VIABILITY AND MORPHOMETRY IN *Agave* SEEDS AS AN INITIAL STUDY FOR SUSTAINABLE MANAGEMENT AND GENETIC PRESERVATION †

[VIABILIDAD Y MORFOMETRÍA EN SEMILLAS DE *Agave* COMO ESTUDIO INICIAL PARA LA GESTIÓN SOSTENIBLE Y LA CONSERVACIÓN GENÉTICA]

Jorge Luis Lechuga-Campuzano[1], Amaury Martín Arzate-Fernández[1], Hilda Guadalupe García-Núñez[1], María Dolores Mariezcurrena-Berasaín[1] and Jesús Ignacio Reyes-Díaz[1, 2, *]

[1]*Centro de Investigación y Estudios Avanzados en Fitomejoramiento, Facultad de Ciencias Agrícolas, Universidad Autónoma del Estado de México, C.P. 50200 Toluca, México. *Email: jird.rd@gmail.com*
[2]*Unidad Académica de Capulhuac, Universidad Tecnológica del Valle de Toluca, C. P. 52044 Capulhuac de Mirafuentes, México.*
[*]*Corresponding author*

SUMMARY

Background: The Tehuacán-Cuicatlán Valley (TCV), located at the crossroads of the Nearctic and Neotropical biogeographic regions, is a critical hotspot for biodiversity, particularly for the genus *Agave*. This region plays a vital role in preserving various species, with *Agave* being one of the most important genus due to its ecological and economic significance. **Methodology:** *Agave angustifolia, A. cupreata, A. marmorata*, *A. potatorum, and A. salmiana* seed collection was conducted in the TCV, followed by dehydration and storage under controlled conditions. Seed viability was assessed using the tetrazolium chloride (TTC) test. The percentage of viable embryos (%V) was calculated. Seed morphometry was analyzed by measuring length, width, weight, and volume. **Results:** Significant interspecific variability in seed viability was found. *A. marmorata* exhibited the highest viability across all time points, reaching 78.49 % at 72 hours, indicating robust physiological mechanisms. In contrast, *A. potatorum* showed the lowest viability, with a maximum of 57.99 %, likely due to genetic limitations or suboptimal conditions during seed development. Morphometric analysis also revealed significant differences among the tested species, with *A. angustifolia* seeds being the largest and *A. marmorata* the smallest. **Implications:** This study underscores the importance of understanding seed viability and morphometric traits in *Agave* species. These factors are crucial for the conservation and sustainable management of both wild and cultivated populations. The variability in seed viability and morphometric traits has significant implications for conservation efforts, as it can influence regeneration strategies and breeding programs aimed at improving crop yields or conserving native species. **Conclusion:** *A. marmorata* showed the highest seed viability, indicating robust physiological adaptations, while *A. potatorum* had the lowest viability and a high percentage of seedless embryos, suggesting potential reproductive challenges. Seed morphometric analysis revealed differences that could affect dispersal and germination success. In the broader context of biodiversity conservation in the Tehuacán-Cuicatlán Valley, these results offer valuable insights into the genetic and physiological diversity of *Agave*, guiding future conservation efforts.

Key words: *Agave* seed viability; Morphometric analysis; *Ex situ* conservation; Tetrazolium test.

RESUMEN

Antecedentes: el Valle de Tehuacán-Cuicatlán (VTC), ubicado en la intersección de las regiones biogeográficas neárticas y neotropicales, es un área clave para la biodiversidad, particularmente para el género *Agave*. Esta región juega un papel vital en la preservación de varias especies, siendo *Agave* uno de los géneros más importantes debido a

† Submitted February 13, 2025 – Accepted April 10, 2025. http://doi.org/10.56369/tsaes.6193

ISSN: 1870-0462.
ORCID = J.L. Lechuga-Campuzano: http://orcid.org/0009-0001-2185-8044; **A.M. Arzate-Fernández**: http://orcid.org/0000-0001-8603-0099; **J.I. Reyes-Díaz**: http://orcid.org/0000-0001-9234-6575; **H.G. Garcia-Nuñez**: http://orcid.org/0000-0002-3758-8114; **M.D. Mariezcurrena-Berasaín**: http://orcid.org/0000-0003-1569-1783

