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Method to overcome dormancy in seeds from unconventional food plant: *Phytolacca americana* (Phytolaccaceae)

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Abstract

Plant species *Phytolacca americana* (Phytolaccaceae) can be classified as UFP (Unconventional Food Plant); it is used for food and/or medicinal purposes due to its analgesic, anti-inflammatory, anti-rheumatic and anti-arthritic properties; moreover, it is suitable to treat different skin diseases. However, its seeds present integumentary dormancy caused by uneven germination. The aim of the present study is to assess the effect of chemical scarification in sulfuric acid on dormancy overcome in *Phytolacca americana* (Phytolaccaceae) seeds. Seeds were immersed in 4 ml sulfuric acid, which was concentrated through different immersion times (2, 5, 8, 11 and 15 min), and compared to the control treatment (without sulfuric acid). The study followed a completely randomized design with six treatments and four repetitions, with 50 seeds per treatment. The following parameters were assessed: days to start germination (DSG), germination (G), germination speed index (GSI), abnormal plantlets (AP), dead seeds (DS), hard seeds (HS) and total dry mass (TDM). Data were subjected to Shapiro-Wilk test for variance normality. The analysis of variance and regression were carried out in RStudio software, version 4.1.0. Means recorded for treatments were compared through Tukey test ($p \leq 0.05$). The treatment based on immersion in sulfuric acid for 2 min was the most efficient in overcoming dormancy in this species, since it led to the best results for germination variables and total biomass accumulation in plantlets.

Keywords: germination; sulfuric acid; UFP; seedlings' production.

Método para superação de dormência em sementes de uma planta alimentícia não convencional: *Phytolacca americana* (Phytolaccaceae)

Resumo

A *Phytolacca americana* (Phytolaccaceae) é uma espécie que pode ser classificada como PANC (Planta Alimentícia Não Convencional), utilizada para fins alimentícios e/ou medicinais com propriedades analgésicas, antiinflamatórias, antirreumáticas, antiartríticas e, também é adequada para o tratamento de várias doenças de pele. Contudo, suas sementes apresentam dormência tegumentar causando desuniformidade na germinação. Objetivou-se com o presente trabalho avaliar o efeito da escarificação química com ácido sulfúrico na superação da dormência em sementes de *Phytolacca americana* (Phytolaccaceae). As sementes foram imersas em 4 ml de ácido sulfúrico concentrado por diferentes períodos de imersão (2, 5, 8, 11 e 15 min) comparadas ao tratamento controle (sem ácido sulfúrico). O delineamento utilizado foi o inteiramente casualizado com seis tratamentos e quatro repetições de 50 sementes por tratamento. Foram avaliados os seguintes parâmetros: dias para iniciar a germinação (DIG), germinação (G), índice de velocidade de germinação (IVG), plântulas anormais (PA), sementes mortas (SM), sementes duras (SD), e massa seca total (MST). Os dados foram submetidos ao teste de Shapiro-Wilk para normalidade das variâncias. E a análise de variância e de regressão por meio do software RStudio versão 4.1.0. Sendo as médias dos tratamentos comparadas pelo teste Tukey ($p \leq 0,05$). O tratamento de imersão em ácido sulfúrico por 2 min mostrou-se o mais eficiente na superação da dormência dessa espécie, proporcionando melhores resultados nas variáveis germinativas e acúmulo de biomassa total das plântulas.

Palavras-chave: germinação; ácido sulfúrico; PANC; produção de mudas.

1. Introduction

Estimates point out that by 2050 the world population must reach 9.1 billion people, and this is a challenge for agriculture when it comes to ensure secure and high-quality food if one takes into account that approximately 80% of human food consumption is represented by plants (DUARTE, 2017). Thus, seeking a healthy life makes people look for new food, based on its functionality and sustainability (LIBERATO *et al*., 2019), as well as increases the demand for studies on plants that have nutritional potential and that are suitable for medicinal purposes.

