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Morphometric characteristics and seed germination of *Dalbergia granadillo* Pittier

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ABSTRACT
Objective: To know the variability and quality of seed in a *Dalbergia granadillo* Pittier (Fabaceae) population from the Soconusco, Chiapas, Mexico.
Design/methodology/approximation: The seed was obtained from six trees during the months of February and March 2020. Two weeks after the seeds were collected, pod and seed variables were determined. Seed length (LS), width (AS), color (CS), weight of 100 seeds (g) (PS) and germination percentage (PG). The results were statistically analysed by analysis of variance using the GLM procedure and the comparison of means by Tukey ($P \leq 0.05$) with the SAS 9.0 program.
Results: There is wide variation in seed content. The pods registered from 1, 2, 3 and up to four seeds, although pods of one predominated. There are seeds of two colours, dark brown and light brown, and the latter are the most abundant; however, light colored seeds registered higher germination (96%). The weight of 100 seeds registered values of 9.6-9.7 g.
Study Limitations/implications: The species has a restricted distribution and presents a low number of individuals in the evaluated populations.
Findings/conclusions: The morphometric characteristics of the seeds allowed to identify the variability and quality, mainly in shape, size and coloration of the seeds, as well as the number of seeds per pod.

Key words: Granadillo, seed quality, weight, and germination.

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INTRODUCTION

Tropical species considered as precious woods have been subjected to various anthropic actions, such as deforestation and fragmentation of their habitat. In addition to the above, it registers low natural repopulation, and they are of slow initial growth. The level of incidence of these factors or their combination, favor vulnerability to their loss (CITES, 2013; Cervantes, 2016). *Dalbergia* sp. (Fabaceae) is a tropical species, with a registered distribution in Chiapas, Guerrero, Jalisco, Michoacán, Nayarit and Oaxaca, (Pittier, 1922; CONABIO, 2017; CITES, 2015; Guala and Döring, 2020). The species *D. granadillo* Pittier known as granadillo (Pittier, 1922), cocobolo or rosewood (Cervantes, 2016) is considered high quality wood. Its contrasting colours, ranging from reddish-dark brown, with almost black veins of its heartwood and yellow sapwood, make it very attractive and increase its preference.

Its acoustic properties have been used since pre-Hispanic times by the Aztecs for the elaboration of musical instruments (Herrera-Castro *et al.*, 2019), and currently in certain regions of Mexico (Suárez-Islas *et al*., 2020), it is used mainly to make studio and concert guitars (Guridi and García-López, 1996). It is also used to make handicrafts, kitchen utensils, castanets, marimba keys and decoration in general (Niembro, 1990).

It registers high natural durability when exposed in the soil compared to other tropical species (Colín-Urieta *et al*., 2019).

In the Soconusco, Chiapas, Mexico, the habitat destruction has been the action with the greatest impact over the *D. granadillo* (Díaz-Gallegos *et al*., 2010) populations, and at present it has been classified as an endangered species, both in NOM-059-SEMARNAT-(2010), as in the appendices of the Convention on International Trade of Species (CITES, 2016 a). An unquantified risk is that the immoderate logging of the habitat has caused the accelerated loss of its genetic diversity. This reduces the possibilities of generating knowledge about the germination process of its seed that facilitates the design of multiplication and reinsertion strategies. Based on the above, it was proposed to know the variability and quality of seed in a population of *Dalbergia granadillo* Pittier (Fabaceae) from the Soconusco, Chiapas, Mexico.

MATERIALS AND METHODS

Location of the study area and biological material

The trees from which the seeds were obtained are located in “La Rioja” Community, in Cacaohatán, Chiapas, Mexico (14° 58’ 37” LN and 92° 16’ 15” LO), at an altitude of 480 m. The climate belongs to the warm humid group Af (w”) i g, with 4720 mm of annual precipitation and an average temperature of 25.4 °C (INEGI, 2017). The soils have a volcanic origin and belong to the mollic Andosol group. They are characterized by the strong phosphate binding but are easy to grow and have good rooting and water storage characteristics (FAO, 2008). The soil of the place presents the following physicochemical characteristics: sand and loam texture (82.5% sand, 12.3% silt, 5.2% clay, 3.7% organic matter) (Walkley-Black), 0.12 ds m^{-1} electrical conductivity, pH 5.71, 0.13% N (Kjeldhal), 4.0 ppm P (colorimetry), 18.5 ppm K^{++} (atomic spectrophotometry), 59 ppm Ca^{++} (atomic spectrophotometry), 9.3 ppm Mg^{++}, 16.8 ppm Na^{++}, y 5 Meq 100 g^{-1} of cation exchange capacity.

