

RESEARCH / INVESTIGACIÓN

Solanum betaceum in vitro seed germination and seedling development in response to pregerminative treatments

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Abstract: Germination and seedling development are critical stages in the life cycle of plants. Insufficient seedling emergence and inadequate establishment of woody plants are significant constraints in producing crops such as tamarillo. The application of pregerminative protocols improves the germination percentage, reduces the seedling emergence time, and improves several species *in vitro*. The present study was conducted to contrast three pregerminative treatments on *Solanum betaceum* seeds. When evaluating the pregerminative treatments, treatment T3 (imbibition for 24 hours) obtained the best results, showing an average of 21.62 days to germination, 73% germinated seeds and 2.31 leaves per explant.

Key words: Tamarillo, imbibition, agriculture, biotechnology, plant tissue culture.

Introduction

Solanum betaceum or tamarillo is a plant species from the Andean territory. This species is mainly grown due to its high nutritional value of edible fruits. Commercial production of plants using micropropagation techniques has several advantages over traditional propagation methods; in addition to being a rapid propagation process, it can lead to pathogen-free plants' production.

Pregerminative treatments can be applied to seed explants before or after a disinfection protocol has been developed. The objective of pregerminative treatments is to improve the germination rate while ensuring the successful development of the seedlings, playing an essential role as the first step in crop cultivation. The rigid structure of seed coats may be the reason for seed dormancy depending on the species, making it difficult to absorb water during germination; physical, mechanical, or chemical scarification treatments help resolve dormancy conditions in the seeds. The use of procedures to end the tegument's dormancy causes a better response in the time, and a higher percentage of germination, one of the common strategies is to hydrate the seeds for 24 h at room temperature.

The scarification procedure's efficiency depends on the technician's skill and practice; if the scarification is too deep, it may damage the embryonic axis, and if it is too shallow, it may not be enough to overcome the mechanical barriers that block seed germination.

Pregerminative treatments induced a particular physiological state in the seeds and had become a promising strategy to improve a plant's behavior in the field; there is great interest for farmers and seed companies to find suitable affordable pregerminative treatments, but also to precisely identify the

improved agronomic properties as a result of their application in crops.

Methods

First, the seeds were soaked in 1.33% v/v iodine for 15 minutes, followed by 3 washes with sterile distilled water. Then they were immersed in sodium hypochlorite at 1.5% v/v for 10 minutes, 5 washes were carried out with sterile distilled water. The disinfection protocol was then performed in a laminar flow cabinet, disinfected in advance with 70% alcohol, and exposed to ultraviolet light for 15 min, adjacent to the instruments to be handled. Basal medium MS (MS519) was prepared with a 50% salt concentration, enriched with 20 g/L of sucrose, 6 g/L of Phyto Agar, and 50 ppm of citric acid. The medium's pH was adjusted to 5.8, and it was sterilized by autoclaving at 121 ° C for 15 minutes. The experimental unit was set as 2 x 15 cm borosilicate glass screw cap culture tubes containing 15 mL of culture medium; 2 seeds were placed per tube. The treatments are described in table 1[NSV4].

Results and Discussion

Germination

Treatment T3 showed the lowest number of days to germination with an average of 21.62 days, besides it reached an average of 73% germination, this response is attributed to the hydration time for 24 hours (Figure 1); it is consistent with several authors who report an increase in germination tanks

TREATMENT	DESCRIPTION
T1	10 vol. Hydrogen peroxide, during 5 min; 48 h hydropriming and changing of water at 24 h
T2	Scarification and hydro priming during 24 h
T3	Hydropriming during 24 h
T0	Control treatment

Table 1. Pregerminative treatments applied to *Solanum betaceum* seeds.

