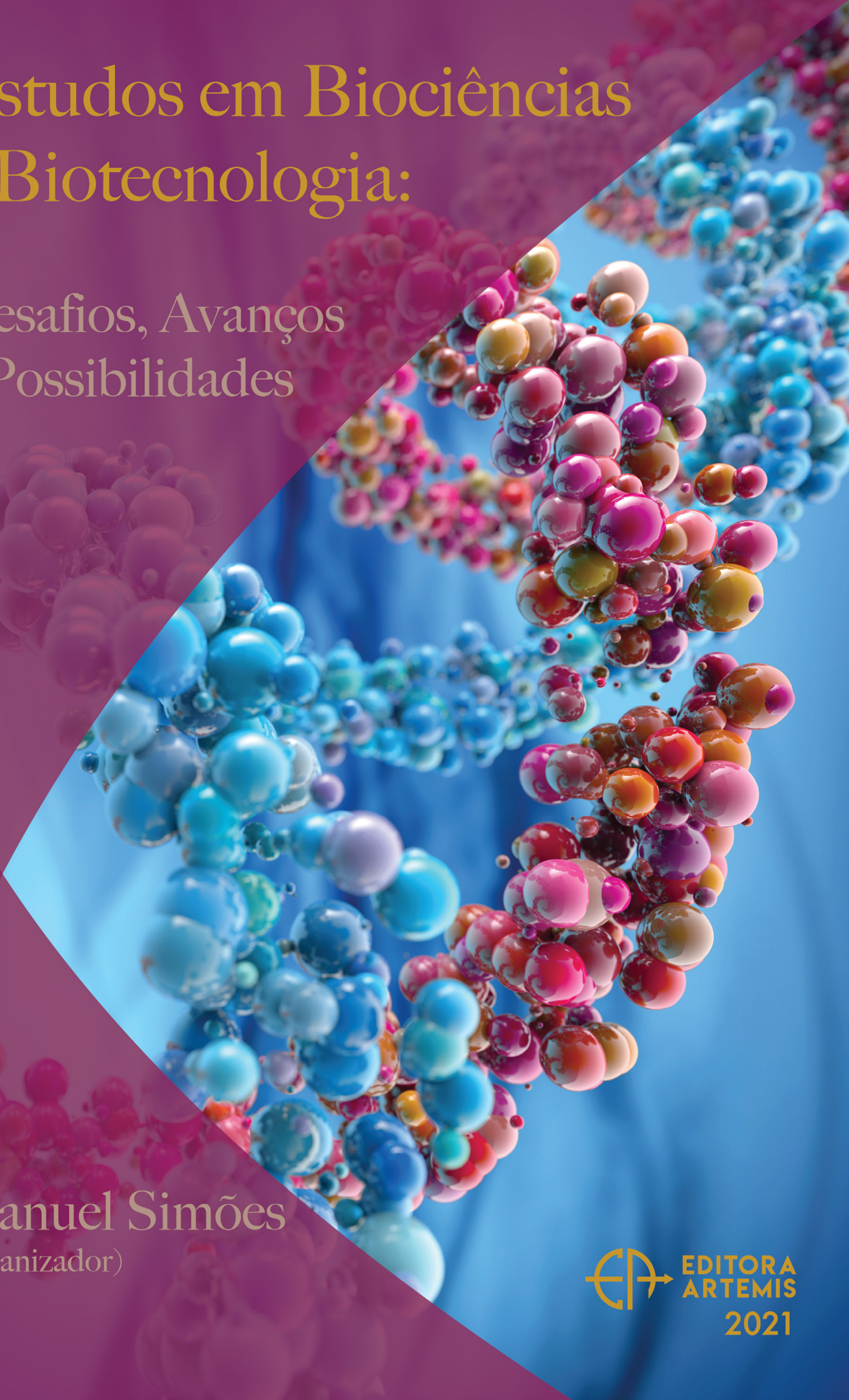
Estudos em Biociências e Biotecnologia:

Desafios, Avanços e Possibilidades



Manuel Simões
(organizador)

