Colegio de Postgraduados

Morphometric, weight, viability, and germination analysis of castor bean seeds (*Ricinus communis*) under two temperature and relative humidity conditions

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ABSTRACT

Objective: To analyze the morphometric, weight, viability, and germination evaluation under two temperature and relative humidity conditions, in sixteen local varieties of castor bean (*Ricinus communis*) from several states of Mexico (E1-E16), as well as two commercial varieties (k75B and k93B).

Design/Methodology/Approach: The following morphometric characteristics were analyzed using a vision system: area, elongation index (EI), and Feret's diameter (FD). Viability and germination tolerance (germination percentage (GP)), germination speed (GS), and emergence speed index (ESI)) were evaluated under two conditions of relative humidity and temperature (T1 - RH 80%/T 20 °C; T2 - RH 30%/T 40 °C), using a completely randomized block experiment design, with four replicates of 75 seeds.

Results: There are morphometric differences (EI, area, and FD) between and within the study varieties. There are significant differences between T1 and T2 regarding the following variables: days of radicle emergence (T1: 44.71 and T2:11.6), germination percentage (T1: 48.37 and T2: 56%), ESI (T1: 34.07±12.72 and T2: 77.02±23.78), and GS (T1: 9.93 and T2: 24.60). The results obtained show a positive correlation between the morphometric properties and the germination percentage in T1; however, there was no correlation in T2.

Study Limitations/Implications: There were no limitations to carry out this study.

Findings/Conclusions: The E4 and E15 local varieties obtained a >93% productive potential in T1, while the E3 and E16 local varieties obtained a >78% productive potential in T2. Meanwhile, the k75B commercial variety had a better performance in T1 (89.75%) than in T2 (78.67%).

Keywords: germination, local varieties, *Ricinus communis*, viability.

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INTRODUCTION

Castor bean (*Ricinus communis* L.) is an allogamous plant (Euphorbiaceae, 2n=20) native to tropical Africa. It spread out from Africa to the Middle East as a wild plant, giving birth to a wide range of different weights, sizes, shapes, and colors (Goytia-Jiménez

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et al., 2011; Peña-Uribe *et al.*, 2021). In Mexico, castor bean is grown in the states of Chiapas, Chihuahua, Coahuila, Colima, Guanajuato, Guerrero, Jalisco, Estado de México, Michoacán, Morelos, Nayarit, Oaxaca, Sinaloa, Sonora, Tabasco, Tamaulipas, San Luis Potosi, Zacatecas, Tlaxcala, Veracruz, and Yucatán (Acosta-Navarrete *et al.*, 2017; Barrios-Gómez *et al.*, 2018; Solis Bonilla *et al.*, 2016; Llaven Valencia *et al.*, 2019; Canul *et al.*, 2021).

From the diversity point of view —and in order to evaluate the variability between and within genotypes or species—, the morphometric characterization of plants (seeds and fruits) is based on the qualitative or quantitative values (Barrios-Gómez *et al.*, 2018; Canul-Ku *et al.*, 2022; Pecina-Quintero *et al.*, 2013), the knowledge about sizes and shapes, and the useful data available. This information can be used to design innovative management and storage techniques in natural ecosystems and farms (Huang *et al.*, 2022; Peña-Uribe *et al.*, 2021). These measurement techniques include vision systems that have been implemented (Modarres Najafabadi and Farahani, 2012) to find out the morphometric dimensions of the objects, in order to generate databases (Acosta *et al.*, 2013). The analysis of images is a less expensive non-invasive technique than hand techniques (Pasato-Guanga and Fuentes-Pérez, 2021).

Mature seeds have a certain latency degree, which can cause a slow, erratic, and low germination, probably as a result of the hard shell that covers the seeds and limits water permeability. Therefore, the plants have developed several adaptative strategies to counteract the adverse effects, enabling their survival (Ricardo Abril-Saltos *et al.*, 2017). These strategies include the control of physical and chemical changes, such as the loss of the cell membrane integrity, the reduction of enzymatic activity, and lipid peroxidation (Ergin *et al.*, 2022). Consequently, the loss of vigor and the germinative potential are fundamental parameters for the certification and commercialization of castor bean seeds (Escobar-Álvarez *et al.*, 2021).

