



Research Article

Viability, germination and in vitro growth of *Caesalpinia spinosa* from seeds at different phenological stages

Myriam Ximena Mancheno Cárdenas[‡], Ines Patricia Malo[‡], Mateo David León[‡], Jessica Tatiana Muñoz[‡], Juan Jose Solorzano[‡], Jorge Santiago Rojas[‡]

[‡] Universidad Politécnica Salesiana, Cuenca, Ecuador

Corresponding author: Myriam Ximena Mancheno Cárdenas (mmancheno@ups.edu.ec)

Academic editor: Gbenga Akomolafe

Received: 18 Mar 2025 | Accepted: 12 May 2025 | Published: 11 Jun 2025

Citation: Mancheno Cárdenas M, Malo I, León M, Muñoz J, Solorzano J, Rojas J (2025) Viability, germination and in vitro growth of *Caesalpinia spinosa* from seeds at different phenological stages. One Ecosystem 10: e153308. <https://doi.org/10.3897/oneeco.10.e153308>

Abstract

Caesalpinia spinosa is a plant species present in South America, capable of adapting to different climatic and edaphic conditions. It is considered a multipurpose species due to its environmental benefits, contributing to the restoration of degraded soils and containing active compounds intended for various industries. Consequently, this study aimed to evaluate the viability, germination and in vitro growth of *C. spinosa* from seeds in different phenological stages. The development of this research was carried out in parts. First, for the viability study, seeds were classified as immature, mature and overripe, from which embryos were extracted and placed in a 1% tetrazolium salt aqueous solution for 5 hours. Second, the water content in the seeds was determined using thermogravimetric analysis to classify the seeds. Third, the seeds were disinfected with Tween 20 for 10 minutes, 70% EtOH for 1 minute and 1.5% NaClO for 10 minutes. Then, germination pre-treatment was performed with a cut in the distal region of the cotyledon (CT) and removal of the seed coat (ST) and they were cultivated in a basal MS medium supplemented with Gamborg vitamins. The highest viability was observed in immature seeds (SI) and mature seeds (SM), with values of 97.72% and 73.45% and corresponding water content of 52.23% and 7.60%, respectively. Regarding germination,

the SI-ST treatment achieved a germination speed of 48.55% at 4 days, while in the interaction of phenological state and scarification, the SI-ST treatment achieved the highest percentage at 93.16%. In terms of growth, the treatments showed no statistically significant differences, with values between 52.26 mm and 64.79 mm. These results indicate that the phenological state of the seeds and the type of scarification are important for obtaining quality seedlings in vitro in a shorter time, which can be used in future reforestation and ecosystem restoration programmes.

Keywords

in vitro cultivation, in vitro seedlings, Fabaceae, Tara

Introduction

The *Caesalpinia* genus includes 150 species, of which 40 are present in South America (Neri Chávez et al. 2018). *C. spinosa*, also known as tara or guarango, is a tree from the Caesalpiniaceae family, native to Peru (Castro et al. 2017) and found in Venezuela, Colombia, Ecuador, Peru, Bolivia and Chile, growing in areas with average annual precipitation between 230 and 500 mm and temperatures ranging from 12 to 18°C (Guerrero et al. 2016).

C. spinosa is a plant species known for its environmental benefits, as it contributes to soil conservation in the sloped areas of the Andean Basin (Murga et al. 2023). It can grow in degraded soils unsuitable for conventional agriculture (De La Torre 2018), suggesting its potential as an interesting species for ecological soil restoration (Sangay and Robin 2018). Additionally, it contributes to fog capture in forests, which improves soil water availability (Cordero et al. 2017). Furthermore, various vegetative parts of *C. spinosa* contain biomolecules used in different industries: polyphenolic compounds from dry pods are used in the cosmetic and pharmaceutical industries (Salirrosas et al. 2021), as biopesticides for controlling phytopathogens in the agricultural industry (León and Mancheno 2020) and tannins are employed in the leather industry (Ibieta and Peñarrieta 2021).

C. spinosa exhibits a low natural regeneration rate in both remnant forests and agroforestry systems (Murga et al. 2023). This population decline is driven by land-use changes caused by deforestation, desertification, invasive species and the expansion of agricultural, livestock and urban boundaries (Isbell et al. 2017). In light of this situation, in vitro plant propagation technology has emerged, where micropropagation refers to the multiplication of plants from germinated seeds or vegetative tissues cultured with the correct combination of nutrients and growth regulators (Kulak et al. 2022). To obtain high-quality seedlings, it is important to consider parameters such as seed viability and germination, as well as seedling growth, with the latter two assessed at the in vitro level. Several authors have conducted research on these parameters in various species of the *Caesalpinia* genus.

Viability studies have been conducted by Guerrero et al. (2016) with mature and immature seeds of *C. spinosa*, Neto and Barbedo (2015) with mature seeds of *C. echinata* and Ferro et al. (2019) with mature seeds of *C. pulcherrima*. These studies evaluated seed physiological quality using the tetrazolium test, which is based on the activity of specific dehydrogenase enzymes and indirectly measures the respiratory capacity of living seed tissues (França and Krzyzanowski 2022).

Germination studies were carried out by Romero et al. (2016) with *C. glabrata* seeds, achieving the highest germination percentage by applying seed coat scarification and by Vishnu (2020) with *C. bonduc* seeds, where mechanical scarification yielded the highest germination percentage. Da Silva et al. (2018a) worked with *C. ferrea* seeds scarified with emery stone and achieved a higher germination rate compared to explants of zygotic embryos.

Several authors have also worked on tissue culture of specific species within the *Caesalpinia* genus. Núñez et al. (2017) established an in vitro propagation protocol for *C. spinosa* from axillary buds obtained from selected trees with superior morpho-agronomic characteristics. In *C. bonduc*, Cheruvathur et al. (2010) achieved shoot organogenesis from callus derived from the epicotyl, while Santosh et al. (2012) established a high-frequency shoot induction micropropagation protocol using cotyledons as explants. Da Silva et al. (2018b) developed a protocol for the rapid in vitro multiplication of *C. ferrea*.

