Sulfuric acid on breaking dormancy seeds and on emergence and morphology of *Canna edulis* seedlings [1]

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ABSTRACT

The objective of this study was to evaluate the efficiency of time scarification of seeds in sulfuric acid to break the dormancy of seeds, seedling emergence and post-seminal morphology of *Canna edulis*. This way, were determined biometric characteristics of seeds. To dormancy breaking, the seeds were immersed in sulfuric acid 98% during 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 hours and sown in sand in a condition greenhouse. After 21 days emergence percentage, emergence speed index (IVE), shoot length and seedling dry weight of plants were recorded. Periodically, digital images was taken and performed the seedlings morphological characterization. The fruit of *Canna edulis* contains an average of 19.27 globoses seeds of black coloration measuring about 5.62 mm in diameter, the average weight of 1000 seeds is 128.8 grams. The highest percentage of seedling emergence of 60% and 90% are obtained from seed treated with 0.5 hours in sulfuric acid at 10 and 21 days after sowing, respectively. The seeds have hypogeal germination with development of fasciculate roots with root axes differentiated at 4 days after sowing. The protophyll is observed from the eighth day and the seedlings have intense purple coloration at 16 days after sowing.

Keywords: Cannaceae, physical dormancy, coat seeds, post-seminal development.

RESUMO

Ácido sulfúrico na quebra de dormência de sementes e na emergência e morfologia de plântulas de *Canna edulis*

O objetivo deste trabalho foi avaliar a eficiência do tempo de imersão das sementes em ácido sulfúrico na quebra de dormência de sementes, emergência de plântulas e morfologia pós-seminal de *Canna edulis*. Assim, foram determinadas características biométricas das sementes. Para superação da dormência, as sementes foram imersas em ácido sulfúrico 98% durante 0,0; 0,5; 1,0; 1,5; 2,0 e 2,5 horas sendo posteriormente semeadas em areia em condição de estufa. Aos 21 dias após a semeadura calculou-se a porcentagem de emergência, índice de velocidade de emergência (IVE), comprimento de parte aérea e massa seca de plântulas. Periodicamente após a semeadura foram capturadas imagens digitais de plântulas e caracterizadas a morfologia. Os frutos de *Canna edulis* contém em média 19,27 sementes globosas de coloração preta medindo cerca 5,62 mm de diâmetro, cujo peso médio de 1000 sementes é de 128,8 gramas. As maiores porcentagens de emergência de plântulas de 60% e 90% são obtidas de semente tratadas por 0,5 horas em ácido sulfúrico aos 10 e 21 dias, respectivamente. As sementes apresentam germinação hipógia com desenvolvimento de sistema radicular fasciculado com eixos radiculares diferenciados após 4 dias de semeadura. O protofilo é observado a partir do oitavo dia e as plântulas apresentam coloração púrpura intensa a partir de 16 dias da semeadura.

Palavras-chaves: Cannaceae, dormência física, escarificação, desenvolvimento pós-seminal.

1. INTRODUCTION

The *Canna* genus is unique to Cannaceae Family, with about 50 species distributed mainly in tropical regions (CASTRO, 1995). *Canna edulis* L., known as canna lily, biri or Indian shot is one of the most popular species of this genus, with representatives spread throughout Brazil (Leonel et al. 2002; LORENZI and SOUZA, 2008, Kessler, 2015). These plants are commonly used as ornamentals in the composition of gardens and squares. These plants are rhizomatous, green or purple foliage slightly depending on the stage of development or the degree of shading where they are grown, its flowers are typically red color, orange or yellow (LORENZI and SOUZA, 2008).

These plants have been used worldwide in parks and gardens, including the road landscaping due to its visual aspect and various colorations of flower petals that value as an ornamental plant (KESSLER, 2015).

These plants also has discretion grown in home gardens with ornamental purposes in folk medicine as well as species whose *Canna edulis* and *C. aurantiaca* are traditionally used to ulcers treatment while *C. glauca* is used to wound treatment (FENNER et al., 2006). Similarly, *Canna indica* has been used as a sedative as tea preparation, and flowers and leaves used for emulsion (TEIXEIRA and MELO, 2006).

