Physiological and enzymatic alterations in sesame seeds submitted to different osmotic potentials

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ABSTRACT. With the imminence of global climate changes that affect the temperature and the rainfall uniformity, it is growing the concern about the adaptation of crops to the water deficit. Thus, the objective of this study was to evaluate alterations in physiological and enzymatic mechanisms during the germination process of sesame seeds under different water availability. To simulate the water restriction we used PEG6000, a high molecular weight molecule that does not penetrate the seed structure but allows different osmotic potentials. The treatments were -0.1, -0.2, and -0.3 MPa, and the control. Germination, first-count germination, germination velocity index, and length and dry mass of the hypocotyl and radicle were performed. The seeds were weighed before and after treatments every 3 h. After each weighing, 100 seeds were taken for analysis of the enzymes alcohol dehydrogenase (ADH), malate dehydrogenase, esterase, catalase (CAT), superoxide dismutase

(SOD), isocitrate lyase (ICL), and glutamate dehydrogenase (GTDH). The statistical design was completely randomized with five replications. PEG6000 prolonged ADH activity during the beginning of germination, maintaining the anaerobic metabolism for longer. Subsequently, their activity was reduced, as well as ICL, favoring the deterioration of the seeds that take the time to germinate. Behavior was evidenced by the appearance of SOD, CAT, and GTDH isoforms after 24 h of imbibition when water restriction was imposed. Therefore, the PEG600 is efficient in simulating water deficit conditions in future scenarios of climate change, offering impotent information regarding the germination behavior of the plants under these conditions.

Key words: Germination; *Sesamum indicum*; Deterioration; Molecular marker

INTRODUCTION

The environmental conditions where the plants are established are fundamental for the species survival. The possibility of alterations in the temperature or in the rainfall regime induced by the human action is one of the biggest threat to the agricultural and ecological systems on the planet, once between all recourses that the plants need to develop, the water is essential and often, limiting (Cochrane et al., 2015). In the process of germination, it is necessary that the environment provides enough water for the activation of all metabolic events necessary for the resumption of embryonic axis growth (Bewley et al., 2013; Ataíde et al., 2014). The availability of water plays a significant role in enzymatic reactions, availability of reserves, transport of metabolites and participates in hydrolytic reactions of proteins, lipids, and carbohydrates (Białecka and Kępczyński, 2010).

Factors like water restriction, salinity, and high temperature are common and can limit the germination of seeds causing higher deterioration and decreases in the physiological quality (Betoni et al., 2011). Besides the delay in the emergence and in the growing of seedlings was also observed in rice under conditions of water restriction (Zheng et al., 2015) and *Banksia* sp (Cochrane et al., 2015).

In some situations, this kind of stress can take to a total inhibition of seedling emergence (Kaya et al., 2006), being this inhibition, mainly caused by the reduction of the absorption of water during the imbibition that affects the activity of enzymes involved in the germination process (Zeid and Shedeed, 2006). The water limitation induces alterations in the morphology of seedlings that in some cases germinate; however, are abnormal, beyond the alterations in the cellular metabolism. Between these alterations, are the overproduction of reactive oxygen species (ROS) that provokes damages in cellular membranes, proteins, DNA and can induce cellular death (Zhang et al., 2007). These alterations affect the plant physiology, generating disturbances in the cellular elongation and expansion (Zheng et al., 2015). The production of ROS provokes serious oxidative damages, considering the effect of dry (Farooq et al., 2009), especially the above normal production of hydrogen peroxide (H_2O_2), which can cross biological membranes and affect distant locals from where they were produced. The excess of harmful molecules could be controlled by anti-oxidative mechanisms, between them, the restrictive enzymes like superoxide dismutase (SOD) and catalase (CAT) (Khaliq et al., 2015).

The germination is considered a screening process to the tolerance to hydric stress. Tolerant plants in experimental conditions, according to the germination test, were also tolerant in field conditions (Khakwani et al., 2011). How is the most part of the cultivated species propagated per seeds, through the risks of climate changes that has been affecting the precipitation patterns, where the predictable scenarios are that approximately one-third of the globe will be prone to drought? To comprehend the mechanisms of the answer to the water restriction during the germination is essential to the adaptation of cultures (Zheng et al., 2015).

The polyethylene glycol (PEG) and the potassium nitrate (KNO_3) are molecules with high molecular weight; therefore, they do not penetrate the seeds and are not toxic to them. The technique proposed to increase the germination and the vigor of seeds is widely used for osmopriming (Chen and Arora, 2011). However, the PEG6000 can also be used as inducer agent to the water stress, simulating the drought. As the soil dries, its water potential becomes increasingly negative, making it more difficult for the seeds to remove water from the soil, delaying or even preventing the germination of seeds (Zheng et al., 2015).

In controlled conditions, this solute binds to the water, reducing the water potential of the solution. This effect induces a reduction in the hydraulic conductivity in the system of the contact point of the membrane, similar to the dry stress in the field (Cochrane et al., 2015). How bigger the concentration of solute is, lower will be the availability of water.

