Colegio de Postgraduados

Evaluation of Different Scarification Methods for Chile Maax Seeds (*Capsicum annum* L. var. *glabriusculum*)

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ABSTRACT

Objective: To evaluate different scarification methods for maax pepper seeds under humid tropical conditions.

Design/methodology/approach: Maax chili pepper seeds collected in greenhouses at the Colegio de Postgraduados Campus Campeche were evaluated. These seeds were subjected to six scarification methods by immersion in a solution of coconut water (*Cocos nucifera* L.) (8 and 12 h); hydrogen peroxide (H_2O_2) (5 and 10 minutes); and sulfuric acid (H_2SO_4) (1 and 5 minutes), plus a control treatment. The evaluation was carried out under greenhouse conditions in plastic planting trays.

Results: The germination percentage and germination time of maax pepper seeds improved with all pre-germination treatments, advancing the onset of germination by up to three days compared to the control. The most effective treatments were seed immersion in coconut water for 8 and 12 h. Early seedling growth of *maax* pepper exceeded that of the control by 26%, 25%, 18%, and 14% in plant height, stem diameter, leaf width, and number of leaves, respectively.

Study limitations/implications: *Capsicum annuum* L. var. *glabriusculum* seeds have difficulty germinating if they are not in their natural state and are not ingested by a bird.

Findings/Conclusions: Due to its plant hormone and other nutrient content, coconut water is a suitable pre-germination treatment for breaking seed dormancy and promoting early growth of maax chili peppers under tropical conditions.

Keywords: *Capsicum annum* L. var. *glabriusculum*; Scarification; Seeds.

INTRODUCTION

Mexico hosts a wide variety of chili peppers that differ in size, color, shape, and flavor, among which is the maax chili (González-Cortés *et al.*, 2015). This ecotype is also regionally known as *piquín*, *chiltepín*, *chilpaya*, *chiltepillo*, *chiltepec*, *chile de monte*, *chile parado*, *pájaro pequeño*, *amomo*, *pico de paloma*, and *pico de pájaro*, among others, and is a highly valued product (Megchún-García *et al.*, 2024). *Maax* chili is a non-domesticated species

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widely consumed in states such as Veracruz, Tabasco, Campeche, Quintana Roo, Yucatán, and Chiapas, although it is broadly distributed throughout Mexico, and currently all indigenous groups interact with this species (Balderas *et al.*, 2023; Solís-Montero *et al.*, 2023). It is rich in nutrients; its fruits contain vitamin A and antioxidants and also possess medicinal properties. Consequently, indigenous communities traditionally used it to treat various ailments such as asthma, cough, sore throat, toothache, and others. Furthermore, it is widely used in various traditional Mexican dishes (Balderas *et al.*, 2023).

Currently, commercial demand has increased due to its exotic characteristics, and it is now available in both national and international supermarkets (Ramírez *et al.*, 2018; Alcalá-Rico *et al.*, 2023). However, to meet this demand, almost all supply comes from wild harvesting, which negatively impacts its distribution and abundance. This species grows at elevations up to 1,300 m above sea level, under the shade of trees and shrubs. However, *maax* chili is characterized by low germination rates, even when seeds are mature and environmental conditions are favorable. This limitation is known as physiological dormancy, in which one or more internal seed conditions prevent germination (Brondo-Ricárdez *et al.*, 2020). Among these factors, seeds of this species possess a hard, waxy, impermeable cuticle and contain natural inhibitors, resulting in germination rates below 5%. Therefore, seed germination represents a major constraint for intensive commercial cultivation. The natural mode of propagation of this chili occurs when mature fruits are ingested primarily by birds, and the seeds are subsequently dispersed through their droppings (Megchún-García *et al.*, 2024). Therefore, to overcome these low germination rates, various methods and techniques have been used to break seed dormancy in *Capsicum* spp., including: pre-soaking for 5 h at 21 °C and pre-drying at 22 °C, 32 °C, and 37 °C; exposure to incandescent and infrared light; potassium nitrate at 0.2%; indole-3-acetic acid at 1,000 ppm; gibberellic acid (GA_3) at 10-100 ppm; kinetin at 10-100 ppm; and removal of seed coat structures (Mireles-Rodríguez *et al.*, 2015).

