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Pollen morphology of four species of *Jatropha* (Euphorbiaceae), including toxic varieties, in Northwestern Mexico

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Abstract: *Jatropha curcas* is a native Mexican plant, distributed in the forests of coastal regions; it has shown potential for the production of biofuel, and its raw protein can be used as animal feed. Nevertheless, its current varieties have low yield and production variability, as it is in domestication period. The knowledge of the pollen grains is fundamental for studies of *Jatropha* reproductive biology and breeding for genetic improvement to generate new hybrids and improve fruit and seed yield. This study compared pollen morphology of four *Jatropha* species, which include two varieties of *J. curcas* (one toxic from India and one non-toxic from Mexico), and two varieties of wild *J. cinerea* (Sinaloa and Baja California Sur); additionally, pollen was collected from wild *J. platyphylla* (Sinaloa) and *J. vernicosa* (Baja California Sur) to characterize them palynologically. We used a scanning electron microscope to describe the size and shape of pollen grains. Pollen grains were observed spheroidal. The diameter of the varieties of toxic and non-toxic *J. curcas* and that of *J. platyphylla* were 58 ± 2.3 , 54 ± 1.7 , and 51 ± 2.4 μm , respectively; the number of clavae was 84 ± 10 , 108 ± 15 , and 180 ± 15.5 , respectively. Pollen grains of *J. cinerea* (Sinaloa) had a diameter of 50 ± 1.7 μm and 220 ± 14 clavae by area; *J. cinerea* (Baja California Sur) had a diameter of 45 ± 1.9 μm and 195 ± 14.7 clavae. Pollen grains of *J. vernicosa* had a diameter of 46 ± 2.1 μm and 231 ± 25.6 clavae. The ornamentation of the exine showed clava with capita gemma shaped for varieties of *J. curcas* species and clavum shaped for *J. platyphylla*, *J. cinerea* and *J. vernicosa* species. The studied varieties have a cross-linked endexine and ectexine intactate. Luminal bacules were observed in the reticular space of *J. curcas* varieties while none were present in the varieties of *J. cinerea*, *J. platyphylla*, and *J. vernicosa*. Rev. Biol. Trop. 65 (2): 799-806. Epub 2017 June 01.

Key words: Palynology, *J. curcas* (toxic and non-toxic var.), *J. cinerea*, *J. platyphylla*, *J. vernicosa*, endemic plant.

The name *Jatropha* (Euphorbiaceae) is derived from the Greek words “*iätros*” meaning physician or doctor and “*trophe*” nutrition or food (Heller, 1996), which is indicative of its potency and widespread use as a medicinal herb during ancient times (Sunil, Kuma, & Varaprasad, 2013). Recently *J. curcas* is considered a source for biodiesel (Bahadur, Murthy, & Sujatha, 2013) and flour production,

with a great potential for animal feed (Escoto-González, Flores-Ceballos, & Maytorena-García, 2013). The genus *Jatropha* contains approximately 170 to 175 known species (Dehgan & Webster, 1979; Heller, 1996), including *J. curcas*, *J. cinerea*, *J. platyphylla*, and *J. vernicosa*.

Jatropha curcas (L) is considered as the most primitive species in the genus (Wilbur,

1954; McVaugh, 1945). The origin of this species has long been debated, but it has been agreed that it is native to Mexico and Continental America (Achten et al., 2010). A non-toxic *J. curcas* variety has been found only in Mexico (Jongschaap, Corré, Bindraban, & Brandenburg, 2007) in the forests of coastal regions. The plants are monoecious with masculine and feminine flowers in the same inflorescence (Chang-wei, Kun, You, & Yongyu, 2007). Normally, inflorescences produce a central female flower surrounded by a group of male flowers, which normally include 1-5 female flowers and 25-93 male flowers (Raju & Bahadur, 2013). Masculine flowers have ten stamens arranged in two tiers of five each. The lower tier is free while the upper tier is united at the base, with yellow anthers. The anthers dehisce by longitudinal slits one hr after flower opening. The pollen grains are yellow, from spheroidal to globular, and measure from 81 to 89 μm (Rianti, Suryobroto, & Atmowidi, 2010).

