DOI: 10.29298/rmcf.v14i77.1345

Research article

Morfometría y viabilidad de semillas de Brosimum alicastrum Sw. en Campeche

Morphometry and viability of Brosimum alicastrum Sw. seeds in the state of Campeche

Alberto Santillán-Fernández[1], Sandra Sugey Castañeda-Alonso[2,3], Eugenio Carrillo-Ávila[3], Alfredo Esteban Tadeo-Noble[1], Jaime Bautista-Ortega[3], Javier Enrique Vera López[3], Fulgencio Alatorre-Cobos[4*]

Fecha de recepción/Reception date: 2 de marzo 2023
Fecha de aceptación/Acceptance date: 16 de mayo del 2023

[1]Investigador por México Conahcyt. Colegio de Postgraduados, Campus Campeche. México.
[2]Instituto Tecnológico Superior de Venustiano Carranza. México.
[3]BIOSAT. Colegio de Postgraduados, Campus Campeche. México.
[4]Investigador por México Conahcyt. Centro de Investigación Científica de Yucatán. México.

*Autor para correspondencia; correo-e: fulgencio.alatorre@cicy.mx
*Corresponding author; e-mail: fulgencio.alatorre@cicy.mx

Abstract

Ramon tree (*Brosimum alicastrum* (*ramón*)) is a species endemic to southern Mexico. Due to its uses in animal and human food and its ability to regenerate degraded ecosystems, this tree has been included by the National Forest Commission in reforestation programs, increasing the demand for its specimens, seeds and fruits. Nevertheless, the species is distributed naturally with an incipient forestry management. The objectives of this research were to characterize the morphometry of seeds of wild populations of Ramon tree in the state of *Campeche*, and to determine the effect of temperature on the viability and germination of seeds under controlled conditions, through the tetrazolium test. Seeds were collected from four municipalities in the north of *Campeche*: *X-Mabén*, *Sahcabchén*, *Hopelchén* and *Calakmul*. Morphometric analysis revealed that the seeds with the best characteristics (size and weight) were those of *Hopelchén* and *Calakmul*. To determine the effect of storage temperature on the viability of the seeds collected, *Calakmul* seeds were used. Viability was found to last up to 70 days at storage temperatures of 4 °C with 90 % germination, while at 28 °C it was reduced to 30 days. It is concluded that 4 °C is an optimal storage temperature to prolong the viability of the seeds and guarantee the highest percentages of germination in the nursery. This information will be important in future forest management plans of the *Ramon* tree, contributing to the conservation and sustainable use of the species.

Key words: *Ramón* tree, *Capomo*, seed germination, Mayan nut, *Ojite*, tetrazolium test.

Resumen

Brosimum alicastrum (ramón) es una especie endémica del sur de México, que por sus usos en la alimentación animal y humana, y su capacidad para regenerar ecosistemas degradados, la Comisión Nacional Forestal la ha incluido en los programas de reforestación, lo que ha incrementado la demanda de sus ejemplares, semillas y frutos. No obstante, el taxón se distribuye de manera natural con un incipiente manejo silvícola. Los objetivos de esta investigación fueron caracterizar la morfometría de semillas de poblaciones silvestres de ramón en Campeche, y determinar el efecto de la temperatura sobre la viabilidad y germinación de las semillas bajo condiciones controladas, mediante la prueba de tetrazolio. Se recolectaron semillas de cuatro distintas localidades del norte de Campeche: X-Mabén, Sahcabchén, Hopelchén y Calakmul. El análisis morfométrico reveló que las semillas con las mejores características (tamaño y peso) fueron las de Hopelchén y Calakmul. Para determinar el efecto de la temperatura de almacenamiento sobre la viabilidad de las semillas recolectadas, se seleccionó el material de Calakmul. La viabilidad se prolongó hasta 70 días en temperaturas de almacenamiento de 4 °C, con 90 % de germinación, mientras que a 28 °C se redujo a 30 días. Se concluye que 4 °C es una temperatura de almacenamiento óptima para prolongar la viabilidad de las semillas y garantizar los mayores porcentajes de germinación en vivero. Esta información será importante en los planes futuros de manejo forestal de *B. alicastrum*, y contribuirá a la conservación y aprovechamiento sustentable de la especie.

Palabras clave: Árbol ramón, capomo, germinación de semillas, nuez Maya, ojite, prueba de tetrazolio.

Introduction

Brosimum alicastrum Sw. (known as *ramón*) is a tree of the Moraceae family, widely distributed in Mesoamerica, where it is considered endemic, which grows wild in evergreen and deciduous forests (Vergara *et al*., 2014). From November to February, and from July to September, the species produces fruits which are berries of 2 to 2.5 cm in diameter, globose with fleshy pericarp, and green when immature and yellowish-green to reddish-orange when ripe; they contain a seed 1.5 to 2 cm in diameter, covered by a yellowish papery *testa*, with two cotyledons mounted one on the other; they are recalcitrant seeds with germination percentages lower than

50 % (Morales and Herrera, 2009).

In Mexico, *B. alicastrum* is distributed from the south of the state of *Tamaulipas* to the state of *Quintana Roo*, and from the state of *Sinaloa* to the state of *Chiapas* (Pennington and Sarukhán, 2005); it is highly appreciated in the *Yucatan* Peninsula where its seeds came to be considered the maize of the Mayans (Espinosa-Grande *et al.*, 2023). In this region, currently, the species is distributed naturally, and the collection of seeds, fruits and forage is used for animal feed (Rojas-Schroeder *et al.*, 2017). The taxon also has potential for human consumption and in industry. The fruit is rich in phenolic compounds with antioxidant activity; its starch has thickening and gelling properties suitable for processed foods and the pharmaceutical industry (Moo-Huchin *et al.*, 2019).

Based on its ability to restore degraded soils, in 2019 *B. alicastrum* was included as a priority species in the Federal Program *Sembrando Vida* of the *Secretaría de Bienestar* (Ministry of Well-being), whose objective is to propagate it for reforestation purposes (Secretaría de Bienestar, 2020). In addition, in a context of climate change and food security, it has become a widely used local resource for animal and human food (Ramírez-Sánchez *et al.,* 2017). Therefore, the demand for specimens (plant, seed and fruit) of *B. alicastrum* has increased, and with it the need to generate research regarding its propagation (Santillán-Fernández *et al.*, 2021a).

