

## Descripción de semillas silvestres de *Euphorbia strigosa* Hook and Arn del estado de Nayarit, México

### Description of seeds of wild *Euphorbia strigosa* Hook & Arn from the state of Nayarit, México

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#### RESUMEN

En México, *Euphorbia strigosa* se ha identificado como una especie con potencial ornamental, por su coloración y su porte pequeño (35 cm, se considera una versión enana de *Euphorbia pulcherrima*). La dificultad en la conservación de *E. strigosa* se encuentra en la gran variabilidad de sus semillas en función de las condiciones ambientales y genotípicas donde la especie crece. Por lo tanto, el objetivo del presente estudio fue describir las semillas de *E. strigosa*, de plantas silvestres del Estado de Nayarit, México (2013-2014). Se midieron variables grupales de peso de 1000 y 100 semillas, así como el número de semillas en 1 g y en 10 mL. Además, una muestra de 130 semillas de cada año se les evaluó individualmente variables físicas. Las semillas se clasifican en función de tres grados de luminosidad del color café (claro, medio y oscuro) y cuatro rangos de peso (3,0-8,0; 8,1-13,0; 13,1-18,0 y > 18,1 mg). Basado en la clasificación de los factores: año de colecta, luminosidad del color café y el peso, se formaron 22 subgrupos. En cada subgrupo se evaluó el porcentaje de germinación. Se presentaron diferencias significativas en todas las características evaluadas, lo que indica la diversidad morfo-genética de las semillas colectadas.

**Palabras clave:** *Euphorbia strigosa*, caracterización, semilla, recursos fitogenéticos, silvestre, germinación.

#### ABSTRACT

In Mexico, *Euphorbia strigosa* has been identified as a species with ornamental potential, as a result of its color and small size (35 cm, is considered a dwarf version of *Euphorbia pulcherrima*). The difficulty in the conservation of *E. strigosa* is found in the great variability of its seeds depending on environmental and genotypic conditions where the species grows. Therefore, the objective of the present study was to describe the seeds of *E. strigosa* wild plants of the State of Nayarit, México (2013-2014). Group variables of 1000 and 100 seeds were measured, as well as the number of seeds in 1 g and in 10 mL. In addition, a sample of 130 seeds from each year were individually assessed for physical variables. The seeds were classified according to three degrees of luminosity of the brown color (Clear medium and dark) and four weight ranges (3.0-8.0; 8.1-13.0; 13.1-18.0 y > 18.1 mg). Based on the classification of factors: Year of collection, lightness of coffee color and weight, 22 subgroups were formed. In each subgroup the percentage of germination was evaluated. There were significant differences in all characteristics evaluated, which indicates the morphogenetic diversity of the seeds collected.

**Key words:** *Euphorbia strigosa*, characterization, seed, plant genetic resources, wild, germination.

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## INTRODUCTION

The family Euphorbiaceae has 300 genera and approximately 7,500 species, most of them herbaceous, although in the tropics they are also present as trees and shrubs. They are monoecious or dioecious, typically with latex; some are succulents that resemble cacti (Steibel 1995). Fruits are capsules that normally open explosively when they mature (Narbona et al. 2005). There are potentially three seeds per capsule with a wide variety of sizes, forms and characteristics of the seed and capsules surface, among species. Among Mexican native Euphorbiaceas, the most remarkable species are *Euphorbia pulcherrima*, known as "poinsettia" and *Euphorbia antisyphillia* Zucc, "candelilla" whose importance lies in the vegetable wax for making cosmetics and electronics industry insulation. In agricultural fields the stems of *Croton* sect. *Eluteria* are used as stakes (Steinmann 2002), while *Jathropha curcas* is being studied worldwide as a source of biodiesel (Huse et al. 2011). Other important non Mexican species are *Euphorbia tirucalli* L, which went from being a medicinal and ornamental plant to a biodiesel source (Kajikawa et al. 2005; Khaleghian et al. 2011), and *Ricinus communis* L, oleaginous plant now also being studied as a biodiesel source (Mazzani y Rodríguez 2009). In Mexico, few researchers study the Euphorbiaceae family in depth, although they are found from coast to coast from border to border and from sea level to 3500 meters above sea level (Cházaro y Mostul 1997).

