Centro de Investigación en Alimentación y Desarrollo, A.C.

SELECCIÓN DE GERMOPLASMAS ÉLITE DE *Jatropha platyphylla* DEL NOROESTE DE MÉXICO

Por:

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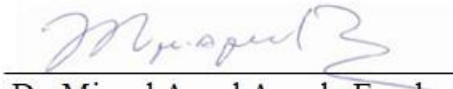

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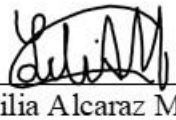

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CONTENIDO

LISTA DE FIGURAS

LISTA DE CUADROS

RESUMEN

La caracterización molecular y morfológica de plantas se ha basado en el uso de rasgos fenológicos y agronómicos debido a su alto poder discriminatorio y su velocidad en la identificación de germoplasmas a nivel de campo para la identificación de híbridos y en programas de mejoramiento. El uso de técnicas estadísticas multivariadas, como el análisis de componentes principales y conglomerados, permite reducir el número de rasgos con alto valor discriminatorio, para caracterizar los materiales con potencial para la producción de biodiesel. Las especies de plantas, que han dado lugar a una alternativa a los combustibles fósiles, han recibido un interés mundial en los últimos años. En esta categoría de plantas se destaca *Jatropha platyphylla*, árbol o arbusto, nativo de América tropical, que crece en suelos infértiles y tiene tolerancia a la sequía. Estas características las hacen de vital importancia para los países en desarrollo. Por lo tanto, el objetivo de este trabajo fue conocer y analizar la diversidad morfométrica, de producción y diversidad genética de 8 germoplasmas de *J. platyphylla* localizados en la selva baja caducifolia de Sinaloa y Durango para establecer estrategias de domesticación. Se realizó un análisis de componentes principales y análisis de conglomerados en rasgos cuantitativos de la planta y mapas de cuadrícula para conocer la diversidad y distribución de *J. platyphylla*. Para determinar la diversidad genética de los germoplasmas se utilizó marcadores moleculares ISSR. Además se evaluó el crecimiento y desarrollo de germoplasmas injertados y no injertados mediante una caracterización morfométrica y de producción. Esta evaluación permitió propagar plantas injertadas de *J. platyphylla* con rendimiento específico y características fenotípicas para la propagación de plantas élite a gran escala en un tiempo relativamente corto. En el análisis de componentes principales, los primeros tres componentes explicaron el 70.2% de la varianza en los datos y los rasgos que más contribuyeron a las diferencias fueron las características morfológicas de las semillas. En el análisis de conglomerados, tres grupos agruparon los germoplasmas con los porcentajes más altos de aceite. El genotipo PR11 produjo los valores más altos en todos los rasgos analizados. La utilización de marcadores morfológicos y de producción, marcadores moleculares y la técnica de injerto permitió identificar plantas con rendimiento y características fenotípicas específicos para su utilización en el mejoramiento genético.

Palabras clave: Germoplasmas, diversidad, *Jatropha*

ABSTRACT

The molecular and morphological characterization of plants has been based on the use of phenological and agronomic aspects due to their high discriminatory power and their speed in the identification of germplasm at a field level for the identification of hybrids and in breeding programs. The use of multivariate statistical techniques, such as the analysis of main components and conglomerates, allows reducing the number of traits with high discriminatory value to characterize the materials with potential for biodiesel production. Plant species, which have resulted in an alternative to fossil fuels, have received worldwide interest in recent years. In this category of plants, *Jatropha platyphylla*, tree or shrub, native to tropical America, stands out, which resists infertile soils and has drought tolerance. These characteristics make them vitally important for developing countries. Therefore, the objective of this work was to know the morphometric diversity, production, and genetic diversity of *J. platyphylla* germplasms located in the deciduous forest of Sinaloa and Durango to establish domestication strategies. There was realized an analysis of principal components and analysis of conglomerates in quantitative features of the plant and maps of grid to know the diversity and distribution of *J. platyphylla.* To determine the genetic diversity of the germoplasmas marking molecular ISSR were used. Also there was evaluated the growth and development of germoplasmas grafted and not grafted by means of a characterization morfométrica and of production. This evaluation allowed to spread grafted plants of *J. platyphylla* with specific yield and phenotypic characteristics for the spread of plants large-scale elite in a relatively short time. In the analysis of principal components, the first three components explained 70.2 % of the variance in the information and the features that more they contributed to the differences were the morphologic characteristics of the seeds. In the analysis of conglomerates, three groups grouped the germoplasmas with the highest percentages of oil. The genotipo PR11 produced the highest values in all the analyzed features. The use of morphologic scoreboards and of production, molecular scoreboards and the skill of graft allowed to identify plants with yield and phenotypic characteristics specifics for his use in the genetic improvement.

Keywords: Germplasm, diversity, *Jatropha*

1. SINOPSIS

1.1. Justificación

En México existe una planta con semillas con alto contenido de aceite, proteína y compuestos antiinflamatorios. Esta planta crece en suelos poco fértiles y es resistente a sequias, es conocida como bonete (*Jatropha platyphylla*) y a pesar de que la planta muestra este potencial, la especie no es explotada y su variabilidad genética permanece desconocida. La demanda de fuentes vegetales de aceite y proteínas para la producción de biocombustibles y productos alimenticios, está aumentando en todo el mundo. En la mayoría de los países en desarrollo, estos productos se importan en grandes cantidades, generando un desequilibrio entre la producción y la demanda. Debido a la baja disponibilidad de insumos agrícolas o limitado poder de compra de los agricultores, los recursos disponibles se utilizan principalmente para cultivos básicos (principalmente suministro de carbohidratos). En muchas partes del mundo, existe una gran necesidad de cultivos adaptados que produzcan aceite y proteína bajo regímenes de bajos insumos. Los aceites y proteínas pueden ser "ideales" en términos de agronomía si satisfacen una demanda, desde este punto de vista es de suma importancia la innovación y desarrollo de nuevas fuentes, como es el caso de *Jatropha platyphylla.*

1.2. Antecedentes

1.2.1. Caracterización de Plantas para su Mejoramiento Genético

El género *Jatropha* pertenece a la familia Euphorbiaceae. Se distribuye en las regiones tropicales de los continentes asiático, africano y americano e incluye aproximadamente 186 especies. Este género es conocido por su uso potencial, como ornamental, medicinal y energético (Fresnedo-Ramírez y Orozco-Ramírez, 2013).

De este género, *Jatropha platyphylla* destaca por su uso potencial como fuente de energía (Sosa-Segura *et al*., 2014). Por lo tanto, la caracterización del germoplasma de esta especie es esencial para maximizar su utilidad y promover una conservación ex situ eficiente.

La evaluación de la morfología y los rasgos agronómicos del germoplasma puede proporcionar información relevante sobre las características y la calidad del rendimiento (Anumalla *et al*., 2015), debido a su alto poder discriminante y su rápida identificación a nivel de campo.

La descripción morfométrica de las estructuras vegetales es un requisito previo para comprender las relaciones entre estructura y función en la evolución y puede contribuir a definir situaciones de desarrollo asociadas con su composición genómica. Los cambios de forma pueden ser el resultado de los programas de desarrollo en un entorno "regular" o la respuesta a los cambios (estrés) en el entorno (Cervantes *et al*., 2016).

Para la caracterización óptima de los recursos genéticos y para las comparaciones válidas de datos entre ensayos, es necesario utilizar rasgos estándar los cuales son los posibles atributos que caracterizan a la planta ideal y en casos donde es posible, se indicarán valores de referencia para los germoplasmas (George *et al*., 2016). Los rasgos morfológicos tienen confiabilidad comprobada como una herramienta para delinear la variabilidad intraespecífica que surge de diferentes procedencias en al menos algunas especies. Por lo tanto, la documentación de la variación en los rasgos morfométricos es necesaria para aprovechar efectivamente la diversidad disponible en el cultivo para una posible mejora genética (Sunil *et al*., 2013). Sin embargo, la evaluación de la diversidad basada únicamente en los rasgos morfométricos no siempre es confiable, ya que *Jatropha* muestra una gran plasticidad (Mazumdar *et al*., 2018).

La domesticación de la *Jatropha* requiere una intervención humana intensiva para que el cultivo sea rentable. Los políticos y promotores de *Jatropha* deben abordar aspectos importantes relacionados con la *Jatropha*, incluido el uso de sus subproductos, mejorar el rendimiento y desarrollar la gestión agronómica (Soto *et al*., 2018). Una estrategia útil para la domesticación es hacer el máximo uso de la variabilidad genética presente en la población seleccionada para obtener nuevos genotipos que no presenten las siguientes desventajas como el aborto de embriones, el bajo rendimiento de frutos y semillas y la baja germinación (Sunil *et al*., 2013). Por lo tanto, el manejo de recursos genéticos intraespecíficos juega un papel importante en la domesticación y también en la determinación de la estabilidad ecológica de los sistemas agrícolas. Investigaciones futuras se deben enfocar en producir fenotipos que cumplan con los requisitos de la visión del agricultor, como aumentar el rendimiento de frutos, semillas y aceite y generar siembras de cobertura las cuales contribuyen a mejorar la fertilidad del suelo y calidad del agua (Lu *et al*, 2000; Achten *et*

al., 2010). Además, es importante comprender el patrón de reproducción (panmíctico / no panmíctico), ya que es fundamental para el diseño de estrategias de domesticación. Para la reproducción de clones e híbridos, se debe dar la máxima prioridad a la identificación y caracterización de grupos heteróticos (Figura 1) (Montes y Mechilger, 2016).

Figura 1. Pasos para el mejoramiento genético de germoplasmas de especies potencialmente útiles con fines energéticos (Loo, 2011)

En los últimos 20 años, el cambio climático ha afectado los patrones de lluvia y la temperatura, ocasionando pérdidas económicas en varios cultivos importantes como algodón, maíz y soya

(López *et al*., 2016). La selección genómica ha resultado ser una oportunidad para aumentar la producción de granos y afrontar el cambio climático. Esta selección genómica puede reducir el ciclo fenológico a la mitad del tiempo convencional y en hibridaciones aumentar significativamente el rendimiento de grano, fenómeno observado en especies de interés económico como la soya, entre otros (Bhat *et al*., 2016).

El uso de marcadores genéticos es una vía para acelerar los procesos de mejoramiento genético. El marcador genético es un gen o secuencia de ADN con una ubicación cromosómica conocida que controla un gen o un rasgo en particular. Los marcadores genéticos están estrechamente relacionados con el gen objetivo y actúan como banderas de señalización. Los marcadores genéticos están ampliamente agrupados en dos categorías: marcadores clásicos y marcadores de ADN o moleculares. Los marcadores morfológicos, citológicos y bioquímicos son tipos de marcadores clásicos y algunos ejemplos de marcadores de ADN son polimorfismo de longitud de fragmento de restricción (RFLP), polimorfismo de longitud de fragmento amplificado (AFLP), repeticiones de secuencia simple y los marcadores ISSR (intersecuencias simples repetidas), entre otros (Nadeem *et al*., 2018). Sin embargo, en cualquier caso, todas las técnicas de análisis de marcadores moleculares deben cumplir con los siguientes criterios: (1) fiabilidad, los marcadores moleculares deben estar muy cerca de un locus investigado. Los resultados se mejoran usando varios marcadores si están flanqueando un loci o intragénicos; (2) ser altamente polimórfico, discriminar entre diferentes genotipos y distribuirse de manera uniforme en el genoma; (3) tiene que ser una técnica simple, barata y rápida; y (4) necesitar de muy poco material de partida genético para llevar a cabo el análisis (Garrido-Cardenas *et al*., 2018).

Los marcadores moleculares son muy valorados en los campos de investigación con una base genética, debido a su capacidad para distinguir entre genotipos (Grover y Sharma, 2016).

Se han desarrollado y aplicado con éxito diferentes tipos de marcadores moleculares de ADN en actividades genéticas y de mejoramiento genético en plantas en diversos cultivos agrícolas y en especies de *Jatropha* no es una excepción. La siguiente sección proporciona información breve relacionada con los marcadores moleculares en función de su método de detección.

1.2.2. Marcadores Morfológicos

Los descriptores, codificadores o marcadores morfométricos son una característica de las plantas que permiten la distinción, uniformidad o establecimiento del germoplasma. Las descripciones morfométricas de alta calidad son críticas para la comprensión de los sistemas biológicos, porque a menudo la apariencia de una estructura (por ejemplo, hoja, pecíolo, entre otros) aumenta la funcionalidad (flujo, transporte de nutrientes, entre otros) (Balduzzi *et al*., 2017)

Las características visibles de una especie no se expresan con la misma intensidad y algunos miembros de la población pueden presentar diferentes grados de expresión que se traducen en diferentes tipos de datos o categorías de variables. Por tanto, los descriptores se pueden diferenciar de acuerdo con el estado que presentan, lo cual es conocido como estados del descriptor y se registran mediante escalas de valor (Franco, 2003). Existen distintas categorías de datos, según la expresión del descriptor que puede ser en forma cualitativa o cuantitativa (George *et al*., 2016). Si se expresa en forma cualitativa, se pueden generar datos binarios (también llamados de doble estado), datos con secuencia (ordinales) y datos sin secuencia (nominales). Si se expresa en forma cuantitativa, los datos generados pueden ser discontinuos (llamados también discretos) y continuos (Franco, 2003).

Se han utilizado descriptores cualitativos para describir las hojas de *Jatropha*: (filotaxis, forma, superficie, textura, margen, tipos y forma de lóbulos, presencia y naturaleza de estípulas, pecíolos, venación y arquitectura de las hojas) (Nwokocha *et al*., 2012; George *et al*., 2016).

Los descriptores cuantitativos son aquellos en los que la expresión cubre todo el rango de variación de un extremo al otro. La expresión se puede dar en una escala unidimensional, continua, discreta o lineal. El rango de expresión se divide en varios estados que se pueden registrar utilizando datos discretos (contables, como "número de plantas") o continuos (datos medibles, como altura, peso, longitud de la planta, entre otros) (Franco, 2003). Esta división busca proporcionar, en la medida de lo posible, una distribución uniforme.

Los datos cuantitativos consisten en mediciones o recuentos que utilizan valores numéricos, lo que permite el análisis estadístico. Los descriptores cuantitativos permiten la clasificación de objetos individuales para la formación de grupos; pueden describir la forma general de los individuos según regiones particulares como el ápice o la base para distinguir variedades; pueden informar la variabilidad de acuerdo con su distribución geográfica; puede permitir el desarrollo de bases de datos para clasificar individuos y distinguir relaciones taxonómicas entre accesiones (Sun *et al*.,

2012).

Algunos descriptores cuantitativos incluyen las características estructurales de la altura de la planta, ganancia de altura post-poda, orden de ramificación, número de ramas primarias, diámetro de ramas primarias, número de ramas secundarias, diámetro de ramas secundarias, amplitud de la corona, dosel este-oeste, dosel sur-norte, volumen de dosel de la planta y diámetro basal del tallo (Sunil *et al* 2011; Wani *et al*., 2012; Aguilera-Cauich *et al*., 2015; Rao *et al*., 2015; Singh *et al*., 2016). Las características de las hojas incluyen longitud y anchura, pedicelo y longitud de pecíolo (Sunil *et al* 2011; Wani *et al*., 2012).

