



Overcoming Dormancy and Influence of Light on the Physiological Quality of *Senna cana* (Fabaceae) Seeds

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ABSTRACT

Environmental factors affect the germination process, like the presence of seed coat and the quality of light; these informations are still scarce for many native species from Brazil, especially for *Senna cana*, which there are no adequate standards and methodologies to be used in germination tests. The aim of this research was to recommend adequate pre-germinative treatment(s) to overcome seed dormancy, and determine the degree of influence of different light regimes in seed germination of *S. cana*. Two experiments were carried out: T1-evaluation of different methods of dormancy overcoming (intact seeds (control), T2-imbibition of the intact seeds for 24 hours (in distilled water), T3-scarified seeds with sandpaper n° 100 in the hilum opposite region, T4-scarified seeds with sandpaper n° 100 in the region the hilo opposite region and imbibition in water (in distilled water) for 24 hours; T5-imbibition in water at 80°C); 2-Influence of light quality on seed germination and vigor (white light, red light, far red light and absence of light). The evaluated parameters were: first

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germination count, percentage of germination, IVG (Germination speed index), MGT (Mean germination time). Treatments were compared by Tukey at 5% probability. The best method for overcoming seed dormancy was mechanical scarification with sandpaper n° 100. The germination of the seeds of *S. cana* can be classified as indifferent to the luminosity, despite germinative response be greater under white and red light.

Keywords: *Fabaceae*; *germination*; *ecophysiology*; *temperature*; *luminosity*.

1. INTRODUCTION

Senna cana (Nees & Mart.) H.S. is a shrub Fabaceae popularly known as Sao Joao, has occurrence in Cerrado and rupestrian fields, presents yellow flowers, showy, without nectars, and yellow, rigid, porous anthers. *S. cana* is a little studied species, and this is one reason why there are no specific protocols for its cultivation [1-2].

For several species, studies have shown that seeds can germinate under different temperature conditions [3-9], on the other hand, informations about the appropriate light requirements for germination of *Senna cana* seeds do not exist.

From an ecological point of view, it is essential to know the development of plants and where they grow [10]. Several factors affect the germination process such as: substrate, dormancy, temperature, humidity, light, oxygen, etc. Thus, the knowledge of these factors is indispensable to adequate conditions to seed germination process [11].

For some seeds the availability of water provides the beginning of germination; however, others present impermeable tegument, preventing the onset of germination, which is called dormancy, so that it will need to overcome its dormancy before germination begins [12].

The prevention of germination can be attributed to other characteristics of the seeds, such as embryo characteristics and other structures, including the endosperm, tegument, or even the action of parts of the fruit. Some authors state that seeds of the Fabaceae family present physical dormancy due to the water impermeability caused by the seed coat [13].

Knowledge of the requirements for germination is important to answer ecological questions about the species, such as how it develops and the environment in which it grows. Thus, the germination of the orthodox seeds involves the resumption and continuity of the metabolic

activities, promoting the development of the embryo structures, with the consequent formation of the seedlings, being necessary the favorable performance of environmental factors such as availability of water, favorable temperature and oxygen and light quality [14].

In many species the presence of light promotes the seeds germination, while others, the germinative performance in the absence of light is more effective. When the light exerts a positive influence, it is said that the species is photoblastic positive or photoblastic negative, and seeds indifferent to this factor, which are neutral photoblasts [15-16]. This light requirement for germination in some species is directly influenced by temperature and according to [17], the determination of optimal temperature provides maximum percentage of germination.

The present work aimed to recommend adequate pre germinative treatment(s) to overcome dormancy and to determine the influence of light on physiological quality of *Senna cana* seeds.

2. MATERIALS AND METHODS

Dry and mature fruits of *S. cana* were collected directly from ten *S. cana* matrices in good health, located in Catimbau National Park, in Catimbau Mountain, Buique-PE, Brazil. Subsequently, they were packed in black plastic bags, labeled, individualized and identified and transported to the Seed Laboratory at Rural Federal University of Pernambuco (UFRPE), after that, the fruits were submitted to processing for seed extraction and the experiments were carried out in the batch composed by the ten matrices.

