

Dormancy break in seeds of “quina” (*Strychnos pseudoquina* A. St.-Hil.)

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ABSTRACT: “Quina” (*Strychnos pseudoquina* A. St. Hil) is a medicinal plant species from the Brazilian Cerrado. As its seeds show dormancy, they were subjected to the treatments pre-cooling at 5°C during 7 days, pre-heating at 40°C during 7 days, pre-soaking in sulfuric acid PA during 5 and 15 min, pre-soaking in boiling water during 5 and 15 min, pre-soaking in 100 and 200 ppm gibberellic acid during 48 h, pre-soaking in distilled water during 24 and 48 h, and mechanical scarification to break dormancy. Counts were daily conducted from the 2nd day after the experiment implementation until the germination stabilization at the 65th day. The germination speed index (GSI) and the germination percentage were evaluated. Germination rates above 96% were reached in seeds pre-soaked in water during 48 h and substrate moistened with water or KNO₃.

Key words: “quina”, medicinal plant, germination, Loganiaceae, seed dormancy

RESUMO: Superação da dormência de sementes de quina (*Strychnos pseudoquina* A. St.-Hil.). A quina (*Strychnos pseudoquina* A. St.-Hil) é espécie medicinal do Cerrado Brasileiro. As sementes apresentam dormência e, por isso, foram submetidas aos tratamentos pré-resfriamento a 5°C durante 7 dias, pré-aquecimento a 40°C durante 7 dias, pré-embebição em ácido sulfúrico P.A. durante 5 e 15 minutos, pré-embebição em água fervente durante 5 e 15 minutos, pré-embebição em ácido giberélico a 100 e 200 ppm, durante 48 horas, pré-embebição em água destilada durante 24 e 48 horas e escarificação mecânica, visando à superação da dormência. Foram realizadas contagens diárias entre o 2º dia após a implantação do experimento até a estabilização da germinação, que ocorreu no 65º dia. Foram avaliados o índice de velocidade de germinação (IVG) e a porcentagem de germinação. Taxas de germinação acima de 96% foram atingidas em sementes pré-embebidas em água durante 48 horas e substrato umedecido com água ou KNO₃.

Palavras-chave: quina, planta medicinal, germinação, Loganiaceae, dormência de sementes

INTRODUCTION

Cerrado is one of the regions of greatest biodiversity in the planet, with a significant degree of endemism. However, human occupation, stimulated by a series of development policies, has transformed the region into a fragmented landscape, an environment where the agro-pasture system prevails (Buschbacher, 2000). The indiscriminate use of medicinal species in popular medicine, as well as by the pharmaceutical industry, has led to a considerable reduction in the population density of some plants in areas of natural occurrence (Nadeem et al., 2000).

“Quina” (*Strychnos pseudoquina*)-Loganiaceae - tree grows to about 4 m and is found mainly in Cerrado *sensu stricto*. This plant has been

widely used in folk medicine for its aphrodisiac, tonic, and antipyretic properties and is also indicated in case of liver problems, stomach disorders and anemia (Almeida et al., 1998; Silva et al., 2005). The study of techniques to facilitate seedling production using this species is therefore of great importance, particularly because to date no such studies exist. Moreover, its leaves contain flavonoids and alkaloids, the latter also in the branches (Nicoletti et al., 1984).

Seed dormancy is a type of latency in which absence of germination is caused by obstacles in the seed itself. It is activated by a combination of specific environmental conditions such as water, temperature, photoperiod and seed position in the

plant. These conditions could cause the interference of one or more blocking mechanisms, preventing the transcription of the genetic message to activate the metabolic sequence that culminates in germination. Dormancy is a defense mechanism of seeds against environmental variations that hinder or prevent the normal metabolic activity during certain periods, in which there is no transcription of the genetic message. This activity is only resumed after the action of an environmental stimulation (Marcos Filho, 2005).

According to Cardoso (2007), dormancy in this species was concluded, since germination took up to three months. The aim of the present study was therefore to evaluate the most appropriate methods to break seed dormancy in *Strychnos pseudoquina*.

