Germination in seeds of *Abrus precatorius* **L., a species with antileishmanial activity1**

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ABSTRACT - *Abrus precatorius* L. has been the subject of research to develop drugs with improved therapeutic profiles for

the treatment of infectious diseases such as visceral leishmaniasis, which has very high mortality. Commercial cultivation

is necessary to meet the demands of the pharmaceutical industry; however, cultivation can be hampered by the presence of

integumentary dormancy in the seeds. The aim of this study was to evaluate the effects of different pre-germination treatments

and different temperatures on germination in seeds of *A. precatorius*. The experiment was completely randomised in a 4 x 3 factorial

scheme corresponding to four methods for breaking dormancy (intact seeds - T1; immersion in sulphuric acid for 30 minutes - T2;

immersion in sulphuric acid for 45 minutes - T3; immersion in 20% caustic soda for 60 minutes - T4) and three temperatures

during the germination process (25 °C; 30 °C; 35 °C). At 25 °C and 30 °C, chemical scarification with caustic soda results in a

lower rate of germination compared to scarification with sulphuric acid, while at 35 °C, germination was higher. The seeds of *A. precatorius*

show higher germination at 30 °C when previously immersed in sulphuric acid for 30 or 45 minutes. This information is

important for viable commercial cultivation.

Key words: Integumentary dormancy. Pre-germination treatments. Temperature. Plants with antileishmanial activity.

DOI: 10.5935/1806-6690.20250045

Editor-in-Chief: Profa. Charline Zaratin Alves - charline.alves@ufms.br

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Received for publication 09/10/2023; approved on 18/10/2024

¹ Article from research work developed in the Agronomy course at the Federal Rural University of Pernambuco, Academic Unit of Serra Talhada (UFRPE-UAST), Serra Talhada - Pernambuco, Brazil

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INTRODUCTION

Abrus precatorius L. is a shrub belonging to family Fabaceae. It occurs in the tropical and subtropical regions of many countries (Garaniya; Bapodra, 2014). In Brazil, the species can be widely found in the Amazon, Caatinga, Cerrado, Atlantic Forest, Pampa, and Pantanal (Queiroz, 2020).

Its roots contain isoflavanquinones, which have anti-inflammatory and anti-allergic properties (Kuo *et al*., 1995), and phenolic compounds, which are chemical constituents widely used in the pharmaceutical industry for their antioxidant and anticarcinogenic properties (Muddathir *et al*., 2017). The hot water extract from the fresh roots is antimalarial and anticonvulsant, while the seeds have an insecticidal and antimicrobial effect (Attal *et al*., 2010).

A. precatorius has been used in studies to develop drugs with improved therapeutic profiles for the treatment of infectious diseases (Sheikh; Hedge, 2017). In many developing and less-developed countries, visceral leishmaniasis is a significant cause of death. Available drugs result in a high level of toxicity when used at effective therapeutic doses, which has prompted the search for safer alternatives. Okoro et al (2020) reported the anti-leishmanial activity of isoflavanquinones from genus Abrus against cutaneous leishmaniasis, validating the use of *A. precatorius* in the case of dermatological disease.

The species, *A. precatorius*, propagates by seed, which, according to Nautiyal *et al*. (2014), present integumentary dormancy. To germinate, it is necessary to break the dormancy in order to resume the metabolic processes (Hudson; Ayre; Ooi, 2015). Various tree species in hot and arid areas present physical dormancy, meaning their seeds are impermeable to water (Araújo; Silva; Ferraz, 2017; Cartes-Rodríguez *et al*., 2022; Jaganathan *et al*., 2016; Menegatti *et al*., 2017; Missio *et al*., 2011; Pinheiro *et al*., 2021).