There are investigations in literature for *B. alicastrum* in botany and ecology (Espinosa-Grande *et al.*, 2023), nutritional properties in animal and human food (Rojas-Schroeder *et al.*, 2017; Subiria *et al*., 2019) and recently, due to its high economic potential, issues related to forest plantations have been incorporated (Hernández-González *et al.*, 2015). However, there is little knowledge about the storage conditions and their effects on the viability (ability to germinate) of their seeds, due to the incipient forest management of the species (Mendoza-Arroyo *et al.*, 2020).

In the sexual propagation of forest species, a recurring problem of nursery management is the determination of the optimal storage time, without affecting the viability of the seeds (Mejenes-López *et al.*, 2019). During the storage period, factors such as humidity, temperature, light and the amount of oxygen affect the deterioration of the seed and directly condition its viability (Valverde-Rodríguez *et al*., 2019). In seeds that do not tolerate dehydration, called recalcitrant, storage is complicated; these, mainly refer to tropical taxa, can be stored for long periods if they are slightly dehydrated and have available oxygen (De Vitis *et al.,* 2020). Valverde-Rodriguez *et al.* (2019) determined that, in storage, temperature is the factor that can be influenced faster to lengthen the viability of recalcitrant seeds.

To quickly calculate the viability of the seeds, the tetrazolium test is the most appropriate due to the simplicity of its application and the reliability of its results (Salazar *et al.,* 2020). It has been used successfully in forest species such as *Pinus caribaea* Morelet var. *caribaea* Barret y Golfari (Dorta, 2020), *Jatropha mollissima* (Pohl) Baill. (Siqueira *et al.*, 2020), *Coffea arabica* L. (Calla *et al.*, 2019), *Guaiacum sanctum* L. (Flores *et al.*, 2019), *Cedrela odorata* L. and *Cariniana pyriformis* Miers (Espitia-Camacho *et al.*, 2017).

In this context, the objectives of this research were to characterize the morphometry of seeds of wild populations of *B. alicastrum* in *Campeche* State, and to determine the effect of storage temperature on seed viability and germination under controlled conditions by means of the tetrazolium test, in order to determine the temperature and maximum storage time that guarantee high germination percentages. The proposed hypothesis is that there is a natural variation in the morphometry of the seeds and that the storage temperature is a factor that directly impacts the viability of the seeds.

Materials and Methods

Fruit collection

The first week of September 2019, *Brosimum alicastrum* fruits were collected in four locations in northern *Campeche* (Figure 1). The collection provenances are characterized by a coverage of medium sub-evergreen forest and high evergreen forest, with clayey soils, rainfall from 600 to 4 000 mm, with dry seasons of three to seven months, average annual temperature of 18 to 27 ºC and altitudes from 20 to 1 000 m (Inegi, 2021). This region has been previously described by Santillán-Fernández *et al.* (2021b) as an area with a high natural distribution of *B. alicastrum*, homogeneous in its edaphoclimatic parameters.

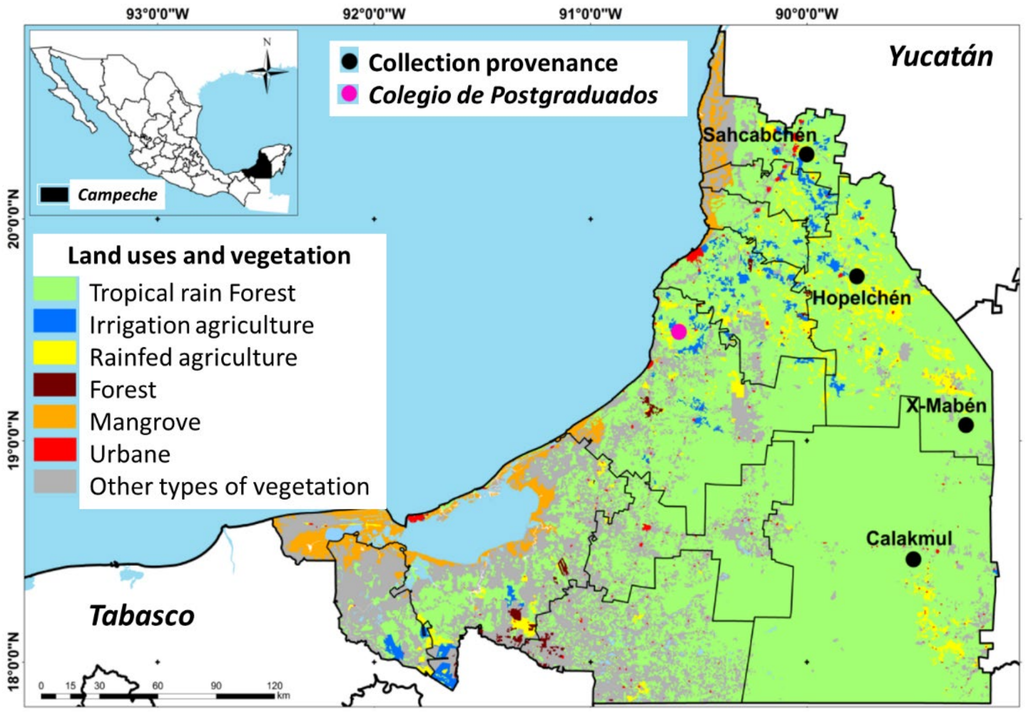


Figure 1. Spatial location of the provenances for collecting fruits of *Brosimum alicastrum* Sw. in the state of *Campeche*.

Fruit collection in *X-Mabén* and *Hopelchén* was carried out in areas disturbed by anthropic activities, unlike *Sahcabchén* and *Calakmul* whose collection was in less disturbed areas and even in the biosphere reserve, in the case of *Calakmu*l. The collection of the fruits was made based on the methodology described by Vallejos *et al.* (2010); 10 trees were selected per provenance with the best phenotypic characteristics (dasometric: greater height, greater *dbh* and straight stem), with a minimum distance between selected trees of 100 m; an average of 50 fruits was obtained from each individual, which were stored in sterilized plastic bags for transfer to the facilities of the *Colegio de Postgraduados* (Graduate Studies College), *Campeche campus* (*Champotón*, *Campeche*, Mexico). The fruit was pulped, exposing the seed, which was washed with water to remove pericarp residues.

