Revista de CIENCIAS AGRÍCOLAS

e-ISSN Online 2256-2273

Received: December 05 2025 Accepted: February 27 2026 Published: April 23 2026

Research article: Plant science

Effect of pretreatments on the viability of seeds from five *Passiflora* species

Efecto de pretratamientos en la viabilidad de semillas de cinco especies de *Passiflora*

Jesús David Quintero-Caleño[1], Yordan Arley Palencia-Delgado[1], Pedro Isaac Salcedo-Garcia[1], Seir Antonio Salazar-Mercado[1*]

[1] Universidad Francisco de Paula Santander, Cúcuta, Norte de Santander, Colombia, jesusdavidqc@ufps.edu.co, http://orcid.org/0000-0002-2011-2638; yordanarleypade@ufps.edu.co, https://orcid.org/0000-0001-9044-2803; pedroisaacsg@ufps.edu.co, https://orcid.org/0009-0006-3998-0853; seirantoniosm@ufps.edu.co, https://orcid.org/0000-0002-3287-703X (*Correspondence*)

Cite: Quintero-Caleño, J. D., Palencia-Delgado, Y. A., Salcedo-Garcia, P. I., & Salazar-Mercado. S.A (2026). Effect of pretreatments on the viability of seeds from five Passiflora species. *Revista de Ciencias Agrícolas, 43*(1), e1288. https://doi.org/10.22267/rcia.2026431.288

ABSTRACT

Given the economic importance of *Passiflora* species in Colombia and the low germination rate caused by their marked dormancy, it is necessary to optimize rapid and accurate quality control protocols that guarantee viable seeds for agriculture. This study evaluated the effect of different pretreatments on the viability of seeds from five species of *Passiflora* spp. (*P. edulis, P. tripartita* var. *mollissima, P. edulis f. edulis, P. quadrangularis,* and *P. vitifolia*) through the application of tetrazolium (TZ) and indigo carmine (IC) biochemical tests, complemented by a germination assay. Six experimental pretreatments were applied: sodium hypochlorite (NaClO), calcium hypochlorite ($Ca(ClO)_2$), gibberellic acid (GA_3), potassium nitrate (KNO_3), distilled water, and an untreated control, evaluating two concentrations of TZ and one of IC at three exposure times. The results showed that GA_3, KNO_3, and $Ca(ClO)_2$ were the most effective pretreatments for increasing the detected viability, with responses above 80% in several species, especially when using TZ at 0.25%. Sodium hypochlorite had the lowest values, even zero in some cases. In general, TZ at 0.25% showed better viability values than 0.1%, while IC showed intermediate values but complemented the evaluation of membrane integrity. The germination test confirmed the trends found in the biochemical assays and showed that in species with pronounced dormancy, physiological viability does not always translate into germination without pretreatment. The results obtained provide technical criteria for optimizing quality control and seed conservation protocols in *Passiflora* spp., with direct implications for agricultural production and biodiversity preservation.

Keywords: dormancy; germination; indigo carmine; preservation; seed quality; tetrazolium; vigor

RESUMEN

Dada la importancia económica del género *Passiflora* en Colombia y la baja tasa de germinación causada por su latencia marcada, es necesario optimizar protocolos de control de calidad rápidos y precisos que garanticen semillas viables para la agricultura. Este estudio evaluó el efecto de diferentes pretratamientos sobre la viabilidad de semillas de cinco especies de *Passiflora* spp. (*P. edulis*, *P. tripartita* var. *mollissima*, *P. edulis* f. *edulis*, *P. quadrangularis* y *P. vitifolia*), mediante la aplicación de pruebas bioquímicas de tetrazolio (TZ) e índigo carmín (IC), complementadas con un ensayo de germinación. Se aplicaron seis pretratamientos experimentales: hipoclorito de sodio (NaClO), hipoclorito de calcio ($Ca(ClO)_2$), ácido giberélico (GA_3), nitrato de potasio (KNO_3), agua destilada y un control sin tratamiento, evaluando dos concentraciones de TZ y una en IC en tres tiempos de exposición. Los resultados mostraron que GA_3, KNO_3 y $Ca(ClO)_2$ fueron los pretratamientos más efectivos para incrementar la viabilidad detectada, con respuestas superiores al 80% en varias especies, especialmente al utilizar TZ al 0,25%. El hipoclorito de sodio presentó los valores más bajos, incluso nulos en algunos casos. En general, el TZ al 0,25% presentó mejores valores de viabilidad que el 0,1%, mientras que el IC arrojó valores intermedios,

pero complementó la evaluación de la integridad de membranas. La prueba de germinación confirmó las tendencias halladas en los ensayos bioquímicos y evidenció que en especies con latencia pronunciada la viabilidad fisiológica no siempre se traduce en germinación sin pretratamiento. Los resultados obtenidos aportan criterios técnicos para optimizar protocolos de control de calidad y conservación de semillas en *Passiflora* spp., con implicaciones directas en la producción agrícola y la preservación de la biodiversidad.

Palabras clave: calidad de semillas; conservación; germinación; índigo carmín; latencia; tetrazolio; vigor

INTRODUCTION

The genus *Passiflora* belongs to the family Passifloraceae, which includes approximately 573 species distributed throughout various tropical and subtropical regions (Mateus-Maldonado *et al*., 2023). Cultivated species are especially valued in the commercial sector for the production of fruits and leaves (Da Silva Francischini *et al*., 2020). Brazil is the leading exporter in this market, followed by Colombia (Hernández-Martínez *et al*., 2023). The anxiolytic and sedative potential of *Passiflora* species. has facilitated its widespread application in traditional medicine worldwide, in addition to its use in the food, pharmaceutical, and cosmetic industries (Santos-Tierno *et al*., 2022; Pereira *et al*., 2023).

In Colombia, *Passiflora* sp. varieties play an important role in the socioeconomic context, representing a viable option for family farming as an alternative crop (Orrego *et al*., 2021; Fischer & Miranda, 2021) and creating many job opportunities in rural areas as their commercialization increases (Osorio Cardona *et al*., 2020). Likewise, their flowers exhibit a wide variety of shapes, sizes, and colors, characteristics that give them outstanding ornamental potential (Eshghi Khas *et al*., 2020). *Passiflora* species stand out for their nutraceutical capacity, attributed to the phytochemicals they contain (Viera *et al*., 2022; Nikolova *et al*., 2024). Finally, their ecological relevance lies in their relationship with *Heliconius*, a genus of butterflies whose larvae feed on these plants, thus contributing to biodiversity (De Castro, *et al*., 2018; Sánchez Melo, 2021).

However, large-scale cultivation of *Passiflora* sp. faces a significant challenge due to the poor quality of its seeds, which are characterized by irregular germination (Loffler *et al*., 2022), attributed to physiological dormancy (Morales Pizarro *et al*., 2023). Furthermore, due to its physiology, adequate metabolic reactivation does not occur during germination, which results in low production quality, decreased germination capacity, and even zero seedling growth (Ruesta-López *et al*., 2024). Therefore, it is crucial to implement techniques that increase seed viability and enable the rapid, simple, and effective identification of seed potential, thereby contributing to the solution of these problems. In this regard, assessing viability through the tetrazolium test is an excellent alternative, as it is one of the most widely used methods (França-Neto & Krzyzanowski, 2019; González Campos *et al*., 2025), along with indigo carmine, which has positioned itself as another efficient and affordable option (Sousa *et al*., 2025). Likewise, the incorporation of pretreatments that counteract dormancy can improve uniformity in germination (Solano Ortiz, 2021; Zembele & Ngulube, 2022).

The tetrazolium test is a biochemical evaluation method that complements germination tests. It is generally applied to seeds that have latency problems or that need to be sown immediately after harvesting (Cordeiro *et al*., 2022). This test is based on the red coloration of the embryo, which is the result of the reduction of tetrazolium salts in the living parts of the seed due to the action of dehydrogenase enzymes that form triphenylformazan (Portuguez-García *et al*., 2021), indicating respiratory activity. In contrast, the indigo carmine test is a method that has been used to determine the viability of smaller groups of plants, proving particularly beneficial in pine species (Jyoti & Malik, 2013). This test demonstrates the permeability of cell membranes by staining dead tissues blue, while viable seeds remain colorless (Sousa *et al*., 2025). Although traditional germination tests remain the benchmark for assessing seed physiological quality, the development and validation of complementary tests such as TZ and IC have increased, improving the accuracy and speed of evaluation (Paim *et al*., 2019).

