

METHODS FOR OVERCOMING DORMANCY IN *Dinizia excelsa* Ducke SEEDS<sup>1</sup>ENIEL DAVID CRUZ<sup>2</sup>, RAFAELA JOSEMARA BARBOSA QUEIROZ<sup>3</sup>, JOSE EDMAR URANO DE CARVALHO<sup>4</sup>

**ABSTRACT** – The impermeability of seed coat to water is common mechanism in Fabaceae seeds. Treatments to overcome hardseededness include scarification with sulphuric acid, scarification on abrasive surface and soaking in water among others. The objective of this study was to identify an effective method to overcome dormancy in *Dinizia excelsa* seeds. A pre-test (untreated seed) and three experiments were carried out: immersion of seeds in acid sulphuric for 10, 20, 30, 40, 50 and 60min (experiment 1); scarification on abrasive surface at the positions distal end, near of the mycophyle and on the lateral tissue and tegument clipping at 1mm of the distal end, near of the mycophyle and on the lateral tissue (experiment 2); scarification on abrasive surface and immersion in water for 0, 12, 24 and 48h (experiment 3). The experimental design was completely with four replications of 50 seeds for each treatment. The statistical analysis was carried out by ANOVA and regression analysis. Seedlings emergence on untreated seeds started on the 8<sup>th</sup> day after sowing and reached 52.5% on the 1,709<sup>th</sup> day. In general, the treatments to overcome dormancy increase emergence. Emergence was higher for seeds treated with sulphuric acid for 20 and 30min with emergence of 93.6% and 86.6%, respectively. For seeds scarified on abrasive surface higher emergences were recorded for scarification on distal end, near of the mycophyle and on the lateral, 82.7%, 74.3% and 75.7%, respectively. Seeds scarified manually showed higher emergence when not immersed in water (75%), or when immersed for 12 and 24h (75%, 73.6% and 65.6%, respectively). Immersion seeds in sulphuric acid for 20 and 30min and scarification on abrasive surface of distal end are effective to overcome dormancy in *D. excelsa*.

Index terms: emergence speed index, hard seed, vigor

MÉTODOS PARA SUPERAÇÃO DA DORMÊNCIA EM SEMENTES DE  
*Dinizia excelsa* Ducke

**RESUMO** – A impermeabilidade do tegumento de sementes a água é um mecanismo comum em Fabaceae. Tratamentos utilizados para superação da dormência consistem de escarificação com ácido sulfúrico, escarificação em superfície abrasiva, imersão em água entre outros. O objetivo deste trabalho foi identificar um método eficiente para superar a dormência em sementes de *Dinizia excelsa*

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e acompanhar seu processo de germinação ao longo do tempo. Foram conduzidos um pré-teste (sementes não escarificadas) e três experimentos: imersão em ácido sulfúrico por 10, 20, 30, 40, 50 e 60min (experimento 1); escarificação em superfície abrasiva na região distal, próximo a micrópila e na lateral da semente e corte de 1mm no tegumento na região distal, próximo a micrópila e na lateral da semente (experimento 2); escarificação em superfície abrasiva e imersão em água por 0, 12, 24 e 48h (experimento 3). O delineamento experimental foi inteiramente casualizado com quatro repetições de 50 sementes por tratamento. A análise estatística consistiu de ANOVA e análise de regressão. Sementes não escarificadas iniciaram a emergência de plântulas oito dias após a semeadura e atingiu 52,5% aos 1.709 dias. Em geral, os tratamentos para superação da dormência promoveram a emergência das plântulas. A emergência foi maior nas sementes escarificadas com ácido sulfúrico por 20 e 30min com 93,6% e 86,6%, respectivamente. Nas sementes submetidas a escarificação abrasiva e corte no tegumento, os melhores tratamentos foram escarificação na região distal, próximo à micrópila e na lateral da semente com 82,7%, 74,3% e 75,7%, respectivamente. Sementes escarificadas em superfície abrasiva e imersas em água apresentaram melhores resultados quando o tempo de imersão foi de 12 e 24h, juntamente com as sementes não imersas, com emergência de 73,6 e 65,6% e 75%, respectivamente. A imersão de sementes em ácido sulfúrico por 20 e 30min e escarificadas em superfície abrasiva na região distal são tratamentos indicados para superar a dormência de *D. excelsa*.

