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Behavior of *Jatropha curcas* L. seeds under osmotic stress: germination and cell cycle activity

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ABSTRACT. *Jatropha curcas* is an oil-rich Euphorbiaceae seed species renowned for its apparent tolerance to environmental stresses. It is considered a promising source of renewable feedstock for biodiesel production in the Brazilian semiarid region where crop establishment requires a better understanding of the mechanisms leading to proper seed and plant behavior under water restrictive conditions. This study describes physiological and cytological profiles of *J. curcas* seeds imbibed in water restriction conditions by means of osmotic stress or osmoconditioning. Seeds were characterized by size, weight, moisture content and dry mass, germinability, and cell cycle activation by means of tubulin and microtubule cytoskeleton accumulation. Osmoconditioning at -0.8 MPa did not induce priming effects as it did not improve the physiological quality of the seed lots. Western blotting and immunocytochemical analysis revealed an increasing accumulation of tubulin and microtubule cytoskeleton in seeds imbibed in water for 48h onwards, culminating in the onset of mitotic configurations after germination. Only cortical microtubules were observed during seed osmoconditioning, whereas mitotic microtubules only occurred after re-imbibition of osmoconditioned seeds in water and subsequent germination.

Keywords: physic nut, germination, drought stress, PEG, tubulin, microtubules.

Comportamento de sementes de *Jatropha curcas* L. submetidas a estresse osmótico: germinação e atividade do ciclo celular

RESUMO. *Jatropha curcas* (Euphorbiaceae) é uma espécie de sementes ricas em óleo e conhecida pela aparente tolerância a estresses ambientais, considerada promissora fonte de matéria-prima para biodiesel no semiárido brasileiro, onde o estabelecimento da cultura requer melhor compreensão dos mecanismos que possibilitam sementes e plantas se estabelecerem em condições restritivas de água. O presente estudo descreve perfis fisiológico e citológico em sementes de *J. curcas* submetidas a condições de restrição hídrica por meio de estresse osmótico ou osmocondicionamento. As sementes foram caracterizadas quanto ao tamanho, peso, teor de umidade e de massa seca, germinação e atividade do ciclo celular por meio de acúmulo de tubulina e citoesqueleto microtubular. O osmocondicionamento a -0,8 MPa não induziu efeito de *priming*, visto que não melhorou a qualidade fisiológica dos lotes de sementes. Análises de *Western blotting* e imunohistoquímica revelaram reativação do ciclo celular por meio do acúmulo de tubulina e citoesqueleto microtubular em sementes embebidas em água a partir de 48h, culminando com o surgimento de configurações mitóticas após a germinação. A reativação do ciclo celular durante o osmocondicionamento induziu somente microtúbulos corticais, sendo que microtúbulos mitóticos ocorreram somente após a re-embebição de sementes osmocondicionadas em água e subsequente germinação.

Palavras-chave: pinhão manso, germinação, estresse hídrico, PEG, tubulina, microtúbulos.

Introduction

Jatropha curcas L., which is known as the physic nut, belongs to the Euphorbiaceae family and naturally occurs in the semiarid region of northeastern Brazil and in equatorial American countries (SANTOS et al., 2005). It is characterized as an arbustive plant with a height of two to five meters. The flowers are small, greenish-yellow, monoecious and produced in the same inflorescence. Its fruits are trilocular capsules with a seed in each locule (CARVALHO et al., 2009).

The seeds of *J. curcas* are particularly important because they may contain approximately 55% oil that is suitable for oil lamps, soap manufacturing,

cosmetics industry, cooking and the production of biodiesel (JONGSCHAAP et al., 2007; OPENSHAW, 2000). This species has the capacity to adapt to diverse environments, it also highlights the apparent rusticity and tolerance to abiotic stress conditions (SIQUEIRA et al., 2012).

Plants in natural and agricultural conditions are frequently exposed to environmental stresses. In the case of seeds, when these conditions do not lead to death, it may alter the speed, time and uniformity of germination, influencing their viability and vigor (SANTANA; RANAL, 2004).

