



**ASIGNACIÓN DE CARACTERES MORFOLÓGICOS COMO
DESCRITORES PARA LA DIFERENCIACIÓN ENTRE ACCESIONES DE
Jatropha curcas †**

**[ASSIGNING MORPHOLOGICAL TRAITS AS DESCRIPTORS FOR
DIFFERENTIATION BETWEEN *Jatropha curcas* ACCESSIONS]**

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SUMMARY

Background. The morphological characterizations and varietal descriptions of *Jatropha curcas* do not consider all morphological variability of this species. The phenotypes of *J. curcas* that have been described represent only a part of the variability of this species, making it difficult to distinguish between accessions based on morphological traits. **Objective.** Propose a method to assign trait states to discriminate between accessions based on their phenotype, and to evaluate the efficiency of these phenotypes as morphological traits. **Methodology.** Thirteen morphological traits were selected, and trait states were assigned according to their variability. **Implications.** The results show that some traits, such as the number of male flowers and the ratio of male to female flowers provide redundant information, while other traits do not provide information with enough discriminatory power. By other hand, trait associated with the frequency of one, two, three or four seeds per fruit set the pattern for the identification of phenotypes. **Conclusion.** The proposed method allowed to use the variability of traits as a descriptor without limiting the use of morphological traits not included in this work.

Keywords: Morphological characterization; Discriminatory power; Trait states; Similarity between accessions

RESUMEN

Antecedentes. Las caracterizaciones morfológicas y las descripciones varietales de *Jatropha curcas* no contemplan toda la variabilidad morfológica de esta especie. Los fenotipos de *J. curcas* que se han descrito representan solo una parte de la variabilidad de esta especie, lo que dificulta la distinción entre accesiones basadas en rasgos morfológicos. **Objetivo.** Proponer un método para asignar estados de carácter para discriminar entre accesiones en función de su fenotipo, y evaluar la eficiencia de estos fenotipos como descriptores morfológicos. **Metodología.** Se seleccionaron trece caracteres morfológicos y se asignaron estados de rasgos según su variabilidad. **Implicación:** Los resultados muestran que algunos caracteres, como el número de flores masculinas y la proporción de flores masculinas a femeninas, brindan información redundante, mientras que otros rasgos no brindan información con suficiente poder discriminatorio. En contraste, el carácter asociado con la frecuencia de una, dos, tres o cuatro semillas por fruto establecen el patrón para la identificación de fenotipos. **Conclusión.** El método propuesto permitió utilizar la variabilidad de rasgos como descriptor sin limitarse al uso de rasgos morfológicos no incluidos en este trabajo.

Palabras clave: Caracterización morfológica; poder discriminatorio; estados de carácter; similaridad entre accesiones

INTRODUCTION

Jatropha curcas is a plant of the family Euphorbiaceae (Orwa *et al.*, 2019), which seeds content between 25% to 52% of oil (Kotoky *et al.*, 2015; Yong *et al.*, 2010); this makes it a useful species for the production of biodiesel (Bojan *et al.*, 2011) and creates interest in applying genetic improvement techniques to accessions in order to obtain varieties with better yield

and improved agronomic characteristics (Gongora-Canul *et al.*, 2017). *J. curcas* is currently cultivated worldwide, mainly in African and Asian countries. However, their advances in domestication and genetic improvement are limited by low genetic diversity. On the contrary, in Mexico it was considered as the center of origin and diversification of *J. curcas* have found greater genetic variability which could contribute important advances in the improvement programs with

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accessions from regions of Central America (Divakara *et al.*, 2010; Li *et al.*, 2017; Sunil *et al.*, 2013b).

The morphological characterization of *J. curcas* was a first step in its domestication and genetic improvement (Prado *et al.*, 2017). It has allowed to make varietal guides (Achten *et al.*, 2010; Montes and Melchinger, 2016; Sunil *et al.*, 2013a; Yi *et al.*, 2014) that can serve to improve the exchange of information between collections from different regions and accelerate the process of domestication of *J. curcas* from a wild crop to an agronomic crop (Prado *et al.*, 2017). However, most of the traits described in the guides do not have a pure line that could be used as reference to determine their importance for the differentiation of varieties. Some traits provide redundant information, such as the length and width ratio of fruits and seeds (Aguilera-Cauich *et al.*, 2015; George *et al.*, 2016; Sunil *et al.*, 2013a); others traits lack a method for measuring them and remain as a subjective interpretation, such as the architecture of the plant (Sunil *et al.*, 2013a). While others correspond to yield characteristics that are affected by the environment, such as the average number of fruits per plant, seed per inflorescence or seed per plant the height of the plant or the diameter of the stem (Behera *et al.*, 2010). Due to this, there are no standardized measurements or a homogeneous evaluation method among the existing works of morphological characterization (Bioversity International, 2007), and it is therefore not possible to compare between varieties and accessions, which hinders the possibility of carrying out genetic improvement programs and furthering the process of domestication worldwide.

