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Effects of pre-germination treatments on *Copaifera langsdorffii* seeds

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Copaifera langsdorffii has been intensely exploited, since it attracts several interests, and is now on the list of endangered species. Due to this risk of extinction, seedling production of this species has important economic and social value. The aim of this study was to evaluate seed germination of _C. langsdorffii_ subjected to different pre-germination treatments for breaking seeddormancy: i) control; ii) immersion in sulfuric acid (98%) for 2 min; iii) immersion in sulfuric acid for 10 min; iv) removal of the seed coat; v) immersion in distilled water for 24 h; and vi) immersion in boiling water at 80°C for 2 minutes. In the period of 34 days after setting up the experiment, there was 70% germination of the seeds subjected to the method of immersion in sulfuric acid (98%) for 10 minutes, 63% of seeds in which the seed coats were removed, and below 27% in the rest. In this context, it may be concluded that the method of immersion in sulfuric acid for 10 min proved to be more effective and advantageous for overcoming dormancy.

Key words: Arboreal species, Copaiba, germination, dormancy.

INTRODUCTION

*Copaifera langsdorffii (*Copaíba) is a species of the *Fabaceae* family, which reaches from 5 to 15 m height and 20 to 60 cm diameter at breast height (Lorenzi, 1992). This species attracts great commercial interest due to the potential production of wood and of resin oil, with a bitter flavor and fragrance and brown coloring, which is extracted from the trunk and may be used as a fuel for diesel engines and also in home remedies as an antiseptic, healing ointment, expectorant, diuretic, laxative, stimulant, emollient and tonic (Veiga Júnior and

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Pinto, 2002). This species may also be used for urban forestry, as well as reforestation for environmental recovery, and is thus highly valued internationally.

Copaiba occurs naturally throughout Brazil and is found from the South to the Northeast. It is also observed in other South American countries, such as Argentina, Bolivia and Paraguay. Since it attracts such diverse interests, Copaiba has been intensely exploited and is on the endangered species list (Carvalho, 2003). Due to this risk of extinction, seedling production of this species has important economic and social value (Toledo and Parente, 1988).

Seed dormancy is a physiological and/or physical state for survival present in forest species that allows longer duration of seed quality and germination and emergence potential in environments unfavorable to the growth and establishment of the seedlings. Although dormancy is advantageous for survival of species in natural conditions, it is not a desirable characteristic for seedling production in a nursery (Fowler and Bianchetti, 2000). Copaiba seeds have dark coloring and an ellipsoid shape, partially covered by a yellowish aril, and they have seed coat dormancy caused by substances present in the seed and also in other structures (Crestana and Beltrati, 1988; Almeida, 1998; Veiga Junior and Pinto, 2002). In the face of this barrier, there is the need for seeking effective pre-germination treatments for breaking seed dormancy. Among the treatments successfully used for breaking seed coat dormancy of forest species, manual and chemical scarification stand out (Fowler and Bianchetti, 2000).

The aim of this study was to evaluate the efficiency of pre-germination treatments for breaking dormancy in Copaiba seeds.

MATERIALS AND METHODS

The experiment was performed in the Seed Analysis Laboratory of the Faculdade de Rondônia/FARO in the months of July and August 2012. To carry out this study, seeds collected in July 2012 were used in a natural state of dehiscence. Seeds were collected from a population of 3 seed trees. The seed trees exhibited stem quality 1 and 2 (1 = straight; 2 = branched) as characteristics. Soil in the region of collection was characterized as Hapludox, which is predominant in the state of Rondonia and equatorial climate.

The collected seeds exhibited medium size, shape and homogeneous morphological characteristics. The experiment was composed of six pre-germination treatments: T1) control (without treatment); T2) sulfuric acid (98%) for 2 min and subsequent washing with distilled water three times; T3) sulfuric acid (98%) for 10 min and subsequent washing with distilled water three times; T4) removal of the seed coat; T5) distilled water at rest for 24 h and subsequent washing with distilled water three times; and T6) hot water at 80°C for 2 min and subsequent washing with distilled water once.