Termos para indexação: índice de velocidade de emergência, semente dura, vigor.

## INTRODUCTION

The impermeability of seed coat to water, known as physical dormancy, is a common mechanism in Fabaceae seeds (Rolston, 1978), caused by the presence of a cuticle and a layer of palisade cells or both (Copeland and McDonald, 1995). Physical dormancy causes slow and non-uniform germination (Cruz and Carvalho, 2006) and several pretreatments have been proved effective to overcome it. High, fast and uniform seed germination is a pre-requisite for the success of underutilized plant species (Travlos et al., 2007)

Treatments used to overcome physical dormancy include scarification with sulphuric acid, scarification by abrasive surface and soaking in water among others (Schmidt, 2007). The effectiveness of the treatment varies with species, concentration acid and treatment duration. The use of sulphuric acid has been recommended for species such as *Tachigalia multijuga* Benth. scarified for 20min (Borges et al., 2004), *Enterolobium contortisiliquum* (Vell.) Morong. for 5 to 10min (Scalon et al., 2006), *Astragalus hamosus* L. for 60min (Patène and Gresta, 2006) and *Vigna* spp. for 15 min (Wang et al., 2007). Scarification on abrasive surface has been effective

on *Bauhinia variegata* L. (Martinelli-Seneme et al., 2006) and *Hymenaea intermedia* Ducke (Cruz et al., 2001a).

*Dinizia excelsa* Ducke, locally known as “angelimpedra”, is native to the States of Pará, Rondônia, Roraima and Amazonas, in Brazil and Guiana (Ducke, 1949). The species produces a very dense wood, which is commercially prized (Parrotta et al., 1995).

The present study was designed to identify an effective method of breaking seed dormancy and establishing the inflexion point of the seedlings emergence curve in *D. excelsa*. The working hypothesis was that *D. excelsa* seedling emergence is improved when seeds are subjected to treatments to overcome dormancy.

## MATERIAL AND METHODS

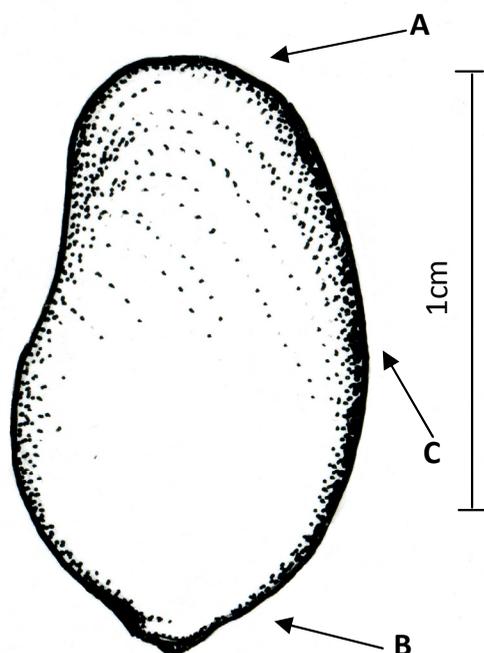
*Dinizia excelsa* seeds were collected from native forest in Belterra (02°38'S; 54°57'W) (pre-test) and Rondon do Pará (04°32'S; 48°12'W) (experiment 1, 2 and 3), State of Pará, Brazil. The experiments were carried out in Belém, Pará, Brazil (1°28'S; 48°27'W) to study *D. excelsa* seedling emergence. Seed moisture was quantified on uncut seeds in two replications of 20 seeds (untreated seed) and four replications of 20 seeds (experiment 1, 2 e

3) in an oven set at  $105\pm 3^{\circ}\text{C}$  for 24hs (Brazil, 1992), prior to the start experiment.