The controlled hydration of seeds by exposure to osmotic solutions up to a limit that allows pre-germinative metabolic events to proceed, but without the radicle protrusion, is called osmoconditioning, osmopriming or osmotic priming (BRADFORD, 1986; BUTLER et al., 2009). This pre-sowing treatment has been used in seed technology to improve the seed quality of agricultural species, allowing a uniform stand of seedlings, reducing the time required for primary root protrusion and reducing financial losses during the crop development, thus promoting its establishment under adverse environmental conditions (VARIER et al., 2010). Despite its potential benefits, several physiological mechanisms of survival to drought stress are not yet known, such as the drying effect and the possibility of reversing the treatment effects during seed storage (CÓRDOBA, 1995).

The germination process is initiated with the imbibition of water by the seed. During this phase, embryo cells change to a metabolically active state in which several biochemical and physiological events occur (SHEORAN et al., 2005). These complex metabolic changes leads to the protrusion of the radicle through the covering structures of the seed, defining at the physiological level the completion of germination and the beginning of seedling growth (CARDOSO, 2004).

It has been reported that cell cycle activities, i.e. accumulation of tubulin and microtubular cytoskeleton are induced during seed imbibition and that cell division may occur as a post-germination phenomenon with radicle protrusion occurring by cell elongation, or as a pre-germination phenomena (DE CASTRO et al., 2000). Whereas, it may also occur in seeds imbibed in water restrictive conditions such as under osmotic pretreatments in seed priming techniques, i.e. osmoconditioning or osmopriming (HUANG; SONG, 2013). Subunits of α- and β-tubulin are involved in the maintenance of the cellular cytoskeleton and are the components of microtubules associated with cell expansion and division and are reported to accumulate as a reflection of cell cycle reactivation during imbibition of water as well as during osmoconditioning and priming in many seed species (DE CASTRO et al., 2000).

The objective of this study was to investigate the suitability of different lots of *J. curcas* seeds produced in the northeastern semiarid region of Brazil by means of osmotic pretreatment and the evaluation of physiological (fresh and dry weight, germination and vigor) and cell cycle (tubulin accumulation and microtubule cytoskeleton configurations) parameters with respect to the quality of the seeds and their germinability under non-stress and under osmotic stress conditions.

Material and methods

Plant material

Two lots of *J. curcas* seeds were used in this study. They were selected as potential feedstock for the production of biodiesel and originated from the Brazilian counties of Irará in the state of Bahia and Janaúba in the state of Minas Gerais.

The lot from Irará consisted of seeds produced in 2009 by the Empresa Baiana de Desenvolvimento Agrícola S.A. (EBDA), at the experimental station of Porteiras farm (12º 03' 00" S; 38º 46' 00" W), where seeds were hand harvested and stored for 2 months within plastic bags at ambient conditions under an open shed, i.e., without humidity and temperature control. The lot from Janaúba consisted of seeds produced in 2008 at the experimental station of the Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), located in Janaúba (15º 48' 09" S; 43º 18' 32" W), where seeds were hand harvested and stored for 12 months within burlap bags also at ambient conditions under an open shed without humidity and temperature control. There were no records of the moisture contents during the storage of both lots.

Laboratory assays

Both seed lots were received at the Laboratory of Biochemistry, Biotechnology and Bioproducts (LBBB) of the Federal University of Bahia (UFBA), where they were identified with respect to their origins and characterized for morphometric parameters (height, width and thickness), 1000 seed weight, seed moisture content and dry weight (BRASIL, 2009). The remaining seeds of both lots were placed in plastic bags, sealed and stored at 4ºC until the beginning of the physiological assays.