Collection date and number of trees

Fruits were collected during February and March 2020 from six trees that constitute the population. Pods were collected from each tree and stored in labelled paper bags. The characterization was carried out in the Laboratory of Forest Biotechnology and Biofertilizers of the Faculty of Agricultural Sciences, Huehuetán, Chiapas (15° 19’ N and 92° 44’ O) where the pods with and without seeds were separated. The pods with seed were grouped according to the number of seeds and kept at room temperature in the laboratory (23.2 °C) in a ziploc® plastic bag.

At 15 days after its collection, basic initial quality tests were determined using the established rules of the International Seed Testing Association (ISTA, 2010), such as morphometric and germination variables (Table 1).

Table 1. Variables of pods and seeds of *D. granadillo* Pittier collected in the Soconusco, Chiapas, Mexico.

Variable	Description
Number of pods per tree	The number of pods with seed of each tree was quantified.
Pod length	It was measured with a digital vernier (Caliper Brand, Stainless Hardened, USA) with a precision of 0.1 mm.
Seeds per pod	The number of seeds per pod in each tree was counted.
Number and colour of seeds per tree	The number of seeds per tree was recorded and separated for germination by colour; dark brown and light brown.
Weight of 100 seeds	100 seeds (g) were weighed with four repetitions on a digital scale (Ohaus Brand, SocutPro Model SP401 USA).
Number of seeds per kg	It was determined by the formula (Number of seeds in the sample / Weight of the sample) * 100. (ISTA, 2010).
Germination	It was measured in percentage (%) according to the Testa colour through the paper germination method (ISTA, 2010) the first germination count was made after 5 days and the last one on day 20.

Statistical analysis

The mean results of the variables in the induction phase were plotted with the Sigma Plot (V. 11.0) program from Jandel Scientific. The data of the variables in the multiplication stage were analysed with the SAS Program for Windows Ver. 8.1 (1999-2000) and the comparisons of means between treatments with Tukey ($P \leq 0.05$).

RESULTS AND DISCUSSION

Number of pods and seeds per pod

The trees show a difference in the number of pods and the number of seeds per pod. The values fluctuated between 65 to 102 pods among the trees and with 1, 2, 3 and up to 4 seeds (Table 2). The immature fruit has a green colour and resembles a leaf.

The number of pods with one seed dominated in all trees, and it appears to be a characteristic of the species. Tree 4, with the lowest number of pods, presented the highest percentage of pods with one seed, while trees 1, 2, 6 and 12, with the highest number of pods, represented on average 25% fewer pods with one seed. In trees 2, 6 and 12, the pods with two seeds fluctuated in percentage from 20 to 23% and only tree 1 registered 40%. In the case of pods with three seeds in most trees, it represented between 3 and 7% of the total;

Table 2. Number of pods and seeds per pod of *D. granadillo* Pittier trees.

Tree and total number of pods	Number of seeds per pod			
	1 (%)	2 (%)	3 (%)	4 (%)
1 (102 pods)	56 (54.9)	40 (39.3)	5 (4.9)	1(0.98)
2 (84 pods)	55 (65.4)	23 (27.3)	6 (7.3)	
4 (65 pods)	56 (86.1)	6 (9.2)	3 (4.7)	
5 (78 pods)	58 (74.3)	14 (18.0)	5 (6.4)	1(1.3)
6 (84 pods)	49 (58.4)	20 (23.8)	15 (17.8)	
12 (88 pods)	60 (68.2)	20 (22.7)	7 (7.9)	1 (1.2)

however, it increased to 15% in tree 6. According to CITES, (2016) these data coincide with those found for *Dalbergia calycina* Benth.