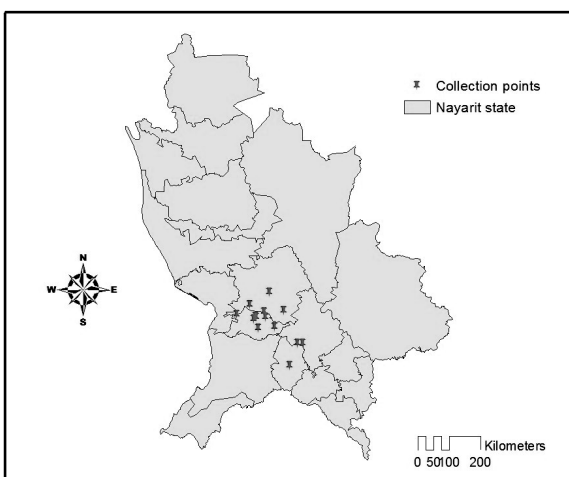
The wild species with ornamental potential, *Euphorbia strigosa* known as "flor de pasquilla" is considered a dwarf version of *E. pulcherrima* (Mostul y Cházaro 1996), because it has red or crimson bracts, in addition to having a size smaller than 35 cm and presents tuberous roots; their seeds are ellipsoid to ovoid, 3.6-3.7 mm and a thickness of 2.5 mm (Mayfield 1997). According to information obtained

from herbal materials of Mexico and the United States, this species is distributed in the States of Colima, Sinaloa, Sonora, Jalisco and especially in Nayarit. It has been reported that sexual and vegetative propagation is possible (Mostul y Cházaro 1996). However, agronomic characteristics of the wild material and the degree of morphological diversity in populations of *E. strigosa*, have not been specifically studied. Because of its wild origin and the explosive nature of the fruit it cannot be possible to select fruits with an advanced maturation level. The maturity of the seed and in a population, where plants are subject to different environmental conditions (temperature, water and nutrients), is expected to vary in weight (Milberg et al. 1996), which has implications for the dispersion, viability, germination, emergence, survival and competitive ability of seedlings (Harper et al. 1970; Aguilar-Carpio et al. 2015), which can eventually generate problems in obtaining plants for their cultivation, since the age of the seed may also present a highly variable germination response (Navarro et al. 2014).

Given the invasive nature of some species of the genus *Euphorbia*, the detection and identification of the seeds is important for agriculture: either for quarantine, prevent an ecological imbalance, the qualification of agronomic products, archaeological and archaeobotanical or productive studies (Pahlevani y Akhiani 2011; Araiza-Lizarde et al. 2016). For seed cultivation some basic parameters are considered (weight, moisture content, density and size), since they are closely related to the viability and vigor of the seedling (Poulsen 2000; Doria 2010). Therefore, the objective of the present study was to morphologically describe the seeds of *E. strigosa* from wild plants of the State of Nayarit, Mexico, and their germination evaluated, to obtain basic information to know whether the seed of the species behaves as orthodox, recalcitrant or intermediate, which could have repercussions on their management.

## MATERIALS AND METHODS

The seeds of *E. strigosa* were collected during 2013 and 2014 in 14 localities of the State of Nayarit, México (Figure 1). The seeds were weighed in separate groups of 100 and 1000 seeds on an analytical balance (Ohaus®, model PA232,  $\pm 0.001$  g); the number of seeds was quantified in 1 g and 10 mL.



**Figure 1** Municipalities in the State of Nayarit where seeds of *Euphorbia strigosa* were collected from wild plants during 2013 and 2014.

A 130 seed sample from each year were individually measured for length (L), width (W) and thickness (T) with a digital micrometer (Neiko®  $\pm 0.01$  mm). Geometric mean diameter (mm) (Dg) was calculated with the formula  $Dg = [(L \cdot W \cdot T)]^{1/3}$  and the arithmetic mean diameter (Da) (mm) with the formula  $Da = [(L + W + T)]/3$  (Mohsenin 1978). The surface area (S) (mm<sup>2</sup>) was determined with the equation  $S = (\pi \cdot W \cdot L^2) / (2 \cdot L - W)$  (Jain and Bal 1997) and sphericity ( $\phi$ ) dimensionless with  $\phi = Dg/L$  (Altuntas and Demirtola 2007). The qualitative variable color was determined using the color charts of the Royal Horticultural Society (RHSCC) by placing each seed under a stereo microscope where the main color (present in over 50% of the seed coat) and secondary (present in less than 50% of the seed coat) was determined.