Seed lot characterization

a) Morphometric parameters and 1000 seed weight

The morphometric parameters of length (measured from the apex to the hilum on the opposite side), width and thickness (middle axis) (BRASIL, 2009) were determined from 5 replications of 50 seeds from each lot using a digital caliper (Marathon) with an accuracy of 0.01 mm.

b) Seed moisture content and dry weight

Water content was obtained from 4 replicates of 5 g of sectioned seeds, i.e., previously cut into four parts with the aid of a stylus and dried in oven at 105 ± 3°C for 24h. The results are expressed as the average percentage of the seed fresh weight (BRASIL, 2009), from which the seed dry weight was concomitantly determined.

Physiological assays

a) Seed surface sterilization

Both seed lots were surface sterilized by soaking in 0.125% sodium hypochlorite (NaClO) solution for 10 min., rinsed 5 times with distilled water, rapidly surface dried with the aid of dry sterile germination paper to remove the excess water (BRASIL, 2009) and then stored dried or transferred to imbibition for the physiological tests.

b) Seed osmoconditioning

The possible effects of seed priming were evaluated as a means to recover and/or improve seed quality in both lots, presuming better performance in germinability and seedling establishment as required under drought stress conditions (BRADFORD, 1986; CHEN, et al., 2010; YOON et al., 1997), as commonly found in the semiarid region of northeastern Brazil.

Priming was evaluated by submitting sterilized dry seeds to osmotic pre-treatment or osmoconditioning in polyethylene glycol solution (PEG 8000, Sigma-Aldrich) at -0.8 MPa for 7 days at 25°C.

The following treatments were applied: (1) sterilized, dry non-osmoconditioned seeds were directly submitted to the imbibition and germination test in distilled water (control); (2) sterilized osmoconditioned seeds were directly (freshly) submitted to the (re)imbibition and germination test in water (Fresh-osmoconditioned), and (3) sterilized osmoconditioned seeds were first re-dried and then submitted to the (re)imbibition and germination test in water (Dried-osmoconditioned). The re-drying applied on treatment 3 was performed by distributing osmoconditioned seeds on top of the lab bench for approximately 3 days until they equilibrated to their initial moisture content (6 to 9%).

c) Seed germinability

Germinability was tested on non-osmoconditioned (control) and osmoconditioned seeds of both *J. curcas* seed lots, as described above, by sowing 5 replicates of 20 seeds from each treatment in 15 x 15 cm plastic germination boxes (20 seeds per box) containing 3 layers of sterilized seed germination paper previously moistened with distilled water or osmotic solution (osmoticum) equivalent to three times the total paper dry weight. The seeds were then incubated in a germination chamber under continuous dark at 25°C (Eletrolab, Mod. EL202). The germinability parameters were scored and calculated daily according to the official Brazilian Rules for Seed Testing (BRASIL, 2009), i.e., the germination percentage (G, as protruded embryo radicle longer than 2 mm), the mean germination time (mGT), the mean germination rate (mGR), the germination rate index (GRI) and the germination uniformity coefficient (GUC).

The data were subject to ANOVA and the mean values compared by the Tukey test at 5% probability using SISVAR software (FERREIRA, 2000).

Immunocytological assays

Embryo radicle tips of ca. 2 mm were isolated from dry seeds, from seeds during imbibition in water or during seed osmoconditioning for specific immunocytological assays intended for the analysis of cell cycle activities as described below. Only seeds of the Irará lot were used, as it presented better initial physiological quality.

a) Immunocytochemical detection of tubulin

For the analysis of the β-tubulin content, 2 replicates of 15 embryonic radicle tips were isolated from dry seeds and from seeds imbibed for different intervals in water or in osmoticum solution, according to the osmoconditioning and germinability assays. Protein extraction, electrophoresis, electroblotting and immunochemiluminescence detection of β-tubulin were performed according to De Castro et al. (1998) with modifications. Electrophoresis was performed in 10 x 10 cm one-dimensional 12.5% SDS-polyacrylamide gels (miniVE) at 250 V and 20 mA (GE Healthcare, EPS 601). The first well of the gel was loaded with pure bovine brain tubulin as a reference (Invitrogen - Molecular Probes). The remaining wells were loaded with 30 μg replicates of

total protein samples. Semi-dry electroblotting was conducted at 80 mA for 2h (GE Healthcare, TE 77 PWR). Photographic films (GE Healthcare, Amersham Hyperfilm-ECL) were exposed for different periods of time from 1 to 20 min.