Unconventional food plants, also known as UFP, are a great source of nutritional and functional human food. The term UFP was created in 2008 by Biologist and professor Valdely Ferreira Kinuppe; it refers to all plants that have one or more edible parts. These plants can be spontaneous or grown, native or exotic, and they are not included in our daily cuisine. Moreover, they play the role of functional food, because they present essential vitamins, fibers, antioxidants and mineral salts in their composition; in other words, all nutrients necessary for our body. They can also be used as spice, dye, and in infusion as herbal and folk medicine (KELEN *et al*., 2015).

Genus *Phytolacca* (Phytolccaceae) holds approximately 20 herbaceous, shrub or arboreal species with tropical, subtropical and temperate distribution. This genus is different from the other ones in its family, because its leaves are not chordate, their sepals are subisomorphic and slightly fleshy; the ovary is superior, sessile and formed by 5 to 16 united carpels (STEINMANN, 2010). This genus is represented by only three species in Brazil, namely: *P. dioica* L., *P. rivinoides* and *P. thyrsiflora* (BFG, 2015), which are considered pioneer species in recently deforested terrains, although they are also invasive in some cultures. Species belonging to this genus are commonly known by imbu, deer kalaloo and Pokeweed. Their leaves can be eaten in salads after they are boiled, their green fruits are purgative and the ripe ones provide material for dyeing (SANTOS; FLASTER, 1967).

P. americana (Phytolaccaceae), which is commonly known as American Pokeweed, is an invasive perennial plant native to North America and widely distributed in Northeastern Iran, mainly in costal zones and forests (ZARGARI, 1981; MIRHAYDAR, 1994). It preferentially grows in Mediterranean weather regions, in wastelands or in sites densely inhabited by plants (PATRA *et al*., 2014; ZHELEVA-DIMITROVA, 2013).

It is a small-sized plant, approximately 1 m tall, but it can reach up to 3 m when weather and soil conditions are favorable, such as little mobilized soils rich in nitrates, and nice temperatures with high rainfall rates. Plant growth, development and proliferation mostly take place in humid forests, riparian forests, edges of cultivated fields, roadsides and clearings (MANESS *et al*., 2014; TAKAHASI *et al*., 2003). In morphological terms, this mid-sized species is featured by reddish trunk, big leaves, and white flowers with initially erect linear racemose inflorescences that tend to fall due to fruit ripening (GUEDES, 2015).

The cooked leaves are used in a popular salad in the American diet called “grandma’s salad” (SANTILLO, 1990; MURRAY, 1999). They are also acknowledged by several medical properties and for being commonly used as laxative. They also present analgesic, anti-inflammatory, anti-rheumatic, anti-arthritic activity, as well as are suitable to treat several skin diseases (GOLDESTEIN *et al*., 1973). This plant is traditionally used in Asia and in America to treat gastrointestinal disorders, inflammatory processes, and skin rashes; anticancer and antiviral properties associated with this plant have been recently investigated (MANESS *et al*., 2014; PATRA *et al*., 2014; IWAKIRI *et al*., 2013).

However, studies about *P. americana* germination are scarce because little is known about this species’ reproductive biology, physiology and ecology (ARMESTO *et al*., 1983). One can observe difference in the germination time of *P. americana* seeds in the same raceme, because this species presents autotoxicity, a fact that inhibits germination in part of the seeds; consequently, it leads to greater dispersion and perpetuation in space and time (EDWARDS *et al*., 1988).

Knowing the germination ability of unconventional food plant seeds helps producing seeds belonging to this species and adopting management practices to its cultivation due to its likely use for multiple purposes. Accordingly, the aim of the present study was to assess the germination aspects of *P. americana* (Phytolaccaceae) seeds in order to overcome dormancy in its seeds through chemical scarification in sulfuric acid.

2. Materials and methods

Study site

The study was carried out in the Didactic Laboratory of Seed Analysis, also known as LABSEM, of Federal Rural University of Amazon (UFRA), Belém City, Pará State, Brazil. Seeds were collected in Bujary County, Pará State, Brazil; fruits were collected and taken to the laboratory. Seeds were processed and divided into batches formed of mature seeds that did not present visual damages – malformed seeds, or the ones attacked by animals, were excluded from the experiment.