To assess whether a morphological trait can be used to discriminate between two accessions, discriminant indices are established, based on the variability and its frequency between a group of accessions (Carović-Stanko *et al.* 2011). The indices are used to determine the potential of a trait to discriminate between two accessions (discriminatory power "Dm"). Morphological traits can be divided into several categories based on the criteria used by the lists that describe the different varieties of commercial crops: passport traits, management traits, traits related to the response to the environment, characterization traits and evaluation traits (Bioversity International, 2007). Of these categories, only the characterization traits were used in the test "Distinction, homogeneity and Stability (DHE)". The other categories, as their name implies, account for variability generated by external factors (environmental conditions, agronomic management, phytosanitation, etc.), not the variability associated with a given genotype or a combination of genotypes (UPOV, 2010); thus, their Dm could be masked by those external factors.

With respect to *J. curcas*, since it is still in the process of domestication, it is necessary first to know which traits are associated with the genotype or combination of genotypes and which traits are the result of interaction with the environment. Other works have reported that some of the traits that best represent the genotype of an accession are: Number of main branches, the plant architecture, the number of lobes in a leaf, the length of the petiole, the number of female and male flowers, the size and shape of fruits and seeds, and the number of seeds produced per fruit (Aguilera-Cauich *et al.*, 2015; George *et al.*, 2016; Sunil *et al.*, 2013a; Wu *et al.*, 2011; Yi *et al.*, 2014). No pure lines of agronomic traits have been produced, as happens with other domesticated species. Even though there is already a record of the variability of these traits, the degree of variability of the accessions that is attributable to genetics or to external factors is still unknown.

The objective of this work was to propose a method to assign trait states that allow to discriminate between accessions based on their phenotype, and to evaluate the efficiency of each morphological trait. To achieve this objective, 13 morphological traits were selected and assigned a trait state according to their variability.

MATERIALS AND METHODS

Plant material

Nine accessions were collected from different regions: JatroP1 and JatroP2 from Nicaragua; JatroP3, JatroP4, JatroP6 and JatroT2 from Chiapas, Mexico; JatroP5 and JatroT1 from Veracruz, Mexico; and JatroT3 from Yucatán, Mexico. Six were chosen (JatroP1, JatroP2, JatroP3, JatroP4, JatroP5, JatroP6) because its agronomic features and oil yield as parental and three accessions were used as control (JatroT1, JatroT2 and JatroT3) to define a better separation of crosses from parents. Parental accessions were crossed, and one line per cross candidate for variety materials were chosen for this work: JatroC1-P1xP2, JatroC2-P5xP2, JatroC3-P2xP4, JatroC4-P1xP4, JatroC5-P1xP5, JatroC6-P2xP6, JatroC7-P1xP6, JatroC8-P2xP3, JatroC9-P1xP3, JatroC10-P4xP5, JatroC11-P4xP6, JatroC12-P4xP3, JatroC13-P5xP6, JatroC14-P5xP3, and JatroC15-P6xP3. In an experimental field located at 21° 08' 00.7" N 89° 46' 49.6" W a total of 24 lines were randomly distributed in three blocks (2015 June). The plants were produced by vegetative propagation and each accession had five specimens in each block, for a total of 15 plants per accession (Sánchez-Velázquez *et al.*, 2018). The plants were pruned 9 months after planting, homogenizing the height of the plants to 70 cm. The management of the plantation was homogeneous in terms of irrigation, fertilization (two applications of fertilizer, one when the plantation was established and another after pruning) and pest control

(monitoring of insect pests and fungi, with application of pesticides). The collection of data was carried out from July to December 2016.

Assignment of trait states

The evaluated traits comprise 13 descriptors usually used for morphological characterization, divided into four vegetative traits and nine reproductive traits (Table 1). The data corresponding to each trait were averaged by accession and later divided into categories (trait states), so that each accession belonged to only one category. The assignment of categories for each trait was performed based on the literature, when the information was available.

When no reference was found about the range of variability of a specific trait, the standard deviation of the averages was calculated, then, range of variability was constructed based on the minimum and maximum value of the standard deviations, grouping 68% of the variability quantified. The range of variability cover 66% of the accessions of the subject population. Later, 25% was added to the range of variability obtained, which was divided into four quartiles. For those accessions whose average and standard deviation were outside of the constructed variability range, was necessary adding quartiles (addition of 25% of the constructed range) as new trait states.