To monitoring the physiological quality of seeds during these conditions, many studies indicate fast tests capable of detecting alterations in the metabolism, which takes to deteriorations, before the visible damages appear (Delouche, 2002). To identify these alterations, biologic molecular techniques associated with the control of seed quality has been useful in the obtainment of different classes of molecular markers that help in the understanding of factors that affect the physiological quality of seeds. Between them, the electrophoretic profile of some enzymes is involved in metabolic reactions of synthesis and degradation of molecules (Albuquerque et al., 2009).

These enzymes can be used like molecular markers to elucidate events that occur during the germination process, like the alcohol dehydrogenase (ADH) and the malate dehydrogenase (MDH), markers used to evaluate the alterations in the respiratory activity of seeds and the esterase (EST) to evaluate the integrity of the membrane system. In oil seeds, such as sesame, the isocitrate lyase (ICL) is the key enzyme in the regulation of the glyoxylate cycle and is directly involved in the lipids stored and degraded during the germination process. Beyond these, the glutamate dehydrogenase (GTDH) acts in the oxidation of storage proteins providing energy to the cells and/or reducing the α-ketoglutarate to the synthesis of amino acids.

When in conditions of dry stress, there is the higher production of ROS that affects the cellular metabolism accelerating the deterioration of seeds, being necessary the activity of enzymes from oxidative stress like CAT and SOD (Scandalios, 2005). The SOD primary action is in the catabolization by the dismutation reaction of free superoxide radicals (O_{2-}), converting it to molecular oxygen (O_2) and H_2O_2, which although less reactive at high concentrations becomes toxic to the seeds. By the CAT action, this H_2O_2 formed can be further converted to H_2O and O_2 (Lehninger, 2006). However, it is expected that the activity of these enzymes is directly related to the seed deterioration level and the need for eliminating reactive and harmful compounds to the seeds. The same behavior is expected for CAT enzyme activity since it acts on the products processing derived from SOD activity. This increased activity of both enzymes during the germination process has been reported in a study by Flores et al. (2014) with seeds of *Melanoxylon brauna*, where they observed increased activity of the two enzymes with longer exposure of the seeds to imbibition, and by Carneiro et al. (2011), with sunflower seeds in NaCl solutions.

The electrophoretic technique is able to assist in the detection of early deterioration stages by evaluating the activity of the enzyme associated with degradation and oxidation of reserve substances, as well as biosynthesis of new substances (Spinola et al., 2000; Santos et al., 2004). The evaluation capacity and the correct interpretation of electrophoretic variation in protein profiles and enzymes can be an effective tool in determining biochemical changes resulting from the deteriorating process.

Sesame (*Sesamum indicum*) is now one of the main oleaginous with the largest area planted in the world (Queiroga and Silva, 2008). Its production is stimulated by its easy cultivation, economic returns to producers, high oil content in the seeds, and rapid germination (Brasil, 2009). However, despite the ecological and economic importance of the species, it appears that there are few studies that address physiological and biochemical changes when these seeds are subjected to adverse conditions for germination. Evaluating alterations that occur during the germination under conditions that simulate the dry is still a challenge in the agriculture.

Thus, the objective of this study was to study the alterations in germination and in the physiological potential of seeds in conditions of limiting water quantity, simulated by the PEG6000.

MATERIAL AND METHODS

The research was conducted in the Central Laboratory of Seeds of the Agricultural Department at Universidade Federal de Lavras (UFLA), in Lavras, MG, Brazil. Sesame seeds harvested in 2014 were used. Until the realization of the experiments, seeds were stored in cold chamber at 10°C.

Initially, preliminary tests were performed to determine the concentrations of PEG solution of molar mass 6000 (PEG6000). The treatments were water (control) and the osmotic concentrations of PEG were -0.1, -0.2, and -0.3 MPa, totalizing four treatments. The following determinations were done.

Germination test

Sesame seeds were placed to germinate in each solution described above. The test was realized in the gearbox with the seeds, in five replications of 50, distributed on germination paper moistened with 3mL of the solutions. The gearboxes were maintained in biochemical oxygen demand (BOD) with an alternate temperature of 20-30°C and a constant presence of light (Brasil, 2009). The sixth day after sowing, the percentage of normal seedlings was evaluated.

First-count germination (FCG)

Consisted of a record of the number of normal seedlings obtained on the third day after sowing, the values are reported as a percentage.

Germination speed index (GSI)

Daily counts were done on the number of seeds that issued a radicle higher than 1.0 mm and were calculated according to Nakagawa (1999).

Length of hypocotyl and radicle

Seeds in five replications of 25 were sown in the gearbox, following the methodology described above for the germination test. A measurement of the length of hypocotyl was done in the seedlings classified as normal with the help of a graduated ruler. The results are reported as cm/seedling.

Hypocotyl (HDM) and radicle dry matter (RDM)

The seedlings were separated in hypocotyl and radicle and after were dried in an oven for 72 h at 65°C they were submitted to a new weighing. The results are reported as mg/seedling.

