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Physiological quality of *Vasconcellea pubescens* A. DC seeds as a function of moisture content and pre-germination treatments

Calidad fisiológica de las semillas de *Vasconcellea pubescens* A. DC en función del contenido de humedad y tratamientos pregerminativos

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Abstract

Understanding seed physiological quality and germination control significantly contribute to use and conservation of native species. *Vasconcellea pubescens* A. DC. is important due to its nutritional value in Peru; however, one of the main limitations of this species is seed dormancy. The objective of this study was to evaluate the influence of seed moisture content and seven pre-germination treatments on the physiological quality of seeds stored for six years at 10 °C and 50% relative humidity. The experiment was conducted under a completely randomized design with a factorial arrangement with two seed moisture contents (8 and 9%) and seven pre-germination treatments (GA_3 200 ppm for 24 h, GA_3 400 ppm for 24 h, KNO_3 2.5% for 24 h, KNO_3 1.5% for 24 h^{-1}, mechanical scarification, hot water at 70 °C for 5 min, and a control). Analysis of variance (ANOVA) was performed at a 5% significance level using Tukey's mean comparison test ($\alpha = 0.05$). The results showed there was a significant effect of ¨moisture content" and "pre-germination treatments" on germination. Mechanical scarification and GA_3 at 200 ppm promoted the highest germination (up to 80%). In contrast, KNO_3 and hot water treatments resulted in high percentages of non-germinated seeds, while the application of GA_3 at 400 ppm caused cotyledon oxidation. In this context, storing *V. pubescens* seeds at 9% moisture content keeps their viability and enhances their response to pre-germination treatments, representing an effective alternative for *ex situ* conservation and ensuring the preservation and sustainable use of this species.

Keywords: dormancy, gibberellic acid, seed preservation.

Resumen

Conocer la calidad fisiológica de las semillas y el control de la germinación contribuye significativamente en el uso y conservación de especies nativas. *Vasconcellea pubescens* A. DC, es importante por su valor nutricional en Perú; sin embargo, uno de los problemas que presenta esta especie es la latencia de su semilla. El objetivo de este estudio fue evaluar la influencia del contenido de humedad de la semilla y siete tratamientos pregerminativos, sobre la calidad fisiológica de las semillas almacenadas durante seis años a 10 °C y 50% de humedad relativa. El estudio se realizó en un diseño completamente al azar con un arreglo factorial, evaluando dos contenidos de humedad de semilla (8 y 9%) y siete tratamientos pregerminativos (GA_3 200 ppm 24 h^{-1}, GA_3 400 ppm 24 h^{-1}, KNO_3 2,5% 24 h^{-1}, KNO_3 1,5% 24 h^{-1}, escarificación mecánica, agua caliente a 70 °C 5 $minutos^{-1}$, y testigo). Los ANOVA se efectuaron a un nivel de signifi-

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cancia del 5% empleando la prueba de medias de Tukey (α = 0,05). Los resultados mostraron que los factores “Contenido de humedad” y “Tratamientos pregerminativos” afectaron significativamente la germinación, siendo la escarificación mecánica y GA_3 a 200 ppm los que promovieron la mayor germinación (hasta 80%). En contraste, el KNO_3 y el agua caliente presentaron altos porcentajes de semillas no germinadas, además que, la aplicación de GA_3 a 400 ppm presentó oxidación de los cotiledones. En este sentido, el almacenamiento de semillas de *V. pubescens* a una humedad de 9% mantiene su viabilidad y mejora su respuesta a los tratamientos pregerminativos, como una alternativa para la conservación *ex situ*, asegurando la preservación y utilidad sostenible de esta especie.

Palabras clave: ácido giberélico, conservación de semillas, dormancia.

1. Introduction

The Caricaceae family comprises four genera, among which *Vasconcellea* stands out with 53 recognized species, including *Vasconcellea pubescens* A. DC (Scheldeman et al., 2007; Kyndt et al., 2005), locally known as ‘papayita serrana’. Its consumption provides numerous nutritional benefits due to its high content of phenolic compounds, β-carotene, and vitamin C, which confer strong antioxidant capacity (Vega-Gálvez et al., 2022). Regarding processed products, Peru exported 25,344 jars of syrup in 2022, according to data from the Ministerio de la Producción (2023). Furthermore, this species is considered an important source of genes associated with tolerance to papaya ringspot virus [PRSV-P] (Ordaz-Pérez et al., 2017; Urtasun et al., 2020).

