horticultura brasileira

Scientific Communication

ZEIST, AR; CARBONERA, MZ; RECH, C; OLIVEIRA, GJA; TOROCO, BR; SILVA JÚNIOR, AD; GARCIA NETO, J; LEAL, MHS. Soaking time in sulfuric acid to overcome sweet potato seeds dormancy. *Horticultura Brasileira* v.42, 2024, elocation e2560. http://dx.doi.org/10.1590/s0102-0536-2023-e2560

Soaking time in sulfuric acid to overcome sweet potato seeds dormancy

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ABSTRACT

To increase genetic variability, sweet potato needs to be sexually propagated in breeding programs. However, its seeds have a hard and thick integument, requiring some dormancy breaking method. Chemical treatment of seeds allows greater efficiency of the relationship between seeds obtained in crosses and those that germinate, providing genotypes to be tested for their potential, aiming at developing a new commercial cultivar. Thus, the objective of this study was to identify the effects of immersion times of seeds in sulfuric acid (98%) on the germination and initial growth of sweet potato seedlings. The seeds were immersed for five different times: 0 (control), 20, 40, 60, and 80 min. The experiment was carried out in a completely randomized design with ten repetitions consisting of 25 seeds each experimental plot. The germination percentage, germination speed index (GSI), dry mass of whole seedlings, and length of the largest root and shoot of the seedlings were evaluated. The germination percentage and GSI increased with immersion time until 53.30 min, with maximum estimated values of 85.39 and 25.10%, respectively. For the parameters dry mass of whole seedlings, length of the largest root, and shoot length, increases were observed up to 55.00, 37.63, and 44.44 min, respectively. In conclusion, the immersion of sweet potato seeds in sulfuric acid (98%) for 53 min is ideal for breaking dormancy and providing better conditions for the initial development of seedlings.

Keywords: *Ipomea batatas*, chemical scarification, seed germination, genetic improvement.

RESUMO

Tempo de imersão em ácido sulfúrico para superar a dormência de sementes de batata-doce

Visando ampliar a variabilidade genética, a batata-doce necessita ser propagada sexuadamente em programas de melhoramento genético. No entanto, devido à presença de tegumento duro e espesso nas sementes, é necessária aplicação de algum método para quebra de dormência. A escarificação química é a técnica que tem demonstrado maior eficiência da relação entre sementes obtidas em cruzamentos e aquelas que germinam, gerando genótipos a serem testados quanto ao seu potencial, visando desenvolver uma nova cultivar comercial. Assim, objetivou-se identificar os efeitos de tempos de imersão das sementes em ácido sulfúrico (98%) na germinação e crescimento inicial das plântulas de batata-doce. As sementes foram imersas em cinco intervalos de tempo: 0 (controle), 20, 40, 60 e 80 min. O experimento foi realizado em delineamento experimental inteiramente casualizado com dez repetições compostas cada uma por 25 sementes. Avaliaram-se a porcentagem de germinação, índice de velocidade de germinação (IVG), massa seca das plântulas inteiras e comprimento da maior raiz e da parte aérea das plântulas. A porcentagem de germinação e IVG cresceram à medida que se aumentou o tempo de imersão, atingindo os valores máximos estimados de 85,39 e 25,10%, respectivamente, após 53,30 min. Para os parâmetros massa seca das plântulas inteiras, comprimento da maior raiz e comprimento da parte aérea foi verificado aumento nos valores até o limite de 55,00, 37,63 e 44,44 min de imersão, respectivamente. A imersão de sementes de batata-doce em ácido sulfúrico (98%) por 53 min é ideal para a quebra de dormência e proporciona boas condições para o desenvolvimento inicial das plântulas.

Palavras-chave: *Ipomea batatas*, escarificação química, germinação de sementes, melhoramento genético.

Received on September 27, 2022; accepted on March 14, 2023

Sweet potato (*Ipomoea batatas*) belongs to the Convolvulaceae family. It is a hexaploid (2n = 6x = 90) and widely known species, representing one of the most important food crops in the world (Katayama *et al.*, 2017). Moreover, sweet potato has socioeconomic relevance since its tuberous roots are widely used in human and animal nutrition, as well as for biofuel production. Roots are an important source of raw material for industry. They have a wide range of minerals, sugars, and vitamin A, C, and B-complex, in addition to having a high carbohydrate content (Akoetey *et al.*, 2017; Laurie *et al.*, 2015). In contrast, the roots have a low glycemic potential. Nevertheless, it has been possible to obtain biofortified roots through plant breeding (Low *et al.*, 2017).

