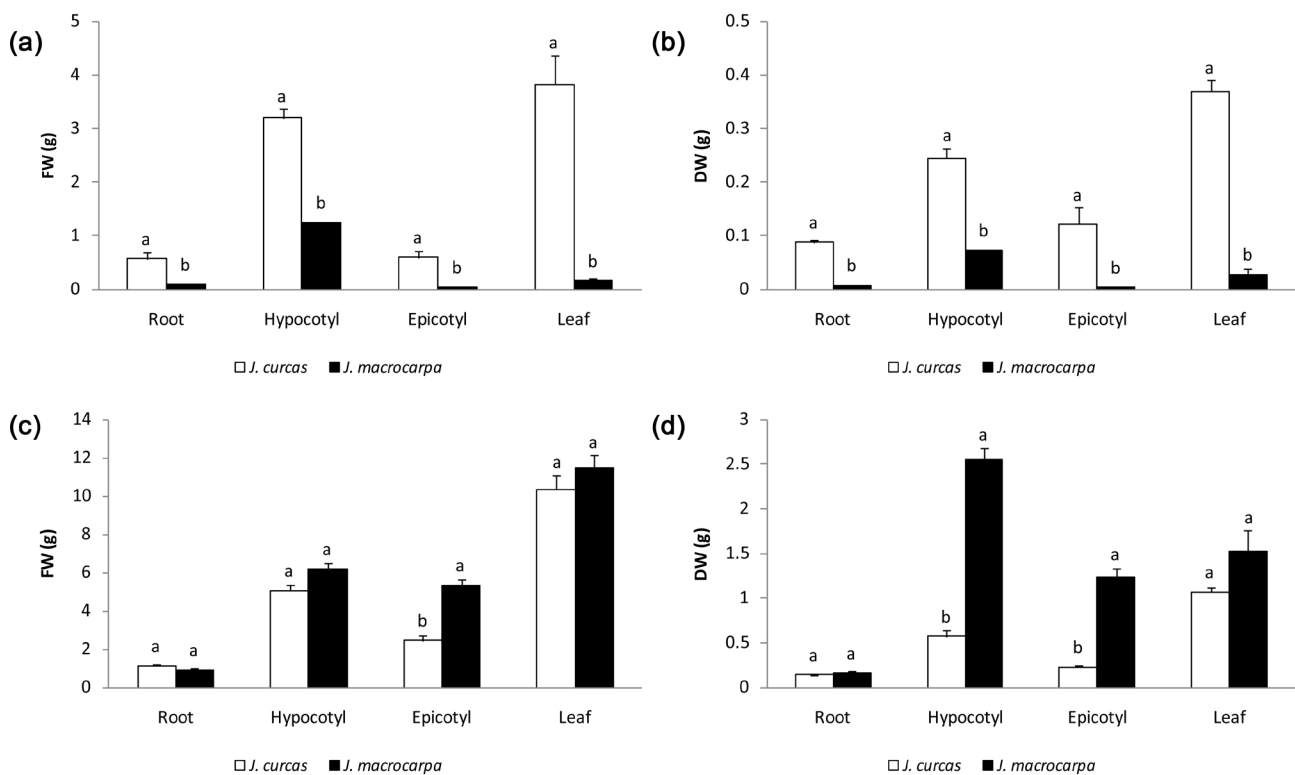
At 40 days (early growth), FW and DW of root, hypocotyl, epicotyl and leaf of *J. curcas* were significantly higher than *J. macrocarpa* (Figure 4(a); Figure 4(b)). At 140 days (late growth), epicotyl FW of *J. macrocarpa* was higher than *J. curcas*. Regards to, FW of root, hypocotyls and leaf not significant differences were registered for both species (Figure 4(c)). Hypocotyl and epicotyl DW of *J. macrocarpa* were significantly higher than that of *J. curcas* (Figure 4(d)).

**4. Discussion**

External morphology of the seeds of *J. curcas* and *J. macrocarpa* allow different

**Table 3.** JA and ABA contents in *J. curcas* and *J. macrocarpa*. Values are mean ± SE from triplicate experiments. Different letters indicate significant difference (p > 0.05).