Therefore, its conservation is essential for programs focused on the genetic improvement of other crops, such as *Carica papaya* (Fielder et al., 2016; Van Treuren et al., 2017; Urtasun et al., 2020). The incorporation of wild relatives into crop breeding programs contributes significantly to global food security and to the adaptation of agricultural systems to future climatic conditions (Urtasun et al., 2020).

One of the main strategies for germplasm conservation is ex situ seed conservation, which is particularly suitable for orthodox seeds due to their tolerance to desiccation and low-temperature storage, allowing them to maintain viability for extended periods under controlled conditions (Gutiérrez and Koch, 2015). Although seed longevity depends on storage temperature and the moisture content of the genetic material, the duration of seed viability is closely related to the biochemical composition of the species of interest, particularly lipid content. Seeds with higher lipid concentrations, such as oilseeds, generally exhibit a greater capacity to withstand water loss. However, lipid content is also an important factor because lipid oxidation may occur during desiccation. In addition, other factors associated with oxidative stress, such as sugar and protein content, may also influence seed longevity (Pirredda et al., 2024; Corbineau, 2024). Although low-temperature storage combined with low seed moisture content is necessary to prolong seed preservation, these conditions alone are insufficient to guarantee the maintenance of physiological quality over the long term.

In contrast, several strategies have been developed to improve the germination of seeds stored for extended periods. In this regard, the application of gibberellic acid [GA_3], which has a favorable effect on seed germination, has been documented in *Vasconcellea cundinamarcensis* (Benítez et al., 2013), *Brassica napus*, *Triticum aestivum*, and *Hordeum vulgare* (Koçak Şahin et al., 2025). Likewise, the positive effect of potassium nitrate [KNO_3] on seed germination has been reported in *Capsicum annuum* (Maphalaphathwa & Nciizah, 2025) and *Solanum lycopersicum* (Ali et al., 2020). In both cases, germination percentages increased compared to the control treatment.

The use of hot water has also been considered an alternative for improving seed germination. This effect was reported by González & Mendoza (2008) in *Leucaena leucocephala* and by Mohammadi et al. (2012) in *Abelmoschus esculentus*. However, the aforementioned pre-germination treatments have not yet been evaluated in *V. pubescens*, which limits the technical options available for improving the conservation and germination of this species.

Therefore, this study aimed to evaluate the influence of two seed moisture contents and seven pre-germination treatments on the physiological quality of *V. pubescens* seeds stored for six years at 10 °C and 50% relative humidity.

2. Materials and Methods

2.1. Study site and plant material

The study was conducted at the Seed Laboratory of the Seed Bank of the Dirección de Recursos Genéticos y Biotecnología of the Instituto Nacional de Innovación Agraria [INIA], Lima, Peru. The experimental period lasted two months, from May to June 2024.

Botanical seeds of mountain papaya or *papayita* *serrana* (*Vasconcellea pubescens* A. DC), in Spanish, were used. The seeds were obtained from the mountain papaya collection maintained at the INIA's Estación Experimental Agraria Arequipa and had been conserved for six years.

2.2. Treatments

In this study, the factor "Seed moisture content" (A) was evaluated at two levels (8 and 9%), while the factor "Pre-germination treatments" (B) was evaluated at seven levels. The combination of both factors resulted in a total of 14 treatments (Table 1).

2.3. Preparation of chemical pre-germination treatments

In the experiment, seeds of mountain papaya were used. The seeds had been stored for six years in hermetically sealed aluminum envelopes at moisture contents of 8% and 9%, respectively, under conditions of 10 °C and 50% relative humidity.

Solutions of GA_3 and KNO_3 were prepared as pre-germination treatments. For the GA_3 solutions, 36 and 72 mg were weighed and diluted in 5 mL of 96% ethanol. Distilled water was then added to obtain 180 mL of solution at concentrations of 200 ppm and 400 ppm, respectively. For the KNO_3 solutions, 2.7 and 4.5 g of the compound were weighed to obtain concentrations of 1.5% and 2.5%, respectively, and subsequently diluted with distilled water to a final volume of 180 mL. Once the concentrations of each pre-germination treatment had been prepared, 840 seeds were soaked for 24 h in each solution.