Seed length and width

The size of the seeds was greater in three trees 1, 2 and 4 (Figure 1) and they do not present a relationship between the total number of pods and the percentages of seeds per pod. It is important to mention that the variation in the size of the seed plays a significant role in the germination process. This indicates that heavier seeds may be more appropriate for their multiplication (Seltmann *et al.*, 2007).

The length and width values of the seeds did not coincide with the *Dalbergia tucurensis* Donn species, where the average value in the seeds was 1.0 cm long and 0.5 cm wide (CITES, 2016b). Changes in seed size also occur in accessions of various tropical forest species and the changes are contrasting, as in *Cedrela odorata* L. where Alderete-Chávez *et al.* (2005) cite differences in size based on length and width of the seeds with values of 2.60 cm and 3.64 mm, respectively.

The size and weight of the seed are closely related to its reserve content, which varies between species. They are also related to the establishment, the growth of the seedlings and when the reserves are high, greater persistence of the species is achieved in a given site (Fenner and Thompson, 2005; Luna *et al.*, 2018), it is also relevant to harvest the seed with optimal humidity to favour its benefit, conservation (Sánchez *et al.*, 2010).

Weight of 100 seeds

The average weight of one hundred seeds per tree (W100S) was in a range of 8.8 and 9.2 g, and the trees that expressed the highest values were 5 and 6 (Figure 2). The differences in the size of the seeds, which induce weight differences, occur in other tropical species, such as *Cedrela odorata* L. (Arce-Córdoba *et al.*, 2018).

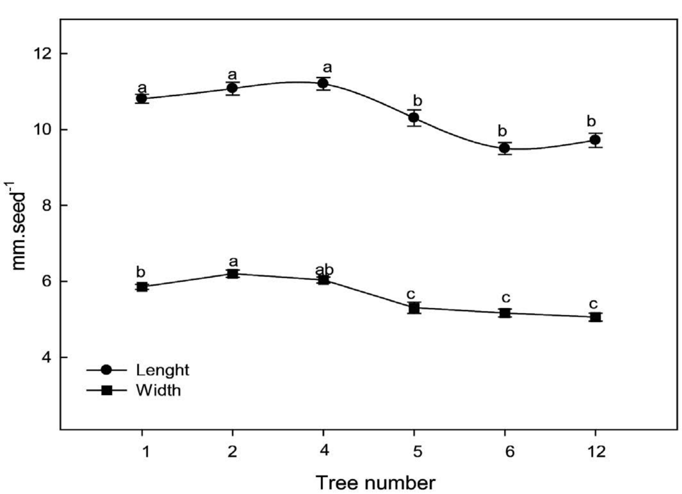

Figure 1. Length and width of *D. granadillo* Pittier seeds from six trees located in Soconusco Chiapas, Mexico. The values are means of 35 repetitions ± standard error and the letters that are not equal indicate statistical difference (Tukey p≤0.05).

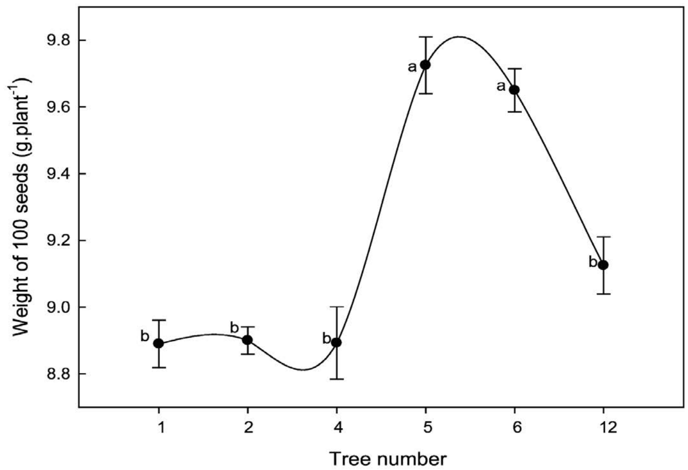

Figure 2. Weight of 100 seeds per tree of *D. granadillo* collected in the Soconusco region, Chiapas Mexico. The values are means of 35 repetitions ± standard error and the letters that are not equal indicate statistical difference (Tukey $p \leq 0.05$). CV = 1.7%.