Germination test

Seeds were immersed in sulfuric acid, which was concentrated in glass beakers and constantly stirred with the aid of glass stick to make its abrasive action uniform at different immersion times (2, 5, 8, 11 and 15 minutes) in comparison to the control treatment (without sulfuric acid). After the determined times were over, seeds were washed in running water and in distilled water for 5 min to allow the sulfuric acid to be fully removed. The experiment followed a completely randomized design with six treatments and four repetitions, with 50 seeds per treatment.

The germination test was carried out in blotting paper (10.5x10.5 cm) in BOD controlled environment (28 °C +/-3), where samples remained under observation for 14 days. The papers were distributed in gerbox sterilized stainless steel vertical chamber autoclave AISI 304. Paper weight (g) was multiplied by 2.5 to determine the necessary amount of water to moisten the paper at sowing, based on the formula suggested by Brasil (2009) – it totaled 317.25 ml of distilled water. Samples were irrigated every two days throughout the experimental time. Studies based on conditions similar to the current one were carried out with other plant species (DIÓGENES *et al*., 2010; NETO *et al*., 2012).

The following parameters were assessed: days to start germination (DST), germination (G), germination speed index (GSI), abnormal plantlets (AP), dead seeds (DS), hard seeds (HS) and total dry mass (TDM). Assessment started at the first germination, 8 days after sowing – plantlets whose essential structures were normal were considered germinated (BRASIL, 2009); this period lasted until the 14th day after sowing. The number of days to first germination was taken into consideration to count the days to start germination (DSG). Normal plantlets, abnormal plantlets, dead seeds and hard seeds were determined based on *Regras para Análise de Sementes* – Rules for Seeds Analysis - (BRASIL, 2009).

Germination speed index was calculated based on the formula suggested by Maguire (1962):

$$\mathbf{SGI=(G1/N1)+(G2/N2)+(Gi/Ni)}$$

Wherein:

SGI – germination speed index

G1, G2, ..., Gi = number of germinated plantlets every day;

N1, N2, ..., Ni = number of days from sowing to the first, second and last counting.

Plantlets were separated in order to find the dry mass biomass of the normal ones, which were stored in paper bags and placed in forced air circulation oven at 65 °C, for 48 hours.

Statistical analysis

Data were subjected to Shapiro-Wilk test to find variances' normality. Analysis of variance and regression were carried out in RStudio software, version 4.1.0. Means recorded for treatments were compared through Tukey test ($p \leq 0.05$).

3. Results and discussion

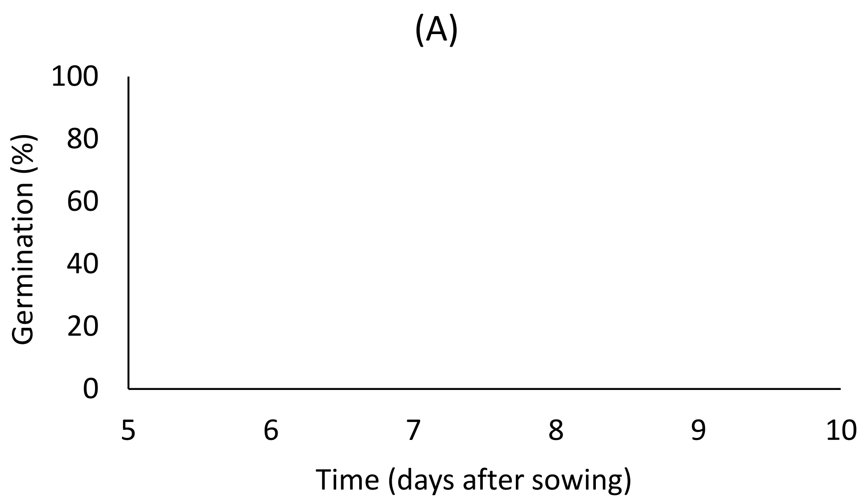
The *P. americana* seeds subjected to treatments based on immersion in sulfuric acid (Figure 1) started to germinate 8 days after the experiment had started. The control treatment did not present germination (seeds that were immersed in sulfuric acid) at the end of the experiment (14 days after sowing); this outcome pointed out that seeds belonging to this species present integumentary dormancy.

