ANTIOXIDANT ACTIVITY AND PHYSIOLOGICAL EFFECTS IN

Peltophorum dubium (Sprengel) Taubert SEEDS AFTER OVERCOMING

DORMANCY TREATMENTS WITH WATER IN DIFFERENT

TEMPERATURES

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SUMMARY

This study aimed to evaluate the physiological behavior, the antioxidant enzymes activity and the lipoperoxide levels in Peltophorum dubium *(Sprengel) Taubert seeds when submitted to different immersion times and water temperatures to overcome physical dormancy.* P. dubium *seeds were submitted to treatments with heated water (25, 50, 75 e 95°C) during 24h, with and without previous scarification, in order to overcome dormancy. Seed physiology, electrical conductivity and germination (percentage,* average time, frequency and synchrony) were analyzed. The enzymes catalase and peroxidase were quantified after 2, 4, 8, 12 and 24h of imbibition. It is concluded that the best methods for overcoming seed dormancy in P. dubium *were the treatments using water at 75 and 95°C because, due to the efficient action of the enzymes catalase and peroxidase, the levels of lipid peroxidation were controlled, allowing the seeds to achieve high germination and coefficient of velocity of germination.*

Introduction

The recovery of degraded areas consists in a complex system comprised by actions that try to reestablish the conditions of the disturbed environment, back to its natural state (Pereira and Rodrigues, 2012). In order to achieve it, one of the used methods is re-vegetation, characterized by the use of native species that act creating a new vegetation cover that is capable of sustaining itself, thereby assisting

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ACTIVIDAD ANTIOXIDANTE Y EFECTOS FISIOLÓGICOS EN SEMILLAS DE *Peltophorum dubium* (Sprengel) Taubert DESPUÉS DE TRATAMIENTOS DE SUPERACIÓN DE LA LATENCIA CON AGUA A DIFERENTES TEMPERATURAS

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RESUMEN

El objetivo fue evaluar el comportamiento fisiológico, la actividad de las enzimas antioxidantes y niveles de lipoperóxidos en las semillas de Peltophorum dubium *(Sprengel) Taubert cuando se someten a inmersión en agua caliente para superar la latencia física. Semillas de* P. dubium *fueron sometidas a tratamientos de superación de latencia con agua calentada a diferentes temperaturas (25, 50, 75 y 95ºC) durante 24h, con y sin escarificación previa. En la etapa fisiológica se evaluó la conductividad eléctrica, y el porcentaje, tiempo promedio,* índice, frecuencia y sincronización de la germinación. Las enzimas catalasa y peroxidasa se cuantificaron después de 2, 4, 8, 12 y 24h de imbibición. Se concluye que los mejores métodos para romper la latencia en P. dubium *fueron los tratamientos con agua calentada a 75 y 95ºC, ya que debido a la acción eficaz de las enzimas catalasa y peroxidasa, los niveles de peroxidación lipídica se controlaron lo que permite que las semillas presentan elevada germinación e índice de velocidad de germinación.*

ATIVIDADE ANTIOXIDANTE E EFEITOS FISIOLÓGICOS EM SEMENTES DE *Peltophorum dubium* (Sprengel) Taubert APÓS TRATAMENTOS DE SUPERAÇÃO DE DORMÊNCIA COM ÁGUA EM DIFERENTES TEMPERATURAS

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RESUMO

O objetivo foi avaliar o desempenho fisiológico, a atividade das enzimas antioxidantes e os níveis de lipoperóxido nas sementes de Peltophorum dubium *(Sprengel) Taubert quando submetidas a diferentes tempos de imersão e temperaturas de água para superação de dormência física. As sementes de* P. dubium *foram submetidas a tratamentos de superação de dormência com água aquecida (25, 50, 75 e 95ºC) por 24h, com e sem escarificação prévia. Na etapa fisiológica foi avaliada a condutividade elétrica, e a porcentagem, tempo médio, índice,* frequência e sincronização da germinação. As enzimas catalase e peroxidase foram quantificadas após 2, 4, 8, 12 e 24h de embebição. Conclui-se que os melhores métodos para superação de dormência em P. dubium *foram os tratamentos utilizando água aquecida a 75 e 95ºC, pois devido a ação eficiente das enzimas catalase e peroxidase, os níveis de peroxidação lipídica foram controlados permitindo assim que as sementes apresentassem elevada germinação e índice de velocidade de germinação.*

in the recovery of the present vegetation (Guglieri-Caporal *et al.*, 2011).

