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2 **Overcoming dormancy and influence of light on**  
3 **the physiological quality of *Senna cana* seeds**  
4 **(Nees & Mart.) H.S. Irwin & Barneby**

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8 **ABSTRACT**  
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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, 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, to determine the degree of influence of different light regimes in seed germination of *S. cana*. Two experiments were carried out: 1- evaluation of different methods of dormancy overcoming (T1-intact seeds, T2-imbibition of the intact seeds for 24 hours, T3-scarified seeds with sandpaper n° 100 in the hilo opposite region, scarified seeds with sandpaper n° 100 in the region the hilo opposite region and imbibition in 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 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* is considered as indifferent to the luminosity, but can be classified as preferential positive photoblastic by the fact of the germinative response be greater under white and red light.

10  
11 *Keywords: Fabaceae, germination, ecophysiology, temperature, luminosity.*  
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14 **1. INTRODUCTION**  
15

16 From an ecological point of view, it is essential to know the development of plants and where  
17 they grow [1]. Several factors affect the germination process such as: substrate, dormancy,  
18 temperature, humidity, light, oxygen, etc. Thus, the knowledge of these factors is  
19 indispensable to adequate conditions to seed germination process [2].

20 For some seeds the availability of water provides the beginning of germination; however,  
21 others present impermeable tegument, preventing the onset of germination, which is called  
22 dormancy, so that it will need to overcome its dormancy before germination begins [3].

23 The prevention of germination can be attributed to other characteristics of the seeds, such  
24 as embryo characteristics and other structures, including the endosperm, tegument, or even  
25 the action of parts of the fruit. Some authors state that seeds of the Fabaceae family present  
26 physical dormancy due to the water impermeability caused by the seed coat [4].

27 Knowledge of the requirements for germination is important to answer ecological questions  
28 about the species, such as how it develops and the environment in which it grows. Thus, the  
29 germination of the orthodox seeds involves the resumption and continuity of the metabolic  
30 activities, promoting the development of the embryo structures, with the consequent

31 formation of the seedlings, being necessary the favorable performance of environmental  
32 factors such as availability of water, favorable temperature and oxygen and light quality [5].

33 In many species the presence of light promotes the seeds germination, while others, the  
34 germinative performance in the absence of light is more effective. When the light exerts a  
35 positive influence, it is said that the species is photoblastic positive or photoblastic negative,  
36 and seeds indifferent to this factor, which are neutral photoblasts [6-7]. This light requirement  
37 for germination in some species is directly influenced by temperature and according to [8],  
38 the determination of optimal temperature provides maximum percentage of germination.

39 For several species, studies have shown that seeds can germinate under different  
40 temperature conditions [9-14], on the other hand, informations about the appropriate light  
41 requirements for germination of *Senna cana* seeds do not exist.

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

## 45 46 **2. MATERIAL AND METHODS**

47  
48 The fruits of *S. cana* were collected directly from *S. cana* matrices, located in Catimbau  
49 National Park, in Catimbau Mountain, Buique-PE, Brazil. Subsequently, they were packed in  
50 black plastic bags, labeled, individualized and identified and transported to the Seed  
51 Laboratory at Rural Federal University of Pernambuco (UFRPE), after that, the fruits were  
52 submitted to processing for seed extraction and the experiments were carried out.

### 53 **Determination of the moisture content of seeds**

54 The water content of *S. cana* seeds was performed by the oven method at  $105\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$  for  
55 24 hours [15], using subsamples of 2 g of seeds, with four replicates. The seeds were  
56 packed in aluminum capsules (6 cm in diameter x 4 cm in height), previously weighed. After  
57 this period, the samples were removed and placed in a desiccator, for approximately ten  
58 minutes and then weighed in an analytical balance with a sensitivity of 0.0001 g. The  
59 resulting water content was given as a percentage.

### 60 **Overcoming dormancy**

61 In addition to the control (T1, seeds that were not subjected to any method to overcome its  
62 dormancy), the following treatments were performed: T2 - imbibition of the intact seeds for  
63 24 hours; T3 - scarified seeds with sandpaper n° 100 in the hilo opposite region; T4 -  
64 Scarified seeds with sandpaper n° 100 and imbibition in water for 24 hours; T5 - imbibition in  
65 hot water at  $80\text{ }^{\circ}\text{C}$  until reaching room temperature.

