Seed oil content and composition of *Jatropha curcas* (L.) and grafted *Jatropha curcas* (L.) on *Jatropha cinerea* (Ortega) Muell. Arg. rootstock

Composición y contenido de aceite en semillas de *Jatropha curcas* (L.) y *Jatropha curcas* (L.) injertada en porta injertos de *Jatropha cinerea* (Ortega) Muell. Arg.

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Abstract

Jatropha curcas has been investigated for its high content of oil, its moderate salinity and drought tolerance, and *Jatropha cinerea* is a species that can withstand long drought periods and tolerate salinity up to 100 mM of NaCl. The aim of this study was to graft *J. curcas* plants on *J. cinerea* and grow them in experimental semiarid conditions, different soil and climate conditions from those of *J. curcas* native area to analyze their effects on oil seed composition and content. The survival of grafted *J. curcas* on *J. cinerea* rootstock was 95%. Seeds from grafted and non-grafted plants were analyzed to determine their oil content. The grafted plants showed greater height (150.7 cm) and oil content (51.3%) than the non-grafted plants (123.5 cm and 49.2%, respectively) without affecting their fatty acid composition. The meteorological information of the experimental plot (Baja California Sur, Mexico) showed values below those necessary for good phenological development; nonetheless, the graft improved its characteristics. Therefore, the use of grafted plants is an option for the establishment of *J. curcas* plantations in other parts of the world with different soil and climate conditions than those where they grow in the wild.

Keywords
Jatropha • fatty acids • grafts • rootstock • climate

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Resumen

Jatropha curcas ha sido investigada como fuente de aceite y es moderadamente tolerante a la sequía y salinidad; *Jatropha cinerea* es una especie que puede resistir largos periodos de sequía y tolerar salinidades hasta 100 mM de NaCl. El objetivo de este estudio fue injertar plantas de *J. curcas* sobre *J. cinerea* y cultivarlas en una parcela experimental en condiciones semi-áridas, condiciones de suelo y clima diferentes del área nativa de *J. curcas*, para analizar los efectos en la composición y contenido de aceite de las semillas. La sobrevivencia de *J. curcas* injertadas sobre el porta injerto de *J. cinerea* fue de 95%. Las plantas injertadas presentaron mayor altura (150,7 cm) y contenido de aceite (51,3%) que las plantas no injertadas (123,5 cm altura y 49,2% aceite) sin afectar la composición de los ácidos grasos. La información meteorológica de la parcela experimental (Baja California Sur, México) mostró valores por debajo de los necesarios para un buen desarrollo fenológico de *J. curcas*; sin embargo, el injerto mejoró las características de la planta. Por lo tanto, el uso de plantas injertadas es una opción efectiva para el establecimiento de plantaciones de *J. curcas* en condiciones de suelo y clima diferentes a aquellos donde se desarrollan en condiciones silvestres.

Palabras clave

Jatropha • ácidos grasos • injertos • portainjerto • clima

Introduction

The genus *Jatropha* comprises approximately 170 to 175 known species, of which 45 are found in Mexico (18), including *J. curcas* that has been investigated in depth as source of raw material for biodiesel production since it produces oil-rich seeds (27 to 40%) (6, 10); its fatty acid composition is mainly linoleic (28.9-47.5%) and oleic (31.7-47.1%) acids (22). *Jatropha curcas* grows in a wide range of climate conditions (annual precipitation from 300 to 3000 mm) with tolerance to high temperatures and a preference for deep and well-drained soils (21) with moderate tolerance to salinity up to 50 mM NaCl (9).

Jatropha cinerea is distributed in wild populations of northwestern Mexico; it is a shrub from 1-3 m in height that can withstand long periods of drought and tolerate up to 100 mM of NaCl (11). Studies have reported medicinal and industrial applications (16, 22, 23). Its latex is useful for healing all types of wounds and burns; it is used as a mordant (color fixative) and also as astringent and a remedy to remove warts (12).

The biological distribution of both species is found in distinct ecological zones. Behera *et al.* (2010) indicated that it was important to know the biological distribution of the species because of its wide variation in oil content. Zamarripa and Díaz (2008) reported an area in Mexico with high and medium potential for the establishment of *J. curcas* in the state of Sinaloa, which had the largest area (557641 ha) for this crop in contrast to the state of Baja California Sur where areas with productive potential had not been detected.