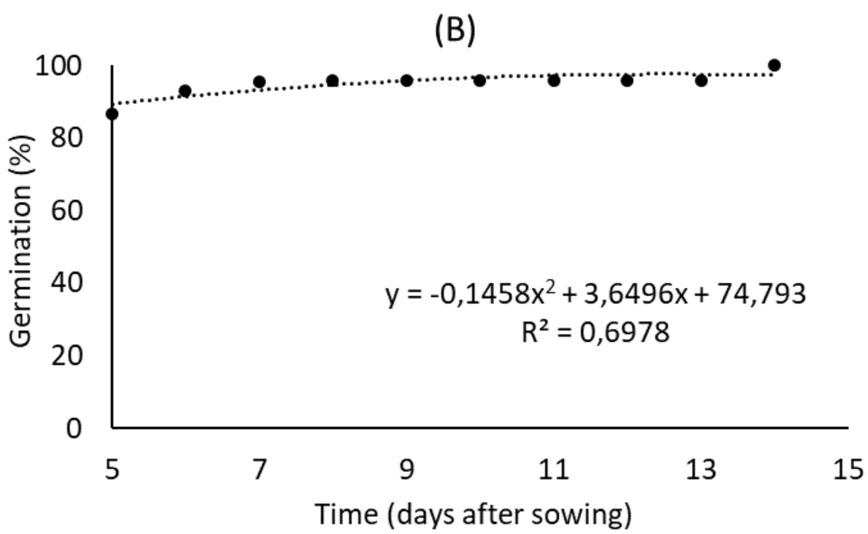
Sulfuric acid application made it feasible boosting *P. Americana* seeds' germination, and this finding evidenced this method's effectiveness in overcoming dormancy and seminal production of this species' seedlings, although with significant difference among the herein assessed treatments. The beginning of germination was recorded 8 days after sowing in treatment based on immersion in acid for 2, 5 and 8 min (Figures 1B, C and D); the other treatments did not germinate until the 14th germination day (Figures 1A, E and F).

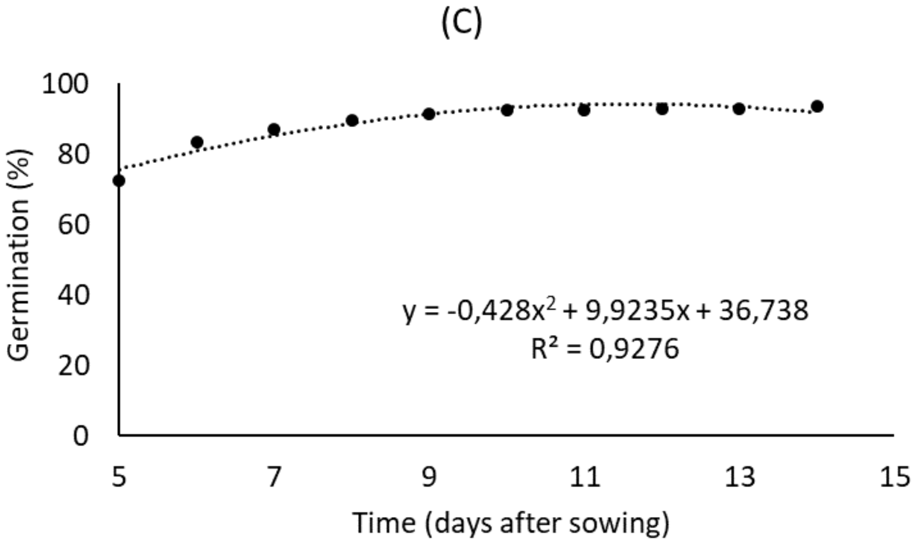
Immersion in acid for 2 min led to fast increase in germination (Figure 1B), which reached 72.5% up to 14 days after sowing; this outcome was similar to that of 5-min immersion in acid (Figure 1C), which reached approximately 40% at the same exposure time. With respect to the control treatment (Figure 1A), there was no water soaking by seeds up to 14 DAS because of integument sealing, which is a dormancy type quite common for species belonging to several botanical families – such a feature impairs permeability to water and oxygen (NETO *et al*., 2012). Integumentary dormancy is broken by mechanical or chemical scarification processes that result in integument disruption or weakening, a fact that allows water and gases to enter in plants, as well as embryo to grow through cell elongation. The cell elongation system results from water capturing and from the accumulation of reserves that trigger the germination process. Among these processes, one can follow the recommendation for using acids or strong bases, such as sulfuric acid and caustic soda, for chemical scarification due to their corrosion capacity (ARAÚJO, 2020).