Recent research has shown the importance these plants as an alternative to production starch due the more higher and regular granules compared with taioba (*Xanthosoma sp.*), arrowroot (*Maranta arundinacea*), arracacha (*Arracacia xanthorrhiza*) and taro (*Colocasia esculenta*) (LEONEL, 2007). In South Asia, northern India and Colombia the *Canna edulis* rhizomes are used as raw material for starch extraction to produce biscuits and other foodstuffs (LEONEL et al. 2002).

Although *Canna edulis* can be used in various areas of interest, there is little information about the propagation

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to commercial cultivation. According Lorenzi and Souza (2008) *Canna edulis* can be propagated by plant division through rhizomes or by seeds. The sexual propagation is important in genetic improvement by enabling the production of more productive hybrids as well as the production of large amount of seedlings without damaging the plant matrix (BARBOSA et al., 2005).

A main limitation of *Canna* seed germination is the presence of physical coat dormancy. This is because the mechanical strength and water restriction is assigned to the exotestal layer of Malpighian cells, which acts as a physical barrier to imbibition, for this the seeds do not imbibe water they remain hard and unswollen (GRAVEN, et al., 1997). These authors reported that *Canna sp*. seeds are small and spherical containing physical hard coat which makes difficult the mechanical scarification (IMANI et al., 2014). Almeida et al. (1991) reported that the germination of seeds *Canna warzeviczzi* in nature is limited by coat numbness, however when treated with acid they can achieve high germination percentage.

Studies show that the immersion *C. indica* seeds in sulfuric acid for 1, 2, 3 or 4 hours enabled the germination percentage of 76%, 79%, 91% and 73%, respectively, at 16-day after sown, while untreated seeds germinated 0% (JOSHI and PAINT (2010). The mechanical scarification of *C. indica* seeds provides germination percentages above 80% under wide range of temperature from 15 to 30 °C, implying that a coat seed is the main limiting factor to germination and the temperature has low influence on this process (SOUZA FILHO et al., 2011).

Breaking seeds dormancy allows high percentages of germination and more homogeneous seedlings emergence, as well as post-seminal characterization is a study contributes important information for conservation of seeds, differentiation of taxonomic groups, conservation and characterization of species as well as the optimization of crop production with a view to commercial production (MELO and VARELA 2006; SIMÃO and SCATENA 2003).

Although the seed dormancy breaking and morphology of seedlings are important for *Canna edulis* propagation, There are few studies on this area of study. Thus, the aim of this study was to evaluate the time in sulfuric acid seed soaking on breaking dormancy of seeds, seedling emergence and characterize the post-seminal morphology of *Canna edulis*.

2. MATERIAL AND METHODS

Canna edulis capsule-shaped fruits, fully ripe, showing dehydrated and brown color with of black color seeds, were manually collected in Viçosa city, Minas Gerais state (20º45' Latitude South, 42º55' Longitude West and altitude of 689,7 m) from plants grown in Floriculture Sector of the Department of Plant Science of Federal University of Viçosa. The fruits and seeds were analyzed to determinate the biometrical characteristics as number of seeds per fruit by direct counting, diameter of the seed using digital caliper and weight of 1000 seeds according rules for seed analysis (BRASIL, 2009b).

After processing to remove inert materials, impurities, the seeds were submitted to different times of immersion in concentrate sulfuric acid (98%) to time of 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 hours. During the immersion time, the containers were agitated every 5 minutes to ensure a homogeneous scarification. After treatment, the seeds were washed in water to remove excess acid and sown at 1.5 cm depth in plastic trays containing washed sand as substrate according emergency test (BARBOSA et al., 2005, BRASIL, 2009b). The substrate was moistened until beginning of drainage and the maintenance of moisture was manually performed through irrigation, every two days for 21 days. After 21 days the following variables were evaluated.

Emergency percentage: were considered emerged the seedlings that had epicotyl or leaflet longer than 0.5 cm above the level of the substrate and the results were expressed as a percentage (BRASIL, 2009b).

Emergence speed index (ESI): the seedless emergency was daily recorded for 21 days, similarly to the proposed methodology to Rules for Seed Analysis (BRASIL, 2009b), and the ESI was calculated according to the formula proposed by Maguire (1962).