b) Immunohistochemical detection of tubulin and microtubule cytoskeleton

For the analysis of the configurations of polymerized tubulin and microtubule cytoskeleton, samples of at least 5 radicle tips were isolated from dry seeds and from seeds imbibed for different intervals in water or in osmoticum solution, according to the osmoconditioning and germinability assays. Radicle samples were chemically fixed in 4% paraformaldehyde, dehydrated in a series of increasing concentrations of ethanol followed by series of ethanol substitution, embedded in butylmethylmethacrylate (BMM, Sigma-Aldrich), and UV polymerized at -20ºC for 24h (BASKIN et al., 1992). Longitudinal sections (1 μm thick) were affixed on slides, and BMM was removed by acetone washing. The slides were then rinsed in phosphate buffered saline (PBS), and sections were blocked in hydroxyl-tetra-ammonium chloride (HAH, Sigma-Aldrich) and bovine serum albumin (BSA) (FARIA et al., 2005). Samples were incubated in 1:100 v/v primary antibody solution (monoclonal Anti-α-Tubulin Clone, Sigma-Aldrich, cod. nº T9026), followed incubation in 1:100 v/v secondary antibody solution (Alexa Fluor® 488 goat anti-mouse IgG (H+L), Molecular Probes, A11001). In all sections, omission of the first antibody was used as the control for every treatment. Tubulin or microtubules were visualized using fluorescence microscopy (Olympus QC Color 3).

Results and discussion

Characterization of seed lots

The dimensions and morphometric analysis of the seeds from the Janaúba lot showed averages of 18.17 mm in length, 10.77 mm in width and 8.69 mm in thickness while the Irará lot was 18.36 mm, 11.37 mm and 8.59 mm, respectively (Figure 1). Statistical analysis demonstrated a significant difference in length and width between the two lots; however, there was no difference in the thickness of the seeds.

These values are similar to those recorded by Aquino et al. (2009), who studied the morphology of *J. curcas* seeds from different sources and found values between 17.03 to 19.08 mm in length and 8.47 to 8.77 mm in thickness, while Liu et al. (2009) observed variations of 15 to 20 mm in length and 10 to 13 mm in width in seeds of the same species. Seed morphometry is a key phenotypical parameter that contributes to the botanical identification of superior genotypes, and it becomes essential for plant breeding when combined with quantitative physiological and agronomic traits. In addition, it may serve as reference for small holder family farmers in selecting superior plant materials (JARVIS, 2000; REGO et al., 2007).

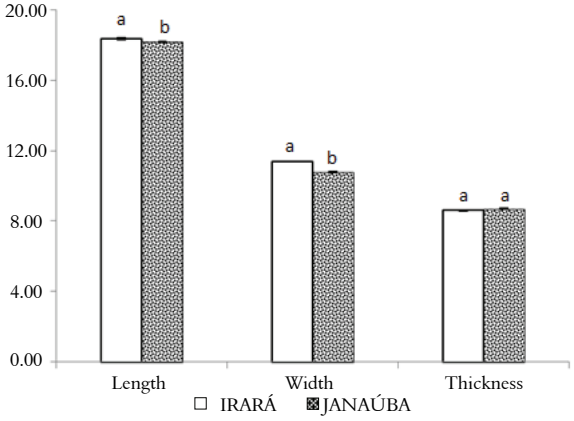

Figure 1. Length, width and thickness in millimeters (mm) of *J. curcas* seeds from the Irará and Janaúba lots.

In fact, differences in morphology and germination have been reported among different accessions of *J. curcas* seeds (GINWAL et al., 2005). In spite the small differences in morphometry observed between the two seed lots, the detailed characterization for selection and identification of physical characteristics that can be correlated with physiological seed quality is of relevance to eliminate unwanted seeds and improve the quality of seed lots (JARVIS, 2000).