Material and methods

Location and Plant Material

Experimental processes were carried out at the Biotechnology Laboratory of the Life Sciences Laboratories at Politécnica Salesiana University, Cuenca campus. Pods were collected from 10 randomly selected trees in the Monay parish of Cuenca (17M 725585 UTM 9679559) and from the only two trees in the Jima parish of Sigsig (17M 0729048 UTM 9645248 - 17M 0727560 UTM 9646970). Both locations are situated in the Azuay Province, Ecuador. The pods were classified into three groups, based on their colour (Fig. 1):

1. Immature pod (red with greenish-yellow coloration);
2. Mature pod (light red with yellow);
3. Overripe pod (dark red).

They were collected between November and December 2022, January and February and May and June 2023, respectively.

Seed Viability

Seeds were manually extracted from *C. spinosa* pods and classified into three groups based on their colour (Fig. 2):



Figure 1.

Colouration of *Caesalpinia spinosa* pods. Immature pod (reddish with greenish-yellow), mature pod (light red with yellow) and overripe pod (dark red).



Figure 2.

Colouration of *C. spinosa* seeds. SI (greenish-yellow), SM (light brown) and SSM (dark brown). [SI: immature seed, SM: mature seed and SSM: overripe seed].

1. Immature seed (yellow-green colouration);
2. Mature seed (light brown colouration);
3. Overripe seed (dark brown colouration).

The seed viability test was conducted using the tetrazolium test (ISTA 2016). A total of 1,000 mature and overripe seeds were submerged in water for 48 hours for hydration, with 30% of the seed coat softening. Following the method established by Salazar et al. (2020), the embryo and cotyledons from the various seeds were extracted using a scalpel and placed in a 1% aqueous tetrazolium salt solution for 5 hours, stored in the dark at room temperature. The test was conducted in triplicate, with 100 immature seeds, 100 hydrated mature seeds and 100 hydrated overripe seeds. After the incubation period, each embryo and the condition of the cotyledons were evaluated individually based on colour.

Seed Water Content

New *C. spinosa* seeds were classified into three groups as mentioned in the previous section. According to ISTA (2016) and Ileleji et al. (2019), the seed water content was determined using the thermogravimetric analysis technique. Seeds were placed in a halogen moisture analyser (HB43-S Halogen Mettler Toledo®) and to reach the dry content of the sample, the temperature was set at 105°C. This is a destructive water measurement technique. The test was conducted in triplicate with 100 seeds of each type.

Pre-germinative Treatments and In Vitro Growth

Immature, mature and overripe *C. spinosa* seeds were disinfected according to the following protocol: Tween 20 for 10 minutes, ethanol (EtOH) 70% for 1 minute and sodium hypochlorite (NaClO) 1.5% for 10 minutes, with three rinses between each disinfectant agent.

The pre-germinative treatments consisted of mechanical scarification, cutting at the distal region of the cotyledon (CT) as described by Alvarez et al. (2014), removal of the seed coat (ST) and intact seeds (control). Previously, mature and overripe seeds were soaked for 48 hours. Following the method established by Koné et al. (2015), the seeds and cotyledons with embryos were placed in a sterile semi-solid Murashige and Skoog (MS) medium, supplemented with Gamborg vitamins, 30 g l⁻¹ sucrose, 7 g l⁻¹ agar and 500 mg l⁻¹ polyvinylpyrrolidone (PVP) (pH 5.8), sterilised at 121°C and 1 atm for 20 minutes.

The in vitro cultures were maintained in a growth chamber with a relative humidity of 60 ± 5%, a light-dark photoperiod of 12 hours each and a temperature of 20 ± 2°C. The light source used in this experiment was LED type with an irradiance intensity of 10 µmol m⁻² s⁻¹ and a light wavelength of 400–700 nm.

Seed germination was evaluated daily for a period of 10 days after sowing. The standard for determining seed germination was that the length of the radicle must penetrate 1 cm through the seed coat. The germination rate over time and the effect of phenological status and scarification on seed germination were evaluated. The growth of new seedlings was assessed 21 days after sowing. The tests were conducted in triplicate with 40 seeds of each type.

Germination rate (%) = number of germinated seeds / total number of seeds considered × 100%.

Finally, the in vitro growth of the seedlings (epicotyl + hypocotyl) was evaluated 21 days post-sowing.

Statistical Analysis

All experiments were analysed using R statistical software, version 4.0.2. The results of seed viability, pre-germinative treatments and growth were presented as means $\pm$ standard deviation. Differences between treatments were evaluated using a one-way Analysis of Variance (ANOVA), based on a Randomised Complete Block Design (RCBA), a two-way ANOVA with a 3 x 3 factorial design, based on a Completely Randomised Design (CRD) and a one-way ANOVA, based on a CRD, with a significance level of 0.05, respectively. The assumptions of normality, homoscedasticity of variances and additivity were assessed using the Shapiro-Wilk test, Levene's test and Tukey's one-degree-of-freedom test, respectively. Pairwise multiple comparisons were conducted using the Tukey's Honest Significant Difference (HSD) test.

On the other hand, the results of water content in seeds were presented as mean ranges. Differences between treatments were evaluated using a one-way ANOVA, based on the Kruskal-Wallis design, with a significance level of 0.05. Pairwise multiple comparisons were performed using the Holm adjustment method.

Results

Seed Viability

The origin of the plant material did not significantly affect the results according to the experimental design; seed quantity and availability were considered, so seeds from *C. spinosa* trees located in the City of Cuenca were used.

According to the evaluation of *C. spinosa* seed staining, variation was found in the three groups, indicating that the condition of the tissues is influenced by exposure to tetrazolium salt. Based on this, the seeds were classified into different categories according to the characteristics of the embryo and cotyledons as viable or non-viable.