Seedling length: the roots and shoots of seedlings were measured with the aid of calipers graduate, and the results are expressed in centimeters per seedling (PEGO et al., 2015);

Dry weight of seedlings: seedlings were dried in a forced-air oven at 70 °C until constant weight of dry matter (PEGO et al., 2015).

The experimental design was completely randomized, with six treatments and five replications of 30 seeds. For the analyzes of length and mass were used 5 repetitions with one plant per plot at random within each treatment. Data were subjected to analysis of variance and the significant effects submitted to polynomial regression.

To post-seminal development of *Canna edulis* seedlings characterization, the seeds were scarified with sulfuric acid for 30 minutes. The seeds were washed in running water to remove excess sulfuric acid and sown according to the method described for seedling emergence. The seedlings were analyzed in periods of 0, 2, 4, 8, 12, 16, 18 and 20 days. For this they have been carefully removed from the substrate with a spatula to help preserve the integrity of root system, washed in running water to remove excess sand and photographed using a digital camera. The main morphological characteristics of color, shape and length were recorded at each time to morphological description.

3. RESULTS AND DISCUSSION

The *C. indica* fruits are a trilocular capsule type developed in a terminal inflorescence, in average each inflorescence produce five fruits (BRASIL, 2009a). Each fruit containing in average 19.27 seeds (Table 1). The Seeds are globular form and black color measuring about 5.6 mm in diameter with an average weight of 1000 seeds is 128.8 grams.

Table 1. Number of seeds per fruit, diameter of seeds and weight of 1000 seeds of *Canna edulis*.

	Number of seeds per fruit	Diameter of seeds (cm)	Weigth of 1000 seeds (g)
Average	19.27	5.62	128.8
Maximum	26.00	6.68	130.7
Mínimum	16.00	4.98	127.0
SD	2.99	0.42	0,13
CV (%)*	15.50	7.48	1,03

* SD = Standard deviation; CV = Coefficient of variation.

* SD = Desvio padrão; CV = Coeficiente de variação.

The biometrics of seeds is important to establish the seeds of harvest time, helps in optimizing the seeding process and to establish criteria harvesting, processing, storage and marketing (HAY and PROBERT, 2013).

The *Canna edulis* seed scarification using sulfuric acid caused noticeable changes in the seed envelope mainly in significant loss of color and intensity of loss of seed coat which allowed the white colored embryo exposure (Figure 1).

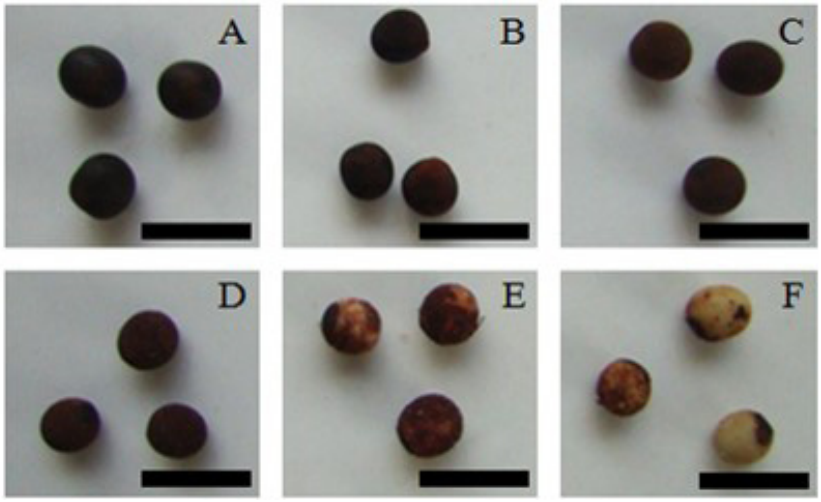

Figure 1. Visual aspect of *Canna edulis* cost of seeds scarified by immersion in sulfuric acid at time 0 (A), 0.5 (B), 1, 0 (C), 1.5 (D), 2.0 (E) e 2.5 (F) hours. Bars = 1cm.