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PREFÁCIO

A biotecnologia baseia-se em conhecimentos multidisciplinares fortemente associados às ciências naturais e exatas, e às ciências aplicadas. As ciências biológicas e o seu enquadramento na biotecnologia têm aplicações em grandes áreas de importância socioeconómica, principalmente na medicina humana e animal, ambiente, agronomia e na indústria. Os processos biotecnológicos são caraterizados por usarem células procariotas ou eucariotas, partes das mesmas ou análogos moleculares - com o objetivo de se obterem produtos e serviços. Avanços significativos na biotecnologia surgiram das sinergias estabelecidas entre engenheiros, cientistas e reguladores para transformar descobertas cientificas em novos processos e produtos, com impacto socioeconómico. A elevada dinâmica académica e industrial no desenvolvimento de conhecimento em ciências biológicas e biotecnologia é revelador da sua importância. Contudo, a necessidade de atualização dos avanços científicos, em conjugação com a transformação desse novo conhecimento em conteúdo curricular técnico-científico relevante são desafios para um eficaz processo formativo de recursos humanos altamente qualificados. O enquadramento ético e regulamentar de novos processos e produtos é igualmente desafiante.

Este livro foi dividido em quatro partes: a primeira parte reúne capítulos (1 a 6) relacionados com as biociências e a biotecnologia na área biomédica. A segunda parte concentra capítulos (7 a 11) na área do ambiente. A terceira parte é composta pelos capítulos 12 a 14 que se enquadram em aspetos da bioprospecção. A quarta parte contém os capítulos 15 e 16 que abordam aspetos do ensino/aprendizagem em biotecnologia e da bioética, respetivamente. Neste contexto, pretende com este livro contribuir para que estudantes e professores do ensino superior, ligados às biociências e à biotecnologia, quer a nível de graduação quer de pós-graduação, possam ter uma perspetiva de avanços na área. Este livro pode ser também útil a profissionais ligados a setores nos quais as biociências e a biotecnologia têm um papel de relevo, bem como para professores do ensino pré-académico.

Manuel Simões

SUMÁRIO

BIOMEDICINA

CAPÍTULO 14

DORMANT RUPTURE AND HORMONES LEVELS IN *Jatropha curcas* L. AND *Jatropha macrocarpa* GRISEB SEED

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ABSTRACT: *J. curcas* L. and *J. macrocarpa* Griseb. are perennial shrub with the greatest importance mainly from its biofuel potential. Several authors consider that seed tegument is one of the factors that induce dormancy. The aim of the work was to study the role of tegument and abscisic acid (ABA) y jasmonic acid (JA) in dormancy and germination in

these species. *J. macrocarpa* present dormancy since it does not germinate by traditional methods. Consequently, seeds of *J. macrocarpa* were subjected to different treatments to break seed dormancy: T1) Control; T2) Scarification with sandpaper; T3) Total elimination of the tegument; T4) Immersion in boiling water; T5) Alternating hot and cold water; T6) Immersion in concentrated H_2SO_4 for 15 min; T7) Immersion in concentrated H_2SO_4 for 30 min; T8) Stratification in wet and cold paper; T9) Stratification in moist sand and cold. After each treatment the seeds were placed in Petri dishes containing distilled water at 30°C temperature. Germination percentages (GP) were determined during 30 days. We used 20 seeds by treatment, with three replications each one. ABA and JA were extracted and purified from both *Jatropha* species tegument. These hormones were identified and quantified from tissue using reverse-phase high-performance liquid chromatography (HPLC)-mass spectrometry (MS). The total removal of tegument showed a 50% increase in germination percentage, with the other treatments achieved between 0-10%. JAs were the most abundant compound detected in tegument. ABA level was higher in *J. curcas* (628%) than in *J. macrocarpa*, for this reason we assume that the tegument ABA level is not directly linked to germination and/or dormancy of these *Jatropha* species. In contrast, level of JAs was higher in *J. macrocarpa* (101%) than in *J. curcas.* In effects JA could have a roll in inhibition of germination of *J. macrocarpa* seeds.
KEYWORDS: Dormancy. Phytohormones. Seeds. Jatropha.

