



# Storage time effect on the germination of *Haematoxylum campechianum* in Campeche, Mexico

## Efecto del tiempo de almacenamiento en la germinación de *Haematoxylum campechianum* en Campeche, México

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### ABSTRACT

*Haematoxylum campechianum* L. (Fabaceae), is an economically and culturally important tropical tree distributed across Central America and the Yucatán Peninsula, Mexico. To contribute to the conservation of this species, it is important to understand the seed germination process; however, research in this subject on *H. campechianum* remains scarce. We evaluated the relationship between the *H. campechianum* seed storage time and its fresh weight and germination. Mature seeds were collected in the municipality of Palizada, Campeche, Mexico, from 2016 to 2019, stored at 25 °C and 60%-80% relative humidity until experimentation after the 2019 collection. Seed replicates (10, comprising 25 seeds) were weighed to estimate fresh weight per collection year. Seed viability was determined by means of the tetrazolium test, while germination was carried out in Petri dishes using cotton as a substrate under a 12-hr photoperiod at 25 °C. The seeds collected in 2019 presented a high viability and germination (100% and 98.5% respectively), in contrast with those collected in 2018, which presented a drastic reduction in the values for these parameters (55% and 54%, respectively). Null viability and germination were observed in seeds collected in 2016 and 2017. The seeds collected in 2016 showed the highest fresh weight values than the other years sampled. The results indicate that the viability and germination of *H. campechianum* seeds were observed to be affected by storage time, the recently harvested seeds presented a higher viability and germination than older seeds. Therefore, the *H. campechianum* seeds could be recalcitrant because they presented an accelerated ageing process over the collection periods.

KEYWORDS: conservation, fresh weight, palo de tinte, recalcitrance, viability.

### RESUMEN

*Haematoxylum campechianum* L. (Fabaceae), es una un árbol tropical de importancia económica y cultural distribuida en Centroamérica y la Península de Yucatán, México. Para contribuir a la conservación de esta especie, es importante entender el proceso de germinación de semillas, sin embargo, las investigaciones sobre este tema en *H. campechianum* siguen siendo escasas. Se evaluó la relación entre el tiempo de almacenamiento de las semillas de *H. campechianum* y su peso fresco y germinación. Se recolectaron semillas maduras en el municipio de Palizada, Campeche, México, de 2016 a 2019, y se almacenaron a 25 °C y 60%-80% de humedad relativa hasta la experimentación después de la recolecta de 2019. Se pesaron lotes de semillas (10, conteniendo 25 semillas) para estimar el peso fresco por año de recolecta. La viabilidad de semillas se determinó mediante la prueba de tetrazolio, mientras que la germinación se realizó en cajas de Petri utilizando algodón como sustrato bajo un fotoperíodo de 12 h a 25 °C. Las semillas recolectadas en 2019 presentaron una alta viabilidad y germinación (100% y 98.5% respectivamente), en contraste con las recolectadas en 2018, que presentaron una reducción drástica en los valores para estos parámetros (55% y 54% respectivamente). Se observó nula viabilidad y germinación en semillas recolectadas en 2016 y 2017. Las semillas recolectadas en 2016 mostraron los valores de peso fresco más altos que los otros años muestreados. Los resultados indican que la viabilidad y la germinación de las semillas de *H. campechianum* se vieron afectadas por el tiempo de almacenamiento, las semillas recién cosechadas presentaron mayor viabilidad y germinación que las semillas más viejas. Por lo tanto, las semillas de *H. campechianum* pudieran ser recalcitrantes debido a que presentaron un proceso de envejecimiento acelerado durante los períodos de recolección.

PALABRAS CLAVE: conservación, peso fresco, palo de tinte, recalcitrancia, viabilidad.

## INTRODUCTION

The Yucatán Peninsula (YP), is a region in south-eastern Mexico, covering 450 000 km<sup>2</sup> bordered by the Gulf of Mexico and the Caribbean Sea, including the states of Campeche, Quintana Roo and Yucatan. It is considered a biotic province in the Neotropical region showing a high diversity (Morrone, 2005). In the YP, 2300 plant species have been documented across 956 genera and 161 families, of which the family Fabaceae is the best represented, with 78 genera and 225 species (Fernández Carnevali *et al.*, 2012).