By submitting seeds of *A. precatorius* to physical and chemical pre-germination treatments, Cortines *et al*. (2010) found that the most efficient methods for breaking dormancy are mechanical scarification and immersion in sulphuric acid (H_2SO_4) for 30 min, with germination of 88% and 52%, respectively. After immersing seeds of *A. precatorius* in H_2SO_4 for 0, 90, 105, 120, 135 and 150 minutes, Nautiyal *et al*. (2014) evaluated germination 5, 10, 15 and 20 days after sowing, and found that with no immersion (control), there was no germination throughout the 20 days of the experiment. Seeds immersed in H_2SO_4 for 135 min showed 90% germination by the fifth day.

After breaking dormancy, the seeds must be placed under favourable conditions so that the germination

process can begin. Temperature is one of the most important environmental factors to start the seed germination process and to promote the establishment and development of normal and vigorous seedlings (Wang *et al*., 2016; Yue *et al*., 2019). There is therefore an optimal temperature for obtaining a high number of germinated seeds in the shortest time (Marchelli *et al*., 2019). An increase in ambient temperature results in a linear increase in seed germination, which, due to protein denaturation and lipid oxidation (Marcos Filho, 2015), tends to decrease as the temperature approaches the maximum (Bradford, 2002). The highest rate of seed germination is reached at an optimal temperature; if germination stops due to excess temperature, this means that the maximum germination temperature has been reached (Alvarado; Bradford, 2002).

Given the potential of this species for exploitation, it is important to have information on the thermal requirements of the germination process. The aim of this study, therefore, is to evaluate the effects of temperature combined with different methods for breaking dormancy in seeds of *A. precatorius*.

MATERIALS AND METHODS

Experimental site

The study was conducted in the district of Serra Talhada, Pernambuco, Brazil. The *A. precatorius* seeds used in the experiment were collected during 2014 in the urban area of the district of Triunfo, Pernambuco (7°50'26'' S, 38°6'1'' W).

Statistical design

The design was completely randomised in a 4 x 3 factorial scheme, corresponding to four methods for breaking dormancy (intact seeds - T1; immersion in sulphuric acid for 30 minutes - T2; immersion in sulphuric acid for 45 minutes - T3; immersion in 20% caustic soda for 60 minutes - T4) and three temperatures for the germination test (25 °C; 30 °C; 35 °C), in four replications, comprising glass Petri dishes with 20 seeds per plot for each treatment, giving a total of 80 seeds per treatment.

Application of the treatments

To break integumentary dormancy, the seeds were subjected to the following treatments: immersion in concentrated sulphuric acid (H_2SO_4) for 30 minutes (T2) (Cortines *et al*., 2010), immersion in concentrated sulphuric acid for 45 minutes (T3), and immersion in 20% caustic soda (NaOH) for 60 minutes (T4); seeds without a method for breaking dormancy were used as the control (intact seeds - T1).

To eliminate residue from the chemical reagents following scarification, the seeds treated with sulphuric acid were washed in running water for ten minutes, while seeds treated with caustic soda were washed for five minutes.

Twenty seeds per replication were then sown on two sheets of blotting paper arranged in glass Petri dishes; the sheets had been previously moistened with distilled water at 2.5 times the dry weight of the paper.

Variables under analysis

To evaluate the effects of the treatments, we calculated the germination percentage (GP) $(\%)$. A seed was considered as having germinated after emitting a primary root of at least 2 mm. The germination speed index (GSI) was determined by counting the number of germinated seeds and dividing this by the number of days between sowing and germination, as per Maguire (1962). The mean germination time (MGT) was evaluated together with the germination test and determined using the formula proposed by Labouriau (1983). At the end of the germination test, we determined the percentage of hard (PHS) and dead (PDS) seeds. The seeds were punctured using tweezers: those that resisted were considered hard, those that crumbled were considered dead.

Statistical analysis

The data were submitted to analysis of variance (ANOVA). Whenever the results of the F-test were significant (at 1% and 5% probability), the mean values were compared by Tukey's test at 5% probability. The SISVAR software (Ferreira, 2011) was used in the analysis.