Immature (SI) and mature (SM) seeds showed uniform embryos of light pink colour, with the tissues exhibiting a firm texture and were considered viable and vigorous seeds. In contrast, overripe seeds (OS) showed deteriorated embryos with a deep red colouration; the cotyledons displayed the presence of deteriorated tissue, characterised by a deep red colour and the tissues exhibited a soft texture (Fig. 3).

The average viability rates for SI, SM and SSM were 97.72%, 73.45% and 26.76%, respectively. The treatments showed a significant effect ($P = 4.32e^{-08}$). The viability percentage of SI was 1.33 and 3.65 times higher compared to SM and SSM, respectively (Fig. 4).

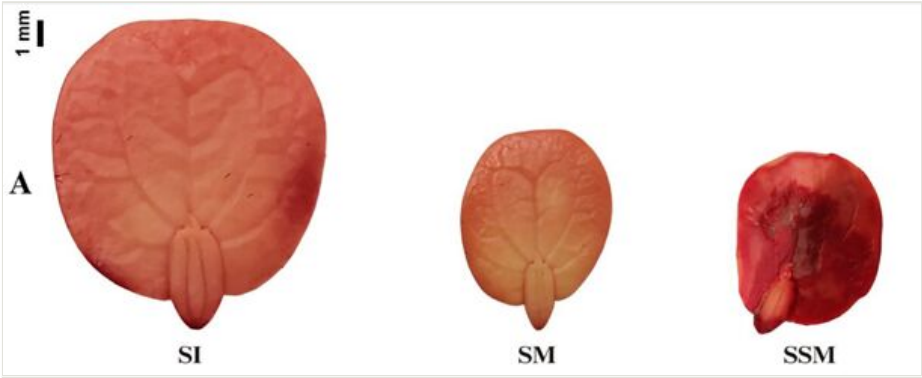


Figure 3.
Seed viability of *C. spinosa* using the tetrazolium test. SI and SM were viable, while SSM was not viable.

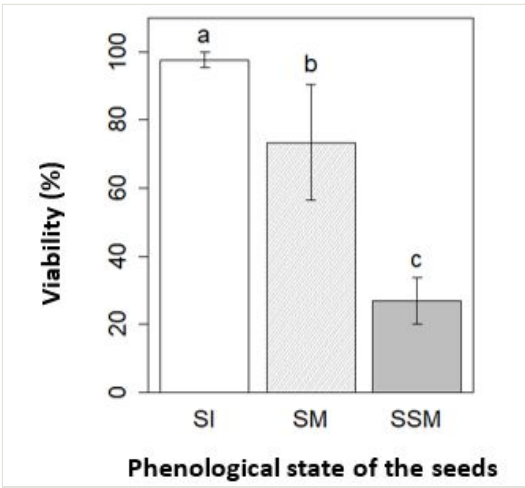


Figure 4.
Effect of 1% tetrazolium salt evaluated after 5 hours on *C. spinosa* seeds. The results are expressed as the mean percentage of seed viability $\pm$ ED. Treatments sharing a common letter do not differ at a 5% level according to Tukey's HSD test [SI: immature seed, SM: mature seed and SSM: overmature seed].

Water Content of Seeds

According to the results obtained for the plant species *C. spinosa*, a higher water content in the seeds ensures a greater percentage of viability; clearly, immature seeds (SI) are better compared to mature (SM) and overripe seeds (SSM).

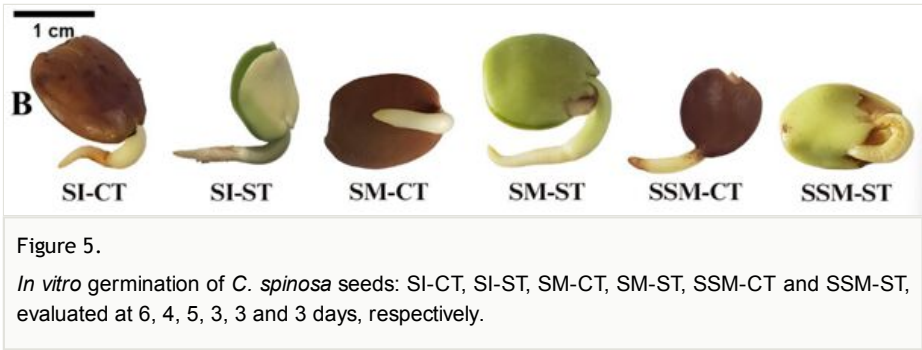
The mean water content ranges for SI, SM and SSM were 15.5%, 9.5% and 3.5%, respectively. The different phenological stages of the seeds showed a significant effect (P

= 0.00051). The percentage of water in SI was 1.63 and 4.42 times higher compared to SM and SSM, respectively (Table 1).

Table 1. Water content in <i>Caesalpinia spinosa</i> seeds.			
Phenological stages of seeds	Mean (%)	Mean ranges (%)	Groups
SI	52,23	15,5	A
SM	7,60	9,5	Ab
SSM	4,59	3,5	B

Germination and In Vitro Growth

The results of this research showed that the phenological state of the seeds, type of scarification and days of evaluation are important factors in the in vitro germination of *C. spinosa* seeds. The highest percentage of germination was observed in immature seeds (SI) with testa removal (ST) at 48.55%, followed by mature seeds (SM) with ST at 33.01%, evaluated at 4 days compared to other treatments. The minimum percentage of germination was recorded in overripe seeds (SSM) with cotyledon cut (CT) and SSM with ST, both at 0%, evaluated on the same day. In the various treatments (SI-CT, SM-CT and SSM-CT), germination was observed between 20.82% and 7.41% over 12 days (Figs. 2B and 4A, Fig. 5 and Fig. 6).