*Haematoxylum campechianum* L. (Fabaceae), commonly known as Palo de Tinte, is a species of tree native to the tropical region of the American continent and distributed across Guatemala, Belize, and the YP in Mexico (Niembro, 2002). In the YP, this species grows on deep clay substrata with poor drainage and a high moisture content, generally known as lowlands (Niembro, 2002). As a significant biocultural resource, *H. campechianum* is raw source for the extraction of dyes from pre-Columbian times, exponentially increasing during the Spanish colonization at the beginning of the 16th century (Dampier, 2004; Villegas & Torras, 2014). It is used for bioprospecting anti-inflammatory, antioxidant, and antiseptic active compounds (Norton, 1996; Duke, 2008), histochemical properties (reviewed by Avwioro, 2011) as well as anticarcinogens (Peng *et al.*, 2014). Moreover, its trunks are used to produce posts for perimeter fences on rural land, coal and as firewood, while its leaves and shoots are used for fodder and ornamental purposes (Plasencia-Vázquez, Villegas, Ferrer-Sánchez, & Zamora-Crescencio, 2017). The latter due to its colorful flowers, nectar-polleniferous and visited by bees of the species *Apis mellifera* L. (Quezada-Euán, May-Itzá, & González-Acereto, 2001); thus, *H. campechianum* is a potential resource in the Mexican meliponiculture to production of honey and wax.

Studies undertaken on *H. campechianum* have mainly focused on aspects of its natural history (Niembro, 2002; Villegas & Torras, 2014), ecological restoration (Zamora-Cornelio, 2010), resilience in degraded environments (Pérez, 2014), as well as its distribution (Plasencia-Vázquez *et al.*, 2017; Chablé-Vega *et al.*, 2019), tree morphometric

structure (Chablé-Vega *et al.*, 2019) and ethnobotanical characteristics (Contreras, 2010). However, research seeking knowledge on the germination of the *H. campechianum* seeds has received little attention (Zamora-Cornelio, Ochoa-Gaona, Vargas Simón, Castellanos Albores, & de Jong, 2010).

The germination of seeds is one of the most crucial stages in plant development (Baskin & Baskin, 2014). Environmental factors, such as temperature, water, light, and pH determine the success of the germination process (Baskin & Baskin, 2014). However, as seeds do not indefinitely conserve their germination capacity, a high viability and germination rate depends, in large part, on storage conditions (Baskin & Baskin, 2014). For example, a high viability rate in dry-stored seeds (45 °C – 50 °C) depends, in large part, on the balance between the storage container moisture and oxygen (Ellis & Hong, 2007). However, this balance varies from species to species and depends on the seeds' chemical composition and seed type.

Various adaptation strategies are observed in seed morphometry (e.g., size, biomass and/or fresh weight) and play an important role in the germination process and the establishment of seedlings (Rojas-Aréchiga, Mandujano, & Golubov, 2013). Large seeds and/or those with greater fresh weight present a higher level of nutrient reserves in the cotyledons, which tends to increase their viability, germination, survival, and emergence speed, making them more tolerant to adverse conditions than small seeds (Khurana & Singh, 2001; Loza-Cornejo, López-Mata, & Terrazas, 2008; Wang, Baskin, Cui, & Du, 2009).

Since many plant species develop under adverse environmental conditions (e.g., water scarcity as well as high temperatures and light intensity; Nobel, 2010), an important aspect to consider in the natural dynamics of the germination process is the differential response observed in seeds' desiccation tolerance over time (Farrant, Pammenter, & Berjak, 1993; De Oliveira-Gentil, 2001). Therefore, desiccation tolerance is a crucial functional characteristic of the ecology of plant regeneration (Tweedle, Dickie, Baskin, & Baskin, 2003).