MATERIAL AND METHOD

The experiment was conducted at the Seed Laboratory of "Instituto Federal de Educação, Ciência e Tecnologia Goiano" - Rio Verde Campus. "Quina" seeds were collected from three trees at Gameleira Farm in Montes Claros de Goiás Municipality, Goiás State, Brazil (latitude S - 16°06'20", longitude W - 51°17'11", 466 m above sea level). The fruit pulp was rubbed off by hand using a steel sieve mesh under running water. Then, the seeds were allowed to dry at room temperature for several minutes until they lost all surface moisture.

The seeds were treated with fungicide Vitavax-Thiram® [Active Ingredient (carboxin + thiram): 200 + 200 g L⁻¹], at a concentration of 300 mL fungicide per 100 kg seed.

The following treatments were applied to the seeds to break dormancy: (i) pre-cooling - on moist substrate, cooling to 5°C in refrigerator for 7 days; (ii) pre-heating - in an oven with free air circulation at 40°C during 7 days; (iii) chemical scarification - pre-soaking in sulfuric acid PA (98%) during 5 and 15 min, according to each treatment. After the established period, the acid solution was removed and the seeds washed in running water to remove all acid; then, they were allowed to dry at room temperature during 60 min. (iv) immersion in boiling water - after the period of 5 and 15 min established for each treatment, the seeds were washed in tap water and allowed to dry at room temperature for 60 min; (v) soaking in gibberellic acid - seeds were immersed in solutions of 100 ppm and 200 ppm gibberellic acid (GA₃) during a period of 48 h; (vi) soaking in distilled water - seeds were immersed in distilled water during 24 and 48 h at room temperature; (vii) mechanical scarification - the seed tegument was abraded with sandpaper No. 80, taking care to avoid any damage to the embryo, sanding the top opposite to the embryo axis; and (viii) control - intact seeds with no pretreatment.

After the treatments described above,

germinated seeds were transferred to a Mangelsdorf germinator, at 25°C ± 4°C.

In all treatments, seeds were allowed to germinate on substrate moistened with a solution of 0.2% KNO₃ (potassium nitrate) or distilled water, using germination boxes (Gerbox) containing 2 sheets of absorbent paper moistened to 2.5-fold the dry substrate weight.

The germination percentage and the beginning of radicle protrusion were daily counted to calculate the germination speed index (GSI), according to the formula of Maguire (1962).

The experiment had a completely randomized block, 2x12 factorial design, with 2 substrates x 12 methods of breaking dormancy and 4 blocks with 15 seeds each, totaling 96 experimental units. The results were subjected to analysis of variance (F 0.05) and the means compared by Scott-Knott test at 5% probability, using the software SAEG (System for Genetic Analysis and Statistics).

RESULT AND DISCUSSION

"Quina" seeds began to germinate from the 13th day onwards and this process extended to 65 days, when germination stabilized.

The highest germination speed indexes (vigour) were observed in seeds pre-soaked in water for 48 h on both substrates (distilled water and KNO₃), pre-soaked in 200 ppm gibberellic acid and pre-soaked in H₂O during 24 h for the water-moistened substrate (Table 1). An inhibitory effect was observed for the KNO₃-moistened substrate compared to water, in seeds pre-soaked in 200 ppm GA₃ and the control. Ferreira & Gentil (2006) also found satisfactory results (70% germination) of germination speed in tucumã (*Astrocaryum aculeatum*) seeds soaked in water, compared to KNO₃. According to Vieira et al. (2002), soaking rice seeds in GA₃ for 36 h is efficient to rapidly break dormancy.

The lowest vigor was observed in seeds soaked in H₂SO₄ or boiling water during 5 and 15 min on the substrates containing water or KNO₃. The treatments of pre-heating at 40°C, mechanical scarification and control yielded no satisfactory results for the substrate KNO₃. Seed treatment with sulfuric acid resulted in a very large number of dead seeds (Table 1).

A similar result was found by Carmona et al. (1986) in a study on *Stylosanthei Macrocephala* seed germination. According to that author, the treatment with sulfuric acid decreased germination due to the increase in dead seeds.

An inhibitory effect of seed treatment with sulfuric acid on germination was observed at all times evaluated, regardless of the substrate (Table 2). Sulfuric acid has a corrosive effect on the seed husk, which is

TABLE 1. Germination speed index of “quina” (*Strychnos pseudoquina*) seeds treated with different methods to break dormancy when allowed to germinate on substrates moistened with water or soaked in KNO₃ solution (0.2%).