The germination of *C. spinosa* seeds is conditioned by the phenological state of the seeds and the type of scarification. It was observed that the highest percentage of germination was achieved with the SI-ST treatment at 93.16%, followed by the SI-CT treatment at 48.84% and the control treatment at 0%. A similar trend was observed in mature seeds, with the highest percentage of germination obtained from the SM-ST treatment at 84.52%, while the SM-CT treatment yielded 44.75% and the control treatment at 0%. For overripe seeds, the percentage of germination did not differ significantly between treatments, with SSM-ST and SSM-CT achieving 32.40% and 27.03%, respectively and the control treatment at 0% (Fig. 7 and Fig. 8).

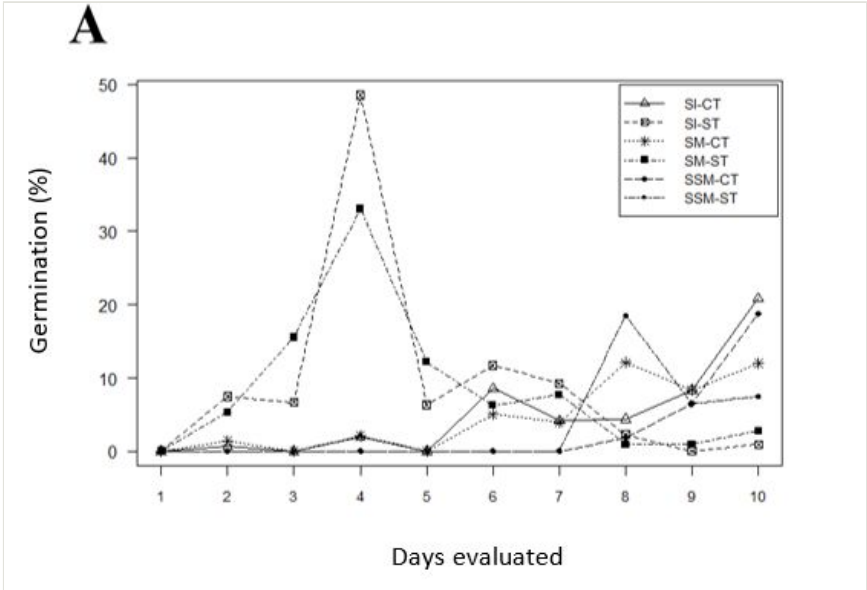


Figure 6.
Germination rate of *C. spinosa* seeds according to phenological state and pre-germination treatment.

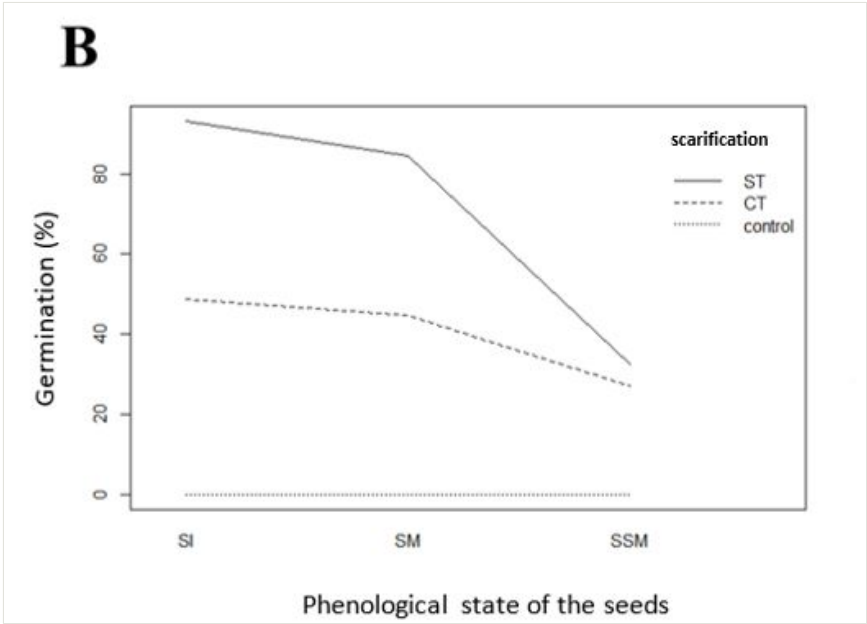


Figure 7.
Germination of *C. spinosa* seeds in relation to phenological state and pre-germination treatment factors.

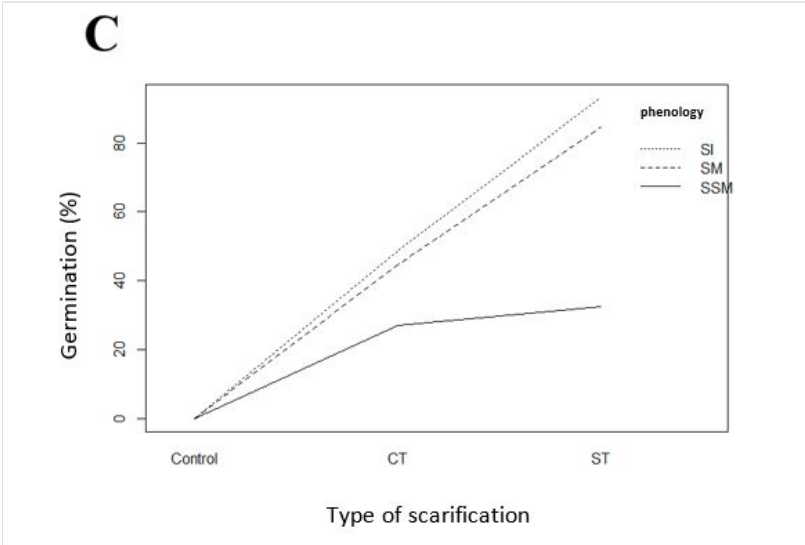


Figure 8.
Germination of *C. spinosa* seeds in relation to phenological state and pre-germination treatment factors.

The average growth for SI-CT, SI-ST, SM-CT and SM-ST was 52.26 mm, 54.06 mm, 64.79 mm and 61.26 mm, respectively. The treatments did not show a significant effect ($P = 0.223$) (Fig. 9 and Fig. 10 - Table 2).

