REVISTA DE CIÊNCIASAGRÁRIAS
Amazonian Journal
of Agricultural and Environmental Sciences
www.ajaes.ufra.edu.br

http://dx.doi.org/10.4322/rca.2013.088

ORIGINAL ARTICLE

Physical and chemical methods to breach seed dormancy of sugar apple

Métodos físicos e químicos na quebra de dormência de sementes de pinha

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KEYWORDS
Annonaceae
Annona squamosal
Gibberellic acid
Scarification

PALAVRAS-CHAVE
Anonáceas
Annona squamosa
Ácido giberélico
Escarificação

ABSTRACT: Cultivation of sugar apple has increased in the Setentrional Amazon, but one of the problems for obtaining quality seedlings is related to overcoming dormancy of its seeds. The objective of the present study was to evaluate the efficiency of physical and chemical methods to break sugar apple seed dormancy. Ripe sugar apple fruit were collected from an orchard located in Boa Vista-RR, pulped and their seeds washed in running water and dried in the shade for 24 h. A completely randomized experimental design was used in a factorial 2 (0 and 1000 mg L^{-1} GA_3 = gibberellic acid) × 4 immersion times (0, 6, 12 and 24 h) × 2 (with and without scarification) with 4 replications and 50 seeds per replication. The seeds were then sown in beds under greenhouse-type shade with 50% brightness in soil and sand mixture (3:1 v / v) subtrate. The following were evaluated 30 days after sowing: percentage of emergence (%) and shoot growth after seedling emergence, at four-day intervals. At fifty-eight days after sowing, the shoot and root lengths, number of leaves, shoot and root dry matter were assessed. The highest sugar apple seedling emergence speed and growth rate were obtained when the seeds were subjected to the method of scarification and soaking in GA_3 for 12-24 h. Scarification and soaking in water for 24 h resulted in greater economy, practicality and financial savings.

RESUMO: *O cultivo comercial de pinheira tem sido crescente na Amazônia Setentrional, porém um dos problemas para obtenção de mudas de qualidade tem sido aqueles relacionados com a superação de dormência de suas sementes. Assim, objetivou-se avaliar a eficiência de métodos físicos e químicos na quebra de dormência de sementes de pinha. Frutas de pinheira maduras foram coletadas em um pomar comercial localizado no município de Boa Vista-RR e despolpadas; suas sementes foram lavadas em água corrente e secas à sombra por 24 h. O delineamento utilizado foi inteiramente casualizado, em esquema fatorial 2 (0 e 1000 mg L^{-1} de ácido giberélico= GA_3) × 4 tempos de imersão (0, 6, 12 e 24 h) × 2 (com e sem escarificação com lixa), totalizando 16 tratamentos, com quatro repetições e 50 sementes por repetição. Em seguida, as sementes foram semeadas em canteiros instalados sob telado tipo sombrite com 50% de luminosidade, com substrato composto por uma mistura de solo e areia (3:1 v/v). Trinta dias após a semeadura, foram avaliados a porcentagem de emergência (%) e o crescimento da parte aérea após a emergência das plântulas, em intervalos de quatro dias. Aos 58 dias após semeadura, se quantificaram o comprimento da parte aérea e do sistema radicular; o número de folhas, e a massa seca da parte aérea e do sistema radicular. Maior índice de velocidade e porcentagem de emergência, e crescimento de plântulas de sementes de pinha é obtido quando estas são submetidas ao método de escarificação e imersão em GA_3, por um tempo de 12 a 24 h. A escarificação e a imersão em água por 24 h proporcionaram maior economia, praticidade e economia financeira.*

Received: 11/15/2013
Accepted: 12/10/2013

1 Introduction

Cultivation of sugar apple (*Annona squamosa* L.), also known as pine, has increased in the Setentrional Amazon, mainly because of its delicious fruit flavor. The plants have excellent adaptation but commercial orchards face difficulties because there is little technology for quality seedling production and orchard management.