2.4. Conditioning of seeds for physical pre-germination treatments

Once the position of the radicle in the seeds had been identified, scarification was performed by making transverse cuts at the seed poles. For seed stratification, distilled water was heated to 70 °C, and the *V. pubescens* seeds were immersed for 5 min.

2.5. Variables evaluated

Root emergence and morphological changes were determined through daily observations. Radicle protrusion, the appearance of root hairs, and seed length were recorded.

Germination percentage was determined based on the number of germinated seeds relative to the total number of seeds sown. Dormancy break was considered to begin with germination or rupture of the seed coat (González-Zertuche & Orozco-Segovia, 1996).

The germination index was evaluated as the relationship between mean germination time and germination capacity (Scott et al., 1984). Mean germination time was defined as the average time required for seed germination (Côme, 1970). Germination rate was calculated as the ratio between the number of germinated seeds and germination time (Maguire, 1962), whereas the coefficient of velocity was considered inversely proportional to the time required for daily germination (Kotowski, 1926).

To determine germination rates, seeds were placed in Petri dishes containing sterilized filter paper. Distilled water was used to maintain filter paper moisture. The properly labeled Petri dishes were then placed in a CIMMSA RGDS/L-425 germination chamber at 25 °C and 80% relative humidity.

2.6. Data analysis

The study was conducted using a completely randomized design with a 2 × 7 factorial arrangement. The evaluated factors were seed moisture content (8 and 9%) and pre-germination treatments (gibberellic acid at 200 and 400 ppm, potassium nitrate at 2.5% and 1.5%, mechanical scarification, hot water treatment, and a control treatment), resulting in a total of 14 treatments. The statistical model described in equation [1] was used:

$$Y_{ijk} = \mu + MC_i + PGT_j + (MC \times PGT)_{ij} + \varepsilon_{ijk} \quad [1]$$

Where: Y_{ijk} = response of the i-th moisture content

Table 1. Treatments under study regarding the physiological quality of *Vasconcellea pubescens* seeds.

	Pre-germination treatment	Seed moisture content	
T1	GA_3 200 ppm 24 ^{h}-1		
T3	KNO_3 2.5% 24 h^{-1}		
T4	KNO_3 1.5% 24 h^{-1}	8%	9%
T5	Mechanical scarification		
T6	Hot water 70 °C 5 minutes^{-1}		
T7	Control		

under the j-th pre-germination treatment; μ = overall population mean; MC = moisture content (i = 1 or 2); PGT = pre-germination treatment (j = 1–7); and ε = experimental error ~ NID (0, σ2).

When statistical significance was detected for the evaluated variables, Tukey's multiple comparison test ($\alpha = 0.05$) was performed to identify significant differences among treatments in terms of seed quality.

3. Results and Discussion

3.1. Morphological changes

According to Li et al. (2017), germination consists of three distinct phases: water imbibition, radicle protrusion, and seedling establishment. In the present study, *Vasconcellea pubescens* seeds exhibited radicle protrusion at 6 days after sowing (DAS). Radicle protrusion represents one of the key stages of germination and corresponds to the visible emergence of the radicle through the endosperm layers as a result of uniform root growth. This process involves reserve mobilization, metabolic activation, DNA, RNA, and protein synthesis, as well as coleoptile elongation (Ding et al., 2024). Furthermore, the overproduction of reactive oxygen species [ROS], such as superoxide radicals [O_2^-], hydrogen peroxide [H_2O_2], and hydroxyl radicals [·OH], has been reported to facilitate radicle protrusion (Li et al., 2017).

Between 8 and 11 DAS, not only was an increase in root length observed, but also an abundant development of root hairs [RH]. Therefore, 11 DAS was considered the germination time for *V. pubescens* seeds. Root hairs play an important role in soil exploration, as they enhance the absorption of water and nutrients and contribute to rhizosphere formation (Rongsawat et al., 2021). In addition, cotyledon emergence was observed at 27 DAS, whereas complete seedling formation of *V. pubescens* occurred at 33 DAS.