The production of native seedlings is an essential step to the recovery of degraded areas. The production of these seedlings requires studies about the species to be used, such as botanical identification, seed processing and storage, and methods for overcoming dormancy, since approximately two thirds of seeds of native tree species used to recover degraded areas present dormancy (Santos *et al.*, 2011; Freire *et al.,* 2016, Abreu *et al.,* 2017).

The physical dormancy is the most common one, mainly in the Fabaceae family. In laboratory, methods are being tested that facilitate to overcome dormancy, therefore enhancing the germination percentage of these species, allowing a high productivity in the nursery (Oliveira, 2013).

The use of heated water is a promising treatment for overcoming dormancy, being a low cost and effective approach for various native species (Galindo, 2006). Its efficiency is verified through the softening of the rigid tegument that helps water absorption, being effective in the germination of several species. However, for some species the continuous use of water heated to high temperatures causes damage to the embryo, impeding the occurrence of germination (Albulquerque *et al.*, 2007; Zucareli *et al.*, 2010). Ribeiro *et al.* (2016) reported that the use of water at 95ºC in *P. dubium* seeds did not promote germination (0%), under the hypothesis that in these conditions a denaturation of proteins and enzymes occurred, causing damages to the embryonic tissue.

The good or bad performance of germination in seeds

subjected to treatments for overcoming dormancy using heated water is being increasingly related to mechanisms of membrane repair present in plant cells, where it is possible to verify the action of antioxidant enzymes as peroxidase, catalase and superoxide dismutase. These enzymes remove and degrade the reactive oxygen species (ROS), since the production of these free radicals causes stress, compromising the integrity of the membranes, cell metabolism and, consequently germination (Nkang and Omokaro, 2000; Ataide *et al.*, 2016).

Taking into account the use of native species in the recovery of degraded areas, the species *Peltophorum dubium* Spreng (Taubert), a member of the Fabaceae family popularly known as canafistula, is highlighted. It is a mediumsized tree,

with alternate and compound semidecidous leaves, that plays the role of secondary species in the ecological succession and has a wide distribution in the Parana territory (Duarte and Krentkowski, 2014).

As most of Fabaceae, *P. dubium* seeds have a thick and rigid tegument, an epidermis formed by macrosclereids, which are covered with a grease cuticle and a layer of hemicellulose, accompanied by a layer of osteosclereids. These cells waterproof the seed tegument, preventing the gas exchange and water entrance that occurs during the germination, resulting in dormant seeds (Souza, 2009; Ribeiro *et al.*, 2016).

The present study aimed to evaluate the physiological behavior, the activity of antioxidant enzymes and the levels of lipoperoxide in seeds of *Peltophorum dubium* (Spren-

gel) Taubert when subjected to immersion in heated water in order to overcome physical dormancy.

Methodology

The experiment was carried out in the Plant Physiology Laboratory of the West of Parana State University (Unioeste), Cascavel campus, from March to September of 2016. The seeds of *Peltophorum dubium* were donated by the Itaipu seedling nursery, Foz do Iguaçu, and presented a 12% of water content.

The seeds underwent an asepsis process according to Brasil (2013) and were subjected to the treatments of dormancy overcoming, in a completely randomized design with a factorial 2×4 scheme (factor 1, seeds with and without previous scarification by sandpaper in the opposite side of the micropyle × factor 2, four different water temperatures for immersion during 24h). The different temperatures employed were 25, 50, 75 and 95ºC, totalizing 8 treatments.

The enzymatic tests were conducted utilizing seeds with or without previous scarification in a factorial 4×5 scheme, with 25 sampling units in each replication. The 20 treatments were composed by the combination of 4 immersion temperatures (25, 50, 75 and 95ºC) × 5 sampling times in each association (2, 4 , 8, 12 and 24h).