Hormone	Species	Content (pmol·g <sup>-1</sup> FW)
JA	<i>J. curcas</i>	8369.26 ± 2682.33 b
	<i>J. macrocarpa</i>	16860.41 ± 1482.22 a
ABA	<i>J. curcas</i>	737.96 ± 68.82 a
	<i>J. macrocarpa</i>	101.29 ± 27.08 b



**Figure 4.** Growth parameters of *J. curcas* (white bars) and *J. macrocarpa* (black bars) at 40 days ((a) and (b)) and 140 days ((c) and (d)) of grown. (a) and (c): root, hypocotyl, epicotyl, and leaf FW. (b) and (d): root, hypocotyl, epicotyl, and leaf DW. Values are mean ± SE from triplicate experiments. Different letters indicate significant difference (p ≤ 0.05).

them easily since they vary in size, shape and coloration of the tegument and the carúncle. Internally also the embryos show evident differences in special, in form, size and thickness of the foliar cotyledons and in length and shape of the radicle. Nevertheless both species are endosperm and this tissue surrounds firmly to the embryo. The internal structure of these seeds is unusual in dicotyledons, but is common in *Euphorbiaceae*. A similar organization has been described for the seeds of other species of this family as *Ricinus communis* L. [38], *Croton floribundus* Spreng y *Croton urucurana* Baill [39], *Jatropha elliptica* Mull. Arg. [40] and *Cnidosc ulus juercifolius* Paxe K. Hoffm [41].

Morphological characteristics found in the seeds of *J. curcas* were in many aspects coincident with those described previously by Loureiro *et al.* [42], although the cotyledon form for these authors is cordiform, with the narrow apex and a broad base excavated and rounded while for us they are ovoid.

The protective seed membrane that is located between the tegument and that tightly binds to the endosperm in *J. curcas*, it carry microorganisms that will hamper the seed germination [26]. Nevertheless the tegument is a major barrier to radicle protrusion for many seeds [43], whose physical properties determine its effect on seed germination [14]. Our results showed that germination percentage of *J. macrocarpa* seeds with intact tegument was very low (4%). However, when the tegument was removed completely increased GP (from 4% to approximately 50%), despite the seed high viability (93%). These results indicate the presence of physical dormancy in *J. macrocarpa* seeds. In fact, it was reported that mechanical or chemical scarification can break physical seed dormancy [15]. Similarly, Zhang *et al.* [44] demonstrated that the tegument of canola (*Brassica napus*) restricted seed germination at low temperature and this inhibitory effect was more apparent in the yellow seed line compared to the black seed line. It is possible that differences in color of tegument of *J. curcas* and *J. macrocarpa* are also related to the differential level of dormancy observed between these seeds. In many species, the presence of tegument pigmentation color is associated with a different degree of permeability and dormancy [45].

The tegument eliminated of *J. macrocarpa* increase the germination of seeds, from 4% to 50%, so the tegument is directly relationally with de dormancy, although other tissues could be involved in the imposition of dormancy. This is a clear example that dormancy in some seeds resides in their teguments with probable intervention of the hormones, JA in this particular case. In this sense, the seed dormancy can be imposed by the embryo, the envelopes (tegument, endosperm, etc.), or a combination of both factors to an extent that depends on the plant species [46]. Recent physiological and molecular studies have shown that physiological dormancy includes an embryo and coat component, and their sum and interaction determine the degree of whole-seed physiological dormancy [15]. In fact, the dormancy attributed to different tissues of the seed has been reported in different species [47] [48].