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 (Tang *et.al*, 2011). *J. curcas* and *J. macrocarpa* growing in semi-arid and arid soils, and their non-edible seeds have high oil content (Achten *et al*., 2008).

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 (Andrade *et al*., 2008) and need annual rainfall is greater than 700 mm for good fruit production (Achten *et al*., 2008). 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 (Wassner *et al*., 2012).

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. 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 (Lee *et al*., 2010) (Smykal *et al*., 2014). 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 (Beuley *et al*., 2013). 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 Debeaujon *et al*., 2000) (Finch-Savage and Leubner-Metzger, 2006). Studies previous showed high variation in *J. curcas* seeds germination (Ginwall *et al*., 2005) (Ahamad *et al*., 2013).

This variation is influenced by genotype, age, storage conditions of the seed and environmental conditions of the crop (Islam *et al*., 2009) (Pompelli *et al*., 2010) (Windauer *et al*., 2012) (Duong *et al*., 2013) (Moncaleano-Scandon *et al*., 2013). 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 (Moncaleano-EsScandon *et al*., 2013). 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 (Marcello *et al*., 2015) *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* (Windauer *et al*., 2012) (Mohan *et al*., 2011); 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 (Linkies and Leubner-Metzger, 2012) (Arc *et al*., 2013). Abscisic acid (ABA) is one of such hormone; that plays a prominent role in dormancy and germination control in coordinated interaction with various others (Nonogaky *et al*., 2014) (Nambara *et al.*, 2010) (Shu *et al*., 2016). Recently, evidences have been provided for an interaction between ABA and jasmonates (JAs) in the regulation of these processes (Dave *et al*., 2016) (Xu *et al*., 2016). In particular, in ABA-JA interaction, Dave *et al*. (2016) 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*. (2016) reported that JAs and ABA have opposing roles in the regulation of dormancy release by stratification in wheat.

We hypothesize that the tegument is one of the factors that induce dormancy. The aim of the work was to study the role of tegument and abscisic acid (ABA) y jasmonic acid (JA) in dormancy and germination in these species.

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).

2.1 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 were obtained.

2.2 SEED TREATMENTS TO BREAK DORMANCY IN *J. MACROCARPA*

The seeds of *J. macrocarpa* were subjected to different scarification and stratification treatments: T1) Control; 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_2SO_4 for 15 min; T7) Immersion in concentrated H_2SO_4 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.

2.3 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*. (2005). 200 mg of tegument were homogenized in a mortar with liquid nitrogen and 5 ml ultra-pure water. D6-ABA (NRC-Plant Biotechnology Institute, Saskatoon, Canada) and D6-JA (Leibniz-Institute of Plant

Biochemistry, in Halle, Germany) were used as internal standards. Extracts were transferred to 50 ml tubes, centrifuged at 1500x 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^{-1}, 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.

2.4 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_{18} 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 $ml.min^{-1}$. 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 D6-ABA (m/z 269/159) and JAs (m/z 209/59), D6-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).

2.5 STATISTICAL ANALYSIS

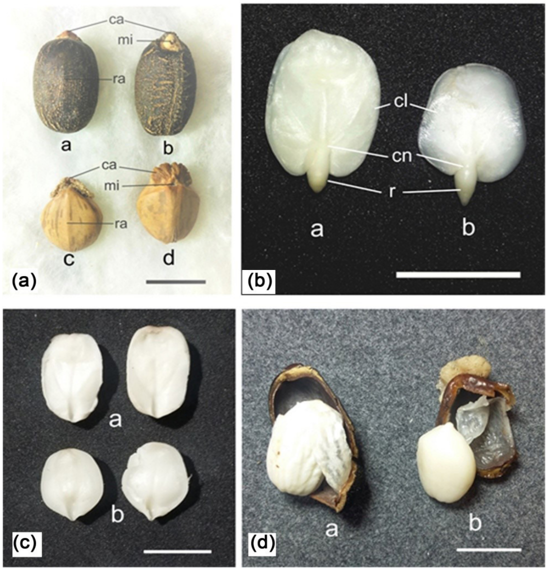
Analysis of variance (ANOVA) was applied and data were subjected to Mul- tiple Range the Duncan. Test using the software INFOSTAT-UNC.