Morphometry of *B. alicastrum* seeds

For the morphometric analysis of the seeds, those that were broken, vain, or with symptoms of damage by pests and diseases were removed. Four samples of 30 seeds per provenance (480 seeds in total) were randomly selected. The quantified variables were: length (cm), width (cm) and the length/width ratio, measured with a Vernier caliper (*Mitutoyo* 530-312CAL™); weight (g), calculated using an analytical balance (*Wellish™*); volume (cm^3) and density ($g\ cm^{-3}$), with the water displacement method (Rashidi *et al.*, 2007) which consists of placing each seed inside a glass cylinder (*Cylinder*-250mL-2PK-Glass™) with a known volume of water (100 mL) and record the displaced volume.

The statistical differences between the morphometric variables by place of origin were determined with the R software (Venables and Smith, 2023) through a means test by Tukey, with a 95 % reliability (α=0.05).

Seed viability analysis

Seed viability was determined with the tetrazolium chloride technique, which is used to differentiate metabolically active from inactive tissues (Berridge, 2005). For this, a completely randomized experimental design was used, with four replicates of 30 seeds for each collection site (480 seeds). Since no previous works were found for *B. alicastrum* in which the viability of its seeds with tetrazolium is analyzed, the preparation of the solution and the evaluation of the staining patterns were made considering what has been cited for similar species.

A 1 % solution of 2,3,5-triphenyltetrazolium chloride (CTT) (*Sigma T8877*-10G) in sterile tridistilled water was used (Espitia-Camacho *et al.*, 2017). The seeds were cut following the equatorial axis and placed in 20 mL of CTT solution in a Petri dish; then, they were incubated (*Labnet*® modelo 222DS) in the dark at 28 °C for 4 h. The incubation time was previously determined from a 24-h staining kinetics with 4-h sampling intervals.

According to the methodology described by Rodríguez *et al.* (2008) and Orantes-García *et al.* (2013), when the living cells of the seed embryo actively respire (they have a higher probability of germination), the CTT test stains them red, and in the case of dead cells, they keep the original color of the embryo. For this analysis, three categories were considered, according to the Pérez-Mendoza (2018) classification system: fully stained seeds (viable and vigorous embryos), partially stained seeds (viable embryos with medium vigor) and unstained seeds (non-viable embryos); in all cases they were counted and expressed as a percentage per replication and provenance. The statistical differences of the proportion of seeds that presented a category of staining by provenance were determined with the R software (Venables and Smith, 2023) through a means test by Tukey with a 95 % (α=0.05) reliability.

Effect of storage time and temperature on seed viability

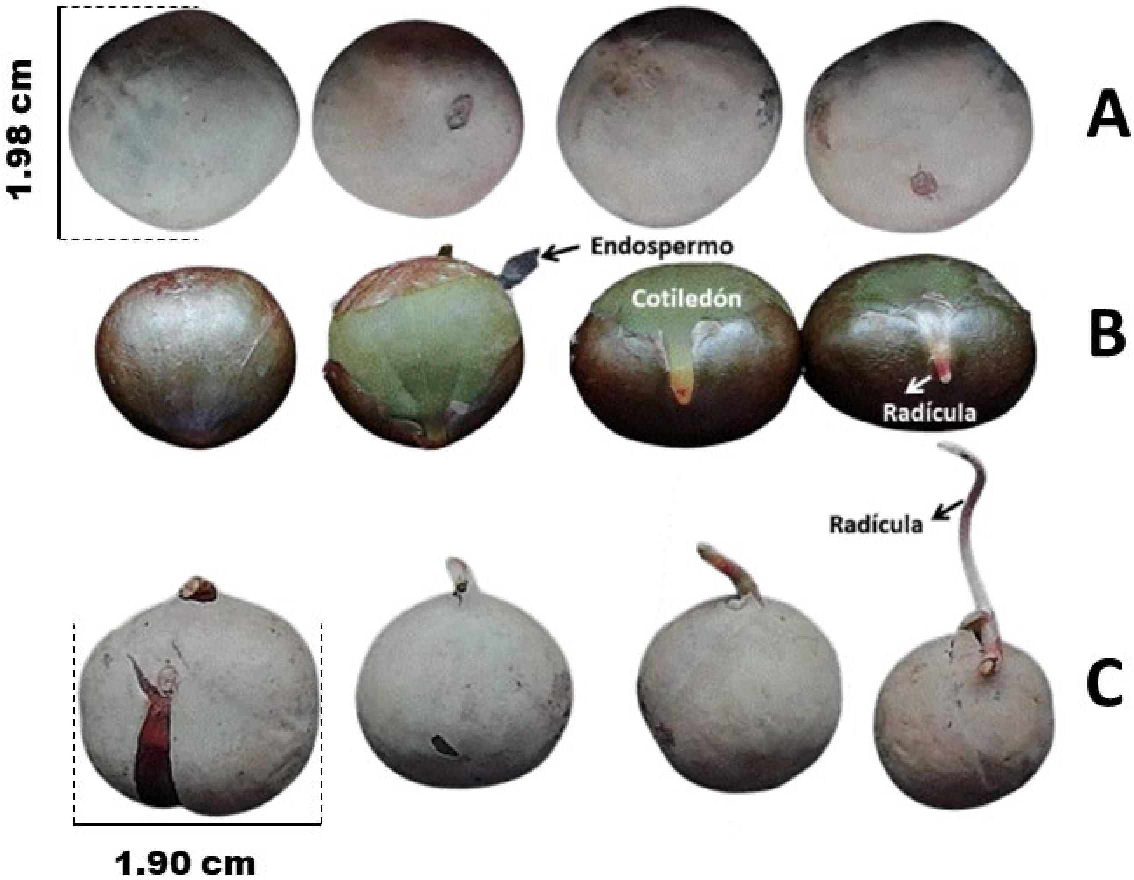
To analyze the effect of storage time and temperature on the viability of *B. alicastrum* seeds, a completely randomized experimental design was established with 24 replicates of five seeds for each storage temperature. The temperature gradients were defined based on preliminary studies for tropical trees such as *B. alicastrum*; in this regard, the temperature of 4 °C is one of the most evaluated in studies on the effect of storage temperature, while that of 28 °C is considered an ambient temperature within the range of 23 to 28 °C (Campbell-Martínez *et al.*, 2022; Park *et al.*, 2022). For this experiment, the seeds from the place of origin that obtained the lowest percentages in the CTT test were selected, in order to study the effect in the worst scenarios.