Los rasgos cuantitativos evaluados de la semilla son el peso de la semilla, la longitud de la semilla, la amplitud, el espesor, el volumen de las semillas, el peso de cien semillas, el contenido de aceite, el rendimiento de aceite por planta, el peso de las semillas frescas, el peso de las semillas secas (Sunil *et al* 2011; Wani *et al*., 2012; Han *et al*. en 2015; Nietsche *et al*.,2015; Aguilera-Cauich *et al*., 2015; Rao *et al*., 2015). Los descriptores se han utilizado para caracterizar el fruto: número de fruto por racimo, número de frutos por rama, número de frutos por planta, longitud del fruto, anchura del fruto, forma de fruto, peso fresco, peso seco, número de semillas por fruto. Los descriptores de flores son el número total de flores por rama, el número total de flores masculinas por ramas, el número total de flores femeninas por rama, la proporción de flores masculinas y femeninas y el número total de flores por planta (Sunil *et al* 2011; Aguilera-Cauich *et al*., 2015, Nietsche *et al*.,2015; Arolu *et al*., 2015).

1.2.3. Marcadores Moleculares

Los marcadores moleculares son muy valorados en los campos de investigación con base genética, debido a su capacidad para distinguir genotipos (Grover y Sharma, 2016).

Se han desarrollado y aplicado con éxito diferentes tipos de marcadores de ADN molecular en actividades genéticas y de fitomejoramiento en diversos cultivos agrícolas y *Jatropha* no es una excepción. La siguiente sección proporciona información breve relacionada con los marcadores moleculares según su método de detección.

Los polimorfismos en la longitud de fragmentos amplificados (AFLP) es una técnica con base en PCR (Reacción en cadena de la polimerasa). Se desarrolló debido a las limitaciones de otras

técnicas como RAPD (Amplificación aleatoria de ADN polimórfico) (baja reproducibilidad) y RFLP (Polimorfismos de longitud de fragmentos de restricción). Los marcadores AFLP son rentables y no se necesita información de secuencia previa. En AFLP, se puede usar ADN de buena calidad y parcialmente degradado; sin embargo, este ADN no debe contener enzimas de restricción ni inhibidores de la PCR (Nadeem *et al*., 2018). Los AFLP consisten en la digestión completa del ADN genómico total con enzimas de restricción, seguida de la amplificación selectiva de los fragmentos obtenidos para detectar polimorfismos debidos a mutaciones en la secuencia de ADN en o cerca de los sitios de restricción. Los polimorfismos se detectan mediante electroforesis como un patrón de fragmentos de ADN amplificados (bandas) que difieren en número y tamaño, este patrón es altamente específico y debido a las restricciones de la técnica es altamente reproducible (Vos *et al*., 1995).

Los marcadores AFLP se han empleado con éxito para estimar variaciones genéticas entre especies del género *Jatropha* en Costa Rica (*J. curcas*, *J. stevensii*, *J. costaricencis* y *J. gossypifolia*) y para establecer las relaciones filogenéticas entre ellas (Avendaño *et al*., 2015). Konan *et al* (2018), evaluaron setenta genotipos de *J. curcas* de África (Senegal, Malí, Burkina Faso y Madagascar) y Ecuador utilizando una combinación de cebadores AFLP, revelaron una alta diversidad genética.

En Brasil, Pioto *et al*. (2015), estudiaron la diversidad genética de 55 accesiones de *Jatropha* utilizando marcadores AFLP y encontraron más del 80% de polimorfismo que proporcionó un alto poder discriminativo para la clasificación de accesiones de germoplasma en diferentes grupos.

Singh *et al*. (2016), empleó marcadores AFLP y poblaciones de retrocruzamiento para estimar las tasas de cruzamiento entre *J. curcas* y *J. integerrima*.

Los marcadores SSR se utilizaron para analizar el alcance de la diversidad genética entre varias colecciones de germoplasma en todo el mundo (Siju *et al*., 2016). Sin embargo, los estudios basados en marcadores moleculares revelaron resultados contrastantes sobre el alcance de la diversidad genética del cultivo. Algunos informes revelaron la existencia de una alta diversidad genética (Santos *et al*., 2016; Wen *et al*., 2010; Ovando-Medina *et al*., 2011) a moderada (Basha y Sujatha, 2007) a nivel bajo (Rosado *et al*., 2010; Zhang *et al*., 2012; Arolu *et al*., 2015; Vischi *et al*., 2013; Ouattara *et al*., 2014) e incluso a ninguna diversidad genética entre las accesiones de *Jatropha* (Yue *et al*., 2014).

Entre los marcadores moleculares disponibles, los microsatélites (repeticiones de secuencia simple - SSR) son los más estables genéticamente, específicos de especie y multialélicos. Las regiones de

microsatélites son muy variables y polimórficas (Santos *et al*., 2016). Los marcadores de microsatélites son robustos en su amplificación y se utilizan ampliamente en la caracterización de bancos de germoplasma, que incluyen cultivos como soya (Qiu *et al*., 2013), olivo (Cicatelli *et al*., 2013), uva (Salmaso *et al*., 2008) y *Jatropha*. Sanou *et al*. (2015), utilizaron marcadores SSR para estudiar la diversidad genética entre las accesiones de *Jatropha* y concluyeron un alto nivel de similitud entre ellas.

Entre los métodos disponibles y ampliamente utilizados para la caracterización de la diversidad genética se encuentran los marcadores moleculares denominados Inter-Secuencias Simples Repetidas (ISSR). Los marcadores ISSR son marcadores multiloci arbitrarios producidos por amplificación por PCR con cebador microsatélite, que ofrecen ventajas únicas sobre otros marcadores moleculares, ya que su aplicación no requiere información genómica previa de la especie en estudio, solo se requiere una pequeña cantidad de ADN molde y esta se realiza rápidamente (Nasir *et al*., 2017).

El método ISSR es útil para evaluar la diversidad genética e identificar cultivares estrechamente relacionados en muchas especies (Soonthornyatara *et al*., 2015). En plantas de *Jatropha* se ha evaluado la diversidad genética de accesiones de India y Brasil (Mastan *et al*., 2012; Grativol *et al*., 2011), Taiwán (Mavuso *et al*., 2016), África y Asia (Trebbi *et al*., 2015) revelando una baja diversidad. En contraste, se ha encontrado una alta variabilidad genética en accesiones en América (Li *et al*., 2017; Vásquez-Mayorga *et al*., 2017).

Cuadro 1. Comparación entre marcadores moleculares más empleados (Adhikari *et al*., 2017).

Característica	**AFLP**	**RAPD**	**SSR**	**ISSR**
Concentración de ADN requerida	0.5-1.0	0.02	0.05	0.05
Calidad de ADN	Moderada	Alta	Moderada	Alta
Basados en PCR	Si	Si	Si	Si
Facilidad de uso	Fácil	Fácil	Fácil	Fácil
Reproducibilidad	No reproducible	Alta	Alta	Alta
Costo por análisis	Moderado	Bajo	Bajo	Bajo

1.2.4. Análisis Multivariados para Clasificar Germoplasmas

El análisis multivariado se ha utilizado con éxito para clasificar y ordenar la variación de rasgos tanto cuantitativos como cualitativos en diversos cultivos energéticos como el ricino (*Ricinus communis*) (Goodarzi *et al*., 2012), la caña de azúcar (*Saccharum officinarum*) (Santchurn *et al*., 2012), pasto energético (*Miscanthus sinensis*) (Slavov *et al*., 2013) y palma aceitera (*Elaeis guineensis* Jacq) (Abimbola *et al*., 2016).

Las técnicas multivariadas son herramientas importantes para predecir la diversidad genética, la clasificación del germoplasma, el orden de variabilidad de las accesiones y el análisis de las relaciones genéticas entre las características y el material genético existente (Abdi y Williams, 2010). El interés en explorar alternativas a la regresión lineal ordinaria, como el modelado predictivo multivariante basado en variables latentes, está aumentando (Costa *et al*., 2014). Entre estas técnicas, podemos destacar el análisis de componentes principales (PCA) y el análisis de conglomerados (Prado *et al*., 2017).

El uso de algoritmos estadísticos multivariados es una estrategia importante para clasificar el germoplasma, ordenar la variabilidad para un gran número de accesiones y analizar las relaciones genéticas entre los materiales de mejoramiento (Mohammadi y Prasanna 2003).

1.2.5. Análisis de Componentes Principales

Es una técnica matemática utilizada para categorizar una gran cantidad de variables en componentes principales y evaluar su contribución a la variación total, incluso para informar qué rasgos tienen mayores contribuciones a la variación total disponible (Basu *et al*., 2017).

Los componentes principales (PC) permiten revelar patrones de características suficientemente discriminatorios para la adecuada selección de progenitores que pueden ser utilizados en programas de mejoramiento para incrementar el rendimiento (Das *et al*., 2017).

El primer componente principal es el que más contribuye a la variación total de la población, seguido de los componentes posteriores. Los primeros tres componentes principales son a menudo los más importantes para reflejar los patrones de variación entre accesiones, y los rasgos asociados con estos son más útiles para diferenciar las accesiones (Das *et al*., 2017). Sin embargo, la interpretación de la salida de un análisis de PC requiere que se determine la importancia de las PC

y las variables asociadas con cada PC. Se utilizan dos criterios para juzgar si un PC es significativo. El primero requiere un examen del valor propio. Si este número es 1.0, entonces, teóricamente, el PC correspondiente tiene inherentemente más información que cualquier variable por sí sola. Todos los PC con un valor propio 1.0 estarían sujetos a interpretación.

El investigador puede tener motivos para creer que cualquier PC es importante si explica un cierto porcentaje de variación total en un conjunto de datos. Si se puede asignar un significado biológico al PC a partir de un examen de los valores propios, entonces aumenta la confianza en la importancia del PC (Iezzoni y Pritts, 1991).

La aplicación de métodos multivariados a descriptores morfológicos en *Jatropha* fue informada por Kumar *et al*. (2008), quienes describieron variaciones intraespecíficas e interrelaciones entre rasgos morfológicos, nutricionales y bioquímicos en 27 accesiones de *Jatropha* curcas. El PCA explicó el 58% de la variación total en los rasgos medidos y reveló que había una correlación negativa entre cuatro rasgos morfológicos y todos los compuestos nutricionales como proteína cruda, fibra detergente neutra, fibra detergente ácido, lignina, hemicelulosa y celulosa.

Singh *et al*. 2016, utilizaronPCA para analizar el patrón de variación a fin de distinguir las mejores características entre accesiones parentales para la reproducción de plantas; al encontrar una correlación negativa en el contenido de aceite y el número de ramas secundarias, mencionan que esto brinda una oportunidad de mejora. Por otro lado, Aguilera-Cauich *et al*. 2015 identificaron alta variabilidad entre accesiones de América, concluyen que puede deberse a que México es el centro de origen del género *Jatropha*.

Vera-Castillo *et al*. 2014, evaluaron la biodiversidad del germoplasma nativo de *Jatropha* curcas utilizando rasgos morfológicos. Identificaron los rasgos más discriminantes a través del PCA, el primer componente estaba más fuertemente asociado con los caracteres relacionados con el tamaño del fruto: peso, longitud y diámetro del fruto entero y peso del pericarpio. El segundo componente estuvo fuertemente asociado con las hojas, específicamente las siguientes: largo del pecíolo y largo y ancho del limbo.

El Análisis de Componentes Principales (PCA) se utilizó como una de las diversas técnicas para la detección de posibles relaciones entre las procedencias de *Jatropha* curcas a pesar de la distribución espacial (Zapico *et al*., 2011).

La agrupación basada en análisis de componentes principales es un procedimiento de dos pasos que implica la descomposición de datos genotípicos en componentes principales ortogonales

ordenados (PC) (o vectores propios) de importancia decreciente (es decir, valores propios) seguido de la agrupación jerárquica de la matriz de distancia euclidiana obtenida de un subconjunto de PC importantes (Odong *et al*., 2013).

1.2.6. Análisis de Clusters o Conglomerados

Las técnicas de agrupamiento jerárquico, como Ward y el método de grupos de pares no ponderados con media aritmética (UPGMA), todavía se encuentran entre los métodos más utilizados para describir la estructura genética de colecciones de germoplasma (Odong *et al*., 2013).

Es un método matemático utilizado principalmente para la formación de grupos con características similares a partir de las similitudes o disimilitudes que se dan entre pares (Núñez-Colín y Escobedo-López, 2015). Este tipo de análisis se compone de dos métodos interrelacionados e igualmente importantes. El primero es el cálculo de los índices de similitud o disimilitud entre pares y el segundo es la aplicación del método de aglomeración adecuado mediante el uso de representaciones gráficas (Núñez-Colín y Escobedo-López, 2011).

Varias investigaciones han realizado análisis de conglomerados para encontrar diferencias y / o similitudes dentro y entre especies del género *Jatropha*, en accesiones de India (Kaushik *et al*., 2007; Gupta *et al*., 2008; Sunil *et al*., 2013; Basu *et al*., 2017); Taiwán (Mavuso *et al*., 2016); China (Cai *et al*., 2010); Egipto (Abou *et al*.,2016); Brasil (Rosado *et al*., 2010; Pioto *et al*., 2015); África, Asia, América del Sur y México (Pecina-Quintero *et al*., 2011; Aguilera-Cauich *et al*., 2015; Trebbi *et al*., 2015).

1.2.7. *Jatropha platyphylla*

Jatropha platyphylla pertenece al Phylum: Magnoliophyta, clase Magnoliopsida, orden:

Malpighiales, familia: Euphorbiaceae, subfamilia: Crotonoidae, tribu: Jatropheae, género: Jatropha y epíteto específico: platyphyllla Müell (Dehgan y Webster, 1979).

Su sinonimia es *Jatropha peltata* Sessé (Steinmann, 2002; ITIS, 2010). El nombre vernáculo es Bonete, por la forma que presenta el fruto (Martínez, 1979). En algunos lugares de México se le llama "Sangregado", sangre de toro y sangre de grado, debido a que el látex exudado tiñe de rojo (Schroeder, 2006).

J. platyphylla es una planta que puede medir hasta 10 m de altura, se distribuye en el bosque tropical caducifolio (Ramírez, 2009). Es nativo de México encontrándose a lo largo de Sinaloa, Nayarit, Jalisco, Michoacán y Durango (Figura 2) (Gutiérrez, 2011; Vega, 2007; SEMARNAT, 2004). Presenta individuos dioicos y monoicos. Su descripción botánica incluye a los tallos que son pardo amarillentos, es una planta poco ramificada, las ramas jóvenes son densamente tomentosas, suaves y suculentas, con hoyos aplanados, extendiéndose, densamente tomentosas, sin descamación, látex copioso, turbios en brotes más jóvenes pero rojizos en las partes más viejas de la planta; tronco de aproximadamente 30 cm de diámetro.

Figura 2. Mapa de la distribución geográfica de *J. platyphylla*. a) Sinaloa, b) Nayarit, c) Jalisco, d) Michoacán e) Durango (Gutiérrez, 2011).

Las hojas son deciduas; con estípulas lineales a lanceoladas, 1.5-2 mm de largo; pecíolos 8-14.5 cm de largo y 2.5-4.5 mm en diámetro, glabro; láminas peltadas, orbiculares, 13-36 x 9-27 cm, rojizas cuando son jóvenes, con 3-5 lóbulos poco profundos, 6-20 cm de ancho, membranosas o más o menos cartáceas, base poco cordadas, márgenes enteros (no glandulares, excepto en las

eofilas) (Martínez, 1979), ápices ampliamente agudos a acuminados, venación palmada, con 7-9 venas rojas rosadas primarias que irradian desde el centro y notablemente engrosadas y levantadas en el lado abaxial; escasamente pubescente en las venas de ambas superficies (Figura 3).

