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Compatibilidad anatómica de *Jatropha curcas*
(L.) injertada en portainjerto de *Jatropha*
cinerea (Ortega) Muell.Arg
Anatomical compatibility of *Jatropha curcas*
(L.) graft on *Jatropha cinerea* (Ortega)
Muell.Arg rootstock

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Resumen

Se evaluó la compatibilidad de injertos de *Jatropha curcas* sobre portainjertos de *Jatropha cinerea* comprobando la compatibilidad de los injertos entre ambas especies con un 95% de supervivencia del total de plantas injertadas y observando la compatibilidad de los tejidos vasculares, su regeneración y desarrollo mediante pruebas histológicas. A nivel anatómico, ambas especies tuvieron los mismos tipos celulares (parénquima en la corteza externa los tipos celulares específicos que forman los tejidos en el floema, cambium vascular y xilema) completamente diferenciados al momento de ser injertadas. Al comparar los diferentes tejidos, las células de parénquima de *J. curcas* tuvieron menor cantidad de granos de almidón que las de *J. cinerea*. Otra diferencia anatómica fue la amplitud del cambium vascular en la que *J. cinerea* presentó mayor área que *J. curcas*. Los resultados en los cortes histológicos realizados a los tallos injertados mostraron la integración del tejido de *J. curcas* con el portainjertos de *J. cinerea* al desarrollarse como una sola planta.

Palabras clave: Injerto, Portainjertos, *Jatropha curcas*, *Jatropha cinerea*

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Abstract

Graft compatibility of *Jatropha curcas* on *Jatropha cinerea* was confirmed by obtaining a high survival (95%) of the total grafted plants. Their regeneration and development was accomplished by histological assays observing compatibility of the vessel tissues. At anatomical level, both species had the same cell types (parenchyma in the external cortex, specific cells forming phloem tissues, vascular and xylem cambium) completely differentiated at the moment of grafting. When comparing different tissues, the parenchyma cells of *J. curcas* had fewer amounts of starch grains than those of *J. cinerea*. Another anatomical difference was vascular cambium amplitude, which was greater in *J. cinerea* than in *J. curcas*. The results of the histological cuts in the grafted stems showed integration of *J. curcas* tissue with *J. cinerea* stock when they developed as one plant only.

Key words: Grafting, Rootstocks, *Jatropha curcas*, *Jatropha cinerea*

Introduction

Propagation by grafting is a frequently used method in agriculture because it shows several advantages, such as reducing the plant juvenile period to obtain fruit in less time; homogenizing and improving fruit quality; achieving strong plant structures; and conserving plants of genetic importance (Rojas et al. 2004).

In grafting, the plant donating and forming the root is called stock, scion, or rootstock; the part of the plant placed on the stock providing the variety for propagation is called graft, scion, or bud (Baraona and Sancho 2000). For successful grafting, both the rootstock and the graft should be compatible. Species of the same genus can be grafted perfectly between them, but those of different genera usually cannot although there are exceptions (Irigoyen and Cruz 2005).

Incompatibility of the graft causes economic loss and delay in launching new cultivations (Pereira et al. 2014). Thus, identifying the compatible rootstock and other benefits from the scion enhances grafting as an adequate propagation technique (Colla et al. 2010). For successful grafting between different plant species or varieties of plants, the ability to produce callus tissue starting from parenchyma cells and differentiating vascular tissue on the callus bridge is of great importance. On the other hand, the reduction of necrotic layers in the union of both grafting members improves the tissue in the graft (Cholid et al. 2014).

Jatropha curcas plants have shown potential for the production of biofuels and other products because the seed is rich in lipids (55-58%) and raw protein (31-34.5%) (Martínez-Herrera et al. 2006). Nevertheless, *J. curcas* is still in domestication period; its current varieties have low yield and production variability, and it shows a moderate tolerance to salinity of up to 50 mM of NaCl (Díaz-López et al. 2012). To improve its cultivation and adapt it to drought and salinity conditions requires genotypes with high yield, homogeneous maturity, resistance to pests and diseases, and tolerance to salinity (Quiroz, 2013).

On the other hand, *Jatropha cinerea* is a species distributed in wild populations of northwestern Mexico in saline soils; it can withstand long drought periods and tolerate up to 100 mM of NaCl (Hishida et al. 2013); it has medicinal and industrial uses (Yetman and Van, 2002; Quattrocchi, 2012). *J. cinerea* could be used as rootstock of *J. curcas* scion improving its tolerance to drought and salinity.