Jatropha curcas and *J. cinerea* are mainly propagated by seeds, cuttings, *in vitro* culture and grafting (7, 8). Soto-Landeros *et al.* (2016) mentioned that the use of rootstocks could be used as an alternative to reduce water and saline stress of the plants cultivated in semi-arid zones and low precipitation. Both the climate of a site where *J. curcas* develops in the wild and that of the experimental field of our research facilities were compared to be able to have a reference of the development of the plants in semi-arid conditions. Therefore, the objective of this study was to graft *J. curcas* plants on *J. cinerea* and grow them in experimental semiarid conditions, different soil and climate conditions from those of *J. curcas* native area to analyze their effects on oil seed composition and content.

Materials and methods

Plant material

Seeds of *J. curcas* from Sinaloa, Mexico were collected from an experimental cultivation field in Estación Dimas (23°46' 35.4" N, 106°46'48.3" W; 42 m a. s. l.) in October 2013 and *Jatropha cinerea* seeds from wild plants located in the town of San Antonio de la Sierra, B. C. S., Mexico (23°45'06.2" N, 110°07'03.5" W; 452 m a. s. l.) in November 2013.

Seed germination

The experiment was performed in the Laboratory of Plant Biotechnology at Centro de Investigaciones Biológicas del Noroeste (CIBNOR), La Paz, BCS, Mexico. The seeds of both species were placed in sterile paper wetted with sterile water for their germination and incubated in a growth chamber at 25 ± 2°C under dark conditions in March 2014. Once the seeds germinated, they were sowed in 250 mL polyethylene cups, perforated on the base and filled with plant transplant growing mix Sphagnum moss Sogemix® (Quakertown Canada).

When seedlings developed, they were placed in a greenhouse under sunlight and temperature of 28 ± 7°C.

Grafting method

Thirty-day old seedlings of both species with similar diameter and stem height were selected; *J. cinerea* seedlings were cut in a "V" shape under the cotyledons to be used as rootstocks, discarding the aerial part to avoid growth of the axillary buds found in the knot of the cotyledon leaves. On the other hand, *J. curcas* (graft) plants were cut in an arrow shape above the cotyledons avoiding the bud union, in such a way that it joined the rootstock, discarding the root (7). The grafting parts were joint perfectly and covered with Parafilm® (American National Can, CT, U.S.A.) tape to hold them together and prevent air entrance.

The seedlings recently grafted (50 plants) were placed in a greenhouse at a temperature of 28 ± 7°C, relative humidity from 70 to 80% and sunlight. Three-month grafted seedlings were transferred to 5 L pots with substrate (Sogemix®, Quakertown, CAN) and perlite (Perlita Group, Torreón, Coahuila, MX) (75% and 25%, V / V, respectively).

Growing conditions

After three months, grafted and non-grafted plants were sown on an experimental plot (CIBNOR) (24°08' 05.0" N, 110°25' 31.0" W; 6 m a. s. l.) at a distance of 2 m between rows and 2 m between plants (12 plants, 8 grafted and 4 non-grafted). Observations were made one month later; the experimental parameters were plant growth, height and stem diameter above the graft union. After four months, the plants were pruned at 1 m in height to homogenize them. Later, the relationships of soil characteristics and seed fatty acid content were evaluated.

The soil analyses consisted on evaluating pH, electrical conductivity, organic matter and texture. Each plant was fertilized with 50 g NPK formula (17-17-17) and watered once a week.

Seed characteristics and oil content

Seeds were harvested from grafted and non-grafted plants three months after planting. Their characteristics, such as number of seeds per plant, mass, length and diameter were assessed. Then, they were sent to Centro de Investigación en Alimentación y Desarrollo (CIAD), Culiacán, Sinaloa, Mexico for oil content and composition analyses. Each of the 25 seeds per plant of each type were measured in length and diameter with a Truper® digital Vernier (Mexico City, MX). Weight was determined using an analytical balance with an accuracy of ± 0.2 mg. The seeds were cracked open using tweezers; the seed coat was carefully removed, and the kernels were stored in a desiccator prior to sample preparation. Kernels, subsequently tarred and milled, were weighed (5 g) in a porcelain crucible. Moisture content of the samples was determined by oven drying to a constant weight. A 2 g sample was placed in a pre-weighed extraction thimble and put in the Soxhlet system for 16 h using hexane. The solvent was distilled under vacuum rotary evaporation (30°C) (BÜCHI, 816HE, BÜCHI Labortechnik AG, Flawil, CH).

The thimble was placed in an oven (Yamato DKN602C, Tokyo, JP) at 103 ± 2°C for 24 h, using method 920.39, A.O.A.C. (1) to remove residual solvent and then weighed on an analytical balance (Sartorius AX124, Göttingen, DE). The result was expressed as the percentage of oil in dry matter. The oil was stored at 5°C in amber bottles for further analysis of fatty acid composition.