Figura 3. Hojas y fruto *de J. platyphylla.*

Inflorescencias: terminal que se vuelve lateral con crecimiento continuo; estaminados grandes y con numerosas paracladias pero pistiladas mucho más pequeñas y con pocas paracladias; pedúnculos 1-19 cm de largo en estaminados y 2-9 cm de largo en pistilados; brácteas ovado-lanceoladas, 1-7 mm de largo; pedicelos de 2-3 mm de largo en flores estaminadas y 4.5-10 mm de largo en pistilados. Las flores estaminadas tienen sépalos ovados, poco pubescentes en ambas superficies, pero a veces glabros en la superficie adaxial, redondos en el ápice, márgenes completos, libres, de 2.5-3.5 x 15-2.2 mm de ancho; corolas tubular-campanuladas, blancas; pétalos de 7-8 x 2,5-3 mm, connados a de longitud, ápices redondos, fuertemente reflexos, tomentosos en la superficie adaxial y glabros en los abaxiales; estambres 10, biseriato monadelfo (5 + 5), filamentos de la serie exterior de 2,5-4 mm de largo y connatos alrededor de ½ de longitud, las de serie interior 4-5 mm y connatas alrededor de ¾ de largo, anteras de 1.1-1.5 mm de largo. Flores pistiladas: como estaminadas pero sépalos foliares, 9-13 x 3-7 mm; pétalos 8-10 x 4-5 mm; carpelos 3 o excepcionalmente 4 en la flor central superior; estilos 3, 1.5-2 mm de largo, connados ¼-½ de longitud. Las flores son blancas a rosadas; las plantas florecen en mayo, junio y julio (Noguera, 2002). Cápsulas: 3-locular (o raramente 4-locular), ovalada, 2.7-3.8 x 2.5-3 cm, más angosta en la parte superior que en la inferior (Standley, 1967) (Figura 3). Semillas: café claro con moteado más oscuro, casi esférico, 1.8-2.2 cm de diámetro, vestigio carúnculo (Dehgan, 2012) (Figura 4).

Figura 4. Semillas y flores de *J. platyphylla.*

1.2.8. Composición química de *Jatropha platyphylla*

Las semillas de plantas silvestres de *J. platyphylla* poseen un alto contenido de aceite (60.3%) en el germen y su composición del perfil de ácidos grasos es basado en la presencia de un 53.7% de ácido linoleico, oleico (23.1%), palmítico (13.2%), esteárico (7.5 %) y en menos proporción palmitoleico (0.7%), mirístico (0.2%), araquídico (0.2%) e icosenoico (0-0.2%). Esta composición de ácidos grasos lo hace similar a los aceites de canola, soya y ajonjolí (Sosa-Segura *et al*., 2014), Además contiene un 27% de proteína aproximadamente, aunque en semillas desgrasadas este porcentaje aumenta (66.4%) (Makkar *et al*., 2011).

Se ha reportado que tanto el aceite como el biodiesel de *J. platyphylla*, cumple con los parámetros establecidos por normas oficiales, el porcentaje de ácidos grasos libres es de 0.3, el índice de yodo de 91.1 $g \cdot 100^{-1}$ de muestra, el índice de peróxido de 3.5 miliequivalente de peróxido kg^{-1} de muestra y el índice de saponificación de 186.4 mg de KOH requeridos para saponificar 1 g de muestra (Altamirano, 2011).

Esta especie *de Jatropha* no presenta niveles detectables de compuestos tóxicos conocidos como esteres de forbol que son los responsables de envenenamiento en ganado y ser promotores de tumores (Makkar *et al.,* 1998). Respecto a los compuestos antinutricionales, el contenido de inhibidores de tripsina osciló entre 13.83 y 24.96 expresado en unidades de inhibición de tripsina (TIUs), el de fitatos fue de 1.69 a 2.44% equivalentes a ácido fitico en base fresca y la cantidad de

saponinas de 1.05-1.23% equivalentes a diosgenina (Gutierrez, 2011).

También se ha detectado compuestos con posible poder antioxidante como compuestos fenólicos y lipófilicos en hoja, pulpa y germen de la planta (Ambriz *et al*., 2016).

En la hoja se han detectado los compuestos fenólicos: Floridzina, Apigenina 8-C-glucósido, Luteolina 6-C-glucósido, Apigenina O-pentosil 8-C-hexosil, Luteolina 6-O-glucósido, Apigenina 7-O-glucósido, Genisteina 7-O-glucósido y Luteolina 8-C-glucósido y los compuestos lipófilicos como n-octadecanol, Ácido petadecanoico, Lanosterol y ζ-sitosterol. En la pulpa se han detectado los siguientes compuestos fenólicos: Apigenina 8-C-glucósido, Apigenina O-pentosil 8-C-hexosil y Apigenina 7-O-glucósido Genisteina 7-O-glucósido y los compuestos lipófilicos Ácido oleico, Hexadecanol y Ceteno. Y en el germen se han detectado el Ácido 3-O-p-cumaroilquinico, Apigenina 7-O-glucósido, Succinato de luteolina 6,8-di-C-glucósido, Apigenina 7-O-neohesperosida, Apigenina 8-C-glucósido y la Apigenina O-pentosil 8-C-hexosil y los compuestos lipófilicos Ácido pentadecanoico, Ácido palmítico, Ácido esteárico, Ácido benceno propanoico y Ácido betulínico (Ambriz *et al.,* 2016).

A través de la técnica de HPTLC (High Performance Thin Layer Chromatography) se logró detectar la presencia de 8 a 13 tipos de flavonoides, 7 a 13 terpenos y un alcaloide en extractos metanólicos de corteza (Leyva, 2018).

1.2.9. Importancia y usos de *Jatropha platyphylla*

En un ensayo con tilapia del Nilo (*Oreochromis niloticus*) se probó la inocuidad de la inclusión de harina de *J. platyphylla*. Se alimentaron los peces durante 12 semanas con una dieta que remplazó el 50% de harina de pescado por harina de *J. platyphylla*, previamente tratada con temperatura (121 °C durante 20 minutos). La harina de pescado sirvió como control y la harina de soya se utilizó para comparación. El crecimiento de los peces en los tres tratamientos fue similar y los parámetros bioquímicos que se utilizaron como marcadores de toxicidad se registraron dentro de los rangos normales (Oyeleye, 2009). Estos resultados sugieren que *J. platyphylla* puede ser considerada como una planta no tóxica y que se puede añadir a la lista de plantas comestibles de México.

J. platyphylla se incluye dentro de la flora útil del municipio de la Huerta, Jalisco, indicando que se utiliza en la medicina tradicional (Rendón, 1999) y en otros usos como el cercado de parcelas o cercas vivas para el control del viento (Rodríguez, 2006). Aunado a esto, esta planta tiene importancia cultural y nutrimental ya que forma parte de alimentos realizados tradicionalmente por pobladores de la región sur de Sinaloa. Los frutos inmaduros y las semillas se preparan en conservas o mermeladas (Makkar *et al*., 2011).

Se han utilizado extractos metanólicos de hojas y corteza de *J. platyphylla* para inhibir el crecimiento micelial hasta un 80.95% en el medio de cultivo de *Aspergillus parasiticus* y 75.5% en *Fusarium verticillioides* durante las primeras 48 h; mientras que la germinación de ambos hongos fue inhibida por al menos 12 h utilizando la IC_{50} a concentraciones de 2.79±0.13 mg/mL para *A. parasiticus* y 8.04±0.66 mg/mL. Estos resultados sugieren que el grupo de alcaloides *de J. platyphylla* poseen actividad fungiestática (Leyva, 2018).

1.3. Hipótesis

1. Las características de las semillas como el peso de semilla, diámetro de semilla, peso de germen y peso de testa son los descriptores con mayor poder discriminatorio para la selección de germoplasmas élite.
2. Los marcadores ISSR permiten detectar polimorfismos entre los germoplasmas
3. La técnica de injerto produce plantas con rendimientos similares o mejores en los descriptores morfológicos y de producción evaluados en comparación con las plantas no injertadas

1.4. Objetivo General

Identificar los individuos élite de *J. platyphylla* localizados en la selva baja caducifolia de Sinaloa y Durango mediante el análisis de la diversidad morfométrica, de producción y diversidad genética

de germoplasmas.

1.5. Objetivos Específicos

- Caracterizar la variabilidad morfométrica y productiva de germoplasmas de *Jatropha platyphylla* e identificar los individuos élite.
- Determinar la diversidad genética de germoplasmas de *J. platyphylla* mediante marcadores ISSR (Inter secuencias simples repetidas).
- Evaluar el crecimiento y desarrollo de germoplasmas injertados y no injertados mediante las caracterizaciones morfométricas y de producción.

1.6. Sección Integradora del Trabajo

Los estudios desarrollados para cumplir con los objetivos propuestos se encuentran plasmados en los diferentes capítulos que conforman esta tesis, los cuales fueron escritos conforme al formato de envío para las diferentes revistas científicas.

El primer artículo es un artículo de investigación original publicado en la revista Scientia Horticulturae en el mes de octubre de 2019. En este artículo se cumplió con el objetivo de analizar la variabilidad morfológica y el contenido de aceite de *J. platyphylla* para determinar las características y establecer estrategias de domesticación. Se realizó un análisis de componentes principales en 11 rasgos morfológicos cuantitativos de la planta: altura de la planta, diámetro del tallo, extensión del dosel, cantidad de ramas, producción total de fruto, peso de fruto seco, peso de la semilla, diámetro de la semilla, peso del germen, peso de la testa y contenido de aceite. Se encontraron diferencias significativas en ocho de los rasgos. Además se realizaron mapas de cuadrícula para conocer la diversidad y distribución de *J. platyphylla* en los rasgos: producción total de fruto y porcentaje de aceite. En el análisis de componentes principales, los primeros tres componentes explicaron el 70.2% de la varianza en los datos y mostraron que los rasgos que más

contribuyeron a las diferencias fueron las características morfológicas de las semillas. En el análisis de conglomerados, se obtuvieron siete conglomerados de los cuáles tres grupos agruparon los germoplasmas con los porcentajes más altos de aceite. Las variaciones en el contenido de aceite y las variables morfológicas sugirieron oportunidades para una selección viable para programas de mejoramiento genético, considerando que *J. platyphylla* es una especie silvestre. La búsqueda de plantas adaptadas a la región y altamente productivas para la generación de híbridos pueden crear valor agregado a estas especies no domesticadas y poco explotadas.

El segundo artículo derivado de esta investigación, es un artículo de investigación original preparado para ser enviado a la revista 3Biotech. En este artículo se cumplió con el objetivo de determinar la diversidad genética de germoplasmas mediante marcadores ISSR (Inter secuencias simples repetidas) y rasgos morfológicos. Siete accesiones de *Jatropha platyphylla* fueron evaluadas por sus rasgos morfológicos y utilizando marcadores moleculares ISSR. Los análisis de conglomerados se realizaron con nueve rasgos: número de ramas por planta; frutos por racimo; racimos por rama; racimos por planta; producción total de semillas; producción total de frutos, contenido de proteína, contenido de aceite y el perfil de ácidos grasos. Los genotipos PR11 (genotipo obtenido de la región sur de Sinaloa, El Rosario) produjeron los valores más altos en todos los rasgos analizados. El análisis de correlación de los rasgos cuantitativos mostró altas correlaciones entre la producción de semilla y la producción total de fruto ($r = 0.99$). Los ácidos grasos más abundantes fueron el ácido linoleico (57.64-52.39%). Las variaciones en el contenido de aceite y las variables morfológicas sugirieron oportunidades para una selección viable en los programas de domesticación y mejora genética. Los resultados de este estudio pueden considerarse un punto de partida para futuras investigaciones destinadas a definir el nivel de diversidad genética para detectar accesiones prometedoras para generar híbridos de *Jatropha platyphylla*. Para hacer esto, se debe analizar un mayor número de poblaciones naturales recolectadas y probar marcadores ISSR adicionales. Además, podrían clonarse y secuenciarse bandas discriminantes. Estos estudios han dado pistas importantes para comprender la relación genotipo-fenotipo, lo que puede ayudar aún más a desarrollar estrategias de reproducción de las plantas.

El tercer artículo es un artículo de investigación original preparado para enviar a la revista European Journal of Agronomy. Para cumplir con el tercer objetivo de este trabajo de investigación, que fue evaluar el crecimiento y desarrollo de germoplasmas injertados y no injertados mediante una caracterización morfométrica y de producción, fue necesario abordar el

uso de esquejes en la propagación vegetativa y el injerto como una herramienta indispensable para la multiplicación en masa de genotipos élite. Se obtuvo un 76% de supervivencia de las plantas injertadas. Las plantas injertadas obtuvieron los valores más altos en los rasgos fenotípicos evaluados. Las correlaciones más altas se encontraron en la producción total de frutos y la producción total de semillas. El ácido graso insaturado más abundante fue el linoleico. Esta evaluación permitió identificar plantas injertadas de *J. platyphylla* con rendimiento específico y características fenotípicas para la propagación a gran escala en un tiempo relativamente corto.

2. MORPHOLOGICAL VARIABILITY AND OIL CONTENT OF *Jatropha platyphylla* MÜLL. ARG. GERMPLASM AS DETERMINED USING MULTIVARIATE ANALYSIS

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Morphological variability and oil content of *Jatropha platyphylla* Müll. Arg. germplasm as determined using multivariate analysis

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ABSTRACT

Jatropha platyphylla is a species of the Euphorbiaceae family with kernels rich in oil and protein with no toxicity. The aim of this study was to analyze the morphological variability and oil content of *J. platyphylla* in tropical deciduous forests of Mexico to determine the characteristics and establish domestication strategies. Principal component and cluster analyses were performed with 11 traits: plant height, stem diameter, canopy spread, number of branches, total fruit production, dry fruit weight, seed weight, seed diameter, kernel weight, shell weight and oil content. Significant differences were found in eight of the traits. Grid maps of the distribution pattern, total fruit production and oil percentage were generated. In principal components analysis the three components explained 70.2% of the variance in the data and showed that the traits that contributed most to the variance were the morphological characteristics of seeds. In the cluster analysis, seven clusters were obtained, of which three clusters grouped the germplasms with the highest oil percentages. The variations in oil content and morphological variables suggested opportunities for viable selection in domestication and genetic improvement programs, considering that *J. platyphylla* is a wild species. Exploring adapted varieties and producing interspecies hybrids can create added value to these little explored species.

1. Introduction

The evaluation and the characterization of germplasm collections have strategic applications to breeders and researches in crop improvement (Nietsche et al., 2015). Among different markers, morphological characters must be recorded for selection of parents and are also the first choice used for describing and classifying the germplasm (Balduzzi et al., 2017; Jingura and Kamusoko, 2015). The prerequisites for the potential use of *Jatropha* species are systematic selection, breeding and domestication to achieve higher productivity and production uniformity (Achten et al., 2010). The use of standard traits is necessary for the optimal characterization of genetic resources and for valid data comparisons among trials (Freitas et al., 2011).