A pre-test to quantify the seedling emergence was carried out on untreated seeds. Emergence was quantified daily for 1,709 days in four replications of 100 seeds. The germination type and seedlings type were also determined according to Duke and Polhill (1981).

**Experiment 1 - Effect of sulphuric acid.** In this trial, the effect of immersing seeds in sulphuric acid (98%) for 10, 20, 30, 40, 50 and 60min on seedling emergence was evaluated. After scarification, seeds were washed for 10 minutes with running tap water to remove any traces of acid.

**Experiment 2 - Effect of scarification on abrasive surface and tegument clipping.** This trial evaluated the effect of manual seed scarification by electric emery (Ferrari ME 5A, 3450 rpm) on the following positions: distal end (Figure 1A), near of the mycophyle (Figure 1B) and on the lateral tissue (Figure 1C). Also, the effect of clipping (using a nail clipper) on seedling emergence was studied in the following positions: clipping at 1mm of the distal end (Figure 1A), 1mm near of the mycophyle region (Figure 1B) and 1mm of the lateral tissue (Figure 1C).



**FIGURE 1.** *Dinizia excelsa* seed. The arrow indicates the spot of manual scarification used in experiment 2.

**Experiment 3 - Effect of scarification on abrasive surface and immersion in water.** This trial investigated the effect of scarification on abrasive surface combined with immersion in water. Seeds were scarified by electric emery close to the mycophyle region (Figure 1B). After scarification and immersing in water for 0, 12, 24 and 48h they were sown. In all experiments the treatments were compared with non-scarified seeds (control treatment).

Seeds were sown in a horizontal position in plastic trays (22 x 13 x 6cm) at a depth of 1.0cm. The substrate used was a mixture of sand and sawdust (1:1), previously heated at  $100^{\circ}\text{C}$  for two hours to reduce microbial contaminants. The trays were irrigated every three days. All emergence tests were conducted in a laboratory under no temperature control and relative humidity (minimum and maximum temperature and relative humidity were  $22^{\circ}\text{C}$  and  $29^{\circ}\text{C}$ , and 74% and 95%, respectively).

The seedling emergence was noted daily. A seedling was considered as emerged when it showed the first pair of definitive leaves. The number of days to the emergence onset, i.e., the number of elapsed days from sowing until emergence of the first seed, emergence speed index (Maguire, 1962), hard seeds, dead seeds and abnormal seedlings were also quantified (Brasil, 1992).

The experimental design was completely randomized with four replications of 50 seeds for each treatment. The data were subjected to homogeneity of variance test (Bartlett test) and data were transformed ( $\arcsin \sqrt{(x+0.5)/100}$ , for percentage data, and  $\log(x+1)$  for other variables) when necessary. The statistical analysis of emergence percentage was carried out by regression analysis. After the last count, ANOVA analyses were carried out for other variables and emergence. Treatment means were compared by Tukey's test ( $P \leq 0.05$ ). All analyses were performed by the software Statistica, 2<sup>nd</sup> Edition (Statsoft, 1999). Transformed values were back-transformed for presentation.

## RESULTS AND DISCUSSION

On the pre-test seed moisture content was 12.1% (fwb) and germination was epigeal and seedlings were phanerocotylar. Seedling emergence was non-uniform and slow, beginning eight days after sowing. The whole emergence process took 1,709 days, when emergence achieved 52.5%. The emergence curve was quadratic, with most increase in emergence between the 8<sup>th</sup> and the 197<sup>th</sup> day (Figure 2).