The grafting combination generally used for *Jatropha* planted in dry areas is a combination between the scion that has a high yield performance and the rootstock selected that is tolerant to the limited water availability (Cholid et al. 2014). Although *J. curcas* and *J. cinerea* belong to the same genus, they are morphologically distinct (leaves, flowers, fruits, and seeds), as well as the environmental conditions where they grow. Compatibility of the rootstock and the graft is essential for optimal growth, water capture, and nutrient transport (Martínez-Ballesta et al. 2010). In this work we have shown the grafting procedure and characteristics between *J. curcas* and *J. cinerea* plants, as well as descriptive anatomical study to allow explaining grafting compatibility of these two species.

Method

Plant material

The experiment was performed in the Laboratory of Plant Biotechnology at CIBNOR, La Paz, Baja California Sur, Mexico (N 24° 08' 05.7'' N, 110° 25' 31.8'' W; 6 m a.s.l.). Seeds of non-toxic *J. curcas* from cultivated plants were collected from an experimental lot in Estación Dimas, Sinaloa, México (N 23° 46' 35.4'' N, 106° 46' 48.3'' W; 42 m a.s.l.). *J. cinerea* seeds were collected from wild plants located in the town El Tambor, Navolato Sinaloa, México (N 24° 44' 43.9'' N, 108° 01' 26.6'' W; 5 m a.s.l.).

Seed germination

First, 200 seeds of each species were washed with detergent Axion® (Colgate-Palmolive, Mexico City, MX) for 10 min; then, they were treated with 10% commercial sodium hypochlorite Cloralex® (concentrated 4-6%) (Industrias Alen, Nuevo Leon, MX) for 15 min and rinsed five times with distilled water; after that, they were soaked in distilled water for a 24 h period at room temperature to soften the seed coat and allow a homogeneous germination (Willan 2000). The seeds were placed in sterile paper wetted in sterile water for their germination and incubated in a growth chamber at $25 \pm 2^\circ \text{C}$ in dark conditions. Once germination occurred, they were sowed in 250 mL polyethylene cups, perforated on the base and filled with vegetable transplant growing mix *Sphagnum sp.* moss Sogemix® (Canada). The germinated seeds were incubated in a growth chamber at $25 \pm 2^\circ \text{C}$ and periods of 12 h light and 12 h darkness. Once seedlings developed, they were placed in a polycarbonate greenhouse in light and temperature conditions at $28 \pm 7^\circ \text{C}$. The germination percentage under these conditions was evaluated in both species.

Top cleft

Thirty-day seedlings (N = 50) of both species were selected similar in stem diameter and height; *J. cinerea* seedlings were cut to use them as rootstock, and a V-shaped cut was made under the cotyledons discarding the aerial part to avoid growth of axillary shoots 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 (Dhillon et al. 2009, Cholid et al. 2014). The grafting parts were joint perfectly and covered with Parafilm® (American National Can, CT, U.S.A.) tape to hold them together and prevent the entrance of air. The seedlings recently grafted were placed in a growth chamber at a temperature of $27 \pm 2^\circ$ C and relative humidity from 70 to 80%. Grafted 3 month seedlings were transferred to 5 L of plastic pots with Sogemix® (CANADA) and perlite (Grupo Perlita, Torreón, Coahuila, MX) substrate (75% and 25%, V/V, respectively).

Plant growth measurement

The observations started 3 months after grafting. The parameters observed included: the percentage of grafted plant success, plant growth (plant height and stem diameter above the graft union) (Cholid et al. 2014). The statistical test ANOVA and Tukey's test of mean comparison were performed on MINITAB 15 software to compare plant height and stem diameter of the *Jatropha* species studied.