Erdtman (1952) was the first one to study *Jatropha* pollen, proposing the descriptive term “crotonoid” to characterize the type of sculptural pattern of the exine found in some members of the family Euphorbiaceae, especially in the subfamily Crotonoideae. Bahadur et al., (2000, 2013) not only described the general aspect of pollen grains of *Jatropha* species found in India, but also from outside India, including *J. curcas*. Our study discusses a comprehensive analysis update of light microscopy (LM) and scanning electron microscopy (SEM) studies of the pollen of all *Jatropha* species to date, including *J. cinerea*.

Jatropha cinerea [(C.G. Ortega) Muell.-Arg] is a drought resistant shrub 1-3 m high distributed from Arizona to the coasts of Sinaloa in Mexico. The species has mainly monoecious plants but also genotypes with dioecious plants are found, a non-typical behavior in some disturbed areas. Heart-shaped leaves are 5 cm diameter with palmate venation; staminate flowers show 2 or 3 individual flowers in the apical bud (Dehgan & Webster, 1979), and the pollen grains are spheroidal of

58 μm in diameter inaperturate (Bahadur et al., 2013). The seeds are small, brown shell, and pearl white kernel. Sosa-Segura et al., (2014) mentioned that *J. cinerea* oils have a fatty acid profile similar to those of *J. curcas* and *J. platyphylla*.

Jatropha platyphylla (Muell.-Arg) is a drought-resistant dioecious perennial tree 2-5 m high found from Sinaloa to Michoacán, México (Sosa-Segura et al., 2014); native *J. platyphylla* from Sinaloa contains 60 % kernel oil, rich in oleic (23 %) and linoleic acids (54 %) (Makkar et al., 2011).

Jatropha vernicosa (Brandege) is a monoecious shrub 2-3 m tall with simple leaves and bright pedicellate. Flowering occurs in August and September; after the summer rains, it develops white flower inflorescence. The fruit is a capsule with three seeds. It is found in Baja California Sur, only in mountain areas of the Sierra de la Laguna and the Sierra de la Giganta. Latex and bark are used to relieve tooth pain and heal wounds (León de la Luz, Domínguez-Cadena, Domínguez-León, & Coria-Benet, 2014).

Jatropha species have cross-pollination that allows a high degree of genetic variation, offering a wide array of possibilities to select the desired characteristics in cultivations (Salinas, Armijos, Jiménez, & Proaño, 2011). Saenz de Rivas (1976) mentioned that pollen sculpture is an important taxonomic characteristic because it is easy to observe, and it is constant within the same taxon. However, some of these *Jatropha* species have not been described palynologically, thus accurate species diagnosis and classification are necessary.

This work describes the pollen morphology of one toxic *J. curcas* species from India, its non-toxic variety from Mexican Sinaloa state, and three natives species of México (*J. cinerea*, *J. platyphylla* and *J. vernicosa*) using the scanning electron microscopy technique, to compare surface shape and exine texture of pollen grains, whose palynodata has not been provided up to date by other researchers of *Jatropha* plants in Mexico.

MATERIALS AND METHODS

Study site: Pollen from the toxic variety of *J. curcas* from India (IND) BIOD1 was collected from an experimental cultivation field of Foundation Produce Sinaloa, (25°49'25.7" N - 108°16'08.0" W, 85 masl). Pollen from the non-toxic *J. curcas* variety from Sinaloa (SIN), México was collected from an experimental cultivation field (23°46'35.4" N - 106°46'48.3" W, 42 masl). Pollen collected from the native species *J. cinerea* (24°44'43.9" N - 108°01'26.6" W, 5 masl), and *J. platyphylla* (23°31'52.7" N - 106°30'12.1" W, 48 masl), were from wild plants variety Sinaloa (SIN). Finally, pollen was collected from wild *J. cinerea* (23°45'06.2" N - 110°07'03.5" W, 452 masl) and *J. vernicosa* (23°42'04.1" N - 110°00'56.2" W, 601 masl), plant variety Baja California Sur (BCS), México.