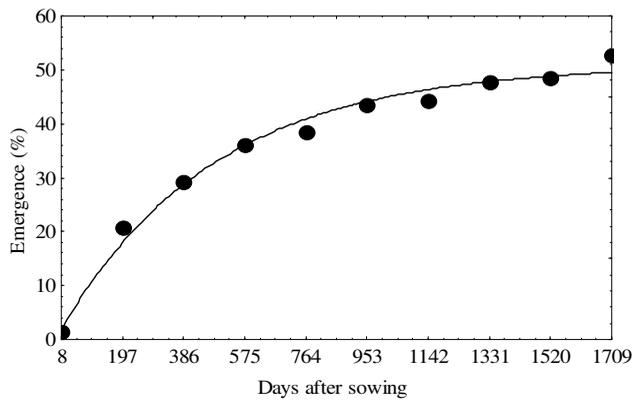


FIGURE 2. Seedling emergence in untreated *Dinizia excelsa* seeds.

**Experiment 1 – Effect of sulphuric acid.** Seed moisture content was 10.4% (fwb). Significant differences ( $P \leq 0.05$ ) between treatments for all variables were observed. Seedlings required six to seven days to begin emergence, except in control in which emergence began 15.2 days after sowing (Table 1). All treatments to overcome dormancy promoted seedling emergence; however, immersion in acid for 20 and 30min were the most efficient, with emergence of 93.6% and 86.6%, respectively. Similar result was reported by Vastano

Júnior et al. (1983). Also, these treatments showed higher emergence speed index and less hard seeds. Seeds scarified for 10min showed higher hard seed percentage (26%) together with control treatment (99%). Regression analyses were significant ( $P \leq 0.05$ ) for all treatments, except for control. Treatments with higher emergence (immersion in acid for 20 and 30min) showed increase on emergence until the 11<sup>th</sup> and the 15<sup>th</sup> day, respectively, whereas for the others treatments the increase was detected during most evaluations (Figure 3). Regression equations and coefficients of determination of the all treatments are show on Table 2.

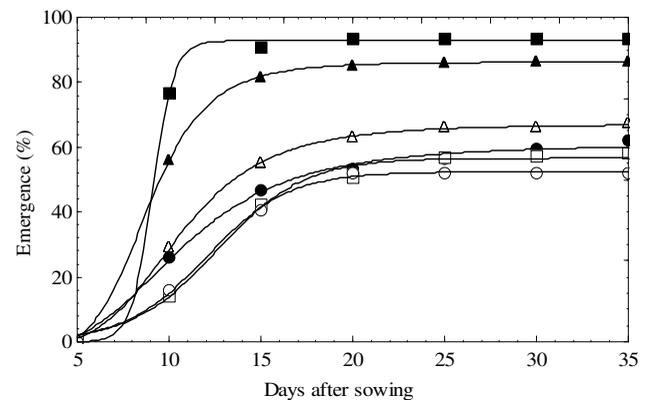


FIGURE 3. Immersion in sulphuric acid for ● 10, ■ 20, ▲ 30, △ 40, □ 50 ○ 60min, in *Dinizia excelsa* seeds.

TABLE 1. Number of days to emergence onset (DEO), emergence (E), emergence speed index (ESI), dead seeds (DS), hard seeds (HS) and abnormal seedlings (AS), in *Dinizia excelsa* seeds scarified with sulfuric acid.

Treatments	DEO	E (%)	ESI	DS (%)	HS (%)	AS(%)
Control	15.2 b	0.8 c	0.0690 d	*	99.0 c	*
Immersion in H <sub>2</sub> SO <sub>4</sub> for 10 min	7.0 a	62.4 b	2.5786 c	4.7 a	26.0 b	6.0 a
Immersion in H <sub>2</sub> SO <sub>4</sub> for 20 min	6.3 a	93.6 a	5.2030 a	2.3 a	1.3 a	2.7 a
Immersion in H <sub>2</sub> SO <sub>4</sub> for 30 min	6.0 a	86.6 a	4.3844 ab	5.9 a	1.5 a	6.0 a
Immersion in H <sub>2</sub> SO <sub>4</sub> for 40 min	6.2 a	67.5 b	2.9630 bc	20.5 b	*	12.0 ab
Immersion in H <sub>2</sub> SO <sub>4</sub> for 50 min	6.0 a	58.1 b	2.4177 c	21.2 b	*	20.5 b
Immersion in H <sub>2</sub> SO <sub>4</sub> for 60 min	6.0 a	55.4 b	2.2050 c	29.5 b	*	14.7 ab