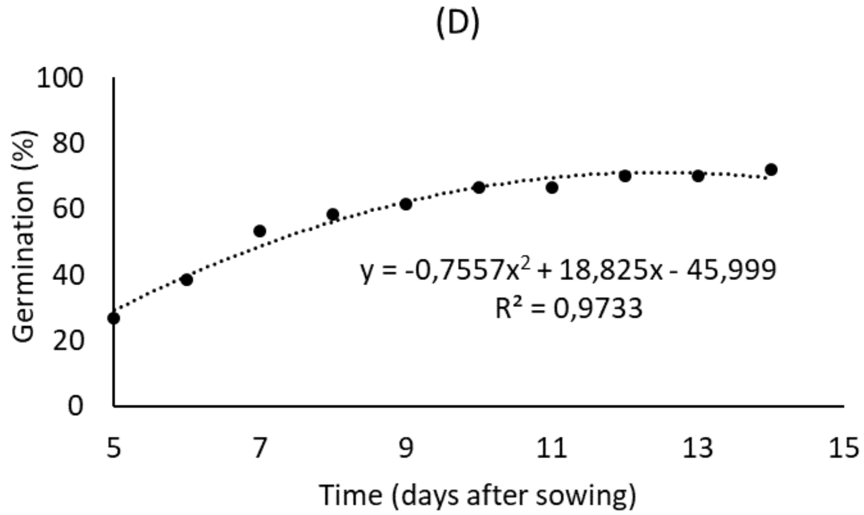
Times longer than 5 min (Figures 1D, E and F) have impaired this species' germination likely due to embryo unfeasibility, if one takes into account the fact that sulfuric acid is a highly corrosive product and that *P. americana* seeds are small. It is known that immersion time in sulfuric acid depends on each species, on its dormancy level or on its size. Therefore, it is essential highlighting the relevance of seeds' exposure time to acids, since exceeding this optimum scarification time can cause damages to the embryonic axis and lead seeds to lose their feasibility and vigor (LIMA; MEIADO, 2017).

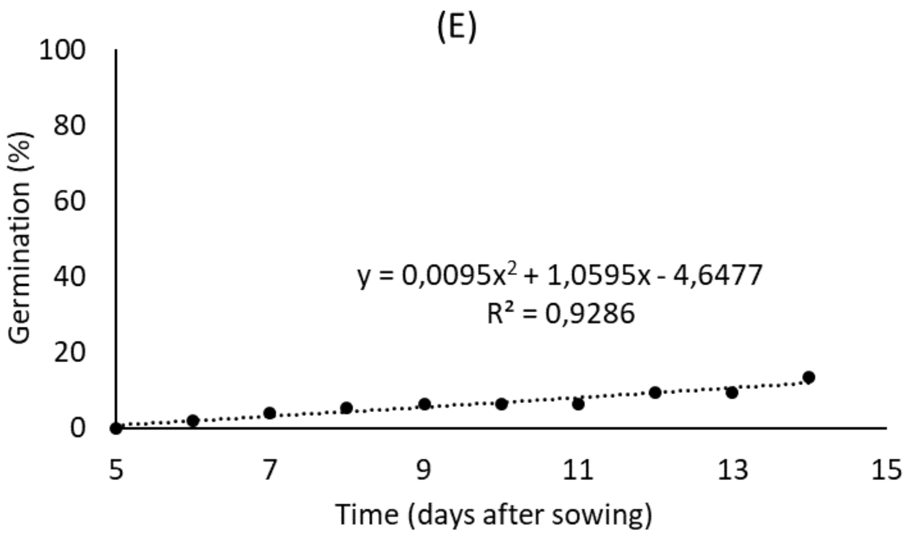
Figure 1. *P. americana* seeds' germination at different sulfuric acid immersion times, control (A), 2 min (B), 5 min (C), 8 min (D), 11 min (E), and 15 min (F).