The traits in the main branches of the accessions were divided into three states based on the variability reported previously (Aguilera-Cauich *et al.*, 2015; Yi

et al., 2014) and that observed in the experimental field: state I = 1 to 2.5 branches on average; state II = 2.6 to 4 branches on average and state III = 4.1 to 5.5 branches on average. The angle of the main branches was measured by taking photographs of the plant branches; the photographs were then simplified into a white background over which the plant shape contrasted in black, using the free software ImageJ 1.50i. Vectors were then drawn between the main stem and the first 5 cm long of the branch to be measured, taking as the Y-axis half of the diameter of the main stem and as intersection vector half of the diameter of the branch under analysis. The intersection angle of the vector with the Y-axis was measured for each branch of each plant of each accession. To calculate the trait states in each accession, the angle of 30 randomly chosen branches from each accession was averaged using the Excel function (Office 2010) "= random 0 - (total branches per accession)". The angle of the main branches was divided into three states based on the variability reported [7,10]. State I = < 30°; state II = > 30° to 60°; state III = > 60°.

With respect to the vegetative traits, length variation of petiole was divided into three states using ranges of 5 cm and modified ranges of 10 cm (Sunil *et al.*, 2013a). State I = < 10 cm; state II = 10.1 to 15 cm; state III = > 15 cm. In the same way, the trait corresponding to the number of leaf lobes was divided into three states based on the variability reported by Sunil *et al.* (2013). State I = 1 to 2; state II = 3 to 4; state III = > 4.

Table 1. Morphological traits evaluated.

Trait	Type of trait	Unit	Trait states	No. of data analyzed per accession
Main branches (MB)	Vegetative	#	3	15
Angle of main branches (AMB)	Vegetative	<°	3	30
Leaf lobes (LL)	Vegetative	#	3	75
Petiole length (PL)	Vegetative	#	3	30
No. of male flowers (MF)	Reproductive	#	4	12
No. of female flowers (FF)	Reproductive	#	4	12
Ratio between female and male flowers (FF:MF)	Reproductive	1:X	4	12
Ratio between width and length of fruits (W/LF)	Reproductive	0.0 - 1	3	204
Ratio between width and length of seeds (W/LS)	Reproductive	0.0 - 1	4	58
Frequency of 1 seed per fruit (FS1)	Reproductive	%	4	300
Frequency of 2 seeds per fruit (FS2)	Reproductive	%	4	300
Frequency of 3 seeds per fruit (FS3)	Reproductive	%	4	300
Frequency of 4 seeds per fruit (FS4)	Reproductive	%	5 (I, II, III, IV, and VII)	300

#: Natural number; <°: degrees; 1:X: one to X; %: percentage.

Regarding the traits of the reproductive organs, the number of male flowers, female flowers and the female/male flower ratio, we counted the total number of flowers in three inflorescences of four plants for each accession. The criteria used was a total opening of the floral buttons and no presence of withered male flowers. Hermaphrodite flowers were counted as female flowers. The number of male and female flowers was divided into four states. For male flowers: state I = 0; state II = 1 to 100; state III = 101 to 200; state IV = 201 to 300. For female flowers: state I = 0 to 5.5; state II = 5.6 to 10.5; state III = 10.6 to 15.5; state IV = > 15.5. The ratio of female to male flowers was divided into four states based on the variability reported by Nietsche *et. al* (2013) and Wu *et al.* (2011). State I = 0M:1F; state II = 1 to 10M:1F; state III = 11 to 20M:1F; state IV = > 21M:1F.

Regarding the trait corresponding to fruit shape and seed shape, the length and width of fruits and seeds were measured transversally, and then the width was divided between the length. The fruits used for the measurements, including seeds, were in mature state, without green spots or signs of senescence, which correspond to the states III or VII reported by Junio da Silva *et al.* (2012) and Sinha *et al.* (2015), respectively.

The fruit shape trait was divided into three states representing rounded shapes on a scale from 0.00 to 1, where values closer to zero indicate more elongated shapes and values closer to 1 indicate more circular shapes. State I = 0.71 to 0.8; state II = 0.81 to 0.9; state III = 0.91 to 1.00. It is possible that some fruits are wider than long and that the measurement results in a value greater than 1. Nevertheless, these fruits were classified into state III. Regarding the categorization of the seeds, they were divided into four states representing oval shapes [8] on a scale of 0.0000 to 1.0000, where a value closer to 0.0000 indicates oblong shapes and a value closer to 1.0000 indicates more obovate shapes, with state I = 0.5488 to 0.5863; state II = 0.5864 to 0.6239; state III = 0.6240 to 0.6615; state IV = 0.6616 to 0.6991.