3.2. Germination and dormancy

The results showed a significant interaction between seed moisture content and pre-germination treatments ($p \leq 0.05$) for the germination percentage; however, for dormancy time, only the pre-germination treatments showed statistically significant differences (Figure 1). Furthermore, it was observed that with seed moisture content of 8 and 9%, scarification (T5) was an effective practice that yielded 80% germination. These results, and in accordance with Bonner & Karrfalt (2008), suggest that *V. pubescens* seeds exhibit physical dormancy due to the presence of impermeable layers (Baskin & Baskin, 2004) that prevent water from penetrating the seed. In this trial, the storage conditions were 10 °C and 50% relative humidity; it can be inferred that relative humidity did not play a significant role, since the seeds were stored in impermeable aluminum bags. In species such as *Glycine max* (Ali et al., 2018; Ali et al., 2015) and *Carica papaya* (Zulhisyam et al., 2013) it is recommended to store the seeds with a moisture content between 6 and 8%, respectively, to maintain high viability during storage; the storage time of orthodox seeds can be up to 60 years (Solberg et al., 2020).

Potassium nitrate is a widely used compound for improving seed germination through the supply of K^+ and NO_3^-. Potassium is associated with osmotic regulation and is primarily located in the cytoplasm and vacuole, whereas nitrate is involved in protein synthesis (Thongtip et al., 2022). In the present study, the application of KNO_3 (T3 and T4) and hot water treatment (T6) had adverse effects on seed physiological quality, as no germination was observed under these treatments.

The effect of KNO_3 has been reported to depend on both species and concentration, which may explain the high percentage of hard seeds obtained with these treatments ($p \leq 0.05$; Figure 1). In *Vasconcellea cundinamarcensis*, KNO_3 application had no effect on germination; however, in *V. goudotiana*, this treatment significantly improved germination (Benítez et al., 2013). Likewise, increased germination percentages following KNO_3 application have been reported in *V. quercifolia* and *Carica chilensis* (Urtasun et al., 2020; Loayza et al., 2023), contrary to the results observed in the present study.

Therefore, it is inferred that the KNO_3 concentrations used in this experiment (1.5% and 2.5%) may have caused deterioration of the *V. pubescens* embryo, resulting in severe damage and subsequent loss of viability.

The use of hot water as a pre-germination treatment, although frequently recommended, does not guarantee favorable results in all species. This has been observed in seeds of *Neonotonia wightii*, *Prosopis ruscifolia*, and *Nolina cespitifera*, in which immersion in hot water did not increase germination percentage, demonstrating a species-dependent response (Castillo-Quiroz et al., 2018; Flores et al., 2020; Abdala et al., 2020).

Although hot water treatment can be used to break seed dormancy, exposure to extreme temperatures may cause severe embryo damage, compromising seed viability and inhibiting subsequent seedling growth and development, as observed in the present study. The ineffectiveness of hot water treatment was confirmed by the high percentage of hard seeds detected in both the 8% and 9% moisture-content treatments ($p \leq 0.05$; Figure 2). In *Abelmoschus esculentus* L., immersion in water at 100 °C for 5 min reduced germination to less than 10% (Musara et al., 2015). Likewise, high temperatures have been reported to damage cell membranes, stimulate hydrogen peroxide accumulation, and reduce the activity of antioxidant enzymes (Rashid et al., 2020; Al-jebory, 2013).

It is important to highlight that the application of GA_3 was effective in promoting germination when seeds were stored at 9% moisture content (Figure 1), possibly due to the improved physiological condition of the seeds at this moisture level. This condition may have enabled the preservation of protective mechanisms through the presence of non-reducing sugars, antioxidant accumulation, and protective compounds such as raffinose, oligosaccharides, flavonoids, lignins, and vitamin E (Ranganathan & Groot, 2023), thereby facilitating a more favorable response to the application of this phytohormone.

Scarified seeds exhibited a shorter dormancy period, followed by those treated with gibberellic acid ($p \leq 0.05$; Figure 1C). In this regard, previous studies have demonstrated that scarification is an effective method for promoting seed germination in *Amburana cearensis* and *Myroxylon peruiferum* (Galíndez et al., 2015), although its effectiveness may vary depending on the plant material evaluated (Priyadharshini & Lekha, 2021).