Castor bean seed can develop under extreme environmental conditions: 18-33.9 °C temperatures, 383-1083 mm annual precipitations, and a 21-69% relative humidity (Hernández-Ríos *et al.*, 2019; Llaven Valencia *et al.*, 2019; Solis Bonilla *et al.*, 2016). The optimal temperatures for the development of this plant range from 20 to 25 °C (Machado *et al.*, 2012). These measurements were recorded using fiber optic sensors, in order to determine the development and behavior of castor bean seeds under the various agro-climatic conditions to which they are exposed (Huerta-Mascotte *et al.*, 2016).

Both in Mexico and in the rest of the world, castor bean (*Ricinus communis*) is a high interest culture, as a result of its oil content —mainly ricinoleic acid, which is a raw material used in the production of high value-added products. Therefore, efforts are aimed to increase the agronomic yield of this crop, under erratic weather conditions (Vasco-Leal *et al.*, 2022). Consequently, the objective of this study was to evaluate the following vigor parameters: the emergence speed, the germination speed, and the germination percentage of 16 local varieties and 2 commercial varieties of castor bean. The vision system proposed takes into account their vigor and the morphometric characteristics, as well as their relationship with the weight of 100 seeds as dimensional reference.

MATERIALS AND METHODS

Castor bean seeds came from a national collection, which was initially made up of 120 wild accessions, stored at 4 °C (Bonner, n.d.) for two years before they were evaluated. Out of this collection, 16 indehiscent varieties (E1-E16) were chosen, because they had obtained a high agronomic yield in Guanajuato. These seeds were grown in the INIFAP-Bajío experimental unit (20° 34' 47" N and 100° 49' 14" W, at 1,767 m.a.s.l.). We used the technological package developed by Hernández Martínez *et al.* (2013). The materials were compared with 2 commercial varieties native to India (k75B and k93B). The offspring of the first generation of the original plants was obtained.

Weight and Morphometry

Seed weight was determined using an Oahus analytical scale (0.0001g accuracy), randomly taking one hundred seeds from each variety. To morphometry, an area (a), minor axis (ma), and major axis (MA) were established and, subsequently, the elongation index (EI) and the Feret's diameter (FD) were determined, using the equations proposed by Quintanilla Carvajal *et al.* (2015) and the image processing algorithm developed by Ojeda-Magaña *et al.* (2010). This process is based on three stages: i) binarization, ii) segmentation, and iii) extraction of morphometry. We used the Matlab R2011 version to process the digital images, with a Dino-lite 311-ST microscope, which was placed 29 cm from the base where the seeds were placed. As dimensional reference, the algorithm validation was confirmed by hand, with a $\pm 0.24\ cm^2$ average error.

In order to evaluate germination viability and tolerance (germination percentage, germination speed, and emergence speed index), indirect tests were carried out in the lab to measure the physiological attributes of the seeds (Martínez-Solís *et al.*, 2010).

Germination percentage (GP)

The test was carried out using polystyrene trays, with peat-moss as a substrate. The seeds were sown at a 2-cm depth. The percentage was determined through a standard germination test, following the International Rules for Seed Testing (International Seed Testing Association, 2016), using a modified Bitoronette Mark II-845 plant growth chamber. The seeds were subjected to two treatments, controlling relative humidity (RH) and temperature (T) throughout the stages of the germination process. The first treatment had 80% RH and a 20 °C T, while the second treatment had a 30% RH and a 40 °C T. Germination tests were carried out for every experiment, using a completely randomized block design, with four repetitions of 75 seeds each.

Germination speed (GS)

GS was determined with the equation proposed by González-Zertuche and Orozco-Segovia (1996). The number of germinated seeds was quantified on a daily basis, during the germination period:

$$GS = \sum \frac{ni}{t} \qquad \text{Equation 1}$$

Where *GS* is the germination speed; *t* is the germination time from the sowing to the germination of the last seed; and *ni* is the number of seeds germinated in a given day.

Emergence speed index (ESI)

The number of plants that emerged was counted every day, considering the day in which they were sown in the trays as the first day. ESI was calculated using the following equation (2) proposed by González-Zertuche and Orozco-Segovia (1996):

$$ESI = \frac{\sum (niti)}{N} \quad \text{Equation 2}$$

Where *ESI* is the emergence speed index; *ni* is the number of seeds that germinated that day; *ti* is the number of days since the beginning of the experiment until day *i*; and *N* is the total number of germinated seeds.

Experimental design and result analysis

The results were statistically processed through an Analysis of Variance (ANOVA), comparing the means through the Tukey-Kramer HSD method (α=0.01 and 0.5), using the JMP software ver 5.0.1 (JUM-Statical Discovery Software, SAS).