Imbibition curve

For each treatment, the seeds were placed on Petri dishes containing two germination papers, moistened with 3.0 mL water and the PEG solutions described above. The dishes were capped and kept in BOD regulated to an alternate temperature of 20-30°C and a constant light (Brasil, 2009). During the evaluation, seeds were removed from Petri dishes, carefully dried with the aim of towel paper and weighed in the analytical balance with a precision of 0.0001 g. To monitoring the absorption of water, seeds were weighed before the beginning of imbibitions and in intervals of time of 3 h. This procedure was followed until occurs the protrusion of primary root of 50% +1 of seeds. The percentage of mass increment (I) was calculated over time in function of initial seeds mass (Justo et al., 2007). $I (\%) = [(Mt - Mi) / M] \times 100$, in which: Mi = initial fresh weight of sample and Mt = mass of the sample in time (t).

Activity of the main enzymes

After each period of weighing during the imbibition process, a quantity of 100 seeds for treatment was removed and conditioned in the deep freezer to posterior analyses of enzymes. However, to optimize the technique of electrophoresis six periods per treatment were determined that were representative of the beginning, middle, and end of the germination process. The enzyme extracts were obtained by maceration of seeds in the presence of polyvinylpolypyrrolidone (PVPP) and liquid nitrogen, and then were stored at a temperature of -86°C.

The electrophoretic run was realized in a discontinuous system of polyacrylamide gels at 7.5% (separating gel) and 4.5% (concentrating gel). The system gel/electrode used was the Tris-glycine, pH 8.9. The channel of the gel was applied to 50 µL supernatant of the sample and the electrophoretic run was realized at 150V by 5 h. At the end, the gels were revealed for the enzymes ADH (EC 1.1.1.1), MDH (EC 1.1.1.37), EST (EC 3.1.1.1), CAT (EC.1.11.1.6), SOD (EC.1.15 .1.1.), ICL (EC 4.1.3.1), and GTDH (EC 1.4.1.2.).

For all determinations, the statistical design was entirely randomized with five replicates. The data were submitted to analysis of variance (ANOVA) with the help of the statistical program SISVAR® (Ferreira, 2011) and the averages obtained for the treatments were compared by the Tukey test at 5% significance. To adjust the curve to the data of imbibition, we tried to establish for each treatment, an equation of 3rd degree that was capable of adjusting to the three-phase standard germination. With respect to the gels, the evaluation was performed on the transilluminator and is considered the variation of intensity of the bands.

RESULTS

Treatments with PEG6000 reduced the vigor and the imbibition rates of sesame seeds

To verify the efficiency of PEG6000 in simulating dry conditions and their effects on the germination process of sesame seeds, the percentage of germination, vigor, and the imbibition rates was evaluated. The germination of sesame seeds (*S. indicium*) under optimal conditions (water) was 98%; 66.8% points higher to that obtained at a concentration of -0.3 MPa PEG. The different values of water potential studied reduced germination, and despite this, the germination at -0.1 MPa is above 60%, considered minimal for the marketing of sesame seeds, according to Normative Instruction No. 45, September 17, 2013 (Brasil, 2013).

The results of FCG and GSI, indicative of seed vigor, showed that the water deficit in any of the tested potential reduced the germination speed of sesame seeds (Table 1). There was a reduction of increasing water stress, i.e, with a decrease in the solution potential of PEG6000.In relation to the hypocotyl length, related to the vigor of plants (Table 1), a decrease was observed as the osmotic potential became more negative. In the potential -0.3 MPa, the hypocotyl length was 75% lower than the one observed in seedlings that have developed under adequate conditions of water availability (0.0 MPa), and this potential also affected more drastically the development of shoots of the sesame seedling.

Table 1. Germination (G%), first-count germination (FCG%), germination speed index (GSI), hypocotyl length (HL cm/seedling), radicle length (RL cm/seedling), hypocotyl dry matter (HDM mg/seedling), radicle dry matter (RDM mg/seedling) of *Sesamum indicum* submitted to imbibitions into water and PEG6000 at -0.1, -0.2, and -0.3 MPa.

Osmotic potential (MPa)	Initial characterization of lot						
	G%	FCG%	GSI	HL	RL	HDM	RDM
0.0	98^{a}	95^{a}	29.72^{a}	2.18^{a}	4.44^{a}	20.07^{a}	19.54^{a}
-0.1	67^{b}	25^{b}	16.70^{b}	0.42^{b}	0.48^{cd}	9.43^{b}	7.43^{b}
-0.2	40^{c}	8^{cd}	6.50^{cd}	0.29^{bc}	0.62^{c}	6.17^{c}	5.12^{bc}
-0.3	32^{d}	3^{d}	4.50^{d}	0.08^{c}	1.08^{b}	3.15^{c}	2.19^{c}
CV (%)	12.2	6.52	10.53	7.34	6.39	8.91	10.32

*Average followed by the same lower case in the column does not differ by the Tukey test at 5%.