When dormancy is shallow, it can be broken using chemical treatments, gibberellic acid, or warm or cold stratification. However, when dormancy is intermediate, it can only be broken after a prolonged period of cold stratification, and gibberellic acid may or may not be effective in overcoming dormancy (Loayza *et al.*, 2023).

Additionally, other solutions can be used to overcome dormancy in *maax* chili seeds, such as coconut water, which contains a variety of hormones with cytokinin-like activity, including isoprenoid-type cytokinins that aid in cell division, as well as aromatic-type cytokinins involved in post-germination processes. Bertolini *et al.* (2014) demonstrated that a culture medium containing copper chelate and coconut water promoted the development and survival rate of *Rossioglossum grande* (Lindl.) Garay & G.C. Kenn. protocorms and reduced chlorosis after 60 days. Similarly, Arana-Paredes *et al.* (2017) reported that using coconut water as a culture medium for Dianthus caryophyllus resulted in higher germination percentages, as well as increased germination rate and index. Loayza *et al.* (2023) demonstrated that ultra-drying (>3% moisture) and treating seeds with sulfuric acid, gibberellic acid, or potassium nitrate are the most effective methods for germination of *C. chilensis*. Therefore, the following research question arises: Which is the most effective method to induce and achieve higher germination of maax chili seeds under humid tropical conditions? Accordingly, the objective of this study was to evaluate different scarification methods for *maax* chili seeds and determine their germination percentage under humid tropical conditions.

MATERIALS AND METHODS

The research was conducted in the Plant Physiology Laboratory and experimental field at the Colegio de Postgraduados, Campus Campeche, located on the Haltunchén-Edzná road, km 17.5, Sihochac, Champotón, Campeche, Mexico.

Seed Treatment

Mature red maax chili fruits were collected from greenhouses at the same institution (Figure 1). Seeds were extracted by decantation, assisted by blending the mature chilies at low speed. The seeds were then placed in a container of water to remove non-viable seeds, which remained floating. Afterwards, the seeds were collected using a sieve and spread on brown paper. The seeds were rinsed three times with sterile water and then evenly distributed in a glass jar. They were subsequently treated with 10 ml of coconut water for 8 and 12 h, sulfuric acid (H_2SO_4) for 1 and 5 min, or hydrogen peroxide (H_2O_2, 3%, JENFARMA ZAGAL) for 5 and 10 min, plus a control consisting of seeds without hydration. These treatments and immersion times were selected because Ruiz-Carranza *et al*. (2024) demonstrated their effectiveness in breaking physical dormancy and promoting germination, including in woody species such as *Ebenopsis ebano*, *Havardia pallens*, *Parkinsonia aculeata*, *Prosopis laevigata*, and *Vachellia farnesiana*.

Sowing

The seeds were sown in 200-cell plastic trays using a substrate composed of garden soil mixed with sugarcane bagasse vermicompost in a 3:1 ratio, prepared on the same campus. One pre-treated seed was placed in each cell at a depth of 0.5 cm. After sowing all seeds,

Figure 1. Mature fruits of *Capsicum annuum* L. var. *glabriusculum* collected for experimentation.

potable water was sprayed using a plastic watering can. The trays were then placed under shade at an ambient temperature of 30 °C. Irrigation was performed every 3 days during the first 3 weeks and thereafter once per week.

Statistical Analysis

A completely randomized design was used with seven treatments plus a control, each with four replicates. The treatments were as follows: T1=unscarified seed (Control), T2=Coconut water (8 h), T3=Coconut water (12 h), T4=H_2O_2 (5 min), T5=H_2O_2 (10 min), T6=H_2SO_4 (1 min), and T7=H_2SO_4 (5 min).

The study variables were the number of germinated plants, plant height at 15 and 30 DAS (days after sowing), stem diameter, leaf width, and number of leaves. Data was analyzed using the SAS statistical software. An analysis of variance (ANOVA) was performed, and when significant differences were detected, Duncan's multiple range test was applied for mean comparison.