Typically, seeds can be classified as orthodox (Roberts, 1973; Tweddle *et al.*, 2003; Wyse & Dickie, 2017), meaning that they are able to tolerate desiccation under low humidity conditions (3% - 7%), thus drastically reducing metabolic activity and, by means of prior rehydration, conserving a high viability rate (Roberts, 1973; Rangel Fajardo *et al.*, 2011). These seeds can be easily conserved either *in situ*, via the formation of persistent seed banks in the soil (Thompson, 2000), or *ex situ*, where they are stored in germplasm banks (Rangel Fajardo *et al.*, 2011).

Seeds can also, in contrast, be classified as recalcitrant when they remain metabolically active and do not tolerate desiccation, rapidly losing their viability below humidity levels of 12% - 31% (Roberts, 1973; Tweddle *et al.*, 2003; Wyse & Dickie, 2017). Consequently, these seeds do not form persistent seed banks in the soil, they are not able to be stored in germplasm banks (Tweddle *et al.*, 2003; Rangel Fajardo *et al.*, 2011). A third seed type can be classified as intermediate, with these seeds capable of tolerating desiccation within a range of 7% - 20% humidity; however, their viability decreases more rapidly when stored at low (0 °C) rather than warm (12 °C - 21 °C) temperatures, over the long term (Roberts, 1973; Pritchard, 2004).

To date, few studies have focused on evaluating the germination of *H. campechianum* seeds. Zamora-Cornelio *et al.* (2010), found a high and speed germination (86%) in freshly harvested seeds of this species. These authors suggest a recalcitrant effect in the seeds of this species. Nevertheless, to test this hypothesis, long-term studies focused on assess variations in fresh weight, viability, and germination of the *H. campechianum* stored seeds are necessary.

## OBJECTIVES

The present study aimed to analyze the effect of storage time on the fresh weight, viability, and germination of *H. campechianum* seeds.

## MATERIALS AND METHODS

### Study species

*Haematoxylum campechianum* L. (Fabaceae) (Palo de Tinte), is a tree species growing to a height of up to 15 m, with leaves that grow to lengths of 3 cm to 10 cm and comprise three to four pairs of sessile leaflets (Durán & Souza, 2014). Its nectar-polleniferous flowers are observed in axillary and terminal inflorescence arrangements, which are up to 12 cm in length, while its fruit is a 3 cm to 6 cm long flattened pod containing 1 to 2 seeds, 12 mm long, 3.9 mm wide, and 1 mm thick flattened seeds (Niembro, 2002). There is no information about seed production per tree, seed size and soil seed bank dynamics in *H. campechianum*. Research about phenology of this species also limited (Durán & Souza, 2014; Niembro, 2002).

### Seed collection

The *H. campechianum* seeds were harvested in the municipality of Palizada, Campeche, Mexico (Fig. 1), in 2016, 2017, 2018, & 2019, between the months of March to July, the species' fructification period (Centro de Investigación Científica de Yucatán [CICY], 2010). The climate is tropical monsoon (Am), the average annual temperature at the site is 26.2 °C, with temperatures of over 46 °C in spring (March-May) and below 18 °C in winter (December-February), and a mean precipitation of 1634.5 mm is observed during the rainy season, occurring between June and October (Comisión Nacional del Agua [Conagua] 2010); Servicio Meteorológico Nacional [SMN], 2020).

In the YP, this species forms dense monospecific groups in clay soils subject to periodic flooding that have poor drainage knowns as "tintales" (Niembro, 2002; Plasencia-Vázquez *et al.*, 2017). For the four collection cycles, the seeds were obtained from pods of 20 mature individuals (at least 20 pods of three branches per mature individual were collected) pertaining to two adjacent tintales

in the study area (Fig. 1). A polyethylene mesh (1.83 m × 6.10 m) at 50% was placed under the tree canopy to retain the collected pods; subsequently, they were cleaned, and the seeds were extracted manually from pods, stored in muslin bags under 60% - 80% relative humidity conditions and a standard temperature of 25 °C, until the experiments inherent to this research began after the 2019 harvest.