The seeds were stored at 4 °C (*Samsung side by side*® refrigerator) and 28 °C (culture room) for a period of 92 days. Every 15 days, four replicates were selected randomly and without replacement by temperature gradient, to which the CTT test was applied to determine the percentage of fully stained seeds, partially stained seeds, and unstained seeds. Due to the number of replicates (24) and analysis period (15 days), the CTT test was performed six times. In addition, a germination test was carried out on 80 seeds (from the provenance that had the lowest percentages in the CTT test) stored for 30 days at 4 °C and 28 °C, four replicates of 10 seeds were made for each gradient of temperature, in each one the percentage of germination was obtained. The statistical differences of the proportion of seeds that presented a category of staining by analysis period, and the percentage of germination by temperature gradient were determined in the R software (Venables and Smith, 2023) by means of a test of means by Tukey with a reliability of 95 % (a=0.05).

Results and Discussion

Morphometry of *B. alicastrum* seeds

An exploratory analysis of the collected seeds showed that they have a spherical or flattened shape, covered with a whitish-brown papyraceous testa that detaches itself when it dries, with a greenish seed with two asymmetric cotyledons (Brechú-Franco *et al.*, 2021) (Figure 2). When analyzing the morphometry of the seeds of the four provenances, the highest averages for length (cm) and width (cm) corresponded to the *Hopelchén* material and the lowest to *Calakmul* (Table 1). However, the *Calakmul* seeds had the best averages in volume (cm^3) and weight (g).



A) Outer testa of brown-grey color that covers the seed, B) Seed without testa showing the endocarp, cotyledons and radicle of the embryo, and C) Seed with germination induced by high relative humidity. *Endospermo* = Endosperm; *Cotiledón* = Cotyledon; *Radícula* = Radicle.

Figure 2. Morphology of *Brosimum alicastrum* Sw. seeds. Seeds with different degrees of radicle development are illustrated.

Table 1. Statistical differences between the morphometric variables of *Brosimum alicastrum* Sw. seeds by collection provenance in *Campeche*.

Collection provenance	Morphometric variables					
	Length	Width	Length/Width	Volume	Weight	Density

	(cm)	(cm)	(cm)	(cm^3)	(g)	(g cm^{-3})
Sahcabchén	1.65 B	1.64 B	1.01 B	2.27 B	2.70 B	1.32 A
X-Mabén	1.61 BC	1.63 B	0.99 B	2.08 B	1.84 C	1.02 B
Hopelchén	1.92 A	1.98 A	0.97 B	2.82 A	3.32 A	1.28 AB
Calakmul	1.50 C	1.38 C	1.10 A	3.03 A	3.16 A	1.15 AB

Means with the same letter per column are not statistically different (Tukey, a=0.05).

The Length (*L*)/ Width (*A*) ratio is one of the factors that define the shape of a seed: $L/A<1$ corresponds to a flattened seed, $L/A=1$ to a round one, and $L/A>1$ to an oblong one (Bezerra *et al.*, 2015). In the present study, although the statistical analyzes showed significant differences between the provenances, all the values of *L/A* are close to 1 (Table 1), that is, they correspond to round seeds, as previously cited (Brechú-Franco *et al.*, 2021).

The seeds with the highest volume were those from *Hopelchén* and *Calakmul*, which also registered the highest weights and densities (Table 1). These morphometric data are slightly different from those recorded by Morales and Herrera (2009), who evaluated seeds of *ramón* trees located in *Yucatán*, with lengths less than 1.4 cm, widths greater than 1.8 cm, and average weights of 2.8 g. The difference in seed sizes may be due to genetic and environmental factors inherent to the regions where the populations of the species are located (Vergara *et al.,* 2014).

Tetrazolium chloride (CTT) staining test

Fully stained embryos and partially stained embryos were observed (Figure 3). According to Mancipe-Murillo *et al.* (2018), staining intensity indicates a higher probability of germination. However, it should be considered that due to the recalcitrant property of *B. alicastrum* seeds, they are more likely to lose viability in the first 30 days (Magnitskiy and Plaza, 2007).