Since the amount of fruit produced per plant can be influenced by agronomic management or environmental factors, the yield was not considered, but the number of seeds per fruit was. Although the number of seeds per fruit is not homogeneous for every plant, the frequency of fruits with the same number of seeds per plant can be considered as a possible varietal descriptor. This descriptor was divided into four traits with their own states: (a) the frequency of fruits with one seed, with four states; (b) frequency of fruits with two seeds, with four states; (c) the frequency of fruits with three seeds, with four states; (d) the frequency of fruits with four seeds, with seven states (Table 2).

Data analysis and Traits Dm determination

Once each trait was categorized, theoretical Dm was estimated by assuming the same frequency for all trait states and the same value of C_j for each state, with the following equation:

$$D_m = 1 - C_j \quad (1)$$

Where D_m is discriminatory power and C_j is the probability that an accession shares the same trait state with the other accessions. The value of C_j was estimated using the following equation:

$$C_j = \sum_{i=1}^j p_i \left(\frac{N p_i - 1}{N - 1} \right) \quad (2)$$

Where N is the number of accessions, p_i is the frequency of the trait state i and j is the sum of all C_j of the trait. Then D_m of traits was calculated using the real frequency of the trait states following the above equation, and results were compared with theoretical D_m .

Correlation between traits

Based on the trait states obtained for each accession, a phenotypic profile was assigned to the accessions, with the trait states as dependent variables of the accessions. A correlation analysis between the traits was carried out using the Pearson index. A PLS regression analysis was also carried out (PAST 3.15; (Hammer *et al.*, 2001)).

Table 2. Traits state assignment according to the frequency with the number of seeds presented per fruit.

Seeds/fruits	Traits state						
	I	II	III	IV	V	VI	VII
	Frequency range of the number of fruits that have n seeds (%)						
FS1	0 to 0.348	0.349 to 0.7394	0.7395 to 1.1299	1.13 to 1.5204			
FS2	0.4 to 7.2913	7.2914 to 14.1827	14.182 a 21.074	21.0742 a 27.972			
FS3	70 to 77.5	77.6 to 85	85.1 to 92.5	92.6 to 100			
FS4	<0.01	0.01 to 0.81	0.82 to 1.62	1.63 to 2.43	2.44	3.25	4.06
					to	to	to
					3.24	4.05	4.86

Determination of the Index of diversity index and similarity among accessions of *J. curcas*

The diversity index of Shanon-Weaver was calculated using the following formula:

$$H' = \sum_{i=1}^n p_i (\log_2 p_i) \quad (3)$$

Where n is the evaluated population and p_i is the frequency of occurrence of trait i.

The similarity between accessions was calculated using Hamming distances for categorical data (Sen, 2005), considering traits as genotype-dependent variables with the morphological profile of each accession. A dendrogram was constructed with the UPGMA method with the help of PAST software 3.15 (Tessier *et al.*, 1999).

RESULTS AND DISCUSSION

Assignment of trait states

Some morphological trait was divided into different states according to the ranges reported or specified by the proposed method (Gongora-Canul *et al.*, 2017). For each evaluated morphological trait, a state was assigned to each accession, so that each accession was assigned only one trait state per morphological trait, forming in this way a phenotypic profile for each accession. This allowed to assess the similarities and differences between accessions, as well as the most frequent phenotype for each trait (Figure 1). For the

trait AMB there are no states differences between accession, since the variability of the AMB values are tight.

Determination of the Dm of the traits

Independently of the number of traits evaluated and the assignment of trait states in the morphological characterization, it is necessary to know if the traits can be used to discriminate one accession from another, since it is possible that the predominance of a trait state over the others could negatively affect the ability of that trait to distinguish between accessions and varieties. Indices have been used in other species to assess the effectiveness of traits as descriptors, as well as their usefulness for standardizing measurements between accessions from different regions, and the possibility of comparing pure lines with their wild relatives (Carović-Stanko *et al.*, 2011). The present work evaluated 13 morphological traits with the Dm index, which has a maximum theoretical range (maxDM) between 0 and 0.92 when using the maximum number of trait states allowed by the International Union for the Protection of New Varieties of Plants (UPOV). Each trait has a maximum theoretical Dm, but the number of theoretical states does not matter, since the final value of Dm only takes into account the frequency of the trait states present in the accessions evaluated, so that trait states that are not present will not influence the Dm of a trait in a group of accessions (Tessier *et al.*, 1999).