Treatments means sharing the same letter within columns are not significantly different by Tukey test ( $P > 0.05$ ).

\*Values omitted from variance analysis for being zero.

**TABLE 2. Regression equations and coefficients of determination for treatments to overcome dormancy in *Dinizia excelsa* seeds.**

Pre-test: Seedling emergence on untreated seeds			
Treatment	Equation		R <sup>2</sup>
Untreated seeds	$Y = 49.8435(1.0206-\exp(-0.0021x))$		0.99
Experiment 1: Effect of sulphuric acid			
Treatments	Equations		R <sup>2</sup>
Imersion for 10 min	$Y = -1.544(5539.916)+60.893(x^{3.617})/5539.916+x^{3.617}$		0.99
Imersion for 20 min	$Y = 92.781/1+6282117.4(\exp(-1.721x))$		0.99
Imersion for 30 min	$Y = -1.498(235390.7)+86.3(x^{5.648})/235390.7+x^{5.648}$		0.99
Imersion for 40 min	$Y = -1.025(40720.321)+67.118(x^{4.495})/40720.321+x^{4.495}$		0.99
Imersion for 50 min	$Y = 56.651/1+213.871(\exp(-0.424x))$		0.99
Imersion for 60 min	$Y = 52.407/1+218.558(\exp(0.447x))$		0.99
Experiment 2: Effect of manual scarification			
Treatments	Equations		R <sup>2</sup>
Scarification on distal end	$Y = 81.751/1+2920701.6(\exp(-1.247x))$		0.99
Scarification near of the mycrophyle	$Y = 72.507/1+187601.09(\exp(-1.031x))$		0.99
Scarification on the lateral	$Y = 73.401/1+1303267.8(\exp(-1.572x))$		0.99
Clipped distal end	$Y = 14.046/1+5926.792(\exp(-0.692x))$		0.99
Clipped near of the mycrophyle	$Y = 41.751/1+2240593.1(\exp(-1.206x))$		0.99
Clipped on the lateral	$Y = 38.127/1+10287847.3(\exp(-1.155x))$		0.99
Experiment 3: Effect of manual scarification and immersion in water			
Treatments	Equations		R <sup>2</sup>
Immersion in water for 0 h	$Y = 71.327/1+2510.211(\exp(-0.663x))$		0.99
Immersion in water for 12 h	$Y = -1.818(6184.563)+72.055(x^{3.582})/6184.563+x^{3.582}$		0.98
Immersion in water for 24 h	$Y = -2.428(412.156)+72.028(x^{2.244})/412.156+x^{2.244}$		0.97
Immersion in water for 48 h	$Y = -0.744(2148380.5)+38.416(x^{5.644})/2148380.5+x^{5.644}$		0.97

Sulphuric acid has been reported as an effective method to overcome dormancy in hard coat seeds in species such as *Bowdichia virgiloides* Kunth (Smirdele and Sousa, 2003), *Caesalpinia leiostachya* (Benth.) Ducke (Biruel et al., 2007), *Dimorphandra mollis* Benth. (Hermansen et al., 2000), *Mimosa caesalpiniaefolia* Benth. (Bruno et al., 2001), *Schizolobium amazonicum* Huber ex Ducke (Cruz et al., 2007), *Senna siamea* (Lam.) H.S. Irwin & Barneby (Dutra et al., 2007) and *Stylosanthes scabra* Vogel (Araújo et al., 2002). However, exposure time to acid is critical and needs to be quantified for each species, since seeds exposed for a long period may be damaged (Schmidt, 2007). In the present study, seed soaked for 40, 50 and 60min suffered reduction in emergence and increase in the percentage of

dead seeds and abnormal seedlings, when compared with scarified seeds for 20 and 30min. This may be due to an excessive exposition of seeds to acid which damage the embryo. Kondo (1993) reported that *Lotus corniculatus* L. seeds soaked for 20min or more in acid showed reduced germination and increase in abnormal seedlings and dead seeds percentage.