Sweet potato is highly linked to environmental sustainability since it is a great alternative for agriculture in less developed regions, with the possibility of cultivation in low- to medium-fertility lands. However, productivity in Brazil is 14.0 t/ha, well below the average of other countries, such as China, Senegal, Australia, and Japan (FAO, 2021). This occurs mostly due to the predominant genotypes in Brazil, which are obsolete, susceptible to pests and pathogens, and unresponsive to improvements in crop management (Leal *et al.*, 2021). In addition, in most cases, producers do not invest the least amount of agronomic techniques indicated for their cultivation.

To strengthen sweet potato cultivation, a crucial step is to develop improved genotypes. Plant breeding is necessary to obtain new cultivars with superior quality and yield stability and without major limitations to express their productive potential (Leal *et al.*, 2021). To conduct a genetic improvement program, the obtention of sexual offspring from the cross between parents is needed, and new genotypes are selected according to the breeder's interest. However, sweet potato seeds have a very hard and thick integument, showing irregular and slow germination (Nair *et al.*, 2017; Brito *et al.*, 2021). Thus, seed scarification is essential to remove part of the integument, facilitating water penetration and enabling rapid and uniform germination.

Even without a mandatory dormancy period, sweet potato seeds can remain viable for many years. To accelerate germination, seed scarification can be performed by mechanical abrasion or chemical treatment (Huamari, 1992). Effective results of chemical scarification were obtained using sulfuric acid (H_2SO_4) at a concentration of 98% and mechanical abrasion of sweet potato seeds (Brito *et al.* 2021). Likewise, mechanical treatments that cause rupture of the external integument and chemical treatments using H_2SO_4 at different concentrations result in high germination percentages (Cha *et al.*, 2011; Nair *et al.*, 2017). Generally, H_2SO_4 is efficient for scarifying sweet potato seeds; however, few studies indicate the ideal time of seed immersion and its effects on seedling development.

The exact determination of the immersion time of seeds in H_2SO_4 can directly contribute to breeding programs, making it possible to increase the efficiency of the relationship between seeds obtained and those that germinate and are evaluated in terms of their agronomic potential and food quality (Katayama *et al.*, 2017). Thus, the objective of this work was to identify the effects of immersion times of sweet potato seeds in sulfuric acid (98%) on germination and initial seedling growth.

MATERIAL AND METHODS

The laboratory stages of the experiment were carried out at the Didactic Laboratory of Seeds at the Plant Science Department of the Agricultural Sciences Center (CCA) at the Federal University of Santa Catarina (UFSC) in Florianópolis-SC.

Sweet potato seeds were obtained from July to September 2021 in polycross blocks in Presidente Prudente-SP, in the experimental area of the Center for Studies in Horticulture at the University of Western São Paulo (UNOESTE). The experimental accession UBD-L1-22 and the commercial cultivar BRS Rubissol were used as female and male parents, respectively. The crossing blocks were composed of two lines with 30 plants from each parent. The plants were grown in a greenhouse in 10-dm^3 pots containing sieved soil and cattle manure in a 3:1 ratio. At 75 days after planting the stem cuttings, flowering was stimulated by suspending irrigation for 15 days. Then, hand pollinations were performed. The flowers that showed fertilization had the seeds collected when the seed capsules were dry.

The study consisted of determining the effects caused by the pre-germination treatment of sweet potato seeds to overcome dormancy. Seeds were immersed in 98% sulfuric acid (H_2SO_4) (Anidrol®) for 0 (control), 20, 40, 60, and 80 min. The study was carried out in a completely randomized design with ten repetitions consisting of 25 seeds each. The seeds used in the experiment had a moisture content of 9.6%, as determined by the greenhouse method (105ºC for 24 h) according to Brasil (1992).

After immersion in H_2SO_4, at room temperature, the seeds were washed in running water. Germination paper (Germitest®) was imbibed in distilled water, 2.5 times the paper dry weight. Twenty-five seeds were distributed on the moistened germination paper and placed in a germination box (Gerbox®) previously cleaned with sodium hypochlorite. The germination boxes were then placed in a germination chamber at 25°C±0.5 and 12 h photophase.