		<i>J. curcas</i> accessions																							
		JatroP1	JatroP2	JatroP3	JatroP4	JatroP5	JatroP6	JatroT1	JatroT2	JatroT3	JatroC1	JatroC2	JatroC3	JatroC4	JatroC5	JatroC6	JatroC7	JatroC8	JatroC9	JatroC10	JatroC11	JatroC12	JatroC13	JatroC14	JatroC15
Trait	MB	II	II	II	I	II	II	I	I	II	II	II	III	II	II	III	II	III	II	II	II	II	II	II	I
	LL	II	III	III	III	III	II	II	II	III	III	III	III	II	II	III	III	II	III	III	II	II	III	II	III
	PL	II	II	II	III	II	I	II	II	III	III	II	II	I	I	II	II	II	II	II	I	I	II	III	III
	FF	III	IV	IV	I	II	IV	II	III	III	II	II	III	II	III	II	IV	II	III	II	II	III	IV	III	IV
	MF	III	I	I	IV	III	I	III	III	III	III	III	IV	III	III	III	IV	III	III	III	III	I	I	III	IV
	FF:MF	III	I	I	IV	IV	I	IV	III	IV	III	III	III	III	III	I	I	III	III						
	W/LF	II	III	II	III	II	II	II	II	II	II	III	II	III	II	III	II	II	II	II	II	III	II	II	II
	W/LS	III	III	III	III	II	II	II	II	III	II	IV	I	III	III	IV	II	I	II	III	II	III	III	I	II
	FS1	III	I	I	I	I	I	IV	II	II	II	I	IV	I	II	I	II	I	II	I	II	II	II	IV	II
	FS2	I	I	II	I	I	I	III	II	III	III	I	II	I	IV	I	IV	I	I	I	I	II	II	III	II
	FS3	III	IV	III	IV	IV	III	II	III	II	II	IV	III	IV	I	IV	I	IV	IV	IV	IV	III	III	II	III
	FS4	II	III	III	III	II	VII	II	III	I	I	I	IV	II	II	II	II	I	III	II	II	I	III	I	I
	AMB	II	II	II	II	II	II	II	II	II	II	II	II	II	II	II	II	II	II	II	II	II	II	II	II

Figure 1. Morphological profile of the 24 accessions through thirteen morphological traits.

We evaluated Dm of 13 traits with a theoretical range of 0.5 for minimum Dm and 0.74 for a maximum Dm, reaching at least 75% of the maxDm (Figure 2). The traits with broader ranges of variability showed the high results of Dm, the best of them being the frequency of FS4, which reached 87.5% of the value of maxDm. Dm evaluation gives us an approximation of which are the last heterogeneous morphological traits among the accessions of *J. curcas*: W/LS, MP and AMP. The results are also reflected in the number of trait states and the predominance of the frequency of one of the states of these traits, which indicates there is a limited range of variability. Although some traits showed minimum Dm values, fruit shape and the number of main branches exceeded the suggested minimum value of 0.33 on a scale of 0.01 to 0.99, so it should be possible to use them as discriminating between accessions in a germplasm bank, however, 10 were very effective for distinguishing between accessions.

Only the trait corresponding to the AMB showed a Dm equal to 0, indicating that the architecture of the plant is predominantly influenced by the environment. Although some individuals may have more closed or open angles, the general behavior of one accession shows the same phenotype as the rest of the accessions. This behavior has been reported for other species, in which a closed architecture is favored by competition between plants when the density of the plantation increases, while more open architectures are favored when the density of the plantation decreases (Van de Peer *et al.*, 2017). The trait "angle of the main branches" was excluded from the following analyzes due to its null ability for discriminate between accessions.

Correlation between traits

It is possible that the information provided by some traits is redundant in terms of its usefulness to discriminate between accessions. Thus, to ensure that some pairs of traits do not mask the information provided by another trait (e.g., number male flowers and female to male flower ratio), a correlation analysis was carried out between traits. Pairs of traits should have a correlation between -1 and 0, since this would indicate that the information, they provide is independent and not redundant. Values closer to 1 would indicate a narrower positive relationship between traits, which would mean that they provide the same discriminatory information. Table 3 shows the results of this analysis; it is possible to see that the relationship between male flowers (MF) and the ratio of male to female flowers (FF:MF) is closest to 1, with

a value of 0.8, indicating that the measurement of both traits could be simplified by taking only one of the two measurements. This is because the ratio between male and female flowers (FF:MF) is derived from the measurement of two other traits. This effect has been reported before for parameters such as the number of fruits and the number of inflorescences or the number of seeds and the number of fruits. The relationship between pairs of traits is less noticeable in non-reproductive traits (Biabani *et al.*, 2012).