Table 2.
Germination percentage of *C. spinosa* seeds in relation to phenological stage and type of scarification.

Phenological stage	Type of scarification		
	ST	CT	Control
SI	93.16% _{Aa}	48.84% _{Ab}	0% _{Ac}
SM	84.52% _{Aa}	44.75% _{Ab}	0% _{Ac}
SSM	32.40% _{Ba}	27.03% _{Aa}	0% _{Aa}

Discussion

Results obtained in this study indicated that the viability and in vitro germination of *C. spinosa* seeds depend on the phenological stage of the seeds. Our results showed that the water content in the seeds is an important parameter. In this context, immature seeds (SI) achieved a higher percentage of viability, germination speed and germination percentage compared to mature (SM) and overripe seeds (SSM). We observed that, as the phenological maturation process of *C. spinosa* pods progresses, the water content in

the seeds decreases, which could affect seed vigour. It is important to understand that water content is specific to each plant species (Li et al. 2020).



Figure 9.
In vitro growth of *C. spinosa* seedlings at 21 days post-sowing [SI: immature seed, SM: mature seed and SSM: overmature seed. CT: cut in the distal region of the cotyledon and ST: seed coat removal].

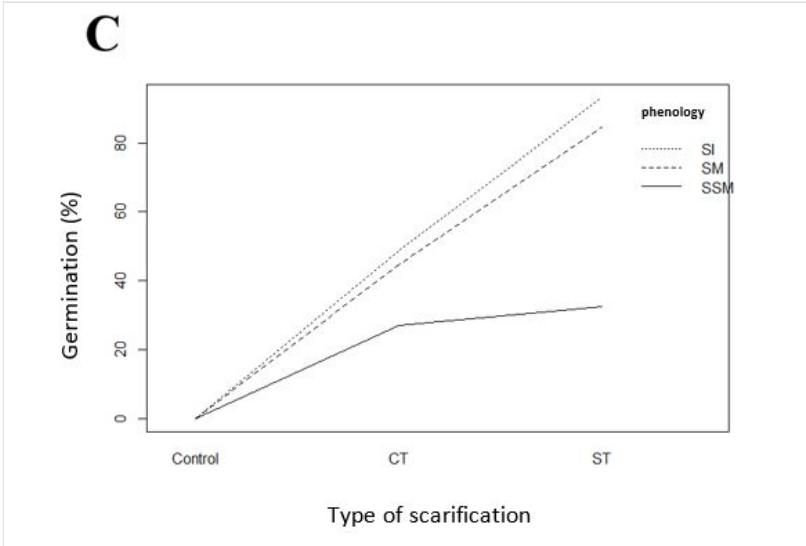


Figure 10.
Germination of *C. spinosa* seeds in relation to phenological state and pre-germination treatment factors.

The tetrazolium test is an efficient alternative for determining seed viability and vigour (Ferro et al. 2019). As shown in Fig. 2A, visual inspection made it possible to assess the state of the embryo and cotyledons of the different types of *C. spinosa* seeds for correct discrimination. Our observations were corroborated by Guerrero et al. (2016) in mature *C. spinosa* seeds and by Neto and Barbedo (2015) in *C. echinata* seeds with 10% water

content. Ferro et al. (2019) evaluated and classified the embryos and cotyledons of *C. pulcherrima* seeds, where a viable seed exhibited a uniform pink colour and firm-textured tissue, while a non-viable seed showed an intense red colour and flaccid tissues with deformation. A dead seed appeared milky white. Similar characteristics have also been observed in other Fabaceae species, as reported by Nunes et al. (2022) in *Tamarindus indica* L. According to Fernandes et al. (2019), red or light pink colours indicate viable tissue and the terminology used to determine the colours of seeds observed in the tetrazolium test is generally established by the authors.

Based on the results in Fig. 3 and Table 1, a relationship could be established between the percentage of viability and water content. SI and SM with average water contents of 52.23% and 7.60% obtained average viabilities of 97.72% and 73.45%, respectively. Contrasting these results with the observations of Guerrero et al. (2016), we found differences; in the present research, using SI resulted in a higher percentage of viability compared to SM seeds, while the aforementioned authors reported average viabilities of 78% and 86% in SI and SM, respectively. Therefore, the differences found in seeds at the SM phenological stage could be attributed to seasonal variations in environmental conditions (De Oliveira et al. 2010). In contrast, the differences found in seeds at the SI phenological stage could be due to the ideal humidity level in our study, resulting in high metabolic activity (Ferreira and Barbedo 2017). Research does not report viability and germination in seeds at the SSM phenological stage, possibly because their dehydration level causes damage to the cytoskeleton (Ranganathan and Groot 2023).

Various studies report that seeds of the genus *Caesalpinia* are recalcitrant, meaning they lose viability and germination capacity if their moisture content is below 20% (Romero and Pérez 2016). These seeds, upon detaching from the tree with high moisture content, are metabolically active and need to remain hydrated (Berjak and Pammenter 2017; Ranganathan and Groot 2023). Water is crucial for their metabolic and enzymatic reactions (Kibinza et al. 2006). Recalcitrant seeds germinate and establish rapidly under humid conditions due to their large energy reserves (Colville 2017; Pritchard et al. 2022). During drying, they experience negative changes such as reduced respiration, toxin accumulation and DNA degradation (Kibinza et al. 2006, Yuniarti 2015), which can lead to dysfunction of cellular organelles and extensive vacuolisation, predisposing the seeds to deterioration (Ranganathan and Groot 2023). In overly dry seeds, extreme dehydration alters the water film interface covering the cellular structure and exposes macromolecules in the membranes, such as lipids and proteins, making them vulnerable to reactive oxygen species (ROS) (Ramtekey et al. 2022). In this context, Li et al. (2016) and Castro et al. (2017) extracted oil from the seeds and fruits of *C. spinosa*. Damage caused by free radicals leads to a breakdown of genomic integrity within the nucleus (Shvachko and Khlestkina 2020). This occurs because antioxidant enzymes and non-enzymatic antioxidants, such as ascorbic acid, decrease during maturation and glutathione acts as the main redox buffer, with its redox state (E GSSG/2GSH) being a marker of viability. As seeds age, this marker becomes more positive; viability is lost when (E GSSG/2GSH) exceeds -160 mV (Nadarajan et al. 2022), allowing us to predict whether a seed will survive and germinate or die. Therefore, E (GSSG/2GSH) is a

universal marker of plant cell viability and allows us to predict whether a seed will survive, germinate and produce a new plant or if it will die (Kranter et al. 2006).