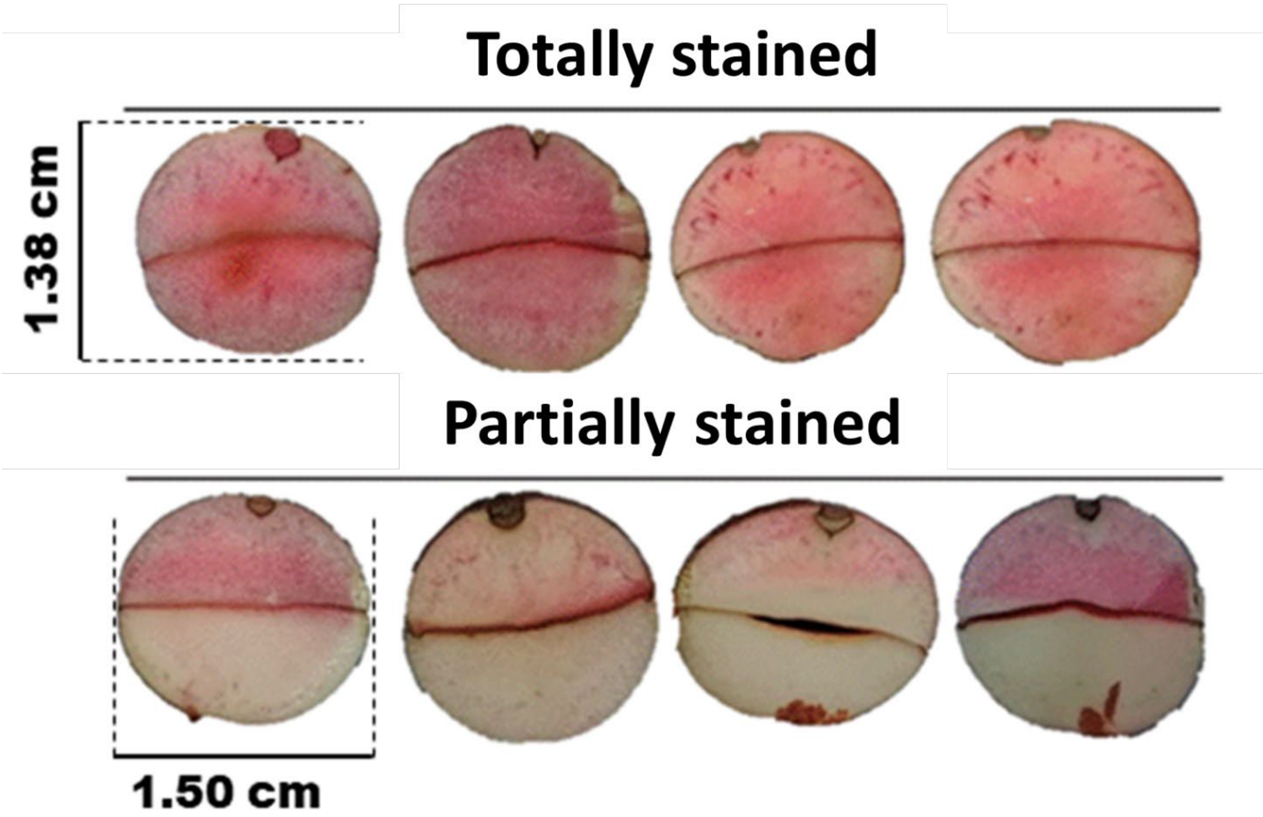


Figure 3. Staining of *Brosimum alicastrum* Sw. seeds by the 2,3,5-triphenyltetrazolium chloride (CTT) test.

Tukey's means test by (a=0.05) showed that the *X-Mabén* and *Hopelchén* provenances had the seeds with the highest percentages of complete staining and were statistically different from those from *Sahcabchén* and *Calakmul* (Figure 4).

The collection in *X-Mabén* and *Hopelchén* was carried out in areas disturbed by anthropogenic activities. According to Romero-Saritama (2018), the viability of a seed is affected by various factors, including the age of the tree and the physiographic conditions of the collection area. However, forest species tend to develop greater resilience under unfavorable conditions, which helps explain why higher percentages of viability were recorded in disturbed sites.

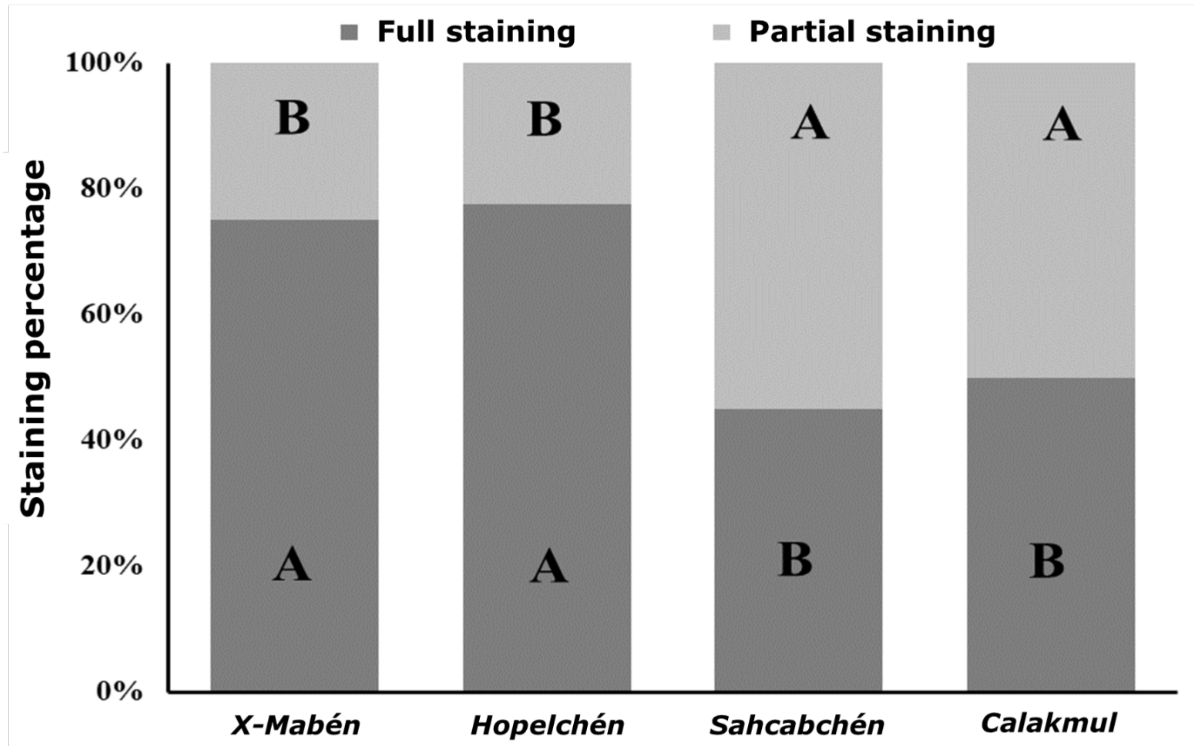


Figure 4. Statistical differences in the staining percentage of *Brosimum alicastrum* Sw. seeds by provenance. Means with the same letter per column are not statistically different (Tukey, α=0.05).

Effect of storage time and temperature on seed viability

Under room temperature conditions (28 °C) around 30 days, the percentage of partially stained seeds was higher than that of fully stained seeds, so it is considered that after 30 days the viability of the seeds is considerably reduced. In the case of seeds stored at 4 °C, the percentage of partially stained seeds exceeded the value of fully stained seeds after 70 days (Figure 5).