Research on the viability of *Passiflora* sp. seeds is still limited, and there is no precise information about the challenges these seeds may present. In this context, this study aims to evaluate the germination potential and quality of *Passiflora* spp. seeds by combining tetrazolium and indigo carmine tests. Additionally, it seeks to observe and generate relevant data on what happens in the embryo through the application of various pretreatments, which help mitigate germination limitations. This approach allows for innovation in the analysis of *Passiflora* seeds by optimizing traditional procedures and offering an alternative to germination tests, with the aim of ensuring success in the production of these crops.

MATERIALS AND METHODS

Plant material

Passion fruit (*Passiflora edulis*), red granadilla (*Passiflora vitifolia*), and badea (*Passiflora quadrangularis*) fruits were collected from the municipality of Tibú, Norte de Santander Department, in the village of La Esmeralda 2. Fruits of curuba (*Passiflora tripartita* var. *mollissima*) and gulupa (*Passiflora edulis f. edulis*) were also collected from the municipality of Chitagá (Figure 1). In all cases, mature fruits were randomly selected to ensure uniformity in the physiological ripening stage across all species. The seeds were manually extracted from the fruits and stored in Kraft paper bags, properly labeled with the name of each species to facilitate identification and avoid confusion. The experimental phase was carried out in the Biology Laboratory of the Faculty of Basic Sciences at the Francisco de Paula Santander University, Cúcuta campus.

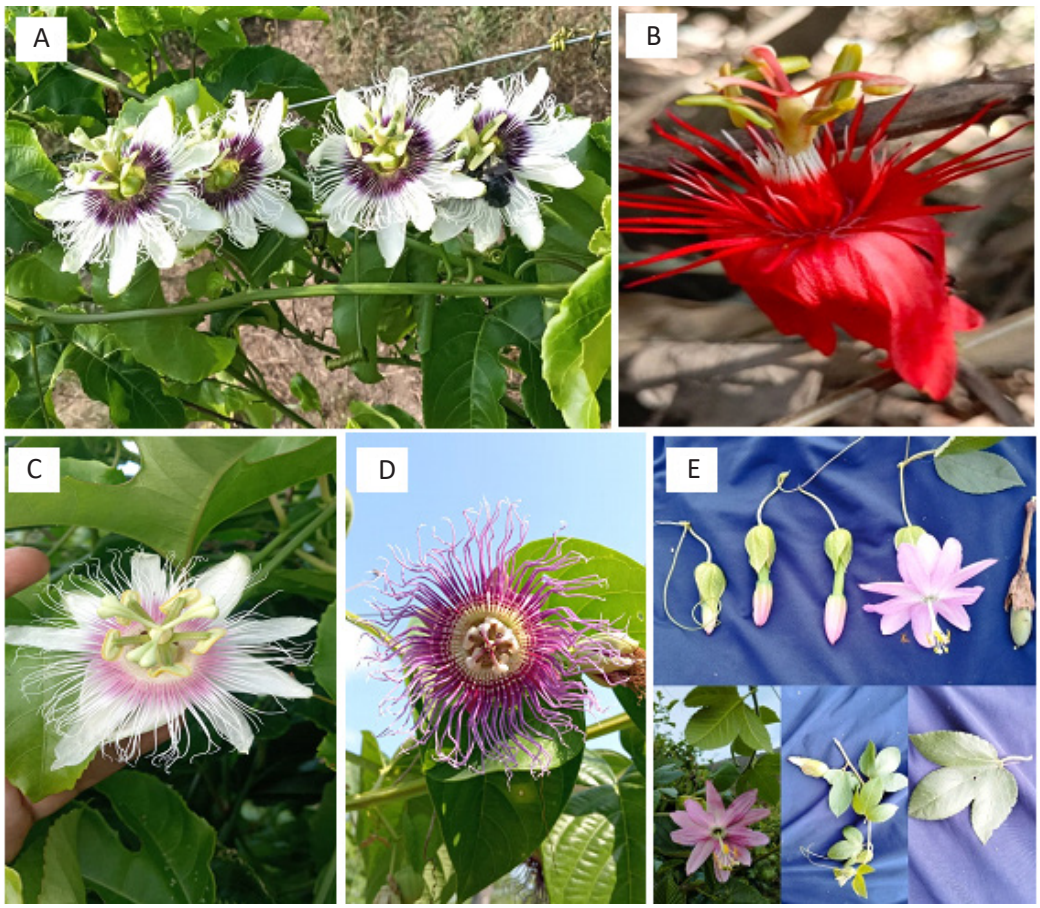


Figure 1. *Flowers of the species investigated (A) Passiflora edulis; (B) Passiflora vitifolia; (C) Passiflora edulis f. edulis; (D) Passiflora quadrangularis; (E) Passiflora tripartita var. mollissima.*

Application of Pretreatments

The seeds of the different species studied were divided into six groups for the application of the pretreatments. Three of these were based on immersing the seeds for 10 min in NaClO (2.5%), $Ca(ClO)_2$ (1%), and distilled water. The fourth and fifth pretreatments involved immersing the seeds for 24 hours in a 0.3M KNO_3 solution and in gibberellic acid (GA_3) at 150 mg/L. Finally, the sixth group represented the control without pretreatment. For this, the syringe method described by Salazar (2012) was implemented, which consists of placing a group of seeds inside a sterile 5 mL syringe with a cloth filter. Finally, all seeds were washed three times with distilled water to remove the residues of the different solutions.

Viability Tests

The embryos were extracted using equipment adapted to the characteristics of the seed coat, consisting of pliers and tweezers. For each experimental assay, a total of 30 seeds were processed, and the embryos were then placed in sterile Petri dishes for analysis.

Tetrazolium Test (TZ). For the tetrazolium (TZ) test, the embryos were exposed to two concentrations of the solution (0.25% and 0.10%) for three periods of time (6, 12, and 24 hours) at a constant temperature of 25°C and in complete darkness. At the end of the exposure period, the seeds were observed under a stereoscopic microscope. Those that showed red coloration in the embryo, resulting from the reduction of tetrazolium to formazan, which indicates respiratory activity, were considered viable (Mercado, Caleño *et al*., 2020; Mercado, Delgado *et al*., 2020). In contrast, embryos with a pale color or no staining were classified as non-viable.

Indigo Carmine Test (IC). For the indigo carmine (IC) test, the same sample size of 30 embryos per treatment was immersed in a 0.5% indigo carmine solution, with three exposure times (6, 12, and 24 h), also in dark conditions. They were then examined under a stereoscopic microscope. Unlike the tetrazolium test, in this case, the blue staining revealed non-viable areas of the embryo; therefore, those seeds whose embryo remained colorless were considered viable (Sousa *et al*., 2025). Finally, the results obtained in both tests were expressed as a percentage of viable seeds in relation to the total evaluated.

Germination Test

The Germination experiment was carried out using three replicates of 30 seeds for each species evaluated without the application of pretreatments. The method used consisted of germination on damp paper towels, maintaining a temperature range between 25 and 30°C and a photoperiod of 8 hours of light and 16 hours of darkness (Salazar & Zambrano, 2025). The tests were carried out in previously disinfected plastic containers. The seeds were placed in the containers and kept for approximately 30 days. After this time, seeds that showed clear signs of growth were considered germinated. The results were expressed as a percentage of germination and compared with those obtained in the control group (without pretreatment).

Experimental Design and Statistical Analysis

A completely randomized design was used for the feasibility tests. Each combination of pretreatment, test type, exposure time, and germination assessment was performed in three replicates, using 30 seeds per replicate. In total, 1,710 seeds per species were used to evaluate the different treatments. Prior to analysis, the assumptions of normality and homoscedasticity were verified. Data obtained were subjected to analysis of variance (ANOVA), and then Tukey's multiple range test was applied to identify significant differences between means, considering a significance level of $p \leq 0.05$. Statistical processing was performed using Infostat software.

RESULTS

Seed Viability Assessment

Viability of *Passiflora edulis*. According to Table 1 and Figure 2, the most effective pretreatment at a concentration of 0.1% TZ was the use of gibberellic acid (GA_3), reaching a maximum viability value of 67.7% after 24 hours of exposure, followed by potassium nitrate (KNO_3, 46.6%) and calcium hypochlorite (43.3%).