### Fresh weight

The seeds obtained in the abovementioned harvests were separated into ten replicates, comprising 25 seeds ( $n = 25$ ) per each year of collection (2016, 2017, 2018, and 2019). Each replica was individually weighed, using a Cole-Parmer Symmetry analytical balance (precision  $\pm 0.2$  mg), to estimate the individual fresh weight (g,  $\pm$  standard error = SE) per seed, per replicate and collection year.



FIGURE 1. Location of the natural populations of *Haematoxylum campechianum* in the study area, in the municipality of Palizada, Campeche, Mexico.



### Seed viability

This test was carried out in 2019 after all seed collections were completed. The tetrazolium test (Peters, 2000) was used with a 0.5% triphenyl tetrazolium chloride solution to determine the viability of the *H. campechianum* seeds. Five 20-seed replicates ( $n = 20$ ) per collection year (2016, 2017, 2018, and 2019) were used to evaluate viability. The seeds in each replicate were placed in beakers and embedded in distilled water for 24 h prior to placement in the tetrazolium solution. Subsequently, each replicate of seeds was placed in a beaker, to which 50 ml tetrazolium solution was added and which was then wrapped in aluminium foil, in order to keep it in dark conditions, and a temperature of 25 °C for 24 h. In order to facilitate the ingress of the solution into the seed, an incision was made parallel to the hypocotyl axis using a scalpel (Aragón-Gastélum *et al.*, 2018). Finally, the seeds were cut transversally with a scalpel to observe the embryos and ascertain whether they were viable. The embryos of the viable seeds presented a reddish hue under the microscope (Baskin & Baskin, 2014). With these data, the percentage seed viability ( $\pm$  SE) for each collection year was obtained.

### Seed germination

The seed germination experiments were carried out in 100 mm  $\times$  15 mm Petri dishes using a sterile cotton layer as a substrate, moistened with 8 mL distilled water, 20 seeds per dish (10 replicas per collection year;  $n = 200$ ), each of which were sealed with plastic film to avoid moisture loss. Once sealed, the Petri dishes were not watered during the entire experiment. The Petri dishes were incubated continuously at 25 °C (optimum temperature for the germination of *H. campechianum* seeds; Sánchez-Rendón, Pernús-Álvarez, Torres-Arias, Barrios, & Dupuig, 2019) and for a 12 h light/darkness photoperiod with 80% relative humidity for 30 days (period in which these experiments are typically monitored; see Baskin & Baskin, 2014) in a grow room. The germination data were recorded daily in this period, with a seed considered germinated when the radicle was visible. The data obtained was used to calculate the final

germination percentage ( $\pm$  SE) for each collection. The *H. campechianum* seeds of each collection year (2016, 2017, 2018, and 2019) did not have any germination pretreatment (Sánchez-Rendón *et al.*, 2019).

### Statistical analysis

The normality assumption for the variables was determined by means of the Kolmogorov-Smirnov and Lillifor tests (Hill & Lewicki, 2006). Since a normal adjustment for the fresh weight and viability data was not possible, in both cases, the  $X^2$  analysis was used for the comparison of medians. As no viability and germination were observed for the seeds collected in 2016 and 2017, germination performance over time was solely considered for the years 2018 and 2019 as treatments and was analyzed by means of the Wilcoxon signed-rank test, taking the germination times (days) as categories and the differences in daily germination values from 2018 to 2019 as  $d_i$  (Daniel & Cross, 2018).

The 2018 and 2019 germination data not adjusted to normal distribution were compared via the application of the Kruskal-Wallis test by ranks under non-parametric ANOVAs, while the data with a higher number of samples than the category size was analyzed via a one-way ANOVA with Newman-Keuls weighting (Daniel & Cross, 2018). The model explaining the germination observed considered the value of the residuals, the coefficient of determination  $R^2$ , and the Fisher's F value (McKellar & Lu, 2004), while the Chow test was used to compare the projections via a generalized logarithmic linear model, taking into account the difference in the residuals (Gujarati & Porter, 2009).