Multivariate techniques are useful tools for determining genetic diversity and classifying germplasm (Uyeda et al., 2015). Among these techniques, principal component analysis (PCA) is a mathematical technique used to categorize large numbers of variables into major components. PCA can be used to identify characters informative for germplasm characterization and to assess their contribution to total variation (Singh et al., 2016). In addition, cluster analysis of phenotypic data can be used to identify geographic regions where certain genetic and/or environmental factors are favorable to seed quality (Montes et al., 2013). It is considered important to collect and introduce as many provenances as possible of valuable species. This practice allows the creation of germplasm banks in different locations where the material increases circumstantially and can be characterized in a convenient way (Senger et al., 2016). Multi-environmental field trials have recently been set up and detected significant effects on various traits in Jatropha germplasm (Rao et al., 2015; Senger et al., 2016). After intensive field testing these banks can serve as necessary bases for the identification and selection of elite germplasm with highest yield performance, yield stability and other relevant traits for future use to be released as cultivars or to serve as parental genotypes for subsequent breeding cycles. Several investigations have focused on phenotypic characteristics to

Abbreviations: DM, Dimas; LC, Chilla; LH, Higuerita; PP, Mocorito; PR, Rosario; QP, Quelite; TP, Tamazula; PH, plant height; DBH, stem diameter; CS, canopy spread; BP, number of branches per plant; TFP, total fruit production; DFW, dry fruit weight (DFW); SW, seed weight; SD, seed diameter; KW, kernel seed weight; SS, shell seed weight; OP, percentage oil content; PCA, Principal component analysis; CVs, coefficients of variation; masl, meters above sea level

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facilitate germplasm selection, such as in *J. curcas* plants (Kaushik et al., 2007; Sunil et al., 2009; Mishra, 2009). These investigations have analyzed the genetic variability associated with phenotypic traits, such as seed characteristics, oil content, plant height, stem diameter, number of primary branches, petiole length, number of fruits per cluster, and pedicel length, as indicators of agronomic quality of Indian germplasm of *J. curcas*.

Jatropha platyphylla (Mull. Arg.) is a multipurpose and drought-resistant shrub or small tree 2–5 m in height that grows in the tropical deciduous forests of Mexico. This plant is able to grow on marginal lands, does not compete with food production and has low ecological impact. The immature fruits and roasted seeds of *J. platyphylla* are consumed within local communities in Mexico. The kernel contains undetected quantities of toxic compounds, such as phorbol esters, as in the case of *Jatropha curcas*. The kernel meal of *J. platyphylla* contains lectins, phytate and trypsin inhibitor. Both lectins and trypsin inhibitor are heat labile, which explains why the local people can eat the roasted seeds without any ill effects; therefore, the seeds are considered a safe source of edible protein and oil (Makkar et al., 2011; Akinleye et al., 2012). The kernel contains 50–60% oil and defatted flour in 75% of crude protein. The leaves and fruits are rich in phenolic compounds with anti-inflammatory activity (Ambriz-Pérez et al., 2016). *J. platyphylla* has nutritional potential, motivating investigations on its chemical and antioxidant contents to find new promising sources of natural antioxidants. In addition, the seeds have high oil contents, with chemical characteristics useful for producing biodiesel.

Due to the great potential of *J. platyphylla*, it is important to perform genotype characterization for domestication and to obtain elite genotypes for future use in breeding programs. Therefore, the present study aimed to evaluate phenotypic diversity in a *J. platyphylla* germplasm using morphological traits and multivariate analyses to allow selection of the most divergent and superior genotypes.

2. Materials and methods

2.1. Collection of plant material and spatial distribution

The present investigation was conducted in April 2017 and involved eight wild populations of *J. platyphylla* growing under the tropical conditions of Sinaloa and Durango States in northwestern Mexico. Sampling of 37 plants was conducted at the following eight sites: Cofradia (CP) (n = 2), Dimas (DM) (n = 2), Chilla (LC) (n = 6), Higuerita (LH) (n = 2), Mocorito (PP) (n = 2), Rosario (PR) (n = 15), Quelite (QP) (n = 4), and Tamazula (TP) (n = 4). The geolocation data of each site were recorded.

Soil samples were collected and analyzed by the Bouyoucos densimetry method (Grossman and Reinsch, 2002). The yearly average values of maximum (Tmax, °C) and minimum (Tmin, °C) temperatures, relative humidity (HR%) and precipitation (Pp, mm) (CIAD, 2017) at the collected sites were recorded. During data collection, germplasms from wild populations, in greater or lesser abundance, that were well ramified and vigorous with abundant branching and little or no evidence of disease were given priority. However, data were also collected from germplasms located along the border fences of paddocks and other crops provided they showed no evidence of successive previous prunings.

To determine the spatial distribution of *J. platyphylla* and evaluate germplasm diversity, grid maps were constructed with the software DIVA-GIS 7.5.0 from latitude and longitude recorded in decimal degrees (Hijmans et al., 2004; Scheldeman and Zonneveld, 2011). Shannon's index (H´) was estimated for total fruit production (TFP) using the formula $H' = -\Sigma P_i \ln P_i$ ($i = 1–S$), where S is the number of species present, ln is natural logarithm and Pi is the proportion of species. $Pi = ni / N$, where ni is the number of individuals of species i, and N is the total number of individuals (Shannon, 1948).

2.2. Measurement of morphological traits and oil content

The morphological characterization of *J. platyphylla* germplasm was based on 11 traits: plant height (PH), stem diameter (DBH), canopy spread (CS), number of branches per plant (BP), total fruit production (TFP), dry fruit weight (DFW), seed weight (SW), seed diameter (SD), kernel seed weight (KW), shell seed weight (SS) and percentage oil content (OP) (Sunil et al., 2013). PH, DBH and CS were recorded using a measuring tape, and BP was obtained by counting each branch (Sosa-Segura et al., 2012). Fruit and seeds (n = 20) were weighed individually (Sartorius), and diameter was measured in millimeters with a Vernier caliper. The data obtained were organized in a matrix to facilitate storage, access and retrieval with Microsoft Excel 2013 (v15.0) software. Oil percentage was determined according to the method recommended by the Association of Official Analytical Chemists (AOAC, 1998) technique 920.39 and reported as a percentage of ether extract.

2.3. Data analysis

The morphological traits and oil content were evaluated through descriptive statistics to obtain the mean, standard deviation, maximum and minimum values and the coefficients of variation (CVs). Analysis of variance (ANOVA $p < 0.05$) was performed to identify significant differences among germplasms for comparison of means followed by Fisher post hoc tests. Variables exhibiting significant differences were subjected to principal components analysis (PCA). In addition, a correlation analysis was performed of morphological characteristics and environmental factors. To evaluate the relationships among the different variables, correlation coefficients were calculated with MINITAB 17. The morphological data and oil content were further analyzed using Euclidian distance coefficients by cluster analysis (Karuri et al., 2010).

3. Results

3.1. Morphological characterization and oil content

To assess the phenotypic diversity of *J. platyphylla*, we evaluated the morphological traits and oil content to allow the selection of the most

Table 1
Information on the sites at which germplasms of *J. platyphylla* were collected and climatological data were recorded. The yearly average values of maximum temperature (Tmax, °C), minimum temperature (Tmin, °C), relative humidity (RH%) and annual precipitation (Pp mm) are presented.

ID	Collection area	Latitude (°N)	Longitude (°W)	Altitude (masl)	Type of soil	Tmax (°C)	Tmin (°C)	RH (%)	Pp (mm)
CP	Cofradia, Sinaloa	24°51'448"	107°11'002"	159	Sandy-Loam	33.4	16	78.8	881.2
DM	Dimas, Sinaloa	23°45'019"	106°46'356"	4	Sandy-Loam	29.5	17.1	79.1	481.2
LC	Chilla, Sinaloa	24°23'276"	107°06'177"	74	Sandy-Clay-Loam	31.2	18.2	74.5	790
LH	Higuerita,Sinaloa	24°45'376"	107°08'391"	200	Sandy-Loam	35.2	22	80.1	881.2
PP	Mocorito, Sinaloa	25°04'058"	107°43'158"	52	Sandy-Clay-Loam	33.6	16.7	65	684.8
PR	Rosario, Sinaloa	23°11'185"	106°09'093"	15	Sandy-Clay-Loam	35.5	19.1	85.9	828.6
QP	Quelite, Sinaloa	23°31'513"	106°30'108"	58	Sandy-Loam	32.2	18.4	81.3	640.9
TP	Tamazula, Durango	24°59'122"	106°59'178"	263	Sandy-Loam	32.2	16.7	86	1031

Table 2
Results of statistical analyses of 11 morphological traits and oil content of *J. platyphylla* germplasms. CV: coefficient of variation (%), SD: standard deviation, PH: plant height, DBH: stem diameter, CS: canopy spread, BP: number of branches, TFP: total fruit production, DFW: dry fruit weight, SW: seed weight, SD: seed diameter, KW: kernel seed weight, SS: shell seed weight, OP: oil percentage, CP: Cofradia, Sinaloa, DM: Dimas, Sinaloa, LC: Chilla, Sinaloa, LH: Higuerita, Sinaloa, PP: Mocorito, Sinaloa, PR: Rosario, Sinaloa, QP: Quelite, Sinaloa, TP: Tamazula, Durango. * Different letters within a row indicate significant differences.

Germplasm	PH	DBH	CS	BP	TFP	DFW	SW	SD	KW	SS	OP
CP	480^{ab*}	14^{a}	364.5^{a}	27.5^{b}	85^{g}	5.5^{ab}	1.4^{abc}	15.1^{ab}	0.7^{a}	0.7^{bc}	56.8^{ab}
DM	455^{ab}	22.5^{a}	362.5^{a}	24.3^{b}	315^{e}	5.3^{ab}	1.6^{abc}	16^{ab}	0.7^{a}	0.9^{ab}	54.6^{b}
LC	506.7^{ab}	25^{a}	287.2^{a}	24.1^{b}	1060^{b}	4.5^{b}	1.4^{c}	15.1^{b}	0.6^{a}	0.7^{bc}	59.5^{a}
LH	446^{ab}	20^{a}	342.5^{a}	38^{b}	52^{h}	8^{a}	1.8^{abc}	16.6^{ab}	0.9^{a}	0.9^{ab}	60.3^{a}
PP	523.5^{ab}	26^{a}	451^{a}	56.5^{a}	850^{c}	5.8^{ab}	1.3^{bc}	15.6^{ab}	0.6^{a}	0.6^{c}	56.4^{ab}
PR	558.9^{a}	24.7^{a}	421.8^{a}	24^{b}	9700^{a}	4.8^{b}	1.8^{a}	16.1^{a}	0.8^{a}	0.9^{a}	59^{a}
QP	344.3^{b}	14.2^{a}	383^{a}	14.2^{b}	385^{d}	4.2^{b}	1.5^{abc}	15.8^{ab}	0.7^{a}	0.8^{abc}	59.5^{a}
TP	486^{ab}	23^{a}	375.8^{a}	43^{b}	205^{f}	4.8^{ab}	1.4^{abc}	15.4^{ab}	0.7^{a}	0.7^{bc}	59.5^{a}
Mean	499	22.8	377.3	27.5	367.1	4.9	1.6	15.8	0.7	0.8	58.7
Minimum	265	9	19	7	10	2.2	1.2	14.5	0.5	0.6	50.8
Maximum	750	43	668	71	2005	8.4	2.4	18.3	1.2	1.1	62.5
SD	128.1	9.21	150.3	18.4	471.2	1.5	0.2	0.7	0.1	0.1	2.3
CV	25.6	40.2	39.8	67.1	128.3	31.1	15.8	4.8	17.3	13.9	3.9

divergent and superior genotypes. Information on each germplasm is presented in Table 1.

The Rosario germplasm (PR) yielded the highest values of PH, DBH, CS, TFP, SW and SS (Table 2). The Mocorito germplasm (PP) was the germplasm with the most branches (highest BP). No significant differences among sites were found in canopy spread (CS), stem diameter (DBH) or oil percentage (OP).

Plant height (PH), stem diameter (DBH), canopy spread (CS), branches per plant (BP), total fruit production (TFP) and dry fruit weight (DFW) showed high CVs (> 20%). Intermediate CV values (10–20 %) were observed for seed weight (SW), kernel seed weight (KW) and shell seed weight (SS). Seed diameter (SD) and oil percentage (OP) had a low coefficients of variation (CV) (< 10%).

The correlation analysis of the morphological traits and oil content showed high correlations between pairs of seed traits: SW and SD, and KW and SS (r = 0.83 and, 0.88). SD was highly correlated with both KW and SS (r = 0.85 and 0.82). KW was highly correlated with SS (r = 0.88). PH had moderate correlations with DBH and CS (r = 0.42 and 0.57). Low correlations were observed between PH and TFP (r = 0.39) and TFP and CS (r = 0.22) (Table 3).

3.2. Influence of environmental factors on the morphological variation of *J. platyphylla*

A correlation analysis was performed with the morphological traits and oil content and environmental factors (Table 4). Moderate negative correlations were observed between both PH and DBH and soil type (r = −0.41 and r = -−0.33 respectively). TFP had moderate negative correlations with altitude (r = −0.62) and soil type (r = −0.69) and a high positive correlation with maximum temperature (r = 0.80). SW, SD, KW and SS were moderately correlated with the environmental variables maximum and minimum temperature and relative humidity (r = 0.322 to 0.587). SS was moderately negative correlated with altitude (r = −0.37).

3.3. Spatial distribution

A DIVA-GIS grid map was generated for the traits of TFP (those with the highest CV values) (Fig. 1). The highest diversity index (> 1.451) was observed for the germplasm collected in Rosario, located in the southern region of Sinaloa State, México.

3.4. Principal component and cluster analyses

In the principal component analysis (PCA) in the present study (Table 5), significant differences were found in eight traits: PH, BP, TFP, DFW, SW, SD, SS and OP. The first three components accounted for 70.2% of the total variation. The first principal component (PC1) accounted for 36.6% of the variability, the second principal component (PC2) accounted for 19.3%, and the third principal component (PC3) accounted for 14.4%. In the PCA, the first two components, PC1 and PC2, explained 55.8% of the variance in the data.

The graphical biplot interpretation of PC1 and PC2 revealed that the germplasms showed differences in a set of 8 traits (Fig. 2). PC1 was composed of two parameter groups; BP had a negative loading on this component, and SD, SW and SS had positive loadings on this component. This latter parameter group was highly collinear; the lines were oriented in the same direction in the plot and were very close to each other. The first group mainly represented traits of plant structure, whereas the second one represented parameters related to seed quality.

Table 3
Correlation coefficient (r) values of the morphological characteristics and oil content of *J. platyphylla* germplasm. PH: plant height, DBH: stem diameter, CS: canopy spread, BP: number of branches, TFP: total fruit production, DFW: dry fruit weight, SW: seed weight, SD: seed diameter, KW: kernel seed weight, SS: shell seed weight, OP: oil percentage. ***P* ≤ 0.05.**

	PH	DBH	CS	BP	TFP	DFW	SW	SD	KW	SS	OP
DBH	0.428*										
CS	0.579*	0.363*									
BP	−0.022	−0.001	0.012								
TFP	0.389*	0.215	0.223	−0.192							
DFW	−0.164	−0.197	−0.241	0.029	−0.125						
SW	−0.041	−0.009	−0.129	−0.053	0.530*	0.161					
SD	−0.173	0.044	−0.105	0.041	0.354*	0.174	0.839*				
KW	−0.175	−0.050	−0.252	−0.099	0.398	0.227	0.960*	0.857*			
SS	−0.012	−0.030	−0.045	0.025	0.580*	0.117	0.961*	0.822*	0.886*		
OP	−0.249	−0.006	−0.321*	−0.362*	0.095	−0.050	0.185	0.115	0.240	0.160	

Table 4
Results of correlation analyses of the morphological traits and oil content of *J. platyphylla* germplasm and environmental factors. PH: Plant height, DBH: stem diameter, CS: canopy spread, BP: number of branches, TFP: total fruit production, DFW: dry fruit weight, SW: seed weight, SD: seed diameter, KW: kernel seed weight, SS: shell seed weight, OP: Oil percentage. *$P \leq 0.05$. Maximum temperature (Tmax, °C) minimum temperature (Tmin, °C), relative humidity (RH%) and annual rainfall (Pp mm).