For the radicle length, a significant variable to tolerance to drought in field conditions, a reduction was verified when the seeds were submitted to water stress compared to those imbibed into water (Table 1). However, comparing different potentials, it is observed that the radicle length of sesame seeds increased the osmotic potential of the solution became more negative, with the lowest values observed in potentials of -0.1 and -0.2 MP.

A higher accumulation of dry matter was observed in control seedlings that showed HDM of 20.07 mg/seedling and RDM of 19.54 mg/seedling (Table 1). When the seeds were submitted to osmotic potential -0.1 MPA there was a reduction of 53 and 62% in HDM and RDM, respectively. In the potential -0.2 MPa, the reduction was 69 and 74%, and in the potential -0.3 MPa there was a reduction (84 and 88%, respectively) in HDM and RDM compared to control. These data are in agreement with Moraes et al. (2005), who found that the dry matter of soybean seedlings decreased by reducing the osmotic potential being lower in potential -0.3MPa.

For imbibition curve, which indicates the speed of absorption of water by seeds (Figure 1), the results of the polynomial regression analysis were observed for the sesame seeds imbibition in different osmotic potentials and water and a cubic tendency for all treatments. The three-phase pattern was most evident in the water potential equal to zero, in the presence of pure water (optimum conditions). Stage 1 was characterized by a significant increase in moisture in the first 12 h of imbibition with gain more than 50% of the dry mass of sesame seeds. The next phase, or phase II, showed slower gain moisture, lasting about 14 h in sesame seeds imbibed into the water. The radicle protrusion was observed in 50% of the seeds +1 after approximately 24 h of imbibition (graphically represented by an arrow), indicating the beginning of phase 3, or visible emission radicle (Figure 1).

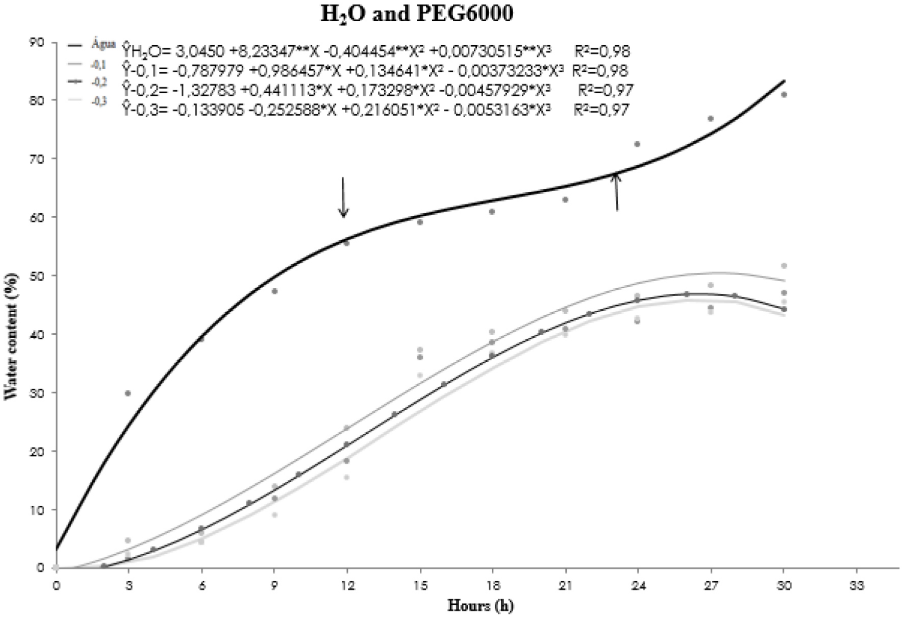

Figure 1. Water absorption curve of seeds *Sesamum indicum* submitted to imbibition with water (control), higher osmotic potential (-0.1 MPa), intermediate osmotic potential (-0.2 MPa), and low osmotic potential (-0.3 MPa).

In treatments with PEG6000 osmotic solutions, there was more time for hydration and less accumulation of seed water during phase I, compared to the imbibition into water (Figure 1). In PEG6000 solutions, there was the beginning of phase II approximately 23 h after the beginning of imbibition for the three treatments, and the end of phase II could not be observed because the evaluation curve was terminated when the seeds were in the stationary phase. Compared to the control, phase II was extended so that seeds have not reached phase III, not occurring the radicle emergence.

Metabolic alterations in enzymes involved in the germination

To verify alterations in the metabolism of enzymes involved in the germinative process that can be related to the reduction of germination, vigor, and imbibition rates, the electrophoretic profile of enzymes of reserve degradation and from the oxidative stress was evaluated. The enzyme systems in sesame seeds submitted to periods of 0-30 h of imbibition and revealed to ADH, MDH, EST, SOD, CAT, ICL, and GTDH are shown in Figures 2 and 3.

The activity of the ADH in the sesame seeds was observed for all treatments (Figure 2A). However, at the beginning of the water absorption, a high enzyme activity was detected with a decreasing trend in the final phases of imbibition.