su relevancia ecológica y económica. **Metodología**: Semillas de *Agave angustifolia, A. cupreata, A. marmorata, A. potatorum y A. salmiana* fueron recolectadas en el VTC, seguida de deshidratación y almacenamiento en condiciones controladas. La viabilidad de las semillas se evaluó mediante la prueba de cloruro de tetrazolio (TTC). Se calculó el porcentaje de embriones viables (%V). Se analizó la morfometría de las semillas se analizó mediante la medición de longitud, ancho, peso y volumen. **Resultados**: Existe una variabilidad significativa en la viabilidad de las semillas entre especies. *A. marmorata* presentó la mayor viabilidad en todos los puntos de tiempo, alcanzando un 78.49 % a las 72 horas, lo que indica mecanismos fisiológicos robustos. En contraste, *A. potatorum* mostró la menor viabilidad, con un máximo del 57.99 %, probablemente debido a limitaciones genéticas o condiciones subóptimas durante el desarrollo de las semillas. El análisis morfométrico también reveló diferencias significativas entre las especies analizadas, siendo las semillas de *A. angustifolia* las más grandes y las de *A. marmorata* las más pequeñas. **Implicaciones**: este estudio resalta la importancia de comprender la viabilidad de las semillas y los rasgos morfométricos en las especies de *Agave*. Estos factores son cruciales para la conservación y el manejo sostenible de las poblaciones silvestres y cultivadas. La variabilidad en la viabilidad de las semillas y los rasgos morfométricos tiene implicaciones importantes para los esfuerzos de conservación, ya que puede influir en las estrategias de regeneración y los programas de cría destinados a mejorar los rendimientos de los cultivos o conservar las especies nativas. **Conclusión**: En *A. marmorata* se observó la mayor viabilidad de semillas, lo que indica adaptaciones fisiológicas robustas, mientras que *A. potatorum* tuvo la menor viabilidad y un alto porcentaje de embriones sin semilla, lo que sugiere posibles desafíos reproductivos. El análisis morfométrico de las semillas reveló diferencias que podrían afectar el éxito en la dispersión y germinación. En el contexto más amplio de la conservación de la biodiversidad en el Valle de Tehuacán-Cuicatlán, estos resultados ofrecen valiosos conocimientos sobre la diversidad genética y fisiológica de *Agave*, orientando los esfuerzos de conservación futuros.
Palabras clave: Viabilidad de semillas de *Agave*; Análisis morfométrico; Conservación *ex situ*; Prueba de tetrazolio

INTRODUCTION

The Tehuacán-Cuicatlán Valley (TCV) is strategically located at the intersection of the Nearctic and Neotropical biogeographic regions, encompassing the physiographic provinces of the Balsas Dry Forests, the Xerophytic Vegetation of the TCV, and, to a lesser extent, the Temperate Forests Province in Mexico. This valley is recognized as a crucial floristic region, covering only 0.65 % of Mexico's national territory yet hosting approximately 13 % of the estimated Mexican flora (Ulloa Ulloa *et al.,* 2017). The TCV stands out as the most diverse arid region in Mexico and is considered the North American arid-semiarid region with the highest biological diversity, boasting over 3,000 plant species within its 10,000 km² area (Dávila *et al.,* 2002).

Within this biodiversity hotspot, the genus *Agave* comprises approximately 200 species (Eguiarte *et al.,* 2013), with Mexico being recognized as the center of origin and diversity for these species. The country harbors between 150 and 159 *Agave* species, with 69 % being endemic (Álvarez-Ríos *et al.,* 2020). The significance of agaves in Mexico extends beyond their ecological importance, as they are integral to the country's cultural and economic landscape. *Agaves* are utilized as raw materials for various purposes, including food products, traditional medicines, pharmacological applications, construction materials, agricultural products, fibers, ornamental uses, and the production of alcoholic beverages such as mescal and fermented sap (Colunga-García and Zizumbo-Villarreal, 2007; Delgado-Lemus *et al.,* 2014; Álvarez-Ríos *et al.,* 2020).

Assessing the morphometry and viability of *Agave* seeds is crucial for the conservation of wild populations and the sustainability of their cultivation, particularly in the face of increasing demand and overexploitation without adequate technical or regulatory controls (Villanueva-Castillo *et al.,* 2021). Seed viability testing, implemented to assess whether seeds are viable and usable after collection or storage, is an integral part of plant research and conservation. A viable seed is considered to have achieved the highest physiological maturity, ensuring germination under appropriate conditions (Copeland. and McDonald., 2002). However, with time, all seed collections gradually age and decline in viability (Popova *et al*., 2016). Thus, seed viability testing it can be used to determine the efficacy of collection health and inform recollection efforts (Dalziell and Tomlinson, 2017).

Although conservation *in situ*, within natural habitats, remains the best option for safeguarding species, *ex situ* conservation efforts, such as seed banking, provide a critical backup for the long-term preservation of species and their genetic diversity (Schofield and Sarasan, 2018). Seed banking optimizes storage conditions by reducing temperature and humidity to

ensure that a significant proportion of seeds remain viable for future regeneration, in species targeted for conservation, overcoming seed dormancy and ensuring germination can be challenging, requiring species-specific knowledge and adequate seed quantities (Pritchard and Nadarajan, 2008; Pradhan *et al.,* 2022). Chemical viability tests, like the tetrazolium chloride (TTC), test which relies on enzymatic activity within live tissues, offer rapid and reliable methods for assessing seed viability, especially for species that are difficult to germinate (Pradhan *et al.,* 2022).
The preservation of genetic variability is vital, especially given that habitat fragmentation and the expansion of monocultures diminish population resilience, increasing species vulnerability (Delgado-Lemus *et al.,* 2014). In this context, traditional morphometric analyses focusing on linear measurements such as length, width, volume, and height-to-width ratio are essential for assessing seed traits and understanding the morphological variation within and among *Agave* species (Villanueva-Castillo *et al.,* 2021). These measurements provide quantitative descriptors that are crucial for comparative studies, enabling the identification of subtle differences that may be linked to ecological adaptability, seed dispersal mechanisms, and overall fitness in different environments. While geometric morphometrics, which captures the shape and structure of seeds beyond simple linear dimensions, offers powerful tools for studying more complex morphological traits, the present study focuses on these conventional measurements due to their applicability in practical conservation strategies and their ability to inform management decisions aimed at preserving both wild and cultivated populations of agaves (Félix-Valdez *et al.,* 2016; Álvarez-Ríos *et al*., 2020; Villanueva Castillo *et al.,* 2021).