In the interaction between the phenological state of the seeds and the type of scarification, germination in this study began 2 days after sowing, achieving its highest germination rate at 4 days. These results are similar to those obtained by Alvarez et al. (2014), who observed germination starting at 3 days in *C. cacaeco* seeds with a scarification that involved a small cut in the testa. Meanwhile, Sánchez et al. (2016) reported germination beginning at 1 day and the peak at 4 days in *C. platyloba* seeds with a scarification involving rubbing the seeds on sandpaper. In contrast, Da Silva et al. (2018b) achieved germination beginning between 7 and 10 days with mechanical scarification by rubbing *C. ferrea* seeds on an abrasive surface. These results suggest that mechanical scarification with CT and ST is effective in removing the dormancy of *C. spinosa* seeds, allowing for a higher germination rate.

Regarding the relationship between the phenological state of the seeds and the type of scarification, although there was no significant difference between treatments, SM-CT achieved a seedling growth of 64.79 mm in 21 days. These results are superior to those reported by Assis et al. (2012) and Marín and Iglesias (2022) with the woody species *Anacardium othonianum* and *Pinus pseudostrobus*, which achieved seedling lengths of 20.00 mm and 30.26 mm evaluated at 30 days, respectively.

Conclusions

This research demonstrated that the phenological state and the scarification applied to *C. spinosa* seeds are important for the parameters of viability, speed and percentage of germination. In immature seeds (SI) and mature seeds (SM), the viability results were 97.72% and 73.45% with water contents of 52.23% and 7.60%, respectively. On one hand, regarding the relationship between phenological state and type of scarification, the germination speed for SI-ST and SM-ST achieved the best averages of 48.55% and 33.01% at 4 days after sowing. On the other hand, in the relationship between phenological state and type, the best results for SI-ST and SI-CT were 93.16% and 48.84%, while for SM-ST and SM-CT, they were 84.52% and 44.75%, respectively; the control treatments showed no germination. Finally, the results of in vitro growth for SI and SM with the different types of scarification evaluated at 21 days after sowing ranged between 52.26 mm and 64.79 mm. This research provides a foundation for selecting SI and SM of *C. spinosa* for efficient in vitro seedling production, which could later be allocated to reforestation programmes, conservation and sustainable economic development.

Acknowledgements

The authors of this research express their gratitude to the Vice-Rectorate of Research at Politécnica Salesiana University, the Research and Biodiversity Assessment Group

(GIVABI), the Rufford Foundation, the Jima Ltda Community Development Cooperative and the Tambillo Forestry Initiative for their support and collaboration, which were instrumental in the successful development of this study.

Hosting institution

Politécnica Salesiana University, Jima Ltda Community Development Cooperative

Conflicts of interest

The authors have declared that no competing interests exist.

References

- Alvarez C, Barradas L, Ponce O, Williams G (2014) Soil seed bank, seed removal, and germination in a seasonally dry tropical forest in Veracruz, Mexico. *Botanical Sciences* 92 (1): 111-121. <https://doi.org/10.17129/botsci.42>
- Assis K, Pereira F, Cabral J, Silva F, Silva J, Santos S (2012) In vitro cultivation of *Anacardium othonianum* Rizz.: effects of salt concentration and culture medium volume. *Acta Scientiarum. Agronomy* 34 (1). <https://doi.org/10.4025/actasciagron.v34i1.10984>
- Berjak P, Pammenter NW (2017) Recalcitrance. *Encyclopedia of Applied Plant Sciences* 532-539. <https://doi.org/10.1016/b978-0-12-394807-6.00048-4>
- Castro A, Ramos N, Juárez J, Ruiz J, Choquesillo F, Ponce J, Santa María O, Castillo A, García D, Escudero J, Navarro A, Huamán S, Machaca M, Gutiérrez P, Ramirez E, Gonzales S (2017) COMPOSICIÓN QUÍMICA DEL ACEITE ESENCIAL DE *Caesalpinia spinosa* "TARA", EVALUACIÓN ANTIOXIDANTE Y EFECTO ANTIBACTERIANO FRENTE A *Streptococcus mutans*. *Ciencia e Investigación* 19 (2): 89-94. <https://doi.org/10.15381/ci.v19i2.13636>
- Cheruvathur M, Britto J, Thomas T (2010) Callus induction and shoot regeneration from epicotyl explants of ethnomedicinally important *Caesalpinia bonduc* (L.) Roxb. *Iranian Journal of Biotechnology* 8 (4): 263-269.
- Colville L (2017) Seed Storage. *Encyclopedia of Applied Plant Sciences* 335-339. <https://doi.org/10.1016/b978-0-12-394807-6.00080-0>
- Cordero I, Ruiz B, Balaguer L, Richter A, Pueyo J, Rincón A (2017) Rhizospheric microbial community of *Caesalpinia spinosa* (Mol.) Kuntze in conserved and deforested zones of the Atiquipa fog forest in Peru. *Applied Soil Ecology* 114: 132-141. <https://doi.org/10.1016/j.apsoil.2017.02.015>
- Da Silva D, Imakawa A, Costa S, Sampaio P (2018a) In vitro culture of zygotic embryos and seeds of *Caesalpinia ferrea* Martius. *Hoehnea* 45 (4): 663-668. <https://doi.org/10.1590/2236-8906-65/2018>
- Da Silva D, Imakawa A, Bruno F, Costa S, Sampaio P (2018b) In vitro propagation and seedling acclimatization of *Caesalpinia ferrea* Mart., a valuable medicinal plant in the Amazon (Fabaceae). *Boletim do Museu Paraense Emílio Goeldi - Ciências Naturais* 13 (1): 57-65. <https://doi.org/10.46357/bcnaturais.v13i1.368>