The seed coat of *Canna* genus have exotesta constituted of palisade malpighian cell layers that provide mechanical strength in addition to silica beads, callose and lignin in its upper layers addition of lipids (GROOTJEN and BOUMAN, 1988; GRAVEN, et al., 1997). These structures limit the absorption of water giving physical dormancy in these plants, thus, the chemical scarification is an alternative to overcome the physical impediment provided the seed coat composition (BASKIN et al., 2000; SOUZA FILHO et al, 2011).

The highest percentage of seedling emergence, 90%, was observed at 21 days after sowing seeds treated by immersion in sulfuric acid for 0.5 hours (Figure 2A). Also was observed the presence of hard tegument limited germination of untreated seeds in which the germination was 8%.

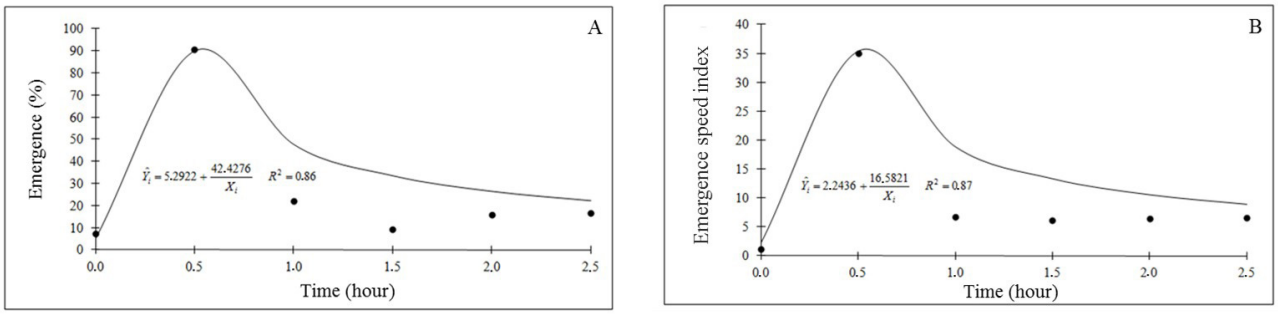

Figure 2. Porcentage of emergence (A) and emergence speed index (B) of *Canna edulis* plants treated by immersion in sulfuric acid at different times.

Seed treatment for longer than 1.0 hours had significantly decreasing of percentage of seedlings emergence, possibly due to phytotoxic or harmfull effect caused by contact of acid sulfuric on embryo tissues. It was observed during the experiment from the 17th day after sowing, the seedlings from seeds treated major that 1.0 hours had chlorotic leaves whose lesions progressed to necrosis causing death of seedlings.

In research laboratories, it is common the use of concentrated acids in order to simulate environmental conditions to overcome numbness due to the impermeability of the integument and promote seed germination (KOSZO et al., 2007). The acid scarification with sulfuric acid was effective in breaking dormancy *C. indica*. These authors observed that the seeds exposed for periods of 1.0, 2.0, 3.0 or 4.0 hours germinated about 76%, 69%, 81% and 63%, respectively; however, untreated seed had no germinate indicating that seeds of this species have hard coat reported (JOSHI and PANT, 2010). In our study was observed that *Canna edulis* seeds also have coat hard since less than 10% of seedlings were obtained at the end of 21 days, confirming the existence of hard seed coat which limits the seedling emergence.

The most emergency speed index obtained was 36 from the seeds scarified for 0.5 hours (Figure 2B). These results are important for understanding the germination behavior of seeds, according to the Rules for Seed Analysis the emergency speed index, as well as the first count are tests used to assess the strength and physiological seed quality and the choice methods for optimization of seed germination and lots for sale classification (BRASIL, 2009b).

Studies shown that *C. indica* exhibit rapid germination in a wide range of temperature to germination of 15°C to 35 °C when scarified, showing that the major limitation to produce rapid and uniform seed is mainly related to overcome dormancy in that with external factors such as temperature (SOUZA FILHO et al., 2011). A occurs in *Canna edulis*, *Strelitzia reginae* seeds, both of Zingiberales order, have higher speed of emergence when treated with sulfuric acid as compared to untreated seeds (BARBOSA et al., 2005).