## RESULTS

### Fresh weight

The statistical results indicate significant fresh weight differences between the treatments (collection year), considering an  $X^2 = 27.20$  and  $p \leq 0.05$ . The highest fresh seed weight (4.94 g  $\pm$  0.03 g, SE) was obtained in 2016. The 2017 seeds showed low mean fresh weight values than 2016, but also statistically differed between collection years

(3.84 g  $\pm$  0.01 g). For the 2018 and 2019 seeds, lower in fresh weight values were found (3.63 g  $\pm$  0.02 g, and 3.67 g  $\pm$  0.06 g, respectively), no statistical differences between both treatments (Fig. 2).

### Seed viability

The highest viability was obtained in the seeds collected in 2019 (100%), while 2 years to 3 years of storage was found to drastically reduce seed viability. The results of the analysis revealed a value of  $X^2 = 220.25$ , and  $p \leq 0.05$ , indicating statistically significant differences between the years of collection. The 2018 sample presented intermediate seed viability values with an average of 55%  $\pm$  3.2%, while the percentage of viability for the 2017 and 2016 samples was zero.

### Seed germination

Significant differences in germination over time between the results obtained for 2018 and 2019 ( $T_{0.05(2),28} = 116$ ) were found. In all categories, the germination observed for

the 2019 sample surpassed the germination of seeds stored for a year (44.5% - 71.5% respectively) (Table 1).

### Germination: 2018 vs 2019

Significant differences in germination were found for both years. The 2019 seeds germinated in a shorter time and showed a higher germination percentage than the 2018 seeds (66.5%  $\pm$  0.76% *vs* 5%  $\pm$  0.21%, respectively) in the first two days of the experiment (Fig. 3). The maximum germination percentage also differed significantly between both years ( $F_{(19,360)} = 41.919$ ;  $p \leq 0.05$ ), with the 2019 sample reaching 98.5%  $\pm$  3.43% in eight days, while the 2018 sample reached maximum germination, 54%  $\pm$  2.84%, in 20 days. The residual difference (SCRR 2018= 16.435, SCRR 2019= 0.868, SCRNR= 17.303;  $F_{(2,102)} = 204$ ;  $p < 0.01$ ) indicate significant differences in accumulated germination between the seeds collected in 2018 and 2019, with the latter presenting rapid germination, in contrast to the former (Fig. 4).

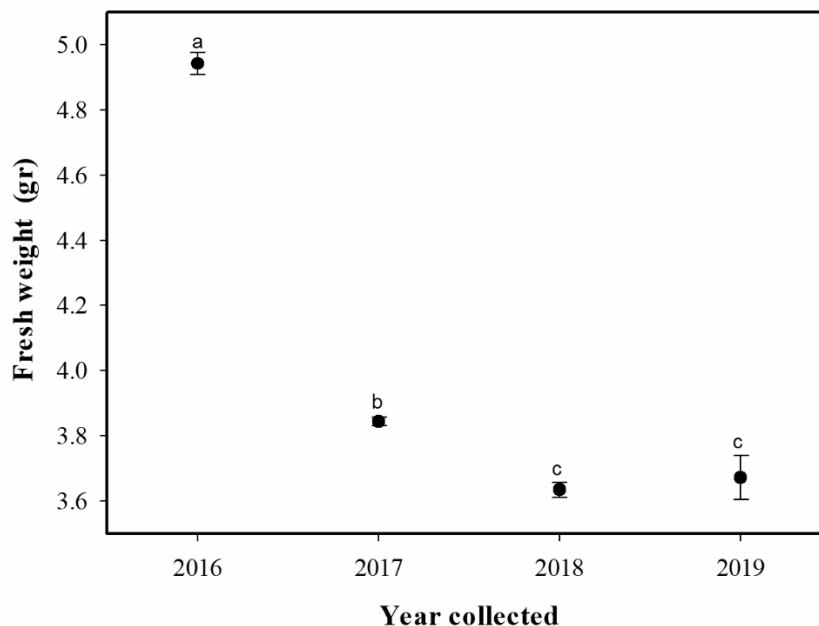


FIGURE 2. Mean fresh weight of *Haematoxylum campechianum* seed batches ( $\pm$  standard error) harvested over four years of collection ( $X^2 = 27.20$  and  $p \leq 0.05$ ).