A. squamosa is usually propagated sexually. Its asexual propagation is by grafting used to multiply the most productive clones (ALMEIDA et al., 2010), but this form of propagation is not yet the most widespread, although it is most correct for quality seedling production. Seeds are used to form the root stock. However, these have problems of dormancy. The dormancy state in seeds is manifested by delay in germination for longer or shorter periods of time even if the environmental conditions (light, temperature, water, oxygen) are favorable for germination (FERREIRA et al., 2009). Nevertheless, dormancy can be overcome and germination started using plant regulators together with imbibitions (ANDRADE et al., 2010). Hormones play a fundamental role in the seed germinating process, especially gibberellins, cytokinins and ethylene (TAIZ; ZEIGER, 2009).

Plant hormones can be used associated to physical and chemical methods for more efficient seed germination and emergence. Sousa et al. (2008) found the best results for *A. squamosa* seeds using gibberellic acid at 50 and 750 mg L^{-1} imbibition for 12 h. However, higher concentrations were used by Oliveira et al. (2010), who verified that higher values for atemoya emergence percentage, germination speed index and normal seedling percentage were obtained using regulators at concentrations of 600 to 1000 mg L^{-1} GA_3 and 75 to 100 mg L^{-1} ethephon.

Ferreira, Erig and Moro (2002a) investigated the effects of different GA_3 concentrations on sugar apple (*Annona squamosa*) seed germination and seedling emergence in different packaging and tested seven GA_3 concentrations ranging from 0 to 1.000 mg L^{-1} for 5 h. They concluded that GA_3 positively influenced seed germination of the species, but did not affect seedling emergence in the packaging tested. In the same context, Ferreira, Erig and Moro (2002b) assessed the effects of different GA_3-based products (Progibb and Promalin) and their concentrations (25; 50 and 75 mg L^{-1}) and a control and verified that using GA_3 gave more effective responses for several parameters when assessed at 50-75 mg L^{-1}. For the stem length and diameter parameters, the same authors calculated the maximum technically efficient GA_3 concentration 65.37 and 65.97 mg L^{-1}, respectively. The Promalin concentration that promoted the greatest increase in stem length was 75 mg L^{-1}, but the results did not permit calculation of the maximum technically efficient concentration. Positive results from applying GA_3 on the germination rate and germination speeds in atemoya and sugar apple seeds were reported by Stenzel, Murata and Neves (2003).

Menegazzo et al. (2012) assessed the effects of chemical, physical and mechanical methods to overcome dormancy in *A. squamosa* seeds and reported that using gibberellic acid at 100 mg L^{-1} for 24 h immersion and 250 mg L^{-1} for 5 h resulted in higher germination percentages and better seedling development. The authors further observed that the sulfuric acid tested was not efficacious in breaking *A. squamosa* seed dormancy.

Interest is growing in the study and production of Annonaceae, especially *A. squamosa* as a commercial fruit tree, justifying the study of methods to break dormancy. Thus several studies have been carried out where mechanical, physical and chemical methods have been tested to overcome dormancy.

The objective of the present study was to assess the efficiency of physical and chemical methods in breaking *A. squamosa* seed dormancy.

2 Materials and Methods

The experiment was carried out at the Fruit Cropping Sector on the Experimental Campus of the Agricultural Technical College at the Federal University of Roraima, in Boa Vista-RR, from December 2012 to March 2013. To set up the experiments, *A. squamosa* seeds were obtained from ripe fruits selected from trees in a commercial orchard. The seeds were extracted from the fruit by washing in running water until the pulp residues were completely removed and dried in the shade for 24 h.

A randomized complete design was used in a 2 × 4 × 2 factorial scheme with four replications and 50 seeds per replication. The seeds were submitted to treatment with and without immersion in gibberellic acid (GA_3) at 1000 mg L^{-1}, in four immersion periods of 0, 6, 12 and 24 h and with and without mechanical scarification with sandpaper in a total of 16 treatments.

The seeds were then sown in beds installed under sombrite-type roofing, with 50% light and soil and sand substrate (3:1 v/v). Thirty days after sowing the following were assessed: emergence percentage (%) and shoot growth after seedling emergence, at four-day intervals using a graduated ruler and the values were expressed in centimeters. Afterwards, the emergence speed index was calculated (IVE) (SILVA; NAKAGAWA, 1995), the emergence percentage and the seedling growth in this period.