Oil composition

Fatty acid composition was determined by gas chromatography (Agilent, 7820, Santa Clara, CA). Oil methylation was performed using method 920.33 A.O.A.C. (1). The oil samples were analyzed using a 30 m x 0.32 mm x 0.2 µm capillary column (Omega wax 320, Sigma-Aldrich Corp., St. Louis MO, U.S.A). The injector temperature was 260°C. The carrier gas was helium, which was maintained at a constant flow of 1 mL/min for 40 min. The analysis was performed in triplicate using a commercial sample (Supelco-Sigma-Aldrich, Bellefonte, PA, U.S.A.) with 37 fatty acids (19). The percentage of individual fatty acids was calculated by comparing the peak areas with the commercial standard and expressed as the total proportion of fatty acids in each lipid fraction (2). The ANOVA statistical test and Tukey's mean comparison test were performed with MINITAB 17 software.

Weather information

Daily environmental conditions (precipitation, relative humidity and temperature) were recorded in an automated Davis Vantage Pro 2 Plus (Davis Instruments, CA, U.S.A.) located at CIBNOR experimental plot, CIBNOR, La Paz, BCS, Mexico (24°08'08.6" N, 110°25'40.1" W 6 m a. s. l.). The meteorological data of the cultivation site located in Estación Dimas, Sinaloa, Mexico were obtained from an automated climate station Adcon Telemetry® (Vienna, AT), located 5 km from where wild *J. curcas* grows (23°44' 01" N, 106°49'11" W; 4 m a. s. l.).

Results

Seed germination

At third day, germination of *J. curcas* seeds were 40% and *J. cinerea* seeds were 10%. The rest of the germination for both species happened heterogeneously, obtaining 60% for *J. curcas* with a total of 120 plants and 25% for *J. cinerea* with a total of 50 plants in a period of 12 days. Seedling emergence was 7 to 10 days after sowing.

Grafts

A survival of 95% of grafted plants was obtained after 25 days. Callus formation was observed in the graft union area when the parafilm (Sigma-Aldrich, St. Louis, MO, U.S.A.) was removed increasing stem diameter. The stems of the grafted plants showed the integration of *J. curcas* tissues with *J. cinerea* rootstock and developed as a single plant.

Crop development

Table 1 shows the parameters recorded in non-grafted and grafted *J. curcas* plants after a 10-month period, displaying significant differences ($p > 0.05$) between the height of the two types of plants, 26 cm in average; in terms of stem diameter, no significant difference ($p \leq 0.05$) was found between grafting and non-grafting. The yield of the number of seeds was significantly different ($p \leq 0.05$), which was higher in grafted plants with an average increase of 36 seeds per plant. Seeds harvested from grafted and non-grafted plants showed significant differences among weights. Comparing the weight of the seeds developed in Estación Dimas (0.63 ± 0.3) (3) and those obtained in this study, the grafted plants had a similar weight (0.56 ± 0.13). However, in terms of length and diameter, no statistical difference was observed. The differences between plant height, weight and number of seeds were due to the fact that *J. cinerea* rootstock benefited from *J. curcas* grafting because it is not a wild plant that grows in BCS, Mexico. In contrast, *J. cinerea* is adapted to semi-arid conditions and distributed naturally throughout northwestern Mexico.

Table 1. Results of *Jatropha curcas* grafted and non-grafted characteristics of plant and seeds.

Tabla 1. Resultados de las características de plantas y semillas de *Jatropha curcas* injertada y no injertada.

Parameters	*Jatropha* Non-grafted	*Jatropha* grafted
	Plants	
Height (cm)	123.5±8.96 ^{b}	150.7±18.63 ^{a}
Diameters (mm)	60.7±2.76 ^{a}	58.2±3.23 ^{a}
	Seeds	
Seeds for plant	142±19.2 ^{b}	178±23.5 ^{a}
Mass (g)	0.49±0.11 ^{b}	0.56±0.13 ^{a}
Length (mm)	16.7±0.93 ^{a}	18.3±0.73 ^{a}
Diameter (mm)	9.5±0.48 ^{a}	10.2±0.39 ^{a}

Value of the mean is ± SD. Different superscripts in the same line indicate significant differences among plant types (Test Tukey, $p < 0.05$).

El valor de la media es ± SD. Diferentes superíndices en la misma línea indican diferencias significativas entre los tipos de plantas (Prueba de Tukey, $p < 0,05$).