RESULTS AND DISCUSSION

The average weight of the sixteen local varieties evaluated was 49.31 g per 100 seeds (0.49 g per seed). Meanwhile, the heaviest varieties were E-15, E-16, and E-4, which recorded 91.65±14.78, 86.95±27.87, and 86.83±23.44 g, respectively. In contrast, both commercial varieties (k75B and k93B) had an average weight of 31.45 g per 100 seeds (0.31 g per seed). This last result is the closest to the findings of Goytia *et al.* (2011) and Acebedo *et al.* (2018), who recorded average values of 48.72 and 32 g, respectively. The different results reported by the authors corroborate the diversity that likely results from the various agricultural and weather conditions. A wide variation range in the weight and shape of the seeds is considered a significant element in the plants' reproductive strategies and adaptations.

Meanwhile, 4.14±0.64 to 4.41±0.44 cm^2 ranges were reported in the area. They belong to the E1, E3, E4, E15, and E16 varieties. For their part, the E7 and E8 varieties reported a 1.36 to 1.38 cm^2 range, respectively, which matches the findings of Goytia-Jiménez *et al.* (2011).

Table 1 shows the results of the morphometric values of sixteen local castor bean varieties (E1-E16), plus two commercial varieties (k75B and k93B). Those values were obtained through a statistical comparison of their average values and standard deviations. The weight of the seed is directly proportional to its dimensions (area and FD). Likewise, Salihu *et al.* (2014) reported that seed weight is related to the total number of seeds produced by the plant.

Table 1. Weight and morphometric characteristics of castor bean seeds.

Variety	Weight in 100 seeds (g)	Area (cm^2)	EI (Φ)	FD
E-1	79.98±0.86	4.30±0.42	0.78±0.07^{cde}	2.76±0.23^{ab}
E-2	48.02±16.03	2.61±0.19	0.77±0.06^{gh}	1.66±0.13^{d}
E-3	78.81±18	4.14±0.64	0.76±0.06^{de}	2.65±0.44^{b}
E-4	86.83±23.44	4.41±0.44	0.68±0.03^{cd}	2.76±0.46^{ab}
E-5	43.09±5.32	2.69±0.31	0.74±0.07^{fg}	1.78±0.42^{cd}
E-6	50.78±13.28	2.89±0.32	0.73±0.07^{ef}	1.84±0.25^{c}
E-7	31.08±18.24	1.36±0.29	0.75±0.16^{de}	0.94±0.18^{ij}
E-8	21.13±17.75	1.38±0.17	0.61±0.06^{k}	0.89±0.14^{j}
E-9	40.94±12.88	2.30±0.31	0.69±0.07^{g}	1.50±0.29^{e}
E-10	33.42±13.99	1.99±0.11	0.66±0.04^{ghi}	1.13±0.08^{h}
E-11	36.65±16.93	1.99±0.18	0.76±0.02^{cde}	1.27±0.11^{fg}
E-12	27.74±18.21	1.69±0.30	0.78±0.07^{cd}	1.08±0.21^{h}
E-13	31.76±15.85	1.80±0.15	0.61±0.04^{jk}	1.15±0.11^{gh}
E-14	38.65±25.94	1.99±0.14	0.79±0.02^{c}	1.26±0.09^{fg}
E-15	91.65±14.78	4.49±0.41	0.76±0.03^{cde}	2.86±0.26^{a}
E-16	86.95±27.87	4.16±0.61	0.75±0.12^{cde}	2.66±0.54^{b}
Average	**51.71**	**2.76**	**0.72**	**1.76**
Standard deviation	**24.32**	**1.16**	**0.05**	**0.73**
k75B	32.7±20.14	1.50±0.27	0.64±0.03^{hij}	1.36±0.07^{f}
k93B	30.2±19.05	1.46±0.12	0.89±0.05 ^{a}	1.37±0.09^{ef}
Average	**31.45**	**1.48**	**0.76**	**1.365**
Standard deviation	**1.76**	**0.02**	**0.17**	**0.007**

Average±Standard Deviation. Means compared using Tukey-Kramer HSD. The same letter indicates that there is no significant difference (95%).
EI: elongation index. FD: Feret's diameter.

The evaluated variables have contrasting morphometries and colors (silver grey, ochre, dark, and matt black). Even within the same variety, there are colors or intensity combinations that hinder their classification.