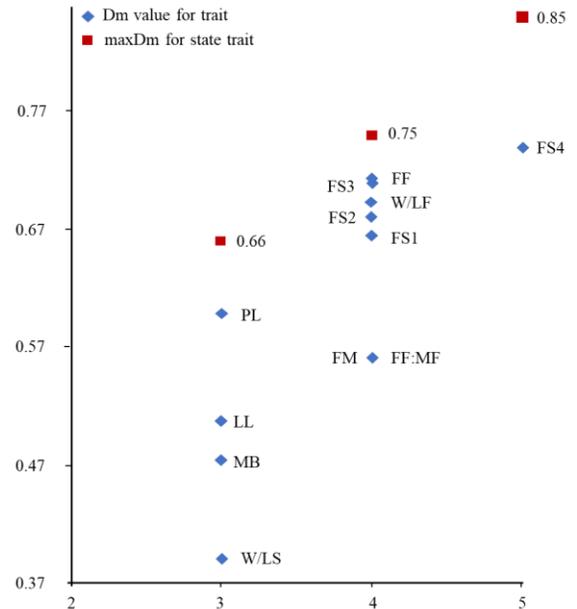


Figure 2. Discriminatory power (Dm) of 12 morphological traits and maximum discriminatory power (maxDM) of the evaluated traits per number of possible states of the trait (the trait AMB was excluded because its Dm = 0).

Some traits showed correlation values above 0.5, which is already considered a strong positive correlation for morphological traits in plants (Conner *et al.*, 2014). Three of these traits are vegetative traits and one is a reproductive trait. Fruit and seed shape showed a correlation of 0.50152. Moreover, the relationships between fruit shape and the frequencies of three and one or two seeds per fruit indicate that although the fruits can determine and limit the shape of the fruit seeds, it is also possible that seeds can modify their shape depending on the amount of seeds that develop in the fruit. Thus, the shape of the fruit is not an indicator of the shape of the seed, and large fruits can generate many small seeds or a few large seeds.

Table 3. Pearson's correlation analysis between 12 morphological traits.

	W/LF	MB	LL	PL	MF	FF	FF:MF	W/LS	FS1	FS2	FS3	FS4
W/LF												
MB	-0.051											
LL	0.268	0.092										
PL	-0.158	0.240	0.524									
MF	-0.277	0.100	0.131	0.400								
FF	-0.101	0.019	0.090	-0.075	-0.460							
FF:MF	-0.119	0.100	0.044	0.333	0.867	-0.709						
W/LS	0.502	0.045	0.098	-0.149	0.050	-0.417	0.149					
FS1	-0.507	0.090	-0.283	0.199	0.277	0.118	0.188	-0.421				
FS2	-0.216	0.170	-0.057	0.196	0.175	0.301	0.044	-0.488	0.501			
FS3	0.219	0.169	0.162	-0.135	-0.113	-0.383	0.023	0.555	-0.529	-0.960		
FS4	-0.108	0.015	-0.033	-0.348	-0.348	0.343	-0.381	-0.037	-0.107	-0.260	0.118	

Bold numbers indicate pairs of traits with the highest positive correlation.

The correlation values between the other pairs of traits suggest that some of them should not be used to discriminate between accessions. Only the most relevant should be used (independently of their agronomic value). A PLS analysis of the covariance between pairs traits was carried out to determine which of them showed greater linearity and closeness to each other and can thus be discarded (Figure 3). The results of the analysis corroborated the information shown in Table 3; the linearity between male flowers and the ratio of male flowers to female flowers is the closest, so it is possible to discard one of these two traits. The PLS analysis also showed that the origin of the accession (either parental or cross), is closely linked to an increase in the frequency of fruits with one or two seeds, which may be due to inbreeding effects and the low genetic diversity of the accessions. With respect to the frequency of four seeds per fruit, an exceptional trait with agronomic value, one of the parents showed frequencies above 4% (Trait state VII). However, the crosses derived from this source remained within the ranges of states 1, 2, 3 and 4, together with the other crosses and parents. Thus, this trait showed the least linearity with the origin of the accessions. The trait corresponding to the frequency of three seeds per fruit showed a negative correlation with the frequency of one seed per fruit (≥ 0.5); the PLS analysis showed a linearity in the opposite direction to the origin of the accession. The trait corresponding to the shape of the seeds was shown to affect the frequency of the trait of one seed per fruit and, at the same time, to be related to the shape of the fruits (Table 2). It also seems to be a trait through which the frequency of the trait of four

seeds per fruit can increase (Figure 3), as it showed the greatest linearity between FS3 and FS4, a relationship that could have agronomic value if it contributes to the improvement of *J. curcas*. It is possible that seeds that are closer to an elliptical shape have a better spatial arrangement within the fruit than more oblong seeds; thus, the elliptical shape of the seeds could increase the frequency of the presence of four seeds per fruit, which could have great agronomic value.