Environmental factors	PH	DBH	CS	BP	TFP	DFW	SW	SD	KW	SS	OP
Altitude (m)	−0.169	−0.120	−0.123	−0.064	−0.628*	0.185	−0.286	−0.213	−0.160	−0.370	0.163
Type of soil	−0.418*	−0.335*	−0.067	0.234	−0.691*	0.146	−0.172	−0.036	−0.067	−0.143	−0.082
Tmáx (°C)	0.301	0.102	0.248	−0.349*	0.809*	0.116	0.507*	0.414*	0.469*	0.483*	0.182
Tmin (°C)	0.073	0.063	0.027	−0.206	0.449*	0.207	0.512*	0.426*	0.476*	0.526*	0.349*
RH (%)	0.119	−0.002	0.148	−0.118	0.609*	−0.119	0.493*	0.322*	0.413*	0.587*	0.224
Pp (mm)	0.210	0.094	0.005	−0.378*	0.152	0.051	0.051	−0.063	0.088	−0.041	0.345*

DFW and OP had a large positive influence on PC2, indicating that this component represented productivity. PH and TFP had large negative influences on PC2.

The cluster analysis was performed using the Ward method and yielded the dendrogram shown in Fig. 3. Seven clusters were generated, with a cut-off level at which an abrupt decrease in similarity was observed. The germplasms in cluster 1 exhibited the highest oil contents, plant heights, stem diameters, canopy spreads and branch numbers, but they exhibited the lowest averages of total fruit production, single seed diameter and dry fruit weight. The germplasms in cluster 2 had the highest dry fruit weights and single seed diameters but not TFP. Cluster 3 was composed of germplasms with the highest single seed weights and SD, KW, SS and OP values but with the lowest branch numbers. Cluster 4 exhibited high values of PH, CS and TFP. The germplasms in Cluster 5 had the lowest values of TFP and OP. Cluster 6, despite exhibiting low values for all of the morphological traits, had high OP values and branch numbers. The germplasms in Cluster 7 had the highest numbers of branches but the lowest SW and SS values. Three of the seven clusters had high OP values.

4. Discussion

4.1. Morphological characterization and oil content

High CVs (> 20%) were found for PH, DBH, CS, BP, TFP, and DFW, indicating variability within the populations of *J. platyphylla* growing in these habitats. Sunil et al. (2009) found high CVs for several morphological characteristics of *J. curcas* plants, including the number of primary branches and height. The high variability in our analyzed traits may be due to high endemism (Steinmann, 2002), because Mexico and Central America are the center of origin of the species (Aguilera-Cauich et al., 2015). This region has the most diverse genetic resources of *Jatropha*, which can contribute to breeding projects (Ovando-Medina et al., 2013; Salvador-Figueroa et al., 2015; Rincón-Rabanales et al., 2016; Li et al., 2017). Knowledge of the variabilities of morphological characteristics and oil content is the basis of genetic improvements in breeding programs, particularly for the selection of elite genotypes with high oil content and high fruit and seed yields (Basu et al., 2017).

The identification of the morphological traits and oil content that

Table 5
Eigenvalues of the first three principal components of *J. platyphylla* germplasm. PC1: first principal component, PC2: second principal component, PC3: third principal component, PH: plant height, BP: number of branches, TFP: total fruit production, DFW: dry fruit weight, SW: seed weight, SD: seed diameter, SS: shell seed weight and OP: oil percentage.

Descriptor	PC1	PC2	PC3
PH	−0.046	−0.647	−0.208
BP	−0.202	−0.123	−0.338
TFP	0.052	0.628	0.006
DFW	0.120	−0.149	0.726
SW	0.569	0.055	−0.014
SD	0.533	0.000	−0.126
SS	0.567	0.084	−0.053
OP	0.105	−0.373	−0.545
Eigenvalue	2.925	1.541	1.151
Proportion	0.366	0.193	0.144
Cumulative	36.6	55.8	70.2

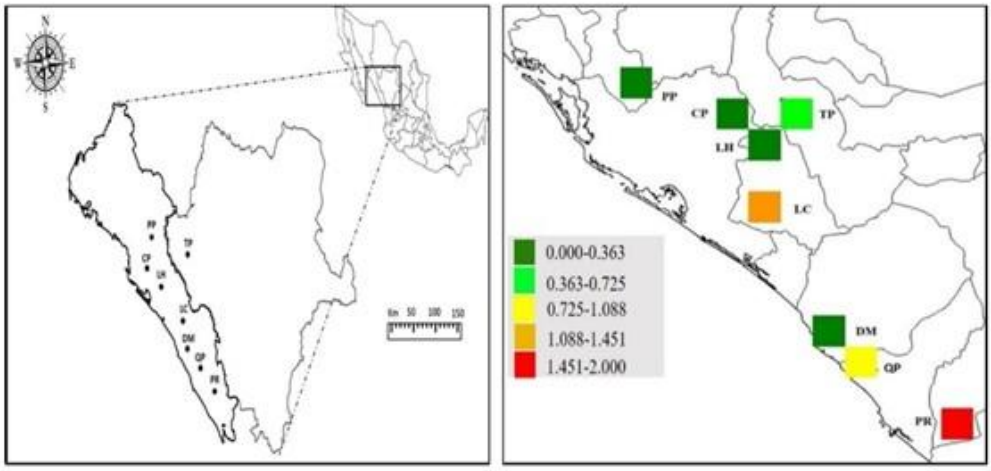

Fig. 1. Mapping of *J. platyphylla* germplasm using DIVA-GIS and the index of diversity for total fruit production (TFP). Colors in the grid indicate the degree of diversity in the germplasm. PR: Rosario, Sinaloa, LC: Chilla, Sinaloa, LH: Higuerita, Sinaloa, QP: Quelite, Sinaloa, TP: Tamazula, Durango, CP: Cofradía, Sinaloa, PP: Mocorito, Sinaloa, DM: Dimas, Sinaloa.

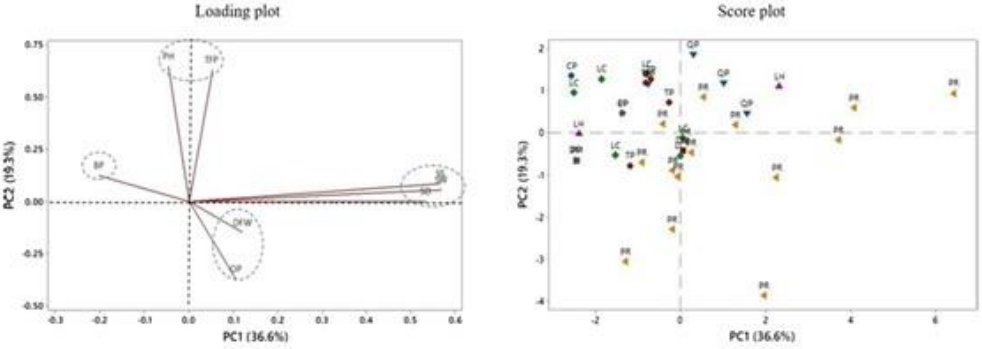

Fig. 2. Loading plot and score plot of the principal component analysis (PCA) of *J. platyphylla*. PR: Rosario, Sinaloa, LC: Chilla, Sinaloa, LH: Higuerita, Sinaloa, QP: Quelite, Sinaloa, TP: Tamazula, Durango, CP: Cofradia, Sinaloa, PP: Mocorito, Sinaloa, DM: Dimas, Sinaloa, PH: plant height, BP: number of branches per plant, TFP: total fruit production (g), DFW: dry fruit weight (g), SW: seed weight (g), SD: seed diameter (mm), SS: shell seed weight (g), OP: oil percentage, PC1: first principal component, PC2: second principal component.

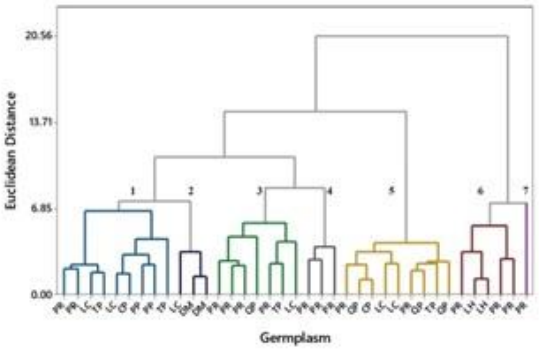

Fig. 3. Hierarchical clustering dendrogram analysis using Euclidean distance and the associations between groups by the Ward method for morphological traits and oil content of *J. platyphylla*. PR: Rosario, Sinaloa, LC: Chilla, Sinaloa, LH: Higuerita, Sinaloa, QP: Quelite, Sinaloa, TP: Tamazula, Durango, CP: Cofradía, Sinaloa, PP: Mocorito, Sinaloa, DM: Dimas, Sinaloa.

directly and indirectly influence on the productivity of the plant can serve as selection criteria for breeding programs. Singh et al. (2016) reported similar results to ours for *J. curcas*, with the number of secondary branches and oil yield being negatively correlated, indicating an opportunity for genetic improvement.

4.2. Spatial distribution

The diversity index obtained by DIVA-GIS suggests that the southern zone of Sinaloa has a high potential for the distribution of *J. platyphylla*. Our observations are similar to those reported by Araiza-Lizarde et al. (2016), who found that *J. curcas* develops best in the central and southern region of Sinaloa State, at lower altitude sites. Among similar studies using spatial analysis tools, Sunil et al. (2009) generated grid maps of the distribution and diversity of *J. curcas* in southern India based on phenotypic traits to identify areas with potential for the development of germplasms with high oil content. In addition, Shabanimofrad et al. (2011) found high indexes of diversity for 100-seed weight and oil content in the central region of Selangor State in Malaysia. DIVA-GIS mapping may be effectively used for documentation, diversity analysis, identification of gaps in collection, and assessment of diversity loss, which can aid the development of new strategies for conservation and sustainable utilization (Dikshit and Sivaraj, 2015).

4.3. Influence of environmental factors on the morphological variation and oil content of J. platyphylla

The results showed that the morphological and oil content variations were related to altitude and maximum temperature and that soil type indirectly affected this variability, as Hernández-Nicolás et al. (2017) reported in a study conducted on *Jatropha* species native to Mexico. *Jatropha* germplasm develops best in the central and southern regions of Sinaloa State, where the temperatures are high and the altitudes are low. According to the Köppen classification, the prevailing climate in the region is classified as a semidry climate with rainfall during the summer (BS1hw) (INEGI, 2015). The reported climate conditions for *J. platyphylla* include temperatures of 29–34.0 °C and an annual precipitation of 800–1500 mm (Cordova-Téllez et al., 2015). The temperature and relative humidity of the region were favorable for the seed traits: SW, SD, KW and, SS. The means of seed yield and the other recorded parameters were significantly different among the locations. Climate factors had significant effects on distribution, productivity, seed yield and oil content of germplasm. The reasons for these differences vary, but factors important for the superiority of germplasm in terms of seed yield include annual temperature and rainfall and soil parameters, which affect the availability of water and nutrients to plants. Plant height and the number of branches per plant are important characteristics for the selection of germplasm; in addition, genetic variability is important to consider because environmental effects can cause too much variation to effectively distinguish between germplasms (Rao et al., 2015). Knowing the relationship between the seed yield and oil content of germplasms under specific environmental and soil conditions is valuable for improving growth and promoting seed and oil yields (Wen et al., 2012).

The collection and identification of wild *Jatropha* germplasms in a wide range of environments is essential for efficient selection in *Jatropha* breeding programs. The performance of genotypes at various locations, which are representative of present and future growing regions of *Jatropha*, and potential genotype-by-environment interactions can be monitored when contrasting testing environments are available to the breeder (Martin and Montes, 2015).

4.4. Principal component and cluster analyses

Principal component analysis is the most common method used to reduce the dimensionality of a data set prior to phylogenetic comparative analysis (Uyeda et al., 2015). The technique increases interpretability while minimizing information loss by creating new, uncorrelated variables that successively maximize variance (Jolliffe and Cadima, 2016). The variables with the same sign act directly; that is,

when an increase in one variable occurs, an increase occurs in the other, those variables with opposite signs act indirectly: when the value of one increases, that of the other one decreases (De Moraes et al., 2017).

The principal component analysis revealed that the first principal component was strongly correlated with the morphological characters SW, SD, and SS. This result indicated the high degree of complementarity in these structural features and, therefore, their suitability for measuring seed quality. The second principal component was negatively related to the variables plant height and total fruit production. Therefore, it can be inferred that plant height is indirect linked with yield attribute traits as well as total fruit production and that these characters together may be pertinent for indirect selection purposes. The third principal component showed the indirect relationships of number of branches per plant, weight of dry fruit and oil percentage.

Our results are consistent with other studies on *Jatropha* in which the first three or four components explained more than 70% of the total variation (Shabanimofrad et al., 2013; Nietsche et al., 2015). The germplasms were distributed along the axes of the main components; thus, germplasms that were closer together could be considered as more similar to one another, whereas those farthest from the axis of the main components could be considered the most discrepant (Prado et al., 2017). *Jatropha* plants located in different components that share certain particular characters can be used as progenitors for the development of elite varieties (Singh et al., 2016).

In cluster analysis, sample units are clustered into groups based on some classification criteria with the aim of obtaining low homogeneity within clusters and heterogeneity among them (Prado et al., 2017). The plants assessed were grouped into seven different clusters according to their characteristics. Clusters 1, 3 and 6 comprised germplasms with the highest average oil contents. Number of branches per plant, dry fruit weight and oil percentage were the most important characteristics that can be used as traits for the selection of germplasm. The clustering pattern revealed a tendency of germplasm lines from diverse geographical regions to be grouped into one cluster, with only a few accessions from the same geographical region clustering into separate groups. Due to the germplasm variability, the generated dendrogram did not reveal clustering by region, which can be attributed to the fact that each group is composed of different accessions. Similar results were obtained in studies of Indian accessions of *Jatropha curcas* (Kaushik et al., 2007; Jun-ling et al., 2010; Tripathi et al., 2015), in which the authors concluded that geographical diversity did not necessarily correspond to genetic diversity.

Phenotypic studies of cultivars of *J. curcas* from India revealed low genetic diversity and that the significant variation in morphological traits (height, diameter, leaf area index and number of primary branches) can contribute to variation in yield (fruits per plant, seeds per plant, weight of seeds and percentage of oil) (Ginwal et al., 2005; Basha and Sujatha, 2007; Patolia et al., 2007).

The observed variation in oil content and morphological variables reveals divergent germplasms that could be used as progenitors and crossed with members of distant clusters to increase heterosis (Shabanimofrad et al., 2013). Two research priorities are the study of phenotypic variation and genetic analysis of populations to identify loci associated with quantitative characteristics of productive interest (Ovando-Medina et al., 2013).