No differences regarding the shape of the seed (SS) were recorded between the castor bean populations evaluated, all of which were elliptical.

For its part, the Feret's Index (FI) shows the correlation of the accessions with a bigger area and a greater FI (1, 3, 4, 15, and 16), which they are elements of the seed shape. Consequently, as the area increases, its parameters record a proportional increase and vice versa, because they are dependent elements, according to the formula proposed by Quintanilla Carvajal *et al.* (2015).

Table 2 shows the parameters of the germination percentage (%), ESI, and GS, when the seeds were subjected to two different temperature and relative humidity conditions.

Table 2. Germination under different Relative Humidity (RH) and Temperature (T) treatments.

Variety	T1 RH 80%, T 20 °C			T2 RH 30%, T 40 °C		
	GP	ESI	GS	GP	ESI	GS
E-1	92.00 ab	11.04 ab	E-1	92.00 ab	11.04 ab	E-1
E-2	64.25 d	6.43 d	E-2	64.25 d	6.43 d	E-2
E-3	88.00 abc	10.56 bc	E-3	88.00 abc	10.56 bc	E-3
E-4	93.75 ab	10.31 bc	E-4	93.75 ab	10.31 bc	E-4
E-5	86.50 abc	6.92 d	E-5	86.50 abc	6.92 d	E-5
E-6	73.50 abc	5.88 e	E-6	73.50 abc	5.88 e	E-6
E-7	83.75 abc	9.21 c	E-7	83.75 abc	9.21 c	E-7
E-8	78.75 bc	6.30 d	E-8	78.75 bc	6.30 d	E-8
E-9	80.00 c	6.40 d	E-9	80.00 c	6.40 d	E-9
E-10	81.25 c	6.50 d	E-10	81.25 c	6.50 d	E-10
E-11	77.75 abc	13.22 a	E-11	77.75 abc	13.22 a	E-11
E-12	82.50 abc	7.43 c	E-12	82.50 abc	7.43 c	E-12
E-13	86.00 abc	7.74 c	E-13	86.00 abc	7.74 c	E-13
E-14	78.50 c	7.07 c	E-14	78.50 c	7.07 c	E-14
E-15	95.75 a	10.53 bc	E-15	95.75 a	10.53 bc	E-15
E-16	93.25 a	11.19 ab	E-16	93.25 a	11.19 ab	E-16
Average	**83.46**	**8.54**	**Average**	**83.46**	**8.54**	**Average**
DS	**8.27**	**2.29**	**DS**	**8.27**	**2.29**	**DS**
k75B	89.75 ab	9.87 c	k75B	89.75 ab	9.87 c	k75B
k93B	87.00 abc	9.28 c	k93B	87.00 abc	9.28 c	k93B
Average	**88.37**	**9.57**	**Average**	**88.37**	**9.57**	**Average**
DS	**1.94**	**0.41**	**DS**	**1.94**	**0.41**	**DS**

GP: germination percentage (%); ESI: emergence speed index; GS: germination speed (number of seeds/day). Means compared with Tukey-Kramer HSD. The same letters indicate that there is no significant difference (95%).

Tenorio-Galindo *et al.* (2008) highlight that bigger seeds have a greater possibility of survival and that they also increase the germination percentage and speed. However, both parameters depend on the chemical composition of the seed, the permeability of the husk, and the difference between the water potential and the thickness of the storage tissues. In this research, bigger seeds recorded greater GP and GS under T1, which provided greater relative humidity to the seeds than T2. There are few studies about castor bean seeds, as a result of their great phenotypic diversity.

According to Escobar-Álvarez *et al.* (2021), high relative humidity accelerates metabolism and, consequently, triggers germination. In their turn, these results are related with the findings of this research, since the emergence speed index (ESI) was higher in bigger seeds; therefore, a greater ESI entails a greater germination speed. These findings contrast with the results of Acevedo-Lara *et al.*, (2018), who determined that the ESI value is the result of the lower water volume required for the germination of smaller seeds (while bigger seeds require more water).

Table 3 shows that T1 had a positive correlation ($r>0.67^{**}$) regarding the GPT1, GST1, and ESIT1 variables. There was a positive correlation ($r>0.88^{**}$) regarding T2 between the A, FI, and W variables, and a negative correlation ($r>-0.50^{*}$) between EI and A.