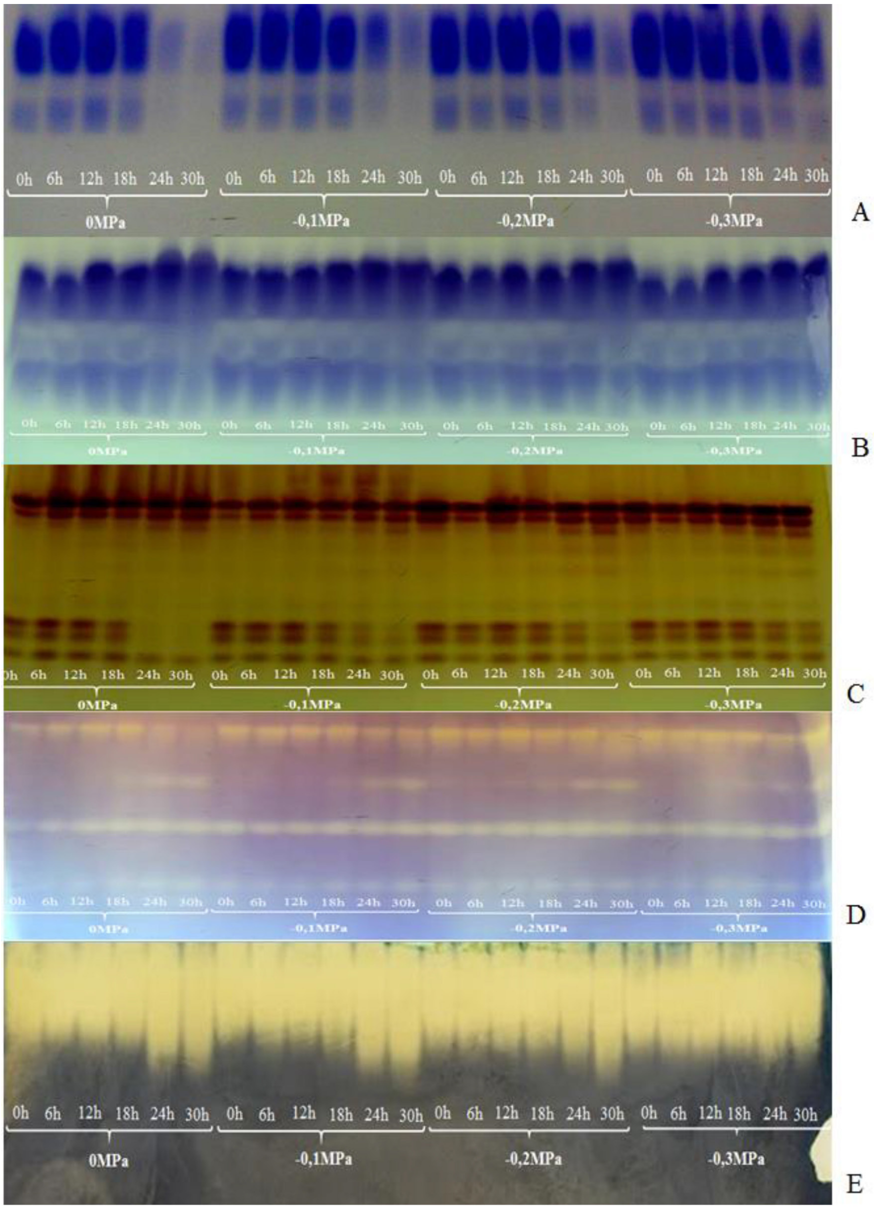

Figure 2. Electrophoretic patterns of alcohol dehydrogenase enzymes (**A**) malate dehydrogenase (**B**), esterase (**C**), superoxide dismutase (**D**), and catalase (**E**) in sesame seeds submitted to imbibition process in pure water (0 MPa) and different potential osmotic (-0.1, -0.2, and -0.3 MPa).

In the MDH enzymatic profile (Figure 2B), contrary to what normally is expected for this enzyme whose activity is most evident in situations of high deterioration, it was observed significant differences in their expression in seeds imbibition into the water and PEG6000 solutions during the germination process.

The EST enzyme expression patterns are shown in Figure 2C. During the absorption process into the water and at different osmotic potentials, there is a decrease in intensity and number of bands as the time approaches the root protrusion. Into the water, this decrease in the intensity of bands was most drastic after 24 h. In different osmotic potential, there is the same trend over the imbibition process, with a less drastic decrease compared to imbibition into the water, but visible in intensity and number of bands.

At zymogram of SOD and CAT enzymes (Figure 2D and E), it was not possible to observe differences in expression until the interval of 24 h in seeds imbibed at different osmotic potentials. However, new isoforms appeared after this period.

About the ICL enzyme (Figure 3A), it can be observed their higher activity before a visible marker of radicle protrusion. However, it notes that with the increase in osmotic potential, the enzyme activity decreases, becoming almost zero in the higher osmotic potential of -0.3 MPa.