The variation in the weight of 100 seeds can be influenced by genetic factors in interaction with the environment, and in this regard, Mendizábal-Hernández *et al.* (2012; 2013) mention that the differences may also be due to pollen viability and fertility, as well as stigmatic receptivity.

In other species, such as *C. odorata* L., Arce-Córdova *et al.* (2018) and *Roseodendron donnell-smithii* Miranda, Agustín-Sandoval *et al.* (2017) variations in weight of 100 seeds between collections in the same region of Soconusco, Chiapas are consigned. It is important to highlight that the seeds of greater size and greater weight present the highest quality in attributes for germination and vigour, therefore, full, healthy and mature seeds are stored better than those that do not reach maturity (Doria, 2010).

The storage of nutrients in the cotyledons has a direct influence on the initial growth of the seedlings (Soriano *et al.*, 2011; Bewley *et al.*, 2013; Soriano *et al.*, 2013) and in the dry tropical forest It has been observed that the species that produce larger seeds produce larger seedlings (Soriano *et al.*, 2011), survival in the field (Khurana and Singh, 2004) and in general, an increase in the content of aerial and root biomass (Velázquez- Rosas *et al.*, 2017).

The establishment and survival of the seedlings depends greatly on the content of reserves stored in the seeds (weight), in such a way that seeds with a lower weight express a low level of vigour and, consequently, a decrease in the probability of their establishment (Rubio *et al.*, 2011). However, those seeds of greater weight are also more susceptible to consumption by local fauna with respect to those of lower weight, although the potential for dispersal (zoocoria) may be greater in the former (Fenner and Thompson, 2005).

Number and colour of seeds per tree

All the trees presented seeds of two colours (light and dark brown), some in the same pod. In trees number 1, 2, 6 and 12, dark brown seeds dominated and in trees 4 and 5, the proportion of the two seed colours was similar (Figure 3).

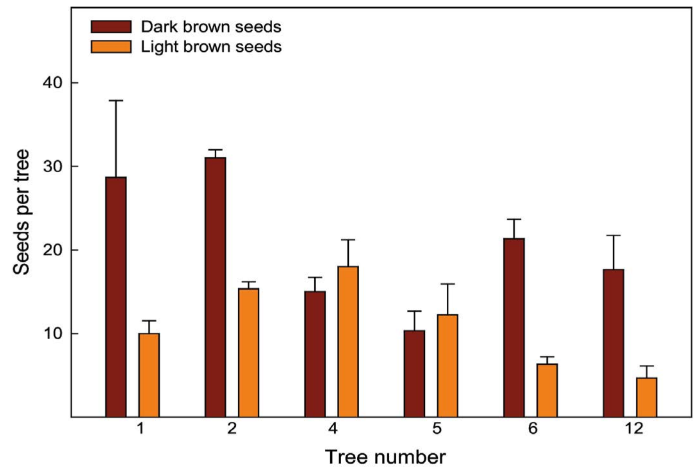

Figure 3. Number and colour of seeds per tree of *D. granadillo* collected in the Soconusco, Chiapas, Mexico.

In this regard, Doria (2010) refers to the importance of seed colour for germination, highlighting that the difference can be an adaptive characteristic for the species as it can germinate in a wider range of environmental conditions.

In the present case, the dark and light brown colours presented a contrasting difference in germination. Authors such as Debeajun *et al.* (2000) mention that the colour of the seed is positively correlated with restriction to germination, due to the phenolic components in the seed coat.

Todd and Vadkin (1996) mention that the seeds with darker colours germinate little due to the pigments of the Testa, while the seeds with lighter colours, in addition to having less pigments, absorb less heat, they can be kept at a temperature closer to optimal and germinate in greater quantity.