Therefore, this study focuses on analyzing the morphological variation and viability of *Agave* seeds as a foundational step in establishing comprehensive management and conservation programs for wild agaves, thereby ensuring the ecological and economic sustainability of this invaluable genus within the broader context of rich Mexican biodiversity.

MATERIALS AND METHODS

Study species, seed collection, and dehydration

Seed collection of *Agave angustifolia*, *A. cupreata*, *A. marmorata*, *A. potatorum*, and *A. salmiana* was conducted in the Tehuacán-Cuicatlán Valley (TCV), Puebla, Mexico, between August and October 2022. These species were selected due to their ecological and economic significance within the region, as well as their representation of a broad range of environmental adaptations within the genus. For each species, fully matured fruits at the verge of dehiscence were sampled per species. The collected fruits were carefully transported in labeled paper envelopes to prevent contamination or mechanical damage.

The capsules were dried in a greenhouse at an average temperature of 27°C for 30 days. Once the capsules opened, the seeds were extracted, and sterile seeds without endosperm or embryos (white sterile seeds), as well as those that were broken or showed signs of pest and disease damage, were removed.

Once extracted, seeds were dried under controlled conditions, utilizing vacuum dryers filled with anhydrous calcium chloride to maintain humidity levels below 10 %. The humidity was measured with the help of a ThermoPro TP55 hygrometer. The dehydration process was conducted at room temperature for three days to ensure the seeds reached a consistent moisture content suitable for long-term storage. Following dehydration, the seeds were placed in sealed vials and stored at 4 °C for 18 months, with blue silica gel used as an indicator of relative humidity, until the analyses were performed.

Seed viability analysis

Seed viability was assessed using the tetrazolium chloride (TTC) test, a widely recognized biochemical assay for determining seed viability based on the enzymatic reduction of TTC to formazan by living tissues. A total of three hundred seeds per species were utilized, distributed across twelve experimental units of twenty-five seeds each. Prior to testing, seeds were rehydrated by immersion in distilled water for 24 hours to restore their physiological activity.

To facilitate the penetration of the TTC solution, a precise incision parallel to the hypocotyl axis was made using a sterile scalpel under aseptic conditions. Each experimental unit was then submerged in 50 mL of 1 % (w/v) TTC solution (J.T. Baker) and incubated in complete darkness at 25 ± 2°C. The incubation period lasted for 24, 48, and 72 hours, with four experimental units (representing four replicates per species) being analyzed at each time point. Upon completion of the incubation, the zygotic embryos were meticulously extracted from the seeds using a scalpel and observed under a stereoscopic microscope (MOTIC, model MAIN). Embryo viability was determined based on the presence of a pink-red coloration, indicative of viable tissues with active respiration. The percentage of viable embryos (%V) was calculated using the following equation: V = [(number of viable seeds/number of seeds analyzed) × 100]. The seed quality was assessed using the standard germination test proposed by ISTA, (2016).

In addition to recording the percentage of viable embryos, the percentage of non-viable embryos (unstained) and the percentage of seeds lacking embryos were also documented to provide a comprehensive assessment of seed quality.

Seed morphometric analysis

The morphometric analysis aimed to quantify key morphological traits of the seeds to elucidate potential variations in seed structure among the species studied. For each species, three random samples of thirty seeds (totaling ninety seeds per species) were selected. The following morphometric variables were measured: a) Seed Length (mm) and Width (mm), measured using a digital caliper brand Steren model 48646 with an accuracy of 0.01 mm. The length-to-width ratio was subsequently calculated to assess the shape of the seeds; b) Seed Weight (mg), determined using a precision analytical balance brand Sartorius model 35501963, ensuring consistent measurements across all samples and c) Seed Volume (mm^3), measured via the water displacement method, where each seed was placed in a glass test tube containing a known volume of water (10 mL). The displaced volume was recorded, providing an accurate measure of seed volume.

All measurements were conducted under controlled laboratory conditions to minimize variability and ensure the reliability of the data. The collected data were subjected to analysis of variance (ANOVA), followed by Tukey's post hoc test ($P \leq 0.05$) using the statistical software package InfoStat, version 2017, to determine significant differences among species. The experimental design used to assess viability and morphometry was a completely randomized design with multiple groups (species), allowing for the analysis of differences between them.

RESULTS AND DISCUSSION

Seed viability in *Agave* species

This study presents a detailed examination of seed viability in five *Agave* species: *A. angustifolia*, *A. cupreata*, *A. marmorata*, *A. potatorum*, and *A. salmiana*. The viability was assessed at intervals of 24, 48, and 72 hours, revealing significant interspecific variability (Figure 1). Statistical analysis using one-way ANOVA identified notable differences among species and across different time points, providing crucial insights into the physiological and genetic variability influencing seed viability.