For the study, four plants of each variety were selected, and five flowers from each one of them were obtained for pollen samples in September 2015; the flower samples were mixed and placed in Eppendorf tubes with

2 ml of 10 % glutaraldehyde in duplicate. In December 2015, the samples were dehydrated gradually with ethanol at 20, 40, 60, 80, and 100 % for 30 min each one of them (Erdtman, 1952) and placed in a critical drying chamber (Samdri-PVT-3D®, MD, USA), where alcohol was substituted by CO₂ to maintain pollen shape. The samples were placed in aluminum sheets coated with gold (Denton Vacuum Desk II®, SC, USA). Observation was performed in the Scanning Electron Microscope at a distance of 15 mm and a voltage of 15.0 kV (Hitachi S-30000N).

Pollen grains characterization was performed according to a glossary of palynological terms (Sáenz-Laiín, 2004). The number of bacula was counted from 60 pollen grains in a scale of 900 µm² where the counting area was the center of the pollen grains and subdivided in 10 µm² squares. Pollen counting was performed by processing the micrographs in the program Paint and dividing it in nine squares (Fig. 1). The statistical test ANOVA was performed to compare the length of the equatorial

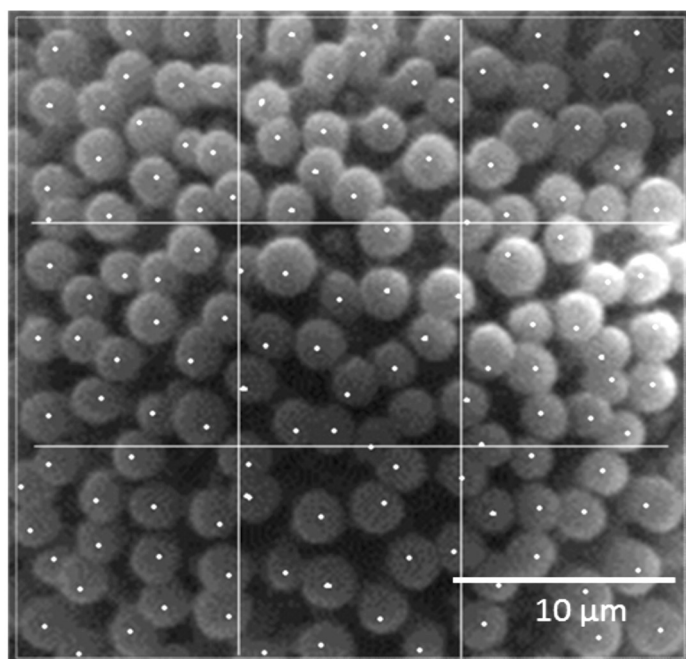


Fig. 1. Pollen micrograph of *Jatropa curcas* count number of clavae per 900 µm².

axis of 60 pollen grains and the number of bacula of the species studied.

RESULTS

All studied varieties (India = IND, Sinaloa = SIN and Baja California Sur = BCS) pollen grains are inaperturate and show crotonoid sculpture; a croton sculpture refers to the presence of polygonal elements (Fig. 1). The variance analysis for clavae count by variety showed a significant difference ($P \geq 0.05$) between the species of *Jatropha* genus; however, between the species of *J. cinerea* and *J. vernicosa* no significant differences were observed (Table 1).

The micrographs obtained from the varieties of India (IND) (toxic *J. curcas*) Sinaloa (SIN) (non-toxic *J. curcas* and *J. platyphylla*) showed that pollen grains had a spheroidal shape and measured in average 58 ± 2.3 , 54 ± 1.7 and $51 \pm 2.4 \mu\text{m}$ in diameter, respectively (Table 1). The pollen grain sculpture, ornamentation or relief was formed by sculptural elements called clava in gemma shape. Clava measured $2 \mu\text{m}$ in height and $2 \mu\text{m}$ in their widest or capita part with 10 to 12 vertical striations slightly visible (Fig. 2), except for the variety *J. platyphylla*, which showed notably that clavae were distributed in pentagon or hexagon manner forming a net. Within this geometrical structure, elements smaller than $1 \mu\text{m}$ called luminal bacules were found only in toxic and non-toxic *J. curcas*.

The pollen grains of toxic *J. curcas* shared a similar shape to those of non-toxic germ plasma and measured $58 \pm 2.3 \mu\text{m}$ in average without visible apertures. Endexine was in reticulated shape, and ectexine was intectate. Clavae were in gemma shape with $2 \mu\text{m}$ in height and $2 \mu\text{m}$ in capita. They showed from 10 to 12 well differentiated striations, different from the clavae of non-toxic Sinaloa variety (Fig. 3).