RESULTS AND DISCUSSION

Germination percentage

The evaluation of scarification methods for *maax* chili seeds began with seven treatments; however, the H_2SO_4 treatments showed no effect, as they resulted in low or no seed germination. Therefore, the analysis was based only on coconut water and H_2O_2 treatments, which were considered more effective for these seeds. Nonetheless, some reports on *E. ebano* seed germination indicate that H_2SO_4 was even more effective than other treatments. The difference observed in this experiment may be attributed to the seed coat of *E. ebano*, which is thicker and harder than that of *maax* chili (Ruiz-Carranza *et al.*, 2024).

Similarly, the results of the scarification evaluation of *maax* chili seeds showed higher germination percentages with H_2O_{22} (75%) and coconut water (70%) treatments ($P>0.05$), exceeding the control treatment by an average of 2% to 25%. In addition, the control treatment required more days to germinate, confirming the positive effect of H_2O_2 and coconut water treatments (Table 1). Likewise, Herrera-Aguilar *et al.* (2017) reported that the application of the commercial soluble powder product Biogib and gibberellic acid

Table 1. Mean comparison by treatment for germination percentage and days to germination of *maax* chili seeds (*Capsicum annuum* L. var. *glabriusculum*) under different scarification methods.

Treatments	Germination (%)	Germination (days)
T1=Unscarified seed (Check),	50 b	10
T2=Coconut water (8 h),	70 a	7
T3=Coconut water (12 h),	70 a	8
T4=Hydrogen peroxide H_2O_2 (5 min);	75 a	8
T5=hydrogen peroxide H_2O_2 (10 min);	75 a	6
T6=Sulfuric acid H_2SO_4 (5 min);	Low germination	
T7=Sulfiric acid H_2SO_4 (10 min)	Low germonation	

Treatments with the same letter are not significantly different (Duncan, $\alpha=0.05$).

promoted seed germination, resulting in higher germination rates at 21 days of evaluation.

In addition, Sandoval-Rangel *et al.* (2018) noted that other factors such as management, production, collection, extraction method, selection, conditioning, drying, and storage of the seed also influence the germination and viability of *maax* chili seeds.

The results confirmed the usefulness of coconut water as a treatment to stimulate seed germination and reduce the number of days to germination, thereby shortening the staggered germination period.

In this way, coconut water represents a viable option as a germination stimulant for *maax* chili seeds in coastal areas. This effect can be attributed to its content of diverse hormones with cytokinin-like activity, such as isoprenoid-type cytokinins involved in cell division processes and aromatic-type cytokinins associated with post-germination processes. As previously mentioned, this cytokinin is a phytohormone that, among other functions, promotes dormancy release and seed germination by stimulating cotyledon cell elongation in response to light (Campos-Ruíz *et al.*, 2014).

Therefore, the addition of coconut water may provide seeds with sufficient hormone levels to break the dormancy observed under natural conditions. It should also be noted that the coconut palm is typical of tropical regions, making coconut water a natural resource for promoting *maax* chili seed germination. Moreover, it is an economical product, with an average price of 30 pesos per liter, compared to hydrogen peroxide at approximately 300 pesos per liter. Coconut water is thus considered not only safer to handle, due to its natural origin, but also more economically accessible than hydrogen peroxide. Actions of this kind should be implemented because the primary habitat of *maax* chili is being lost, highlighting the need to establish germination and production protocols for this ecotype (Solís-Montero *et al.*, 2023).

Plant Growth

When evaluating the effect of seed scarification treatments on germination, their influence on seedling growth was also assessed ($P>0.05$). A positive effect of the pre-germination treatments was observed on plant height, stem diameter, leaf width, and number of leaves. Plant height was greater with scarification treatments compared to the control, with 8-hour coconut water immersion showing the highest values, followed by the 12-hour coconut water treatment, at 15 and 30 DAS (Figure 2). Although the type of seed scarification was evaluated in *maax* chili, it is always recommended to use organic substrates for growth and development, as noted by Palma-López *et al.* (2020), since they lead to higher production yields.

Similar results were found for the basal stem diameter of the seedlings. Seedlings from scarified seeds exhibited greater stem diameter, particularly those treated with coconut water for eight hours (Table 2). These results indicate that the hormonal composition of coconut water, specifically cytokinins, promoted vegetative growth, providing an advantage for plant production under nursery conditions.