- De La Torre L (2018) La Tara beneficios ambientales y recomendaciones para su manejo sostenible en relictos de bosque y sistemas agroforestales. In Condesan URL: <https://condesan.org/wp-content/uploads/2018/10/Libro-Tara-Condesan-2.pdf>
- De Oliveira J, Barbedo C, Salatino A, Figueiredo R (2010) Reserve carbohydrates and lipids from the seeds of four tropical tree species with different sensitivity to desiccation. *Brazilian Archives of Biology and Technology* 53 (4): 889-899. <https://doi.org/10.1590/s1516-89132010000400019>
- Fernandes P, Cavalcante L, Albuquerque A, Rodrigues A, Andrade L, Melo J (2019) Preparo da semente de colubrina para execução do teste de tetrazólio. *Revista Verde de Agroecologia e Desenvolvimento Sustentável* 14 (5): 625-628. <https://doi.org/10.18378/rvads.v14i5.7599>
- Ferreira A, Barbedo C (2017) Changes in desiccation tolerance and respiratory rates of immature *Caesalpinia echinata* Lam. Seeds. *Journal of Seed Science* 39 (2): 123-132. <https://doi.org/10.1590/2317-1545v39n2167788>
- Ferro J, Rocha D, Junior J, Neto J, Silva J, Pavão J (2019) Germination and Viability of Seeds of *Caesalpinia pulcherrima* Newly Harvested and Stored. *Journal of Agricultural Science* 11 (7). <https://doi.org/10.5539/jas.v11n7p73>
- França J, Krzyzanowski F (2022) Use of the tetrazolium test for estimating the physiological quality of seeds. *Seed Science and Technology* 50 (2): 31-44. <https://doi.org/10.15258/sst.2022.50.1.s.03>
- Guerrero R, Lombardi I, Gonzales H, Figueroa C, Calderón A (2016) Determinación de la viabilidad de semilla de *Caesalpinia spinosa* (Molina) Kuntze y su correlación con el contenido de goma y tanino. *Revista Forestal del Perú* 31 (2). <https://doi.org/10.21704/rfp.v31i2.1028>
- Ibieta G, Peñarrieta J (2021) CARACTERIZACIÓN QUÍMICA Y CUANTIFICACIÓN DE TANINOS DEL POLVO DE *Caesalpinia spinosa*: TARA BOLIVIANA. *Revista Boliviana de Química* 38 (1). <https://doi.org/10.34098/2078-3949.38.1.3>
- Illeleji K, Garcia A, Kingsly A, Clementson C (2019) Comparison of Standard Moisture Loss-on-Drying Methods for the Determination of Moisture Content of Corn Distillers Dried Grains with Solubles. *Journal of AOAC INTERNATIONAL* 93 (3): 825-832. <https://doi.org/10.1093/jaoac/93.3.825>
- Isbell F, Adler P, Eisenhauer N, Fornara D, Kimmel K, Kremen C, Letourneau D, Liebman M, Polley H, Quijas S, Scherer M (2017) Benefits of increasing plant diversity in sustainable agroecosystems. *Journal of Ecology* 105 (4): 871-879. <https://doi.org/10.1111/1365-2745.12789>
- ISTA ISTA (2016) Reglas internacionales para el análisis de las semillas. In *International Rules for Seed Testing* 16 (1). <https://doi.org/10.15258/istarules.2016.f>
- Kibinza S, Vinel D, Côme D, Bailly C, Corbineau F (2006) Sunflower seed deterioration as related to moisture content during ageing, energy metabolism and active oxygen species scavenging. *Physiologia Plantarum* 128 (3): 496-506. <https://doi.org/10.1111/j.1399-3054.2006.00771.x>
- Koné M, Koné T, Silué N, Soumahoro A, Kouakou T (2015) In Vitro Seeds Germination and Seedling Growth of Bambara Groundnut (*Vigna subterranea* (L.) Verdc. (Fabaceae)). *The Scientific World Journal* 2015 (1). <https://doi.org/10.1155/2015/595073>
- Kranner I, Birtić S, Anderson K, Pritchard H (2006) Glutathione half-cell reduction potential: A universal stress marker and modulator of programmed cell death? *Free*