66 The seeds were disinfested with 5% sodium hypochlorite solution for five minutes, then  
67 washed with deionized water. The sowing was carried out in trays with dimensions of 30 x  
68 22 x 7 cm in length, width and depth, respectively. The substrate used in the pre-germination  
69 test was vermiculite of fine granulometry. The wetting was carried out with deionized water,  
70 adopting 60% of substrate retention capacity, according to [15]. The trays were placed on  
71 countertops of the greenhouse. The mean, minimum and maximum temperatures were  
72 recorded daily in the greenhouse by a digital thermohygrometer during the experiment.

### 73 **Light quality on seed germination process and vigor of *S. cana***

74 The seeds were submitted to the pre-germinative treatment of mechanical scarification with  
75 sandpaper nº 100 and disinfested with 5% sodium hypochlorite for five minutes and washed  
76 with deionized water and kept in a germinating chamber, type Biochemical Oxygen Demand  
77 (BOD), with four white light fluorescents (4 x 20W) located inside the germinator. Black  
78 boxes were used to obtain the continuous dark. The germinative behavior of the seeds  
79 submitted to four light conditions was evaluated: white light (WL), far red light (FRL), red  
80 (RL) and absence of light (AL).

81 To obtain the light waves were used combinations of cellophane paper filters and fluorescent  
82 and incandescent lamps. To obtain the white light, transparent gerbox boxes were used; for  
83 red light, the boxes were lined with two red sheets of cellophane paper; for red distant, were  
84 coated with red and blue cellophane paper, superimposed according to the methodology  
85 described by [16]. The absence of light was obtained using the gerbox boxes of black  
86 coloration.

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

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

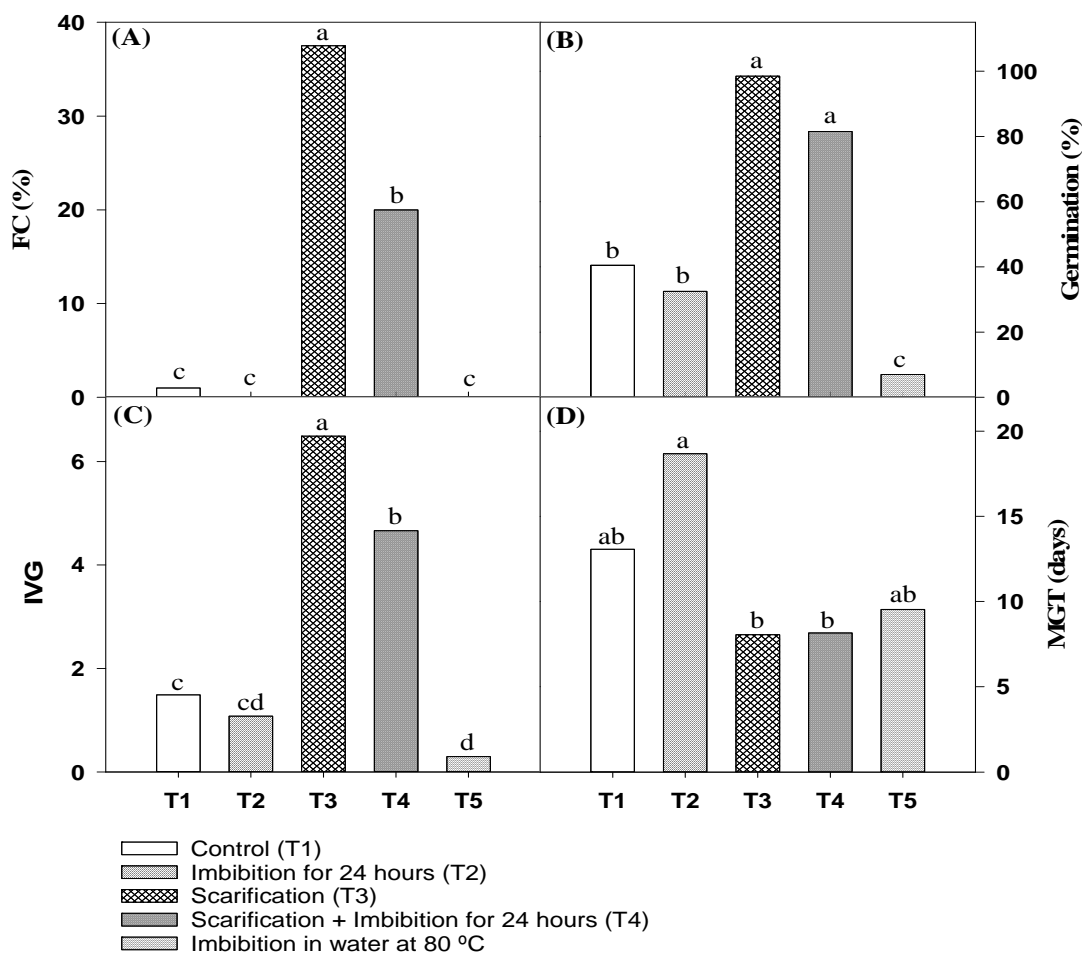
93 The vigor was determined through the evaluation of the first germination count (FC),  
94 germination speed index (IVG), mean germination time (MGT). The first count corresponded  
95 to the percentage of seeds germinated in the period of the first normal seedlings, which  
96 occurred on the fifth day after sowing. Germination speed index (IVG) was evaluated with  
97 the germination test, in which the normal seedlings were counted daily according to [16].  
98 Mean germination time was calculated according to [17], with the results expressed in days  
99 after sowing.