2.1 Determination of the Moisture Content of Seeds

The water content of *S. cana* seeds was performed by the oven method at 105°C ± 3°C for 24 hours [15], using subsamples of 2 g of seeds, with four replicates. The seeds were packed in aluminum capsules (6 cm in diameter

x 4 cm in height), previously weighed. After this period, the samples were removed and placed in a desiccator, for approximately ten minutes and then weighed in an analytical balance with a sensitivity of 0.0001 g. The resulting water content was given as a percentage.

2.2 Overcoming Dormancy

In addition to the control (T1, seeds that were not subjected to any method to overcome its dormancy), the following treatments were performed: T2 - imbibition of the intact seeds for 24 hours; T3 - scarified seeds with sandpaper n° 100 in the hilo opposite region; T4 - Scarified seeds with sandpaper n° 100 and imbibition in water for 24 hours; T5 - imbibition in hot water at 80 °C until reaching room temperature.

The seeds were disinfected with 5% sodium hypochlorite solution for five minutes, then washed with deionized water. The sowing was carried out in trays with dimensions of 30 x 22 x 7 cm in length, width and depth, respectively. The substrate used in the pre-germination test was vermiculite of fine granulometry. The wetting was carried out with deionized water, adopting 60% of substrate retention capacity, according to [18]. The trays were placed on countertops of the greenhouse. The mean, minimum and maximum temperatures were recorded daily in the greenhouse by a digital thermohygrometer during the experiment.

2.3 Light Quality on Seed Germination Process and Vigor of *S. cana*

The seeds were submitted to the pre-germinative treatment of mechanical scarification with sandpaper n° 100 and disinfected with 5% sodium hypochlorite for five minutes and washed with deionized water and kept in a germinating chamber, type Biochemical Oxygen Demand (BOD), with four white light fluorescents (4 x 20W) located inside the germinator. Black boxes were used to obtain the continuous dark. The germinative behavior of the seeds submitted to four light conditions was evaluated: white light (WL), far red light (FRL), red (RL) and absence of light (AL).

To obtain the light waves were used combinations of cellophane paper filters and fluorescent and incandescent lamps. To obtain the white light, transparent gerbox boxes were used; for red light, the boxes were lined with two

red sheets of cellophane paper; for red distant, were coated with red and blue cellophane paper, superimposed according to the methodology described by [19]. The absence of light was obtained using the gerbox boxes of black coloration.

The evaluations for FRL, RL and AL were performed daily in a dark room under a security light, using a fluorescent lamp covered with two sheets of green cellophane paper.

The number of germinated seeds was evaluated daily up to the 17th day after sowing and the results expressed as a percentage, using as germination criterion the appearance of the hypocotyl and the consequent emergence of the cotyledons, as well as the beginning of epicotyl emission.

The vigor was determined through the evaluation of the first germination count (FC), germination speed index (IVG), mean germination time (MGT). The first count corresponded to the percentage of seeds germinated in the period of the first normal seedlings, which occurred on the fifth day after sowing. Germination speed index (IVG) was evaluated with the germination test, in which the normal seedlings were counted daily according to [16]. Mean germination time was calculated according to [20], with the results expressed in days after sowing.

2.4 Statistical Analyzes

The data were analyzed in software R, version 3.5.1, with the aid of the ExpDes package, version 1.2 [21]. The Shapiro-Wilk tests for normality of the ANOVA and Bartlett residues were used for homogeneity among the variances at 5% probability. Afterwards, analysis of the variance (ANOVA) was performed, and Tukey's test was applied at a 5% probability.

3. RESULTS AND DISCUSSION

3.1 Seed Coat Ormancy Overcome

The first germination count refers to the germinated seeds observed in all treatments at the beginning of germination test. Thus, after the third day of assembly of the experiment, the highest number of germinated seeds was observed for the treatment with mechanical scarification (T3), with 36% of germination, followed by the treatment of scarification + imbibition for 24 h (T4) (Fig. 1A).