Regarding leaf width and number of leaves, similar to plant height and stem diameter, scarification treatments on *maax* chili seeds had positive effects, observable from 15 DAS when using coconut water ($P>0.05$) (Figures 3 and 4). Authors such as Origenes and

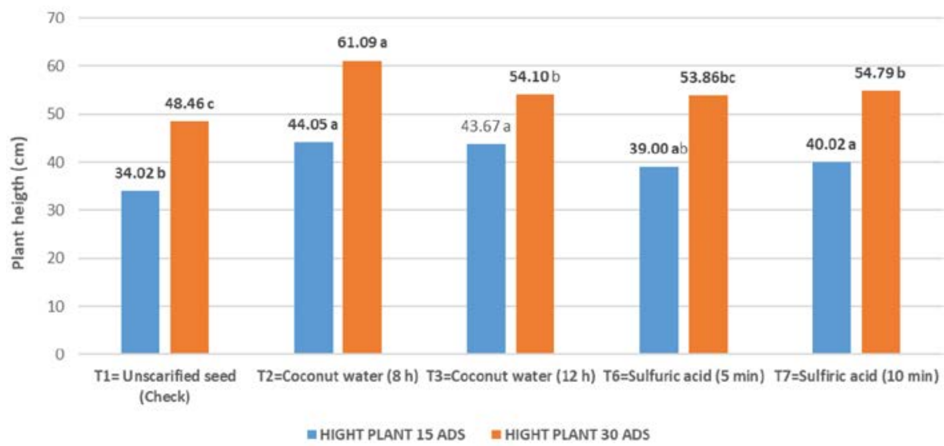

Figure 2. Mean comparison by treatment for plant height of *maax* chili (*Capsicum annuum* L. var. *glabriusculum*) under different scarification methods. Treatments with the same letter are not significantly different (Duncan, α=0.05).

Table 2. Mean comparison by treatment for seedling stem diameter of *maax* chili (*Capsicum annuum* L. var. *glabriusculum*) under different scarification methods.

Treatments	15 DAS	30 DAS
T1=Unscarified seed (Check),	0.42 c	0.82b
T2=Coconut water (8 h),	0.66 a	1.03 a
T3=Coconut water (12 h),	0.53 bc	0.95 ab
T6=Sulfuric acid H_2SO_4 (5 min);	0.47 c	0.95ab
T7=Sulfiric acid H_2SO_4 (10 min)	0.63 ab	1.00 ab

Treatments with the same letter are not significantly different (Duncan, α=0.05). DAS=days after sowing.

Lapitan (2020) also found that coconut water promoted the greatest height and average number of leaves in *Diospyros discolor* seedlings.

Leaf width was enhanced by scarification of *maax* chili seeds with H_2O_2 during the first 15 DAS, followed by coconut water treatments with 8- and 12-hour seed immersion. At 30 DAS, the effect of all scarification treatments was statistically similar (Duncan, α=0.05) (Figure 3).

Regarding the number of leaves, similar effects were observed at 15 DAS, with the best treatment being 10-minute H_2O_2 scarification, followed by coconut water scarification ($P>0.05$). At 30 DAS, the 8-hour coconut water treatment on maax chili seeds proved to be the most effective (Figure 4).

The effect of coconut water observed can be attributed to its richness in plant hormones such as gibberellins and cytokinins (González-Cortés *et al.*, 2015). These characteristics are associated with faster initial seedling growth, reflected in plant height, stem diameter, number of leaves, and leaf width. Although coconut maturity can also be a determining factor in promoting germination, Quinto *et al.* (2009) reported higher germination percentages using tender coconut water in *Tabebuia rosea* seeds, indicating that lower maturity is associated with higher nutrient concentration. However, in this study, coconut