## 100 **Statistical analyzes**

101 The data were analyzed in software R, version 3.5.1, with the aid of the ExpDes package,  
102 version 1.2 [18]. The Shapiro-Wilk tests for normality of the ANOVA and Bartlett residues  
103 were used for homogeneity among the variances at 5% probability. Afterwards, analysis of  
104 the variance (ANOVA) was performed, and Tukey's test was applied at a 5% probability.  
105

## 106 **3. RESULTS AND DISCUSSION**

107  
108 The first germination count refers to the germinated seeds observed in all treatments at the  
109 beginning of germination test. Thus, after the third day of assembly of the experiment, the  
110 highest number of germinated seeds was observed for the treatment with mechanical  
111 scarification (T3), with 36% of germination, followed by the treatment of scarification +  
112 imbibition for 24 h (Fig. 1A).



113

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

117 In the imbibition treatment for 24 hours at environmental temperature and at 80 °C (T2 and  
 118 T3, respectively) was not observed germination in the first germination count, what make the  
 119 averages of such treatments do not present statistical difference between them, evidencing  
 120 physical dormancy in *S. cana* seeds (Fig. 1A).

121 In relation to the imbibition in water at 80 °C (T5), it can be inferred that this probably caused  
 122 the death of the embryo, since, at the end of the experiment, was observed that the seeds  
 123 were deteriorated, what caused a soft and rotted tegument. Similar behavior was observed  
 124 other studies [19-22].

125 The highest percentages of final germination were verified for the seeds of *S. cana*  
 126 submitted to the mechanical scarification treatment with sandpaper (T3), followed by the  
 127 treatment of scarification + imbibition for 24 h (T4), control (T1), imbibition for 24 h (T2) and  
 128 the treatment in which the seeds were submitted to imbibition in water at 80 °C (T5). As can  
 129 be seen in Fig. 1B, the germination of the non-scarified seeds was relatively low (40%), what  
 130 caused a non-imbibition of the seeds.

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

136 According to results observed in the literature and in the present research, it is possible to  
137 verify the versatility of the methodologies for the performance of tests to overcoming  
138 dormancy in seeds of the genus *Senna*, in view of the satisfactory results that were obtained  
139 using tests such as mechanical scarification [23] and the use of sulfuric acid [24].

140 The highest value of IVG (Fig. 1C) was observed for the scarification treatment, followed by  
141 the treatment with scarification + imbibition for 24 h (T3 and T4, respectively) and the lowest  
142 was obtained by the treatment using hot water (T5). It is worth mentioning this variable refers  
143 to the maximum number of germinated seeds, in the shortest possible time, which is  
144 required in all germination tests, thus, the higher the value, the better the result and the  
145 treatment (T3), showed a significant difference in relation to the others, with IVG of 6.5, as  
146 well as the shortest germination time (Fig. 1D) was also obtained by T3 and T4, respectively,  
147 and did not differ statistically.