3 RESULTS

3.1 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 ± 0.03 cm, width 1.0 ± 0.01 cm and thickness 0.8 ± 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 subspherical, on average they are 1.5 ± 0.05 cm long, 1.3 ± 0.04 cm wide and 0.9 ± 0.02 cm thick. The dorsal area is slightly convex and is traversed by an evident 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 with compactly arranged isodiametric parenchyma cells (Tavecchio *et al*., 2019). 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)).

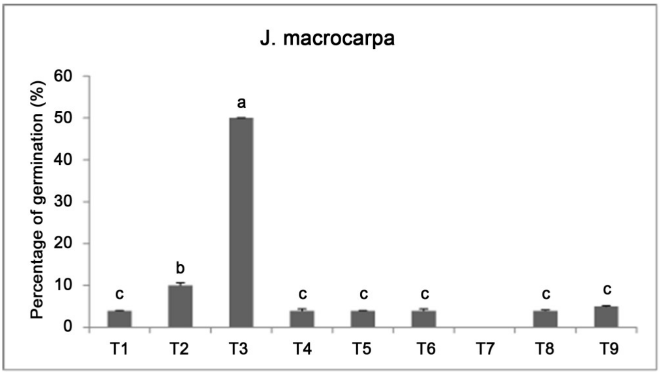
Figure 1.(a) Visual external aspect of seeds of *J. curcas* (a and b) and *J. macrocarpa* (c and d). (b) Embryo of *J. curcas* (a) and *J. macrocarpa* (b). (c) Internal surfaces of the endosperm in contact with the embryo of *J. curcas* (a and b) and *J. macrocarpa* (c and d). (d) Seeds with broken tegument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



3.2 BREAK DORMANCY

J. macrocarpa presents 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.*

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_2SO_4 for 15 min; T7) Immersion in H_2SO_4 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 ($p \leq 0.05$).



3.3 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* (101%) than in *J. curcas*. In contrast, ABA level was higher in *J. curcas* (628%) than in *J. macrocarpa* (Table 1).

Table 1. 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).

Hormones	Species	Content (pmol·g^{-1} FW)
JA	*J. curcas*	8369.26 ± 2682.33 b
	J. macrocarpa	16860.41 ± 1482.22 a
ABA	*J. curcas*	737.96 ± 68.82 a
	J. macrocarpa	101.29 ± 27.08 b

4 DISCUSSION

External morphology of the seeds of *J. curcas* and *J. macrocarpa* allow different them easily since they vary in size, shape and coloration of the tegument and the caruncle. 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. (Sing, 1954), *Croton floribundus* Spreng y *Croton urucurana* Baill (Paoli *et al*., 1995), *Jatropha elliptica* Mull. Arg. (Añes *et al.,* 2005) and *Cnidosculus juercifolius* Paxe K. Hoffm (Silva *et al*., 2007).

Morphological characteristics found in the seeds of *J. curcas* were in many aspects coincident with those described previously by Loureiro *et al*., (2013) 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 (Mohan *et al*., 2011). Nevertheless, the tegument is a major barrier to radicle protrusion for many seeds (Zhang *et al*., 2008), whose physical properties determine its effect on seed germination (Debeaujon *et al*., 2000). 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%). 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 (Finch-Savage and Leubner-Metzger, 2006). Similarly, Zhang *et al*. (2008) 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 (Mac Gregor *et al*., 2015).

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 (Bewley, 1997). 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 (Finch-Savage and Leubner-Metzger, 2006). In fact, the dormancy attributed to different tissues of the seed has been reported in different species (Brunick, 2007) (Gu *et al*., 2015).

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 (Finkelstein *et al*., 2002). 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 (Loscacio *et al*., 2014). There is considerable evidence that ABA is an important positive regulator of both the induction of dormancy and their maintenance (Nambara *et al*., 2010). 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 (Lee *et al*., 2010) (Ali-Rachedi *et al*., 2004) that suggest that ABA abundance or signaling, or both, play an indirect role in promoting seed dormancy during seed development (Chahtane *et al*., 2017). In contrast, different studies have shown that the seed structures surrounding the embryo contain compounds possessing germination inhibitory activities including ABA (Bewley *et al*., 2013). For example, Jin *et al*. (1995) 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*., 2009 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*., 2015 showed a high JAs content in pericarp of dormant B123 sunflower cypselas. In agree to the findings of Andrade *et al*., 2015, 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.