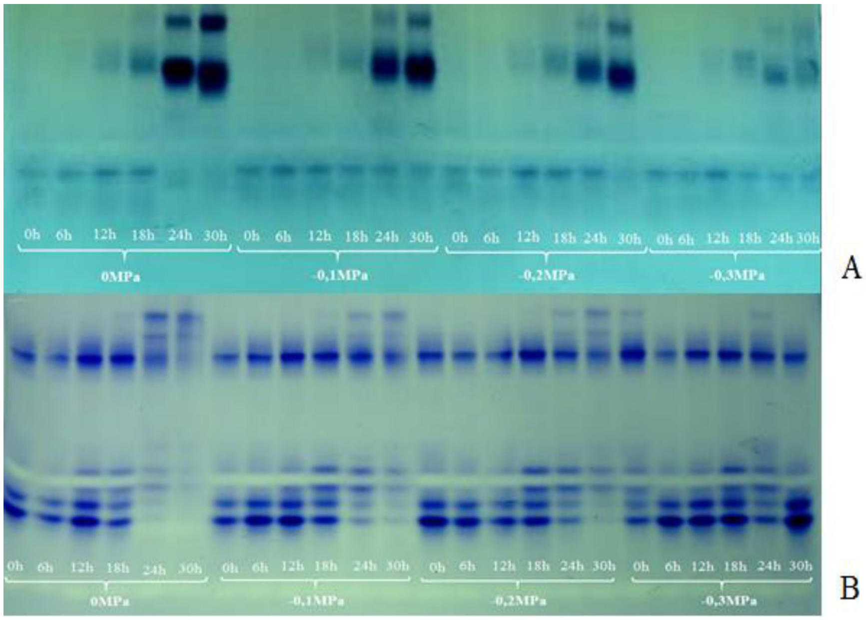

Figure 3. Electrophoretic patterns of isocitrate lyase enzyme (**A**) and glutamate dehydrogenase (**B**) in sesame seeds submitted to imbibition process in pure water (0 MPa) and different osmotic potentials (-0.1, and -0.2, -0.3 MPa).

Another enzyme, which has been used to detect seeds deterioration, is GTDH. Its activity, in accordance with Figure 3B, presented to be constant in the four treatments, with high activity up to 24 h and decreased in the following periods.

DISCUSSION

The global agriculture can be affected by the climatic changes that, according to the predictable scenarios, will suffer from the reduction and the de-standardization in the rainfall patterns. These alterations can negatively affect the production in values upper than 50%, affecting mainly the seedling growth (Wu et al., 2011; Muscolo et al., 2014). The germination is the stage of the vegetable cycle life with a higher sensibility to the climate. The reduction in the percentage of seed germination in drought conditions is attributed to lower kinetic in the water absorption across the tegument. Moreover, the presence of soluble salts causes a reduction in water potential in the substrate, favoring the production of toxic products to the embryo, leading to less water absorption capacity of the seed, and consequent decrease in germination. According to Pelegrini et al. (2013), the decrease in seed germination submitted to water stress is directly related to the decrease in the activity of some enzymes, including the GTDH used as signaling to the osmotic adjustment of seeds cells.

The speeding of germination is an essential factor to the survival of species being fundamental to know their behavior front the water limitation in future scenarios. How slower is the germination, more time the radicle will be in touch with soil microorganisms and could die by contamination. With a rapid germination, the chance of establishment, resource exploration of soil, and survival is higher (Cochrane et al., 2015). After starting the germination process and the radicle emission, the desiccation is not anymore tolerating. The simulation of the drought (water stress) with PEG allowed to demonstrate the divergence in the tolerance to the stress between and into the same species (Cochrane et al., 2015), once PEG6000 is efficient in simulating these conditions because it reduces the hydric potential of the solution.

The negative effects of water restriction on oilseed germination have been reported in several studies. Ávila et al. (2007) found an accentuated reduction in germination of canola seeds when submitted to the more negative osmotic potential. Pereira and Lopes (2011) observed a drastic germination reduction, germination speed, and performance of *Jatropha* seeds in potential -0.2 MPa and Teixeira et al. (2011), working with *Crambe* seed, found a significant reduction of germination and vigor in seeds submitted to more negative osmotic potential, with no formation of normal seedlings in potential inferior to -0.6 MPa.

This effect can be attributed to the lower availability of water, which is essential to the enzymatic reactions, solubilization, and transport of metabolites. Beyond this, the water works like reagent in the hydrolytic degradation of proteins, lipids, and carbohydrates (Białecka and Kępczyński, 2010).

Overall, the radicles were less compromised by water restriction than the development of shoots. These results can be explained by the fact that seedlings submitted to the most severe water stress, tend to invest greater biomass and develop larger root system as a survival strategy. The root distribution in depth/length due to the water insufficiency is considered as a drought tolerance parameter indicator and can give adaptation in some species (Braga et al., 1999; Pires et al., 2016). The reduction in the development of tissues in roots and shoots can be attributed to the lower availability of water to the enzymatic activity involved in the breakdown of reserve substances. This break provides energy to the division and to the cellular elongation, what becomes limited with the lower absorption of water (Muscolo et al., 2014). Water stress affected the root growth and hypocotyl of sesame seedlings. Similar results were found in studies with sunflower (Kaya et al., 2006), canola (Ávila et al., 2007), peanuts, sesame, and castor beans (Pinto et al., 2008).