When the TZ concentration was increased to 0.25%, a significant increase in viability percentages was observed, with $Ca(ClO)_2$ (98.8%), GA_3 (94.4%), and KNO_3 (88.9%) standing out as the most efficient treatments after 24 hours of exposure. The control and NaClO showed values below 65.5% and 45%, respectively, confirming the lower efficacy of NaClO. The results of the test with 0.5% IC were in an intermediate range, with GA_3 (83.9%) and $Ca(ClO)_2$ (84.4%) again leading in effectiveness.

Table 1. ***Viability of Passiflora edulis seeds***

Pretreatments	TZ (0.1%) 6h	TZ (0.1%) 12h	TZ (0.1%) 24h	TZ (0.25%) 6h	TZ (0.25%) 12h	TZ (0.25%) 24h	IC (0.5%) 6h	IC (0.5%) 12h	IC (0.5%) 24h
Control	18.9±1.9a,b	33.3±3a	37.7±5a	44.4±1.9a	63±3.3a	65.5±1.9a	64.4±6.9a	62.2±6a	62.2±8.3a
H_2O	23.3±4.8b	35.5±4a	38.8±6a	25.5±5b	73.3±8.8a	74.4±7.2a	78.8±7.6b	77.7±6.9a	76.6±8.8a,b
GA3	42.2±5.2c	66.6±3,2b	67.7±3.8b	45.5±5a	93.33±3.3b	94.44±1.9b	81.1±5b	81.1±5a	78.8±6.9ª,b
KNO3	40.7±4.4c	44.4±5a	46.6±6a	41.1±1.9a	87±9.3b	88.1±5b	76.6±6.6b	74.4±8.3a	74.4±8.3a,b
NaClO	8.8±2.2a	8.9±2c	10±2c	20±6b	42.2±6c	44.4±4.2c	51.1±1.9c	44.4±4.2b	41.1±1.9c
Ca(ClO)2	15.8±1.3a	42.2±5a	43.3±3.3a	48.8±5a	95.5±5b	98.8±1.9b	85.5±3.8b	84.4±5a	84.4±5b

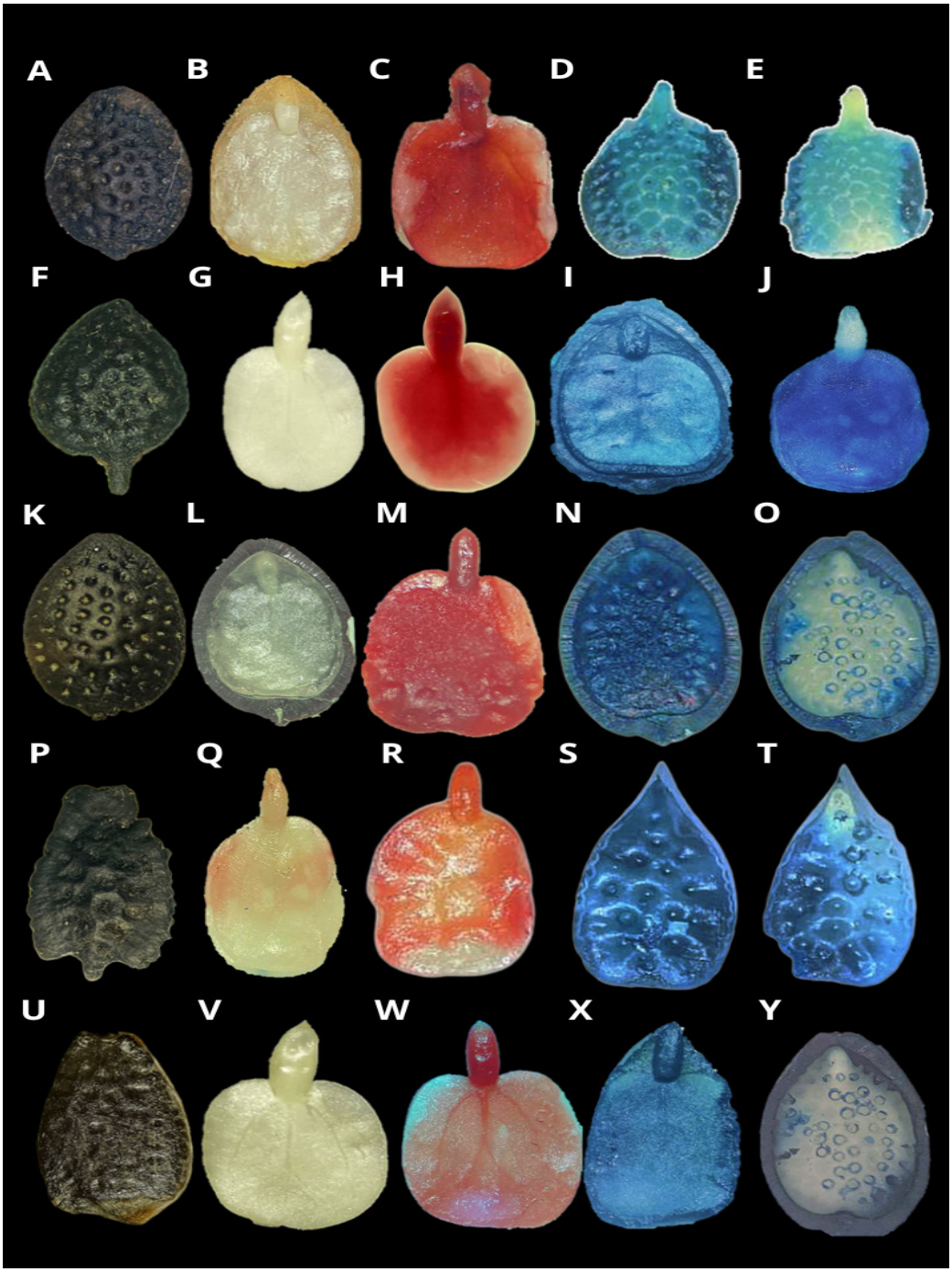
Note. Means with different letters differ significantly (Tukey, $p \leq 0.05$)

Viability of *Passiflora tripartita var.* molíssima. In *Passiflora tripartita* var. mollissima, the viability percentages obtained were lower compared to the other *Passiflora* species evaluated using tetrazolium (TZ) and indigo carmine (IC) assays. Pretreatments with KNO_3, GA_3, and distilled water achieved the best results in TZ at 0.25%–24h (74.4%, 63.3%, and 62.2%, respectively), favoring seed coat permeability and stimulating cellular metabolic activity. In IC, KNO_3 also stood out (50.6%), reinforcing its usefulness as a viability promoter, yielding a statistically similar result to that obtained with TZ at 0.1% (Table 2; Figure 2).

Viability of *Passiflora edulis f. edulis.* In *Passiflora edulis f. edulis*, seed viability varied significantly depending on the pretreatment applied. Potassium nitrate (KNO_3) was the most effective, reaching up to 100% viability with 0.1% TZ for 24 hours and also showing high rates with indigo carmine. Similarly, GA3 promoted high viability levels, particularly with 0.25% TZ at 24 hours (91.1%), confirming the high physiological quality of the embryos. Conversely, NaClO treatments drastically inhibited the staining reaction in both tests, showing negligible viability values that reached 0% in the indigo carmine test at 24 hours (Table 3; Figure 2).

Viability of *Passiflora quadrangularis.* In this species, pretreatment with gibberellic acid (GA_3) resulted in the highest levels of viability, reaching up to 80% with 0.25% tetrazolium after 24 hours. The viability obtained at 12 hours was statistically similar (75.5%). Furthermore, the indigo carmine (IC) test reached its maximum detection peak at 6 hours (52.2±4b with GA3), showing a gradual decline in effectiveness as exposure time increased (Table 4; Figure 2).

Viability of *Passiflora vitifolia*. In *Passiflora vitifolia*, pretreatments with gibberellic acid (GA_3), potassium nitrate (KNO_3), and calcium hypochlorite $Ca(ClO)_2$ were the most effective in improving viability, reaching values above 80% in 0.25% tetrazolium after 12 and 24 hours of exposure (Table 5). In contrast, water, sodium hypochlorite (NaClO), and the control showed significantly lower results, demonstrating that the absence of stimuli or exposure to strong oxidants limits germination capacity.