Germination was assessed daily for six days. Seeds with a ±3-mm-long radicle protrusion were considered germinated. To evaluate the GSI, the methodology described by Cetnarski Filho & Carvalho (2009) was used: GSI = Σ (ni/ti), where *ni* is the number of seeds that germinated at time *i*, and *ti* refers to the time after test installation (from 2 to 6 days). At the end of the observation period, the dry mass of five whole seedlings of each treatment was quantified, and the lengths of the shoot and the largest root were measured.

The data of the evaluated traits were subjected to analysis of variance and tested for normality and homogeneity of residual variances by the Lilliefors and Bartlett tests, respectively. When the assumptions of normality and homogeneity were met and the F test was significant (5% significance), the means were subjected to regression analysis that was derived to estimate the maximum or minimum points. Statistical analyses were performed using the SISVAR program, version 5.6 (Ferreira, 2014).

RESULTS AND DISCUSSION

For all studied parameters, the data fit the quadratic regression model (Figures 1 and 2). The germination percentage and **GSI** increased with immersion time until 53.30 min, with maximum estimated values of 85.39 and 25.10%, respectively (Figure 1). In most cases, H_2SO_4 weakens the integument and removes the cuticle,

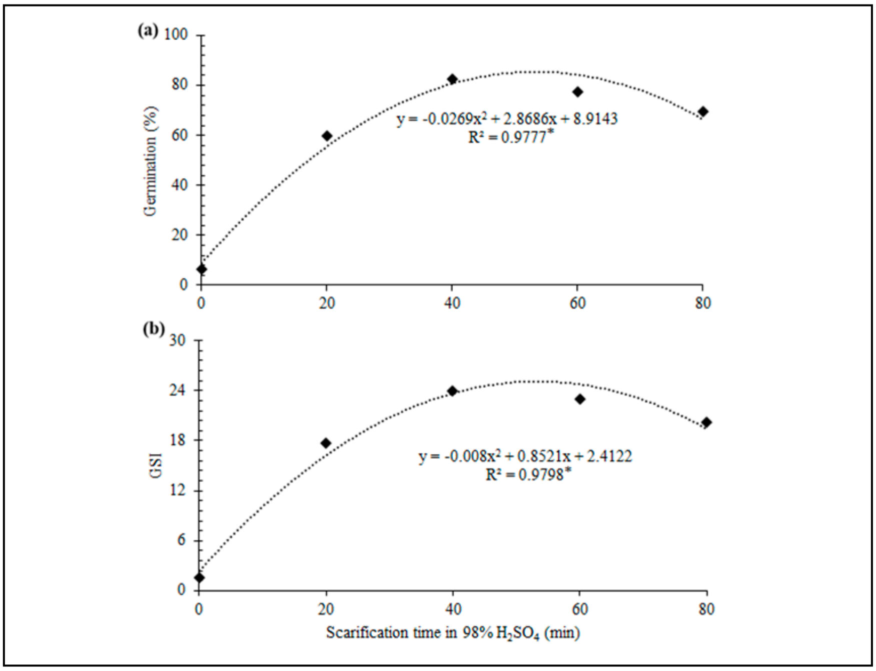

Figure 1. Germination percentage (A) and germination speed index (GSI) (B) of sweet potato (*Ipomoea batatas*) seeds subjected to different immersion times in 98% sulfuric acid (H_2SO_4). The results represent the mean of ten replicates (n= 10). *Significant at 0.05 level (quadratic regression). Florianópolis, UFSC, 2022.