The following were measured 58 days after sowing: a) the shoot and root system lengths, where the plants were removed from the substrates, carefully washed in water and measured using a graduated ruler and the values were expressed in centimeters; b) the shoot and root system dry matter, where the material was placed in paper envelopes and transferred to a forced air chamber at 60 °C, until it reached constant weight after 72 h and the values were expressed in g/plant.

The results were submitted to analysis of variance and the means of the quantitative data were submitted to the regression test using the computer program System for Analysis of Variance - SISVAR®.

3 Results and Discussion

The highest emergence speed index was observed for treatment 10 (T10), where the seeds were scarified and imbibed in water for six hours, followed by T16, where seeds

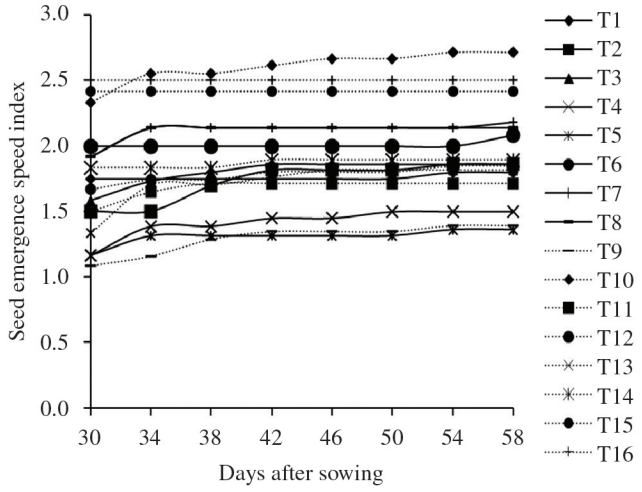

Figure 1. *A. squamosa* seed emergence speed index when submitted to scarification and immersion times in GA_3 to break dormancy: T1 = SE + water for 0 h; T2 = SE + water for 6 h; T3 = SE + water for 12 h; T4 = SE + water for 24h; T5 = SE + GA_3 for 0 h; T6 = SE + GA_3 for 6 h; T7 = SE + GA_3 for 12 h; T8 = SE + GA_3 for 24 h; T9 = CE + water for 0 h; T10 = CE+ water for 6 h; T11 = CE+ water for 12 h; T12 = CE + water for 24 h; T13 = CE + GA_3 for 0 h; T14 = CE + GA_3 for 6 h; T15 = CE + GA_3 for 12 h and; T16 = CE + GA_3 for 24 h. SE = without scarification; CE = with scarification.

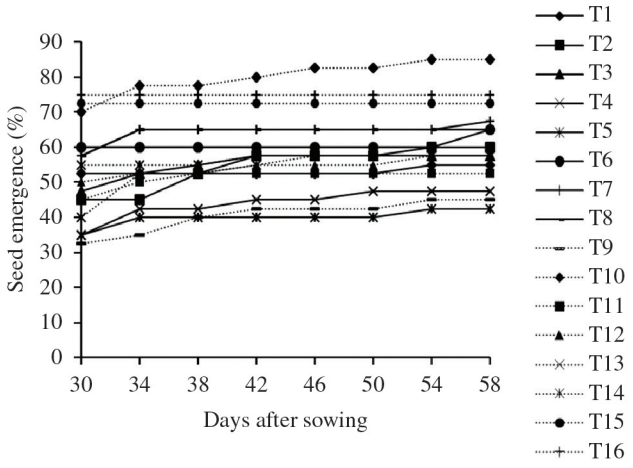

Figure 2. *A. squamosal* seed emergence when submitted to scarification and immersion times in GA_3 to break dormancy: T1= SE + water for 0 h; T2= SE + water for 6 h; T3= SE+ water for 12 h; T4= SE+ water for 24 h; T5 =SE+ GA_3 for 0 h; T6= SE+ GA_3 for 6 h; T7=SE+ GA_3 for 12 h; T8 = SE+ GA_3 for 24 h; T9=CE + water for 0 h; T10= CE+ water for 6 h; T11= CE+ water for 12 h; T12= CE+ water for 24 h; T13= CE+GA_3 for 0 h; T14= CE+GA_3 for 6 h; T15= CE+ GA_3 for 12 h and; T16= CE+ GA_3 for 24 h. SE= without scarification; CE= with scarification.