Note. A) Seed coat of *P. edulis*; B) Non-viable seed-TZ; C) Viable seed-TZ; D) Non-viable seeds-IC; E) Viable seed-IC; F) Seed coat of *P. tripartita* var. *mollissima*; G) Non-viable seed-TZ; H) Viable seed-TZ; I) Non-viable seeds-IC; J) Viable seed-IC; K) Seed coat of *P. edulis f. edulis*; L) Non-viable seed-TZ; M) Viable seed-TZ; N) Non-viable seeds-IC; O) Viable seed-IC; P) Seed coat of *P. quadrangularis*; Q) Non-viable seed-TZ; R) Viable seed-TZ; S) Non-viable seeds-IC; T) Viable seed-IC; U) Seed coat of *P. vitifolia*; V) Non-viable seed-TZ; W) Viable seed-TZ; X) Non-viable seeds-IC; Y) Viable seed-IC.

Figure 2. *Seed staining after viability tests with tetrazolium (TZ) and indigo carmine (IC)*

Table 2. *Viability of Passiflora tripartita var. mollissima seeds*

Pretreatments	TZ (0.1%) 6h	TZ (0.1%) 12h	TZ (0.1%) 24h	TZ (0.25%) 6h	TZ (0.25%) 12h	TZ (0.25%) 24h	IC(0.5%) 6h	IC(0.5%) 12h	IC(0.5%) 24h
Control	18.8±1.9a,b	23.3±3.3a	24.4±1.9a	31.1±5a	47.7±1.9a,b	48.9±1.9a	36.6±3.3a,b	311±3ab	30±3.3a,b
H_2O	34.3±6.2a,b,c	43.9±3.8a,b	48.1±5a	54.4±5.4b	60±3.3b,c	62.2±3.8b,c	27.7±5a	25.5±5a	25.5±5a
GA_3	42.2±5b,c	55.5±5c	57.7±3.8b	48.8±3.8b	62.2±5b,c	63.3±5,7c	42.2±3.8b,c	40±3abc	38.8±3.8abc
KNO_3	46.6±5c	50±6.6c	51.1±8.3b	53.3±8.8b	74.4±6.9b,c	74.4±6.9c	53.1±5b,c	50.6±6bc	50.6±5bc
NaClO	12.2±3d	22.4±4.2c	24.9±5a,b,c	21.1±5a	33.3±3.2a	34.4±3.4d	4.4±1d	4.4±4d	2.2±1d
$Ca(ClO)_2$	7.7±3a	32±3.8b	36.1±1.9d	28.8±4a	51.1±5.1b	53.3±3a,b	44.2±6c	34.6±6.9c	34.6±6c

Table 3. *Viability of Passiflora edulis f. edulis seeds*

Pretreatments	TZ (0.1%) 6h	TZ (0.1%) 12h	TZ (0.1%) 24h	TZ (0.25%) 6h	TZ (0.25%) 12h	TZ (0.25%) 24h	IC(0.5%) 6h	IC(0.5%) 12h	IC(0.5%) 24h
Control	16.6±3.3a	33.3±3.3a	43.3±3.3a	17.7±3.8a	44.4±6.9a,c	47.7±8.3a	51.1±6.9a	48.8±8.3a	47.7±8.3a
H_2O	20±6.6a	37.7±5a	48.8±6.9a,b	17.8±1.9a	34.4±1.9a,b	37.7±1.9a,b	56.6±8.8a	52.2±3.8a	50±3.3a
GA_3	54.4±6.9b	72.2±6.9c	90±3.3c	53.3±3.3b	88.8±5d	91.1±5c	63.3±3.3a	61.1±1.9a	58.8±5a
KNO_3	58.8±5b	94.4±5d	100c	71.1±5c	84.4±5d	93.3±6.6c	85.5±5b	84.4±5b	82.2±6.9b
NaClO	2.2±0.3c	7.7±1.2f	18.8±2c	11.1±3a	17.7±3b	23.3±6.6b	4.4±1c	2.2±0.1c	0c
$Ca(ClO)_2$	17.7±2.3a	54.4±5e	55.5±5b	32.2±5d	52.2±5c	72.2±5d	60±8.8a	57.7±5a	56.6±5.7a

Table 4. *Viability of Passiflora quadrangularis seeds*

Pretreatments	TZ (0.1%) 6h	TZ (0.1%) 12h	TZ (0.1%) 24h	TZ (0.25%) 6h	TZ (0.25%) 12h	TZ (0.25%) 24h	IC(0.5%) 6h	IC(0.5%) 12h	IC(0.5%) 24h
Control	14.3±1a	18.8±3.1a	24.4±2a,b	17.7±3a	28.8±3a	32.3±3a	42.2±5a,b	37.7±3a	36.6±3a
H_2O	16.6±3a	26.6±3b,c	33.3±3b,c	41.1±4b	48.8±3.1b,c	51.1±3.8b	51.1±5b	47.7±3a	44.4±2a
GA_3	27.7±3a	41.1±3d	45±6c	5.6±3b	75.5±7e	80±6b,c	52.2±4b	47.8±4a	46.6±3a
KNO_3	20±2a	37.3±3.2c,d	40±3.3c	53.3±4b	68.9±6d,e	71.1±5b,c	50±6b	47.7±2a	45.5±3a
NaClO	6.6±3a	14.4±2ª,b,c	16.6±2a	23.3±2a	32.2±3a,b	33.3±3a	17.7±3a	11.1±1.7b	8.9±1b
$Ca(ClO)_2$	24.4±2.3a	37.3±4c,d	38.8±3c	48.8±4b	55.5±6c,d	57.7±4b,c	43.3±4a,b	40±3a	38.9±3a

Table 5. *Viability of Passiflora vitifolia seeds*

Pretreatments	TZ (0.1%) 6h	TZ (0.1%) 12h	TZ (0.1%) 24h	TZ (0.25%) 6h	TZ (0.25%) 12h	TZ (0.25%) 24h	IC(0.5%) 6h	IC(0.5%) 12h	IC(0.5%) 24h
Control	27.7±5a,b	34.4±4a,b	36.6±3a,b	36.6±3a	41.1±6ª	44.4±4a	38.8±1.9a,b	38.8±3.8a,b	37.7±3a,b
H_2O	38.8±5a,b,c	47.7±3b,c	50±6.6b	40±4a	42.2±8.3a	46.6±6a	40±1a,b	40±3a,b	10±1a,b
GA_3	48.8±4c	71.1±5d	72.2±5c	72±3b	84.4±5b	85.5±5b	57.7±6b	57.7±5b	55.5±5b
KNO_3	42.2±3.8b,c	62.2±5c,d	66.6±3c	66.6±2b	80.8±7b	83.3±6b	55.5±5b	55.5±6b	53.3±6b
NaClO	25.5±3a	26.6±3a	27.7±5a	33.3±3a	35.5±7a	37.7±3a	24.4±8a	22.1±2a	18.8±1a
$Ca(ClO)_2$	44.4±3c	64.4±5d	67.7±5c	67.7±6b	82.2±8.2b	87.7±7b	65.5±7b	60±3b	58.8±4b