Hormones found in the dry seed are generally provided from the mother plant

during seed maturation; in some cases, hormones leak from the embryo during late embryogenesis [49]. On the other hand, the mechanisms that lead to the definition of the structures composing the seed are highly coordinated and extremely complex and they involve a tight hormonal control and a continuous interchange of signals from and to the maternal tissues [50]. There is considerable evidence that ABA is an important positive regulator of both the induction of dormancy and their maintenance [29]. We found that the tegument of *J. macrocarpa* dry seed have a significantly lower ABA content than *J. curcas*, for this reason we assume that the tegument ABA level is not directly linked to germination and/or dormancy of these *Jatropha* species. Indeed, in *Arabidopsis thaliana* the final ABA levels present in mature dry seeds are unrelated to the depth of dormancy [11] [51] that suggest that ABA abundance or signaling, or both, play an indirect role in promoting seed dormancy during seed development [52]. In contrast, different studies have shown that the seed structures surrounding the embryo contain compounds possessing germination inhibitory activities including ABA [13]. For example, Jin *et al.* [53] showed that high concentrations of ABA in pericarp and seed coat of rose achene could be inhibiting germination. Respect to JAs, there is no functional evidence supporting a role for a correlation between endogenous content in dry seed and level of seed dormancy. For example, Preston *et al.* [54] showed that in dry seed of *Arabidopsis thaliana*, the JAs content in non-dormant seeds was ten-fold higher than in dormant seeds. On the contrary, Andrade *et al.* [55] showed a high JAs content in pericarp of dormant B123 sunflower cypselas. In agree to the findings of Andrade *et al.* [55], we found higher JA levels in tegument of *J. macrocarpa* respect to *J. curcas*. In effects JA could have a roll in inhibition of germination of *J. macrocarpa* seeds.

The seeds of *J. curcas* and *J. macrocarpa* differ in the germination physiology. Our work showed that GP of *J. curcas* seed was 83% at 7 days, similarly to seeds collected from Mision Laishi, Formosa, Argentina [21]; whereas the viability (86%) was similarly to reported for seeds from Río Largo, Brasil by Moncaleano-Scandon *et al.* [23]. In fact, different researches showed that the GP of *J. curcas* changed according to provenance, for example, between 50% to 89% in seeds from Africa [56], 98.96% in hybrid from Malaysia and Indonesia and 53.43% in hybrid form Cape Verde and South Africa [19]. Also, the germination percentage can varies according to the substrate used for germination test [57], being superior in sand than in clay [58]. In addition, it was observed that the GP change from 74% to 88% in fruits with different ripening state [58].

On the other hand, the storage of *J. curcas* seeds for a period of 12 months decreased the GP and oil content; however the use of carbon dioxide and nitrogen during storage prolonged the seed viability and minimized its deterioration [59]. Nevertheless, it was reported that the cold storage ( $10 \pm 2$ ,  $55^\circ\text{C} \pm 5\%$  RH) is the most adequate condition to maintain its viability for a year [60].

The loss of seed viability subjected to accelerated aging is due to the fact that

while the metabolism remains active, the seed show low water levels, the seed reserves are consumed, there is a marked decrease in the levels of starch and soluble proteins, an increase of reducing sugars which leads to protein glycosidation and lipid peroxidation, increasing the loss of electrolytes and consequently causes deterioration in the embryo [23]. In this sense, the aging tests by Tetrazolium, allowed to know the viability and the PG of the seeds of *J. curcas* and *J. macrocarpa* at different time.

At seven days after the aging process, all the viable *J. curcas* seeds germinated, which indicates a high synchronicity in the germination seeds from Formosa. However, the seed GP of *J. curcas* in the early stages of aging was higher than that found by Moncaleano-Scandon *et al.* [23] and Oliveira *et al.* [61]. On the other hand, at 96 h of aging treatment, the GP showed values similar to those determined by Oliveira *et al.* [61]. However, our results showed that in the first times of aging treatment the seed viability of *J. curcas* was superior to them reported by Moncaleano-Scandon *et al.* [23]. On the other hand, studies using varieties of *J. curcas* from Africa, Asia, Central America and South America showed that seed germination was completed 14 days after sowing [62].

On the contrary, *J. macrocarpa* seeds have less synchronicity than those of *J. curcas*. The strong decrease of GP throughout the experiment indicates the greater sensitivity of this species to conditions of accelerated aging.