On the one hand, seeds (E1-E16) under T1 (RH 80%, T 20 °C) recorded an 83.46%±8.27 germination percentage, an 8.54±2.29 ESI, and a 7.39±3.31 GS. On the other hand, when they were exposed to T2 (RH 30%, T 40 °C), the germination percentage was 52.34%±19.14, the ESI was 8.32±3.14, and the GS was 11.64±7.52.

Meanwhile, the germination percentage and ESI of commercial varieties (k75B and k93B) recorded similar values than other varieties (E1-E16), although their GS values were different (3.81±1.32). Under T2, the germination percentage (71.33±10.37), the ESI (13.10), and the ES (24.48±11.36) were higher than the same parameters for the E1-E16 varieties. These results prove that a greater index results in greater germination speed —an attribute that is related to morphometric dimensions—, since small seeds require less water, most of which is assimilated by the contact surface (Acevedo-Lara *et al.* 2018). Meanwhile, ESI was 77.02±23.78, while GS was 24.60.

Table 3 shows the results of the correlation analysis. There are positive correlations between the evaluated parameters (EI, area, germination percentage, FI, ESI, and GS), as well as a high correlation between area and germination percentage (0.678, $p<0.05$), area and FI (0.997, $p<0.05$), ESI and germination percentage (0.946, $p<0.05$), GS and ESI (0.892, $p<0.05$), GS and germination percentage (0.782, $p<0.05$), and ESI and area (0.851, $p<0.05$).

Table 3. Correlation between the evaluated parameters.

Variety	T1 RH 80%, T 20 °C	T1 RH 30%, T 40 °C	Variety	T1 RH 80%, T 20 °C	T1 RH 30%, T 40 °C	Variety	T1 RH 80%, T 20 °C
GPT1	**GP**	**ESI**		**GP**	**ESI**		**GP**
E-1	92.00 ab	11.04 ab	E-1	92.00 ab	11.04 ab	E-1	92.00 ab
E-2	64.25 d	6.43 d	E-2	64.25 d	6.43 d	E-2	64.25 d
E-3	88.00 abc	10.56 bc	E-3	88.00 abc	10.56 bc	E-3	88.00 abc
E-4	93.75 ab	10.31 bc	E-4	93.75 ab	10.31 bc	E-4	93.75 ab
E-5	86.50 abc	6.92 d	E-5	86.50 abc	6.92 d	E-5	86.50 abc
E-6	73.50 abc	5.88 e	E-6	73.50 abc	5.88 e	E-6	73.50 abc
E-7	83.75 abc	9.21 c	E-7	83.75 abc	9.21 cW	E-7FI	83.75 abc
E-8	78.75 bc	6.30 d	E-8	78.75 bc	6.30 d	E-8	78.75 bc
E-9	80.00 c	6.40 d	E-9	80.00 c	6.40 d	E-9	80.00 c
E-10	81.25 c	6.50 d	E-10	81.25 c	6.50 d	E-10	81.25 c
E-11	77.75 abc	13.22 a	E-11	77.75 abc	13.22 a	E-11	77.75 abc
E-12	82.50 abc	7.43 c	E-12	82.50 abc	7.43 c	E-12	82.50 abc
E-13	86.00 abc	7.74 c	E-13	86.00 abc	7.74 c	E-13	86.00 abc
E-14	78.50 c	7.07 c	E-14	78.50 c	7.07 c	E-14	78.50 c

FI=Feret's index; SI=sphericity index; GPT1=germination percentage (%) for T1; GPT2=germination percentage (%) for T2; W=weight of 100 seeds (g); ESIT1=emergence speed index for T1; ESIT2=emergence speed index for T2; GST2=germination speed for T2; A=area (cm^2).

This information contributes to the phenotypic characterization of the seeds. This process would enable the identification of desirable characteristics for seed improvement. Based on their handling, the resulting seeds can be useful for producers, as well as for the design of rolling, pressing, and milling equipment (Tönshoff *et al.*, 2002).

CONCLUSIONS

There were weight, size, and color variations between both the castor bean seeds varieties themselves and the commercial seeds. The proposed algorithm helped to determine the area, elongation index, and Feret's diameter dimensions, applying the image analysis with the adjustments required by the color diversity.

Although catalase can be considered a seed deterioration indicator, some seeds that have been stored for more than two years have managed to maintain an adequate germination percentage.

The relative humidity and temperature conditions may determine seed vigor, as a consequence of their potential for a quick and uniform emergence, producing a high number of normal seedlings (although they might not be optimal for the species). Seed vigor enables the identification of the desirable characteristics for seed improvement.

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