Soil analysis in BCS showed pH 7.5, alkaline soil, sandy-clay texture, organic matter of 0.4% and with respect to electrical conductivity; it is classified as slightly saline soil (14).

Weather information

Annual precipitation was 223 mm in the experimental plot (CIBNOR), distributed mainly from July to October whereas for the field at Estación Dimas where *J. curcas* grows in wild conditions, it occurred from June to October with an annual precipitation average of 700 mm. In the experimental plot (CIBNOR), relative humidity data showed an annual average of 62 ± 1.4% while at the field Estación Dimas it showed an annual average of 81 ± 2%. The average monthly maximum and minimum temperatures ranged from 25.5± 1.1°C to 38.95 ± 1.6°C and from 10.2 ± 1.6°C to 25 ± 1.4°C, in the experimental plot (CIBNOR) respectively, and average monthly maximum and minimum temperatures ranged from 26.4 ± 0.4°C to 33.2 ± 0.3°C and from 13.7 ± 0.7°C to 25.3°C, respectively in Estación Dimas. CIBNOR experimental field had lower precipitation, higher temperature and lower percentage of humidity than the field at Estación Dimas.

Oil content and composition

The oil content of grafted and non-grafted *J. curcas* plants was 51.3% and 49.2%, respectively with significant differences ($p \leq 0.05$). The highest amounts of fatty acids observed in grafted and non-grafted plants were linoleic and oleic acids with an average of 45.83% and 40.30%, respectively, and lower proportions of palmitic (12.20%) palmitoleic (0.56%), linolenic (0.34%) arachidonic (0.40%) and myristic (0.20%) acids. The saturated fatty acids (myristic, palmitic and arachidonic) represented approximately 12.70% of the total content. Significant differences ($p \leq 0.05$) were only observed in palmitic acid content with respect to non-grafted plants. Unsaturated fatty acids, (palmitoleic, oleic, and linolenic) constituted 87.29%. (table 2).

Table 2. Composition of fatty acids in oil obtained from seeds of *Jatropha curcas* harvested in an experimental plot (CIBNOR) in La Paz, Baja California Sur, Mexico.

Tabla 2. Composición de ácidos grasos en aceite obtenido de semillas de *Jatropha curcas* cosechadas en una parcela experimental (CIBNOR) en La Paz, Baja California Sur, México.

Fatty acids	Treatments	
	Non-grafted plants (%)	Grafted plants (%)
Myristic (C14:0)	0.21±0.05 [a]	0.20±0.04 [a]
Palmitic (C16:0)	12.72±0.44 [a]	11.49±0.36 [b]
Palmitoleic (C16:1n7c)	0.58±0.05 [a]	0.54±0.11 [a]
Oleic (C18:1n9c)	40.30±2.67 [a]	42.08±0.68 [a]
Linoleic (C18:2n6c)	45.83±2.36 [a]	44.67±0.40 [a]
Linolenic (C18:3n3a)	0.33±0.05 [a]	0.34±0.04 [a]
Arachidonic (C20:0)	0.32±0.04 [a]	0.49±0.45 [a]

Value of the mean is ± SD. Different superscripts in the same line indicate significant differences among plant types (Test Tukey, $p < 0.05$).

El valor de la media es ± SD. Diferentes superíndices en la misma línea indican diferencias significativas entre los tipos de plantas (Prueba de Tukey, $p < 0,05$).

Discussion

Hishida *et al.* (2013) observed that the beginning of *J. curcas* germination occurred on day three, reaching its maximum rate on day six compared with *J. cinerea* that began at day four with a maximum rate at day 10. Two factors could have affected this difference; the first one might have been due to the seed coat of *J. curcas*, which was thinner than that of *J. cinerea*; a large number of forest species seeds do not germinate because the hard seed coat prevents water ingression (physical latency), and the seed does not germinate unless the seed coat is scarified (15). Another factor that could have been affecting germination percentage was seed quality since the seeds of *J. curcas* were from cultivated plants obtaining a better physiological maturation. As mentioned by Budi *et al.* (2012), who studied the viability of *J. curcas* seeds in different maturity stages of plants grown in an experimental field, they found that the best stage for seed germination was physiological maturity (yellow fruit). For the same reason, because *J. cinerea* seeds came from wild plants, heterogeneous fruits and seeds were obtained.