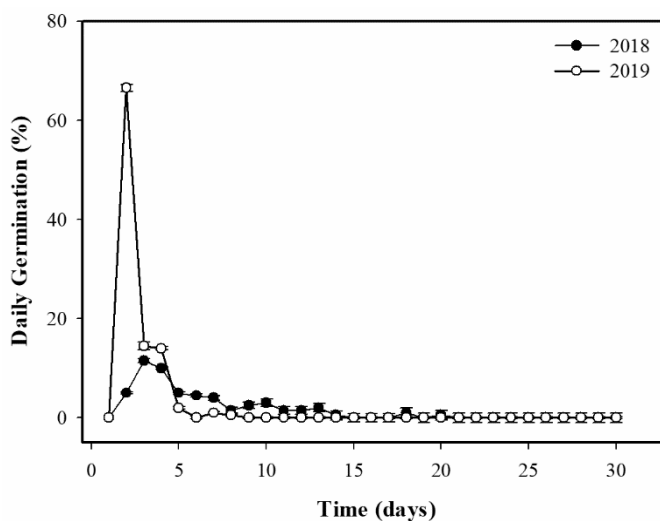


FIGURE 3. Comparison of the daily percentage of *Haematoxylum campechianum* seed germination from 2018 to 2019.

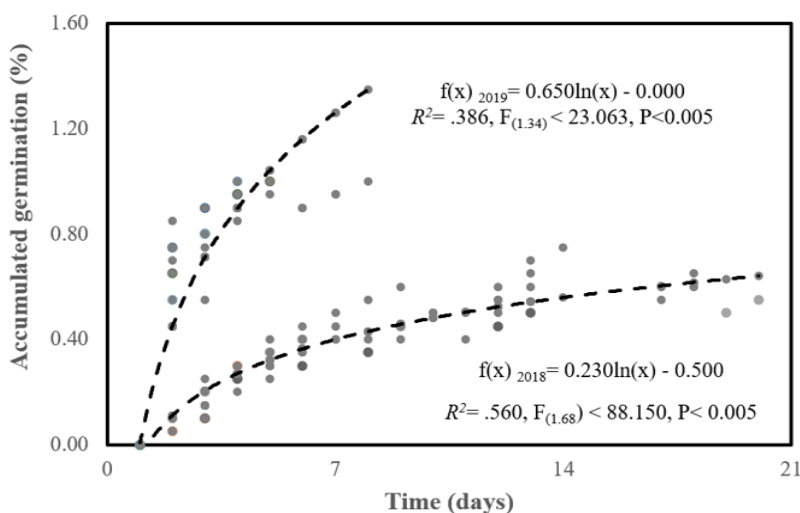


FIGURE 4. Adjusted models for the germination of *Haematoxylum campechianum* seeds for 2018 and 2019.

The difference between models concurs with the comparison undertaken via the Chow test.

## DISCUSSION

The seed viability and germination of *H. campechianum* were affected by storage time, with our results showing that the seeds collected in 2019 displayed higher viability and germination percentages than those collected in 2018 while viability and germination were null for the 2016 and 2017 samples.

The morphometric attributes of seeds, such as size, fresh weight, and biomass are important to the germination process, as they influence seedling development (Rojas-Aréchiga *et al.*, 2013). In this sense, in some species of the family Fabaceae, seeds with greater biomass present differential responses in germination (Huyghe, 1993; Lobos, Miranda, & Mera, 2008), although they also have





greater commercial value (Lobos *et al.*, 2008); however, few seed morphometry studies have been conducted on this plant family.