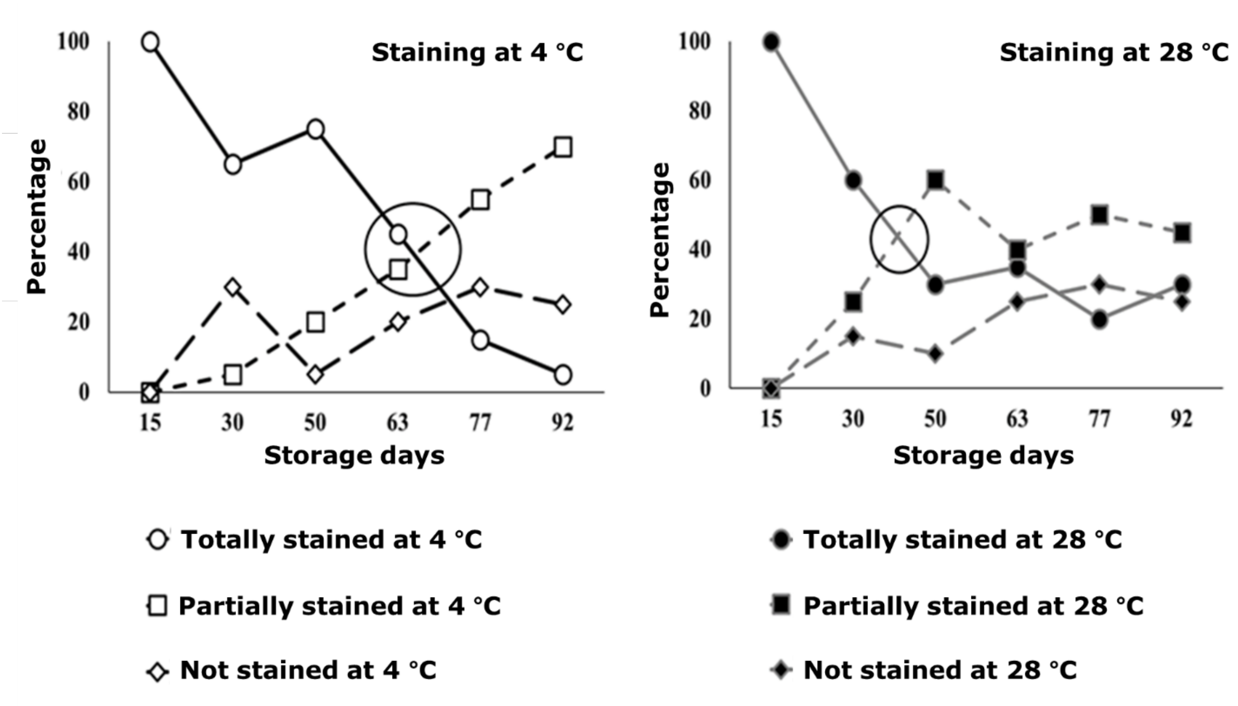


Figure 5. Effect of temperature and storage time on the viability of *ramón* (*Brosimum alicastrum* Sw.) seeds.

From day 77, the seeds stored at different temperatures registered the lowest staining percentages. Due to the recalcitrant property of *ramón* seeds, a gradual increase in the percentages of partially stained seeds and non-stained seeds was expected for both storage temperatures throughout the evaluation period.

The effect of temperature and storage time on the viability of recalcitrant seeds has been described by Magnitskiy and Plaza (2007), who postulated that temperature is the factor that most rapidly affects the increase in the viability of recalcitrant seeds. In this regard, Valverde-Rodríguez *et al.* (2019) pointed out that the longevity in the viability of recalcitrant seeds is greater with storage temperatures below 10 °C. However, given the null forest management applied to *B. alicastrum*, there is little information on the effect of temperature on the viability of its seeds (Vergara *et al.*, 2014).

Finally, the germination test of seeds stored for 30 days at 4 °C and 28 °C revealed 90 % germination in seeds stored at 4 °C, and 60 % for seeds stored at 28 °C (Table 2). The root system of the seeds stored at 4 °C was greater than that of the seeds at 28 °C (Figure 6). These results coincide with those obtained by Del Amo *et al.* (2002) who documented germination percentages higher than 75 % for the species. Although, unlike the study here described (controlled environment), this work was carried out in a nursery, with seeds without a prior storage period.

Table 2. Statistical differences of the effect of temperature at 30 days of storage on the germination of *Brosimum alicastrum* Sw. seeds.

Temperature	Germinated seeds (%)	Non-germinated seeds (%)
4 °C	90 A	10 B
28 °C	60 B	40 A

Means with the same letter per column are not statistically different (Tukey, α=0.05).

Figure 6. *Brosimum alicastrum* Sw. seeds germinated after 30 days of storage under controlled temperatures: 4 °C and 28 °C.

Conclusions

In northern *Campeche*, there is morphometric variability among *Brosimum alicastrum* seeds, whose viability can be extended up to 70 days when the storage temperature is 4 °C. Despite the fact that the germination test was conditioned with seed samples with the lowest viability values, a germination percentage of 90 % was achieved when the storage temperature was 4 °C, which probably corresponds to the optimal temperature to keep the seeds viability of this forest species and guarantee high germination percentages in the nursery stage. However, it should be considered that in this study the humidity factor was not analyzed. The information generated is valuable for the forest management of *B. alicastrum*, which is very important given the high demand for its specimens, seeds and fruits and its scarce forestry management.

Acknowledgments

To the *Consejo Nacional de Humanidades Ciencias y Tecnología (*Conahcyt*)* for the professorship awarded to the first author. To the authorities of the *Instituto Tecnológico Superior de Venustiano Carranza* for the facilities provided for the defense of the thesis of the Bachelor's Degree in Forest Engineering of the second author. This work is part of the project *"Relación de la morfometría con la viabilidad en las semillas, calidad de plántula en vivero y adaptación a una plantación de Brosimum alicastrum Swartz en la Península de Yucatán, México"* (Relation of morphometry with seed viability, seedling quality in the nursery and adaptation to a *Brosimum alicastrum* Swartz plantation in the Yucatan Peninsula, Mexico) with code CONV_RGAA_2023_06, financed by the *Colegio de Postgraduados* (Graduate Studies College) within the framework of the 2023-03 call to support research and advocacy projects aimed at strengthening activities for the conservation, preservation and sustainable use of genetic resources for food and agriculture (RGAA).

Conflict of interest

The authors declare no conflict of interest.

Contribution by author

Alberto Santillán-Fernández: conceptualization and design of the study, statistical analysis and writing of the final manuscript; Sandra Sugey Castañeda-Alonso: field data collection and information analysis; Eugenio Carrillo-Avila: information analysis and writing of the original manuscript; Alfredo Esteban Tadeo-Noble: preparation of cartographic maps and data review; Jaime Bautista-Ortega: analysis of the information and writing of the original manuscript; Javier Enrique Vera López: statistical analysis and drafting of the final document; Fulgencio Alatorre-Cobos: review, monitoring of results and writing of the final manuscript.

References

Berridge, M. V., P. M. Herst and A. S. Tan. 2005. Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction. Biotechnology Annual Review 11:127-152. Doi: 10.1016/S1387-2656(05)11004-7.

Bezerra P., M. A., A. Torquato T., E. H. Costa S., A. Ferreira A., S. Márcio A. and I. Rodrigues N. 2015. Postharvest conservation of structural long shelf life tomato fruits and with the mutant rin produced, in edaphoclimatic conditions of the southern state of Tocantins. Ciência e Agrotecnologia 39(3):225-231. Doi: 10.1590/S1413-70542015000300003.