RESULTS AND DISCUSSION

There was a significant effect ($p < 0.05$) for germination percentage (GP) and germination speed

index (GSI) when the seeds were subjected to the different methods for breaking dormancy and different temperatures during germination. This shows that both factors together affect the physiological quality of seeds of *A. precatorius* (Table 1). The interaction was not significant for mean germination time (MGT), which was influenced by the factors alone.

There was no difference in germination percentage (GP) (Table 2) between the different temperatures when evaluating each dormancy-breaking method, where intact seeds (T1) of *A. precatorius* had average values ranging from 20% to 26%. Scarification with sulphuric acid for 30 minutes (T2) together with a temperature of 30 °C resulted in higher germination, followed by 25 °C. The lowest germination occurred at 35 °C, with a 37% reduction in relation to the germination percentage at 30 °C. The same was seen with the seeds scarified in sulphuric acid for 45 minutes (T3), with higher germination at 30 °C and lower germination at 35 °C. The seeds immersed in caustic soda for 60 min (T4) showed higher germination at 35 °C and lower germination at 25 °C, which differed from the other treatments.

Higher temperatures can increase the fluidity of lipids and reduce the stability of cell membranes, leading to ion loss and even membrane rupture (Taiz; Zeiger, 2017). The optimal temperature for many species that multiply by seed varies from 20 °C to 30 °C. In the present study, germination was higher at 30 °C, behaviour that may be related to the habitat (Marcos Filho, 2015). *A. precatorius* is native to the Indian subcontinent and the East and West Indies, both characterised by higher temperatures (Chadha, 1988). The germination temperature of 35 °C was therefore beneficial to the species, which is naturally adapted to warm climates.

Table 1 - Analysis of variance of germination percentage (GP), germination speed index (GSI), mean germination time (MGT), percentage hard seeds (PHS) and percentage dead seeds (PDS) in seeds of *Abrus precatorius* as a function of the germination temperature (T) and methods for breaking dormancy (MBD)

** Significant at 1%; * significant at 5%; ^{ns} not significant; coefficient of variation (CV)

		GP(%)					
Temperature (°C)	Methods for Overcoming Dormancy						
	T1	T2	T3	T4			
25	20 Ab	51 Ba	44 Ba	26 Cb			
30	20 Ac	60 Aa	54 Aa	36 Bb			
35	26 Ac	23 Cc	31 Cb	60 Aa			
GSI							
25	0.35 Bb	1.07 Ba	1.15 Ba	0.55 Bb			
30	$0.41\,\text{Be}$	1.86 Aa	1.87 Aa	0.79 Bb			
35	0.72 Ab	0.72 Cb	0.95Bb	1.59 Aa			

Table 2 - Germination percentage (GP) and germination speed index (GSI) in seeds of *Abrus precatorius* subjected to different temperatures (25 °C; 30 °C; 35 °C) and different methods for breaking dormancy (intact seeds - T1; immersion in sulphuric acid for 30 minutes - T2; immersion in sulphuric acid for 45 minutes - T3; immersion in 20% caustic soda for 60 minutes - T4)

Mean values followed by the same uppercase letter in a column and lowercase letter on a line do not differ significantly by Tukey's test at 5% probability

By evaluating the effects of different temperatures, storage periods, and pre-germination treatments on dormancy breaking in seeds of *Mimosa scabrella* Benth. (Fabaceae), Menegatti *et al*. (2017) found a higher germination percentage at 30 °C with a pre-germination treatment of three hours immersion in water at 80 °C, regardless of storage. Similarly, Ramos *et al*. (2018) found that seed germination in *Enterolobium contortisiliquum* (Vell.) Morong (Fabaceae) was highest at 30 °C. These findings are in line with our observations that the use of sulphuric acid for 30 or 45 minutes resulted in the highest germination at 25 °C, while the seeds immersed in 20% caustic soda solution for 60 minutes and the intact seeds presented the lowest germination values. The same behaviour was seen at 30 °C, meaning that optimal germination temperatures and effective dormancy-breaking treatments are crucial for enhancing seed germination across different species of family Fabaceae. This method allows seeds to exchange water or gas with the environment, triggering the germination process more quickly (Oliveira, 2012).