3.3. Hard seeds and damaged tissue

Hard seeds are those unable to absorb water during the imbibition process; therefore, they remain in the same condition as at the beginning of the experiment (Latorre, 2015). Seeds treated with KNO_3 remained almost entirely (approximately 100%) unchanged

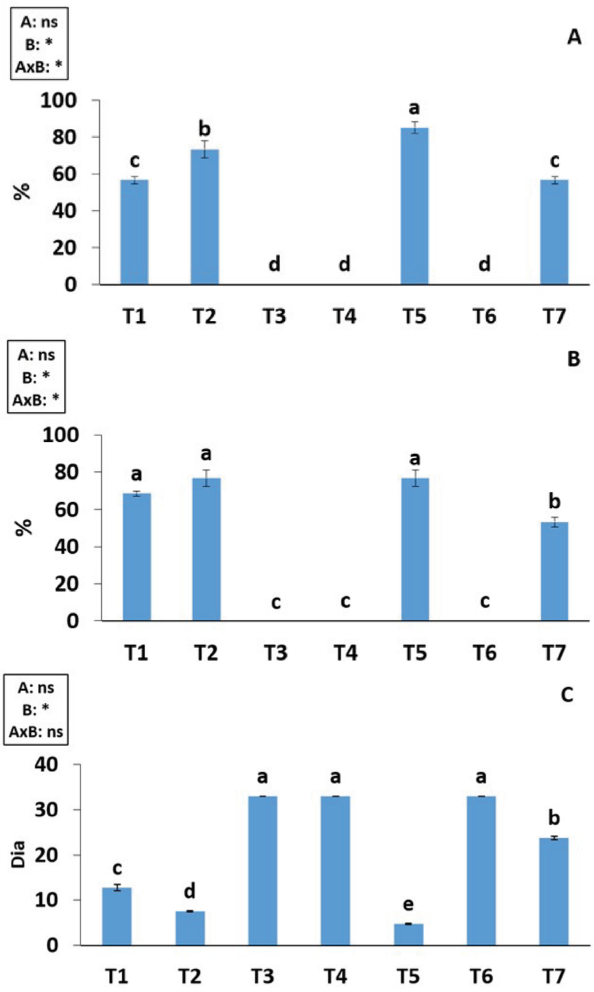

Figure 1. Germination and dormancy of mountain papaya (*Vasconcellea pubescens* A. DC.) seeds stored for 6 years. (A) Germination percentage at 8% moisture content. (B) Germination percentage at 9% moisture content. (C) Dormancy. T1: 8% moisture content + GA_3 200 ppm for 24 h; T2: 8% moisture content + GA_3 400 ppm for 24 h; T3: 8% moisture content + KNO_3 2.5% for 24 h; T4: 8% moisture content + KNO_3 1.5% for 24 h; T5: 8 % moisture content + mechanical scarification; T6: 8% moisture content + hot water at 70 °C for 5 minutes; T7: 8% moisture content. ns: not significant. *: significant difference. Within each figure: A: factor “seed moisture content”; B: factor “pregerminative treatments”.

from their initial condition, suggesting that under the conditions evaluated in this study, this compound was not effective in promoting germination. One possible explanation may be related to the concentration used, since high concentrations of KNO_3 have been reported to reduce germination percentage in rice (Javed et al., 2020). Likewise, Hernández et al. (2022) reported that elevated KNO_3 levels negatively affect antioxidant enzyme activity. In addition, it is possible that the seed coat acted as a physical barrier, limiting the penetration of this compound.

The use of hot water and GA_3 at 200 ppm for 24 h resulted in a lower percentage of hard seeds, particularly in seeds stored at 9% moisture content. In contrast, the application of GA_3 at 400 ppm caused tissue damage in the seeds without completely inhibiting germination (Figure 2). However, phytotoxic effects are often associated with a marked reduction in germination percentage. Similar findings have been reported by Chauhan et al. (2019), who observed that high concentrations of GA_3 inhibited radicle growth in oat seeds. Likewise, Kaewkhieo-ngam et al. (2023) documented harmful effects of high GA_3 doses on seed germination.

3.4. Physiological quality

At both 8% and 9% seed moisture contents, the germination index [GI] was higher in the control treatment (Figure 3 A and B). It is important to note that an interaction between the evaluated factors was observed. For both moisture contents, treatments without pre-germination application doubled the values obtained with mechanical scarification.

Regarding germination rate [GR], seeds with 8% moisture content showed values of 0.26 and 0.24 in the control treatment and in seeds treated with GA_3 at 200 ppm for 24 h, respectively. When seeds had 9% moisture content, the control treatment showed the lowest value (0.24).