Seeds from each year were divided into three grades of brown color brightness [(L) light, (M) medium and (D) dark] (Figure 2) and four weight ranges: T1) 3.0 - 8.0 mg, T2) 8.1 - 13.0 mg, T3) 13.1 - 18.0 g and T4)  $> 18.1$  mg; combination of these classifications (weight-color, collection-year) resulted in 22 subgroups; since no T4C13 (size four-light-2013) with T4O14 (size four-dark-2014) combinations occurred. From each subgroup a 30 seeds sample was taken except for the groups T3C13, T4M13 and T1O13 in which were used 20, 8 and 16 seeds respectively. The seeds were placed in germination trays with a mixture of perlite and peat (2:1), at an average temperature of 33 °C, with a photoperiod of 16:8 (light-dark), for 20 days. A seeds were considered germinated and emerged when cotyledons were observed on the substrate surface outside the seminal envelope as a result of elongation and hypocotyl erection (Laskowsky y Bautista, 2002).

The experiment treatments were distributed using a complete randomized design.



**Figure 2.** Classification of the seed coat of the seeds of *Euphorbia strigosa* based on the brightness of color brown; dark (a), medium (b) and light (c).

To evaluate weight of 100 seeds eight replicates were measured, weight of 1000 seeds three replicates, number of seeds in 1g and 10 mL four replicates respectively. For weight, length, width, thickness, arithmetic diameter, geometric diameter, surface area, sphericity and color, 130 experimental units were used, (one experimental unit consisted of a seed). For all quantitative variables an analysis of variance (ANOVA) and Tukey ( $p < 0.05$ ) was used with Statistical Analysis System (SAS) version 9.0.

The data of germination percentage were transformed with the arcsin formula  $\sqrt{x_2}$ ; which subsequently was applied an ANOVA in which the treatments were considered independently with the GLM procedure and Tukey ( $p < 0.05$ ). A second ANOVA was applied to statistically detect significant differences on germination due to seed size, color (brightness) or collection year.

## RESULTS AND DISCUSSION

Significant differences were found ( $p < 0.05$ ) in three groups of the seed characteristics: weight of 1000 seeds ranged from 8.97 to 12.95 g, the lowest weight (9.3 g) was recorded in seeds from 2013. Weight of 100 seeds varied from 0.89 to 1.23 g; seeds from 2013 recorded 1.0 g average while those collected in 2014 scored 1.2 g. The number of seeds in 10 mL ranged from 319 to 422 seeds, with seeds from 2013 averaged 415 and those of 2014, 327 seeds. No significant differences were found for 1 g number of seeds variable ranging between 87 and 112 seeds with an average of 103 seeds collected in 2013 and 95 seeds collected in 2014 (Table 1).

**Table 1.** Characteristics of *Euphorbia strigosa* seeds collected in 2013 and 2014 in 14 locations in Nayarit, México.

Variation factor	Min	Min	2013 mean	2013 mean
Weight of 1000 seeds (g)	2.67	12.95	9.3 b	12.2 a
Weight of 100 seeds (g)	0.89	1.23	1.0 b	1.2 a
Number of seeds in 10 mL	319.00	422.00	415.0 a	327.0 b
Number of seeds in 1 g	87.00	112.00	103.0 a	95.0 a
Weight (mg)	2.67	25.10	10.9 b	13.0 a
Length (mm)	3.30	4.80	3.8 b	4.0 a
Width (mm)	3.18	3.84	2.8 b	3.1 a
Thickness (mm)	2.42	3.86	2.7 b	3.0 a
Geometric diameter (mm)	2.27	3.87	3.1 b	3.3 a
Arithmetic diameter (mm)	2.74	3.84	3.1 b	3.4 a
Surface area (mm <sup>2</sup> )	2.68	45.15	26.3 b	31.2 a
Sphericity (dimensionless)	19.74	0.97	0.8 a	0.73 a

