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Assessment of Germination of Plukenetia Volubilis L. (Sacha Inchi)

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Abstract
Plukenetia volubilis L. is an Amazon species commonly known as sacha inchi, considered a high agro-industrial potential plant due to high nutritional values. Despite some published propagation studies of sacha inchi, to the best of our knowledge, there is little information about its dormancy especially in Malaysia. Thus, this study aimed to assess seed germination of sacha inchi. The germination test used four different treatments: acid scarification, mechanical scarification, an overnight soak in water, and hot water. Two different scarification methods were conducted using sulphuric acid with a concentration of 20% (98.08g/mol) and mechanical scarification using sandpaper (grade 80) sanding gently about 30 sec to 1 min. Hot water treatment was done by soaking the seeds in 82ºC of hot water until the temperature drops to room temperature and control treatment, an overnight soak in water. In this study, the best way to break the seed dormancy of *Plukenetia volubilis* L. was by using mechanical scarification (64% germinated seeds) to promote germination and considered a feasible method.

Keywords: sacha inchi; dormancy; germination

1. Introduction

Sacha Inchi (*Plukenetia volubilis* L.) is a genus of plants in the Euphorbiaceae family, found in various regions around the world and consists of 37 species of climbing plants and vines (Henao, et al., 2022). It is an Amazon rainforest plant that is being recognised for its nutritional value and potential contributions to sustainable agriculture (Castillo, et al., 2019). It is also commonly referred to by various names such as sacha inchi, inca peanut, mountain peanut, and many more (Khine, et al., 2020). There has been an increase in the utilisation of sacha inchi over the past decade (Henao, et al., 2022).

Sacha inchi is a perennial climbing plant that has different chemical constituents from different parts of the plant (Puangpronpitag, et al., 2021). Its star-shaped fruits, which turn brownish black from green when ripe, contain four, five, or six lenticular seeds covered in shells (Norhazlindah, et al., 2023). Indigenous people roasted its seeds to remove the beany flavour and prevent constipation (Goyal, et al., 2022). Its seeds contain lipids comprising omega-3 (α-Linolenic acid), omega-6 (linoleic acid), and omega-9, which make up 35 to 60% of their composition (Rahim, et al., 2023). The seeds are known to contain edible proteins (25 ~ 60%) such as threonine, tryptophan, cysteine, and tyrosine, as well as polyphenols and vitamin E (Zhan, et al., 2021). These properties make the seeds suitable for dietary purposes, as they help eliminate the beany taste and prevent constipation (Goyal, et al., 2022). Its leaves are heart-shaped and consist of phenolic compounds, terpenoids, and saponins but it is only edible once it is cooked (Sethuraman, et al., 2020). The valuable chemical components found in sacha inchi leaves have led to its extensive utilisation in the pharmaceutical, cosmetic, nutraceutical, and food sectors (Kittibunchakul, et al., 2022). On top of that, the plant also has been categorised as a high agro-industrial crop (Valente, et al., 2017).

In 2017, the Malaysian government introduced sacha inchi to small farmers in Perak as part of their initiative to increase their income and has gained popularity in Malaysia (Berita Harian, 2017). Nevertheless, the small-scale farmers have encountered difficulties in achieving rapid seed germination (Supriyanto, et al., 2022). The Plant Database states that seeds usually start to germinate 14 days after planting (Plant Database, 2019). This species typically reproduces through seeds, which enter a period known as seed dormancy during which they cannot germinate despite favorable environmental conditions (Egley, 2017). Although numerous published studies on sacha inchi exist, there is a dearth of published data regarding methods to overcome the seed dormancy of sacha inchi seeds (Keawkim, et al., 2021). Thus, the aim of this study was to identify the best treatment to break the seed dormancy of sacha inchi.

2. Material and Method

Location of study

The experiments were conducted at Molecular Laboratory and Unit Pertanian Bersepadu of Department of Agricultural Science, Faculty of Technical and Vocational, Universiti Pendidikan Sultan Idris, Tanjung Malim, Perak.

Sample of Study

Approximately 1000 seeds were used for this study. The seeds were of a sizable size, 15 to 20 millimeters in diameter. Their shape is also rather enormous. It is possible to describe them as oval. Each seed possesses a rigid, dark brown coating that preserves the kernel within. Before the experiment started, the seeds were pre-treated with a solution of 50% sodium hypochlorite. Some seeds were eliminated because they were desiccated, shrunken, and affected by insects.

Methodology

Four hundred seeds were used to evaluate the effect of germination treatments on breaking the seed dormancy. The seeds were divided into four groups of treatments. The first treatment was mechanical scarification. The seeds were scarified by using sandpaper (grade 80), sanding gently about 30 sec to 1 min. The second treatment was chemical scarification. The seeds were immersed in 20% sulphuric acid with concentration of 98.08g/mol. The third treatment was hot water treatment. The seeds were soaked in 82°C of hot water until the water temperature dropped to room temperature (27±2°C) (Kimura, et al., 2012). For the control treatment, the seeds were soaked overnight in tap water. Each treatment was replicated four times to ensure reliability and accuracy of the data. The seeds were washed with sterilized distilled water for each germination treatment before sown on moistened paper towels with an equal volume of sterilized distilled water. Each petri dish was hermetically sealed, to prevent excessive water losses. They were kept at a temperature of 25-30ºC with continuous light for 10 days.