In the seed germination process, the radicle protrusion is the beginning of the third stage according to the three-phase standard set by Bewley et al. (2013). To this occurs the seeds need to reach an adequate level of hydration to allow the reactivation of metabolism and consequent growth of the embryonic axis. However, the presence of salts significantly influences the response of seed to germination, since the increase in osmotic pressure and the reduction of water potential lead to lower water absorption by the seeds. PEG6000 has been effectively used to simulate the drought in an efficient way than other compounds, which also reduces the osmotic potential of the solution, causing interference with the absorption of water, and limiting the metabolic reactions without penetrating into seeds or offering toxicity. This occurs due to their higher molecular weight (≥6000). Solutions that simulate the conditions of a dry soil, overcoming other compounds with less molecular weight, like mannitol, sorbitol, inorganic salts, or PEG with molecular weight less than 6000, could penetrate the cellular wall and cause damages to the membrane (Verslues et al., 1998).

The three-phase pattern was most evident in the water potential equal to zero; in the presence of pure water (optimum conditions) this pattern is best seen, confirming the observation by Bewley et al. (2013). In phase I, there was metabolism and enzyme activation in addition to a rapid increase in proportional breathing to the increased tissue hydration. It is characteristically short for some species such as *Jatropha* that Borges et al. (2009) observed mean duration of phase I for 4 to 6 h and castor beans with a maximum duration of 15 h (Pimenta et al., 2014).

The water restriction imposed on the seeds in the potential of -0.1, -0.2, and -0.3 MPa reduces the speed of physiological and biochemical processes, restricting the embryo growth, which can be determined by the results of germination, FCG, and GSI (Table 1). A delay in the germination process also occurs when seeds were submitted to more negative osmotic potential, showing that imbibition occurred more slowly due to the potential difference between the seeds and the solution. Similar behavior was observed in the study of Andréo-Souza et al. (2010), with *Jatropha* seeds, when they noticed a delay in the germination process when submitted to water stress condition in the imbibition phase.

Silva et al. (2006) stated that drought causes a prolongation of the stationary phase imbibition process, because of the reduction enzyme activity and consequently a greater delay in the radicle protrusion. Bewley et al. (2013) associate water restriction to decreased metabolism to digestion reserves and translocation of metabolized products.

The higher activity of ADH enzyme at the beginning of imbibition can be attributed to the more impermeability of the tegument to oxygen, which makes breathing of fully anaerobic seeds and prevents seed exposure to the deleterious acetaldehyde effects. As soon as the tegument is broken up (according to Figure 1, after 24 h of imbibition into water), the aerobic process of energy generation begins to be predominate. At this moment, enzyme ADH expression decreases to almost zero since its activity is no longer required. In the other treatments, the high ADH enzyme activity at the beginning of imbibition is explained by the presence of PEG6000 with high viscosity, which restricts the entry of oxygen and induces anaerobic and subsequent ethanol production, toxic to the seed. In the course of germination process, a reduced and delayed presence of PEG6000, according to Table 1, caused accelerated deterioration of seeds making them more susceptible to the acetaldehyde action and reducing its viability (Taiz and Zeiger, 2013). Thus, the bands' intensity decrease was directly related to low ADH enzyme activity, which makes the seeds less protected to the acetaldehyde action, suggesting an important role of this enzyme in response to environmental stresses.

An enzyme used as a marker of deterioration in seeds is the MDHC that as ADH also operates in the respiratory processes and is responsible for the catalyzing conversion from malate to oxaloacetate, by having a relevant role in the Krebs cycle (Lakshmanan et al., 2014).

Concerning MDH enzymatic profile, it was observed significant differences in its expression in seed imbibition into the water and PEG6000 solutions during the germination process that are possible to infer the physiological quality of sesame seeds on the conditions by the activity of this enzyme.

As a result of the deterioration process, there is compromised the respiratory activity of seeds that should present less intense bands, which was not observed in this study even with the obvious reduction of the physiological seed quality submitted to higher osmotic potential (Table 1). However, its activity in the early stages of germination process is high, since the high amount of energy is required for the reserves consumption and synthesis of new seed tissues.

One of the first events recognized of seed deterioration is the damaging of membrane system (Marcos Filho, 2015). Today it is known the existence of markers capable of evaluating the degenerative process of breakdown of these membranes, such as esterase, which is directly involved in the hydrolysis reactions and lipid metabolism. Into the water, this decrease in the intensity of bands was most drastic after 24 h, indicating a less lipid peroxidation and increased permeability of the tegument at the time which tegument is broken up for radicle protrusion and higher water intake. As the deterioration process progresses when the seeds are exposed to adverse environmental factors, such as the excess of salts, the activity of this enzyme decreases, being directly related to breaking up of the membranes and low physiological seed quality. Similar results were obtained by Aung and McDonald (1995) who showed a decrease in esterase activity with increased deterioration in both imbibed and not imbibed seeds. They also observed a decrease in esterase activity with an increasing deterioration process in corn seeds.