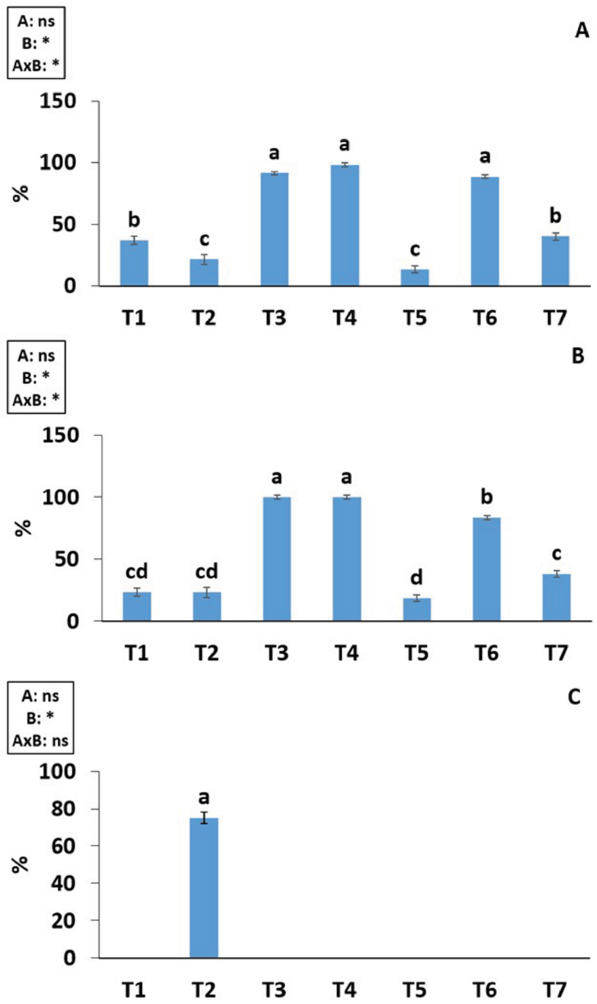

Figure 2. Hard seeds and cotyledon phytotoxicity in mountain papaya (*Vasconcellea pubescens* A. DC.) seeds stored for 6 years. (A) Percentage of hard seeds at 8% moisture content. (B) Percentage of hard seeds at 9% moisture content. (C) Percentage of seeds with damaged tissue. T1: + GA_3 200 ppm for 24 h; T2: + GA_3 400 ppm for 24 h; T3: + KNO_3 2.5% for 24 h; T4: + KNO_3 1.5% for 24 h; T5: + mechanical scarification; T6: + hot water at 70 °C for 5 minutes; and T7: without pre-germinative treatment. ns: not significant. *: significant difference. Within each figure: A: factor "seed moisture content"; B: factor "pre-germinative treatments".

For the velocity coefficient [VC], only the pre-germination treatments showed a significant effect. Mechanical scarification produced the highest VC value (11.7), whereas the control treatment showed the lowest value (3.4). According to Souza & Marcos-Filho (2001), the seed coat plays an important role in germination, vigor, and longevity. In the present study, a cut made in the seed coat facilitated water absorption and gas exchange (Maeda et al., 2021).

4. Conclusions

The results demonstrate that a seed moisture content of 9% promotes better preservation of seed viability, facilitating a more favorable response to pre-germination treatments. Mechanical scarification and GA_3 at 200 ppm were the most effective methods for promoting higher germination rates. However, the phytotoxic effects observed with GA_3 at 400 ppm highlight the importance of balanced phytohormone application to avoid negative effects on seedling development.

The use of KNO_3 and hot water treatments negatively affected germination, emphasizing the importance of adjusting treatment concentrations and conditions to prevent seed damage.

These findings are relevant for the *ex situ* conservation of *Vasconcellea pubescens* A. DC, since adequate seed moisture content and the appropriate selection of pre-germination treatments can significantly improve seed physiological quality, thereby contributing to crop adaptation and sustainability under environmental stress conditions.