were scarified and imbibed in GA_3 for 24 h. Good emergence speed was also observed when the seeds were scarified and imbibed in GA_3 for 12 h (Figure 1). These data showed that the scarification process is important to increase the *A. squamosa* seedling emergence speed. This statement in the present research is evident because lower emergence speed indexes were observed, respectively, in treatments T5, T9 and T4. In treatments T5 and T4 the seeds were not scarified. In T9, although the seeds had been scarified, they were not imbibed.

The *A. squamosa* seeds submitted to scarification and immersion in water for 6 h (T10) had 80% emergence and 72.5% emergence when scarified and imbibed in 1000 mg L^{-1} GA_3 for 12 h (T15). The treatment with scarification and imbibition in 1000 mg L^{-1} GA_3 for 24 h (T16) resulted in 75% emergence. When there was no immersion in GA_3 and no scarification (T5), only 42.5% of the seeds emerged. Decreasing values were also observed for T9 of 45% emergence with scarification and immersion in water for 0 h and T4 with 57 % germination when the seeds were scarified and immersed in water for 24 h. The other treatments presented similar emergence values.

Sousa et al. (2008) used some physical and chemical methods to break dormancy in *A. squamosa* seeds. These authors reported that greater seed emergence was obtained when scarification with sandpaper was combined with imbibition of the scarified seeds in water and with a biostimulant (Stimulate®), and the increases were 22 to 43% in the germination percentage, respectively. These authors observed that when gibberellic acid was used at doses of 50 to 750 mg L^{-1}, the germination percentage ranged from 83 to 98% and mechanical scarification with sandpaper was not necessary.

Regarding the variables shoot length, leaf number, plant dry matter and root system dry matter, there was no significant difference in relation to scarification. These data are in agreement with those reported by Ferreira et al. (2002a) who studied the imbibition curves of *A. squamosa* and *A. Cherimola* Mill. X *A. squamosa* L. (atemoya) seeds and reported that the seeds of these species may not present physical impediments to water entry, thus discarding the possibility of dormancy being due to seed coat impermeability. However, in the present study, it was observed that although it did not influence the variables mentioned above, the scarification and imbibition process was important to increase emergence speed (Figure 1) and the seed emergence percentage (Figure 2).

For the factors scarification and immersion time, the shoot length variable had significant interaction (Figure 3). When there was scarification and imbibition in GA_3 for 24 h, the seedlings obtained greater growth compared to the other treatments, with a height of 15.02 cm. The seeds treated with immersion in water with scarification, regardless of the immersion time, obtained similar seedling height. The treatment where the seeds were not scarified, but immersed in GA_3, resulted in similar shoot lengths while the same treatment when immersed in water for 24 h presented lower shoot height than the others, only 7.83 cm.

These results are similar to those reported by Lima-Brito et al. (2006), who studied the effect of using gibberellic acid on seed emergence of three Annonaceae species, one of which was *Anonna squamosa*. Using gibberellic acid at concentrations ranging from 250 to 1000 mg L^{-1}, increased the germination percentage and emergence speed index of the *A. squamosa* seeds.

Andrade et al. (2010) worked with scarified and imbibed *Hymenaea courbaril* seeds and reported better results in all the variables analyzed, emergence percentage, IVE, shoot and root system length, when compared to intact seeds without scarification. This result reinforces the theory that, according to Esquinca, Moctezuma and Pérez (1997), the dormancy mechanism found in some *Annona* species is not due to the hardness of the seed coat or immaturity but rather to their dormancy mechanism that is related to seasonal survival.