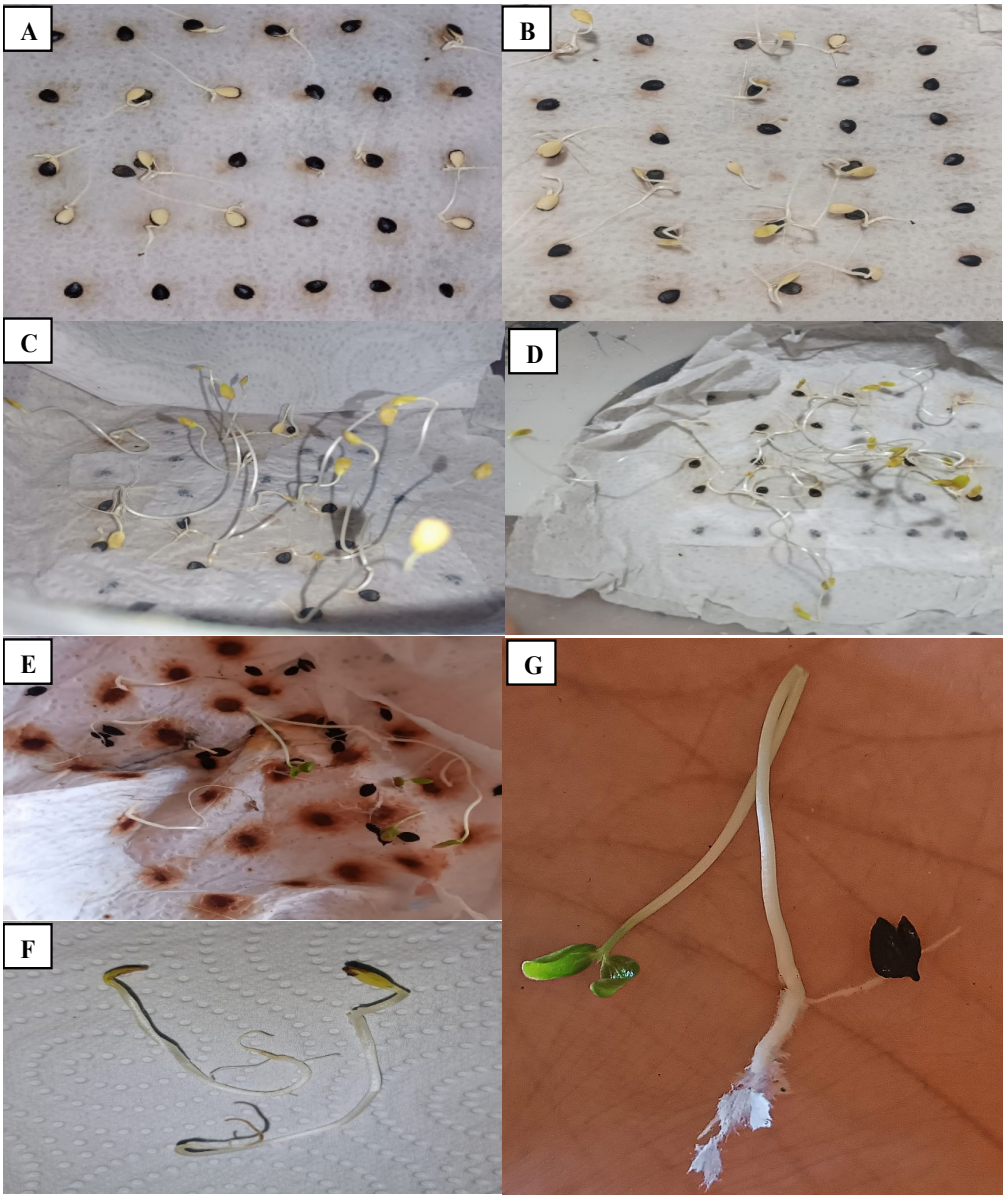
Germination Test

In *Passiflora edulis*, germination was 58.9%, with no significant differences from the viability estimated by IC and TZ at 0.25% (Table 1). In *P. tripartita var. mollissima*, germination reached 38.9% (Table 6; Figure 3), exceeding the viability detected with TZ at 0.1% and similar to that of IC at 0.5% (Table 2).

For *P. edulis f. edulis* and *P. quadrangularis,* the germination percentages were 46.7% and 31.1%, respectively, values slightly higher than the TZ control, but lower than the average values of IC and TZ at 0.25%. Finally, in *P. vitifolia*, the highest correlation between germination and viability was observed, with a germination rate of 40%, equivalent to that estimated by the TZ and IC tests (Table 5).

Table 6. *Seed Germination Percentages on Paper Towels*

Species	Germination percentage
Passiflora edulis	58.89
Passiflora tripartita var. *mollissima*	38.89
Passiflora edulis f. *edulis*	46.67
Passiflora quadrangularis	31.11
Passiflora vitifolia	40



Note. A) *P. edulis* (5 days); B) *P. tripartita* var. *mollissima* (8 days); C) *P. edulis* (15 days); B) *P. tripartita* var. *mollissima* (20 days); E) *P. vitifolia*; F) *P. quadrangularis*; G) *P. edulis* f. *edulis.*

Figure 3. *Seed germination on paper towels*

Seed Viability Assessment

Passiflora edulis. Gibberellic acid (GA_3) promotes germination in physiologically dormant seeds through enzymatic activation and mobilization of reserves (Bewley *et al*., 2013). The results obtained confirm that this phytohormone not only stimulates seed emergence, as previous research has shown (Angelini *et al*., 2021; Castrillón-Pineda *et al*., 2024; Luo *et al*., 2025), but also contributes to the maintenance of cell viability, as evidenced by the intensity and homogeneity of embryo coloration (Figure 2). Domingues Neto *et al*. (2024) reported greater vigor and germination in *Passiflora edulis* seeds treated with GA_3, supporting their recommendation for physiological evaluations and pretreatments in nurseries.

Calcium hypochlorite $Ca(ClO)_2$ demonstrated significant potential for enhancing the viability assessment in *Passiflora edulis* seeds. Compared to sodium hypochlorite (NaClO), $Ca(ClO)_2$ exhibits a more controlled release of active chlorine, which results in reduced oxidative damage to delicate embryonic tissues. This lower phytotoxicity is attributed to the presence of calcium ions, which contribute to the stabilization of cell membranes and the maintenance of wall integrity during the disinfection process (Mulaudzi *et al*., 2020). Furthermore, $Ca(ClO)_2$ pre-treatments help modulate the activity of antioxidant enzymes that protect cellular metabolism from precocious degradation (Issam *et al*., 2012). Spectroscopic studies indicate that $Ca(ClO)_2$ can modify the surface of the seed coat, facilitating the entry of TZ and IC solutions and improving the detection of viable tissue (Xu *et al*., 2024).

***Passiflora tripartita var.* molíssima**. GA_3, although it increased viability, had less effect on structural integrity according to IC. However, various studies have shown that this phytohormone is a useful resource for breaking seed dormancy (Sourki *et al*., 2019; Nedunchezhiyan *et al*., 2023; Johnson *et al*., 2023), which promotes imbibition and metabolic activation, significantly increasing the results in biochemical tests such as those used in this study. Likewise, immersing the seeds in water softens the testa of these particularly hard seeds and allows for the partial elimination of chemical inhibitors present in it, facilitating the entry of reagents and directly influencing the germination process (Campos-Hermosillo *et al*., 2022). In contrast, sodium hypochlorite (NaClO) was the least effective, drastically reducing viability, probably due to damage to the embryo's membranes and proteins (Khayat 2017). The control group also showed low values, highlighting the importance of using appropriate pretreatments to optimize physiological evaluation in this species.

Passiflora edulis f. edulis. In *Passiflora edulis* f. *edulis*, it has been shown that the concentration of TZ can be reduced while maintaining assay sensitivity, thereby reducing costs and toxicity (França-Neto & Krzyzanowski, 2022; Silva *et al*., 2019). KNO_3 promotes cellular respiration and the mobilization of reserves, facilitating the evaluation of viability by breaking dormancy and improving testa permeability (Sakariyawo *et al*., 2020; Salomão *et al*., 2023). In contrast, sodium hypochlorite (NaClO) again showed the lowest values, and even zero values, probably due to its oxidizing effect and direct damage to the embryo, as reported in previous studies on other plant species (Lallana & García, 2013; Silva *et al*., 2019; Salazar Mercado *et al*., 2020; Salazar Mercado & Palencia Delgado, 2025). In addition, treating seeds with NaClO before IC can induce false negatives by discoloring the reagent, confusing viable seeds with non-viable ones (Iwabuki *et al.*, 2018). Despite reports of some usefulness in specific species (Xu *et al*., 2024), this pretreatment was not suitable for *P. edulis f. edulis.*

Passiflora quadrangularis. In *P. quadrangularis,* shorter exposure periods are sufficient to assess viability, optimizing analysis times and facilitating production decisions (Vega-Corrales *et al*., 2022). Studies on other crops such as rice and soybean confirm that

using lower concentrations and shorter times does not affect the accuracy of the test (Carvalho *et al*., 2017).

Potassium nitrate (KNO_3) also showed high efficacy (71.1%), although somewhat lower than GA_3. Gibberellic acid promotes the breaking of dormancy by activating dehydrogenase enzymes that intensify tetrazolium staining (Domingues Neto *et al*., 2024) (Figure 2). In contrast, treatments with $Ca(ClO)_2$ showed intermediate responses, while water and controls reached moderate values between 33 and 51%.

The indigo carmine test stains dead cell tissues and does not reflect metabolic activity. This test showed stable patterns from 12 to 24 hours, although it tends to underestimate viability compared to tetrazolium, and its combined use is recommended for a comprehensive assessment (Salazar Mercado & Gélvez Manrique, 2015; Álvarez Cisneros *et al*., 2020; Moreno Álvarez *et al*., 2001; ISTA, 2022).