The shoot length of seedlings obtained from seeds scarified for 0.5 hours was significantly higher than in untreated seedlings or immersed for periods longer than 1.0 hours (Figure 3A).

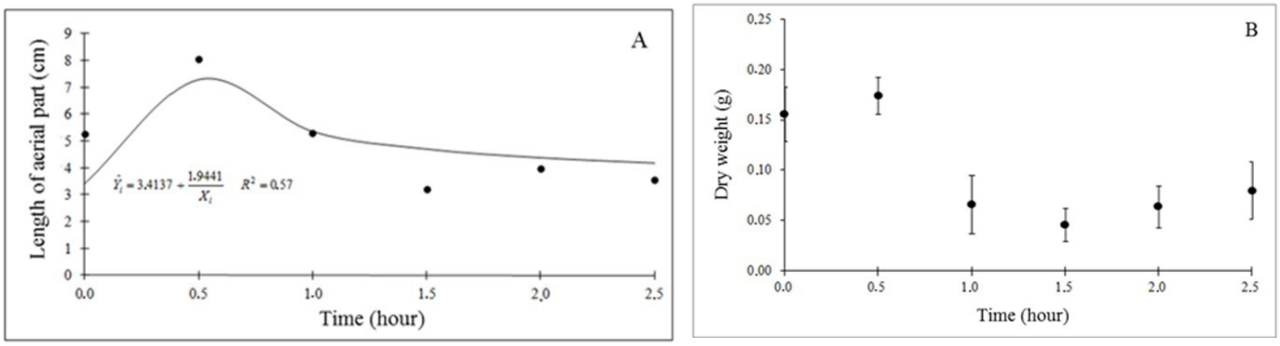

Figure 3. Length of aerial part (A) and dry weight of *Canna edulis* plants (B) treated by immersion in sulfuric acid at different times.

Seedlings obtained from scarified seeds of *C. indica* showed on average 2.7 cm shoot length to 16 days after sowing, but there was no significant difference between treatments (JOSHI and PANT, 2010). The length of seedlings is a test used to assess vigor of the seed lots classification and to evaluate the effectiveness of breaking dormancy of ornamental species seeds as *Bowdichia virgilioides* and *Strelitzia Reginae* (SAMPAIO et al., 2001; BARBOSA et al. 2005).

There was no mathematical model to adjust for the variable fresh weight of seedlings; there was no difference between the dry weight of seedlings from untreated seeds and those obtained from seeds treated by 0.5 hour; however these seedlings were significantly higher than weight fresh seedlings obtained from seeds treated for periods longer than 1.0 hours (Figure 3B).

Although the emergence percentage of *Canna edulis* seedlings from untreated seeds was low when compared to seeds scarified for 0.5 hour, the emerged seedlings have similar development, that because no other effect was observed for limiting the fresh weight of seedling development. On the other hand, the scarification of the seeds for longer than 1.0 hours was damaging to seedlings possibly due to excessive corrosion of acid or soak this comprises the embryo causing leaf chlorosis due to toxicity, which the delayed seedlings development. A similar effect was observed by Joshi and Pant (2010) which *C. indica* seedlings indicates treated with sulfuric acid, these showed no significant differences in dry weight when treated with sulfuric acid having an average of 0.19 mg per plant, the authors also inferred the possible phytotoxic effect of limiting the development sulfuric acid.

The post-seminal development of *C. edilis* seedling performed during 20 day enabled to observe that the seedlings have hypogeal germination. The root protrusion starts at 2 days after sowing by opening the operculum due pressing the embryo axis (Figure 4B), this stage was observed differentiation any structures but only a visible axle white coloring of 0.1 to 0.2 cm of length. After 4 days, was observed the presence of primary root to 0.6 cm long and starting the differentiation of adventitious roots, both have white color; in this stage were accounted on average nine adventitious roots per plant (Figure 4C).