Brechú-Franco, A. E., A. F. Larqué-Saavedra, G. Laguna-Hernández, K. Pasillas-Rodríguez and S. Espinosa-Matías. 2021. Morphology, structure, and histochemistry of the inflorescences, fruit, and seed of the Ramón nut, *Brosimum alicastrum* Sw. subsp. *alicastrum* CC Berg (*Moraceae*). Brazilian Journal of Botany 44(2):457-466. Doi: 10.1007/s40415-021-00708-w.

Calla T., C., J. C. Huarcacho N. y D. Cruz C. 2019. Determinación de concentración de tetrazolio y tiempo de tinción adecuado para el análisis de viabilidad en semillas de café. Apthapi 5(3):1671-1682. https://apthapi.umsa.bo/index.php/ATP/article/view/39. (17 de noviembre de 2022).

Campbell-Martínez, G. E., C. Steppe, S. B. Wilson, M. Thetford and D. Miller. 2022. Effect of temperature, light, and seed provenance on germination of *Paronychia erecta* (squareflower): a native plant with ornamental potential. Native Plants Journal 23(1):56-64. Doi: 10.3368/npj.23.1.56.

De Vitis, M., F. R. Hay, J. B. Dickie, C. Trivedi, J. Choi and R. Fiegener. 2020. Seed storage: maintaining seed viability and vigor for restoration use. Restoration Ecology 28(S3):S249-S255. Doi: 10.1111/rec.13174.

Del Amo R., S., M. C. Vergara T., J. M. Ramos P. y C. Sainz C. 2002. Germinación y manejo de especies forestales tropicales. Universidad Veracruzana. Xalapa, Ver., México. 182 p. https://www.uv.mx/personal/sdelamo/files/2012/11/Germinacion-y-manejo-de-especies.pdf. (14 de noviembre de 2022).

Dorta H., L. M. 2020. Influencia del tiempo de almacenamiento sobre la calidad fisiológica de semillas de *Pinus caribaea Morelet* var. *caribaea* Barret y Golfari del Huerto clonal Malas Aguas. CIFAM Ciencias Forestales y Ambientales 5(1):9-20. https://cifam.upr.edu.cu/index.php/cifam/article/view/138/pdf. (28 de noviembre de 2022).

Espinosa-Grande, E., A. Santillán-Fernández, B. M. Chávez-Vergara, A. A. Vargas-Díaz, A. E. Tadeo-Noble and J. Bautista-Ortega. 2023. Space-time analysis of scientific research on *Brosimum alicastrum* Swartz. Revista Facultad Nacional de Agronomía Medellín 76(1):10247-10261. Doi: 10.15446/rfnam.v76n1.101008.

Espitia-Camacho, M., H. Araméndiz-Tatis y C. Cardona-Ayala. 2017. Características morfométricas, anatómicas y viabilidad de semillas de *Cedrela odorata* L. y *Cariniana pyriformis* Miers. Agronomia Mesoamericana 28(3):605-617. Doi: 10.15517/ma.v28i3.26287.

Flores B., A., L. Ferrufino A. y V. López C. 2019. Viabilidad de semillas de guayacán (*Guaiacum sanctum* L., Zygophyllaceae) posterior a dos tratamientos pregerminativos. Portal de la Ciencia (16):25-37. Doi: 10.5377/pc.v0i16.8093.

Hernández-González, O., S. Vergara-Yoisura y A. Larqué Saavedra. 2015. Primeras etapas de crecimiento de *Brosimum alicastrum* Sw. en Yucatán. Revista Mexicana de Ciencias Forestales 6(27):38-48. Doi: 10.29298/rmcf.v6i27.279.

Instituto Nacional de Estadística y Geografía (Inegi). 2021. *Uso del suelo y vegetación, escala 1:250000, serie VII*. Portal de Geoinformación, Sistema de Información sobre Biodiversidad. http://www.conabio.gob.mx/informacion/gis/?vns=gis_root/usv/inegi/usv250s7gw. (18 de junio de 2022).

Magnitskiy, S. V. y G. A. Plaza. 2007. Fisiología de semillas recalcitrantes de árboles tropicales. Agronomia Colombiana 25(1):96-103. http://www.scielo.org.co/pdf/agc/v25n1/v25n1a11.pdf. (29 de julio de 2022).

Mancipe-Murillo, C., M. Calderón-Hernández y L. V. Pérez-Martínez. 2018. Evaluacion de viabilidad de semillas de 17 especies tropicales altoandinas por la prueba de germinacion y la prueba de tetrazolio. Caldasia 40(2):366-382. Doi: 10.15446/caldasia.v40n2.68251.

Mejenes-López, S. M. A, G. R. Chi-Sáenz, C. Flota-Bañuelos, B. Candelaria-Martínez y R. A. Chiquini-Medina. 2019. Germinación y características de plántulas de Uspí (*Couepia polyandra*: Chrysobalanaceae) en condiciones de vivero rústico en Campeche, México. Polibotánica 48:111-120. Doi: 10.18387/polibotanica.48.9.

Mendoza-Arroyo, G. E., A. Morón-Ríos, M. González-Espinosa, J. A. Alayón-Gamboa y P. A. Macario-Mendoza. 2020. La supervivencia y desarrollo de plántulas de *Brosimum alicastrum* (Moraceae) y *Psidium sartorianum* (Myrtaceae) difieren en condiciones de inundación. Acta Botánica Mexicana 127:e1548. Doi: 10.21829/abm127.2020.1548.

Moo-Huchin, V. M., J. C. Canto-Pinto, L. F. Cuevas-Glory, E. Sauri-Duch, E. Pérez-Pacheco and D. Betancur-Ancona. 2019. Effect of extraction solvent on the phenolic compounds content and antioxidant activity of Ramon nut (*Brosimum alicastrum*). Chemical Papers 73(7):1647-1657. Doi: 10.1007/s11696-019-00716-x.

Morales O., E. R. y L. G. Herrera T. 2009. Ramón (*Brosimum alicastrum* Swartz) protocolo para su colecta, beneficio y almacenaje. Comisión Nacional Forestal (Conafor). Mérida, Yuc., México. 15 p. http://www.conafor.gob.mx:8080/documentos/docs/19/1301RAMON%20(Brosimum%20alicastrum%20Swartz.)%20Yucat%C3%A1n.pdf. (13 de octubre de 2022).

Orantes-García, C., M. Á. Pérez-Ferrera, T. M. Rioja-Paradela y E. R. Garrido-Ramírez. 2013. Viabilidad y germinación de semillas de tres especies arbóreas nativas de la selva tropical, Chiapas, México. Polibotánica 36:117-127. https://www.scielo.org.mx/pdf/polib/n36/n36a8.pdf. (15 de octubre de 2022).