Clavae distribution was in similar shape to those in non-toxic varieties (pentagonal or hexagonal). The bacules among the clavae were in a size less than $1 \mu\text{m}$, and they were found

TABLE 1
Clavae count and comparative pollen study of *Jatropha*, two cultivated and four wild varieties

Species	<i>J. curcas</i> (toxic)		<i>J. curcas</i> (Non-toxic)		<i>J. platyphylla</i>	<i>J. cinerea</i>	<i>J. cinerea</i>	<i>J. vernicosa</i>
Morphological characteristics	Cultivated (IND)*		Cultivated (SIN)**		Wild (SIN)**	Wild (SIN)**	Wild (BCS)***	Wild (BCS)***
Clavae Number	84±10 ^a		108±15 ^b		180±15.5 ^c	220±14 ^d	195±14.7 ^{cd}	231±25.6 ^d
Shape					Spheroidal			
Diameter (µm)	58±2.3 ^c		54±1.7 ^d		51±2.4 ^b	50±1.7 ^b	45±1.9 ^a	46±2.1 ^a
Endexine					Reticulated			
Ectexine					Intectate (no tectum)			
Clava	In Gemma of 2 x 2 µm				In Clavum 2 x 1 µm	In Clavum 2 x 1 µm	In Clavum 2 x 1 µm	In Clavum 2 x 1 µm
Striation	Visible		Slightly visible		Slightly visible	Slightly visible	Non visible	Non visible
Bacules	Present		Present		Not present	Not present	Not present	Not present

*IND = India, **SIN = Sinaloa, ***BCS = Baja California Sur.



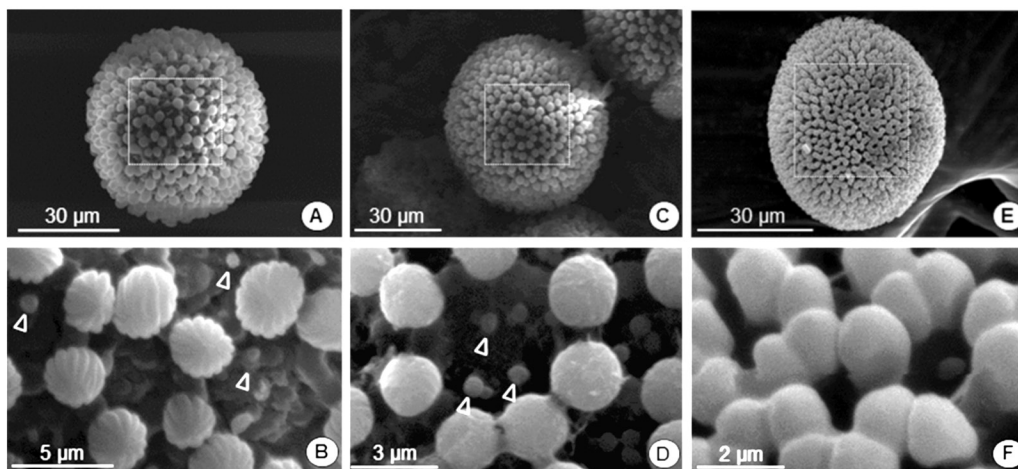


Fig. 2. Pollen micrographies of toxic *Jatropha curcas* variety India: A, B; of non-toxic *Jatropha curcas*: C, D; and *Jatropha platyphylla*: E, F, varieties of Sinaloa, México; verrucae: Δ.