As physiological parameters, the analysis of the 1000 seed fresh weight resulted in 719.15 g for the Janaúba lot and 684.29 g for the Irará lot, within which the water content represented 9.37 and 6.53%, respectively (Table 1). The values of the moisture content represent significant differences between the two lots and are higher than those reported by Goldfarb et al. (2008). In addition, for *J. curcas* seeds from the semiarid region, the 1000 seed fresh weight was 468.95 g with 9.47% water content (SILVA et al., 2008). However, there was no significant difference between the dry weights of both seed lots (Table 1), implying that the difference between them for the 1000 seed weight was directly related to their water content.

Table 1. Average values of 1000 seed weight, dry weight and moisture content of *J. curcas* seeds originating from the Irará and Janaúba lots.

Lot	1000 seed weight (g)	Dry weight (g)	Moisture content (%)
Irará	684.29 b	3.95 a	9.37 a
Janaúba	719.15 a	4.03 a	6.53 b
	701.72	3.99	7.95
C.V. (%)	1.23*	3.93 $^{n.s}$	5.04*

Means with the same letter do not differ by the Tukey test at 5% of probability. C.V. = coefficient of variation. * significantly at 5%, $^{n.s}$ not significantly.

The water content observed for the Janaúba and Irará lots are within the range of 5 to 10%. These values are indicated for the maintenance of seed viability in the dehydrated state for common to orthodox seed species (HONG; ELLIS, 1996). The variation in the water content between the two seed lots may have been related to the environmental conditions in the different locations where the seeds were produced as well as due to differences in seed drying, processing and storage conditions (BEWLEY; BLACK, 1994). In fact, the seed lots were stored in their local origins within plastic or burlap bags at ambient conditions under open sheds without humidity and temperature control until they were transferred to the laboratory, where seeds of both lots were placed into plastic bags and subjected to low temperature storage (4°C) until subsequent laboratorial analyses.

The higher water content found in the Irará lot (9.37% compared with 6.53%) appears to be directly related to the younger age of the lot, which was composed of freshly harvested seeds (2 months old), i.e., seeds submitted to a shorter storage period, compared with seeds of the older Janaúba lot (12 months old). In contrast, the maintenance of seeds for longer periods after harvesting under an open shed could have favored the reduction of water content in the Janaúba seed lot due to the low relative humidity, which is common in the semiarid region where seeds are produced and stored.

Physiological quality and osmoconditioning

In spite the lower 1000 seed weight and the higher water content of the *J. curcas* seeds from Irará (Table 1), their germination percentage appeared to be higher (96%) compared to that of the Janaúba lot (53%) (Table 2), indicating better physiological quality, which reflects the status of a younger seed lot. In contrast, the lower germination percentage of the Janaúba seed lot appears to be primarily related to deterioration resulting from age and the exposure of seeds to a longer period of storage under unfavorable storage conditions.

Aged seeds usually show weakening of cell walls and damage to cell membranes resulting from seed drying processes and the time and conditions of storage, which further implies a delay or loss of seed ability to reorganize and repair the damage upon subsequent imbibition and germination. Such seeds are more sensitive to additional damage caused by imbibition and as a consequence of the leaching of intracellular compounds during the germination process, showing a lower germination response (BEWLEY; BLACK, 1994; FILHO, 2005). In spite of the fact that seeds submitted to a long storage period can lose their germinability and vigor, particularly under inappropriate conditions, it is also known that osmoconditioning pre-treatments may lead to invigoration or priming by recovering and enhancing the vigor of aged seeds (ROSSETTO et al., 2002).

However, the results in this study showed that osmoconditioning might not always lead to such positive effects among *J. curcas* seed lots with varying quality levels. The germination percentages of the Irará seed lot were 96, 67 and 57% for the control, fresh- osmoconditioned and dry-osmoconditioned seeds, respectively, and those of the Janaúba lot were 53, 14 and 34% for the same treatments (Table 2). A reduction in seed quality was observed in the osmoconditioned Irará lot, reflected by the decline of G, mGT, mGR and GRI, which were negatively affected by this treatment, except for the GUC, where the dry-osmoprimed seeds resulted in superior values to those of the control and fresh-osmoprimed seeds.