Physiological analysis of seeds subjected to treatments to overcome dormancy

The electrical conductivity of the seeds subjected to treatments to overcome dormancy was evaluated utilizing four replicates of 25 seeds in each treatment. The seeds were weighted and placed into plastic cups containing 50ml of deionized water and kept at 20ºC for 24h in a biological oxygen demand B.O.D. germination chamber. After, the electrical conductivity of the water where the seeds were soaking was determined using a previously calibrated conductivity meter,

with electrode of 1.0 constant. The results were expressed in $\mu S \cdot cm^{-1} \cdot g^{-1}$, according to the methodology described in AOSA (1983) and Perez and Negreiros (2001).

The germination test was carried out according to Brasil (2009). The seeds were accommodated in 'Germitest' paper rolls, and all the treatments stored in a B.O.D. germination chamber, with constant temperature of 25ºC and photoperiod light:dark of 12:12h.

Germinated seeds counts were carried out daily until the 14º day after the installation of the test (Brasil, 2013). The seeds were considered germinated if they presented 2mm of primary root length (Laboriau, 1983). The analyzed parameters were: germination percentage (G), mean germination time (MGT; Nakagawa, 1994), germination speed index (GSI), germination frequency and synchronization (U), (Santana and Ranal, 2004).

Specific antioxidant enzyme and lipoperoxide activity

The peroxidase (POD) activity in seeds of *P. dubium* treated with heated water to overcome dormancy, with and without scarification, was determined according to Teisseire and Guy (2000), with the addition of 20μl of enzymatic extract, 500μl of potassium phosphate buffer 50mmol·l⁻¹, pH 6.5, 250μl pirogalol (1,2,3-benzenotriol) 20mmol·l⁻¹, and hydrogen peroxide (H_2O_2) 5mmol·l⁻¹, totaling a volume of 1.0ml. The formation of purpurogallin was measured with a UV-visible spectrophotometer at 430nm. The coefficient of molar extinction (2.5mmol·l⁻¹·cm⁻¹) was applied to calculate the specific activity of the enzyme, which was expressed in μmol purpurogallin per min⁻¹·mg⁻¹ of protein.

The catalase (CAT) enzyme activity was established according to Peixoto *et al.* (1999), with the addition of 20μl of the enzymatic extract, 980μl sodium phosphate 0.05mol·l⁻¹ buffer, pH 7.0 and H_2O_2 12.5mmol·l⁻¹ to final volume of 1ml. The absor-

bance readings at 240nm were measured using a spectrophotometer. The coefficient of molar extinction of H_2O_2 (39.4 mmol·l⁻¹·cm⁻¹) was calculated and the result was expressed in μmol of consumed H_2O_2 per min⁻¹·mg⁻¹ of protein.

The lipoperoxide was determined according to Heath and Packer (1968), cited by Rama Devi and Prasad (1998). Solutions of tiobarbituric acid (TBA) 0.25% and trichloroacetic acid (TCA) 10% were used, with 100 mg of material. The absorbance readings were measured using a spectrophotometer UV-visible at 560 and 600 nm. For the calculations, the coefficient of molar extinction of malondialdehyde (155mmol·l⁻¹·cm⁻¹) was used, being expressed in nmol of tiobarbituric acid (TBARS) per g of reactive substances of fresh matter.

Statistical analysis

The physiological data were analyzed following normality assumptions (Shapiro-Wilk) and homoscedasticity (Levene Test). If p>0.5 they were subjected to the analysis of variance (ANOVA - F Test) and the averages compared by the Skott-Knott test with 5% probability, using the program RStudio 3.2.2.

In the biochemical study, the data were subjected to normality test (Shapiro-Wilk), with p<0.5. Since the data did not adjust to any parametrical statistic method, graphics with their standard deviation were presented, and the averages compared in a descriptive manner.

Results and Discussion

Physiological analysis of seeds treated to overcome dormancy

The analysis of the factorial design for the variable electrical conductivity (Table I) demonstrated there was no significant interaction between the analyzed factors (with and without previous scarification × temperatures). However, on

average it was observed that seeds that did not go through the mechanical scarification presented smaller rates for this variable (~145,78μS·cm⁻¹·g⁻¹). This occurs because when there is no mechanical scarification of the seeds of *P. dubium*, the imbibition is slower, as compared to seeds that went through previous mechanic scarification. Thus, there time needed for the cell membranes to go back to its crystalline state without cell damage, decreasing lixiviation of solutes (Ataide *et al.*, 2016).