**Experiment 2 – Effect of scarification by abrasive surface and tegument clipping.** Significant differences ( $P \leq 0.05$ ) between treatments for all variables were observed (Table 3). Seed scarified by abrasive surface and tegument clip required 8.2 to 9 days to begin emergence, while seeds from the control treatment required 26.3 days. Scarification by abrasive surface was most effective,

with emergence ranging from 74.3% to 82.7%. Also this treatment showed higher speed index and lower dead seeds percentage. For clipped seeds, emergence was affected by the clippings position, ranging from 14.2% (clippings on distal end) to 42% (clipping near the mycophyle). In the tegument clipping treatments there was a high percentage of abnormal seedlings, ranging from 12% (clip near

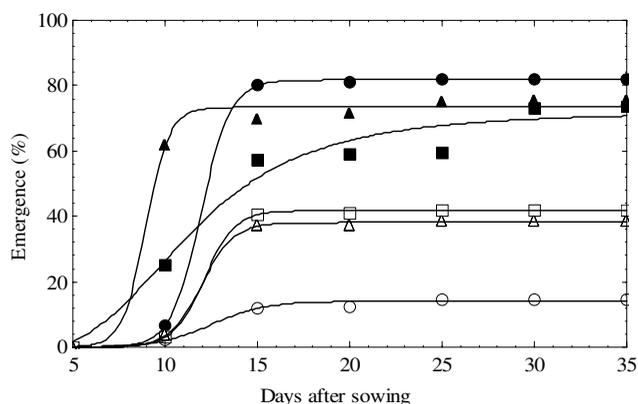
mycophyle) to 54% (clipping on distal end). This was mainly due to permanence adhered tegument on cotyledons hindering seedling development. Regression analyses were significant ( $P \leq 0.05$ ) for all treatments, except for control. Emergence increase until the 15<sup>th</sup> day, the exception was for scarification on the lateral in which the increase was observed only until the 10<sup>th</sup> day (Figure 4).

**TABLE 3. Number of days to emergence onset (DEO), emergence (E), emergence speed index (ESI), dead seeds (DS), hard seeds (HD) and abnormal seedlings (AS), in *Dinizia excelsa* seeds scarified and clipped.**

Treatments	DEO	E (%)	ESI	DS (%)	HS (%)	AS (%)
Control	26.3 b	2.5 d	0.0640 d	*	96.2 b	*
Scarification distal end	8.7 a	82.7 a	3.5859 a	10.7 ab	2.2 a	4.0 a
Scarification near the mycophyle	8.2 a	74.3 a	3.1573 a	8.5 a	9.3 a	7.5 a
Scarification on lateral	8.2 a	75.7 a	3.1854 a	13.5 ab	5.3 a	5.5 a
Clip of the distal end	9.0 a	14.2 c	0.5665 c	31.5 bc	*	54.0 b
Clip near the mycophyle	8.7 a	42.0 b	1.7289 b	46.0 c	*	12.0 a
Clip of the lateral	8.2 a	38.1 b	1.5990 b	42.0 c	*	19.5 a

Treatments means sharing same letter within columns are not significantly different by Tukey test ( $P > 0.05$ ).

\* Values omitted from variance analysis for being zero.