To set up the experiments, the seeds were disinfected with a sodium hypochlorite solution at 2.5% for 10 min and washed with distilled water to remove excess hypochlorite. The substrate used for seeding was germitest paper, which were moistened with distilled water (2.5 times the weight of dry paper) and stored under the form of rolls of paper in a seed germinator at a constant temperature of 25°C (Brasil, 2009). Each treatment was placed in a plastic bag for the purpose of maintaining substrate moisture. Twenty seeds were used in each treatment, with four replications, for a total of 80 seeds per treatment.

Evaluations were performed considering the following germination parameters: 1) first germination count (GC), corresponding to the percentage of seeds germinated in the 15th day after setting up the experiment; 2) total germination percentage (G), corresponding to the total percentage of seeds germinated up to the 34th day after setting up the experiment; 3) germination speed index (GSI), determined according to the formula presented by Maguire (1962), $GSI = G_1/N_1 + G_2/N_2 + ... + G_n/N_n$, where: G_1, G_2, and G_n are the numbers of germinated seeds on the first, second, and last count; and N_1, N_2, and N_n are the numbers of days between the count and n are the numbers of days between the count and n the beginning of the experiment; 4) primary root length (RL), corresponding to theroot length, in centimeters, on the last day of the experiment.

A completely randomized experimental design was used, with the treatments distributed in four replications of 20 seeds each. Data normality was analyzed by means of the Lilliefors Test. Percent values were transformed by arcsine-square root prior to analysis. Analysis of variance (ANOVA) was applied to test the effect of treatments. A Tukey test was applied to compare the above effects between homogeneous groups. Tests of significance were made at a $p \leq 0.05$ confidence level. Analyses were processed by using the GENES statistical software (Cruz, 2006).

RESULTS

Significant differences were detected among the treatments for all the parameters evaluated, which may be seen through the results of the analyses of variance, in which the mean squares of the treatments were significant ($P<0.01$). As of this result, mean value tests were performed to determine these differences among the treatments (Table 1).

For the parameters of first germination count (GC), total germination (G) and germination speed index (GSI), it was seen that the seeds subjected to the pre-germination treatments of immersion in sulfuric acid for 10 min (T3) and those in which the seed coats were removed (T4) did not differ statistically from each other. Thus, it was verified that the two pre-germination treatments were efficient in breaking dormancy in Copaiba seeds.

Seeds treated with sulfuric acid for 10 min showed higher root length, reaching six centimeters length on average, while there was no significant difference among the other treatments.

As for treatment 4, in which the seed coat was removed, the result was not the same, possibly due to the presence of pathogens, which reduced their vigor. Damages on the cotyledon during seed coat removal can be the most probable cause. Loss of nutrients during imbibitions might have caused low vigor. In relation to seed handling, care wasgiven to cleaning the material used for removing the seed coat for each seed.

It is interesting to observe that both the control (T1) and treatment 2 (H_2SO_4 2’), did not lead to germination at 15

Table 1. Mean values of first germination count (GC), total germination (G), germination speed index (GSI) and primary root length (RL) of Copaiba.

Treatment	GC (15days) (%)	G (34 days) (%)	GSI Number germination/week	RL (35 days) (cm)
ANOVA	**	**	**	**
T1 (control)	0.0[b]	21.2[b]	1.0[b]	10.8[b]
T2 (H_2SO_4 2')	0.0[b]	1.3[b]	0.1[b]	0.3[b]
T3 (H_2SO_4 10')	50.0[a]	70.0[a]	6.1[a]	60.8[a]
T4 (without seedcoat)	61.2[a]	63.8[a]	6.4[a]	16.3[b]
T5 (H_2O 24h)	1.3[b]	27.5[b]	1.4[b]	7.8[b]
T6 (H_2O 80ºC 2')	5.0[b]	26.3[b]	2.0[b]	16.8[b]

When treatment factor is significant (ANOVA - * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns = $p > 0.05$ not significant), different letters show significant differences among means (Tukey HDS test, $p \leq 0.05$, N=4).

days after setting up the experiment. In relation to the control, this was already expected because no pre-germination treatment was applied. Nevertheless, for the treatment in which seeds were subjected to immersion in sulfuric acid for a period of two minutes, it was observed that most of the seeds died, for they exhibited a whitish coloring and softening. This probably occurred due to some type of activation of substance(s) that impede germination. However, reports were not found in the literature in regards to this occurrence.