In addition, dark brown seeds are more prone to phytosanitary problems. They are attacked by insects of the Bruchidae family, and *Ctenocolum salvini* that deposit their larvae in the young fruits to complete their development and destroy the interior of the seed. Fungi such as *Alternaria* sp. and *Aspergillus* sp. have been reported in the seed of *Dalbergia retusa* Hemsl which is possibly the same that happens with *Dalberia granadillo* Pittier (CATIE, 2000; Cordero & Boshier, 2003).

According to Baskin and Baskin, (2001) the variation of sizes in seeds, colours and shapes is controlled by the environment, genetics and their interaction. Among the environmental factors, nutrients, light, shade, time of year, defoliation, temperature and humidity intervene, as well as the position in the fruit. Such variation is related to differences in the requirements for germination and the breaking of dormancy.

Germination

The highest germination (90 and 96%) was observed in the light brown seeds in trees number 2 and 12 (Figure 4). However, in the rest of the trees the same trend was presented, that is, greater germination in the light brown seeds. The dark brown seeds, which were the dominant ones in the seed population, presented the lowest germination percentage.

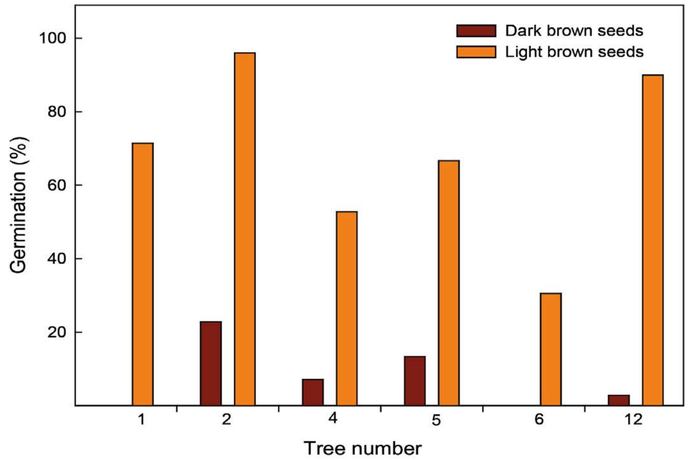

Figure 4. Seed germination by colour of the testa of *Dalbergia granadillo* Pittier collected in the Soconusco Chiapas, Mexico.

The variation in the colour of the seed may be due to the content and distribution of pigments such as anthocyanins, which are present in some Fabaceae seeds as well as glycosides, flavonoids and condensed tannins (Lobova *et al.*, 2003). This is what can happen with *D. granadillo* Pittier with regarding the amount and distribution of flavonoids that can affect the colour of the cover, as it happens with other tropical species.

In the case of the *Dalbergia* genus, in *D. retusa* Hemsl. the seeds present high germination percentage (80-90%), and when extracting the seeds from the pod, Knoblauch (2001) cites a 2.14-fold increase in germination, improving the viability value.

In other species such as *C. odorata* L. without separating seeds by Testa colour, high germination values similar to those found in this study have been recorded (Arce-Córdova *et al.*, 2018). However, in Mahogany (*Swietenia macrophylla* King), red cedar (*Cedrela odorata* L.) and oak or maculís (*Tabebuia rosea* Bertol) contrasting germination percentages of 76%, 54% and 37% respectively are cited (Quinto *et al.*, 2008).

While *D. congestiflora* Pittier, *P. acatlense* Benth and *M. benthamii* J.F. Macbr. which are species of the same family (Fabaceae), showed germination percentages similar to *D. granadillo* Pittier with 90% germination (González *et al.*, 2019). Other authors such as Rojas and Torres (2014) point out the importance of moderate irrigation in greenhouse conditions in *C. odorata* L. plantations for seed production, which under these conditions can achieve up to 80% germination when applying irrigation.

CONCLUSIONS

There are variations in number of pods, seeds per pod, seed size and weight of 100 seeds among the *D. granadillo* Pittier tree population. Pods with a dark brown seed predominate in all trees. The germination of the seeds was differential to the colour of the Testa. The highest percentage was registered in light brown seeds and they represent the smallest number of the total (96%).

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