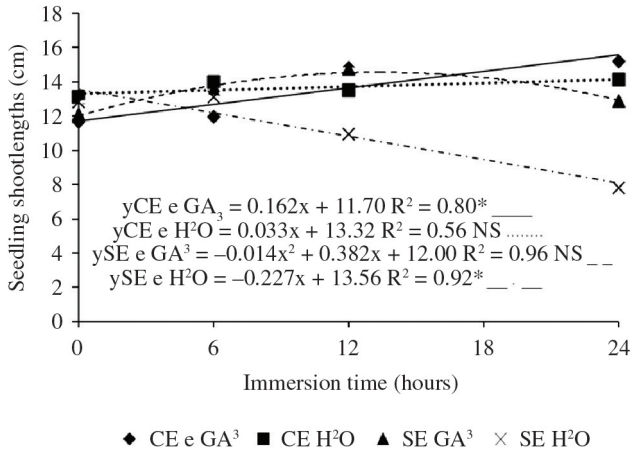

Figure 3. Seedling shoot lengths in function of partitioning, using scarification methods for *A. squamosa* seeds (CE+GA_3) = seeds with scarification and immersed in GA_3; (CE +H_2O) = seeds with scarification and immersed in water; (SE+GA_3) = without scarification and immersed in GA_3 e (SE + H_2O) = seeds without scarification and immersed in water. *=0.05.

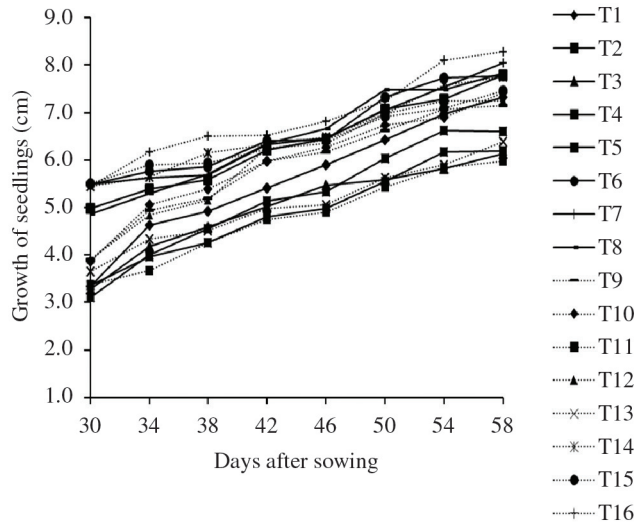

Figure 4. Growth of seedlings submitted to scarification and immersion times in doses of GA_3 to break dormancy in *A. squamosa* seeds: T1= SE + water for 0 h; T2= SE + water for 6 h; T3= SE+ water for 12 h; T4= SE+ water for 24h; T5 =SE+ GA_3 for 0 h; T6= SE+ GA_3 for 6 h; T7=SE+ GA_3 for 12 h; T8= SE+ GA_3 for 24 h; T9=CE + water for 0 h; T10= CE+ water for 6 h; T11= CE+ water for 12 h; T12= CE+ water for 24 h; T13= CE+GA_3 for 0 h; T14= CE+GA_3 for 6 h; T15= CE+ GA_3 for 12 h and; T16= CE+ GA_3 for 24 h.

Weaver (1987) reported that dormancy may be the result of the hormone balance among growth promoters and inhibitors.

Plant growth was linear (Figure 4) when scarified seeds immersed in 1000 mg L^{-1} GA_3 resulted in growth of 8.29 cm in height at 58 days after emergence. Similarly, the treatments without scarification and immersion in GA_3 for 12 h (T7 = 8.05 cm height) and with scarification and immersion in GA_3 for 12 h (T15 = 7.46 cm), also presented linear growth.

When scarification was used with immersion in water for 12 h (T11), the seedlings did not develop well (5.18 cm). Similar performance was observed for T3 with 6.13 cm, without scarification and immersion in water for 12 h. Scarification with sandpaper followed by treatment with hot water was also studied by Shimizu et al. (2011) in *Schizolobium amazonicum* seeds. The authors reported that the best germination results were obtained when seeds were scarified with sandpaper. Nagashima et al. (2010) observed that the different responses, both in seedling growth and emergence associated to the use of physical and chemical dormancy breaking methods, also depend on genetic and environmental factors.

Seedling submitted to treatments where GA_3 was used presented an increasing number of leaves up to 18.73 h immersion time, when 8.5 leaves per plant were observed (Figure 5). This result indicated that imbibing the seeds contributed to increasing the number of leaves.