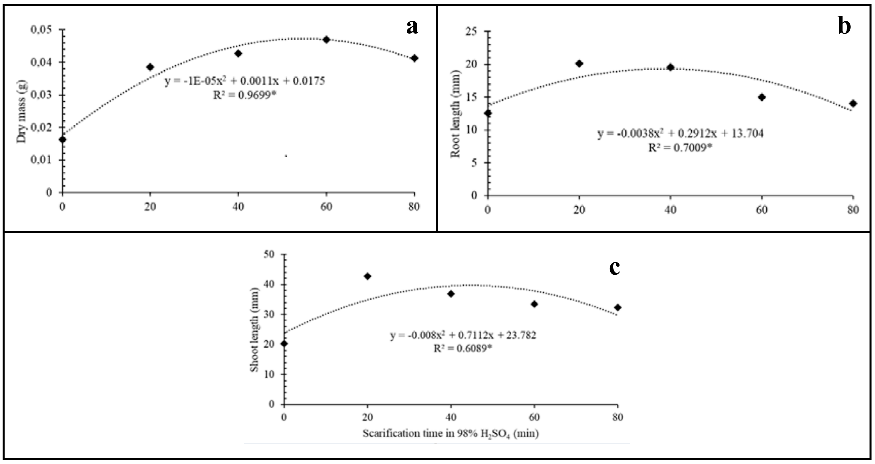

Figure 2. Dry mass of whole seedlings (A), length of the largest root (B), and shoot length (C) of sweet potato (*Ipomoea batatas*) seeds subjected to different immersion times in 98% sulfuric acid (H_2SO_4). For dry mass, values are the sum of five seedlings. The results represent the mean of ten replicates (n= 10). *Significant at 0.05 level (quadratic regression). Florianópolis, UFSC, 2022.

exposing the inner layers of seeds (Abubakar & Muhammad, 2013). The quadratic adjustment of the regressions demonstrates that very long immersion times in H_2SO_4 (above 53.3 min) can damage the seeds, reducing germination again. Chemical scarification degrades the seed coat; however, the increased exposure of seeds to sulfuric acid can cause ruptures in essential cells, impairing germination and emergence and favoring contamination and invasion by fungi (Melo Junior *et al.*, 2018). At the same time, lower exposure times (<53 min) do not provide maximum scarification efficiency of sweet potato seeds and are not the most suitable. Even with this recommendation, it is possible to infer that more prolonged exposure of seeds to H_2SO_4 can contribute to better germination rates, even above 80%, without compromising seed quality and vigor.

H_2SO_4 is commonly used by industry in several applications. It is a strong, colorless, and odorless diprotic mineral acid (Xiao *et al.*, 2022). Furthermore, its degree of ionization is quite high (α = 61%). Thus, when put in contact with organic surfaces, it causes oxidation and dehydration reactions, leading to corrosion. Thus, chemical scarification processes imply direct actions on the seed coat, balancing the entry and exit of water and gases (Long *et al.*, 2015). Generally, H_2SO_4 provides better results than other seed scarification techniques (Niang *et al.*, 2015; Melo *et al.*, 2022). Its high efficiency is also observed in other species of the genus, such as *I. indivisa*, *I. purpurea* (Pazuch *et al.*, 2015), *I. grandifolia*, *I. hederifolia*, and *I. quamoclit* (Azania *et al.*, 2003). In these studies, it was also found that the immersion time of seeds in sulfuric acid greatly influences the parameters related to germination.

The seeds that did not undergo the scarification process (control) obtained a germination rate and GSI of only 6.40 and 1.64%, respectively (Figure 1). Thus, most of the control seeds were dormant. Previous works found that sweet potato seeds, not scarified and only soaked in water, reached 30% maximum germination, which is relatively low and compromises the efficiency of breeding programs (Nair *et al.*, 2017). Results similar to the present work for the control treatment were obtained by Melo *et al.* (2022), where only 6.0 and 7.5% germination for two sweet potato genotypes were found. For Morning Glory (*I. grandifolia*), even lower germinations were observed on water-soaked seeds for 48 h without any additional treatment for scarification (Voll *et al.*, 2003).

For dry mass of whole seedlings, length of the largest root, and shoot length, exponential increases occurred up to 55.00, 37.63, and 44.44 min, respectively (Figure 2). The effects of seed immersion times in sulfuric acid on seedling development and initial growth have been reported for several species

(Silva *et al.*, 2018; Nourmohammadi *et al.*, 2019). Nevertheless, the results found in literature are generally contrasting and inconclusive.

Seedlings are part of an important phase of the entire plant cycle. Thus, seedlings that emerge with perfect essential structures develop morphological, physiological, and biochemical processes with higher quality (Tian *et al.*, 2014). Good seedling root structures are essential in the agronomic performance of sweet potato seedlings and adult plants (Alhadidi *et al.*, 2021). Therefore, the results observed in the present work are relevant, showing that immersion in H_2SO_4 provides better seedling development compared to non-immersion.

Therefore, the immersion time of sweet potato seeds in sulfuric acid influences the germination and initial growth of the seedlings. Sweet potato seeds immersed in sulfuric acid (98%) for 53.3 min show a higher germination percentage and germination speed index. Immersion times between 37.63 and 55.00 min allow greater growth and initial development of seedlings.

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