First, at the 24 hours, *A. marmorata* demonstrated the highest average viability (28.12 %), which was significantly greater than that of *A. cupreata* (17.56 %) and *A. potatorum* (16.45 %) (ANOVA, $F=12.34$, $p<0.05$). *A. angustifolia* and *A. salmiana* exhibited intermediate viabilities of 21.42 % and 19.24 %, respectively. After 48 hours, a significant increase in viability was observed across all species. *A. marmorata* maintained the highest viability (58.61 %), significantly different from all other species ($F=15.76$, $p<0.01$). The viabilities of *A. cupreata* (53.41 %) and *A. angustifolia* (49.64 %) were comparable and significantly higher than those of *A. potatorum* (37.28 %) and *A. salmiana* (46.52 %). These increases suggest an improvement in physiological conditions, potentially due to the progressive activation of germination mechanisms. Finally, at the 72 hours, *A. marmorata* reached the highest viability (78.49 %), significantly surpassing all other species ($F=20.12$, $p<0.001$). The viabilities of *A. cupreata* (61.73 %) and *A. angustifolia* (60.60 %) were high and comparable, yet significantly greater than those of *A. potatorum* (57.99 %) and *A. salmiana* (60.21 %).

On the other hand, the proportion of non-viable seeds varied significantly among species (Figure 2) ($F=8.45$, $p<0.05$). *A. salmiana* had the highest percentage of non-viable seeds (27.95 %), significantly higher than *A. marmorata* (4.30 %) and *A. potatorum* (8.97 %). *A. angustifolia* and *A. cupreata* exhibited intermediate levels of non-viable seeds, with 25.25 % and 18.14 %, respectively. The high proportion of non-viable seeds in *A. salmiana* suggests potential issues during seed formation or resistance to adverse storage conditions.

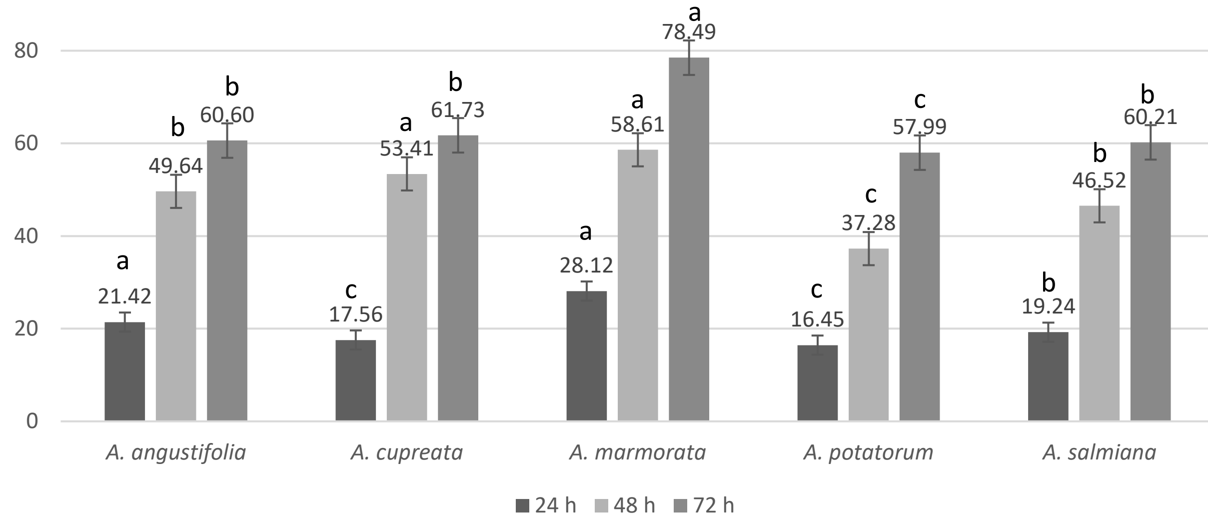

Figure. 1. Seed viability percentage of five *Agave* species at 24, 48, and 72 hours. Same letters indicate no statistically significant difference at 5 % (P ≤ 0.05), according to the Tukey test of comparison of means.