Immersion in caustic soda was inefficient at breaking dormancy in seeds of *A. precatorius* at 25 °C and 30 °C. However, when left to germinate at 35 °C, the seeds treated with caustic soda achieved higher germination compared to the other treatments. This may have been because the immersion time was insufficient to cause integument rupture or because the concentration was low (20%), since the percentage of hard seeds was high. However, when the germination temperature was 35 °C, it was found that seeds previously immersed in 20% caustic soda for 60 min, in addition to presenting higher germination than the seeds of the other treatments, germinated more quickly. The high temperature may have increased the abrasive effect

of immersion in caustic soda, as heat is also considered one way of breaking dormancy.

Furthermore, since the integument was no longer whole after immersion, the high temperature, which favoured a higher rate of imbibition, digestion and mobilisation of reserves (Marcos Filho, 2015), resulted in an accelerated germination process. Studies on the effect of temperature on dormancy are ecologically relevant, as they aim to get closer to natural conditions and encourage a better understanding of the effects of global warming (Xia *et al*., 2018). Sodium hydroxide, also known as caustic soda, which is considered a strong base, has proved effective at breaking seed dormancy. For the species *Libidibia ferrea* (Mart. ex Tul.) L. P. Queiroz (Fabaceae), immersion in 20% caustic soda for 10, 15 and 20 minutes afforded an emergence of 75%, 66% and 80%, respectively, while seeds that had undergone no treatment showed 52% germination (Araújo; Silva; Ferraz, 2017).

Evaluating the germination speed index (GSI) (Table 2) of intact seeds (T1) of *A. precatorius*, a temperature of 35 °C afforded a higher GSI. For seeds immersed in sulphuric acid for 30 or 45 minutes (T2 and T3, respectively), a temperature of 30 °C gave the highest values, while the lowest values were obtained at 35 °C. Seeds immersed in 20% caustic soda solution for 60 min. (T4) had a higher GSI when left to germinate at 35 °C . The lowest values for germination and the germination speed index occurred when the seeds were exposed to a lower temperature, i.e. 25 °C. A gradual decrease in temperature, due to the effects on the rate of soaking, digestion and mobilisation of reserves, causes a marked reduction in the rate of germination. This makes the seeds more susceptible to adverse environmental

factors, particularly to harmful microorganisms during seedling establishment.

In terms of the methods for breaking dormancy, the treatments that included sulphuric acid (T2 and T3) had the highest GSI values at temperatures of 25 °C and 30 °C. At 35 °C, seeds immersed in 20% caustic soda for 60 min (T4) germinated in a greater number per day, resulting in a higher GSI. Nautiyal *et al*. (2014) found that seeds of *A. precatorius* immersed for 135 min in H_2SO_4 presented 90% germination by the fifth day after sowing, while with no immersion (control), the seeds did not germinate during the 20 days of the experiment.

Although the interaction between the germination temperature and the methods for breaking dormancy was not significant for the mean germination time (MGT), the germination process was faster at 35 °C, requiring an average of 1.78 days to germinate when each factor was evaluated individually (Table 3), whereas at the other two temperatures there was no statistical difference. In terms of the methods for breaking dormancy, the intact seeds (T1) and those immersed in 20% caustic soda solution for 60 min (T4) needed more time to germinate, while seeds treated with sulphuric acid had a shorter MGT.

With regard to the percentage of hard seeds (PHS) at each temperature, the intact seeds (T1) achieved a higher PHS at a temperature of 25 °C, while immersion in sulphuric acid for different periods (T2 and T3) resulted in a lower PHS. Similar behaviour was seen when using a temperature of 30 °C. When the germination test was conducted at a temperature of 35 °C, the use of caustic soda (T4) resulted in a lower number of hard seeds, while the highest values were seen in the intact seeds (T1).