Similarity between accessions

It is important to know whether the differences and similarities between accessions are reflected in the phenotypic profiles shown in Figure 1, since this can simplify the identification of accessions with agronomic interest in the field. The visualization of trait states as a temperature chart makes it easier to identify the predominant states among the group of accessions evaluated. To make the chart, the genetic distance between the accessions was calculated and a dendrogram of the separation between the accessions was constructed (Figure 4). The dendrogram was then compared with the percentage of simultaneous trait states that can be shared using the 13 traits and the percentage of trait states effectively shared between the accessions (Figure 1) As a result, the accessions were divided into five groups, using a similarity threshold of 0.6. Five crossed accessions, out of a total of 15, were not grouped with their parents (Figure 4), which indicates that the combination of their parents originates new trait states and intermediate states (Figure 1; traits RP and FS1 as an example) that differ

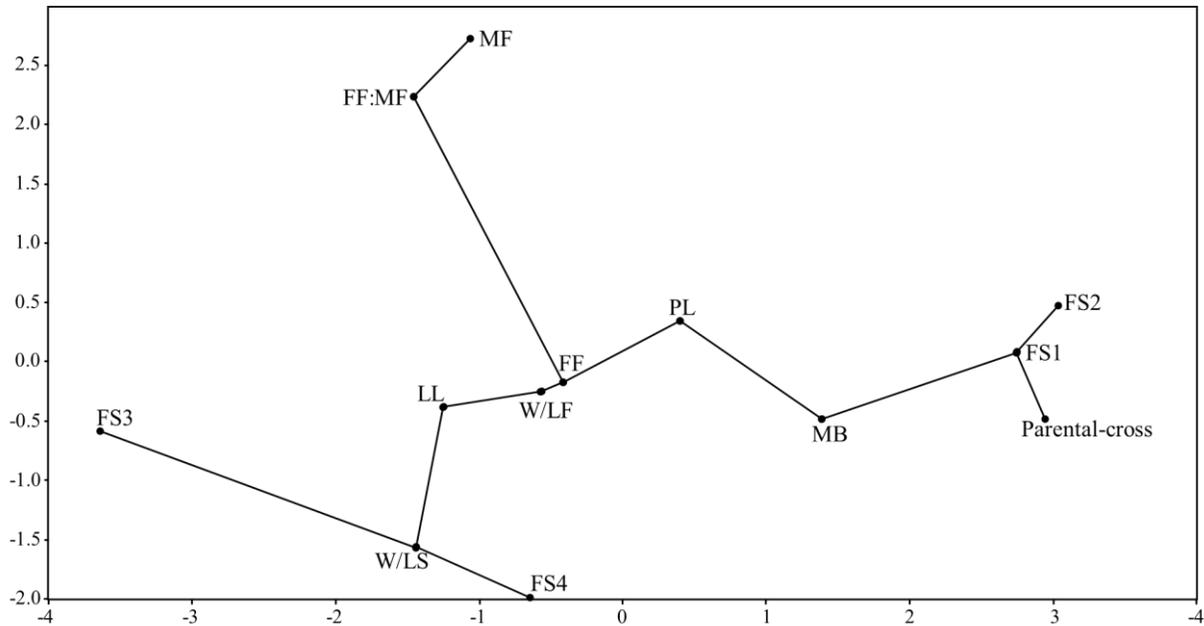


Figure 3. PLS analysis of covariance between the traits and the origin of the accessions ("Parental-Cross") introduced as a variable with three states: I = Parental; II = Control; III = Cross.