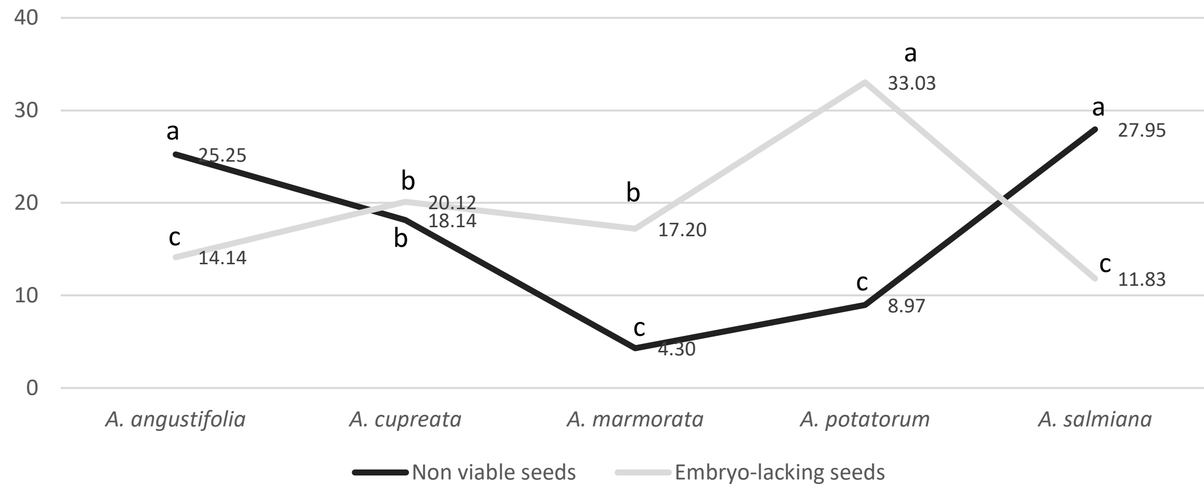

Figure. 2. Percentage of nonviable seeds and embryo-lacking seeds in five *Agave* species. Same letters indicate no statistically significant difference at 5 % (P ≤ 0.05), according to the Tukey test of comparison of means.

The observed differences in seed viability among the five *Agave* species can be attributed to a combination of genetic, physiological, and environmental factors, the role of genetic diversity in seed viability has been well documented in other species, such as *Arabidopsis thaliana*, where genetic differences have been linked to variations in seed dormancy and germination rates (Finch-Savage and Leubner-Metzger, 2006). Genetic variability between plays a critical role, as evidenced by the significantly higher viability in *A. marmorata* compared to other species. *A. marmorata* exhibited the highest seed viability across all time points, with a notable increase from 28.12 % at 24 hours to 78.49 % at 72 hours. This substantial viability increase suggests that *A. marmorata* possesses robust physiological mechanisms, possibly linked to more efficient metabolic activation upon imbibition. This ability to rapidly increase viability under the tested conditions indicates that it might have evolved structural or biochemical adaptations that confer a superior capacity to initiate and sustain germination processes. This finding aligns with studies on other xerophytic species, such as *Opuntia ficus-indica* and *Cereus cacti*, where rapid seed germination is crucial for survival in arid environments (Nobel, 1984; Mandujano, and Rojas-Aréchiga, 2005). Likewise, physiological factors, including the maturity of seeds at harvest and storage conditions, also influence seed viability. The conditions under which seeds are stored, particularly temperature and humidity, are crucial in maintaining viability. In this study, short-term storage at ambient conditions (22°C and 70.9 % relative humidity) did not adversely affect seed viability. However, prolonged storage under these conditions could lead to accelerated seed deterioration due to increased respiratory activity and susceptibility to pathogens, as has been observed in other species (Larios *et al.,* 2014; Pirredda *et al.,* 2023).

Additionally, the percentage of seeds without embryos (Figure 2) was highest in *A. potatorum* (33.03 %), significantly more than in all other species (F=10.67, p<0.01). *A. cupreata* followed with 20.12 %, which was significantly higher than the percentages observed in *A. marmorata* (17.20 %), *A. angustifolia* (14.14 %), and *A. salmiana* (11.83 %). The high percentage of seeds without embryos in *A. potatorum* further supports the hypothesis of reproductive challenges, possibly due to genetic factors or suboptimal conditions during seed development because *A. potatorum* consistently showed the lowest viability at all time points, with a maximum of 57.99 % at 72 hours reflecting inherent limitations in seed quality or a less efficient response to the imbibition process. Similar challenges have been observed in other *Agave* species, such as *A. tequilana*, where high seed abortion rates are attributed to genetic bottlenecks and environmental stress during flowering (Gil-Vega *et al.,* 2006).

Seed morphometry in *Agave* species

The morphometric analysis of *Agave* seeds across five species, *A. angustifolia*, *A. cupreata*, *A. marmorata*, *A. potatorum*, and *A. salmiana*, revealed notable differences in physical seed properties, which have implications for their conservation and propagation.

The seed length, width, ratio, weight, and volume varied significantly among the species studied. *A. angustifolia* seeds are bigger, measured an average length of 9.17 mm and width of 6.82 mm, with a length-to-width ratio of 1.34, a weight of 9.35 mg, and a volume of 21.41 mm³. In contrast, *A. marmorata* seeds were smaller, averaging 3.18 mm in length and 2.26 mm in width, with a ratio of 1.41, a weight of 3.57 mg, and a volume of 8.27 mm³ (Table 1).

The high variability in seed characteristics across species reflects their adaptation to different ecological niches and evolutionary pressures. For instance, *A. angustifolia* and *A. salmiana* seeds, with larger sizes and higher volumes, may indicate adaptations to environments where seedling establishment requires greater initial reserves (Trejo *et al.,* 2024). Hernández-Castro *et al.*, (2021) suggest that seed size is closely linked to its ability to store energy, potentially indicating seed quality.

Conversely, the smaller seed size of *A. marmorata* may represent a strategy to increase dispersal efficiency or reduce resource investment per seed. These variations in seed size and volume are consistent with findings from other studies on seed morphology in the *Agave* genus, which have reported a wide range of seed dimensions depending on the species and environmental factors (Huerta-Lovera *et al.,* 2018). The differences in seed weight and volume among the species in this study are indicative of their potential variations in germination rates and seedling vigor, which are crucial for effective conservation and propagation strategies (Larios *et al.,* 2014; Trejo *et al.,* 2024).