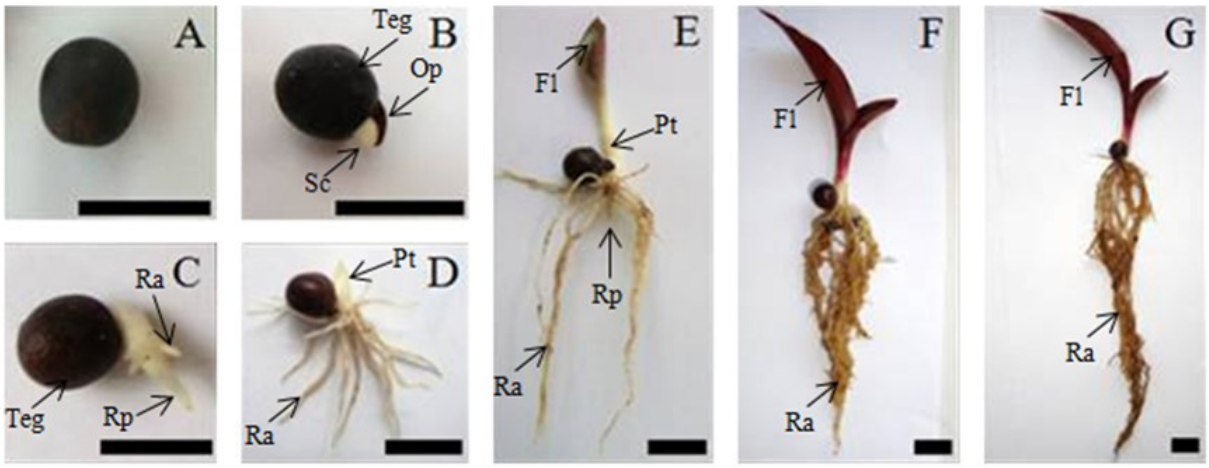

Figure 4. Post-seminal morphology of *Canna edulis* plants at 0 (A), 2 (B), 4 (C), 8 (D), 12 (E), 16 (F) e 20 (G) days after sowing. Teg = seed coat, Op = operculum, Ra = adventitious roots, Rp = primary roots, Pt = protophyl, Fl = leaf. Bars = 1cm.a

Eight days after sowing, on average 15 white color roots measuring on average 1.7 cm long were accounted. At this stage was observed early chlorosis of the primary root (Figure 4D). A leaflet of white color measuring 8mm long was observed; some of them presented green color due exposure to light and synthesis of chlorophyll pigment.

After 12 days, the seedlings had an average 18 roots measuring 6.5 cm in length were observed (Figure 4E). Necrosis on main root was observed in many seedlings being the development of fascicular root system, characteristics of monocotyledonous plants. These seedlings had shoot 1.0 mm containing one leaf with green color on adaxial leaf surface and purple on abaxial leaf surface.

Canna edulis plant 16 days old had an average of 1.7 roots measuring 8.4 cm roots and shoots of 1.4 cm height. In this stage, the plants presented two leaves of purple color on both sides (Figure 4F). In the final analysis, after 20 days of development, few morphological changes were observed, the most significant differences the largest root length, 11.5 cm in length on average of slightly brownish color; aerial parts of 2.3 mm in length composed of 2 to 3 fully purple leaves (Figure 4G).

The remaining structures of the seeds remained attached seedlings during the whole period; however, these seedlings can be transplanted into pots or beds with high percentage of survival.

In synthesis, studies show that the seeds of *Canna edulis* are globular and have hard seed coat that causes physical numbness limiting germination. Soaking in concentrated sulfuric acid is a promising method to improve the germination since the treatment time not exceeding 30 minutes. About 20 days after sowing the plants can be replanted.

4. CONCLUSIONS

The fruit of *Canna edulis* contains an average of 19.27 globoses seeds of black coloration measuring about 5.62 mm in diameter, the average weight of 1000 seeds is 128.8 grams.

The highest percentage of seedling emergence of 60% and 90% are obtained from seed treated with 0.5 hours in sulfuric acid at 10 and 21 days after sowing, respectively.

The seeds have hypogeal germination with development of fasciculate roots with root axes differentiated at 4 days after sowing.

The plants protophyll is observed from the eighth day and the seedlings have intense purple coloration at 16 days after sowing and are completely developed at 20 days.

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