5 CONCLUSION

This study has demonstrated the characteristics of dormancy of both species proposed for the production of biodiesel. It was shown that *J. macrocarpa* have physical dormancy. These studies are fundamental to face studies in relation to the crop establishment.

REFERENCES

Achten, W.M., Verchot, L., Franken, Y.J., Mathijs E., Singh V.P., Aerts R. and Muys B. (2008). Jatropha Bio-Diesel Production and Use. *Biomass and Bioenergy, 32*, 1063-1084. https://doi.org/10.1016/j.biombioe.2008.03.003

Ahamad, S., Joshi, S.K., Arif, M. and Ahmed, Z. (2013. Performance of *Jatropha curcas* L. in Semiarid Zone: Seed Germination, Seedling Growth and Early Field Growth. *Notulae Scientia Biologicae, 5*, 169-174. https://doi.org/10.15835/nsb528961

Ali-Rachedi, S., Bouinot, D., Wagner, M.H., Bonnet, M., Sotta, B., Grappin, P. and Jullien, M. (2004). Changes in Endogenous Abscisic Acid Levels during Dormancy Release and Maintenance of Mature Seeds: Studies with the Cape Verde Islands Ecotype, the Dormant Model of *Arabidopsis thaliana*. *Planta, 219*, 479-488. https://doi.org/10.1007/s00425-004-1251-4

Almeida, A.Q., Souza, R.M.S., Silva, M., Silva, S.M.S.E., de Oliveira Junior, I.S., de Sousa Cavalcante, F. and da Silva Melo, J.A. (2016). Space-Time Dependence of Jatropha Growth Parameters Grown in Brazilian Semiarid. *Revista Caatinga, 29*, 358-366. https://doi.org/10.1590/1983-21252016v29n212rc

Andrade, A., Riera, N., Lindström, L., Alemano, S., Alvarez, D., Abdala, G. and Vigliocco, A. (2015). Pericarp Anatomy and Hormone Profiles of Cypselas in Dormant and Non-Dormant Inbred Sunflower Lines. *Plant Biology, 17*, 351-360. https://doi.org/10.1111/plb.12244

Andrade, G.A., Caramori, P.H., De Souza, F.S., Marur, C.J. and de Arruda Ribeiro, A. (2008). Temperatura mínima letal para plantas jovens de pinhão-manso [Mini- mum Lethal Temperature for Seedlings of the Oil Seed Plant]. *Bragantia, 67*, 799-803. https://doi.org/10.1590/S0006-87052008000300031

Añes, L.M.M., Coelho, M.F.B., Albuquerque, M.C.F. and Dombroski, J.L.D. (2005). Caracterização morfológica dos frutos, das sementes e do desenvolvimento das plântulas de *Jatropha elliptica* Müll. Arg. (Euphorbiaceae). *Revista Brasileira de Botânica, 28*, 563-568. https://doi.org/10.1590/S0100-84042005000300012

Arc, E., Secher, J., Corbineu, F., Raijjou, L. and Marion-Poll, A. (2013). ABA Cross- talk with Ethylene and Nitric Oxide in Seed Dormancy and Germination. *Frontiers in Plant Science, 4*, 63. https://doi.org/10.3389/fpls.2013.00063

Bewley, J.D. (1997). Seed Germination and Dormancy. *Plant Cell, 96*, 1055-1066. https://doi.org/10.1105/tpc.9.7.1055

Bewley, J.D., Bradford, K.J., Hilhorst, H.W.M. and Nonogaki, H. (2013). Seeds: Physiology of Development, Germination and Dormancy. *Springer, New York, NY*. https://doi.org/10.1007/978-1-4614-4693-4

Brunick, R.L. (2007). Seed Dormancy in Domesticated and Wild Sunflowers (*Helianthus annuus* L.): Types, Longevity and QTL Discovery. *Doctoral Dissertation, Department of Horticulture, Oregon State University, 63.*

Chahtane, H., Kim, W. and Lopez-Molina, L. (2017). Primary Seed Dormancy: A Temporally Multilayered Riddle Waiting to Be Unlocked. *Journal of Experimental Botany, 68*, 857-869.