Sample preparation for histological analyses

For the histological analysis, 1 cm of stem fragments were taken from *J. curcas*, *J. cinerea* (60 d after emergence), and grafted plants (30 d after grafting). The samples were preserved in formaldehyde-acetic acid-alcohol solution (FAA). Samples were processed to be cut with the paraffin inclusion technique. Fragments were dehydrated in series of ethyl alcohol in watery dilution at 70, 80, 90, 96°, and finally in absolute alcohol (ethanol) in 1 h periods for each concentration (D' Ambrogio 1986). To clarify the material, pure xylol was used for 12 h. Paraffin infiltration was performed in a Thermo Scientific® (Thermo Fisher Scientific, Waltham, MA, U.S.A.) stove at 60 °C for 12 h. The paraffin inclusion system used with the LEICA® EG 1150 (Leica Biosystems, Germany) model in stainless steel molds; samples were embedded in paraffin and cooled down at 5° C; then, they were set in the freezer to obtain blocks.

The histological cuts were performed with a microtome LEICA® RM 2155 (Leica Biosystems, Germany) obtaining 4 µm cuts in thickness, transversally and longitudinally. The sections were mounted in slides and treated with alcohol. Dye was combined using fast green-safranin, especially for dyeing plant structures. The mounting medium used was distilled water and glycerine at 50% (D' Ambrogio 1986). Observation was performed with an optical microscope Olympus® BX50 (Olympus Co. Japan) with lenses of 4X, 10X, 20X magnification with digital camera CoolSNAP-Pro (Roper Scientific, Inc. U.S.A.).

Results and discussion

Seed germination

Germination of *J. curcas* seeds (40%) occurred at day three while that of *J. cinerea* was only 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 d. Hishida et al. (2013) observed that seed germination of *J. curcas* started at day three, reaching its maximum germination rate at day six, compared with *J. cinerea* where germination started at day 4 with a maximum rate at day 10. Two factors could be affecting this difference. The first one because the seed cover of *J. curcas* is thinner than that of *J. cinerea* since a great number of seeds of forest species do not germinate due to the hardness of the seed cover preventing the entrance of water (physical latency) and germinating unless seed scarification is performed (Poulsen and Stubsgaard 2000). The second factor could be seed quality; because *J. curcas* seeds are from cultivated plants, they reach physiological maturity faster. Budi et al. (2012), who studied *J. curcas* seed viability in different maturity stages in an experimental field, found that the best seed germination stage is physiological maturity (yellow fruit). *J. cinerea* seeds are collected from wild plants without any agronomic practice, obtaining heterogeneous fruits. Seedling emergence was from 7 to 10 d after sowing. *Jatropha* seed viability is reduced to storage periods greater than five months (Budi et al. 2012).

Graft

We were observed a survival of 95% of the grafted plants. As the stem grew, callus formation in the graft union and detachment of the parafilm® (American National Can, CT, U.S.A.). The grafting success is determined by the scion and rootstock ability to develop a composite plant in

the graft union (Figure 1). The transversal and longitudinal cuts performed to the stems of the grafted plants showed *J. curcas* tissue integration with *J. cinerea* rootstock when they developed jointly in only one plant. These macroscopic cuts (Figure 2), where we could confirm that the stems in the grafted area were completely joint at day 15 after grafting, showed successful compatibility with the stem tissues of both species. As mentioned previously, this union was formed by callus proliferation on the grafting area. Furthermore, dark parts or parts without tissue with holes were observed where a necrosis area appeared, typical of regeneration characteristics of mechanical plant tissue damage, including incompatible tissues (Poessel et al. 1996). Cholid et al. (2014) evaluated graft and rootstock compatibility of *J. curcas* using two grafting methods, whip grafting in diagonal cut and top cleft grafting in V-shaped cut with 1, 2, and 3 month rootstock, obtaining a grafted plant survival of 78.6%, they report that the best method for grafting was the V-shaped cut and the rootstock from 2-3 months with a survival percentage of 89.5 and 93.8%, respectively. The whip grafting method and the 1-month rootstock showed the lowest engraftment percentage of 66.5% (Cholid et al. 2014).