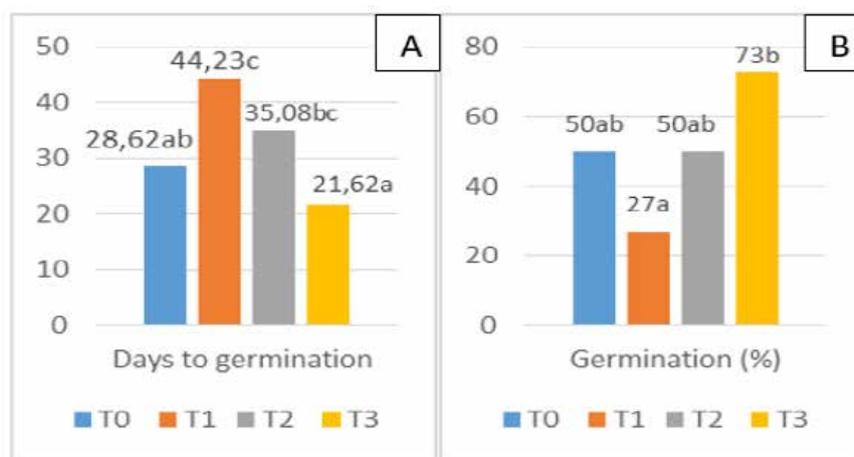


Figure 1. Effects of pregerminative treatments on *Solanum betaceum* seeds *in vitro*. A. Days to germination B. Germination percentage.

to pregerminative treatments that involved hydration of the seeds¹⁻³. In previous articles, it is pointed out that during the seed's imbibition, the meristematic activity is limited; however, preparation for cell division already occurs⁴. Hydro priming activates metabolic processes such as hydrolysis, the activation of enzymes, and the breaking of dormancy in seeds, favoring the expansion of the embryo and significantly decreasing the time of emergence of the radicle⁵. Treatment T3 shows a higher germination percentage than those presented by Murillo-Gómez, Hoyos, & Chavarriga⁶, who reported 57.5% of *Solanum betaceum* seeds germinated; these were scarified and seeded in MS medium with 3 ppm of BAP. This induced improvement in the germination process is based on hydro priming on the activation of DNA repairing mechanisms, synchronization of the cell cycle in the G2 phase, and cell division prepping⁷.

Although scarification was expected to optimize the germination response by facilitating water absorption, T2 did not show a better response than the control treatment, producing only 50 % of germination and 35.08 days to germination (Figure 1B). This fact agrees with authors who state that scarification can damage the embryo, abnormal seedlings, and dead seeds⁸. Furthermore, in most of the species studied, scarification was not the treatment that achieved 100% germination, requiring other pregerminative procedures, or the combination with other factors that break dormancy, such as lighting, refrigeration, thermal shock, among others⁹.

T1 showed the lowest germination percentage; this characteristic could be attributed to the amount of imbibition time, which was higher compared to the other treatments; Trigiano & Gray¹⁰, point out that an excess of the amount of water available in the imbibition can negatively affect the suppression of dormancy speed in germinated seeds *in vitro*; agreeing with Trimble¹¹. It indicates that imbibition of the seed initiates germination, in which water and oxygen are necessary to keep the process going. However, an excess of water around the seed can cause an insufficient oxygen supply for the cells that were growing and dividing rapidly, which would stop the germination process.

Number and color of leaves

Treatment T3 showed the highest number of leaves, with 2,31 leaves per explant, and the best color, with an average of 2,65, which means that the leaves presented a dark green color (Figure 2). The highest number of leaves (2,31) showed by T3 can be attributed to this treatment's advantage due to the early germination, which handed an advantage in metabolic and morphogenetic mechanisms to the *Vitro plants*. Early germination has been considered a characteristic to be avoided by some authors¹²; however, research works suggest that plants produced by induced early germination were not affected morphologically, and the germination time was favorably reduced¹³.