Passiflora vitifolia. GA_3 promotes germination by stimulating the production of enzymes such as α-amylase and activating key metabolic pathways, rapidly activating seeds with physiological dormancy (Domingues Neto *et al*., 2024). KNO_3 acts as a nutritional signal that improves water absorption, regulates hormonal balance, and enhances antioxidant mechanisms, increasing functional viability (Hernández *et al*., 2023; Mahmood Ur Rehman *et al*., 2024). Finally, although the exact mechanism of $Ca(ClO)_2$ is less studied, research suggests that it improves the permeability of the seed coat and does not affect embryo viability, promoting initial growth (Phuong Quynh *et al*., 2022). In addition, it was confirmed that increasing the tetrazolium concentration from 0.1% to 0.25% enhances sensitivity without the exposure time between 12 and 24 hours, modifying the results, allowing for faster and more reliable evaluations. This underscores the importance of combining appropriate pretreatments with optimized methodologies to accurately estimate germination potential in species with physiological dormancy such as *Passiflora vitifolia*.

Germination test

The germination test is a functional tool for elucidating the underlying factors associated with the absence of root emergence, such as physiological or morphological dormancy states and structural alterations of the embryo. This test also allows for the corroboration and complementation of the interpretation of results derived from viability tests by integrating direct phenotypic evidence of germination performance (Salazar & Botello Delgado, 2018; Tola *et al*., 2019; Buendía Contreras *et al*., 2022).

The pattern observed in *P. edulis* corresponds to seeds with moderate dormancy, where the tetrazolium test may overestimate viability by detecting living tissues, even though not all of them germinate; therefore, pretreatments to break dormancy are key to transforming that latent viability into effective germination (Wang *et al*., 2023; ISTA, 2022). In the case of *P. tripartita* var. *mollissima*, the data suggest that the sensitivity of the test depends on the concentration and exposure time, and that its optimization together with IC allows for a more accurate diagnosis (Marrero *et al*., 2007; Rodrigues *et al*., 2025).

For *P. edulis f. edulis and P. quadrangularis*, the relationship between the values indicates that membrane integrity is a limiting factor and that the viability detected translates into germination when the embryo maintains its structures intact (Lamont & Pausas, 2023). In contrast, the results for *P. vitifolia* reflect low dormancy and confirm the usefulness of both biochemical tests in predicting germination potential under standard conditions (Fantazzini *et al*., 2020).

CONCLUSIONS

The results obtained in this study demonstrate that the viability of *Passiflora* spp., seeds can be significantly optimized by combining pretreatments such as gibberellic acid, potassium nitrate, and calcium hypochlorite, achieving percentages above 80% in several species, while the use of sodium hypochlorite is not recommended due to its negative effects on the embryo. The reduction in tetrazolium concentration and exposure time improved the efficiency and practicality of the tests. Tetrazolium has established itself as a reliable method for assessing viability, and indigo carmine is useful as a complementary test. These methodological advances strengthen the production and conservation of *Passiflora* spp., validating comprehensive and efficient protocols for the propagation and quality control of these species, which are important for national agriculture.

AUTHOR CONTRIBUTIONS

Conceptualization, S.A.S.M.; Methodology, J.D.Q.C.; Software, P.I.S.G.; Formal analysis, P.I.S.G. and Y.A.P.D.; Investigation, J.D.Q.C. and Y.A.P.D.; Resources, S.A.S.M.; Data curation, Y.A.P.D.; Writing – original draft preparation, Y.A.P.D.; Writing – review and editing, S.A.S.M. and Y.A.P.D.; Visualization, Y.A.P.D.; Supervision, S.A.S.M.; Project administration, S.A.S.M.; Funding acquisition, S.A.S.M

ACKNOWLEDGMENTS

To Universidad Francisco de Paula Santander for their valuable collaboration.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Angelini, L. G., Clemente, C., & Tavarini, S. (2021). Pre-Germination Treatments, Temperature, and Light Conditions Improved Seed Germination of *Passiflora incarnata L*. *Agriculture*, *11*(10), 937. https://doi.org/10.3390/agriculture11100937

Álvarez Cisneros, O., Pérez-Reyes, C. M., & Bonilla-Vichot, M. (2020). Evaluación de la viabilidad en semillas de *Pinus tropicales Morelet* con diferente tiempo de almacenamiento. *Avances*, *22*(1), 97-109.

Bewley, J. D., Bradford, K. J., Hilhorst, H. W. M. & Nonogaki, H. (2013) Seeds: Physiology of Development, Germination and Dormancy (3rd Edition). Springer: New York. http://dx.doi.org/10.1007/978-1-4614-4693-4

Buendía Contreras, J. D., Moreno Rozo, L. Y., & Salazar Mercado, S. A. (2022). Viability of seeds of two varieties of Coffea arabica L. using different pretreatments in the tetrazolium test. Acta Agriculturae Slovenica, 118(3), 1–6. https://doi.org/10.14720/aas.2022.118.3.2363

Campos-Hermosillo, L. E., Montero-Cortés, M. I., Qui-Zapata, J. A., Castañeda-Nava, J. J., & Gutiérrez-Mora, A. (2022). EFECTO DE TRATAMIENTOS PREGERMINATIVOS EN SEMILLAS DE GRANADO. Revista Mexicana de Agroecosistemas, 9, 11-18.

Carvalho, I. L., Meneghello, G. E., Tunes, L. M. D., Jácome, C. C., & Soares, V. N. (2017). Methodological adjustments to the tetrazolium test in rice seeds. Journal of Seed Science, 39(1), 41-49. https://doi.org/10.1590/2317-1545v39n1169643

Castrillón-Pineda, A. I., Rendón, C. A. H., Jaimes, E. P. G., & Betancur, L. F. R. (2024). Estandarización de un protocolo de micropropagación de Passiflora maliformis L (Passifloraceae) a partir de semillas y segmentos nodales. Revista Colombiana de Biotecnología, 26(2), 21-32.

Cordeiro, L. N., Bruno, R. L. A., Lima, L. K. S., Andrade, A. P., Belarmino, K. S., & Alves, E. U. (2022). Suitability of tetrazolium test for Tamarindus indica L. seeds. Semina: Ciências Agrárias, 43(2), 509-522. https://doi.org/10.5433/1679-0359.2022v43n2p509

Da Silva Francischini, D., Lopes, A. P., Segatto, M. L., Stahl, A. M., & Zuin, V. G. (2020). Development and application of green and sustainable analytical methods for flavonoid extraction from Passiflora waste. BMC Chemistry, 14, 56. https://doi.org/10.1186/s13065-020-00710-5

De Castro, É. C. P., Zagrobelny, M., Cardoso, M. Z., & Bak, S. (2018). The arms race between heliconiine butterflies and Passiflora plants–new insights on an ancient subject. Biological Reviews, 93(1), 555–573. https://doi.org/10.1111/brv.12357

Domingues Neto, F. J., Pimentel Junior, A., Putti, F. F., Rodrigues, J. D., Ono, E. O., Tecchio, M. A., Leonel, S. & Silva, M. d. S. (2024). Effect of Plant Growth Regulators on Germination and Seedling Growth of Passiflora alata and Passiflora edulis. Horticulturae, 10(10), 1087. https://doi.org/10.3390/horticulturae10101087

Eshghi Khas, M., Abbasifar, A., & ValizadehKaji, B. (2020). Optimization of in vitro propagation of purple passion fruit (Passiflora edulis), an important medicinal and ornamental plant. International Journal of Horticultural Science and Technology, 7(3), 305-314.