In seeds of *Brachiaria brizantha* that also show physical dormancy, Cardoso *et al.* (2014) used the treatment of acid scarification for 5min to overcome dormancy during the accelerated ageing, and similarly observed that those seeds led to a higher electrical conductivity during the process, presenting a significant increase of 38.24μS·cm⁻¹·g⁻¹ compared to the control without scarification.

It was verified that, on average, for seeds of *P. dubium* the water temperature of 95ºC utilized to overcome the dormancy led to significantly greater electrical conductivity than the others, with an increase of 50,00μS·cm⁻¹·g⁻¹ in comparison to 25ºC (Table I). Ataíde *et al.* (2016) also observed that the higher temperature, concurrent with longer imbibition time, might have caused conformational changes in membrane phospholipids of of *B. brizantha* seeds and, thus, induced solute lixiviation, about 50,00μS·cm⁻¹·g⁻¹ higher than the control.

In terms of germination percentage, we observed that there was significant interaction between the analyzed factors in of *P. dubium* seeds. Those with no scarification when subjected to water at 75 and 95ºC showed a significant increase of this variable, up to 26% higher than at 25ºC (59%). In contrast, the seeds with previous mechanical scarification did not show an increase in germination percentage when subjected to the imbibition in heated water (Table I). Similar results where reported for the same species by Dutra *et al.*

ELECTRICAL CONDUCTIVITY, GERMINATION, MEAN GERMINATION TIME (MGT) AND GERMINATION SPEED INDEX (GSI), IN SEEDS OF *Peltophorum dubium* SUBJECTED TO TREATMENTS WITH AND WITHOUT SCARIFICATION, COMBINED WITH WATER IMMERSION AT 25, 50, 75 AND 95ºC

ºC	Non-scarified	Scarified	Average
	Electrical conductivity ($\mu S \cdot cm^{-1} \cdot g^{-1}$)		
25	121.95	208.44	165.20 b
50	134.80	186.42	160.61 b
75	146.79	211.65	179.22 b
95	179.59	228.79	204.19 a
Average	145.78 B	208.83 A	
C.V. (%)	9.93%		
	Germination (%)		
25	59 b A	69 a A	-
50	58 b B	83 a A	-
75	79 a A	80 a A	-
95	85 a A	70 a A	-
Average	-	-	
C.V. (%)	12.43%		
	MGT (days)		
25	2.54	0.92	2.88 a
50	2.41	2.32	2.36 a
75	2.10	2.96	2.57 a
95	2.69	2.57	2.63 a
Average	2.197 A	2.437 A	-
C.V. (%)	16.93%		
	GSI		
25	38.58 b A	32.66 b A	-
50	36.22 b B	52.57 a A	-
75	54.27 a A	38.14 b A	-
95	54.97 a A	34.73 b B	-
Average	-	-	-
C.V. (%)	22.28%		

Average yields followed by the same small letters for different temperatures and capital letters for the previous treatments: with and without overcoming dormancy; same letters are not significantly different at the 0.05 level by the Scott-Knot test.

(2012) and Santos *et al.* (2016), who observed increases of 92,2% in emergence and 93,8% in germination, in seeds soaked in heated water at 95ºC.

Examining the water temperatures in relation to the scarification of seeds, it can be seen that the result of hot water at 75 and 95ºC is equivalent to the mechanical scarification treatment, as the seeds that were not scarified showed germination percentages statistically equal to those which went through that process (Table I). This is a satisfactory result with regard to seedlings production in nursery, since overcoming dormancy by using heated water is an easier method to use, compared to mechanical scarification, with low cost and the possibility of being employed at large scale by nurserymen, optimizing *P. dubium* seedlings production.

However, Oliveira *et al.* (2003) and Ribeiro *et al.* (2016) showed that germination of *P. dubium* seeds diminished when subjected to water heated at 95ºC. The authors considered that this change in the response to the method for overcoming dormancy may occur because of the climate conditions to which the matrix producer was exposed during seed maturation, which may alter the degree of dormancy presented by the species.

In relation to the MGT, there was no significant interaction between the analyzed factors and, on average the seeds took 2.5 days to complete their germinative process. Yet, for the GSI variable there was a significant interaction between the factors; seeds with no scarification subjected to temperatures at 75 and 95ºC showed the highest germination speed. According to Carvalho and Nakagawa (2012), the higher the water temperature used for overcoming dormancy, the fastest the germination will be up to a certain degree, where the temperature instead of increasing it will impede germination.