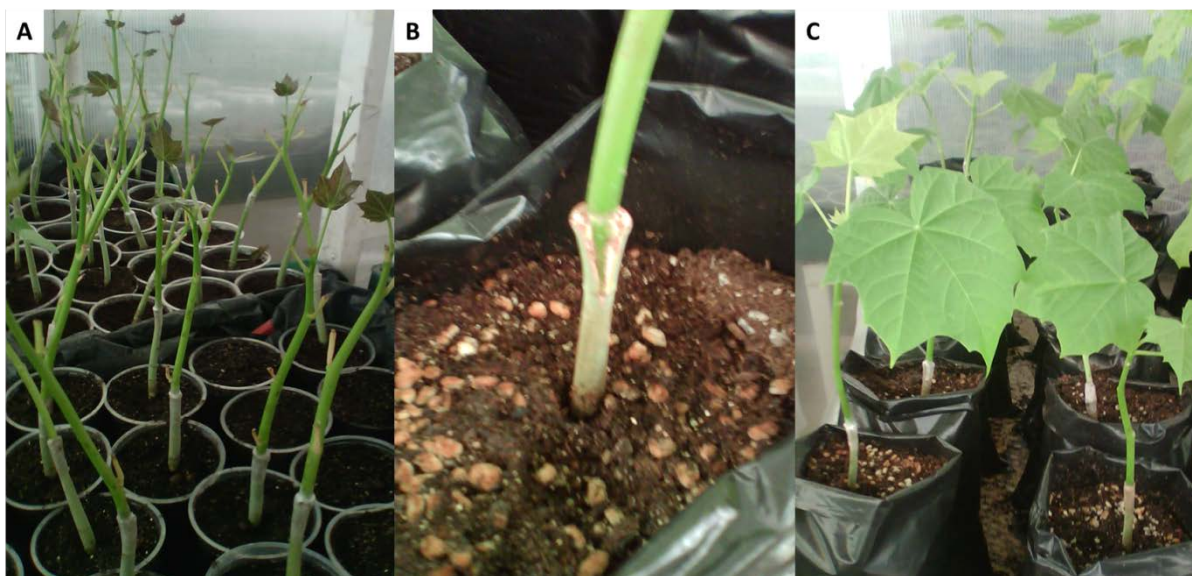


Fig. 1. (A) Grafted *Jatropha* plants in polyethylene cups, (B) Detail of stem grafted *Jatropha*, (C) Composite of *Jatropha* plants in 5 L pots with substrate.

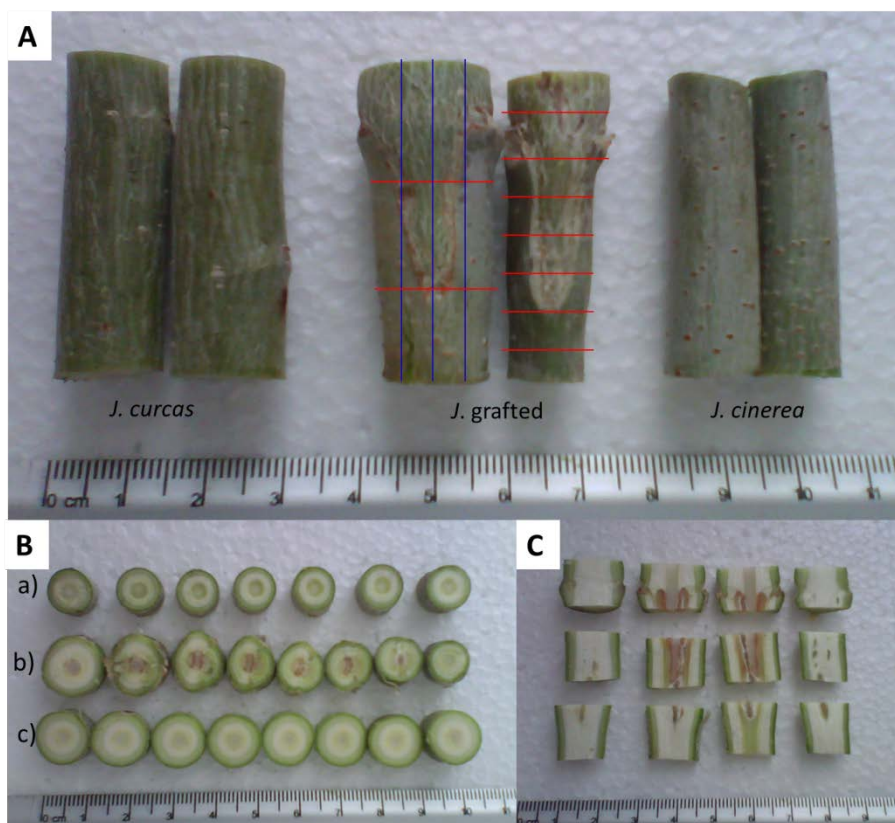


Fig. 2. (A) Stem fragments from *Jatropha* species and (B) Transversal cuts of *Jatropha cinerea*, grafted *Jatropha*, and *Jatropha curcas*, respectively; (C) longitudinal cut of grafted *Jatropha* plants.