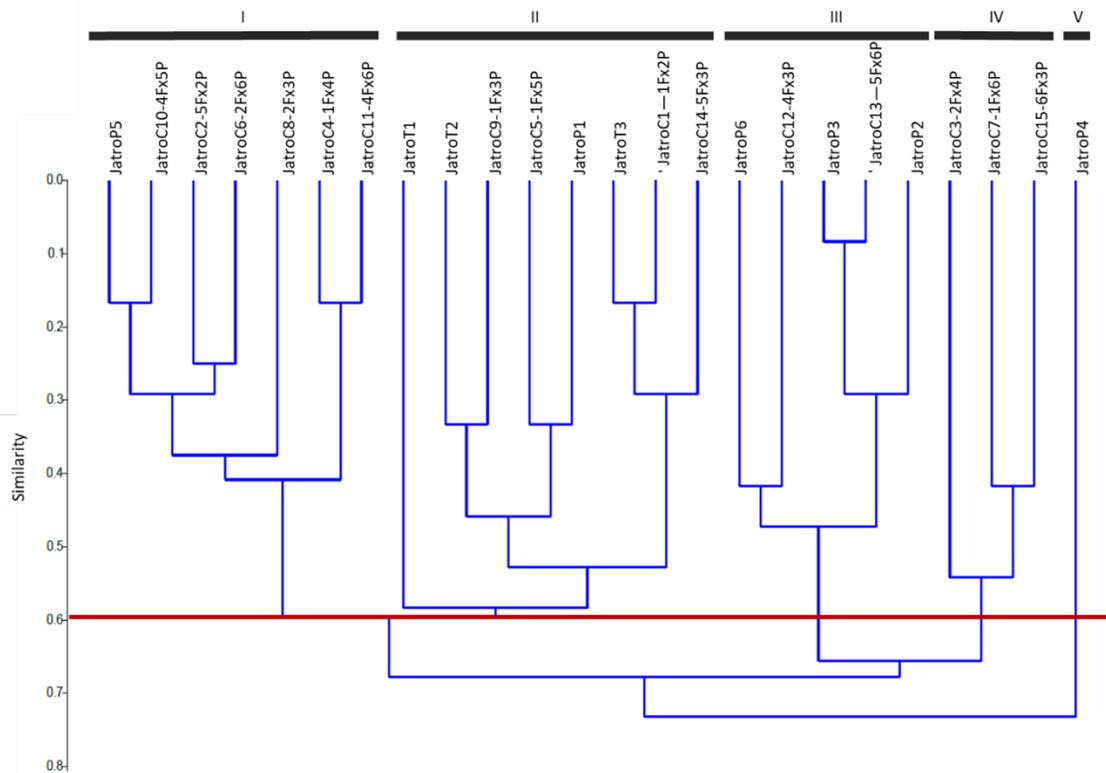


Figure 4. Grouping accessions according to their morphological profile, using 13 morphological traits. The red line indicates a threshold at a distance of 0.6 and an effective separation of the accessions into five different phenotypes. Cohen's correlation = 0.7002

from their parents (Wang et al., 2015); this is the case of JatroC4, JatroC6, JatroC8, JatroC11 and JatroC14. In these accessions, the traits that generated the states that differed the most with respect to the parental states were number of female flowers (FF), followed by the ratio of female to male flowers (FF:MF) and the frequency of four seeds per fruit (FS4), all of them reproductive traits. The FF trait was associated with intermediate states between the parents, while the trait FF:MF was associated, in some cases, with higher states with respect to the parents, while the FS4 trait was associated in general with a decrease in the frequency of fruits with four seeds from the parents to the crosses.

The phenotypic plasticity of the traits is a way to adapt to different environmental circumstances. This adaptability allows individuals to survive until conditions are favorable (Gratani, 2014); however, variability due to the environment is unreliable for distinguishing between accessions, as is evident in the evaluation of the trait angle of main branches (AMB) and to a lesser extent of the trait number of main branches (MB) and fruit shape (A/LF) (Figure 2).

CONCLUSIONS

The present work categorized 13 traits (Figure 1) according to their range of variability, assigning discrete values to the range of variability of each trait (trait states) when doing the transformation of the continuous data to discrete data. The results showed that the construction of morphological profiles based on trait states allows distinguishing quickly between accessions by trait or groups of traits. This method allowed to realize the differences and similarities between parents, between parents and crosses and between crosses, regardless of the agronomic value of each accession. The analysis of 13 morphological traits in accessions of *J. curcas* showed that these could be categorized to form morphological profiles that lead to the establishment of phenotypes, each represented by a trait state. We concluded the assignment of traits states it is necessary to assess the efficiency of a trait for discriminating between accessions, which can save time and resources when characterizing new accessions. The analysis also showed that the assignment of trait states can help select sets of traits to increase or decrease the frequency of other traits through their correlation and linearity, as in the case of the number of seeds per fruit and the ratio between male and female flowers.

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Compliance with ethical standards. The authors are part of the research, are familiar, and are agree with the contents of the manuscript.

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