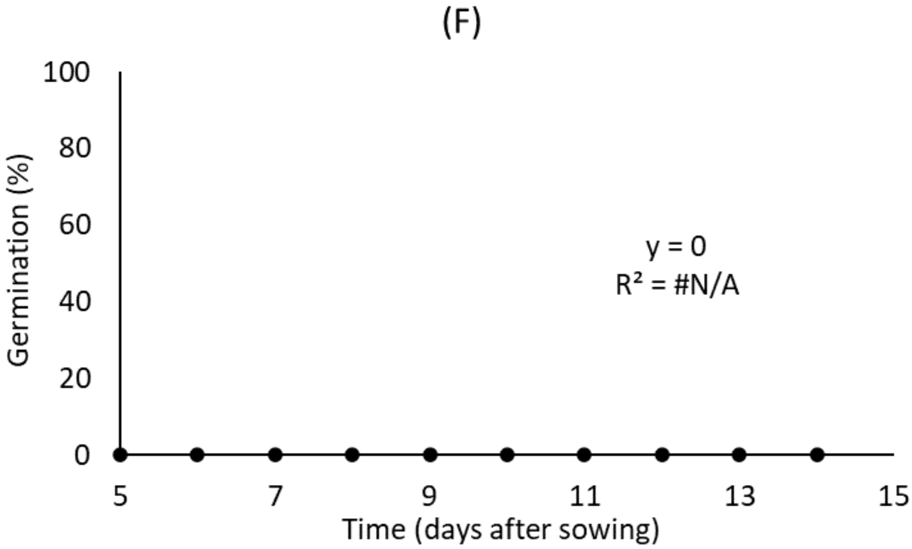





(C)
100
80
60
40
20
0
Germination (%)
5
7
9
11
13
15
Time (days after sowing)
y = -0,428x2 + 9,9235x + 36,738
R² = 0,9276


(D)
100
80
60
40
20
0
Germination (%)
5
7
9
11
13
15
Time (days after sowing)
y = -0,7557x2 + 18,825x - 45,999
R² = 0,9733


(E)
100
80
60
40
20
0
Germination (%)
5
7
9
11
13
15
Time (days after sowing)
y = 0,0095x2 + 1,0595x - 4,6477
R² = 0,9286



Source: Elaborated by the authors.

Table 1 shows that treatments based on 2 and 5-min immersion in sulfuric acid led to 72.5% and 40% germination (G), respectively. They also recorded the best outcomes for germination speed index (GSI): 3.63 and 2.86, respectively. Assumingly, these outcomes result from cracks caused by these treatments in the integument, since they increase seeds' permeability and allow faster soaking, as well as seeds' emergence and germination (OLIVEIRA *et al*., 2017). However, there were similarities among 8, 11 and 15-min treatments; this outcome evidenced the ineffectiveness of these three last action times, likely due to physiological damages in seeds' inner structure. It is so, because the necessary time to promote integument wear and to make it permeable can change depending on the batch of seeds, as well as on species and integument features, such as the structure and conformity of the inhibitory integumentary layer and species' dormancy level (CIPRIANI, 2019).

When one assesses the amount of hard seeds (HS) per treatment, it is possible observing that the control treatment, along with the treatment based on immersion in sulfuric acid for 2 and 5 min recorded 100%, 24% and 43 % HS, respectively. These outcomes corroborate the germination barrier in this species, the so-called 'hardness', which decreases depending on time exposed to sulfuric acid, since treatments that do not promote integument disruption lead to a larger number of hard seeds (PINHEIRO *et al*., 2019). Since 100% of seeds that were not subjected to immersion in acid remained at HS condition, it is fair using the 2 and 5-min treatment times, because they present reduced number of total non-germinated seeds due to such a condition.