Dave, A., Vaistj, F.E., Gilday, A.D., Penfield, S.D. and Graham, I.A. (2016). Regulation of *Arabidopsis thaliana* Seed Dormancy and Germination by 12-Oxo-Phytodienoic Acid. *Journal of Experimental Botany, 67*, 2277-2284. https://doi.org/10.1093/jxb/erw028

Debeaujon, I., Léon-Kloosterziel, K.M. and Koornneef, M. (2000). Influence of the Testa on Seed Dormancy, Germination, and Longevity in *Arabidopsis*. *Plant Physiology, 122*, 403-414. https://doi.org/10.1104/pp.122.2.403

Duong, T.H., Shen, J.L., Luangviriyasaeng, V., Ha, H.V. and Pinyopusarerk, K. (2013). Storage Behaviour of *Jatropha curcas* Seeds. *Journal of Tropical Forest Science, 22*, 193-199.

Durgbanshi, A., Arbona, V., Pozo, O., Miersch, O., Sancho, J.V. and Gómez Cadenas, A. (2005). Simultaneous Determination of Multiple Phytohormones in Plant Extracts by Liquid Chromatography-Electrospray Tandem Mass Spectrometry. *Journal of Agricultural and Food Chemistry, 53*, 8437-8442. https://doi.org/10.1021/jf050884b

Finch-Savage, W.E. and Leubner-Metzger, G. (2006). Seed Dormancy and the Control of Germination. *New Phytologist, 171*, 501-523. https://doi.org/10.1111/j.1469-8137.2006.01787.

Finkelstein, R.R., Gampala, S.S. and Rock, C.D. (2002). Abscisic Acid Signaling in Seeds and Seedlings. *Plant Cell, 146*, 15-45. https://doi.org/10.1105/tpc.010441

Ginwal, H.S., Phartyal, S.S., Rawat, P.S. and Srivastava, R.L. (2005). Seed Source Variation in Morphology, Germination and Seedling Growth of *Jatropha curcas* Linn. in Central India. *Silvae Genetica, 54*, 76-80. https://doi.org/10.1515/sg-2005-0012

Gu, X.Y., Zhang, J., Ye, H., Zhang, L. and Feng, J. (2015). Genotyping of Endosperms to Determine Seed Dormancy Genes Regulating Germination through Embryonic, Endospermic, or Maternal Tissues in Rice. *G3 (Bethesda), 5*, 18 3-193. https://doi.org/10.1534/g3.114.015362

Islam, A.K.M.A., Anuar, N. and Yaakob, Z. (2009). Effect of Genotypes and Pre-Sowing Treatments on Seed Germination Behaviour of *Jatropha*. *Asian Journal of Plant Sciences, 8*, 433-439. https://doi.org/10.3923/ajps.2009.433.439

Jin, B., Dong, H. and Yang, X. (1995). Shortening Hybridization Breeding Cycle of Rose a Study on Mechanisms Controlling Achene Dormancy. *Acta Horticulturae, 404,* 40-47.

Lee, K.P., Piskurewicz, U., Turecková, V., Strnad, M. and Lopez-Molina, L. (2010). A Seed Coat Bedding Assay Shows That RGL2-Dependent Release of Abscisic Acid by the Endosperm Controls Embryo Growth in Arabidopsis Dormant Seeds. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 19108-19113. https://doi.org/10.1073/pnas.1012896107

Linkies, A. and Leubner-Metzger, G. (2012). Beyond Gibberellins and Abscisic Acid: How Ethylene and Jasmonates Control Seed Germination. *Plant Cell Reports, 31*, 253-270. https://doi.org/10.1007/s00299-011-1180-1

Locascio, A., Roig-Villanova, I., Bernardi, J. and Varotto, S. (2014). Current Pers- pectives on the Hormonal Control of Seed Development in Arabidopsis and Maize: A Focus on Auxin. *Frontiers in Plant Science, 5*, 412. https://doi.org/10.3389/fpls.2014.00412