For the Janaúba lot, mGR and GUC were not significantly affected by the treatments, although there was a slight increase in the mGT of the fresh-osmoconditioned seeds compared to dry-osmoconditioned seeds, a slight increase in the germination percentage of the dry-osmoconditioned seeds compared to the fresh-osmoconditioned seeds, and a decrease in the GRI value compared to the control (Table 2). However, according to Santana and Ranal (2004), the GRI value is influenced by the total number of germinated seeds and therefore is suitable for comparisons only when samples or treatments exhibit the same number of germinated seeds. Seeds originating from Irará showed significant differences in the evaluated parameters when subjected to osmoconditioned treatment. It was observed that Janaúba dry-osmoconditioned seeds had an increase higher than 50% in the G values in comparison with fresh-osmoconditioned seeds (Table 2).

Thus, it can be inferred that the applied osmoconditioning treatment at -0.8 MPa might have not been appropriate to lead to proper priming effects because it did not improve the germinability parameters, except for GUC, for

either of the *J. curcas* seed lots (Table 2). These results imply that vigorous seeds apparently respond inversely in such conditions to what is expected from proper 'osmotic priming' because it led to a reduction in seed vigor parameters. Therefore, the effectiveness of osmoconditioning as a priming method in *J. curcas* seeds depends on the initial quality of the seeds, where lots with good physiological quality did not respond to the osmopriming treatment applied (ROSSETTO et al., 2002). Alternatively, further studies on osmoconditioning might be required for the establishment of characteristic positive effects of invigoration or priming in *J. curcas* seeds, perhaps by testing other combinations of osmotium, time and temperature during osmoconditioning, which is corroborated by the studies of Pereira and Lopes (2011), which evaluated the effect of osmopriming in *J. curcas* from -0.2 to -1.2 MPa and concluded that the pre-treatment did not contribute to the uniformity of germination and seed vigor, independently of the seed lot and the osmotic potential solution. Similarly, Pinho et al. (2009) found that osmopriming of *Anadenanthera peregrina* L. seeds at -0.4 MPa decreased the viability and the vigor of seeds previously stored at 5 and 20°C and assumed that hydration and dehydration by osmopriming may have damaged the regeneration system of the membranes and thereby caused the decrease in the viability and the vigor of *A. peregrina*.

The hydration of mature seeds results in germination, and several physiological and biochemical changes occur in the embryo during this process. A long imbibition period, mainly under low osmotic potentials, can influence the speed, uniformity and germination percentage of the seeds (BRACCINI et al., 1999). In this study, *J. curcas* seeds did not respond positively to osmopriming, independent of the initial physiological quality of seeds.

Cell cycle activity in embryos of imbibed *J. curcas* seeds

The embryos of dry and imbibed seeds of *J. curcas* were processed for immunodetection of β-tubulin at different imbibition periods. An accumulation in the level of β-tubulin was found as the imbibition period in water increased. Low levels of β-tubulin were detected in the embryos of dry seeds (Figure 2A, lane 1'), which started to increase at 24h (Figure 2A, lane 2') and increased significantly at 48h (Figure 2A, lane 3'), reaching maximum levels at 72h of imbibition (Figure 2A, lane 5') in germinated seeds. The use of pure bovine brain tubulin confirmed that the signal corresponds to β-tubulin (55 kDa molecular mass). β-tubulin also accumulated in embryos of osmoconditioned seeds at -0.8 MPa. It was observed that the level of β-tubulin quantitatively increased only at 72h (Figure 2B, lane 7') and remained stable up to 120h of imbibition in osmoticum (Figure 2B, lane 8').