- Radical Biology and Medicine 40 (12): 2155-2165. <https://doi.org/10.1016/j.freeradbiomed.2006.02.013>
- Kulak V, Longboat S, Brunet N, Shukla M, Saxena P (2022) In Vitro Technology in Plant Conservation: Relevance to Biocultural Diversity. *Plants* 11 (4). <https://doi.org/10.3390/plants11040503>
 - León M, Mancheno M (2020) Actividad antifúngica de compuestos fenólicos de tara (*Caesalpinia spinosa*) frente a *Fusarium graminearum*. *Revista de Investigación Agraria y Ambiental* 12 (1): 39-50. <https://doi.org/10.22490/21456453.3755>
 - Li X, Simpson W, Song M, Bao G, Niu X, Zhang Z, Xu H, Liu X, Li Y, Li C (2020) Effects of seed moisture content and *Epichloe* endophyte on germination and physiology of *Achnatherum inebrians*. *South African Journal of Botany* 134: 407-414. <https://doi.org/10.1016/j.sajb.2020.03.022>
 - Li Z, Yang F, Yang L, Zu Y (2016) Ultrasonic Extraction of Oil from *Caesalpinia spinosa* (Tara) Seeds. *Journal of Chemistry* 2016: 1-6. <https://doi.org/10.1155/2016/1794123>
 - Marín L, Iglesias L (2022) Effect of LED lights on the in vitro growth of *Pinus pseudostrobus* Lindl., plants. *Journal of Forest Science* 68 (8): 311-317. <https://doi.org/10.17221/43/2022-jfs>
 - Murga H, Abanto C, Fernandes L, Chu-Koo F, Schwartz G, Nuñez E, Stewart P, Santos R, Vourlitis G, De Almeida F, Bardales R (2023) Tara (*Caesalpinia spinosa*) in Natural and Agroforestry Systems under an Altitudinal Gradient in the Peruvian Andes: Responses to Soil and Climate Variation. *Agronomy* 13 (2). <https://doi.org/10.3390/agronomy13020282>
 - Neri Chávez JC, Collazos Silva R, Oliva M, Huamán Huaman E, Vásquez García J (2018) Aplicación de la escarificación física y mecánica en la emergencia y crecimiento de semillas de tara (*Caesalpinia espinosa*). *Revista de Investigación de Agroproducción Sustentable* 2 (2). <https://doi.org/10.25127/aps.20182.392>
 - Neto N, Barbedo C (2015) Viability of Brazilwood seeds (*Caesalpinia echinata* Lam.) stored at room temperature in controlled atmospheres. *Journal of Seed Science* 37 (2): 93-101. <https://doi.org/10.1590/2317-1545v37n2142340>
 - Nunes L, Alcântara R, Lucas S, De Andrade A, Da Silva K, Ursulino E (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>
 - Núñez J, Quiala E, Feria M, Mestanza S, Teanga S (2017) Propagación in vitro de *Caesalpinia spinosa* (Mol.) O. Kuntz a partir de yemas axilares de árboles plus seleccionados. *Biotechnología Vegetal* 17 (2). URL: <https://revista.ibp.co.cu/index.php/BV/article/view/543>
 - Pritchard H, Serphen, Tsan FY, Wen B, Jaganathan G, Calvi G, Pence V, Mattana E, Ferraz I, Seal C (2022) Regeneration in recalcitrant-seeded species and risks from climate change. *Plant Regeneration from Seeds* 259-273. <https://doi.org/10.1016/b978-0-12-823731-1.00014-7>
 - Ramtekey V, Cherukuri S, Kumar S, V. SK, Sheoran S, K. UB, K. BN, Kumar S, Singh AN, Singh HV (2022) Seed Longevity in Legumes: Deeper Insights Into Mechanisms and Molecular Perspectives. *Frontiers in Plant Science* 13 <https://doi.org/10.3389/fpls.2022.918206>
 - Ranganathan U, Groot S (2023) Seed Longevity and Deterioration. *Seed Science and Technology* 91-108. https://doi.org/10.1007/978-981-19-5888-5_5

- Romero J, Pérez C (2016) Seed morphological traits and their implication in the ex situ conservation of woody species in Tumbesian dry forests. *Ecosistemas* 25 (2): 59-65. <https://doi.org/10.7818/ecos.2016.25-2.07>
- Romero J, Orellana V, Balseca M (2016) Morfología, imbibición y germinación de semillas de *Caesalpinia glabrata* Kunth (Fabaceae) distribuida en un bosque seco tropical. *Revista Chapingo Serie Zonas Áridas* 15 (2): 89-101. <https://doi.org/10.5154/r.rchsza.2016.05.004>
- Salazar S, Quintero J, Bustos V (2020) Implementación de la prueba de tetrazolio en las semillas de *Raphanus sativus* L. *Revista Facultad de Ciencias Básicas* 15 (2): 7-15. <https://doi.org/10.18359/rfcb.3831>
- Salirrosas D, Reategui N, Crespo JP, Sánchez L, Arqueros M, Cabrera A, Martínez R, Ayala C, Baby A, Prieto Z (2021) Safety Profile of *Caesalpinia spinosa* Aqueous Extract Tested in *Oreochromis niloticus* Toward Its Application in Dermocosmetics. *Frontiers in Sustainability* 2 <https://doi.org/10.3389/frsus.2021.696289>
- Sánchez B, Pacheco E, Reyes A, Lugo G, Casillas P, Saucedo C (2016) RUPTURA DE LATENCIA FÍSICA EN SEMILLAS DE *Caesalpinia platyloba* S. WATSON. *Interciencia* 10 (10): 691-695. URL: <https://www.redalyc.org/articulo.oa?id=33947690007>
- Sangay S, Robin D (2018) Ecological characteristics of Tara (*Caesalpinia spinosa*), a multipurpose legume tree of high ecological and commercial value. In: Gorawala P (Ed.) *Agricultural Research Updates*. 22. Nova Science Publishers, Lima, Perú. URL: https://www.researchgate.net/publication/324220472_Ecological_characteristics_of_Tara_Caesalpinia_spinosa_a_multipurpose_legume_tree_of_high_ecological_and_commercial_value
- Santosh S, Krishna V, Venkatesh K, Girish K (2012) Micropropagation of *Caesalpinia Bonduc* Roxb., Through High Frequency Shoot Induction From the Cotyledonary Explants. *International Journal of Recent Scientific Research* 3 (12): 1050-1054. URL: <http://www.recentscientific.com>
- Shvachko N, Khlestkina E (2020) Molecular genetic bases of seed resistance to oxidative stress during storage. *Vavilov Journal of Genetics and Breeding* 24 (5): 451-458. <https://doi.org/10.18699/vj20.47-o>
- Vishnu A (2020) Seed germination studies in *Caesalpinia bonduc* (L) Roxb. *JETIR* 7 (2). URL: <https://www.jetir.org/papers/JETIR2002363.pdf>
- Yuniarti N (2015) Viability and Biochemical Content Changes in Seed Storage of Jabon Putih (*Anthocephalus Cadamba* (Roxb) Miq.). *Jurnal Manajemen Hutan Tropika* (Journal of Tropical Forest Management) 21 (2): 92-98. <https://doi.org/10.7226/jtfm.21.2.92>