Pérez (2014), found differences in several attributes, including mass and germination, in the seeds of wild species of the genus *Lupinus* (*L. campestris*, *L. exaltatus*, *L. hintonii*, and *L. montanus*) collected in 2011 and 2012. He documented that these four species presented intraspecific variations in seed weight (*L. hintonii* =  $35.51 \text{ g} \pm 0.087 \text{ g}$ , *L. montanus* =  $1.8 \text{ g} \pm 0.002 \text{ g}$ , *L. campestris* =  $1.6 \text{ g} \pm 0.002 \text{ g}$ , and *L. exaltatus* =  $1.33 \text{ g} \pm 0.014 \text{ g}$ ) and germination (with and without mechanical scarification), which was higher in treated seeds (90%).

Ecologically, all seeds differing in their desiccation tolerance (Farrant *et al.*, 1993; De Oliveria-Gentil, 2001); thus, seeds can be classified into orthodox, recalcitrant, and intermediate (Roberts, 1973; Pritchard, 2004). Desiccation tolerance in seeds of species in the Fabaceae family has also been little researched. Giamminola, Morandini, and de Viana, (2012), did not find significant differences in the germination of *Prosopis nigra* Hieron., with a storage time of three months applied prior to scarification. The seeds of this species were germinated 90% and presented a shorter germination time (4.8 days) and a higher moisture content (CH = 10% - 12%) than desiccated seeds (CH = 3% - 5%), which germinated at a level of 80%, results which indicate that the seeds of *P. nigra* are orthodox. Galíndez *et al.* (2015), identified the presence of physical dormancy and the effect of storage on two subtropical species of Fabaceae, *Amburana cearensis* (Allemão) A.C. Sm and *Myroxylon peruiiferum* Lf. seeds, using different scarification methods. They found that the *A. cearensis* seeds stored at  $-18 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$  for three and 12 months were orthodox, while the *M. peruiiferum* seeds were sensitive to desiccation and storage at  $-18^\circ\text{C}$ .

Furthermore, Morandini, Giamminola, and de Viana (2013), determined both desiccation tolerance and germination in *Prosopis ferox* Griseb and *Pterogyne nitens* Tul., subjecting the seeds to mechanical scarification and storing them for six months at  $-20 \text{ }^\circ\text{C}$  and at CH = 10% - 12% and 3% - 5%, respectively. Their results show that maximum

germination occurred in seeds with a lower 3% - 5%, and that the effect of storage for six months at  $-20 \text{ }^\circ\text{C}$  did not influence germination; therefore, seeds of both species are classified as orthodox, given that the germination level was over 80% when CH was reduced to 3% - 5% and after storage for six months at  $-20 \text{ }^\circ\text{C}$ .

The findings found here are contrary to most results documented above. This is due to the fact that the studies described indicates that the majority of the Fabaceae species studied showed different strategies for increasing their perpetuation potential over time, despite being subject to adverse environmental conditions, as is the case with the induction of dormancy. Although the moisture content of the *H. campechianum* seeds was not evaluated due to poor seed availability over the years of collection, it is assumed that this was high in the collection years, given that *H. campechianum* trees in the YP are normally distributed in lowland areas (tántales) characterized by a high-water content in the soil (Niembro, 2002; Plasencia-Vázquez *et al.*, 2017), and that moisture content reduced as storage time increased; however, this hypothesis should be tested in future research.

The results obtained in the present study indicate that the *H. campechianum* seeds could be considered as recalcitrant due to the high germination percentage in recently harvested seeds in 2019 and an accelerated ageing process, which was characterized by a low viability and germination rate in seeds stored in previous years. Based only in germination data during one collected year, Zamora-Cornelio *et al.* (2010), suggested that the *H. campechianum* seeds were recalcitrant because both a high and speed germination (86%). Our research is novel and incorporates variations in the fresh weight, biochemical viability tests as well as germination essays under a multi-year stored approach of the *H. campechianum* seeds and support the assumption proposed by Zamora-Cornelio *et al.* (2010).