5. Conclusions

Among the studied morphological characteristics, the seed traits had the highest correlations with one another. Climate variables, such as maximum temperature, with total fruit production, were observed to influence the variation in morphological characteristics among the provenances/collection sites. One of the most important priorities for conservation in domestication programs is the evaluation of the genetic resources of wild species. The variability found in the morphological traits and oil content offers extensive opportunities for the selection of superior plant germplasms. Furthermore, this variability offers the possibility of selecting populations with specific traits for agroindustrial and domestication purposes, such as oil production and the development of vegetable protein sources. From a conservation perspective, the high phenotypic diversity in the studied germplasms is promising.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

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Appendix A. Supplementary data

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3. GENETIC DIVERSITY IN *Jatropha platyphylla* ACCESSIONS BASED ON MORPHOLOGICAL TRAITS AND INTER-SIMPLE SEQUENCE REPEAT (ISSR) MOLECULAR MARKERS

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ABSTRACT

Seven accessions of *Jatropha platyphylla* were evaluated for their morphological traits and genetic diversity using ISSR. Cluster analyses with nine traits were performed: number of branches per plant; fruit per bunch; bunch per branch; bunch per plant; total seed production; total fruit production, protein content, oil content, and the fatty acid profile. Genotypes from Rosario, Sinaloa (PR11) yielded the highest values in all traits. Correlation analysis of the quantitative traits showed high correlations between seed production and total fruit production (r=0.99). Unsaturated linoleic acid was the most abundant fatty acid (57.64-52.39 %). Within a genetic improvement program, two of the most important variables to be considered are the oil content and the morphological characteristics of the plant. *J. platyphylla* has shown viable selection traits that provide a possibility of producing interspecies hybrids and give them added value. ISSR primers generated variable banding patterns that were found to be polymorphic and the polymorphic information content (PIC) of these loci ranged from 0.21 to 0.45 with an average of 0.34.UPGMA cluster analysis of the data showed the formation of three groups, with the most divergent accession pair being genotype from Quelite (QP11) and Rosario (PR11).

Key words: Fatty acids, Oil, Plant breeding, Protein, Seed

INTRODUCTION

Jatropha platyphylla is, a non-toxic wild plant in Mexico that promises to be an alternative in the production of oil and protein for energy and food purposes (Salazar-Villa *et al*. 2019). There are a few known about this species like its geographical distribution in the low deciduous forest, close to the Mexican Pacific coast (Sosa-Segura *et al*. 2014). The kernel seed of *J. platyphylla*, has a high oil content (60%) and the oil extraction residue cake contains 75% crude protein, wich does not contain phorbol esters knows as the responsible compounds for the toxicity of *J. curcas* (Makkar *et al*. 2011). Despite this potential, *J. platyphylla* is a wild plant that has so far not been domesticated. Therefore, the establishment of crops that meet the needs of stable and commercial cultivars with high oil content and tolerance to pests and diseases, requires the development of a genetic improvement program (Díaz *et al*. 2017). However, there is a lack of information on the molecular characterization of this plant. The determination of genetic variability is essential for this program. Molecular markers constitute an important technological tools useful in the processes of selection and increase of genetic variability, especially when they are associated with phenotypic population analysis (Pazeto *et al*. 2015).

Among available and widely used methods to characterize genetic diversity, the molecular markers Inter Simple Sequence Repeats (ISSR), technique, offers unique advantages over other molecular markers, since its application does not require prior genomic information of the species under study, only a small amount of template DNA is required and this is done quickly (Nasir *et al*. 2017). Therefore, the aim of this work was to evaluate the morphological traits and the genetic diversity of *J. platyphylla* accessions by molecular markers and Inter Simple Sequences Repeated (ISSR).

MATERIALS AND METHODS

Plant Material

From different geographical regions, cuttings of 7 accessions of *J. platyphylla* were obtained (Table 1). The cuttings were disinfected (Blindaje 50^{TM}, 0.5 g ·L) and kept in rooted solution for 24 hours (Rooting ^{TM}, 200 mg · L). Subsequently, rooted cuttings were planted in plastic bags (20 x 10 cm) with substrate [sand (40%), coconut fiber (30%) and vermicompost(30%)]. Three months later (July 2017), they were transferred on an experimental plot at "La Campana", Culiacán, Sinaloa, Mexico (N 25 ° 30´; W 108 ° 22´) in a completely randomized block design with a distance of 3x3 m. The plants received integrated management for pest control and fertilization with NPK (17-17-17), compost, drip irrigation and pruning. The soils contained sandy loamy soils with pH of 7.2. Daily environmental conditions (temperature, relative humidity, precipitation) were recorded at an automated station of the brand Adtcom Telemetryc ® (Klosterneuburg, Austria), which was located on the study site.

Morphological Traits Characterization

The variation observed in nine quantitative traits viz., number of branches per plant (BP); fruit per bunch (FI); bunchs per branch (NI); bunchs per plant (NF); total seed production (SWP); total fruit production (PFP), protein content (P%), oil content (% O) and fatty acid profile (FAP) (Sunil *et al*. 2011; Sosa *et al*. 2012) was recorded on two-year-old plants.

The fatty acid content was performed by extraction, separation, methylation, purification, and quantification according to Folch method (Folch 1956; AOAC 969.33).

DNA Extraction

Total genomic DNA was extracted from the youngest leaves of three plants of each accession of *J. platyphylla* according to the CTAB protocol with minor modifications (Basha y Sujatha 2007). Five grams of leaves were ground in liquid nitrogen, then homogenized in 20 mL CTAB (2% CTAB, 20 mM EDTA, 2% PVP, 1.4 M NaCl, 100 mM Tris-HCl pH 8.0 and 1% β-mercaptoethanol) and incubated at 65 °C for 15 min, then centrifuged at 12,000 *g* for 5 min where, the supernatant was collected and extracted twice with chloroform: isoamyl alcohol (24:1 v/v). The DNA was precipitated with isopropanol and washed twice with 70% ethanol. The sedimented DNA was air-dried and resuspended in 500 µL of ultrapure water and 1 µL of RNase was added. It was stored overnight at -20 °C. The integrity of the DNA was determined by electrophoresis in 1% agarose gel using a known amount of DNA as standard.

ISSR-PCR

ISSR 841, 836, 880 and 827 primers were used (UBC Num. 9, University of British Columbia, Canada). PCR mixture consisted of 50 ng DNA, 25 µL Amplitaq Gold 360 Master Mix and ultrapure distilled water. DNA amplification was performed by PCR in a Biorad Thermal Cycler with an initial denaturation at 95 °C for 10 min followed by 39 cycles at 92 °C, 1 min anneling temperature (Ta), 2 min elongation at 72 °C and final extension at 72 °C for 7 min. PCR products were subjected to 1.5% agarose gel electrophoresis in TAE buffer and stained whit ethidium bromide at 70V, 200 mA for 1h. A gel was photographed on UV light Axygen® Gel Documentation System.

Data Analysis

Morphological traits were evaluated using descriptive statistics to know the mean, standard deviation, maximum and minimum values and the coefficient of variation. An analysis of variance (ANOVA $p<0.05$) was performed to find significant differences between genotypes for mean comparison followed by Fisher post hoc tests and they were subjected to Principal Components Analysis (PCA).In addition, a correlation analysis was performed between morphological traits used with MINITAB 17.

A binary matrix (absence = 0 and presence of the marker = 1) was created from digitized banding profile of agarose gels using the software Image Lab. Two replications were performed per accession. Blurred bands were discarded. This matrix was used to calculate the similarity between the accessions using the Dice and Jaccard index. Afterwards, the accessions were grouped according to UPGMA, using the software PAST v.3.17 (Harper and Ryan 2001). The Polymorphism Information Content (PIC) value was calculated in accordance with Tanya *et al*. 2011. The number of bands, the number of polymorphic markers and the percentage of polymorphism were calculated. Primers ISSR that had a minimum PIC value of 0.3 were preserved for analysis.

RESULTS AND DISCUSSION

The Environmental Conditions and Morphological Traits

Soil pH of the crop was 7.2 and soil type in this area is not limiting for the good development of the plantations. *Jatropha* adapts to a wide variety of soils, including those with low nutrient content although it prefers light and well-drained soils. Mixed clay and sandy soils provide a texture that promotes better aeration, facilitates gas exchange and increases photosynthetic activity (Sunil *et al*. 2013). It usually develops in arid and semi-arid soils and responds well to wide range of pH levels although it prefers them slightly acidic (Montes and Melchinger 2016).

Relative humidity data show an average of 75 ± 5%. The monthly average of maximum temperature ranged between 25.5 ºC- 38.9 ºC and minimum between 10.2 ºC-25 ºC. The mean temperature from December to January was 19.1ºC, while the minimum mean temperature was 4.9 ºC and the, maximum temperature from April to February was around 31.7 ºC. There were two rains peaks, one from June to August and another peak from October to December, which was scanty. Overall, the mean annual precipitation ranged between 570 mm. The accumulated annual precipitation of the area was below the optimum level established for the *Jatropha* crop, which requires between 800 and 1500 mm (Cordova-Téllez *et al*. 2015), hence it was necessary to complement the water requirements with assisted irrigation. The reported temperature for *J. platyphylla* includes temperatures of 29 at 34.0 °C (Cordova-Téllez *et al*. 2015). The maximum average temperature at the study site was 32.2 °C. The annual minimum relative humidity was 55%, while the annual maximum was 79%. At this relative humidity, the area is assumed to be in the optimal range for the establishment of crops, and should be supported by irrigation during the driest months (April to June) to reduce the vapor pressure deficit. Climate factors had significant effects on distribution, productivity, seed yield and oil content of genotypes (Salazar-Villa *et al*. 2019). The most important factors for the superiority of genotypes in terms of seed yield include annual temperature and precipitation and soil parameters, which affect the availability of water and nutrients to plants.

The data recorded for all quantitative traits are presented in Table 2. There was significant variation in all the morphological traits recorded ($p<0.05$). Morphological variation indicated the existence of diversity for quantitative traits. Morphological traits are important characteristics for genotype selection; in addition, genetic variability is important to consider because environmental effects can cause high variation to distinguish effectively between genotypes (Rao *et al*. 2015). Knowing the relationship between genotypes under specific environmental and soil conditions is valuable for improving growth and promoting seed and oil yields (Wen *et al*. 2012).

The highest values were number of branches per plant (BP) (2.58); fruit per bunch (FI) (6.83); bunchs per branch (NI) (2.33); bunchs per plant (NF) (7.75); total seed production (SWP) (204.20 g); total fruit production (PFP) (336.30 g), protein content (P%) (60.23% and 59.90%), and oil content (O%) (27.29% and 26.79%). PR11 genotypes yielded the highest values of BP, FI, NI, NF, SWP, PFP and P%., while the LH3 and QP11 genotypes yielded the highest values of oil content

(O%). PP1 yielded the highest value of protein content (P%), similar to PR11. BP, FI, NI, NF, SWP and PFP, showed high CVs (> 20%). Oil (O%) and protein content (P%) had low coefficient of variation (CV) (< 10%) (Table 2). The population evaluated in this study has sufficient variability for selection of genotypes of superior agronomic performance (Silva *et al*. 2017). This fact is important for the establishment of a genetic improvement program. The coefficients of variation (CV's) show the variability between the accessions. The morphological differences suggest genetic variation and/or variation in response to different environmental conditions, since the influence of the genotype and the environment on phenotypic variation can occur simultaneously (Laviola *et al*. 2010). The high endemism found in Mexico could be responsible for the high variability between genotypes (Steinmann 2002).

The correlation analysis of the quantitative traits showed high correlations between SWP and PFP (r=0.99). The traits that showed moderate correlation were BP with NF (r=0.42); FI with NI (r=0.64), NF (r=0.63), PFP (0.49), SWP (r=0.48) and O% (0.57); NI with NF (r=0.61), PFP (r=0.44), SWP (r=0.43) and O% (0.44); NF with PFP (r=0.65) and SWP (r=0.66). PFP with P% (r=0.35); O% with P% (r=-0.49). Low correlations were observed between BP and NI (r=0.23), PFP (r=0.28) and SWP (r=0.30) (Table 3).

Correlation coefficient for seed traits is presented in Table 4. All correlations were significant. KW: kernel weight (KW) had a high and significant correlation with seed weight (SW) and seed diameter (SD) (r=0.95 and 0.97).

The highest direct effect on SWP was obtained by PFP (0.99), which is an estimate close to the morphological correlation (Table 3). Thus, SWP is the main determinant in the variation of PFP and evidences the cause and effect relationship between these traits, i.e., the higher the fruit production, the higher is the seed production. The identification of traits that have high morphological correlation and high direct effect in the same direction on the main trait is desirable, since the correlated response by means of indirect selection can be effective (Borah *et al*. 2018). Therefore, selection of genotypes with higher fruit and seed production aiming to increase oil or protein yield is a promising strategy due to the cause and effect relationship between these traits, as evidenced in this study. The negative correlation between seed protein and oil contents has been documented in other crops such soybean and castor bean (Kambhampati *et al*. 2020; Wang *et al*.

2019; Zhang *et al*. 2015). Current evidence indicates that seed storage protein and oil are synthesized during seed development, following stored-starch breakdown (Wang *et al*. 2019). Seed protein and oil content are both complex quantitative traits, controlled by multiple genes and affected by environmental factors.

In the present study the principal component analysis (PCA) showed significant differences in all traits evaluated. The first three components accounted for 81.5% of the total variation. PC1 accounted for 47.1% of the variability, PC2 accounted for 22%, and PC3accounted for 11%. The first factor had high contributing factor loadings from FI, NI, NF, PFP and SWP. The second factor had high negative contributing loadings from O% and positive loading in P%. The third factor had high negative contributing loadings from BP (Table 5). The graphical biplot interpretation of PC1 and PC2 revealed that the germplasms showed differences in a set of 8 traits (Fig. 1).

The most divergent accessions pair was QP11 and PR11 (Figure 2). The highest similarity was observed between QP11 and LH3. The branching patterns in the dendrogram resulted in three major groups. Group I, formed by QP11, LH3, and PP3; group II, formed by QP6, TP3, and PP1; and group III, formed by PR11.

Fatty acid profile (FAP) was similar for the genotypes, although concentration of individual fatty acids differed significantly ($p<0.05$) (Table 6). The most abundant fatty acids were the unsaturated linoleic (57.64-52.39%) and oleic (26.07-21.44%) acids, and the saturated palmitic (16.55-12.07%) and stearic (9.65-4.93%) acids. The composition of fatty acids plays an important role in the selection of oils with fuel and nutritional potential. The fatty acid profile is dominated by palmitic and stearic saturated acid and linoleic and oleic unsaturated acids. Linoleic acid was the main fatty acid that may have potential as an edible oil for the food industry (Sosa-Segura *et al*. 2014). By contrast, soybean, and *J. curcas* oils present similar chemical profiles regarding main fatty acids content, mainly oleic acid (Ustra *et al*. 2013), for biodiesel production.

ISSR Molecular Marker Diversity

Determination of genetic variation among accessions of *J. platyphylla* using morphological traits and molecular analysis is essential to select parents to be crossed for generating appropriate populations for breeding purposes (Duarte *et al*. 2018). The objective of the present study was to assess the molecular variation that could be corroborated with phenotypic variation so that distinct genotypes, if any, identified phenotypically could be utilized in the breeding programs.