Significant differences were found for all quantitative variables except for sphericity (Table 1). Seed weight ranged from 3.3 to 25.1 mg with about 60 % of the seeds between 9 and 13 mg, these weights were found in the seeds of both years, a correlation (0.05) was found between weight and variables: Length (0.45),

width (0.62), depth (0.64), arithmetic diameter (0.62), geometric diameter (0.63), surface area (0.62) and sphericity (0.28). The length of the seed varied between 3.19 mm and 4.80 mm, no correlation was found between years, although a correlation between the width and the thickness was detected (0.6); the width ranged from 2.42 to 3.84 mm, the thickness from 2.27 to 3.87 mm, the geometric diameter from 2.74 to 3.87 mm, the arithmetic diameter from 2.68 to 3.84 mm and the surface area from 19.74 to 45.15 mm<sup>2</sup>. In terms of sphericity, which ranged from 0.73 to 0.97, no differences were found, confirming the ellipsoid-ovoid shape of the seeds. For the variables weight, width, depth, arithmetic diameter, geometric diameter and surface area, significant differences were found between the two years, with seeds from 2013 scoring lower values than those from 2014.

Of the 47 colors identified, including 12 color ranging from white to black (155-NN155ABCD to 202-203 ABCD) (Table 3), the most frequent were in the orange-gray range (164-177 ABCD) with 15 tones, followed by the white (164-177 ABCD) with seven tones. White tones were more frequently present in the main color in the seeds from both years; however, brown tones (200 ABCD) were identified in 32% seeds from 2013, and 39% of seeds from 2014.

In both years the most frequent main color was identified as NN155A and was presented in 34% of the seeds collected in 2013 and 33% in 2014. The least frequent main color (less than 1%) was identified as 155A (0.70%) in seeds from 2013 and for 2014 those identified as 155B, 155D, 156C, 157B, 164A, 164B, N167A, 177A, N187C, N200B, 201A and 202B (0.30% each). The most frequent secondary color was N200A (33%) in 2013 and NN155A (32%) in 2014. The lowest incidence (less than 1%) in 2013 was 156A, 158D, 165C and N200B (0.35%) and in 2014 with NN155B, 158C, 158D, 159B, 164B, 164C, 165C, 166A, 166B, 166C, 177A, 177C, 177D, 198A, 200B, 202B and 203C (0.31%

each). In seeds from 2013, 43 combination colors were present (NN155A-N200A white-coffee presented the highest frequency, 15%) while in seeds from 2014 102 combinations were present (165A-NN155A Greyed-orange to white had the highest frequency, 15%).

Of these colors were presented 43 combinations in the year 2013, of which NN155A-N200A (white-coffee) presented the highest frequency (15%) and in 2014 102 combinations were presented, of which 165A-NN155A (Greyed-orange to white) had the highest frequency (15%).

Significant differences in seed germination were found among the 22 subgroups, subgroups of seeds from 2014 except T1O14 and T2C14 had > 50% germination, 7% more than the highest percentage of germination registered seeds from 2013. The factorial analysis of germination data (Table 2) indicated that there are significant differences

in the germination of both years, in 2014 the superior presented an average of 47.3 and in 2013 presented an average of 25.8. Regarding the size of the seeds according to the mean test the highest germination was recorded in T3 (13.1 mg-18.0 mg) and T4 (> de 18.0 mg) with an average of 54 y 51.9 respectively, while the lowest was presented in T1 (3.0 -8.0 mg) with an average of 6.8. In seed color difference was observed only when using statistical error type III.

As for interaction with significant differences (size-year), the means test indicates that the interactions statistically are those of T3 and T4 of the year 2014, with an average germination of 74.2 and 68.2, while the lower ones are the T1 of both years, with an average germination of less than 10% (Table 3).

It was demonstrated that there is great diversity in *E. strigosa* the seeds collected in the State

**Table 2.** Color ranges and frequency (%) present in seeds of *Euphorbia strigosa* collected in 2013 and 2014 in 14 localities of Nayarit, México.