Fantazzini, T. B., Rosa, S. D. V. F., Carvalho, G. R., Liska, G. R., de Carvalho, M. L. M., Coelho, S. V. B., Cirillo, M. Â., & Ribeiro, F. A. S. (2020). Correlation between historical data of the germination test and of the tetrazolium test in coffee seeds by GAMLSS. Seed Science and Technology, 48(2), 179–188. https://doi.org/10.15258/sst.2020.48.2.05

Fischer, G., & Miranda, D. (2021). Review on the ecophysiology of important Andean fruits: Passiflora L. Revista Facultad Nacional de Agronomía Medellín, 74(2), 9471-9481. https://doi.org/10.15446/rfnam.v74n2.91828

França-Neto, J., & Krzyzanowski, F. C. (2019). Tetrazolium: an important test for physiological seed quality evaluation. Journal of Seed Science, 41(3), 359-366. https://doi.org/10.1590/2317-1545v41n3223104

França-Neto, J., & Krzyzanowski, F. C. (2022). Use of the tetrazolium test for estimating the physiological quality of seeds. Seed Science and Technology, 50(1), 31–44. https://doi.org/10.15258/sst.2022.50.1.s.03

González Campos, J., Koch Zúñiga, L., Vera Castro, T., Ortiz Toledo, M., Ipinza carmona, R., & García Rivas, E. (2025). Variabilidad espacial y temporal del tamaño de embrión, viabilidad de semillas y emergencia de plántulas en Araucaria araucana. Ciencia e Investigación Forestal. 31(1), 33-43 https://doi.org/10.52904/0718-4646.2025.624

Hernández, J. R., Iguaran-Diaz, C. J., Araméndiz-Tatis, H., Cardona-Ayala, C. E., & Espitia-Camacho, M. M. (2023). Tetrazolium test to estimate the physiological quality of tamarind (Tamarindus indica L.) seeds. Revista De Ciencias Agrícolas, 40(2), e2205. https://doi.org/10.22267/rcia.20234002.205

Hernández-Martínez, A. X., Lozano-Puentes, H. S., Camacho-Montealegre, C. M., Costa, G. M., & Díaz-Ariza, L. A. (2023). Establishing the Relationship Between Flavonoid Content, Mycorrhization, and Soil Nutritional Content in Different Species of the Genus Passiflora in Colombia. ACS omega, 8(43), 40647–40656. https://doi.org/10.1021/acsomega.3c05606

International Seed Testing Association - ISTA. (2022). International rules for seed testing. Bassersdorf, Suiza: ISTA. https://www.seedtest.org/en/publications/international-rules-seed-testing.html

Issam, N., Kawther, M., Haythem, M., & Moez, J. (2012). Effects of CaCl2 pretreatment on antioxidant enzyme and leaf lipid content of faba bean (Vicia faba L.) seedlings under cadmium stress. Plant Growth Regulation, 68(1), 37-47. https://doi.org/10.1007/s10725-012-9691-5

Iwabuki, H., Matsumoto, Y., & Urano, H. (2018). Study on Decoloring of Indigo by Sodium Hypochlorite Aqueous Solution. Journal of the Japan Society of Colour Material. 91(7), 215-219. https://doi.org/10.4011/shikizai.91.215

Johnson, A., Geary, B., Hulet, A., & Madsen, M. (2023). Breaking Dormancy and Increasing Restoration Success of Native Penstemon Species Using Gibberellic Acid Seed Coatings and U-Shaped Furrows. Plants, 12(23), 4005. https://doi.org/10.3390/plants12234005

Jyoti, C. & Malik, C. P. (2013). Seed deterioration: A review. International Journal of Life Sciences Biotechnology and Pharma Research, 2(3), 373-385.

Khayat, M. (2017). Assessment Regression Relation between Wheat Seed Germination Characteristics Affected Sodium Hypochlorite (NaClO). Journal of Crop Nutrition Science, 3(4), 70-74.

Lallana, V. H., & García, L. F. (2013). Efecto de pretratamientos en la prueba de viabilidad de semillas de Trichocentrum jonesianum (Orchidaceae). Investigación Agraria, 15(2), 129-132.

Lamont, B. B. & Pausas, J. G. (2023). Seed dormancy revisited: Dormancy-release pathways and environmental interactions. Functional Ecology, 37, 1106–1125. https://doi.org/10.1111/1365-2435.14269

Loffler, J., Lima, B., Sobrinho, S., Tavares, A., & Luz, P. (2022). Overcoming seed dormancy in Passiflora species. Idesia, 40, 67-73. https://doi.org/10.4067/s0718-34292022000300067

Luo, Y., Wang, K., Yao, Y., & Nan, L. (2025). Exogenous GA3 Promotes Germination by Reducing Endogenous Inhibitors in Sainfoin (Onobrychis viciifolia) Seeds. Plants, 14(10), 1464. https://doi.org/10.3390/plants14101464.

Mahmood Ur Rehman, M., Liu, J., Nijabat, A., Alsudays, I. M., Saleh, M. A., Alamer, K. H., Attia, H., Ziaf, K., Zaman, Q., & Amjad, M. (2024). Seed priming with potassium nitrate alleviates the high temperature stress by modulating growth and antioxidant potential in carrot seeds and seedlings. BMC Plant Biology, 24(1), 606. https://doi.org/10.1186/s12870-024-05292-1

Marrero, P., Padilla, D. P., Valdés, F., & Nogales, M. (2007). Comparison of three chemical tests to assess seed viability: the seed dispersal system of the Macaronesian endemic plant Rubia fruticosa (Rubiaceae) as an example. Chemoecology, 17, 47-50 http://doi.org/10.1007/s00049-006-0360-x

Mateus-Maldonado, J. F., Díaz-Gallo, S. A., Albornoz-Molano, A. J., Pedroza-Rodríguez, A. M., Costa, G. M., & Díaz-Ariza, L. A. (2023). Efecto de factores bióticos y abióticos en el contenido de flavonoides en hojas de Passiflora sp. L.: Una revisión sistemática. Scientia Agropecuaria, 14(4), 465-478. https://doi.org/10.17268/sci.agropecu.2023.039

Mercado, S., Caleño, J., & Rozo, L. (2020). Improvement of the methodology of the tetrazolium test using different pretreatments in seeds of the genus Epidendrum (Orchidaceae). Journal of Seed Science. 42, e202042013. https://doi.org/10.1590/2317-1545v42231028

Mercado, S., Delgado, E., & Caleño, J. (2020). Optimización de la prueba de tetrazolio para evaluar la viabilidad en semillas de Solanum lycopersicum L. Ciencia & Tecnología Agropecuaria, 21(3), 1-12. https://doi.org/10.21930/rcta.vol21_num3_art:1344

Morales Pizarro, A., Rivas Chero, A. A., Zapata Córdova, A. C., García Guevara, E., Ruesta López, M. & Peña-Castillo, R. (2023). Efecto de diferentes dosis de ácido giberélico en la germinación de papaya (Carica papaya L.) variedad criolla. Chilean Journal of Agricultural & Animal Sciences, 39(3), 392-400. https://doi.org/10.29393/CHJAA39-35EDAM60035

Moreno Álvarez, M. T., Benito Matías, L. F., Herrero Sierra, N., Domínguez Lerena, S., & Peñuelas Rubira, J. L. (2001). Estudio de nuevos métodos de determinación de la viabilidad de las semillas forestales: test de electroconductividad e índigo carmín. Comparación con el test del tetrazolio y su aplicación a Pinus pinaster y Pinus halepensis. Montes para la Sociedad del nuevo milenio, 3(3), 653-658.

Mulaudzi, T., Hendricks, K., Mabiya, T., Muthevhuli, M., Ajayi, R. F., Mayedwa, N., Gehring, C., & Iwuoha, E. (2020). Calcium improves germination and growth of Sorghum bicolor seedlings under salt stress. Plants, 9(6), 730. https://doi.org/10.3390/plants9060730

Nedunchezhiyan, V., Palanivel, M., Jabeen, P. A., Thangavel, P., Ramakrishnan, B., Velusamy, M., Muthusamy, S., & Edm, I. (2023). Effects of gibberellic acid on seed dormancy of black gram (Vigna mungo L.). Journal of Applied Biology & Biotechnology, 11, 256-259. https://doi.org/10.7324/jabb.2023.45506

Nikolova, K., Velikova, M., Gentscheva, G., Gerasimova, A., Slavov, P., Harbaliev, N., Makedonski, L., Buhalova, D., Petkova, N., & Gavrilova, A. (2024). Chemical Compositions, Pharmacological Properties and Medicinal Effects of Genus Passiflora L.: A Review. Plantas, 13(2), 228. https://doi.org/10.3390/plants13020228

Orrego, C. E., Rodríguez, Y., Zemanate, K., & Rodríguez, L. J. (2021). Productividad y competitividad frutícula andina. Producto 3: Informe técnico de resultados sobre la productividad alcanzada por cultivo y por país. Fontagro, p 41.