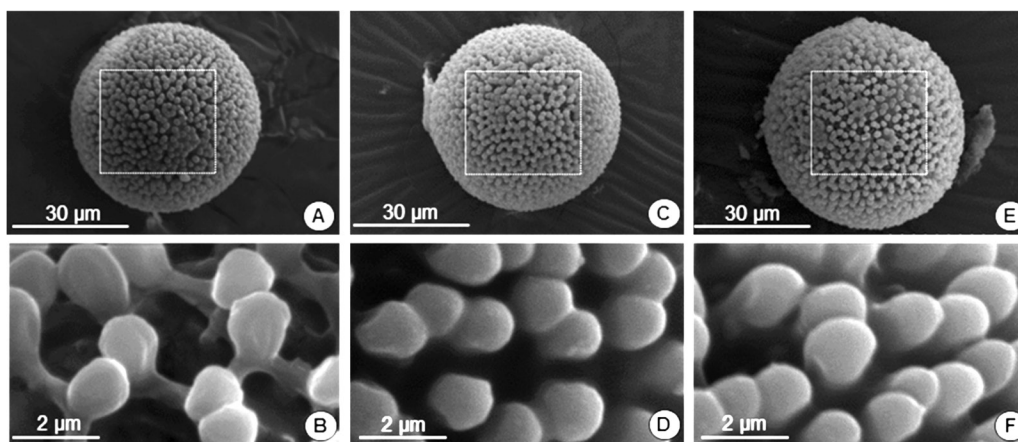


Fig. 3. Pollen micrographies of *Jatropha cinerea* variety Sinaloa: A, B; *Jatropha cinerea*: C, D; and *Jatropha vernicosa*: E, F, varieties of Baja California Sur.

in fewer amounts when compared with the non-toxic varieties of *J. curcas*.

Pollen of *J. cinerea* measured 50 µm in diameter. Its pollen grains were smaller than those of *J. curcas* toxic and non-toxic varieties. They showed a higher number of clavae in the surface, 220 ± 14 in average per 900 µm² (Table 1), and no apertures were observed. Endexine was reticulated, and ectexine was intectate. Clava measured 2 µm in height and 1 µm capita in width, showing a clavum shape

with six striations slightly visible (Fig. 3). Clavae were distributed in groups of five, six, and seven, inserted on the reticula and not showing bacules among them.

A comparative study was performed to concentrate information on pollen differences and similarities of the toxic and non-toxic *J. curcas* varieties and those of the wild *J. cinerea*, *J. platyphylla*, and *J. vernicosa* species (Table 1). Pollen grains of the four species showed a great similarity in shape without

apertures, characters, and ornamentation. The pollen grains differed only in minor points between species. *J. cinerea*, *J. platyphylla*, and *J. vernicosa* showed a great similarity in shape and size, and the exine characters were very much alike. The most distinctive differences were found in toxic and non-toxic *J. curcas*. Differences were found among *Jatropha* varieties as to pollen shape and clava dimensions.

DISCUSSION

Bahadur et al., (2000, 2013) reported that *J. curcas* pollen was spheroidal with 61.6 µm in diameter, inaperturate, highly sculptured with clavae aligned reticulately (hexa or polygonal) with a crotonoid process. The clava or stack heads were more or less rounded with vertical striation. *J. cinerea* pollen was spheroidal with 58.0 µm in diameter without apertures, highly sculptured with reticular clavae or stack alignment (penta or hexagonal) with crotonoid pattern. The clavae heads were triangular or polygonal vertical striation; no images were provided though.

No germination apertures were observed. According to Saenz de Rivas (1976), exine is composed by endexine, which is an internal reticulated layer, and by an external gemmate layer called ectexine that shows a striate without tectum. Bahadur et al., (2013) mentioned that *J. curcas* varieties from India were related with *J. curcas* from Africa and some endemic varieties from Mexico.

Dehgan (1984) mentioned hybrid development among *J. curcas* genotypes as parental female and *J. cinerea* as parental masculine. This fertilization could be due to the fact that the pollen grain is smaller than that of *J. curcas* and could be easily trapped by the style of the feminine flower and germinate. Successful hybridization between members of different sub-sections indicates that the various taxa in *Jatropha* are phylogenetically related (Sujatha, Bahadur, & Reddy, 2013).

Pollination experiments indicated that *J. curcas* was capable of both self and cross-pollination. Observation has shown that a single

female stigma of a *J. curcas* was usually provided with two-four lobes, and the stigma surface was provided with many convexes and concaves, which helped to adhere and embed pollen. In general, a plant will choose pollen from another plant to complete fertilization (Wang & Ding, 2012). The pollen-stigma interactions in *Jatropha* crosses are a clear indication of an active pollen-stigma recognition-rejection phenomenon. In crosses with *J. curcas* as female, pollen grains of *J. podagrica*, *J. hastata* and *J. multifida* failed to germinate. These crosses showed strong cross-incompatibility and pollen germination was confined to the stigmatic surface which was arrested with coiled or spatulate pollen tips. Other motives were interspecific gene transfer is generally limited by crossability barriers, ploidy differences, and genetic distance of taxa (Sujatha et al., 2013).