Color ranges	Code RHSCC	Colors identified	2013		2014	
			Primary (%)	Secondary (%)	Primary (%)	Secondary (%)
White	155-NN155ABCD	7	36	12	42	39
Greyed-white	156 ABCD	4	0	0	8	15
Green-white	157 ABCD	1	0	0	0	0
Yellow-white	158 ABCD	4	19	26	8	4
Orange-white	159 ABCD	2	0	0	0	0
Greyed-orange	164-177 ABCD	15	20	22	25	17
Grayed-purple	N187 ABCD	1	0	0	0	0
Grayed-green	197-198 ABCD	2	0	0	0	1
Gray-brown	199 ABCD	1	0	0	0	0
Brown	200 ABCD	4	22	32	10	15
Gray	201 ABCD	1	0	0	0	1
Black	202-203 ABCD	5	4	8	6	6
<b>Total number of colors</b>		47	17	15	32	45
<b>Total number of combinations</b>			<b>43</b>		<b>102</b>	

**Table 3.** Results to detect the size influence, color and year of collection on germination of seeds of *Euphorbia strigosa* collected in Nayarit, during 2013 and 2014.

Variation factor: media					
Weight	T1:	6.8 c	T2:	50.20 b	T3: 54.3 a T4: 51.9 ab
Color+	Light:	30.4 b	Medium:	40.38 a	Dark: 39.9 ab
Year	2013:	25.8 b	2014:	47.3 a	
+ error type III (Different superscript within a row are significantly different according to Tukey's test p<0.05)					
Interaction Weight-year					
	<b>T1</b>	<b>T2</b>	<b>T3</b>	<b>T4</b>	
<b>2013</b>	6.6 d	33.7 c	31.9 c	32.5 c	
<b>2014</b>	7.0 d	46.6 bc	74.2 a	68.2 ab	

of Nayarit, Mexico, during years 2013 and 2014, which may be due to their geographical origin. At each collection site, the plant populations established there are subject to different environmental conditions, such as temperature, moisture and nutrients which can generate difference even in the contents of the chemical composition of the seed (Ayerza 2010; Franco-Mora et al. 2015) (Milberg et al. 1996; Hernández-Verdugo et al. 2012). In addition, it has been documented that wild plants produce variable seed size to ensure prevalence in the habitat as well as dispersion (Baiges et al. 1991). In addition to the explosive feature of the fruits of the Euphorbias, some types of ants present preferences for plants of this family (Narbona et al. 2005; Beaumont et al. 2009; Webster 2013) (Leal et al. 2007; Beaumont et al. 2009; Lôbo et al. 2011). The ants subject the seeds to different conditions (under ground, humidity or variable temperatures or can cause physical damages) that sometimes can favor the germination and formation of seed banks (Martins et al. 2006). All these mechanisms are necessary to ensure the continuity of a plant species within the ecosystem; since ensuring a high dispersion also helps to avoid competition with their parents and to find different levels in seed banks (Beaumont et al. 2009). Another mechanism in the case of *E. strigosa* is the production of tuberous roots.

Size variability may allow coexistence through a competition-colonization, since large seeds may have a low percentage of germination and high tolerance to stress, whereas small seeds have a higher percentage of germination and a lower tolerance to stress (Muller-Landau 2010). It has been found that the size of Arabidopsis seeds may be genetically delimited by cytochrome P450 KLUH (KLU)/CYP78A5, which acts as a stimulator of organ growth by the expression patterns of specific genes or crosstalk between maternal inheritance and zygotic controls that regulate in coordinated seed size (Luo et al. 2005) (García et al. 2005) (Anastasiou 2007), by the expression patterns of specific genes

(Luo et al. 2005) or crosstalk between maternal inheritance and zygotic controls that regulate in coordinated seed size (García et al. 2005). It is suggested that integument growth limits final seed size (Adamski et al. 2009). On the other hand, there is the hypothesis that seed size evolves as part of a spectrum of life history traits, including plant size, plant longevity, juvenile survival rate and time to reproduction (Moles y Westoby 2006). Or that gene complexes seek adaptation to the environment (Xu et al. 2016). It is also thought that a reduction in the number of fruits causes an increase in the mass of individual seed (Jofuku et al. 2005).