Loureiro, M.B., Clarissa Abreu Santos Teles, C.S., Argolo Colares, C.C., Nascimento de Araújo, B.R., Gonzaga Fernández, L. and Delmondez de Castro, R. (2013) Caracterização morfoanatomica e fisiológica de sementes e plântulas de *Jatropha curcas* L. (Euphorbiaceae) 2013. *Revista Árvore, 37*, 1093-1101. https://doi.org/10.1590/S0100-67622013000600011

Mac Gregor, D.R., Kendall, S.L., Florance, H., Fedi, F., Moore, K., Paszkiewicz, K., Smirnoff, N. and Penfield, S. (2015). Seed Production Temperature Regulation of Primary Dormancy Occurs through Control of Seed Coat Phenylpropanoid Metabolism. *New Phytologist, 205*, 642-652. https://doi.org/10.1111/nph.13090

Marcello, L., Nicla, C., Luca, G. and Maurizio, M. (2015). Effects of Pre-Sowing Treatments on *Jatropha curcas* Seed Germination and Seedling Growth. *African Journal of Agricultural Research, 10*, 2553-2561. https://doi.org/10.5897/AJAR2015.9788

Mohan, N., Nikdad, S. and Singh, G. (2011). Studies on Seed and Embryo Culture of *Jatropha curcas* L. under in Vitro Conditions. *Biotechnology, Bioinformatics and Bioengineering, 1*, 187-194.

Moncaleano-Scandon, J., Silva, B.C.F., Silva, R.S., Granja, J.A.A., Alves, M.C. and Pompelli, M.F. (2013). Germination Responses of Jatropha curcas L. Seeds to Storage and Aging. *Industrial Crops and Products, 44*, 684-690. https://doi.org/10.1016/j.indcrop.2012.08.035

Nambara, E., Okamoto, M., Tatematsu, K., Yano, R., Seo, M. and Kamiya, Y. (2010) Absicic Acid and the Control of Seed Dormancy and Germination. *Seed Science Research, 20*, 55-67. https://doi.org/10.1017/S0960258510000012

Nonogaki, H. (2014). Seed Dormancy and Germination-Emerging Mechanisms and New Hypotheses. *Frontiers in Plant Science, 5*, 233. https://doi.org/10.3389/fpls.2014.00233

Paoli, A.A.S., Freitas, L. and Barbosa, J.M. (1995). Caracterização morfológica dos frutos, sementes e plântulas de *Croton floribundus* Spreng. e de *Croton urucurana* Baill. (Euphorbiaceae). *Revista Brasileira de Sementes, 17*, 57-68. https://doi.org/10.17801/0101-3122/rbs.v17n1p57-68

Pompelli, M.F., da Rocha Gomes Ferreira, D.T., da Silva Cavalcante, P.G., de Lima Salvador, T., de Hsie, B.S. and Endres, L. (2010). Environmental Influence on the Physic-Chemical and Physiological Properties of *Jatropha curcas* Seeds. *Australian Journal of Botany, 58*, 421-427. https://doi.org/10.1071/BT10102

Preston, J., Tatematsu, K., Kanno, Y., Hobo, T., Kimura, M. and Nambara, E. (2009). Temporal Expression Patterns of Hormone Metabolism Genes during Imbibition of *Arabidopsis thaliana* Seeds: A Comparative Study on Dormant and Non-Dormant Accessions. *Plant and Cell Physiology, 50*, 1786-1800. https://doi.org/10.1093/pcp/pcp121

Silva, L.M.M., Aguiar, I.B. and Tertuliano, S.S.X. (2007). Morfología de frutos, sementes e plântulas de *Cnidosculus juercifolius* Pax & K. Hoffm (Euphorbiaceae). *Revista de Biologia e Ciências da Terra, 7*, 1519-5228.

Singh, R.P. (1954). Structure and Development of Seeds in Euphorbiaceae: Ricinus communis L. Phytomorphology, 4, 118-123.