In these conditions, the plants drastically accelerate the ROS production and its excess can cause oxidative damages degrading macromolecules (Jaspers and Kangasjärvi, 2010; Hossain et al., 2011). To control the excess of ROS and protect from the oxidative damages, the antioxidant system is activated, represented mainly by the enzymes SOD and CAT. These two enzymes are also known as enzyme “scavengers”.

At zymogram of the SOD enzyme, it was not possible to observe differences in expression until the interval of 24 h in seeds imbibed at different osmotic potentials. However, the emergence of new isoforms after this period suggests sensibility of this kind of species to adverse situations of high salt concentration and water deficit and the greatest ability to develop stress tolerance mechanisms in a relatively short period. Another fact to be considered is that in normal conditions, oxygen reactive species can be formed without necessarily cause damage to the seed, as is the imbibition case into the water, where it is observed the appearance of bands after 24 h, the moment that preceding germination and mobilization and reserve degradation is intensified. Probably the delay in seed germination submitted to the treatments with PEG allowed conditions to higher production of ROS (Xu et al., 2011). For this reason, we observed the appearance of new isoforms of SOD after this period. When the germination process suffers a delay, which occurred here, there is an increase of deterioration and consequently of respiration, increasing the productions of ROS (Huang and Song, 2013). This relation also applies to the increase of water content that allows the higher production of ROS due to the resumption of metabolism and the increase in respiration. Similar behavior was observed to CAT, in view that they are enzymes with complementary action.

The low expression of bands of these two enzymes during the drought stress can be also related to the less efficient mobilization of antioxidant enzymes in roots than in leaves under these conditions (Grzesiak et al., 2013). The evaluation of seeds during the germination, the speed, and enzyme activity could be inferior to the expected in other plant tissues. Besides this, the lower activity of enzymes like SOD and CAT was observed in seeds submitted to treatments with PEG, showing that PEG reduces the metabolic activity of embryos during the germination (Huang and Song, 2013).

In oilseeds, such as sesame, ICL is a key enzyme in the regulation of glyoxylate cycle and is directly involved in the metabolism of lipids stored. During the germination process, these seeds can metabolize their stock of lipids to obtain sugar, especially in the form of sucrose, and distribute it for seedlings in growing, which explains the high activity of this enzyme at the time prior to germination (Salway, 2009). According to Costa et al. (2007), sesame seeds have high lipid content, varying from 41 to 63%, and these lipids in abundance are considered the base of reserve material for the germination process; thus, it is expected high activity of this enzyme just before radicle protrusion.

The low activity of this enzyme in higher osmotic potentials can be explained by harmful effects on the synthesis “again” of the enzyme, which may have occurred during the germination and development of seedlings grown under high stress, making this a responsible factor for negative interference in the enzyme activity. It is noteworthy that oilseeds are highly dependent on ICL to germinate and their low activity in higher osmotic potential is consistent with the results in Table 1, i.e., the low viability of seeds submitted to rigorous stress. In addition, once during the germination process, there is the production of ROS, mainly next to the root protrusion; then, the higher intensity of bands in periods more advanced of sesame seed germination is justified (24 and 30 h). This behavior disturbs the metabolic equilibrium and takes to the degradation of membrane lipids, delaying the germination.

The GTDH enzyme is responsible for the amino acid oxidation and provides energy for the Krebs cycle and consequent production of NADPH (Lehninger, 2006). This enzyme presented constant for four treatments with higher activity up to 24 h following by decreases. This behavior is expected since the moments prior to germination; this enzyme works to provide intermediates for the Krebs cycle, so there are breathing and subsequent amino acids’ formation. However, at the germination time, the seeds begin to require more breaking of fatty acids for energy generation than the intermediates of the Krebs cycle, which explains the decrease in GTDH, due to the increase of ICL activity.

According to Dash and Panda (2001), salt and water stress can have an influence on the isoenzymatic system during the germination process, increasing or reducing GTDH enzyme activity. Within 30 h at the higher osmotic potential, we can observe that the effect was more drastic for sesame seed at the time that it increases the respiratory activity and high production of intermediate Krebs cycle. In this way, we can suggest that despite this study had been realized in the laboratory, relevant information about the behavior of sesame seeds under drought conditions were provided. Although the efficiency of PEG6000 in simulating the drought has been already demonstrated, the extrapolation of this study in natural conditions, in the soil, could fill the possible gaps about the behavior of plants in natural conditions. Following what was demonstrated in this study could reduce time in the determination of hydric potentials to be used and the variables that could efficiently provide relevant data.

CONCLUSIONS

The sesame seed germination is affected by increased osmotic potential, showing that this species is sensitive to salinity stress. The sesame seed imbibition in optimal conditions is approximately 30 h. There is a lower water absorption in seeds submitted to water deficit, and there was no radicle protrusion in treatments with water deficit due to the delay of phase II. ADH, EST, SOD, CAT, GTDH, ICL, and MDH enzymes are considered good molecular markers for evaluation of changes during this process.

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