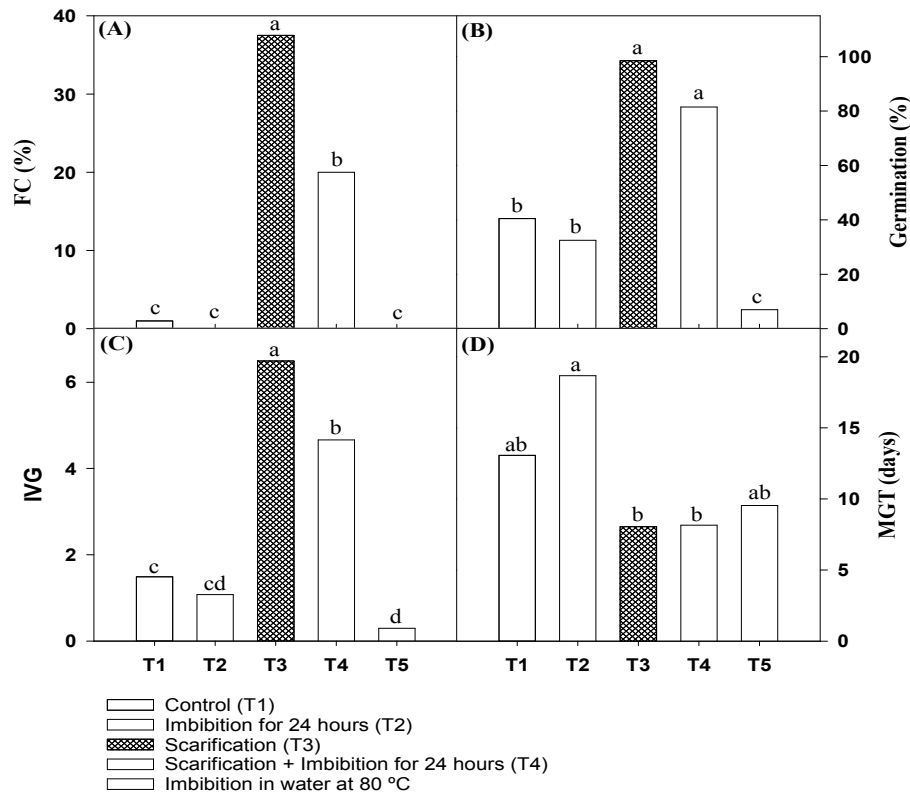


Fig. 1. First germination count (FC) (A); Final germination (%) (B); Index of germination speed (C) and Mean germination time (MGT) (D) of *S. cana* seeds submitted to different pre-germination treatments to overcome its dormancy

In the imbibition treatment for 24 hours at environmental temperature and at 80°C (T2 and T5, respectively) was not observed germination in the first germination count, what make the averages of such treatments do not present statistical difference between them, evidencing physical dormancy in *S. cana* seeds (Fig. 1A) [22,23]. In relation to the imbibition in water at 80°C (T5), it can be inferred that this probably caused the death of the embryo at the end of the experiment, was observed that the seeds were deteriorated, what caused a soft and rotted tegument, since under abiotic stress reactive oxygen species are formed and cause oxidative damages to many cellular components like cell wall and cell membranes, what could explain the embryo death [22-26].

The highest percentages of final germination were verified for the seeds of *S. cana* submitted to the mechanical scarification treatment with sandpaper (T3), followed by the treatment of scarification + imbibition for 24 h (T4), with respect to control (T1), imbibition for 24 h (T2) and the treatment in which the seeds were

submitted to imbibition in water at 80°C (T5). As can be seen in Fig. 1B, the germination of the non-scarified seeds was relatively low (40%), what caused a non-imbibition of the seeds.

In *S. cana*, mechanical scarification promotes a rapid germination, with approximately 70% from the seventh day and, although it did not show statistical difference in the treatment of scarification + imbibition for 24 h, it presented at the end of the test, 20% more germinated seeds, as well as a higher germination speed index (IVG) and a lower mean germination time (MGT) (Fig.1C, 1D).

According to literature the lower the mean germination time, the higher the germination speed [27]. However, for *S. cana* seeds this was not observed for the best treatment, which was mechanical scarification (T3). Thus, the high value of MGT and low IVG, presented by the seeds of the species studied, may indicate that they need a greater intensity in the scarification or even another treatment that provides an increase in the IVG.