Grafted plant growth

The statistical analysis result at 3 months after grafting showed there were no significant differences between grafted and non-grafted plants on plant height and stem diameter (Table 1). The high compatibility of grafted plants showed similar growth in grafted and non-grafted plants. These results differ from those obtained by Cholid *et al.* (2014) who reported an average plant height of 74.42 cm and 2.74 cm of stem diameter, which might have resulted because the readings were made in plants 4 months of age of the same species.

Table 1. Effects of rootstock and graft on plants of *Jatropha curcas* and *Jatropha cinerea*.

Species	Plant height (cm)	Stem diameter (mm)
<i>Jatropha curcas</i>	50.16 ^a	14.41 ^a
<i>Jatropha cinerea</i>	50.50 ^a	14.71 ^a
Grafted plants	49.91 ^a	14.69 ^a

Values with same letters were not significantly different at 5%.

Histological cuts

In the transversal cuts of *J. curcas* and *J. cinerea* (Figure 3), well-differentiated tissue stem were observed from the exterior toward the interior: the epidermis was composed of cork bark in the most external part; the phellogen is the cork cambium. The phellodermis had several layers of cells in strata characterized by the presence of chloroplasts in its interior. The bark was composed of collenchyma and parenchyma cells in non-defined division patterns, which can be divided at random almost at any level and that could be observed up to where extra phloem fibers started. The phloem was located toward the interior with different cell types among the fibers, parenchyma, phloem parenchyma, eschlereid, phloem member elements, and their companion cells characterized by being in groups and with angular cells that have their origin in the vascular cambium. The vascular cambium showed continuously forming a cylinder around the stem, indicating the plant had reached a secondary development stage. The next layer was the xylem with few very large and solitary capillary vessels and radial cells with abundant starch grains dyed in purple, which could be functioning as osmolites to maintain the cellular volume and avoid hydric stress. Seki et al. (2007) mentioned osmotic adjustment is an important physiological mechanism by which plants synthesize and accumulate compounds acting as osmolites in the cells in response to hydric deficit.

On the other hand, latex channels were observed from the peridermis up to the medulla; because the latex function is not clear, researchers suggest some of its components could have an important role in the mechanisms of healing wounds and/or providing mechanical defence against predators and pathogens (Hagel et al. 2008). It is known that the family Euphorbiaceae has resiniferous channels from which latex of some genera as *Euphorbia*, *Hevea*, *Jatropha*, and *Manihot* is used in traditional medicine because of its healing properties. Thus, the presence of articulated and non-articulated laticifers in Euphorbiaceae has been reported (Demarco et al. 2013).

While comparing the different tissues in both species, collenchyma cells of *J. curcas* lacked starch grains, and starch amount was lower in parenchyma cells compared to the cells of *J. cinerea* in both tissues where they were present in greater amount. Another difference was the width of the vascular cambium where *J. cinerea* doubled the number of cambium cells compared to those of *J. curcas* (Figure 3).