Alternatively, in the seeds treated with scarification and subjected to heated water, an increase of GSI took place only at 50ºC, while at higher temperatures (75 and 95ºC) germination speed was statistically similar to the control (Table I). A hypothesis raised by Joshi and Chinnusamy (2014) is that when seeds are scarified, fissures in the tegument are produced, enabling water inflow to occur faster, and the heated water at high temperatures (75 and 95ºC) may have led to cell damage, delaying germination, as observed in this experiment.

With regard to the frequency and synchronization it is observed that the germination of *P. dubium* showed more synchrony and uniformity when the seeds were scarified, presenting greater initial unimodal peaks, compared to when they were not (Figures 1 b, d, f e h). Ribeiro *et al.* (2016) also observed that the utilization of mechanical scarification was enough to achieve germination synchrony, showing unimodal peaks between the second and fourth day of the germination. This demonstrates that in seeds of *P. dubium* there is a physical resistance that prevents water intake, and with mechanical scarification of this rigid tegument, a more synchronized germination is possible.

Antioxidant enzyme and lipoperoxide activity in seeds treated to overcome dormancy

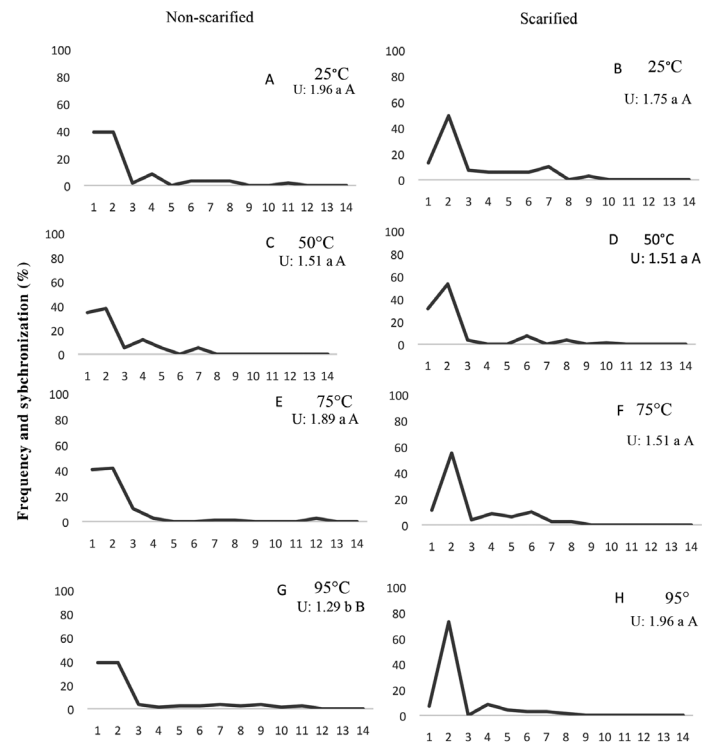The analysis of specific peroxidase activity throughout the

Figure 1. Germination frequency and synchronization in seeds of *Peltophorum dubium* (Sprengel) Tauber, subjected to treatments with and without scarification, combined with immersion in water at 25, 50, 75 and 95ºC.

process of seed germination of *P. dubium* subjected to different methods of overcoming dormancy is shown in Figure 2. When soaked at 25ºC, the seeds showed a similar behavior with and without previous scarification (Figures 2a, b). A peroxidase activity peak was observed 4h after imbibition, with an increase of ~0.20umol·min⁻¹·mg⁻¹ in comparison to 2h. In the seeds immersed at 50ºC (Figures 2c, d) with no scarification, two activity peaks are observed for this enzyme, at 4h and 8h, with an increase of ~0.40umol·min⁻¹·mg⁻¹, while in seeds that went through previous scarification, there is only one activity peak of the peroxidase enzyme after 4h of water treatment, followed by a decrease in the following hours.