The status of the genetic diversity of *J. platyphylla* is not clear yet. Therefore, this study provides the first assessment of the genetic diversity of *J. platyphylla* accessions using ISSR markers. Despite the high genomic similarity, the profiles of the molecular markers show different patterns of amplification in the accessions. In our study the study pattern of specific alleles was observed whereby the population had specific amplification to its accessions.

The number of bands formed by different ISSR primers ranged from 5 to 8 with an average 7 bands per primer. The maximum number of amplified product (8) was observed in the profiles of the primer UBC 827 and primer UBC 836. The minimum number of amplified product (5) was observed in the profiles of primer UBC 841. In the seven accessions, a total of 122 bands were obtained. Molecular weights ranged from 225.22 to 1500 bp. The percentage of polymorphic bands ranged between 40 and 100%. PIC values were in a range from 0.21 to 0.45, with a mean value 0.34. ISSR primers 836 presented the lowest PIC value, while ISSR 880 showed the highest value (Table 7). The PIC value provides a measure influenced by the number and frequency of alleles. The maximum value of PIC for an ISSR marker is 0.5, since the presence of two alleles per locus (Chesnokov *et al*. 2015; Nagy *et al*. 2012). The PIC value reveals the informativeness level and accordingly defined into categories: low (0 to 0.10), medium (0.10 to 0.25), high (0.30 to 0.40) and very high (0.40 to 0.50) (Serrote *et al*. 2020). The moderate values of PIC for the ISSR primers could be attributed to the diverse nature of the accessions and/or highly informative ISSR markers used in this study (Najaphy *et al*: 2011).

The generated mean Jaccard's coefficient of similarity was of 0.53. The maximum coefficient of similarity (0.76) was found between accessions PR11 and PP3. The lower coefficient of similarity (0.28) was found between accessions LH3 and QP11. Dice index was of 0.72. The maximum coefficient of similarity (0.86) was found between accessions PR11 and PP3. The lower coefficient of similarity (0.43) was found between accessions LH3 and QP11.

Polymorphism and genetic information provided by ISSR technique can be complemented with information from morphological and biochemical characterization, and thus be able to elucidate in a clearer way the intricate relationships and interactions that occur in most materials and assess their intraspecific diversity on a much finer scale (Morillo-Coronado *et al*. 2015). In plants of *J. curcas* the genetic diversity of accessions of India and Brazil has been evaluated (Araujo *et al*. 2019; Mastan *et al*. 2012; Grativol *et al*. 2011), Taiwan (Mavuso *et al*. 2016), South America (Costa Rica)(Vásquez-Mayorga *et al*. 2017), Africa and Asia (Trebbi *et al*. 2015), Indonesia (Saptadi *et al*. 2017), revealing a low diversity attributed to the origin of plant material via vegetative propagation, which increases the possibility that germplasm banks store plants of identical provenance (Gomes *et al*. 2018).

The high diversity found in mexican accessions of *J. curcas* agree with our investigation (Diaz *et al*. 2017; Li *et al*. 2017), this may be because Mexico is considered the center of origin of the *Jatropha* genus (Aguilera-Cauich *et al*. 2015), and has a high endemism (Steinmann 2002).. Polymorphism founded indicates that inter-simple repeat sequences are abundant and highly dispersed through the genome (Patil *et al*. 2020).

CONCLUSIONS

The results of this study can be considered a starting point for future research aimed at defining the level of genetic diversity to detect promising accessions to generate hybrids of *Jatropha platyphylla*. To do this, a greater number of natural populations collected from the entire range should be analyzed and additional feeders tested. In addition, discriminating bands could be cloned and sequenced. These studies have given important clues to understanding the genotype-phenotype relationship, which can further help develop and plant reproduction strategies.

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TABLES AND FIGURES

Table 1 Sampling sites of *J. platyphylla* genotypes.

Accession	Collection area	Latitude (N)	Longitude (W)	Altitude (masl)
LH3	Higuerita, Sinaloa	24°45'37.6"	107°08'39.1"	200
PP1	Mocorito, Sinaloa	25°04'11.4"	107°43'10.7"	51
PP3	Mocorito, Sinaloa	25°04'11.4"	107°43'10.7"	51
PR11	Rosario, Sinaloa	23º11'18.5"	106º09'09.3"	15
QP11	Quelite, Sinaloa	23°31'51.3"	106°30'10.8"	58
QP6	Quelite, Sinaloa	23°31'51.3"	106°30'10.8"	58
TP3	Tamazula, Durango	24°59'12.2"	106°59'17.8"	263

Table 2 Results of statistical analyses of morphological traits and oil and protein content of *J. platyphylla* genotypes.

Accession	BP	FI	NI	NF	SWP	PFP	O%	P%
LH3	1.50b*	2.66d	1.50bc	2.33bc	65.00b	124.20b	60.23a	22.36d
PP1	1.63b	1.54e	1.00d	1.81c	43.64b	81.80b	54.59c	26.79a
PP3	2.50ab	1.00e	1.00cd	2.00bc	85.00b	153.80b	60.47a	25.73b
PR11	2.58a	6.83a	2.33[a]	7.75a	204.20[a]	336.30a	59.15ab	27.29[a]
QP11	2.42ab	3.14cd	1.87b	3.57b	59.60b	110.10b	59.90a	24.33c
QP6	1.50b	4.00c	1.33cd	1.33c	35.83b	69.17b	59.43ab	25.64b
TP3	1.87ab	5.37b	1.50bc	3.37b	34.00b	78.30b	57.71b	22.67d
Mean	2.01	3.85	1.57	3.61	150.1	84.5	58.78	24.97
Minimum	1.00	1.00	1.00	1.00	20.00	10.00	52.60	22.04
Maximum	5.00	8.00	3.00	13.00	605.00	360.00	61.25	28.19
SD	0.94	2.28	0.63	2.81	139.3	86.9	2.17	1.87
CV	46.64	59.33	40.18	77.85	92.81	102.87	3.69	7.50

Number of branches per plant (BP); fruit per bunch (FI); bunches per branch (NI); bunches per plant (NF); total seed production (SWP) (g); total fruit production (PFP) (g); protein content (% P) (%); and oil content (% O) (%). CV: coefficient of variation (%), SD: standard deviation. * Different letters within a row indicate significant differences $p<0.05$.

Table 3 Correlation coefficient (r) values of the morphological characteristics and oil content and protein content of *J. platyphylla* genotypes. Number of branches per plant (BP); fruit per bunch (FI); bunches per branch (NI); bunches per plant (NF); total seed production (SWP) (g); total fruit production (PFP) (g); protein content (% P) (%); and oil content (% O) (%). *p≤0.05

	BP	**FI**	**NI**	**NF**	**PFP**	**SWP**	**O%**
FI	0.15						
NI	0.23*	0.64*					
NF	0.42*	0.63*	0.61*				
PFP	0.28*	0.49*	0.44*	0.65*			
SWP	0.30*	0.48*	0.43*	0.66*	0.99*		
O%	0.06	0.57*	0.44*	0.25	0.08	0.08	
P%	0.02	-0.08	0.05	0.20	0.35*	0.36*	-0.49*

Table 4 Correlation coefficient (r) values of the morphological characteristics of *J. platyphylla* genotypes. SW: seed weight (g), SL: seed longitude (mm), SD: seed diameter (mm); TW: Shell weight (g); KW: kernel weight (g). *P≤0.05

	SW	**SD**	**TW**	**SL**
SD	0.44*			
TW	0.85*	0.46*		
SL	0.89*	0.48*	0.78*	
KW	0.95*	0.97*	0.66*	0.71*

Table 5. Eigenvalues of the first three principal components of *J. platyphylla* germplasm. PC1: First principal component, PC2: second principal component, PC3: third principal component. Number of branches per plant (BP); fruit per bunch (FI); bunches per branch (NI); bunches per plant (NF); total seed production (SWP) (g); total fruit production (PFP) (g); protein content (% P) (%); and oil content (% O) (%).

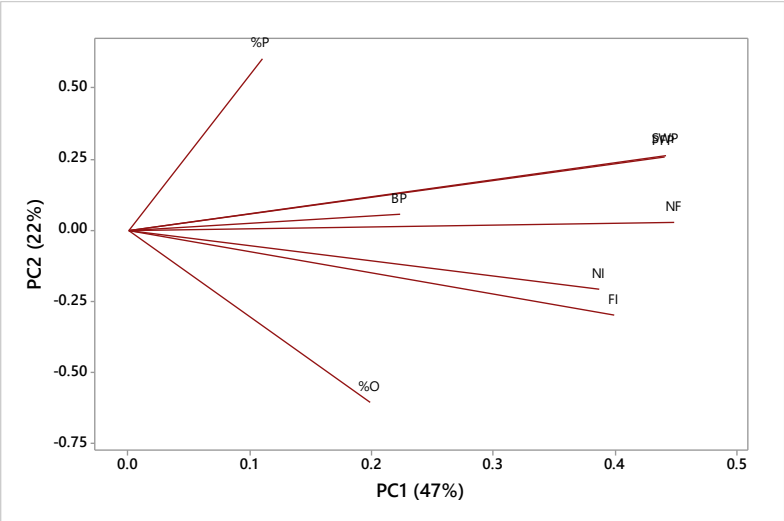
Variable	PC1	PC2	PC3
BP	0.22	0.055	−0.92
FI	0.40	−0.29	0.21
NI	0.38	−0.20	0.10
NF	0.44	0.02	0.11
PFP	0.44	0.25	0.11
SWP	0.44	0.26	0.09
O%	0.19	−0.60	0.07
P%	0.11	0.60	0.21
Eigen value	3.76	1.83	0.91
Proportion	0.47	0.22	0.11
Cumulative	47.1	70.1	81.5

Table 6. Fatty Acid Composition (%) of *J. platyphylla*. Means in a row followed by the same letter are not significantly different by Fisher post hoc tests at the 5% level. SFA, UFA, MUFA, PUFA denote saturated, unsaturated, monounsaturated and polyunsaturated fatty acids, respectively.

Fatty acid	LH3	PP1	PP3	PR11	QP11	QP6	TP3
Myristic C14:0	0.23ab	0.17b	0.23ab	0.30ab	0.36a	0.35a	0.19b
Palmitic C16:0	13.20bc	14.16abc	12.07c	16.55a	15.26ab	14.31abc	7.41d
Palmitoleic C16:1	0.45b	0.44bc	0.29e	0.58a	0.40cd	0.37d	0.40cd
Heptadecanoic C17:0	0.14abcd	0.13bcd	0.15abc	0.11d	0.16ab	0.16ab	0.12cd
Stearic C18:0	9.65a	6.50cd	9.38ab	5.39d	5.32d	4.93d	7.71bc
Oleic Cis 9 C18:1	21.44c	26.18a	24.76b	23.52b	23.38b	23.60b	26.07[a]
Linoleic C18:2	53.95bc	52.39c	53.79bc	54.47b	54.64b	55.29b	57.64[a]
Linolenic C18:3	0.10cd	0.11bc	0.12bc	0.09d	0.09d	0.13ab	0.15ab
Arachidic C20:0	0.10b	0.15a	0.09c	0.10b	0.10b	0.10b	0.15[a]
SFA	24.51	21.20	21.25	20.31	21.22	20.62	17.20
UFA	75.55	79.30	78.62	79.68	78.77	79.27	82.79
MUFA	21.41	27.07	24.66	26.82	24.09	23.87	26.40
PUFA	54.13	51.78	54.10	52.85	54.67	55.28	56.38

Table 7. Genetic diversity parameters for *Jatropha platyphylla* genotypes

Primer ISSR	Sequence	Number of bands	Number of polymorphic markers	% of polymorphism	PIC value
UBC880	GGAGAGGAGAGGAGA	7	5	71.42	0.45
UBC827	ACACACACACACACACG	8	8	100	0.36
UBC836	AGAGAGAGAGAGAGAGYA	8	4	50	0.21
UBC841	GAGAGAGAGAGAGAGAYC	5	2	40	0.34



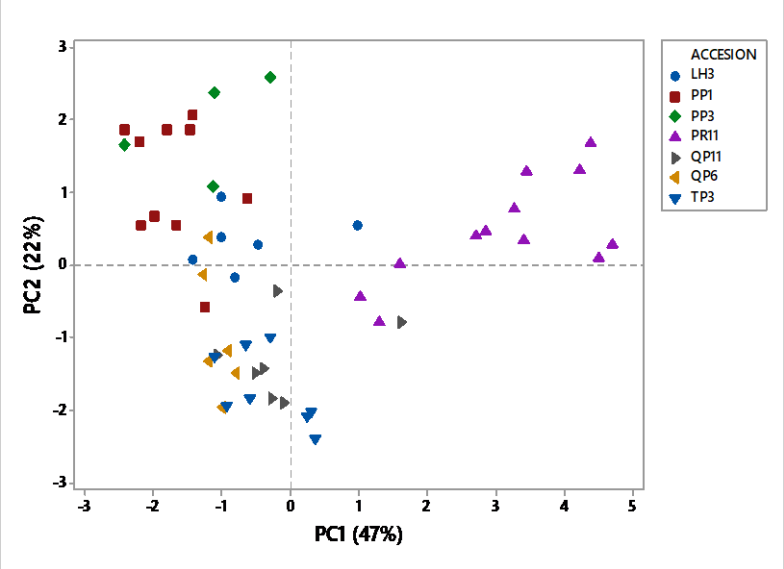


Figure 1. Loading plot and score plot of the principal component analysis (PCA) of *J. platyphylla*. Number of branches per plant (BP); fruit per bunch (FI); bunches per branch (NI); bunches per plant (NF); total seed production (SWP) (g); total fruit production (PFP) (g); protein content (% P) (%); and oil content (% O) (%).PC1: first principal component, PC2: second principal component.

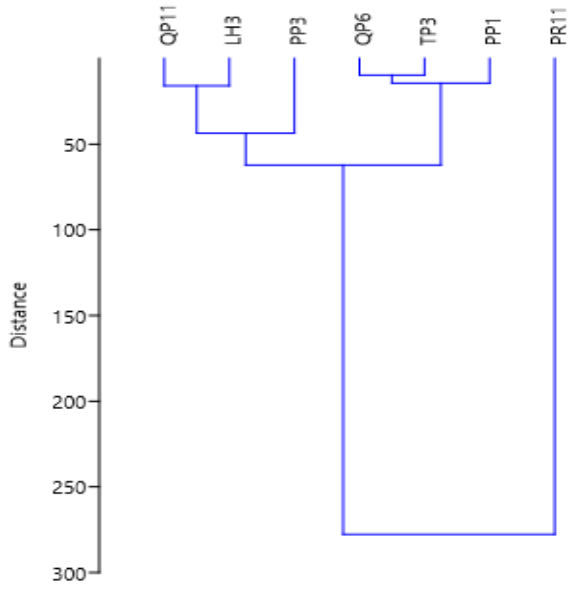


Figure 2. Dendrogram of *Jatropha platyphylla* genotypes based on the Jaccard index calculated from data of 122 ISSR loci, using the UPGMA as the clustering method. Groups are described in the section of result and discussion. Table 1 shown the accession codes.