Treatment	Substrate	
	Water	KNO ₃ (0.2%)
Pre-cooling (5°C during 07 days)	0.22 Ca ^z	0.26 Ba
Pre-heating (40°C during 07 days)	0.18 Ca	0.04 Ca
Pre-soaking in H ₂ SO ₄ PA (98%) during 5 min	0.01 Da	0.03 Ca
Pre-soaking in H ₂ SO ₄ PA (98%) for during 15 min	0.03 Da	0.02 Ca
Pre-soaking in boiling water for during 5 min	0.00 Da	0.00 Ca
Pre-soaking in boiling water for 15 min	0.00 Da	0.00 Ca
Pre-soaking in 100 ppm gibberellic acid for 48h	0.40 Ba	0.32 Ba
Pre-soaking in 200 ppm gibberellic acid for 48h	0.63 Aa	0.34 Bb
Pre-soaking in H ₂ O for 24 h	0.51 Aa	0.47 Ba
Pre-soaking in H ₂ O for 48 h	0.67 Aa	0.77 Aa
Mechanical scarification	0.31 Ba	0.16 Ca
Control	0.30 Ba	0.07 Cb

^zMeans followed by the same uppercase letter among dormancy breaking methods and lowercase letter between substrates did not differ according to Scott-Knott test at 5% probability.

TABLE 2. Germination percentage of “quina” (*Strychnos pseudoquina*) seeds treated with different methods to break dormancy when allowed to germinate on substrates moistened with water or soaked in KNO₃ solution (0.2%).

Treatments	Substrate	
	Water	KNO ₃ (0.2%)
Pre-cooling (5°C for 07 days)	50.00 Ca ^z	55.00 Ba
Pre-heating (40°C for 07 days)	36.50 Da	5.00 Db
Pre-soaking in H ₂ SO ₄ PA (98%) during for 5 min	1.75 Ea	5.25 Da
Pre-soaking in H ₂ SO ₄ PA (98%) for 15 min	6.75 Ea	3.50 Da
Pre-soaking in boiling water for 5 min	0.00 Ea	0.00 Da
Pre-soaking in boiling water for 15 min	0.00 Ea	0.00 Da
Pre-soaking in 100 ppm gibberellic acid for 48h	76.50 Ba	74.75 Ba
Pre-soaking in 200 ppm gibberellic acid for 48h	93.00 Aa	40.00 Cb
Pre-soaking in H ₂ O for 24 h	91.50 Aa	88.00 Aa
Pre-soaking in H ₂ O for 48 h	96.50 Aa	96.75 Aa
Mechanical scarification	70.75 Ba	33.50 Cb
Control	46.75 Ca	18.50 Cb

^zMeans followed by the same uppercase letter among dormancy breaking methods and lowercase letter between substrates did not differ according to Scott-Knott test at 5% probability.

favorable in the case of many species, since the change in the membrane permeability allows water uptake, an essential factor that starts the germination process. The increased permeability also allows gas exchange, reduces mechanical resistance to radicle protrusion and facilitates embryo expansion (Dousseau

et al., 2007). Studies with different species of other families using sulfuric acid have shown that the germination percentage effectively increased and / or the average germination time reduced, even when the time of contact with the seed was longer than that tested here. Seed germination percentage was over

90% for *Leucaena leucocephala* pre-treated for 15 or 20 min (Teles et al., 2000), *Enterolobium contortisiliquum* (Vell.) Morong for 10 min (Meneghello et al., 2000), and *Dimorphandra mollis* for 45-90 min. However, periods longer than 90 min resulted in a marked drop of viability (Hermansen et al., 2000). On the other hand, corroborating the present results, the percentage of damaged *Ormosia nitida* seeds, treated with sulfuric acid, strongly increased, indicating that such jeopardizing treatments should be avoided whenever possible. Despite overcoming the phenomenon of dormancy, the percentage of damaged seeds and abnormal seedlings increase significantly, leading to high incidences of bacteria and fungi (Lopes et al., 2006). For other species, even with a short exposure period (60 seconds), germination was not effectively increased by sulfuric acid, but rather harmful (Boscagli & Sette, 2001).