The 8, 11 and 15-min treatments did not present hard seeds; however, they would not be the most recommended ones for seeds' germination; the last three treatments based on immersion in acid showed 82%, 98.5% and 100% dead seeds (DS), respectively. As previously mentioned, these acid immersion times likely caused physiological damages in seeds, which could have reached the embryo and led to their death. This outcome is explained by the fact that the study carried out with soybeans has already reported that damages close to the hilum or in the embryo can lead to abnormal plantlets or to dead seeds (DORNELES *et al*., 2021). It is important having in mind that the control treatment and treatments with 2 and 5 acid immersion minutes did not show dead seeds.

With respect to variable "abnormal plantlet" (AP), both the control treatment and the 15-min one, which is based in immersion in sulfuric acid, did not present any outcome, given the fact that the control did not show seeds' germination due to seed hardness; and that the 15-min treatment only presented dead seeds. Nevertheless, 2, 5, 8 and 11-min treatments recorded AP values 3.5, 17, 18 and 1.5, respectively. We can define abnormal plantlets as those that do not show the potential to generate normal plants and to keep their normal development, even if they are growing under favorable conditions (BRASIL, 2009). Assumingly, damage to soaking might have led to the presence of abnormal plantlets at seeds' germination. There are studies reporting that at water excess conditions seeds absorb water too quickly and it leads to tissue disruption (VANZOLIN *et al*., 2007 apud REGAZOLLI, 2019).

Using sulfuric acid as pre-germination treatment led to positive responses for variable "biomass" in comparison to the control – without sulfuric acid (Table 1). Seeds' immersion for 2 and 5 min led to higher mean total dry mass (TDM), and it implied in 0.0959 and 0.0759 g/plantlet, respectively. The disruption of the integumentary dormancy, which was made feasible by seeds' chemical scarification, helped seeds' germination by making it possible for the embryo to rehydrate – embryos rehydrate at initial germination phase and enter the quiescent phase. Thus, germination demands rehydration, which can be defined as growth resumption of mature seeds' embryonic axis and of germination events that take place from the very beginning of this dry seeds' soaking, and of the subsequent embryonic emergence, which will, overall, generate the radicle and plumules – essential structures composing the initial biomass of normal plantlets (TAIZ *et al*., 2017).

Table 1. Days to start germination (DSG), germination speed index (GSI), germination (G), abnormal plantlets (AP), hard seeds (HS), dead seeds (DS) of *P. Americana* subjected to different in sulfuric acid (H_2SO_4) immersion times and non-treated seeds.

Treatment (time immersed in H2SO4)	DSG	GSI	G	AP	HS	DS	SM (g/plantlet)
			%				
Control	0 b	0 b	0 c	0 b	100 a	0 c	0 c
2min	8.25 a	3.63 a	72.5 a	3.5 b	24 c	0 c	0.0959 a
5min	8.25 a	2.86 a	40 b	17 a	43 b	0 c	0.0759 b
8min	8.25 a	0.86 b	0 c	18 a	0 d	82 b	0 c
11min	7.5 a	0.075 b	0 c	1.5 b	0 d	98.5 a	0 c
15min	0 b	0 b	0 c	0 b	0 d	100 a	0 c

Means followed by the same letter did not significantly differ from each other in the Tukey test at p<0.05.

The pre-germination treatment based on chemical scarification for 2 min in concentrated sulfuric acid accounted for the best outcomes; it accelerated germination and improved seeds' vigor, a fact that has helped seedlings' seminal production and management of this species, which has significant nutritional and medical potential. This finding points towards a promising way to produce unconventional food plants that can further help ensuring food security.

4. Conclusion

The treatment based on immersion in sulfuric acid for 2 min was the most effective in overcoming dormancy in *P. Americana* seeds; it led to the best outcomes for germination variables and total biomass accumulation in plantlets.

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