148 According to [25], the lower the mean germination time, the higher the germination speed.  
149 However; for *S. cana* seeds this was not observed for the best treatment, which was  
150 mechanical scarification (T3). Thus, the high value of MGT and low IVG, presented by the  
151 seeds of the species studied, may indicate that they need a greater intensity in the  
152 scarification or even another treatment that provides an increase in the IVG.

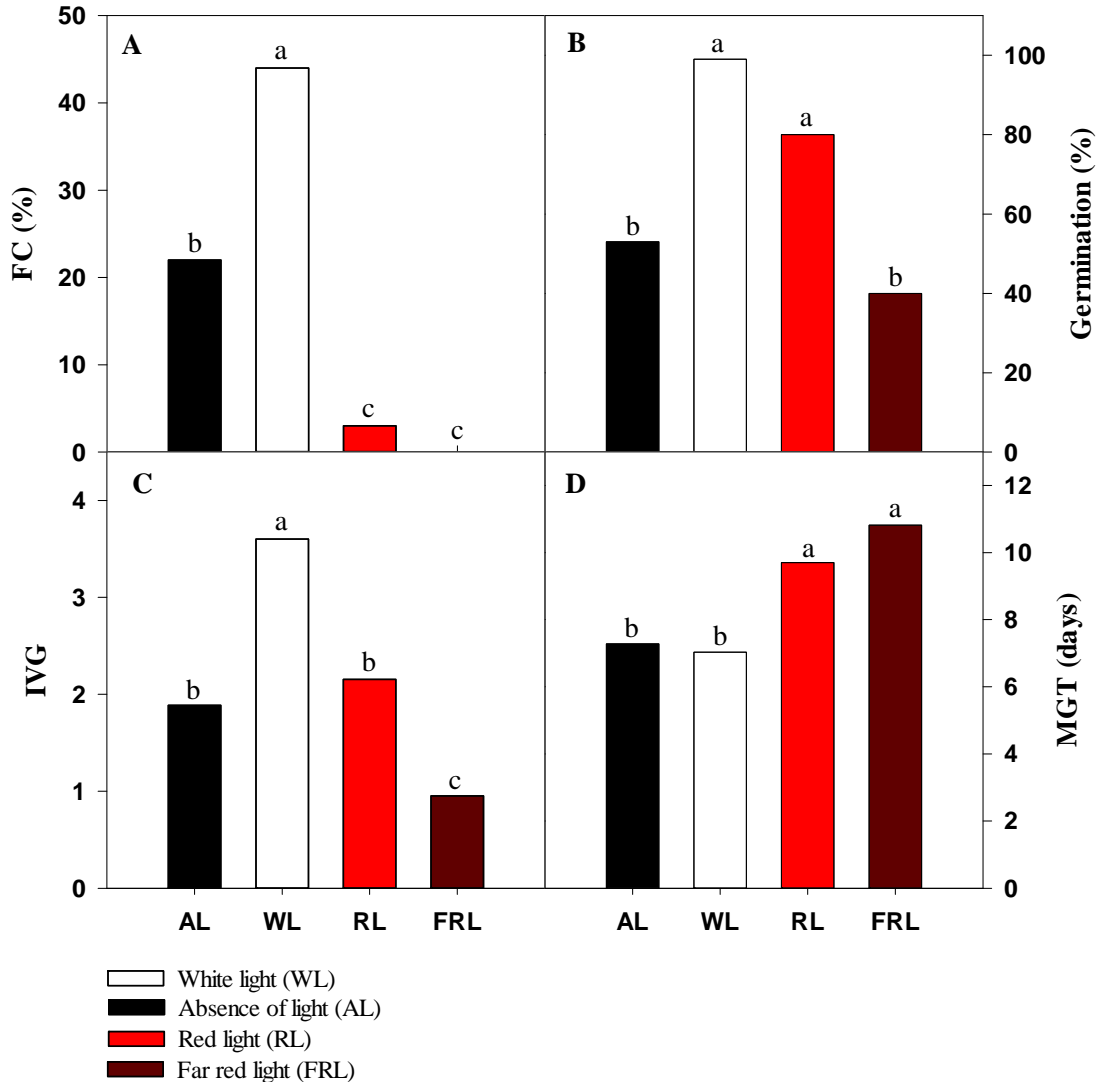
153 This fact corroborates with other studies found in the literature for species of the genus  
154 *Senna*. [26-27], in which different methods, such as immersion in hot water or acid, were  
155 efficient, as obtained in this study using the scarification with sandpaper for mass n° 100,  
156 which provided a percentage of germination greater than 80%.