About graft, Cholid *et al.* (2014) assessed grafting compatibility on *J. curcas* rootstock, following two methods, using lateral plating, joining a diagonal cut and slit-grafting cutting the rootstock and scion in "V" shape with a survival rate of 89.5 and 93.8% after two to three months, respectively, similar as the results. Soto-Landeros *et al*. (2016) have reported that *J. cinerea* rootstock accumulated a greater number of starch granules in its cells, which functioned as osmotic regulators preventing the plant from water deficit. In the results, this characteristic lead it to more biomass production reflected in plant height and seed production.

The seeds produced by grafted plants showed greater weight, which meant higher oil content in their germ. The rootstock favored development and prevented the plant from water deficit, affected by weather conditions (solar radiation, temperature, relative humidity, precipitation and wind speed). Araiza-Lizarde *et al.* (2015) mentioned that environmental conditions (temperature and wind speed) influenced *J. curcas* seed oil content but not its physic-chemical properties.

The fatty acid results obtained in this study were consistent with those reported by Araiza-Lizarde *et al*. (2015) where they recorded 44.1 ± 0.09% of oleic acid and 42.63 ± 1.06% of linoleic acid at Estación Dimas. They also mentioned that high temperatures influenced oil content. Sosa-Segura *et al.* (2014) evaluated oil yield and germ fatty acid composition of three species of *Jatropha* (*J. curcas*, *J. platyphylla* and *J. cinerea*). The results obtained for *J. curcas* showed higher oil content (61.5%) compared to those reported in this study, probably because climate conditions were more favorable since they were developed in a climate with heavier rainfall and higher relative humidity.

However, in the fatty acid profile analysis, the results were similar, mainly in palmitic, linoleic and oleic acids. The fatty acid profile showed that the oil from grafted and non-grafted *Jatropha* was dominated by unsaturated fatty acids (oleic, linoleic and linolenic acids) with a significant amount ($p \leq 0.05$) in palmitic and oleic acids. These data were consistent with those reported by Mazumdar *et al.* (2013), who studied the production of biodiesel from *J. curcas* seed oil and found that vegetable oil with a high content of unsaturated fatty acids was an alternative to replace fossil fuels since it met the requirements of ASTM (American Society for Testing and Materials) international standards.

Environmental conditions were important; *J. curcas* could survive with only 250 to 300 mm of annual rainfall; however, 600 mm were needed for flowering and fruit production (5). In this study, although weather conditions were not favorable, it was observed that grafted plants produced more seeds because *J. cinerea* rootstocks helped for seed production.

Climate factors (temperature, precipitation, relative humidity, etc.) had a significant effect ($p \leq 0.05$) on plant growth, distribution, productivity, seed yield and oil content. Rodrigues *et al*. (2016) evaluated the ability of high relative humidity, associated with the supply of K^+ to mitigate the harmful effects caused by saline stress on the physiological parameters of *J. curcas* plants and two different levels of relative humidity, low (40%) and high (80%); they concluded that the combined effects of high relative humidity and a supply of K^+ were able to improve growth, leaf gas exchange and ionic homeostasis of *J. curcas* plants. It is important to consider relative humidity because the experimental plot in this study had a lower humidity percentage than that in the plot where wild *J. curcas* grows.

The optimum temperature range is from 18 to 28°C. Higher temperatures can reduce yields. Some plants change sex in flowering (protandria) with few immature seeds at conditions of 40 ± 2°C during summer, so it is important to investigate in future studies if it happens in *J. curcas* (12). Wassner *et al.* (2016) studied the quality and composition of *J. curcas* oil under subtropical conditions and found that environmental conditions (precipitation, temperature and relative humidity) modified seed quality and oil composition during grain filling while its concentration was not affected.

Conclusion

The compatibility of *J. curcas* grafts on *J. cinerea* rootstocks was 95% of the total grafted plant survival, which ensured plant breeding and the possibility of establishing commercial plots. The grafted plants showed greater height (150.7 cm) and more oil content (51.3%) than the non-grafted plants, 123.5 cm and 49.2%, respectively, without affecting the composition of fatty acids in both cases. The grafting method was beneficial because increased plant height and seed weight, without affecting the fatty acid profile of the seed germ. Therefore, the use of grafted plants is a good option for the establishment of commercial plantations in low quality soils (from moderate to high salinity).

According to the climate data shown for the development of *J. curcas* under wild conditions, the use of grafting with *J. cinerea* rootstock improved the production and development of *J. curcas* under unfavorable conditions as less rainfall, relative humidity, and rainy season with higher temperatures. On the other hand, sowing grafted plants in Baja California Sur, Mexico or in semi-arid areas in other parts of the world with similar weather conditions offers great advantages especially because they are pest-free and despite the extreme dry summer conditions, the rest of the year is a temperate climate with the possibility of rain.

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