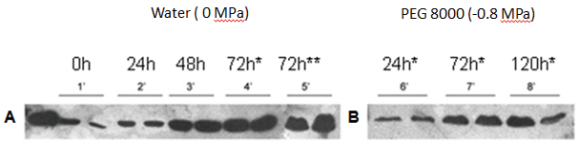

Figure 2. β-tubulin accumulation in embryos of dry and imbibing *J. curcas* seeds. A: First single lane (pure bovine-brain tubulin), Double lane 1' (0h, control-dry seed), Double lanes 2' to 5' (24, 48 and 72h imbibition in water); B: Double lanes 6' to 8' (24, 72 and 120h imbibition in osmotic conditions). *Radicle not protruded; **Radicle protruded, i.e., germinated seed.

The embryos of dry seeds did not show an organized microtubule cytoskeleton (Figure 3A) but did contain fluorescent fragments or granules of β-tubulin at 24h of imbibition (results not shown). From 48h of imbibition, these granules became clearly evident, showing short and irregularly oriented arrays (Figure 3B). When the radicle protruded at 72h of imbibition, a well organized cortical microtubule cytoskeleton was observed (Figure 3C), when several mitotic microtubule arrays were observed as pre-prophase bands, mitotic spindle and phragmoplast. Fluorescent granules also accumulated in embryos during seed osmoconditioning (-0.8 MPa) at all analyzed times (Figure 3D-F), which was more evident after 120h of imbibition in PEG (Figure 3E) and showed an increasing presence of fluorescent granules. At 168h, less prominent fluorescent spots appeared while fluorescent granules were less or disappeared (Figure 3F).

Table 2. Germination (G), mean germination time (mGT), mean germination rate (mGR), germination rate index (GRI) and germination uniformity coefficient (GUC) of non-osmoconditioned and osmoconditioned *J. curcas* seeds from the Irará and Janaúba lots imbibed in water.

Treatments	Germination (%)		mGt (days)		mGR (days $^{-1}$)		GRI (seed.day $^{-1}$)		GUC (days $^{-2}$)	
	Irará	Janaúba	Irará	Janaúba	Irará	Janaúba	Irará	Janaúba	Irará	Janaúba
Control (water)	96aA	53bA	3,8 aA	4,0 aA	0,2aA	0,2aA	5,4 aA	2,7 bA	0,7aB	1,3aA
Fresh-osmoconditioned	67aB	14bC	4,2 aB	4,7aB	0,2aB	0,2aAB	3,3 aB	0,6bB	0,7 aB	1,3aA
Dry-osmoconditioned	57aB	34bB	4,9 aC	5,3 aC	0,2aC	0,1aB	2,3 aC	1,2bB	1,7 aA	1,3 aA

Means followed by the same capital letters in the column and lowercase letters in lines do not differ significantly by the Tukey test at 5% of probability.

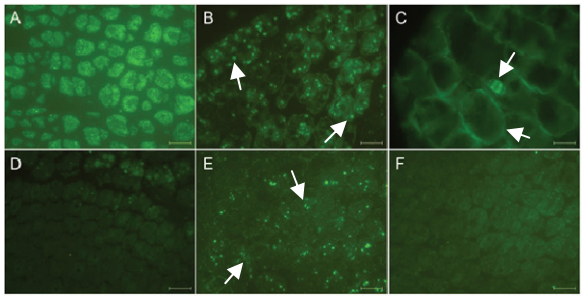

Figure 3. Development and configurations of the microtubule cytoskeleton in *J. curcas* seeds during the imbibition of non-osmoconditioned (control) seeds in water (A-C) and during osmoconditioning at -0.8 MPa (D-F). Fluorescent micrographs of longitudinal sections of embryo radicles labeled with Alexa Fluor 488 are shown (bars indicate 25 µm). (A): 0h (dry seed); (B): 48h (imbibition in water); (C): 72h (radicle protrusion in water); (D-F): 72, 120, and 168h imbibition in osmotic conditions, respectively. Arrows indicate accumulation of tubulin granules, cortical and mitotic microtubules.