The number of leaves is important because they are mainly responsible for solar energy capture and organic material production through photosynthesis that contribute to obtaining healthy and vigorous saplings. It was also observed that immersion treatment for more than 18.73 h presented a small decrease in the number of leaves but this decrease could have been caused by external factors, causing a slight fall in the number of leaves and not because of the product used.

A similar result to the number of leaves was also observed for root system dry matter. Significant difference was observed for this variable for immersion time. Root system dry matter increased as the immersion time increased up to 14.28 h, when 0.044 g were obtained, after which the values of this variable

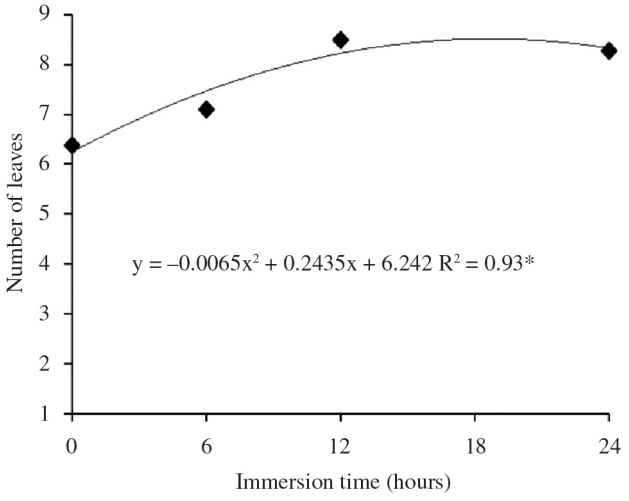

Figure 5. Number of leaves on seedlings submitted to scarification and immersion times in GA_3 to break dormancy in *A. squamosa* seeds. *=0.05.

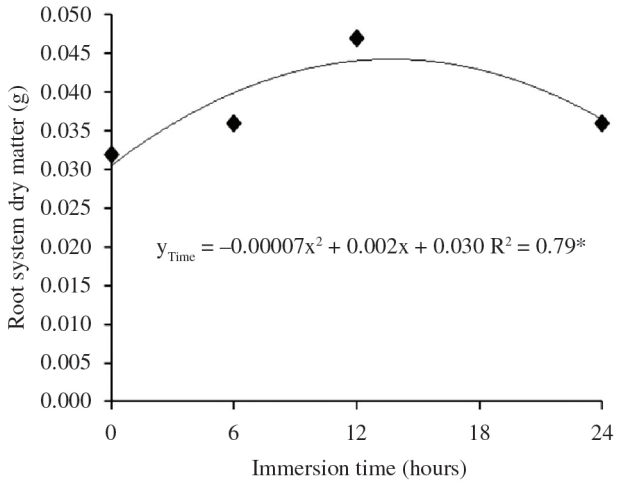

Figure 6. Root system dry matter of seedlings submitted to scarification and immersion times in GA_3 to break dormancy in *A. squamosa* seeds.*=0.05.

decreased (Figure 6). The root system dry matter is directly related to good root system development and is influenced by the substrate used in the planting bed. The plants from treatments with imbibition had better developed root systems.

The root system dry matter is a characteristic of interest because a well-formed root system is desirable when the intention is to obtain quality saplings. The study of applying gibberellic acid (GA_3) in the present research was justified because results in other species have also been variable in function of the dose used. Scalon et al. (2009) used different gibberellic acid doses on *Campomanesia adamantium* Camb seeds and did not observe significant differences in root length or shoot height. However, Prado Neto et al. (2007) used Stimulate® in Jenipano seeds and observed greater root length with 10 mL L^{-1} in pre-imbibition of the seeds for 12 h.

4 Conclusions

The highest speed index, emergence percentage and seeding growth were obtained in *A. squamosa* seeds when they were submitted to the method of scarification and immersion in GA_3 for 12 to 24 h.

Scarification and immersion in water for 24 h resulted in greater economy, practicality and financial savings.

Acknowledgements

The authors thank CAPES, CNPq and FEMARH for funding.

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