The highest value of IVG (Fig. 1C) was observed for the scarification treatment, followed by the treatment with scarification + imbibition for 24 h (T3 and T4, respectively) and the lowest was obtained by the treatment using hot water (T5). It is worth mentioning this variable refers to the maximum number of germinated seeds, in the shortest possible time, which is required in all germination tests, thus, the higher the value, the better the result and the treatment (T3), showed a significant difference in relation to the others, with IVG of 6.5, as well as the shortest germination time (Fig. 1D) was also obtained by T3 and T4, respectively, and did not differ statistically. This fact corroborates with other studies found in the literature for species of the genus *Senna* [28,29], in which different methods, such as immersion in hot water or acid, were efficient, as obtained in this study using the scarification with sandpaper for mass n° 100, which provided a percentage of germination greater than 80%.

The impermeability of the seed coat is associated to several botanical species, being more frequent in species of the family Fabaceae [30]. According to [31], the physical dormancy represents the type of dormancy most observed in seeds that occur in the savannah Biome.

The physical dormancy prevents the water imbibition and consequently the onset of germination process, but when happens the overcoming physical dormancy by any treatment, results in seed coat rupture or weakening and the visible germination begins. What was observed in the seeds of *S. cana* submitted to mechanical scarification, constituting the best pre-germinative treatment for their seeds, being indicated as the most efficient method for the promotion of germination, besides being a simple and low method cost [9,10].

The ecological advantages that dormancy provides refer to their reproductive success and the possibility of occurrence in ecosystems that present limiting and stressful environmental factors to their development and establishment, such as high temperatures, high radiation and mainly water deficit in the ground. Another advantage that these rigid and impermeable teguments provide to the seeds is related to the protection of the embryo through stressful environmental factors and can develop under favorable conditions for germination [1]. It reduces the attack of seed predators in the post-dispersion period and allows these seeds to be

manipulated and/or consumed by different animals without significant damage to the embryo [32].

3.2 Light Quality on Seed Germination

The best result for first germination count was observed for the control (45% of germination), followed by the continuous dark treatment (23%), as well as the seeds that were sown under continuous white light, also presented higher percentage for germination in relation to the other treatments (Fig. 2A). The *S. cana* germinated satisfactorily under a white light (99%) and red light (80%) environment, differing significantly from the far red light environment (40%) and absence of light (50%) (Fig. 2B). Thus, it is verified that the light quality interfered in the germination of *S. cana* seeds, being considered in classificatory term as positive photoblastic [33].

The incidence of red light resulted in a considerable percentage of germination in *S. cana* seeds, increasing gradually until the end of the experiment (17 days), unlike far red light. By absorbing the red light, the phytochromes present in the seeds convert between the active and inactive forms, resulting in stimulation or inhibition of the germinative process [34]. The red light is reported by [35] as a stimulator of seed germination of various species, and this response may be related to the regulation of biosynthesis of gibberellins by active phytochrome, since gibberellins act directly to promote germination.

For some authors the positive photoblastic character would be considered as "preferential" when the occurrence of at least some germination in the condition of absence of light was verified and "absolute" when the seeds did not present the capacity to germinate under absence of light [36-37]. The seeds of *S. cana* germinate both in the presence and absence of light, in this way, they can be considered preferential positive photoblasts, by means of the obtained results, since it obtained percentage of germination of 99% in white light quality (Fig. 2B).

Some authors [35] verified similar behavior when studying seeds of *Mimosa caesalpinifolia* Benth. and classified them as indifferent to light during germination. It was also observed for seeds of *Clitoria fairchildiana* R. A., considered neutral photoblasts, which germinated in all the light regimes provided [38].