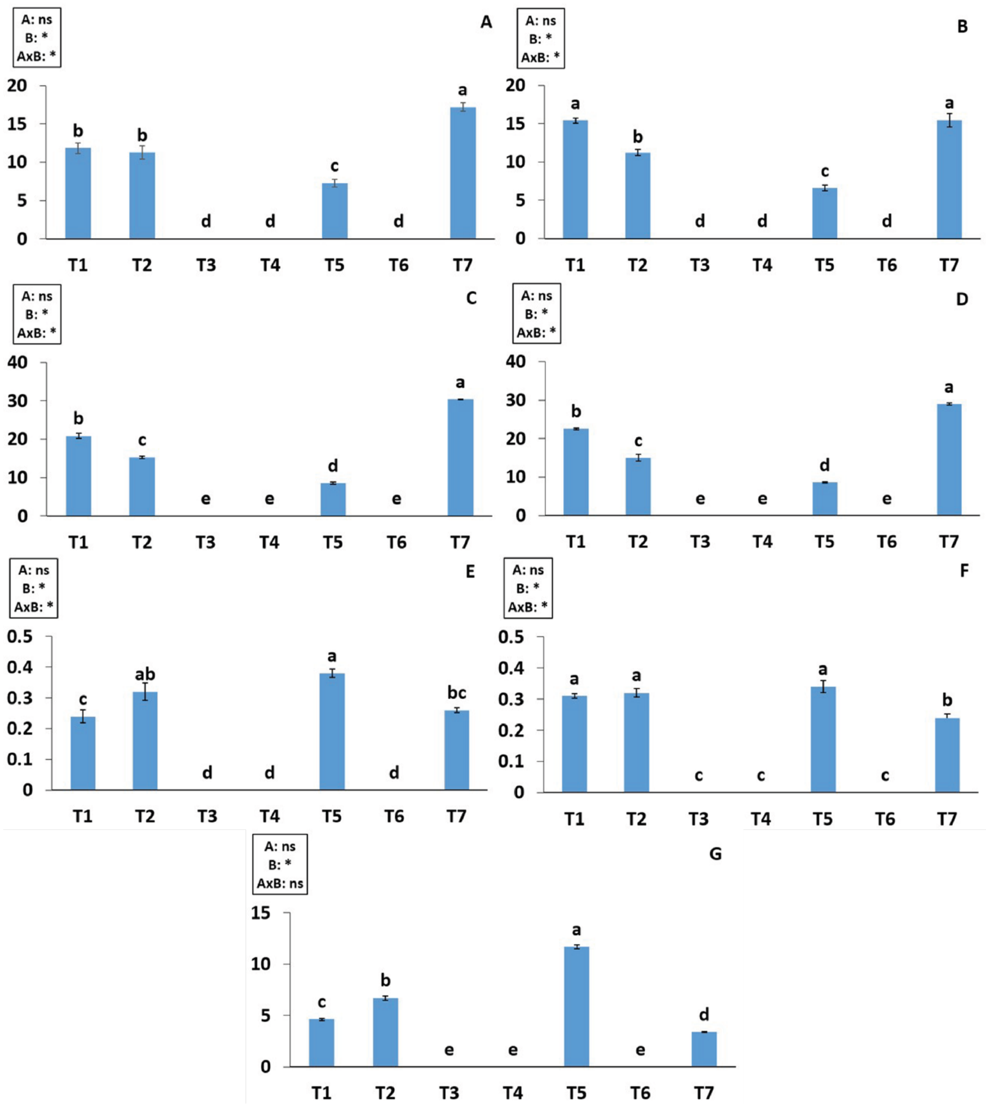

Figure 3. Rates of seed vigor of *Vasconcellea pubescens* A. DC. Germination index (A: 8%) (B: 9%); mean germination time (C: 8%) (D: 9%); germination rate (E: 8%) (F: 9%); coefficient of velocity (G). T1: + GA_3 200 ppm for 24 h; T2: + GA_3 400 ppm for 24 h; T3: + KNO_3 2.5% for 24 h; T4: + KNO_3 1.5% for 24 h; T5: + mechanical scarification; T6: + hot water at 70 °C for 5 minutes; and T7: without pregerminative treatment. ns: not significant. *: significant difference. Within each figure: A: factor "seed moisture content"; B: factor "pregerminative treatments".

Contributor roles

- Jhojana Lorenzo Quispe: conceptualization, investigation, methodology, resources.
- Fredesvinda Carrillo Castillo: validation, writing – review& editing.
- Ana Laura Rucabado Miranda: validation, writing – review & editing.
- Ricardo Borjas Ventura: investigation, software, writing – original draft.

Data Availability

Data will be made available on request.

Use of Artificial Intelligence

The authors declare that no artificial intelligence has been used in the preparation of the manuscript.

Ethical Implications

The authors declare that no ethical conflicts or concerns arose during interactions with the farmers, as participation was voluntary and no personal data were collected. The conversations focused on the importance of conserving native potatoes within their territory.

Conflict of Interest

The authors declare that they have no affiliation with any organization with a direct or indirect financial interest that could have appeared to influence the work reported.

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