For the intact seeds (T1), the percentage of hard seeds was higher when the germination test was carried out at 35 °C, while at 25 °C and 30 °C, the values were lower. The same behaviour was seen for seeds immersed in sulphuric acid for 30 or 45 min (T2 and T3), which had a lower PDS when the seeds were left to germinate at 25 °C and 30 °C compared to 35 °C. By conducting the germination test at 35 °C, the percentage of hard seeds in the treatment that included immersion in caustic soda (T4) was lower compared to the other temperatures.

Efficiency in breaking dormancy can be assessed by the percentage of hard seeds, i.e. those unable to absorb water due to the impermeability of the seed coat. An effective dormancy-breaking treatment reduces the percentage of hard seeds, allowing more seeds to absorb water and germinate. By measuring the percentage of hard seeds before and after each treatment, it is possible to evaluate its effectiveness in breaking dormancy. A significant reduction in the percentage of hard seeds following a treatment means that the method was effective in making the seeds permeable to water, facilitating germination.

In terms of percentage dead seeds (PDS), a temperature of 35 °C resulted in the highest number of dead seeds for each method of breaking dormancy (Table 4).

When evaluating each temperature (Table 4), seeds immersed in acid for 45 min at 25 $^{\circ}$ C (T3) showed the highest percentage of dead seeds (PDS), as did the seeds immersed in 20% caustic soda solution for 60 min (T4). At 30°C, T3 also showed a higher PDS, with no difference from the intact seeds (T1). When the germination process was carried out at 35 °C, the PDS was higher in treatments that included sulphuric acid (T2 and T3). High temperatures alter membrane permeability through protein denaturation and lipid oxidation (Bewley; Black, 1994), culminating in the death of the seed.

Table 3 - Mean germination time (MGT) in seeds of *Abrus precatorius* subjected to different temperatures (25 °C; 30 °C; 35 °C) and different methods for breaking dormancy (intact seeds - T1; immersion in sulphuric acid for 30 minutes - T2; immersion in sulphuric acid for 45 minutes - T3; immersion in 20% caustic soda for 60 minutes - T4)

Mean values followed by the same letter do not differ Tukey's test at 5% probability

		PHS $(\%)$					
Temperature $(^{\circ}C)$	Methods for Overcoming Dormancy						
	T1	T2	T ₃	T4			
25	65 Aa	36Ac	31Ac	56 Ab			
30	61 Aa	25 Bc	21 Bc	48 Bb			
35	44 Ba	34 Ab	24 Abc	14 Cd			
PDS $(\%)$							
25	15 Bb	13Bb	25 Ba	17 Bab			
30	19 Bab	15Bb	25 Ba	17Bb			
35	30 Ab	44 Aa	45 Aa	26Ab			

Table 4 - Percentage hard seeds (PHS) and percentage dead seeds (PDS) in seeds of *Abrus precatorius* subjected to different temperatures (25 °C; 30 °C; 35 °C) and different methods for breaking dormancy (intact seeds - T1; immersion in sulphuric acid for 30 minutes - T2; immersion in sulphuric acid for 45 minutes - T3; immersion in 20% caustic soda for 60 minutes - T4)

Mean values followed by the same uppercase letter in a column and lowercase letter on a line do not differ significantly by Tukey's test at 5% probability

Immersion in sulphuric acid for 45 min at a temperature of 35 °C resulted in the highest number of dead seeds, albeit with no difference to T2, which presented the same behaviour. It should be noted that the efficiency of chemical scarification is related to the time of exposure: if this exceeds the ideal time, it can cause damage to the embryonic cells and result in a high percentage of dead seeds (Missio *et al*., 2011).

CONCLUSION

Seeds of *Abrus precatorius* show higher germination at 30 °C when previously immersed in sulphuric acid for 30 or 45 minutes.

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