Park, K., S. Yeob L., B. Ji, B. K. Jang, … and J. S. Cho. 2022. Seed longevity and germinability of *Pulsatilla dahurica* (Fisch. ex DC.) spreng after storage and accelerated aging test. Horticultural Science and Technology 40(2):147-156. Doi: 10.7235/HORT.20220014.

Pennington, T. D. y J. Sarukhán. 2005. Árboles tropicales de México. Manual para la identificación de las principales especies. Universidad Nacional Autónoma de México-Fondo de Cultura Económica. Coyoacán, D. F., México. 523 p.

Pérez M., C. 2018. Conservación de semillas de algodón nativo (*Gossypium* spp.): análisis físico, fisiológico y bioquímico. Tesis de Doctorado. Programa de Postgrado en Recursos Genéticos y Productividad. Colegio de Postgraduados, Campus Montecillo. Texcoco, Edo. Méx., México. 178 p. http://colposdigital.colpos.mx:8080/jspui/bitstream/10521/4198/1/Perez_Mendoza_C_DC_RGP_Produccion_Semilla_2018.pdf. (18 de abril de 2023).

Ramírez-Sánchez, S., D. Ibáñez-Vázquez, M. Gutiérrez-Peña, M. S. Ortega-Fuentes, L. L. García-Ponce y A. Larqué-Saavedra. 2017. El ramón (*Brosimum alicastrum* Swartz) una alternativa para la seguridad alimentaria en México. Agroproductividad 10(1):80-83. https://www.revista-agroproductividad.org/index.php/agroproductividad/article/view/943. (13 de diciembre de 2022).

Rashidi, M., K. Seyfi and M. Gholami. 2007. Determination of kiwifruit volume using image processing. ARPN Journal of Agricultural and Biological Science 2(6):17-22. https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=182e39bed65bf443a71bc25b74c9448645feee5e. (17 de mayo de 2022).

Rodríguez, I., G. Adam y J. M. Durán. 2008. Ensayos de germinación y análisis de viabilidad y vigor en semillas. Agricultura Revista Agropecuaria (912):836-842. https://orbi.uliege.be/bitstream/2268/37372/1/articulo%20definitivo%20agricultura%20nov.pdf. (11 de septiembre de 2022).

Rojas-Schroeder, J. A., L. Sarmiento-Franco, C. A. Sandoval-Castro y R. H. Santos-Ricalde. 2017. Utilización del follaje de ramón (*Brosimum alicastrum* Swarth) en la alimentación animal. Tropical and Subtropical Agroecosystems 20:363-371. https://www.redalyc.org/pdf/939/93953814001.pdf. (10 de agosto de 2022).

Romero-Saritama, J. M. 2018. Conservación de semillas: Una alternativa inmediata para almacenar germoplasma forestal y recuperar los bosques secos amenazados del Ecuador. Neotropical Biology and Conservation 13(1):74-85. Doi: 10.4013/nbc.2018.131.09.

Salazar M., S. A., J. D. Quintero C. and J. P. Rojas S. 2020. Optimization of the tetrazolium test in three species of orchids of the Andean forest. Australian Journal of Crop Science 14(5):822-829. Doi: 10.21475/ajcs.20.14.05.p2276.

Santillán-Fernández, A., A. Calva-Castillo, N. Vásquez-Bautista, Z. G. Huicab-Pech, A. Larqué-Saavedra y J. Bautista-Ortega. 2021b. Balance hidro-climático de *Brosimum alicastrum* Sw. y su variabilidad ante escenarios de cambio climático en la península de Yucatán, México. Revista Fitotecnia Mexicana 44(1):41-49. Doi: 10.35196/rfm.2021.1.41.

Santillán-Fernández, A., O. V. Santiago-Santes, E. Espinosa-Grande, Z. G. Huicab-Pech, F. A. Larqué-Saavedra y J. Bautista-Ortega. 2021a. Propagación sexual y asexual de *Brosimum alicastrum* Swartz en Campeche, México. La Granja: Revista de Ciencias de la Vida 34(2):105-116. Doi: 10.17163/lgr.n34.2021.07.

Secretaria de Bienestar (SB). 2020. *Programa Sembrado Vida*. https://www.gob.mx/bienestar/acciones-y-programas/programa-sembrando-vida. (27 de septiembre de 2022).

Siqueira W., L., M. Moreno G., M. Almeida da S., A. C. Nogueira and D. Kratz. 2020. Adjustments in the tetrazolium test methodology for assessing the physiological quality of Jatropha mollissima (Euphorbiaceae). Bosque 41(1):77-82. Doi: 10.4067/S0717-92002020000100077.

Subiria-Cueto, R., A. Larqué-Saavedra, M. L. Reyes-Vega, L. A. de la Rosa, … y N. R. Martínez-Ruíz. 2019. *Brosimum alicastrum* Sw. (Ramón): An alternative to improve the nutritional properties and functional potential of the wheat flour tortilla. Foods 8(12):613. Doi: 10.3390/foods8120613.

Vallejos, J., Y. Badilla, F. Picado y O. Murillo. 2010. Metodología para la selección e incorporación de árboles plus en programas de mejoramiento genético forestal. Agronomía Costarricense 34(1):105-119. https://www.scielo.sa.cr/pdf/ac/v34n1/a11v34n1.pdf. (10 de junio de 2022).

Valverde-Rodríguez, K., C. O. Morales y E. G. García. 2019. Germinación de semillas de *Crescentia alata* (Bignoniaceae) en distintas condiciones de temperatura, luminosidad y almacenamiento. Revista de Biología Tropical 67(2):S120-S131. Doi: 10.15517/rbt.v67i2supl.37211.

Venables, W. N. and D. M. Smith. 2023. An Introduction to R. Notes on R: A Programming Environment for Data Analysis and Graphics. Version 4.3.0. R

Foundation for Statistical Computing. Vienna, W, Austria. 105 p. https://cran.r-project.org/doc/manuals/r-release/R-intro.pdf. (22 de abril de 2023).

Vergara Y., S., C. I. Briceño S., J. V. Pérez B., O. Hernández G., L. G. Rosado L. y A. Larqué S. (Comps). 2014. Publicaciones de *Brosimum alicastrum*. Ramón, Oox, Ojoche, Capomo, Mojote. Centro de Investigaciones Cientificas de Yucatán. Mérida, Yuc., México. 102 p.