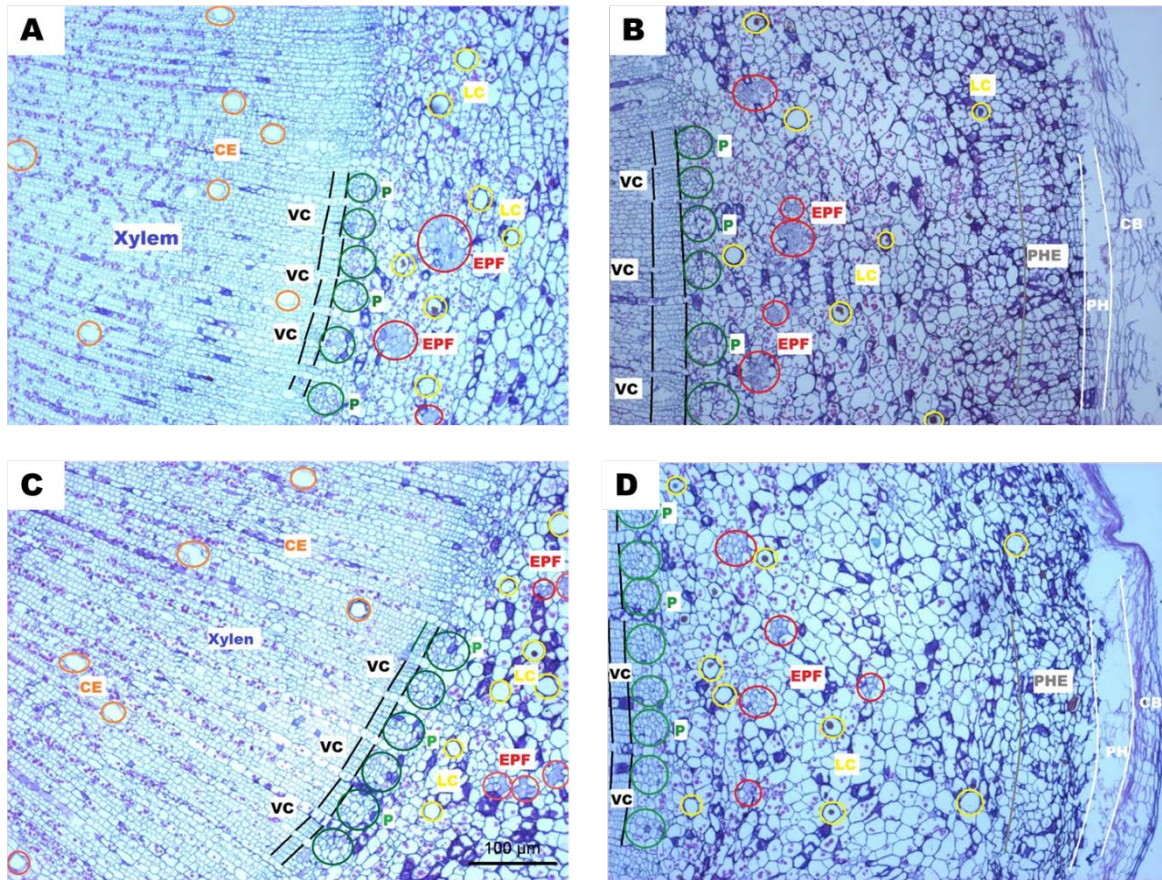


Fig. 3. (A), (B) Transversal cuts of *Jatropha cinerea* stem; (C) and (D) transversal cut of *Jatropha curcas* stem; (CE = conducting elements, VC = vascular cambium, P = phloem, EPF = extra phloem fibers, LC = latex channels, PHE = phellodermis, PH = phellogen and CB = cork bark).

We can conclude that although *J. curcas* and *J. cinerea* plants showed different size in tissues when the cut for grafting was performed at 30 d after emergence, they anatomically showed the same tissues well differentiated, formed by the same cell types, and fixed on similar strata. Some cellular contents as starch grains were present in radial parenchyma cells and chloroplasts in layers not deep from the phelloderm.

The longitudinal cuts performed to *J. curcas* showed a phloem with cells containing starch grains larger than those observed in *J. cinerea* cells although as mentioned before, the amplitude of the vascular cambium is greater in this species. Groups of radial medullary cells in *J. curcas* xylem were observed horizontally in greater quantity compared to those of *J. cinerea*, which showed a smaller number of radial cells piled up diagonally (Figure 4). The grafted plants showed a high survival percentage because *J. cinerea* rootstock has a wider vascular cambium than that of *J.*

curcas. When the vascular cambium area of *J. cinerea* made contact with the graft, there was a high probability of matching with the cambium area of *J. curcas*, allowing a fast forming callus for tissue regeneration in the wound.