Osorio Cardona, J. A., Martínez Lemus, E. P., Hío, J. C., Aguirre Rodríguez, J., Vergara Ávila, J. A., Luque Sanabria, N. Y., Rojas Zambrano, E. D., & Cruz Castiblanco, G. N. (2020). Caracterización sanitaria de los cultivos de granadilla, gulupa y maracuyá en Colombia, con especial referencia a la secadera causada por Fusarium solani f. sp. passiflorae. Corporación Colombiana de Investigación Agropecuaria – AGROSAVIA. https://doi.org/10.21930/agrosavia.investigation.7403381

Paim, L. P., Avrella, E. D., Luchesse, J., Freitas, E. M., Lazarotto, M., & Fior, C. S. (2019). Seed analysis of Lupinus albescens Hook. & Arn. Iheringia, Série Botânica, 74, e2019010. https://doi.org/10.21826/2446-82312019v74e2019010

Pereira, Z. C., Cruz, J. M. D. A., Corrêa, R. F., Sanches, E. A., Campelo, P. H., & Bezerra, J. A. (2023) Passion fruit (Passiflora spp.) pulp: A review on bioactive properties, health benefits and technological potential. Food Research International, 166, 112626. https://doi.org/10.1016/j.foodres.2023.112626

Phuong Quynh, T., Lan Xuan, H. T., Ai My, T., Thanh Thao, N., Phuong Thao, N., & Thien Quang, N. (2022). Effect of calcium hypochlorite on surface sterilization and seedling growth of Vietnamese coconut varieties. Vietnam Journal of Biotechnology, 20(4), 663–673. https://doi.org/10.15625/1811-4989/16221

Portuguez-García, M. P., Rodríguez-Ruiz, A. M., Porras-Martínez, C., & González-Lutz, M. I. (2021). Viability of Ischaemum rugosum Salisb. determined by the tetrazolium test. Agronomía Mesoamericana, 32(1), 316-325. https://doi.org/10.15517/am.v32i1.40318

Rodrigues, M. H. B. S., Alves, E. U., Barbosa, L. D. S., Silva, J. N. D., Bezerra, A. C. & Pádua, G. V. G. D. (2025). Adaptation of the tetrazolium test for assessing the seed quality of Crateva tapia L. Journal of Seed Science. 47: e20. https://doi.org/10.1590/2317-1545v47296471

Ruesta-López, M., Zurita-Chinguel, L., Lizano-Pintado, M., Delgado-Vite, M., Zapata-Durand, D., Jiménez-Castillo, J., Peña-Castillo, R., Galecio-Julca & Pizarro, D. A. M. (2024). Biostimulant and imbibition times on passion fruit (Passiflora edulis S., Passifloraceae) seed germination. Tropical and Subtropical Agroecosystems, 27(2).

Sánchez Melo, C. S. (2021). Coevolución entre Heliconius y Passiflora: una búsqueda de evidencia desde su distribución geográfica y riqueza de especies. https://doi.org/10.48713/10336_32646

Sakariyawo, O. S., Oyeledun, K. O., Adeyemi, N. O. & Atayese, M. O. (2020). Nitrogen use efficiency and performance of maize (Zea mays L.) cultivars as influenced by calcium carbide and inorganic nitrogen application rates in a derived savanna. Journal of Plant Nutrition, 43(6), 784–797. https://doi.org/10.1080/01904167.2020.1711947

Santos-Tierno, R., Garcia, R., Fonseca, E., FALEIRO, F., Moreira, D., Pacheco, G., & Mansur, E. (2022). Flavonoid content and antioxidant potential of leaf extracts of Passiflora setacea cv BRS Pérola do Cerrado, a new wild passion fruit cultivar. Journal of Medicinal Plants research. 16(2):26–34. https://doi.org/10.5897/JMPR2021.7190

Salazar, S. A. M. (2012). Germinación asimbiótica de semillas y desarrollo in vitro de plántulas de Cattleya mendelii Dombrain (Orchidaceae). Acta agronómica, 61(1), 69-78.

Salazar Mercado, S. A., & Gélvez Manrique, J. D. (2015). Determinación de la viabilidad de semillas de orquídeas utilizando la prueba de Tetrazolio e Índigo carmín. Revista de Ciencias, 19(2), 59-69.

Salazar, S. A., & Botello Delgado, E. A. (2018). Viabilidad de semillas de glycine max (l.) Utilizando la prueba de tetrazolio. Revista De Investigación Agraria Y Ambiental, 9(2), 89-98. https://doi.org/10.22490/21456453.2270

Salazar, S. A., & Zambrano, C. (2025). Colchicina como inductora de cambios morfológicos y estomáticos en Kalanchoe × laetivirens Desc. Revista De Investigación Agraria Y Ambiental, 16(1), 213-228. https://doi.org/10.22490/21456453.8115

Salazar Mercado, S. A., Quintero Caleño, J. D., & Moreno Rozo, L. Y. (2020). Improvement of the methodology of the tetrazolium test using different pretreatments in seeds of the genus Epidendrum (Orchidaceae). Journal of Seed Science, 42, e202042013. http://doi.org/10.1590/2317-1545v42231028

Salazar Mercado, S. A. & Palencia Delgado, Y. A. (2025). Manejo de diferentes pretratamientos para optimizar la viabilidad de semillas de orquídeas. Acta Agronómica, 72(4), 346–352. https://doi.org/10.15446/acag.v72n4.112433

Silva, A. F., Hilst, P. C., Dias, D. C., & Rogalski, M. (2019). Overcoming dormancy of Passiflora elegans Mast. (Passifloraceae) seeds. Revista Verde De Agroecologia E Desenvolvimento Sustentável. 14(3), 406-411. https://doi.org/10.18378/rvads.v14i3.6552

Salomão, A. N., José, S. C. B. R. & Santos, I. R. I. (2023). Effect of pre-germination treatments on seed germination of Passiflora setacea DC (Passiflora setacea). REVISTA DELOS, 16(43), 580-597. https://doi.org/10.55905/rdelosv16.n43-007

Solano Ortiz, K. J. (2021). Tratamientos pregerminativos en semillas de Lagenaria siceraria Molina Standl. La Libertad: UPSE. https://repositorio.upse.edu.ec/handle/46000/5819

Sourki, A., Hosseni, Z., & Fallah, S. (2019). Effect of Stratification and Its Combination with Gibberellic Acid on Seed Dormancy Breaking of Echinophora platyloba. Iranian Journal of Seed Research, 5(2), 91-104 https://doi.org/10.29252/YUJS.5.2.91

Sousa, J. M. de, Tremea, M. G., Rossetti, C., Madruga, N. P., Scher, L. G., & Tunes, L. V. M. de. (2025). Ajuste Metodológico do Índigo Carmim na Viabilidade de Sementes de Soja. Revista De Gestão E Secretariado, 16(5), e4940. https://doi.org/10.7769/gesec.v16i5.4940

Tola, C. C., Ninacanchi, J. C. H., & Choque, D. C. (2019). Determinación de la concentración de tetrazolio y tiempo de tinción adecuado para el análisis de viabilidad en semillas de café. Apthapi, 5(3), 1671-1682.

Vega-Corrales, E., Campos-Sánchez, V., Monge-Vargas, A. A., Bertsch-Hernández, S., & Vargas-Ramírez, E. (2022). Morphology and viability test optimization in seeds of Passiflora spp. from Costa Rica. Agronomía Mesoamericana, 33, 51567. https://doi.org/10.15517/am.v33iEspecial.51567

Viera, W., Shinohara, T., Samaniego, I., Sanada, A., Terada, N., Ron, L., Suárez-Tapia, A., & Koshio, K. (2022). Phytochemical composition and antioxidant activity of Passiflora spp. Germplasm grown in Ecuador. Plants, 11(3), 328. https://doi.org/10.3390/plants11030328

Wang, S., Wu, M., Zhong, S., Sun, J., Mao, X., Qiu, N., & Zhou, F. (2023) A Rapid and Quantitative Method for Determining Seed Viability Using 2,3,5-Triphenyl Tetrazolium Chloride (TTC): With the Example of Wheat Seed. Molecules, 28(19):6828. https://doi.org/10.3390/molecules28196828

Xu, J., Xiao, P., Li, T., Chen, C., Wang, X., Wang, Z., & Chen, B. (2024). Tetrazolium viability test method for micro-seeds of the endangered plant Deinanthe caerulea. Seed Science and Technology, 52(1), 57-66 https://doi.org/10.15258/sst.2024.52.1.06

Zembele, E. R., & Ngulube, E. S. (2022). Effect of Seed Pretreatment Methods on Germination and Early Seedling Growth of Senna spectabilis. International Journal of Forestry Research, 2022(1), 6731479 https://doi.org/10.1155/2022/6731479