Implications for conservation and propagation

The significant differences in seed viability and the morphometric variation among *Agave* species provides valuable insights into their reproductive biology and conservation needs, guiding effective strategies for preserving these economically and ecologically important plants.

Table 1. Morphometric analysis of five *Agave* seeds.

Specie	Length (mm)	Width (mm)	Ratio	Weight (mg)	Volume (mm^3)
A. angustifolia	9.17 a	6.82 a	1.34 b	9.34 a	21.41 a
A. cupreata	6.51 c	4.82 b	1.35 b	7.12 b	15.3 c
A. marmorata	3.18 d	2.26 d	1.41 a	3.57 d	8.27 d
A. potatorum	5.08 c	3.59 c	1.42 a	6.56 c	14.37 c
A. salmiana	7.93 b	5.83 a	1.35 b	9.43 a	17.51 b

Means with the same letter within a column are not statistically different (Tukey, α=0.05). Same letters indicate no statistically significant difference at 5 % (P ≤ 0.05), according to the Tukey test of comparison of means.

A. marmorata with a smaller seed size, emerges as a prime candidate for large-scale propagation, particularly in commercial contexts such as mescal production. The species ability to maintain high viability across different time intervals indicates its suitability for mass propagation from seeds, thereby reducing reliance on clonal propagation methods that often result in diminished genetic diversity. High seed viability is essential for preserving genetic variability within cultivated populations, which can enhance resilience to diseases and environmental stresses (Escobar-Guzmán *et al.,* 2008). Moreover, the robust seed performance of *A. marmorata* aligns with sustainable agricultural practices, which focus on minimizing input while maximizing outputs. Given these attributes, *A. marmorata* should be prioritized in breeding programs and habitat restoration projects aimed at promoting genetic diversity and ecological resilience. This is particularly relevant in the context of *A. tequilana* cultivation, where genetic uniformity has increased vulnerability to diseases and environmental stresses (Colunga-García and Zizumbo-Villarreal, 2007). Thus, the high seed viability of *A. marmorata* suggests it could offer greater resilience to environmental changes and be better suited for sustainable cultivation practices in agave-based industries.

A. angustifolia and *A. cupreata* also demonstrated favorable viability rates, though not as pronounced as *A. marmorata.* These species are economically significant, particularly in regions where they are cultivated for traditional alcoholic beverages (Félix-Valdez *et al.,* 2016). However, their moderate viability suggests that propagation efforts should focus on optimizing seed storage and germination conditions to enhance their reproductive success. For instance, cryopreservation or controlled storage conditions could be employed to maintain seed viability over extended periods, as has been effective in other species with similar seed traits (Pritchard and Nadarajan, 2008). *In situ* conservation efforts, such as the protection of natural populations and their habitats, should also be emphasized to ensure that these species continue to contribute to the genetic pool and biodiversity of their ecosystems (Rivera-Lugo *et al.,* 2018).

A. salmiana exhibited intermediate seed viability and intermediate values in terms of length, width, weight, and volume, making it suitable for both conservation and commercial use. This species is often used in traditional fermented sap production and has ecological importance in its native habitats. While its viability rates suggest that it can be effectively propagated from seeds, the variability observed over time indicates that careful management of seed resources is necessary. Conservation strategies for *A. salmiana* should include both *in situ* and *ex situ* methods, combining habitat protection with seed banking and possibly tissue culture techniques to safeguard against environmental changes and genetic erosion (Juárez-López *et al.,* 2023). The ecological role of the species as a keystone plant in arid ecosystems further underscores the need for a balanced approach that supports both its use and conservation (Delgado-Lemus *et al.,* 2014; Félix-Valdez *et al.,* 2016; Álvarez-Ríos *et al.,* 2020).

In contrast, *A. potatorum* exhibited relatively low seed viability and the highest ratio among the species, with a length-to-width ratio of 1.42, indicating a more elongated seed shape compared to others, indicating significant reproductive challenges that could hinder its long-term survival. The high proportion of non-viable seeds and those without embryos suggests that natural regeneration may be severely limited, necessitating more targeted and intensive conservation strategies. *Ex situ* techniques, such as seed banking, tissue culture, and advanced biotechnological approaches like somatic embryogenesis and cryopreservation, should be prioritized to preserve its genetic material (Cuéllar-Martínez, Galindo-González and Andrade-Torres, 2024). Additionally, habitat restoration and the establishment of seed orchards could enhance the species reproductive success, ensuring its availability for future generations. These combined efforts are essential to maintaining the genetic diversity and ensuring the continued existence of *A. potatorum.*

CONCLUSIONS

This study highlights the significant interspecific variability in seed viability and morphometric traits among five *Agave* species, underscoring the complexity of their conservation and propagation. *A. marmorata* exhibited the highest seed viability across all time points, suggesting its robust physiological adaptations that may enhance its survival in arid environments. Conversely, *A. potatorum* showed the lowest viability and a high percentage of seeds without embryos, indicating potential reproductive challenges that may be linked to genetic factors or environmental stress during seed development.