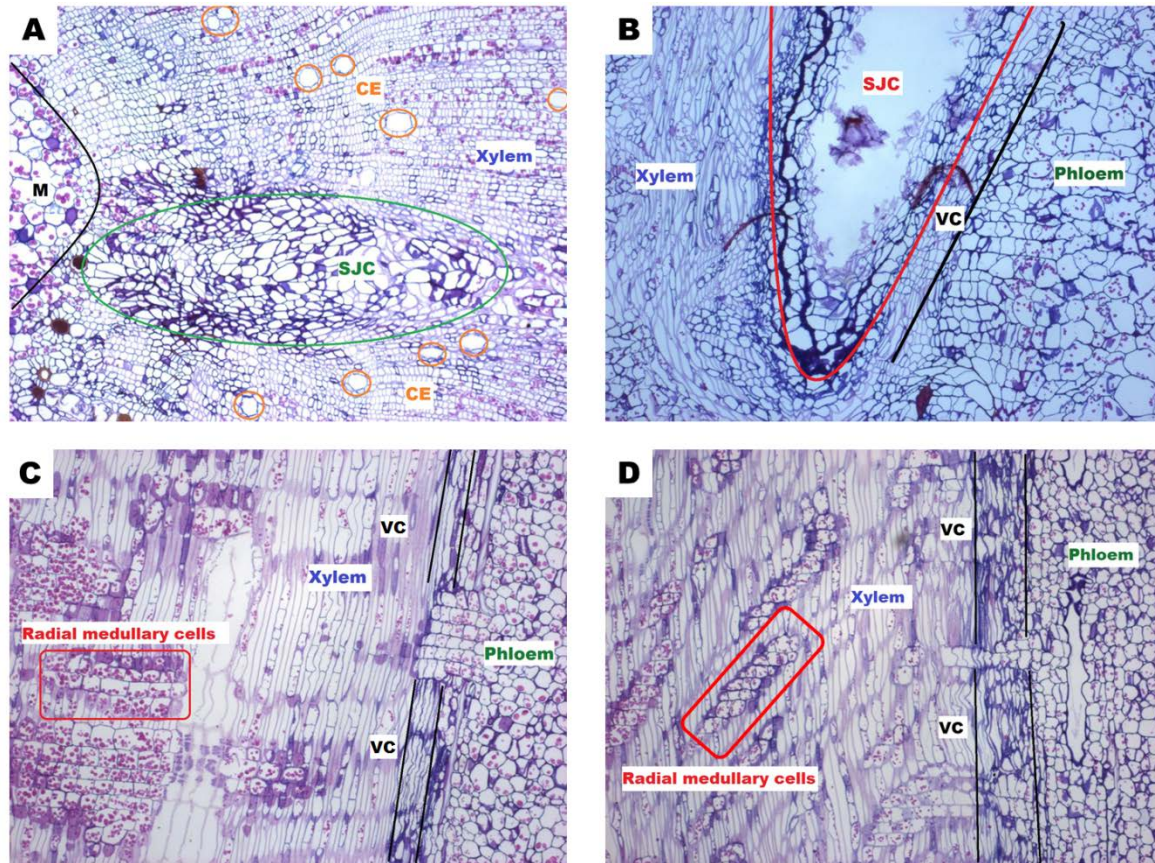


Fig. 4. (A) Transversal cut of grafted *Jatropha* stem; (B) longitudinal cut of grafted *Jatropha* stem; (C) longitudinal cut of *Jatropha curcas* and (D) longitudinal cut of *Jatropha cinerea* stem; (CE = conducting elements, VC = vascular cambium, M = medulla, SJC = scion *J. curcas*).

In general terms, grafting between *J. curcas* on *J. cinerea* was successful mainly because the following characteristics took place: (1) rootstock and graft belonged to the same genus; (2) plants of the same age and diameter were used; (3) anatomically both species showed tissues formed by similar cells distributed in the same order in the stem, which increased the probability of forming interconnections in less time. Another possibility was the amplitude of the vascular cambium and the invariable presence of parenchyma cells in almost all the tissues in both species that could split and generate layers, which jointly formed the callus. The callus can differentiate itself in other more specialized cell types or take an active role in transport by the presence of connections that make lateral transport possible without the need of conducting elements.

According to Cholid et al 2014, the causes of grafting incompatibility can be (1) physiological and biochemical factors; (2) modifications of cells and tissues in the graft union; and (3) cellular recognition between grafting parts. They also mentioned that grafting success depends on compatibility of the graft union in terms of rapid formation of the conductor vascular tissues between the two sections that allow recovery of the root and the aerial part of the grafted plant.

Conclusions

Grafting compatibility between genotypes of *J. curcas* and *J. cinerea* was achieved by histological assays, obtaining a survival 95% of grafted plants and compatibility of the vascular tissues to regenerate and develop composite plants. Considering that each of the species studied developed in different conditions of hydric and saline stress, in future studies we will show adaptation of grafted plants in these stress conditions to evaluate if this methodology will benefit the development of these species and favor seed production.

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