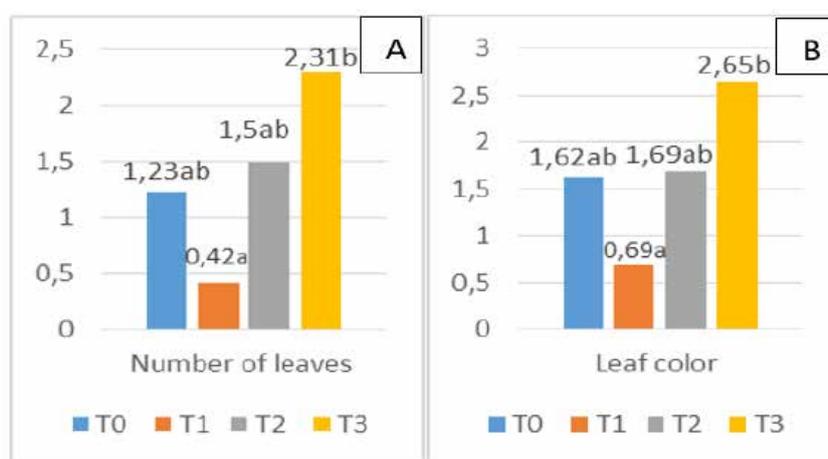


Figure 2. Effects of pregerminative treatments on *Solanum betaceum* seedlings *in vitro*. A. Number of leaves. B. Leaf in color.

A deep green color is considered a sign of health in *Solanum betaceum* plants. The color of the leaves of a plant is an indicator of health and vigor, as a deeper green indicates a higher concentration of chlorophyll, which allows higher efficiency in the transformation of solar energy into glucose¹⁴. Casierra-Posada, Peña-Olmos, & Ulrichs¹⁵, also conclude an essential link between coloration and the chlorophyll index. Due to insufficient chlorophyll content, a low-intensity green color in the leaf blade can signify stress or senescence in plants¹⁶. Besides that, the leaf's color is one of the main parameters for fruit producers, playing a crucial role in the choice, preference, and acceptability of the plants¹⁷.

Bacterial contamination

T1 was the only treatment that displayed bacterial contamination (42 %) (Figure 3A), this characteristic could be due to an exogenous microbial source that could have come into contact with the seeds when the changing of distilled water after

24 hours occurred (this process was not performed in the rest of the treatments); Farooq, Wahid, & Siddique¹⁸, consider the imbibition process with leachate washing as a possible source of contamination. Likewise, Ma¹⁹ indicates that storing seeds that keep their coverage under hydration generally maintains high microbial viability.

Conclusions

The best pregerminative treatment was T3 (imbibition for 24 hours), applied on *Solanum betaceum* seeds, showing an average of 21.62 days to germination, 73% germinated seeds, and 2.31 leaves with a deep green color (2.65/5).

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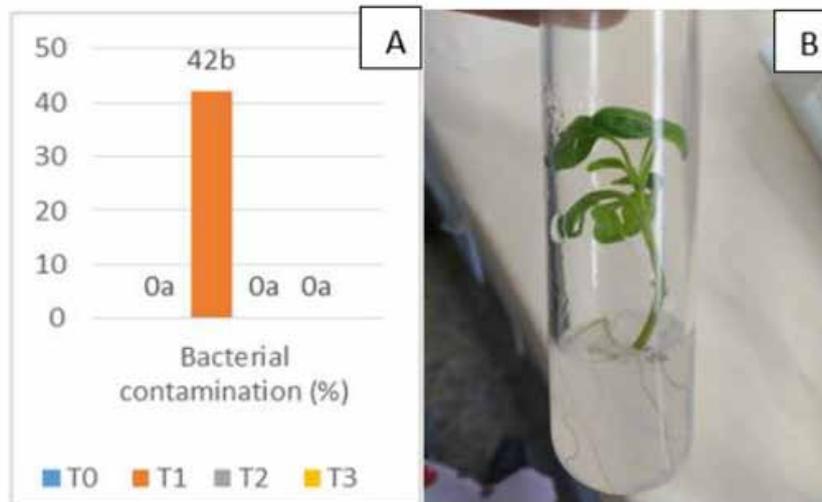


Figure 3. Effects of pregerminative treatments on *Solanum betaceum* in vitro. A. Bacterial contamination percentage. B. 45 days seedling.

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