When finding the correlation between seed weight and variables such as length, width, thickness, geometric diameter, arithmetic diameter and surface area of *E. strigosa*, volume and dimension could be used for subsequent studies for the application of some models as predictors of weight (Sánchez et al. 2002). On the other hand, the values of length and width of the seeds of *E. strigosa* are greater compared to the 35 Euphorbias studied by Pahlevani y Akhani (2011), where the nearest in long and wide seed to *E. strigosa* are: *E. turkestanica* (Length 3.51- 3.42 mm and width 2.84- 2.61 mm) and *E. turczaninowii* (Length 3.03-2.20 mm and width 1.83-1.12 mm), it should be noted that in all species in this study (35 species) only in *E. sheirolepsis* (Std 0.33 long and 1.40 wide) such a wide variability was observed as in the case of *E. strigosa* (Std 0.31 long and 0.26 wide). When comparing the results of *E. strigosa* with the work of Salmaki et al. (2011) with 85 species of *Euphorbia* are similarities with the seed dimensions of other 15 species.

In the case of seed color, its variation may be due to the fact that it is an adaptive strategy to produce seeds that can germinate over a wider range of environmental conditions (Tenorio-Galindo et al. 2008). It is also suggested that the color of the seed varies according to the type of soil in which it grows as a strategy of local

camouflage, probably driven by the predators of these seeds which gives rise to selection and adaptive divergence in pigmentation (Porter 2013). It has also been found a relation of the color of the seed, with the color of the flower and the resistance to some pathogens such as *Xanthomonas campestris* pv *phaseolis* in *Phaseolus vulgaris* (Duncan et al. 2007). In the case of wheat, the color of the seed affects the yield and quality of the flour (Kuraparthy et al. 2008); in *Brassica rapa* the yellow seeds have a thinner covering which also contributes to 5-7% more oil and flour as well as a greater amount of protein (Rahman et al. 2007). In the black lines of *Phaseolus vulgaris* the protein content, iron and zinc are higher, in lines of other colors are richer in calcium, while the "carioca" is high in magnesium and manganese (Silva et al. 2012). For the case of sesame, color is an important aspect as it is associated with biochemical properties, antioxidants and resistance to diseases (Zhang et al. 2013). In *Brassica juncea* the yellow color was found in the genotype of the mother, and brown the father, the latter being the dominant (Xu et al. 2010). However, for other species such as *Salvia hispanica*, no difference was found in seed composition associated with color (Ayerza 2013). In the morphological study developed by Pahlevani and Akhiani (2011) with 35 species of Euphorbias, a single color was identified for each species, while for the present study, 12 general shades were found, including 45 colors.

As for the results of the percentage of germination, it is possible that the seed of *E. strigosa* is of the orthodox type like other Euphorbias (Guzmán-Pozos and Cruz-Cruz 2014), since although the percentage of germination decreased over time, it was still viable in the storage conditions (room temperature) to which was submitted and under the established conditions of germination.

It was found that heavier seeds had a higher percentage of germination, which coincides with results reported by Lobos et al. (2008) in *Lupinus* and Baraloto et al. (2005) in forest species,

since they indicated that the size of the seed influences the growth of the seedling and the density of planting. It is possible that large seeds have a slower growth rate and greater longevity of the plant, whereas small seeds germinate faster, but they die prematurely (Baraloto et al. 2005; Moles et al. 2007). Another explanation might be that smaller wild seeds accumulate lower amounts of biomass in the hypocotyl as observed by Celis-Velazquez et al. (2010), in *Phaseolus vulgaris* L; hence, the low germination percentage of the small seed (3.0-8.0 mg) in both years for *E. strigosa* was less than 10%.

The influence of coat color was detected on germination, similar to the one reported in a study with *Brassica napus*, where it was established that there is a relation between the color of the seed, the content of melanin in the coat and the tolerance to the floods, since the red and black colors presented greater content of melanin and slow absorption of water, whereas the yellow ones to absorb the water more quickly resulting in a lower tolerance to flooding (Zhang et al. 2008). Van Mólken et al. (2005), also found no relationship between seed color and germination in their work with *Tragopogon pratensis*, but they agree that color can be an adaptation to escape predation.

## CONCLUSIONS

There were significant differences in all characteristics evaluated, which indicates the morphogenetic diversity of the seeds collected. Some attributes are associated with modifications acquired to guarantee the survival of the species in the wild, which may be useful for future studies of genetic improvement and conservation of the species.

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