In the case of this study, it was found that independent of the year of collection, the seeds presented an inverse relationship between seed fresh weight and both viability and germination. The fresh weight results are contrary to those previously reported for some Fabaceae species

(Huyghe, 1993; Lobos *et al.*, 2008) and show the great variability of morphometric and physiological attributes in these species. Moreover, seeds of this family show a great range of ecophysiological, genetic, and micro-environmental adaptations which enable them to establish themselves in most of the ecosystems worldwide (Langer & Hill, 1991).

A possible explanation for the greater percentage fresh weight found in *H. campechianum* seeds subject to a longer storage time may be related to the increased number of toxic radicals at a cellular level, such as reactive oxygen species (ROS). They consist in free radicals ( $O_2^{\bullet-}$ , superoxide anion radical;  $OH^{\bullet}$ , hydroxyl radical;  $HO_2^{\bullet}$ , perhydroxy radical and  $RO^{\bullet}$ , alkoxy radicals) and non-radical (molecular) forms ( $H_2O_2$ , hydrogen peroxide and  $^1O_2$ , singlet oxygen (Halliwell, 1987; Huang, Ullah, Zhou, Yi, & Zhao, 2019). ROS are produced naturally during cellular metabolism in different parts of the plant cell (Huang *et al.*, 2019). In seeds, during the first stages of germination, the principal sources for the generation of ROS are mitochondria, peroxisomes, and cell membranes. Considering that, in most cases, the first stages of germination occur in darkness, chloroplasts do not contribute to ROS generation (Huang *et al.*, 2019); however, once the seedling is produced, it will begin to form chloroplasts, which are also a source of ROS.

According to the oxidative window model proposed by Bailly, El-Maarouf-Bouteau, and Corbineau (2008), an adequate and optimal ROS level is required for germination to occur. Therefore, very high ROS levels, for example in seeds that have aged due to prolonged storage periods or those subject to high temperatures, would cause damage at an embryo level and prevent said seeds from germinating (Bailly *et al.*, 2008). It is possible that the higher percentage of fresh weight in older *H. campechianum* seeds documented in the present study is related to a potentially higher ROS level, and that this caused structural damage to the embryos and consequently, negatively affected the viability and germination in stored seeds of this species. However, more research is required to support this hypothesis.

Given the current high deforestation rate from anthropogenic activities documented in the YP (Rueda, 2010), the present study seeks to promote the use and sustainable exploitation of *H. campechianum* within its natural area of distribution and contributing to the creation of effective conservation and restoration strategies as well as biotechnological purposes. Moreover, it could be the key to a paradigm shift about the exploitation of natural resources, not only of this species but also any other timber and non-timber resources in the state of Campeche and the YP. Future research on *H. campechianum* may be focused on evaluating different seed storage mechanisms considering variations in germination between a same year of collection in order to maximize the propagation both *in vitro* and *ex situ* of its natural populations; in addition, include other variables such as length, thickness, colour, number of seeds per pod and/or tree among others. It may also seek to analyze the effect of various environmental factors, such as drought, thermal tolerance thresholds, magnetism, photoblastism, salinity and dynamic of the ROS in different organelles of plant, on germination and seedlings establishment under both laboratory and field conditions. Future studies may also explore the capacity of *H. campechianum* as a soil restorer, evaluating its potential as a nitrogen fixer in the soil, which is typical of many Fabaceae species. This would require long-term research in the field, evaluating the regeneration cycle and reproductive characteristics of the natural populations of this species, the production and dispersal of seeds, dynamic of the seed bank in the soil as well as the establishment, survival, and performance of seedlings and young individuals.

## CONCLUSIONS

The viability and germination of *H. campechianum* seeds were observed to be affected by storage time. The recently harvested seeds presented a higher viability and germination percentage than older seeds. As the findings of the present study indicate an accelerated ageing process in this species, it could be considered recalcitrant. The present research is the first long-term seed ecology study of *H. campechianum*, presenting an evaluation of the seed



germination dynamics and delineating preliminarily the thresholds desiccation tolerance in *H. campechianum*, a charismatic and key forest species with high sociocultural value to the state of Campeche and the YP, Mexico.

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