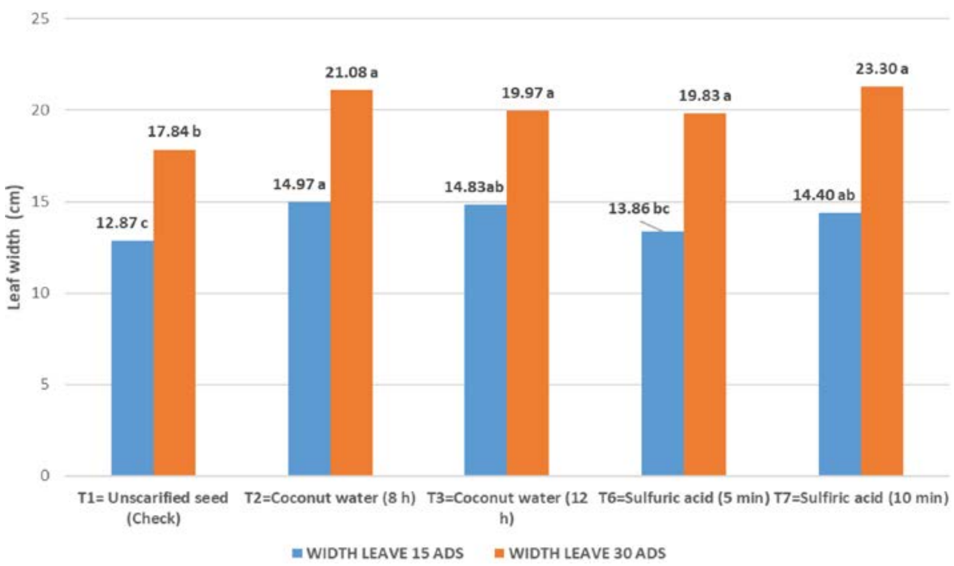

Figure 3. Mean comparison by treatment for seedling leaf width of *maax* chili (*Capsicum annuum* L. var. *glabriusculum*) under different scarification methods. (Treatments with the same letter are not significantly different, Duncan, α=0.05).

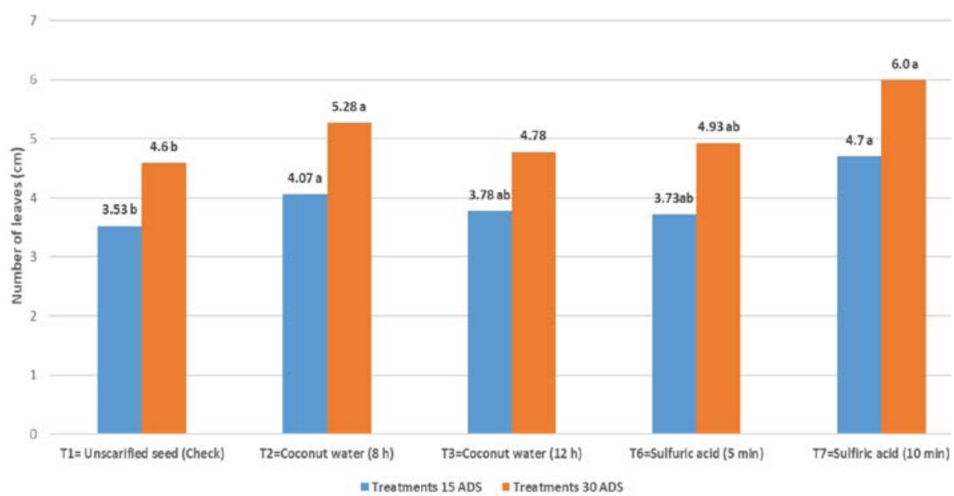

Figure 4. Mean comparison by treatment for seedling number of leaves of *maax* chili (*Capsicum annuum* L. var. *glabriusculum*) under different scarification methods. (Treatments with the same letter are not significantly different, Duncan, α=0.05).

maturity was not evaluated as a variable. Furthermore, it is important to consider that there is considerable variation among *maax* chili ecotypes. According to various studies, some ecotypes have hard seed coats that require acidic substances to promote germination, whereas others have softer seed coats that can germinate when treated with plant hormone-containing substances, such as coconut water (phyto-regulators), as in this case.

CONCLUSIONS

Scarification of *maax* chili seeds by immersion in coconut water proved to be the most effective method for promoting germination and stimulating early seedling growth.

Under the study conditions, the results suggest that the dormancy of the evaluated *maax* chili seeds was primarily physiological rather than physical, although variations in seed coat characteristics due to genotype may induce physical dormancy. Therefore, it is recommended to conduct similar experiments evaluating the effect of these treatments with variations in coconut maturity and across different *Capsicum annuum* varieties and genotypes.

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