J. curcas and its allied taxa grow in tropical mesic forest regions whereas the taxa with reduced vascular bundles are most advanced and occur in dry, warm deserts (Dehgan & Webster, 1979). Interestingly, the monoecious *J. curcas* crosses readily with the dioecious species *J. cinerea* (Sujatha et al., 2013), *J. platyphylla*, and *J. vernicosa*. Most of the *Jatropha* species, with the exception of *J. curcas*, produce flowering flushes continuously throughout the year, whose attribute can be transferred to *J. curcas* to enhance its seed/oil yield potential (Sujatha et al., 2013). Also, *J. platyphylla* with its larger seed size (two–three folds higher than *J. curcas*) and high oil content (60 % on kernel basis) could be exploited in an interspecific hybridization program for improved seed traits (Makkar et al., 2011).

This work reports the first palynological descriptions of four species of *Jatropha* in Northwestern Mexico, non-toxic *J. curcas*, *J. cinerea*, *J. platyphylla*, and *J. vernicosa*. The similarities found in the morphological features in the species pollen phylogenetically matched those assigned to the genus. According to the palynological morphological characteristics, exine shape, size, and ornamentation are elements that make the taxonomic identification

of the genus *Jatropha* easier among species. Knowledge about the degree of genetic diversity among and within natural populations is a prerequisite to find the genetic material necessary to breed high yielding cultivars.

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RESUMEN

Morfología del polen de cuatro especies de *Jatropha* (Euphorbiaceae), incluyendo variedades tóxicas, en el noroeste de México. *Jatropha curcas* es nativa de México, y se distribuye en los bosques de las regiones costeras; las plantas han mostrado potencial para la producción de biodiesel, y la proteína cruda puede utilizarse para alimentación animal. Sin embargo, sus variedades actuales tienen bajo rendimiento y variabilidad en la producción, por estar en periodo de domesticación. El conocimiento de los granos de polen es fundamental para el estudio de la biología reproductiva de *Jatropha* y mejoramiento genético para generar nuevos híbridos y mejorar el rendimiento de frutos y semillas. Este estudio compara la morfología del polen de cuatro especies de *Jatropha*, que incluyen dos variedades de *J. curcas*, (una tóxica de India y una no tóxica de México) y dos variedades silvestres de *J. cinerea* (Sinaloa y Baja California Sur), por último, se obtuvo polen de *J. platyphylla* (Sinaloa) y *J. vernicosa* (Baja California Sur) silvestres para caracterizarlos palinológicamente. Para describir el tamaño y la forma de los granos de polen, se utilizó un microscopio electrónico de barrido. Los granos de polen tienen forma esferoidal. El diámetro de las variedades de *J. curcas* tóxica y no tóxica, y *J. platyphylla* fueron 58 ± 2.3 , 54 ± 1.7 , y 51 ± 2.4 micras, respectivamente; el número de clavos fue de 84 ± 10 , 108 ± 15 y 180 ± 15.5 , respectivamente. Los granos de polen de *J. cinerea* (Sinaloa) tienen un diámetro de 50 ± 1.7 micras y 220 ± 14 clavos por zona; *J. cinerea* (Baja California Sur) tiene un diámetro de 45 ± 1.9 micras y 195 ± 14.7 clavos. Los granos de polen de *J. vernicosa* tienen un diámetro de 46 ± 2.1 micras y 231 ± 25.6 clavos. La ornamentación de la exina mostró clavos con cápsula en forma de gema y pequeños

báculos en el lumen para ambas *J. curcas* y en forma de clavum para *J. cinerea*, *J. platyphylla* y *J. vernicosa* y no presentan báculos en el lumen. Las variedades estudiadas tienen una endexina reticulada y ectexina intactada.

Palabras clave: Palinología, *J. curcas* (tóxica y no tóxica var.), *J. cinerea*, *J. platyphylla*, *J. vernicosa*, planta endémica.

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