Immersion of the seeds at 75ºC with no scarification (Figure 2e, f) led to an increase in peroxidase activity after 4h (1.49umol·min⁻¹·mg⁻¹), which was maintained after

24h. However, after scarification, under the same conditions, the peroxidase activity already increased (1.55umol·min⁻¹·mg⁻¹) within 2h, and during imbibition the activity declined. In seeds subjected to 95ºC without previous scarification (Figures 2g, h), the peroxidase had an increment of 0.54umol·min⁻¹·mg⁻¹ after 4h of water treatment, keeping similar rates in the following hours. In contrast, when previous scarification was done, two peaks of that enzyme activity took place, at 4 and 12h, with raise of ~0.50 and 0.80umol·min⁻¹·mg⁻¹, respectively.

Analyzing the catalase specific activity in *P. dubium* seeds (Figures 3a to h), it was observed that the seeds that were not scarified presented higher rates of this enzyme activity, when compared to the scarified ones. The seeds soaked at 25ºC without scarification, showed a greater catalase specific activity when compared to the scarified ones at

4, 8, 12 and 24h (Figure 3a). In contrast, at 75ºC the specific activity of catalase was about 30 times higher after 8h, and 4 times greater after 24h of imbibition, when compared to the scarified seeds (Figure 3e). In the non scarified seeds soaked for 12h at 95ºC the rate of the catalase activity was 10 times higher, and at 24h nearly 24 times higher when compared to the scarified ones (Figure 3g).

However, at 50ºC higher catalase rates were found in seeds subjected to mechanical scarification, differing from other treatments, where after 24h of imbibition, the enzymatic activity was of 54.97mkat·mg⁻¹, while in the seeds without scarification the rate was of 32.06mkat·mg⁻¹ (Figures 3c, d). It was observed that in the first hours of water treatment (2, 4 and 8h), there was a higher activity of peroxidase, while at 12 and 24h the catalase had a greater action of

eliminating the free radicals in the *P. dubium* seeds.

Under normal conditions, a balance between the production of ROS and the activity of the antioxidant enzymes occurs, especially the peroxidase; yet, the increase of ROS causes cell damage that can lead to cell death. Therefore, Ribeiro *et al.* (2014) reported that the increase in peroxidase activity demonstrates an adaptive response to the cell metabolism in regard to the elimination of ROS, allowing the seeds to tolerate stress conditions. For example, the shock imposed by the high water temperatures used to overcome seed dormancy, culminates in higher rates of germination, in accordance with the observations in the *P. dubium* seeds (Table I).

The use of high temperatures to overcome dormancy of seeds may initiate a thermal stress in the cells, both in the reserve tissues and in the embryo axis, increasing the production and
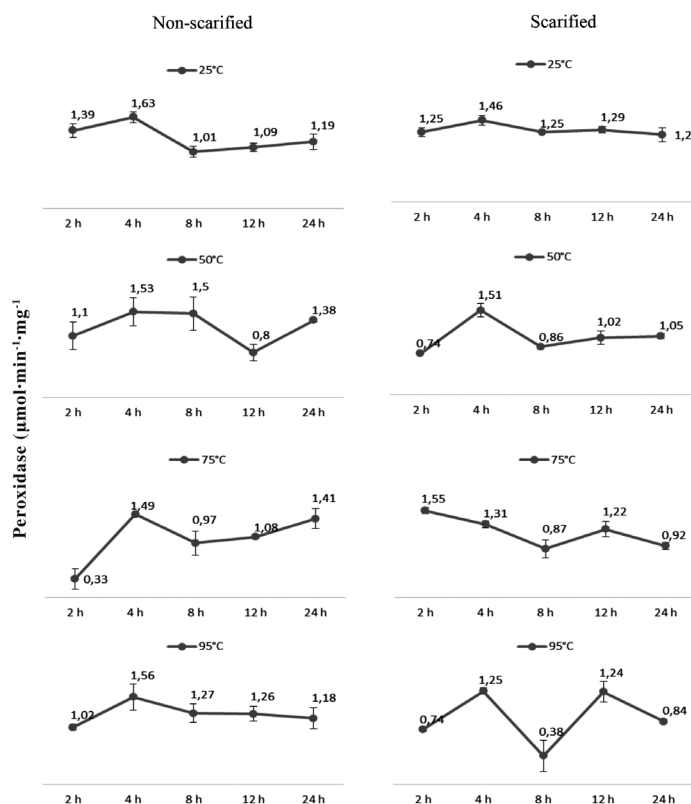
Figure 2. Specific peroxidase activity (umol·min⁻¹·mg⁻¹) in seeds of *Peltophorum dubium,* with and without previous scarification, at different temperatures (25, 50, 75 and 95ºC) after 2, 4, 8, 12 and 24h.