The seed morphometric analysis revealed distinct differences which could influence their dispersal mechanisms and germination success in different ecological settings. These findings emphasize the need for species-specific conservation strategies, particularly for those with lower viability and greater morphometric variability.

In the broader context of biodiversity conservation in the Tehuacán-Cuicatlán Valley, these results contribute valuable insights into the genetic and physiological diversity of *Agave* species, informing future efforts to develop effective management programs that ensure the long-term sustainability of these ecologically and economically important plants.

Funding. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement. The authors declare that no competing interests exist.

Compliance with ethical standards. It does not apply.

Data availability. Data are available with Dr. Reyes-Díaz (jird.rd@gmail.com) upon reasonable request.

Author contribution statement (CRediT). **J.L. Lechuga-Campuzano** – Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **J.I. Reyes-Díaz** – Formal analysis, Investigation, Methodology, Project administration, Writing – review & editing. **A.M. Arzate-Fernández** – Conceptualization, Investigation, Resources, Writing – review & editing. **H.G. García-Núñez** – Investigation, Writing – review & editing. **M.D. Mariezcurrena-Berasaín** – Writing – review & editing.

REFERENCES

Álvarez-Ríos, G.D., Pacheco-Torres, F., Figueredo-Urbina, C.J. and Casas, A., 2020. Management, morphological and genetic diversity of domesticated agaves in Michoacán, México. *Journal of Ethnobiology and Ethnomedicine*, 16(1), p.3. https://doi.org/10.1186/s13002-020-0353-9

Colunga-García, P. and Zizumbo-Villarreal, D., 2007. Tequila and other Agave spirits from west-central Mexico: current germplasm diversity, conservation and origin. *Biodiversity and Conservation*, 16(6), pp.1653–1667. https://doi.org/10.1007/s10531-006-9031-z

Copeland, L.O., S. and McDonald, M.B., 2002. Principles of seed science and technology. *Annals of Botany*, 89(6), pp.798–798. https://doi.org/10.1093/aob/mcf127

Cuéllar-Martínez, M., Galindo-González, J. and Andrade-Torres, A., 2024. Germination of Agave obscura seeds: effects of storage time and crossing systems. *Plant Ecology*, 225(3), pp.189–199. https://doi.org/10.1007/s11258-023-01389-z

Dalziell, E.L. and Tomlinson, S., 2017. Reduced metabolic rate indicates declining viability in seed collections: an experimental proof-of-concept. *Conservation Physiology*, [online] 5(1). https://doi.org/10.1093/conphys/cox058

Dávila, P., Arizmendi, M.D.C., Valiente-Banuet, A., Villaseñor, J.L., Casas, A. and Lira, R., 2002. Biological diversity in the Tehuacán-Cuicatlán Valley, Mexico. *Biodiversity and Conservation*, 11(3), pp.421–442. https://doi.org/10.1023/A:1014888822920

Delgado-Lemus, A., Torres, I., Blancas, J. and Casas, A., 2014. Vulnerability and risk management of Agave species in the Tehuacán Valley, México. *Journal of Ethnobiology and Ethnomedicine*, 10(1), p.53. https://doi.org/10.1186/1746-4269-10-53

Eguiarte, L.E., Aguirre-Planter, E., Aguirre, X., Colín, R., González, A., Rocha, M., Scheinvar, E., Trejo, L. and Souza, V., 2013. From Isozymes to Genomics: Population Genetics and Conservation of Agave in México. *The Botanical Review*, 79(4), pp.483–506. https://doi.org/10.1007/s12229-013-9123-x

Escobar-Guzmán, R.E., Hernández, F.Z., Vega, K.G. and Simpson, J., 2008. Seed production and gametophyte formation in *Agave tequilana* and *Agave americana*. *Botany*, 86(11), pp.1343–1353. https://doi.org/10.1139/B08-099

Félix-Valdez, L.I., Vargas-Ponce, O., Cabrera-Toledo, D., Casas, A., Cibrian-Jaramillo, A. and De La Cruz-Larios, L., 2016. Effects of traditional management for mescal production on the diversity and genetic structure of Agave potatorum (Asparagaceae) in central Mexico. *Genetic Resources and*

Crop Evolution, 63(7), pp.1255–1271. https://doi.org/10.1007/s10722-015-0315-6

Finch-Savage, W.E. and Leubner-Metzger, G., 2006. Seed dormancy and the control of germination. *New Phytologist*, 171(3), pp.501–523. https://doi.org/10.1111/j.1469-8137.2006.01787.x

Gil-Vega, K., Díaz, C., Nava-Cedillo, A. and Simpson, J., 2006. AFLP analysis of Agave tequilana varieties. *Plant Science*, 170(4), pp.904–909. https://doi.org/10.1016/j.plantsci.2005.12.014

Hernández-Castro, E., López-Sandoval, Y.Y., Escobar-Álvarez, J.L. and Ramírez-Reynoso, O., 2021. Análisis morfométrico de semilla y desarrollo de plántulas de maguey sacatoro (*Agave angustifolia* Haw.). *Ecosistemas y Recursos Agropecuarios,* 8(3), pp.e2964. https://doi.org/10.19136/era.a8n3.2964