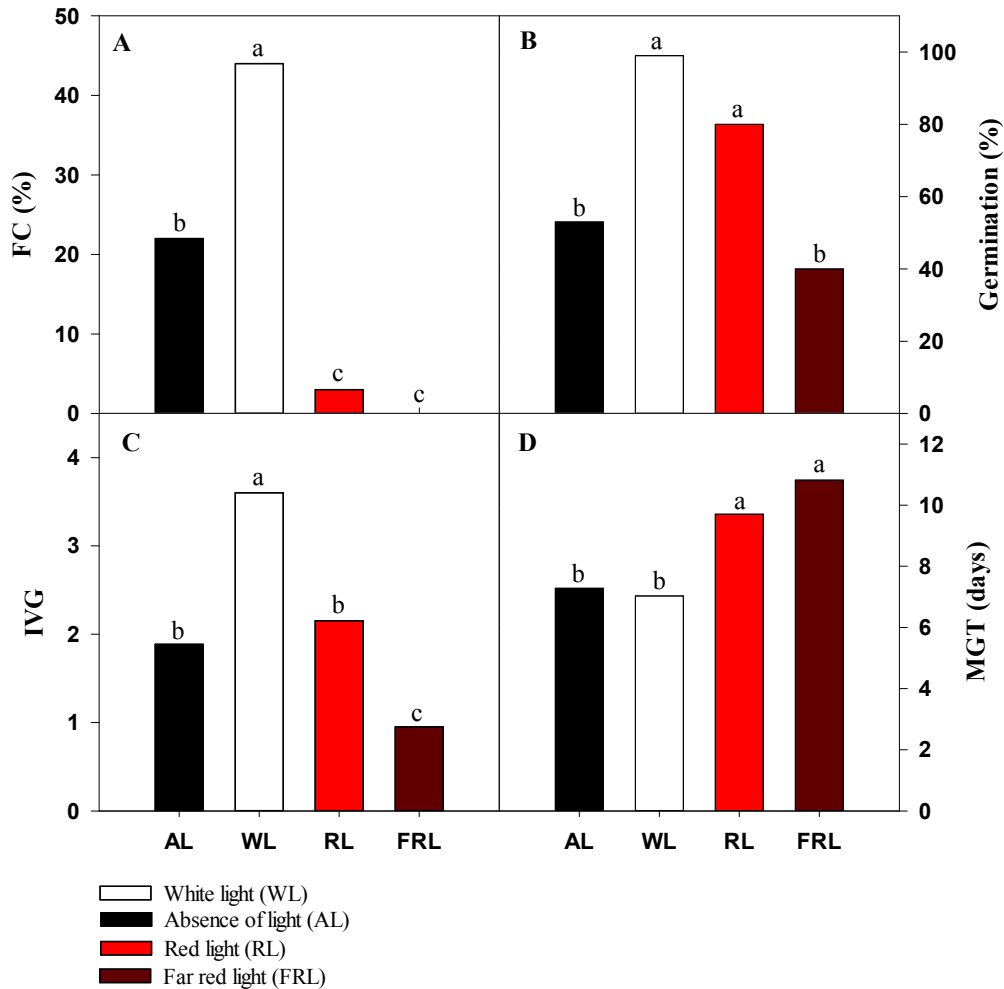


Fig. 2. Effect of different light quality on the first germination count (FC %), percentage of germination (%), germination speed index (IVG) and mean germination time (MGT) of *S. cana* seeds

The highest IVG occurred in the quality of white light and far red light, consequently the highest number of seeds germinated in the shortest time. Thus, the smallest number of seeds germinated in a longer period of time and occurred in the quality of far red light and in the absence of light (Fig. 2C).

The understanding of the IVG contributes to the understanding of the survival and development of the species, since the higher the index, the shorter the exposure time of the seed to the adverse conditions and to the bad weather [27]. The reduction of the IVG, according to [36] is one of the consequences of the physiological potential of the seeds with the condition of the environment in which it is inserted.

This ability of variation in germination represents a very useful ecological strategy for the species *S. cana*, therefore, some seeds must germinate in any light conditions of the environment in where they are, also demonstrating there is no influence of light on germination, and this may occur in areas with different successional stages. Although there is germination in all light qualities, there is greater intensity under the white light spectrum, indicating that the germination is faster when it occurs under a clearing or full sun, where larger thermal amplitudes predominate.

The requirements of the seeds to different qualities of light are related to the ecological groups to which they belong like pioneers, secondary and climax. In general, the pioneer

species germinate under great luminosity, for example in clearings, since the climax species germinate and establish themselves in conditions of little availability of light, like under the forest canopy, while the secondary ones germinate in conditions of light and shade [39].

These characteristics confer to *S. cana* greater germination capacity and consequent establishment of seedlings in the field even though adverse conditions of the environment where it occurs, making it able to withstand wide adverse conditions, especially in semi arid climates.

4. CONCLUSIONS

The seeds of *S. cana* present physical dormancy caused by the seed coat, what results in a low germination speed index. Can be affirmed that the mechanical scarification promotes a high germination rate.

The *S. cana* seeds can be classified as preferential positive photoblast because the germinative response is greater for the qualities of white and red light.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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