Figure 3. Specific catalase activity (nmol of H_2O_2 consumed/min/mg protein), in seeds of *Peltophorum dubium* with and without previous scarification, at different temperatures (25, 50, 75 and 95ºC) after 2, 4, 8, 12 and 24h.
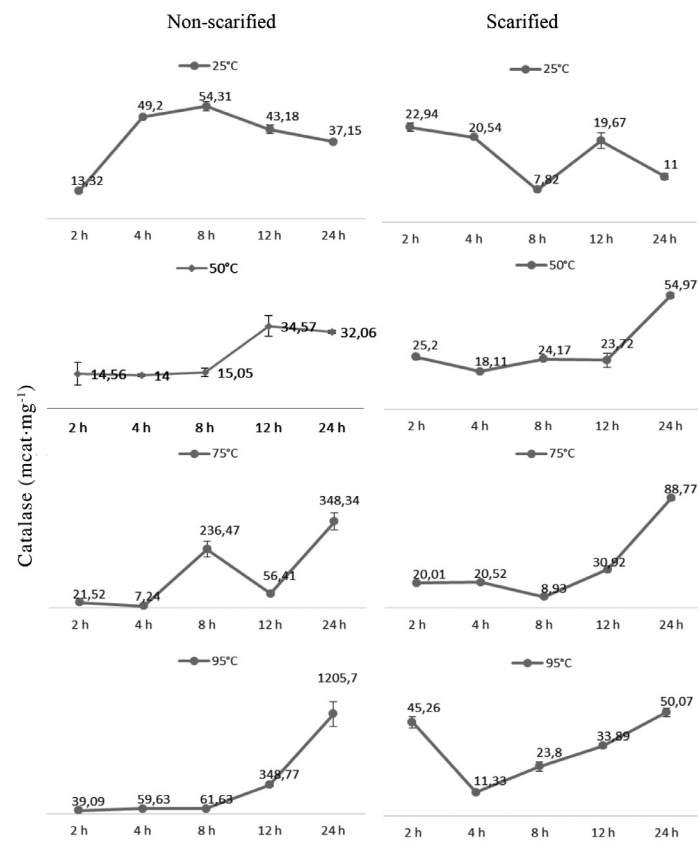
the accumulation of ROS, which in turn will react with lipids, proteins and nucleic acids, resulting in the membrane lesions caused by lipid peroxidation. Plants developed a complex system of protection through oxidant detoxification, where the actions of antioxidant enzymes (peroxidase, catalase) are essential to neutralize the effects of ROS on the cells (Joshi and Chinnusamy 2014; Harsh *et al.,* 2016).

The H_2O_2 radical can act as a cell marker when present in low concentrations in the cells of the seeds. On the other hand, in high concentrations its effect is harmful to the cells, since the radical can cross membranes, mainly through protein channels as aquaporins and cause oxidative damage to the cell (Das and Roychoudhury, 2014). The action of this radical in low concentrations in seeds that are in physical dormancy, as reported by Gill and Tuteja (2010) in seeds of *Capsicum* spp., can be beneficial, acting as a cell wall loosening regulator. It has been observed that this radical can oxidize polysaccharide bonds in the cell wall, leading to its loosening and, thus, water inflow is facilitated, initiating the germination process (El-Maarouf-Bouteau and Bailly, 2008; Barbosa *et al.,* 2014).

Gursky and Santiago (2005) and Ribeiro *et al.* (2016) observed that the tegument of the seeds of *Peltophorum dubium* is rigid, having a layer of macrosclereids in the exotesta, a layer of braquiesclereids in the mesotesta and a layer of osteosclereids in the endotesta, providing its hardness, indicative of physical dormancy.

The increase of peroxidase and catalase activity in overcoming dormancy treatments with heated water at 75 and 95ºC is indicative that these enzymes acted to keep low levels of the H_2O_2 radical in the apoplast of the tegument cells, enabling in this way the loosening of their walls and facilitating the water inflow and germination. These treatments led to the higher germination percentages (79 and 85%, respectively)

without previous scarification (Table I).