Shu, K., Liu, X.-D., Xie, Q. and He, Z.-H. (2016). Two Faces of One Seed: Hormonal Regulation of Dormancy and Germination. *Molecular Plant, 9*, 34-45. https://doi.org/10.1016/j.molp.2015.08.010

Smýkal, P., Vernoud, V., Blair, M.W., Soukup, A. and Thompson, R.D. (2014) The Role of the Testa during Development and in Establishment of Dormancy of the Legume Seed. *Frontiers in Plant Science, 5*, 351.

Tang, M., Liu, X., Deng, H. and Shen, S. (2011). Over-Expression of JcDREB, a Putative AP2/EREBP Domain-Containing Transcription Factor Gene in Woody Biodiesel Plant *Jatropha curcas*, Enhances Salt and Freezing Tolerance in Transgenic *Arabidopsis thaliana*. *Plant Science, 181*, 623-631. https://doi.org/10.1016/j.plantsci.2011.06.014

Tavecchio, N., Dardanelli M., Reguera Y., Reinoso H., Terenti O., Garbero M. and Pedranzani H. (2019). Potencial Technological Use of Reserves of *Jatropha curcas* L. and *J. macrocarpa* Griseb. *American Journal of Plant Sciences, 10*, 1444-1456. https://doi.org/10.4236/ajps.2019.108102

Windauer, L., Martinez, J., Rapoport, D., Wassner, D. and Benech-Arnold, R. (2012). Germination Responses to Temperature and Water Potential in *Jatropha curcas* L. Seeds. A Hydrotime Model Explains the Difference between Dormancy Expression and Dormancy Induction at Different Incubation Temperatures*. Annals of Botany, 109*, 265-273. https://doi.org/10.1093/aob/mcr242

Xu, Q., Truong, T.T., Barrero, J.M., Jacobsen, J.V., Hocart, C.H. and Gubler, F. (2016). A Role for Jasmonates in the Release of Dormancy by Cold Stratification in Wheat. *Journal Experimental of Botany, 67*, 3497-3508. https://doi.org/10.1093/jxb/erw172

Zhang, X.K., Chen, J., Chen, L., Wang, H.Z. and Li, J.N. (2008). Imbibition Behavior and Flooding Tolerance of Rapeseed Seed (*Brassica napus* L.) with Different Testa Color. *Genetic Resources and Crop Evolution, 55*, 1175-1184. https://doi.org/10.1007/s10722-008-9318-x

SOBRE O ORGANIZADOR

Manuel Simões é licenciado em Engenharia Biológica e doutorado em Engenharia Química e Biológica. Atualmente é Professor Associado com Agregação e Pró-Diretor da Faculdade de Engenharia da Universidade do Porto (FEUP), e investigador sénior do Laboratório de Engenharia de Processos, Ambiente, Biotecnologia e Energia (LEPABE) do Departamento de Engenharia Química da FEUP. Nos últimos anos esteve envolvido em 10 projetos nacionais (5 como investigador principal) e 6 projetos europeus. Foi membro do comité de gestão da ação COST BACFOODNET (Rede Europeia para Mitigação da Colonização e Persistência Bacteriana em Alimentos e Ambientes de Processamento de Alimentos) e esteve envolvido em outras 2 ações: iPROMEDAI e MUTALIG. Manuel Simões tem mais de 190 artigos publicados em revistas indexadas no Journal of Citation Reports, 4 livros (1 como autor e 3 como editor) e mais de 40 capítulos em livros. Ele é Editor Associado para o jornal Biofouling - The Journal of Bioadhesion and Biofilm Research (o periódico mais antigo sobre pesquisa em biofilme), Editor Associado para o jornal Frontiers in Microbiology e Section Editor-in-Chief para o jornal Antibiotics. Seus principais interesses de pesquisa estão focados nos mecanismos de formação de biofilme e seu controlo com agentes antimicrobianos, particularmente usando novas moléculas antimicrobianas, e no uso de microalgas para tratamento de efluentes. É um dos investigadores mais citados do mundo (top 1%), tendo sido distinguido nos últimos dois anos no índice Essential Science Indicators, um dos mais prestigiados indicadores da qualidade de investigação.

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ÍNDICE REMISSIVO

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