DISCUSSION

The results obtained in this study agree with those presented by Bezerra et al. (2002), who, evaluating diverse pre-germination treatments, found that chemical scarification with sulfuric acid in Copaiba seeds speeded and increased the percentage of germination. This fact may also be verified by means of the results of the germination speed indices (GSI) in which, for seeds subjected to chemical scarification for 10 minutes, Durigan et al. (1997) and Almeida et al. (1998) attribute slow and uneven germination of this species, extending up to 70 days, to an occasional dormancy. Thus, it is fitting to mention that in this study, the treatments with sulfuric acid for ten minutes (T3) and the treatment with removal of the seed coats (T4) led to a high germination index (70 and 63.8%, respectively), in a time period of 34 days, confirming that seed coat dormancy was overcome by these treatments (Table 1).

It is also important to highlight that the control did not differ from T5 treatment (seeds immersed in water for 24 h) and T6 treatment (seeds immersed in boiling water for 2 min), implying that these treatments were not sufficient to promote and accelerate germination of Copaiba seeds. These results are in contrast to those presented by Prado and Perez (1993), in which the authors found good germination in these treatments for the same species. Borges et al. (1982), for their part, tested some methods for overcoming dormancy in copaiba seeds and, among the most efficient, immersion in water at rest for 24 h at ambient temperature stands out, also in opposition to the results found in this study.

Although the treatment of seeds with mechanical scarification by removal of the seed coat (T4) showed a visual result inferior to chemical scarification with sulfuric acid for 10 min (T3), it showed a greater mean value for primary root length. Machado et al. (1992) state that scarified Copaiba seeds exhibit high germination power, in agreement with this study.

The control (T1) exhibited the first germinated seed on the 18th day after setting up the experiment and, on the last day of the experiment, had only 21.2% of the seeds germinated. Carvalho (1994) cites that Copaiba seeds not treated to overcome dormancy germinate from 12 to 59%, in agreement with the results of study.

On the 19th day, most of the seeds in which the seed coat was removed (T4) had already germinated, exhibiting lengthening of the primary root. In treatment 3, the peak of germination occurred on the 25th day after setting up the experiment. At the end of the experiment (34th day after setting up the experiment), some seedlings already had primary foliage in the treatments in which the seeds were immersed in sulfuric acid for ten minutes (T3) and in the treatment in which the seed coats had been removed (T4) and, in greater number, in the treatment with sulfuric acid for ten minutes (T3).

The present study is in disagreement with the results found by Cruz et al. (2005) in that which refers to the best treatment for overcoming seed coat dormancy in Copaiba, in which mechanical scarification exhibited the best result for overcoming dormancy, exceeding chemical scarification with sulfuric acid. In this study, it was observed that T3 (immersion in H_2SO_4 for 10 min) obtained better results than mechanical scarification through removal of seed coats (T4).

From the results obtained in this study for Copaiba seeds, the pre-germination methods of mechanical scarification (seed coat removal) and chemical scarification (sulfuric acid for 10 min) increased the permeability of the seed coat, allowing water absorption and acceleration of the germination process. However, the seed coat removal method is difficult to perform and makes for more susceptible to contamination by pathogens.

Conclusion

For Copaiba seeds, the pre-germination method of chemical scarification (sulfuric acid for 10 min) most efficiently overcomes seed coat dormancy, accelerating germination speed and providing greater uniformity in seedling development.

Conflict of Interests

The authors have not declared any conflict of interests.

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