157  
158 The impermeability of the seed coat is associated to several botanical species, being more  
159 frequent in species of the family Fabaceae [28]. According to [29], the physical dormancy  
160 represents the type of dormancy most observed in seeds that occur in the savannah Biome.

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

168 The ecological advantages that dormancy provides refer to their reproductive success and  
169 the possibility of occurrence in ecosystems that present limiting and stressful environmental  
170 factors to their development and establishment, such as high temperatures, high radiation  
171 and mainly water deficit in the ground. Another advantage that these rigid and impermeable  
172 teguments provide to the seeds is related to the protection of the embryo through stressful  
173 environmental factors and can develop under favorable conditions for germination [1]. It  
174 reduces the attack of seed predators in the post-dispersion period and allows these  
175 diaspores to be manipulated and/or consumed by different animals without significant  
176 damage to the embryo [30].

177 The best result for first germination count was observed for the control (45% of germination),  
 178 followed by the continuous dark treatment (23%), as well as the seeds that were sown under  
 179 continuous white light, also presented higher percentage for germination in relation to the  
 180 other treatments (Fig. 2A).



181  
 182

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

186 The *S. cana* germinated satisfactorily under a white light (99%) and red light (80%)  
 187 environment, differing significantly from the far red light environment (40%) and absence of  
 188 light (50%) (Fig. 2B). Thus, it is verified that the light quality interfered in the germination of  
 189 *S. cana* seeds, being considered in classificatory term as positive photoblastic [31].

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

194 The understanding of the IVG contributes to the understanding of the survival and  
195 development of the species, since the higher the index, the shorter the exposure time of the  
196 seed to the adverse conditions and to the bad weather [25]. The reduction of the IVG,  
197 according to [32] is one of the consequences of the physiological potential of the seeds with  
198 the condition of the environment in which it is inserted.

199 The incidence of red light resulted in a considerable percentage of germination in *S. cana*  
200 seeds, increasing gradually until the end of the experiment (17 days), unlike far red light. By  
201 absorbing the red light, the phytochromes present in the seeds convert between the active  
202 and inactive forms, resulting in stimulation or inhibition of the germinative process [33]. The  
203 red light is reported by [34] as a stimulator of seed germination of various species, and this  
204 response may be related to the regulation of biosynthesis of gibberellins by active  
205 phytochrome, since gibberellins act directly to promote germination.

206 For some authors the positive photoblastic character would be considered as "preferential"  
207 when the occurrence of at least some germination in the condition of absence of light was  
208 verified and "absolute" when the seeds did not present the capacity to germinate under  
209 absence of light [32]. The seeds of *S. cana* germinate both in the presence and absence of  
210 light, in this way, they can be considered preferential positive photoblasts, by means of the  
211 obtained results, since it obtained percentage of germination of 99% in white light quality  
212 (Fig. 2B).

213 Some authors [35] verified similar behavior when studying seeds of *Mimosa caesalpinifolia*  
214 Benth. and classified them as indifferent to light during germination. It was also observed for  
215 seeds of *Clitoria fairchildiana* R. A., considered neutral photoblasts, which germinated in all  
216 the light regimes provided [36].

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

224 The requirements of the seeds to different qualities of light are related to the ecological  
225 groups to which they belong like pioneers, secondary and climax. In general, the pioneer  
226 species germinate under great luminosity, for example in clearings, since the climax species  
227 germinate and establish themselves in conditions of little availability of light, like under the  
228 forest canopy, while the secondary ones germinate in conditions of light and shade [37].

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

233 **4. CONCLUSIONS**

234

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

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

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241

242 **COMPETING INTERESTS**

243

244 Authors have declared that no competing interests exist.

245

246

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