Prodanović *et al.* (2007) demonstrated that in seeds of *Picea omorika*, which also have rigid tegument, the increase of catalase and peroxidase might be involved in the protection of the seeds against free radicals, assisting germination. The authors observed ~90% of the germination for this species when the catalase and peroxidase activity doubled, helping in the protection against the formation of ROS and promoting the germinative process.

In relation to the lipid peroxidation, we observed the highest rates in non scarified seeds after 24h at temperatures of 25, 50 and 75ºC. On the other hand, when the seeds were previously scarified the lipid peroxidation takes place after 4h of water treatment; except at 95ºC, where the non-scarified seeds after 12h of imbibition was observed, the highest lipoperoxide contents (22.67 nmol·g⁻¹ MDA), and in scarified seeds and at 95ºC, there were two peaks in

the MDA contents, at 2h (18.91nmol·g⁻¹ MDA) and 24h (20.44nmol·g⁻¹ MDA).

The production of ROS leads, to lipid peroxidation, leading to cell damage. Feng Cai *et al.* (2011) observed that the endosperm of seeds of *Jatropha curcas* presented an increase in the levels of malondialdehyde associated to the increase of peroxidase after 4 and 6 days of germination, followed by catalase peaks after 6, 8 and 10 days. The authors also reported that the enzymes doubled their activity during these peaks, highlighting an integrative activity of the enzymes as lipid peroxidation increases, mainly in the endosperm, leading to the combat of free radicals and consequently to a decrease in cell damage.

This work corroborates previously described data showing a higher lipid peroxidation for the seeds soaked for 12h without scarification at 75 and 95ºC, without any increase in the following hours (Figure 4). This is related to the enhancement of

peroxidase and catalase specific activities, which were able to revert the lipid peroxidation and in this way promote germination (79 and 85% respectively).

Conclusion

The best methods for overcoming dormancy in *P. dubium* were the treatments using heated water at 75 and 95ºC in non-scarified seeds since, due to the efficient action of the enzymes catalase and peroxidase, the levels of lipid peroxidation were controlled, allowing the seeds to present high germination rates and germination speed index.

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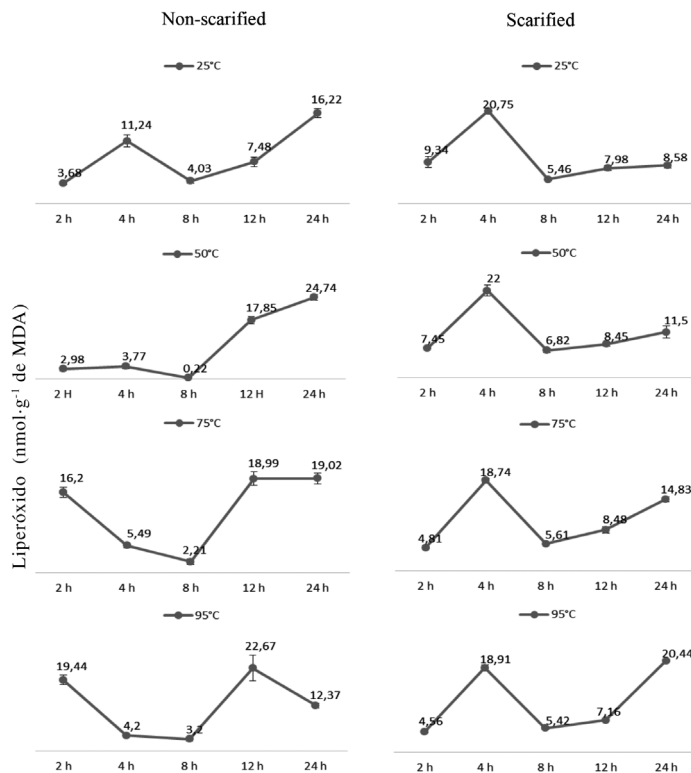
Figure 4. Specific lipoperoxide activity (nmol·g⁻¹·of malondialdehyde) in seeds of *Peltophorum dubium* with and without previous scarification, at different temperatures (25, 50, 75 and 95ºC) after 2,4,8,12 and 24h.

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