The pattern of β-tubulin accumulation detectable on western blots was compared with the pattern of the microtubule cytoskeleton detected by immunohistochemistry. The increase in the β-tubulin level is associated with cell cycle progression. The embryos of dry seed did not show organized microtubules (Figure 3A). Barrôco et al. (2005) working with embryos of *Arabidopsis* found that the visualization of microtubules in dry embryos seeds is infeasible for this species. However, as the seeds were imbibed, the microtubule arrays gradually increased. In dry seeds of coffee, only tubulin granules were found (SILV et al., 2008).

Based on the results of this study, it can be inferred that the accumulation of β-tubulin occurs concomitantly with the assembly of the cortical microtubule cytoskeleton and that embryo elongation occurs exclusively by cell expansion, in agreement with observations in *Medicago truncatula* seeds by Faria et al. (2005). Fujikura et al. (1999) found that tubulin levels in *Vicia faba* seeds remain constant throughout imbibition, and microtubules may be depolymerized until visible germination. In contrast, De Castro et al. (2000) observed accumulation of β-tubulin and microtubules, which lead to the progression of the cell cycle and the occurrence of cell divisions prior to radicle protrusion in tomato seeds (*Lycopersicon esculentum* Mill.). In *Acer platanoides* L., cell cycle events and β-tubulin accumulation preceded cell expansion, division and subsequent growth of the radicle through the seed. In these species, activation of the cell cycle and β-tubulin accumulation were associated with breaking the embryo dormancy and could be used as indicators of the end of seed dormancy (PAWŁOWSKI, 2004).

According to Bewley and Black (1994), it is generally accepted that the cell cycle M phase or cell division occurs concomitantly with or after the radicle protrusion. Therefore, only the initial phases of the cell cycle are needed for seed germination. Vázquez-Ramos and Sánchez (2003) reported that the complete cell cycle events are not essential for germination to occur. Thus, radicle protrusion is not metabolically the end of the process of germination because cell proliferation, a requirement for the establishment of seedlings, may not have been fully completed.

The present results indicate that the cortical microtubules are involved in the adaptation of plants to environmental stresses. However, it is not known how they control the ability of the plant to overcome stress conditions. According to Wang et al. (2007), cortical microtubule depolymerization is not only a passive consequence of stress but also plays an active role to initiate the cascade of reactions. Thus, the hypersensitivity of the microtubule cytoskeleton could be a simple and useful parameter for estimating the intensity of environmental stress.

Preventing germination through inhibitors of protein synthesis or RNA has been taken as proof that new proteins/messengers should be synthesized *de novo* during imbibition. Perhaps there is some type of control that inhibits the establishment of cell cycle S phase, if the conditions for germination are not met. Different species may have developed unique control mechanisms more suitable for their germination and habitat characteristics (VÁZQUEZ-RAMOS; SÁNCHEZ, 2003).

The results described herein lead us to conclude that tubulin accumulation occurs concomitantly with the development of the microtubule cytoskeleton in *J. curcas* seeds during the period of imbibition in water, whereas the process is delayed under water restriction, inhibiting the progress of the cell cycle, the expansion of the embryo radicle and protrusion. The embryonic radicle elongation in this species occurs exclusively through cell expansion, while cell divisions occur only after root protrusion.

Conclusion

Osmoconditioning at -0.8 MPa did not lead to seed priming effects as a possible means of seed envigoration aimed at proper seedling and crop establishment under water restrictive conditions typical of the Brazilian semiarid region, because it was not efficient in restoring and/or improving the physiological quality and better stand stablishment of the distinct *J. curcas* seed lots.

The accumulation of tubulin in *J. curcas* seeds occurs concomitantly with the assembly of the cortical microtubule cytoskeleton during imbibition in water, whereas imbibition under water restriction delayed such processes and inhibited the progression of the cell cycle and cell expansion towards radicle protrusion, i.e., inhibiting germination *per se*.

Embryo radicle elongation and protrusion in *J. curcas* seeds occurs exclusively through cell expansion, while cell division occurs only after root protrusion.

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