Pre-heating the seeds at 40°C during seven days had an inhibitory effect on germination, regardless of the analyzed substrate. Different results were observed for seeds of velvet bean (*Mucuna aterrima* Piper et Tracy Holland) at a temperature of 55°C for periods between 16 and 24 h (Wutke et al., 1995), and exposing seeds of *Brachiaria brizantha*, cv Marandu, to 70°C between 5 and 30 h proved to be an efficient method to break dormancy (Martins & Silva, 2006).

Pre-soaking seeds in boiling water for 5 or 15 min, regardless of the substrate, also caused a reduction in the germination percentage. Similar results were found for *Satureja montana* (Boscagli & Sette, 2001). Furthermore, studies of Teles et al. (2000) with *Leucaena leucocephala* (Lam.) Wit, Barbosa et al. (2004) with *Ochroma lagopus* SW. Bombacaceae, and Gupta (2001) with *Argyre nervosa* and *Urena lobata*, pre-soaked in hot water at 80°C until cooling off, showed that this is a very effective method of breaking dormancy in these species. Again, the results for *O. lagopus* differ from those for "quina"; soaking the seeds of the former in water for 24 and 48 h had no effect on germination, whereas "quina" showed very satisfactory results, with a mean germination of over 96% when pre-soaked for 48 h (Teles et al., 2000).

However, cooling the seeds to 5°C during 7 days, regardless of the substrate, subjected to 200 ppm gibberellic acid and mechanical scarification, had no effect on the percentage of germinated seeds. On the other hand, results of seeds pre-soaked in gibberellic acid at 100 or 200 ppm, immersed in water for 24 or 48 h and kept on water-soaked substrate, and the same treatments except for 200 ppm gibberellic acid on the substrate with KNO₃, were better than those of the control. Among the positive results for both substrates, seeds pre-soaked in water for 24 or 48 h and gibberellic acid in the water-soaked substrate had higher germination rates. Contrary to the results found here,

in *Caesalpinia ferrea* Mart. ex Tul. the mechanical scarification with sandpaper No. 40 was efficient to break dormancy (Lima et al., 2006), as also observed in "chichá" (*Sterculia foetida* L.) seeds (Santos et al., 2004). Unlike the results for "quina", pre-soaking the endocarp of "umbuzeiro" (*Spondias tuberosa* His Câm.) in water during 0, 48, 96 and 144 h did not positively influence germination (Costa et al., 2001).

When potassium nitrate was used in the germination substrate, "quina" seeds showed no response to this salt. On the other hand, seeds of "mate" (*Ilex Paraguariensis*) (Cuquel et al., 1994) and *B. brizantha* cv. Marandu (Garcia & Cicero, 1992) responded positively to this treatment.

Potassium nitrate stimulates germination by helping suppress the tegument impermeability to gases, besides being a co-factor for the phytochrome action. Since germination success requires a combination of environmental factors, the interaction among temperature, light and nitrate has been amply studied. In contrast, gibberellin weakens the cell walls by hydrolases species, such as cellular mannanases, arabinosidases and expansins. They also stimulate the synthesis of α -amylase, which mobilizes endosperm reserves for the embryo development, besides boosting the transcription of α -amylase mRNA, demonstrating hormonal control on the gene expression (Guimarães, 1999, Resende et al., 2009).

These results show that the dormancy of "quina" seeds is most likely of morphological and physiological nature. It is physiological, as shown by the positive effect on germination when seeds were soaked in 200 ppm gibberellic acid, which increased the endogenous concentration of this germination-promoter hormone. It is also morphological since pre-soaking in water during 24 to 48 h caused seed tegument softening, facilitating radicle protrusion.

CONCLUSION

Pre-soaking seeds in water during 24 or 48 h, regardless of the substrate, or pre-soaking in 200 ppm gibberellic acid during 48 h on water-moistened substrate, led to the best results. Of the two possibilities, immersion in water is more appropriate, for being economically more viable.

ACKNOWLEDGEMENT

The authors are indebted to CNPq for the undergraduate research program scholarship to the first author; to Mr Arlindo Thomaz da Silva and his family for donating the plant material used here; and to Dr. Fernanda Ribeiro Queiroz de Oliveira for her critical reading and correction of the manuscript in Portuguese.

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