Huerta-Lovera, M., Peña-Valdivia, C.B., García-Esteva, A., Kohashi-Shibata, J., Campos-García, H. and Aguirre-Rivera, J.R., 2018. Maguey (Agave salmiana) infructescence morphology and its relationship to yield components. *Genetic Resources and Crop Evolution*, 65(6), pp.1649–1661. https://doi.org/10.1007/s10722-018-0641-6

ISTA. 2016. *International Rules for Seed Testing*, 2016(1), pp.1–384. https://doi.org/10.15258/istarules.2016.F

Juárez López, C.R., Chiñas Castillo, F., Alavéz Ramírez, R. and Caballero Caballero, M., 2023. Mezcal Agroindustry Contribution to Climate Change in Oaxaca. In: W. Leal Filho, G.J. Nagy and D. Ayal, eds. *Handbook of Nature-Based Solutions to Mitigation and Adaptation to Climate Change*. [online] Cham: Springer International Publishing. pp.1–28. https://doi.org/10.1007/978-3-030-98067-2_60-1

Larios, E., Búrquez, A., Becerra, J.X. and Lawrence Venable, D., 2014. Natural selection on seed size through the life cycle of a desert annual plant. *Ecology*, 95(11), pp.3213–3220. https://doi.org/10.1890/13-1965.1

Mandujano, M.C., Montaña, C. and Rojas-Aréchiga, M., 2005. Breaking seed dormancy in Opuntia rastrera from the Chihuahuan desert. *Journal of Arid Environments*, 62(1), pp.15–21. https://doi.org/10.1016/j.jaridenv.2004.10.009

Nobel, P.S., 1984. Productivity of Agave deserti: measurement by dry weight and monthly prediction using physiological responses to environmental parameters. *Oecologia*, 64(1), pp.1–7. https://doi.org/10.1007/BF00377535

Pirredda, M., Fañanás-Pueyo, I., Oñate-Sánchez, L. and Mira, S., 2023. Seed Longevity and Ageing: A Review on Physiological and Genetic Factors with an Emphasis on Hormonal Regulation. *Plants*, 13(1), p.41. https://doi.org/10.3390/plants13010041

Popova, E., Kim, H.H., Saxena, P.K., Engelmann, F. and Pritchard, H.W., 2016. Frozen beauty: The cryobiotechnology of orchid diversity. *Biotechnology Advances*, 34(4), pp.380–403. https://doi.org/10.1016/j.biotechadv.2016.01.001

Pradhan, N., Fan, X., Martini, F., Chen, H., Liu, H., Gao, J. and Goodale, U.M., 2022. Seed viability testing for research and conservation of epiphytic and terrestrial orchids. *Botanical Studies*, 63(1), p.3. https://doi.org/10.1186/s40529-022-00333-0

Pritchard, H.W. and Nadarajan, J., 2008. Cryopreservation of Orthodox (Desiccation Tolerant) Seeds. In: B.M. Reed, ed. *Plant Cryopreservation: A Practical Guide*. [online] New York, NY: Springer New York. pp.485–501. https://doi.org/10.1007/978-0-387-72276-4_19

Rivera-Lugo, M., García-Mendoza, A., Simpson, J., Solano, E. and Gil-Vega, K., 2018. Taxonomic implications of the morphological and genetic variation of cultivated and domesticated populations of the Agave angustifolia complex (Agavoideae, Asparagaceae) in Oaxaca, Mexico. *Plant Systematics and Evolution*, 304(8), pp.969–979. https://doi.org/10.1007/s00606-018-1525-0

Schofield, E., Jones, E.P. and Sarasan, V., 2018. Cryopreservation without vitrification suitable for large scale cryopreservation of orchid seeds. *Botanical Studies*, 59(1), p.13. https://doi.org/10.1186/s40529-018-0229-7

Trejo, L., Soriano, D., Romano-Grande, E., Sánchez-Carmona, B. and Dávila-Navarro, D.E., 2024. Diversity of reproductive characters, seed set, and viability of Agave seeds used for pulque production and their wild relatives in Tlaxcala, Mexico. *Genetic Resources and Crop Evolution*, 71(6), pp.2877–2903. https://doi.org/10.1007/s10722-023-01803-5

Ulloa Ulloa, C., Acevedo-Rodríguez, P., Beck, S., Belgrano, M.J., Bernal, R., Berry, P.E., Brako, L., Celis, M., Davidse, G., Forzza, R.C., Gradstein, S.R., Hokche, O., León, B., León-Yánez, S., Magill, R.E., Neill, D.A., Nee, M., Raven, P.H., Stimmel, H., Strong, M.T., Villaseñor, J.L., Zarucchi, J.L., Zuloaga, F.O. and Jørgensen, P.M., 2017. An integrated assessment of the vascular plant species of the Americas. *Science*, 358(6370), pp.1614–1617. https://doi.org/10.1126/science.aao0398

Villanueva Castillo, D.M., Velasco Velasco, V.A., De Los Santos Romero, R.B., Ruiz Luna, J. and Rodríguez Ortíz, G., 2021. Variación morfométrica en semillas de agaves silvestres de Oaxaca. *Revista Mexicana de Ciencias Agrícolas*, 12(1), pp.155–162. https://doi.org/10.29312/remexca.v12i1.2426