Aging accelerated by Tetrazolium test allowed a comparative analysis of VP and GP. *J. curcas* maintains both to 96 h, while *J. macrocarpa* the seed viability is registered along the all treatment. The loss of capacity to germinate at 48 h of aging would be indicating the presence of seed dormancy. The difficult germination of *J. macrocarpa* makes its cultivation less promising for productive purposes. In addition, Wassner *et al.* [2] reported that this species presents a low productivity per plant and the explosive dehiscence of fruits decrease its yield, for this reason its genetic improvement would be necessary for their use for biodiesel production. In contrast, the high germination percentage post-aging of *J. curcas* seeds indicates their good germinative behavior under a wide range of environmental conditions, which facilitates the establishment of the crop through mechanized sowing.

The early growth seedlings study is important to investigate seed development following germination. The morphological and biochemical parameters determination such as fresh weight, dry weight, leaf area and photosynthetic pigments content during the early plant growth stage is frequent and very important [63]. In previous research, it was studied the growth of *J. curcas* seedlings, and parameters such as leaf number, root length and aerial part were measured [64].

These authors emphasize the importance of study early growth because it determines the success in seedling establishment. In the same way, Montes Osorio *et al.* [62] reported the importance of velocity of seedling early growth for the plant establishment. In this study, they determined variations of different biomass variables during the early stages of growth of *J. curcas* seedlings from Asia

and Africa. Also, Achten *et al.* [65] showed the importance to study *Jatropha* genus growth and their behavior under stress using varieties from Ethiopia, India and Thailand. In addition, Maes *et al.* [66] reported the presence of succulent stems in *Jatropha* varieties from Ethiopia, India and Thailand, which counteract the water loss through the leaves during periods of drought, because the leaves remain in the plants and fall gradually in the time. These studies correlate with our observations where the hypocotyl and epicotyl of *J. curcas* from Formosa showed higher early growth than *J. macrocarpa*. In addition, the higher content of chlorophyll a in *J. curcas* during the early growth stages provides interesting data since the chlorophylls of the photosynthetic apparatus are in direct relation to the physiological state for the photosynthesis process, which determines the plant biomass (Data not shown).

In late growth, we observed that the aerial part contributed most to the fresh weight and total dry weight of the plant. In agreement Matsumoto *et al.* [67] reported that the aerial part contributed mostly to the dry weight of the *J. curcas* seedlings originated from seeds from Tanzania grown under different potentials water.

Measurements of fresh weight and dry weight can determine the biomass, allometric relationships and some important features of the seedlings.

It was reported that in *J. curcas* seedlings, under optimal conditions of humidity, the biomass inversion is medium in shoots and low in roots [65]. In this study, the aerial dry biomass of *J. curcas*, represented by the leaves, is twice-fold high than that of Mediterranean trees and shrubs [68]; it is a species relatively tolerant to drought and under these conditions carries out a high translocation of biomass to the roots, especially towards the finer ones [69]. Almeida *et al.* [70] studied late growth and analyzed stem diameter and plants height of *J. curcas*, from Pernambuco, Brazil and reported that these parameters remained constant with respect to previous research carried out in the semi-arid region of this country. This indicates the absence of spatial and temporal variability of these growth parameters indicated the interest of these data for crop management.

Our results showed that, the early growth of *J. curcas* is faster and higher than *J. macrocarpa*. After in late growth stage *J. macrocarpa* showed a greater investment in biomass of epicotyls and hypocotyls than *J. curcas*. This is correlates with the anatomy observed in both species. Both hypocotyls and epicotyls of *J. macrocarpa* develop more protective tissue, cortex and vascular tissue than *J. curcas* [71].

## 5. Conclusion

This study has demonstrated the characteristics of seeds, germination, dormancy and early and late growth of both species proposed for the production of biodiesel. It was shown that *J. macrocarpa* have physical dormancy, low PG, greater tolerance to environmental stress, and low growth compared to *J. curcas*. These studies are fundamental to face studies in relation to the abiotic stress and of

crop establishment.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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