**FIGURE 4. ● Scarification on distal end, ■ near of mycophyle, ▲ on the lateral, and ○ clipped distal end, △ near of mycophyle and □ clipped on the lateral, in *Dinizia excelsa* seeds.**

Seedlings emergence on seeds scarified on abrasive surface was higher than the one reported by Vastano Júnior et al. (1983) for the same species. This was probably due to genetic variation among plants (Borges et al., 2004; Koornneef et al., 2002). This scarification method has been recommended to overcome the physical dormancy

in species such as *H. intermedia* (Cruz et al., 2001a), *S. amazonicum* (Cruz and Carvalho, 2006) and *Trifolium riograndense* Burkart (Suñé and Franke, 2006), however on applying this method it is necessary to avoid damage to the embryo (Perez, 2004). The lower emergence of clipped seeds compared to scarified seeds by abrasive surface, might be related to a more intense scarification of the clipped seeds. This scarification method may cause damage to cotyledons favoring the development of fungi, increasing the number of dead seeds and abnormal seedlings. Cruz et al. (2001b) reported increase on dead seeds and abnormal seedlings in *Parkia nitida* Miquel, when seeds were scarified by abrasive surface and not treated with fungicide.

**Experiment 3 – Effect of manual scarification on abrasive surface and immersion in water.** Significant differences ( $P \leq 0.05$ ) between treatments for all variables were observed (Table 4). Emergence on scarified seeds started between the 6<sup>th</sup> and the 7.5<sup>th</sup> day after sowing, while on the control treatment emergence began after the 15.2 days. All treatments to overcome dormancy increase seedling emergence that ranged from 40.4% (immersion after 48h) to 75% (sowed immediately); while on control treatment it was 2.4%. However, immersing seeds in water after manual scarification did not increase emergence, and when immersion period was 48h was actually reduced. Increase

in seedling emergence was probably caused by manual scarification, since seeds sowed immediately after manual scarification showed similar emergence to the ones that received manual scarification and sowed after 12 and 24h and higher emergence when immersion lasted 48h. Similar performance was observed for emergence speed index. The increasing exposition to water after manual scarification increase seed death, mainly when seeds were soaked for 48h (54.5%). Abnormal seedling percentage was low, ranging

from 4% to 8.5%. Regression analyses were significant ( $P \leq 0.05$ ) for all treatments, except for control. Emergence increase was observed until the 18<sup>th</sup> day, for seeds sowed immediately. For the other treatments (except control), in general, increase on emergence was observed during most evaluations (Figure 5). Cruz et al. (2006) also reported the ineffectiveness of scarification on abrasive surface, followed soaking seeds in water to promote tropical tree seedling emergence.

**TABLE 4. Number of days to emergence onset (DEO), emergence (E), emergence speed index (ESI), dead seeds (DS), hard seeds (HD) and abnormal seedlings (AS), in *Dinizia excelsa* seeds submitted to manual scarification and immersed in water.**

Treatments	DEO	E (%)	ESI	DS (%)	HD (%)	AS (%)
Control	15.2 b	2.4 c	0.1149 c	*	97.5	*
Manual scarification + IS	7.5 a	75.0 a	3.4918 a	17.0 ab	*	8.5 a
Manual scarification + I12h	6.2 a	73.6 a	3.2350 a	22.5 b	*	4.0 a
Manual scarification + I24h	6.0 a	65.6 a	2.8582 a	30.5 b	*	4.0 a
Manual scarification + I48h	6.2 a	40.4 b	1.5617 b	54.5 c	*	5.0 a

Treatments means sharing same letter within columns are not significantly different Tukey test ( $P > 0.05$ ).

IS (mechanical scarification and immediate sowing); I12h, I24h and I48h (mechanical scarification and immersion in water for 12h, 24h and 48h, respectively).

\* Values omitted from variance analysis for being zero.

## CONCLUSIONS

The immersion of seeds in sulphuric acid for 20 and 30min and scarification on abrasive surface of the distal end are effective to overcome dormancy in *D. excelsa*.

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