4. EFFECT OF GRAFTING ON PHENOTYPIC CHARACTERISTICS, YIELD AND FATTY ACIDS PROFILE IN *Jatropha platyphylla*

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ABSTRACT

Jatropha platyphylla has been shown to have antioxidant activity, food and energy use and although the plant shows its potential, the species is not exploited. Propagation by grafting allows to increase uniformity, vigor, resistance to pests and diseases and to preserve its genetic composition. Six grafted plants of *Jatropha platyphylla* of two years old were evaluated for their phenotypic traits: basal diameter, total fruit production, total seed production, seed weight, seed longitude, seed diameter, shell weight, kernel weight, oil content, protein content, and fatty acid profile The percentage of grafted plant success was 76%. The grafted plants obtained the highest values in phenotypic traits evaluated. Highest correlations were found in total fruit production and total seed production unsaturated linoleic was the most abundant fatty acid. This evaluation has helped to identify grafted plant of *J. platyphylla* with specific yield and phenotypic features for large scale propagation in relative short time.

Key words: Fatty acids, *Jatropha platyphylla*, Plant breeding, protein

INTRODUCTION

Jatropha platyphylla has nutritional potential, due to its high protein content, motivating research on its chemical structure and antioxidant action. Its seeds have a high oil content, which can be useful for the production of biodiesel (Salazar-Villa *et al*., 2019). Seedlings, cuttings and tissue culture are propagated methods of *Jatropha* (Islam *et al*. 2015). Choosing the best method of propagating *Jatropha* is one of the challenges for its large-scale planting (Severino *et al*. 2011). Propagation by seed can have a low survival rate, however they have high yields and are more tolerant to pests and diseases (Heller 1996). To maintain the consistent traits, vegetative propagation through cutting or by tissue culture is desirable. The propagation with stem cuttings is recommended for faster establishment and faster production and maintain homogeneity among the progeny and provide early production (Montes and Melchinger 2016). Nevertheless, these plants show a lower longevity and possess a lower drought and disease resistance than those propagated by seeds (Singh *et al*. 2010).

Plants produced from cuttings do not produce true taproots that may penetrate only one-half the depth of the soil compared to taproots produced on seed grown plants (Parvaiz and Rafiq 2013). The possibility of combining sexual and asexual reproduction systems in oil crops is the key strategy to develop new cultivars (Bisognin 2011). In agriculture, propagation by grafting is frequently used to increase uniformity, vigor and resistance to biotic and abiotic stresses. Grafting conserve plants of genetic importance provide and earlier fruit production (Singh and Agrawal 2017). Grafting is the method of inserting a part of the plant (scion part) onto the root system (rootstock lower part of the graft that possess a root system, it is often categorized according to tree vigor, in their resistance to pests and disease, and in their influence on fruiting) of another plant (Louws 2010). Grafting has been contributed to the domestication of certain woody plants that were highly heterozygous (Melnyyk 2017). In addition, genetic stability has been demonstrated in grafted plants, obtaining 100% genetic stability between the mother and the grafted plant (Jaganath *et al*. 2014). The experiments reported in this article aimed to evaluate the growth and development of grafted and non-grafted *J. platyphylla* germplasm through phenotypic traits, yield, and oil content.

MATERIALS AND METHODS

Plant material

Stem cuttings of germplasm of *J. platyphylla* of 40 cm in length with at least three buds, were selected with vigorous stems and apparently pest and disease free. The cuttings were disinfected (Blindaje 50^{TM}, 0.5 g · L) and kept for 24 h in rooted solution (Rooting^{TM}, 200 mg · L). They were planted in plastic bags (20 x 10 cm) with substrate [sand (40%), coconut fiber (30%) and vermicompost (30%)] until the formation of axillary bud.

Grafting

Scions were obtained from thirty-day seedling of *J. platyphylla* and grafted to V-shaped cut root stocks of axillary buds ((N=20). The scion/rootstock junction was wrapped with Parafilm to prevent the dehydration and safeguard from pests. When the scions were fused with the root stocks, the Parafilm were removed. all plants were allowed to grow in a greenhouse and it was recorded the percentage of grafted plant success (Soto-Landeros *et al*. 2016) .

Experimental set-up

Three months plant grafted (July 2017) and a non-grafted plant (T0) were transferred to an experimental plot in the “La Campana” town, Culiacán, Sinaloa, Mexico (N 25 ° 30´ W 108 ° 22´) in a randomized Latin square design of germplasm (iPR14,iPR11, iPR10, iPR2, iDA50, iTP4) 8 replicates was used.

The plants received an integrated management to control pests and fertilization with NPK (17-17-17), compost, drip irrigation and pruning. The soils contained sandy loam type with pH of 7.2. Daily environmental conditions (temperature, relative humidity, and precipitation) were recorded (Adtcom Telemetryc ®).

The phenotypic traits, basal diameter (DBH) (mm); total fruit production (PFP) (g), total seed production (SWP) (g), seed weight(SW) (g), seed longitude (SL) (mm), seed diameter (SD) (mm); Shell weight (TW) (g); kernel weight (KW) (g), oil content (%O), protein content (%P) and fatty acid profile (Sunil *et al*., 2009; Sosa *et al*., 2012) were recorded on two-year-old plants of *J. platyphylla*. The fatty acid content was performed according to Folch method (Folch 1956; AOAC 969.33).

All traits were evaluated through descriptive statistics to know the mean, standard deviation, maximum and minimum values and the coefficient of variation. An analysis of variance (ANOVA $p < 0.05$) was performed to find significant differences between germplasm for comparison of means followed by Fisher post hoc tests used with MINITAB 17.

RESULTS AND DISCUSSION

Phenotypic Traits of Grafting Plants

The percentage of grafted plant success was 76%. The data recorded on phenotypic traits are presented in Table 1. There was significant variation in traits recorded ($p< 0.05$) DBH, PFP, SWP, SW, SL, SD, TW, and %P. No significant difference was found in KW and O% traits between non-grafted (T0) and grafted plants. The highest values were basal diameter (DBH) (375mm); total fruit production (PFP) (69.5g); total seed production (SWP) (43.30g), seed weight(SW) (2.06g), seed longitude (SL) (18.35mm), seed diameter (SD) (16.40mm); Shell weight (TW) (1.07g); and protein content (%P) (29.24%). The non-grafted plant (T0) obtained the highest value of DBH; grafted plant iPR14 obtained the highest value of SW, SD, and TW; iPR2 obtained the highest value in PFP, and SWP. iDA5(0) obtained the highest value of SL and iPR10 obtained the highest value of %P. Although no significant difference was found in the production variables such as kernel weight (KW) and oil percentage (%O) between grafted and non-grafted plants, it is important to note that in non-grafted plants their phenotypic traits were recorded until the second phenological cycle, thus demonstrating, that the grafted plants allow to obtain earlier fruit production.

The percentage of grafted plant success showed high compatibility according to Singh and Agrawal (2017). The compatibility found was to be expected due the success rate of any graft was dependent on the sympathy between the stock and scion. Generally, the stock and scion of the same genera form more compatible grafts in comparison to the scion and stock of different genera (Sharma and Zheng 2019).

The highest values in phenological traits were found in grafted plants. This phenomenon may be due to the presence of a taproot that allows it to anchor to the soil and a correct absorption of nutrients (Peng *et al*. 2020). The basal diameter of the grafted plants was smaller as compared to non-grafted plant.

Analysis of correlation coefficients among phenotypic traits

Correlation coefficient for 10 phenotypic traits is presented in table 2. All correlation registered were positive and high. Highest correlations were SWP with PFP (r=0.99), KW with SW (r=0.98), TW with SW (r=0.96). KW had correlation with SL, SD and TW (r=0.87, 0.89, 0.94), respectively. TW presented correlation with SL and SD (r=0.87, 0.82), respectively. SD has correlation with SL (r=0.86).

The highest positive correlation of SWP and PFP (0.99) is a clear evidence of the cause and effect relationship between these traits, showing thereby that if the fruit production increases seed production increases in the same way (Salazar-Villa *et al*. 2019). The whole *Jatropha* seed is divided into constituent parts therefore with the increase of the weight and area of the seed, increases the weight of the shell and the kernel (Srivastava *et al*. 2011). To have an effective genetic improvement program it is necessary to know the correlation between the phenological traits of *Jatropha* (Shabanimofrad *et al*. 2013).

Table 1. Basal diameter (DBH) (mm); total fruit production (PFP) (g); total seed production (SWP) (g), seed weight(SW) (g), seed longitude (SL) (mm), seed diameter (SD) (mm); Shell weight (TW) (g); kernel weight (KW) (g), oil content (%O), and protein content (%P). SD: standard deviation.*Different letters within a row indicate significant differences P<0.05.

Accession	**DBH**	**PFP**	**SWP**	**SW**	**SL**	**SD**	**TW**	**KW**	**%O**	**%P**
T0*	375a*	30.91b	18.36b	1.97ab	17.90ab	16ab	1.04ab	0.93a	61.37a	25.83bc
iPR14	187.5b	41.7ab	25ab	2.06a	18.10ab	16.40a	1.07a	0.98a	56.44a	27.18abc
iPR11	264.3ab	45ab	24.36b	1.80bc	17.20bc	15.65ab	0.98ab	0.81a	60.90a	25.04c
iPR2	300ab	69.5a	43.30a	1.90ab	17.65ab	15.60ab	1.01ab	0.89a	60.66a	24.94c
iDA5(0)	266.7ab	31.80b	17.80b	1.98ab	18.35a	16.45a	1.02ab	0.95a	61.90a	25.24c
iTP4	230b	33.57b	16.71b	1.65c	17.35bc	15.25b	0.83c	0.82a	59.57a	28.41ab
iPR10	300ab	32.50ab	19ab	1.79bc	16.70c	15.55ab	0.94b	0.84a	59.24a	29.24a
Mean	266.3	42.09	24.28	1.88	17.60	15.84	0.98	0.89	59.76	26.62
Minimum	100	35.39	2	0.90	15	13	0.60	0.15	49.07	24.14

Maximum	500	5	110	2.38	20	19	1.28	1.37	64.10	30.27
SD	89.4	175	22.07	0.28	1.11	1.03	0.14	0.19	3	1.80

Table 2. Correlation of phenotypic traits of grafted and non-grafted plants of *J. platyphylla*. Basal diameter (DBH) (mm); total fruit production (PFP) (g); total seed production (SWP) (g), seed weight(SW) (g), seed longitude (SL) (mm), seed diameter (SD) (mm); Shell weight (TW) (g); kernel weight (KW) (g), oil content (%O), and protein content (%P). SD: standard deviation.* indicate significant differences $p<0.05$.

	SW	SL	SD	TW	KW	DBH	PFP	SWP	%P
SL	0.84*								
SD	0.89*	0.86*							
TW	0.96*	0.87*	0.82*						
KW	0.98*	0.87*	0.89*	0.89*					
DBH	0.52	0.36	0.62	0.54	0.49				
PFP	0.15	0.13	0.19	0.12	0.17	0.07			
SWP	0.09	0.09	0.25	0.06	0.10	0.12	0.99*		
%P	0.03	0.42	0.01	0.12	0.03	0.26	0.25	0.31	
%O	0.42	0.02	0.29	0.41	0.41	0.67	0.07	0.04	0.63

Fatty acid profile (FAP) was similar for the grafted and non-grafted plants, although concentration of individual fatty acids differed significantly ($p<0.05$) (Table 3). The most abundant fatty acids were the unsaturated linoleic acid (57.64-52.39%) and oleic acid (26.07-21.44%), and the saturated palmitic (16.55-12.07%) and stearic (9.65-4.93%) acids. The total amount of average unsaturated fatty acids was 78.96%, of which 26.52% are monounsaturated acids, and 52.49% are polyunsaturated acids, being linoleic acid the main component. No significant difference was found in palmitic and linolenic fatty acids between the grafted and non-grafted *J. platyphylla* plants. Non grafted plants obtain the highest value or palmitoleic acid (0.68%). The graft facilitates the absorption and translocation of mineral nutrients in plants such as Mn, which activates one or more enzymes in the synthesis of fatty acids (Wang *et al*. 2006).

For the selection of vegetable oils with energy and nutritional potential, it is of utmost importance to know the composition of the fatty acid profile. *J. platyphylla* oil turned out to be predominantly polyunsaturated, which has been reported as an important oil in the food industry (Sosa-Segura *et al*. 2014). Linoleic fatty acid can lower blood cholesterol levels and reduce the risk of heart disease (Froyen and Burns-Whitmore 2020). The American Heart Association recommends that they constitute at least 5-10% of total energy (Jandacek 2017) By contrast, the profile of soybean and *J. curcas* oils present similar chemical regarding main fatty acids content, mainly oleic acid (Ustra *et al*. 2013), for biodiesel production.

Table 3. Fatty Acid Composition (%) of *J. platyphylla* grafted plants. Means in a row followed by the same letter are not significantly different by Fisher post hoc tests at the 5% level. SFA, UFA, MUFA, PUFA denote saturated, unsaturated, monounsaturated and polyunsaturated fatty acids, respectively.

Fatty acid (%)	iPR14	T0	iTP4	iDA5(0)	iPR11	iPR2	iPR10
Myristic C14:0	0.30a	0.28ab	0.24bc	0.29ab	0.22c	0.25abc	0.27abc
Palmitic C16:0	14.36a	15.26a	7.34a	13.63a	13.78a	10.10a	13.72a
Palmitoleic C16:1	0.59ab	0.68a	0.51bc	0.50bc	0.60ab	0.56ab	0.37c
Heptadecanoic C17:0	0.00b	0.12a	0.00b	0.00b	0.12a	0.00b	0.00b
Stearic C18:0	6.88a	0.81e	4.06d	6.96a	6.05c	6.49b	6.47b
Oleic Cis 9 C18:1	26.66a	26.76a	25.81ab	26.33a	24.90b	26.14ab	25.93ab
Linoleic C18:2	51.71bc	52.69abc	55.28ab	51.06c	51.06c	56.11a	52.98abc
Linolenic C18:3	0.00a	0.07a	0.57a	0.00a	0.00a	0.11a	0.00a
Arachidic C20:0	0.00b	0.13b	0.00b	0.36a	0.36a	0.07b	0.13b
SFA	21.54	16.6	11.64	21.24	20.53	16.91	14.12
UFA	78.96	80.02	82.17	77.89	76.56	82.92	79.28
MUFA	27.25	27.44	26.32	26.83	25.50	26.70	26.30
PUFA	51.71	52.89	55.85	51.06	51.06	56.22	52.98

The present study reveals that this method will be helpful in generating planting material with better traits (high yielding and taproot) for large scale propagation in relative short time.

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5. CONCLUSIONES GENERALES

- A partir de la utilización de marcadores morfológicos y de producción, como el contenido de aceite y proteína y los marcadores moleculares, se pudo identificar germoplasmas potencialmente útiles para una selección viable para programas de mejoramiento genético, considerando que *J. platyphylla* es una especie silvestre. La búsqueda de plantas adaptadas a la región y altamente productivas para la generación de híbridos pueden crear valor agregado en estas especies poco explotadas.
- La utilización de técnicas moleculares, como los ISSR, para la identificación de la variabilidad genética para la selección de germoplasmas resultó ser una técnica viable. La variabilidad reportada en nuestro estudio sirve como inicio para comprender la relación fenotipo-genotipo de poblaciones silvestres y así poder desarrollar estrategias de reproducción de las plantas. De acuerdo a los resultados obtenidos, se sugiere analizar un mayor número de plantas y probar marcadores ISSR adicionales.
- El uso de esquejes en la propagación vegetativa *de J. platyphylla* y el injerto como una herramienta para la multiplicación en masa de genotipos élite permitió identificar plantas injertadas de *J. platyphylla* con rendimiento y características fenotípicas específicos, logrando su propagación a gran escala en un tiempo relativamente corto.

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