Statistical Analysis

The data were subjected to analysis of ANOVA single factor where variables with significant F ($\rho <$ 0.05 treatment's degree of freedom is bigger than one. LSD test was performed by using GLM procedures in Statistical Analysis System (SAS) for significantly different at $\rho < 0.05$ between treatments (SAS Institute, 1989).

Results and discussion

After sowing for over 10 days, seed germination of *Plukenetia volubilis* L. began on the second day. Treatments vary in the germination rate. Sulphuric acid and sandpaper treatments promoted significantly higher germination percentages (70% and 64%) in comparison to water and hot water treatments (48% and 2%). Despite the chemical scarification's successful promotion of seed germination, the warm and moist conditions could potentially contaminate the seeds with fungus, and the moistened tissue towel, potentially contaminated by bacteria, hindered some of the seeds' growth. Table 1 shows that seeds treated with sandpaper produced the highest germination rate index (GRI) at 4.75, compared to the other treatments. In terms of mean daily germination (MDG), the seeds treated with sulphuric acid exhibited the highest value of 1.75 when compared to the other treatments (Table 1). Regarding the peak value (PV), sandpaper-treated seeds achieved the highest value of 3.33 over a 10-day period, surpassing that of sulphuric acid (2.25), water (0.84), and hot water (0.15), as shown in Table 1. In terms of germination value (GV), the sandpaper treatment produced the highest value (5.43) among the treatments (Table 1).

Table 1: The percentage of germination, germination rate index, mean daily germination, peak value and germination value of *Plukenetia volubilis* L.

Treatment	Percentage of germination (10 days)	Germination rate index (10 days)	Mean daily germination (10 days)	Peak value (10 days)	Germination value (Mean ± SE)
Sulphuric Acid	70 ± 5.28 a	2.90 ± 0.32 b	1.75 ± 0.23 a	2.25 ± 0.40 b	4.50 ± 1.26 b
Sandpaper	64 ± 3.94 b	4.75 ± 0.37 a	1.60 ± 0.12 b	3.33 ± 0.61 a	5.43 ± 1.21 a
Water	48 ± 1.43 b	1.95 ± 0.33 b	1.20 ± 0.25 b	0.84 ± 0.19 b	0.98 ± 0.22 b
Hot water	2 ± 0.13 c	0.15 ± 0.09 c	0.05 ± 0.03 c	0.15 ± 0.09 c	0.01 ± 0.01 c

Pre-treatment with sulphuric acid has thinning the palisade layers on the seed in which the process has allowed the water to enter the embryo through thin palisade layers (Kimura, et al., 2012). Sulphuric acid possesses the potential to expand and form openings in the seed, resulting in the creation of pores (Miranda, et al., 2011). The treatment also induced the formation of cavities in the seed coat, which facilitate the diffusion of oxygen within the seed (Ayumi, et al., 2009). Meanwhile, pre-treatment with sandpaper scarification breaks the dormancy by removing the palisade layers and exposing the embryo to water (Acosta, 2023). Water is an important factor that activates the initial biochemical changes for protein synthesis in the process of germination (Bhatla, et al., 2023). In addition, previous findings on the concept of the water impermeable seed coat are majorly responsible for seed-coat dormancy and removal of the layer can initiate the germination (Seif-El Yazal, 2014).

Consequently, in this study, the application of acid and mechanical classification has led to an increase in the germination percentage of *Plukenetia volubilis* L. seeds. Sacha inchi is well-known for its hard seed coat, classified as coat-imposed dormancy, caused by the hard seed coat that blocks the water from entering the embryo (del-Castillo, 2019). The seed dormancy due to the impermeable seed skin can be overcome by peeling the skin, it triggers the hydrolysis process (Abu Bakar, et al., 2013). Based on the results, the best way to break the dormancy in sacha inchi seed is by acid scarification or mechanical scarification. However, to achieve the study's target in providing the best solution for small farmers in cultivating on time, this study believes that mechanical scarification on sacha inchi seeds was the best method in breaking the dormancy than acid scarification in a matter of costs and efficiencies.

Conclusion

The seed germination of *Plukenetia volubilis* L. was successfully improved by scarification treatment using sulphuric acid of 20% 98.08g/mol. The result showed the highest percentage of germination was 70% over 10 days after sowing. However, mechanical scarification using sandpaper has shown the highest GRI, PV and GV in compared to sulphuric acid treatment. Accordingly, this study recommends the best way to break the seed dormancy of *Plukenetia volubilis* L. by using mechanical scarification to promote germination for mass production and commercial purposes. An alternative way by using acid scarification but not recommended as an efficient method.

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