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Article

Repetibilidad de características útiles como descriptores morfológicos en clones de *Cedrela odorata* L.

Repeatability of useful characteristics as morphological descriptors in *Cedrela odorata* L. clones.

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Resumen

En México hay avances en el mejoramiento genético de *Cedrela odorata*. El material mejorado debe tener características distinguibles, homogéneas y estables cuando crece en diferentes sitios para registrar la propiedad intelectual. El objetivo del presente estudio fue evaluar la repetibilidad de descriptores morfológicos en clones de *C. odorata* cuya reproducción vegetativa ha sido más exitosa por injertos, con el fin de identificar genotipos superiores para el registro de su propiedad intelectual. Se evaluaron 90 clones producidos por injerto en dos sitios contrastantes (ensayos clonales) y en un banco clonal. Se seleccionaron 19 caracteres: 12 cuantitativos y 7 cualitativos relacionados con crecimiento, fuste, copa y hojas; en el banco clonal solo se evaluaron hojas. La mayoría de los descriptores presentaron coeficientes de variación > 20 %, o alta variabilidad fenotípica entre clones. El Análisis de Componentes Principales reveló que los descriptores que contribuyeron significativamente y resultaron consistentes en los tres sitios de evaluación fueron longitud y ancho de hoja, longitud del peciolo y ancho de folíolos secundarios. También, contribuyó el diámetro y la rectitud del fuste. El análisis de agrupamiento evidenció poca relación entre los descriptores morfológicos con los grupos de clones y procedencias en cada sitio de evaluación. Los grupos creados por el análisis de agrupamiento fueron distintos en los tres sitios, y ningún clon se discriminó de manera consistente entre sitios. La interacción genotipo x ambiente y el portainjerto diferente en cada rameto pudieran reducir la posibilidad de repetibilidad en los caracteres y el registro de clones superiores.

Palabras clave: Agrupamiento, Análisis de componentes principales, injertación, procedencias, registro de clones superiores, propiedad intelectual.

Abstract

There are advances in the genetic improvement of *Cedrela odorata* in Mexico. The improved material must have distinguishable and stable characteristics when it grows in different places to obtain intellectual property. The objective of this study was to assess the repeatability of morphological descriptors in *C. odorata* clones whose vegetative reproduction has been more successful by grafting, as superior genotypes can be identified for the registration of their intellectual property. 90 clones produced by grafting at two contrasting evaluation sites (clonal trials) and in a clonal bank were assessed. 19 characters were selected: 12 quantitative and 7 qualitative; related to growth, stem, crown and leaves; only leaves were evaluated in the clonal bank. Most of the characters showed variation coefficients >20 %, which is a high level of phenotypic variability among clones. Principal Component Analysis revealed that the descriptors that contributed significantly and were consistent in the three sites were leaf length and width, petiole length and width of secondary leaflets; in the two trials diameter and straightness of the stem also contributed. The cluster analysis showed little relationship between the morphological characters with the groups of clones and provenances formed in each evaluation site. The clusters formed by the cluster analysis were distinctive at the three sites, and no clones could be consistently discriminated between sites. Genotype by environment interaction and the different rootstock in each ramet could reduce the possibility of repeatability in the characters and the registration of superior clones.

Key words: Clustering, principal component analysis, grafting, provenances, superior clones registration, intellectual property.

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Introduction

In the establishment of genetic improvement programs is necessary to have a morphological description of the outstanding materials and to determine the variability of their characteristics (Núñez-Colín and Escobedo-López, 2011). The purpose of the morphological characterization is oriented to weigh the consistency and variability of a study group through defined, easily visible and measurable traits (Franco and Hidalgo, 2003). In both sexually produced trees and clones there must be consistency in morphological characteristics between progeny or ramets in contrasting environments. It is expected that in clonal propagation there will be greater morphological homogeneity between individuals and that it will be easy to identify (Bonga, 1982).

Commercial forest plantations need to have quality germplasm to have high productivity (White *et al.*, 2007), which is obtained with forest improvement programs. Morphological characterization is an important step in this sense (Núñez-Colín and Escobedo-López, 2014), since the improvement cycles in tree species have long periods depending on their life cycle, and thus, the intellectual protection of plant material produced by breeding is important (Bennadji, 2003). In species without morphological descriptors, it is necessary to choose characters that allow to discriminate groups (López-Santiago *et al.*, 2008), for which grouping techniques and multivariate statistical methods are used (Crossa and Franco, 2004).

When improved plant material is obtained, it can be registered for intellectual property protection purposes, which enables it to be legally used by producers, planters and researchers. In Mexico, the registry of outstanding genotypes is carried out before the National Seed Inspection and Certification Service (SNICS, for its acronym in Spanish), which is regulated by the Federal Law of Plant Varieties (LFVV, for its acronym in Spanish). In Mexico there are no records of forest trees, probably because they are wild materials.

Cedrela odorata L. is a forest species appreciated in Mexico for commercial plantations (Ramírez-García *et al.*, 2008). Superior growth clones propagated by grafts have been developed without any registration, but morphological descriptors have been proposed for this purpose (Méndez and Sánchez, 2012). The SNICS and the International Union for the Protection of New Varieties of Plants (UPOV, for its acronym in Spanish) do not have description guides for forest species. For this reason, the aim of this study was to evaluate the repeatability of morphological descriptors in *Cedrela odorata* clones whose vegetative reproduction has been more successful through grafting, in order to identify superior genotypes for the registration of their intellectual property. The hypothesis was that if there is repeatability in the morphological descriptors between ramets of the same clone, and in turn, these are distinguished from other clones, it is possible to identify a superior clone, for intellectual property purposes.

Materials and Methods

Study area

The study was carried out in two clonal test with different environments. The first is located on land owned by *Agropecuaria Santa Genoveva*, in *San Francisco*, state of *Campeche* (Trial 1) at 19°33'26.53" N, 90°01'33.96" W, at 82 masl. The soil is of the Litosol type, with a clay texture. Rain precipitation is 1 300 mm per year and the average annual temperature is 23 °C. The second test was established on a private property in the *Isla* municipality, state of *Veracruz* (Test 2) at 18°04'50.21" N, 95°32'0.75" W, at an altitude of 56 m. The soil of the place is of the Cambisol type, with a sandy texture, precipitation of 2 000 mm per year and average annual temperature of 25 °C (Sánchez-Monsalvo *et al.*, 2019).

To compare some characters, the same genotypes from the clonal bank of the *El Palmar* Experimental Field of the *Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP)* in *El Palmar, Tezonapa, Veracruz* were used, which is

located at 18°30'19.29" N, 96°45'15.48" W at an altitude of 180 m. The type of soil is Acrisol with a sandy clay loam texture, annual precipitation of 2 888 mm and mean annual temperature of 24.4 °C (Sánchez and Velázquez, 1998).

Design and establishment of clonal tests

The plant material used to establish the clonal trials was propagated by grafting outstanding genotypes from different provenances: (*Bacalar, Quintana Roo; Calakmul, Campeche; Cardel, Cárdenas and Teapa, Tabasco; Comalapa and Palenque, Chiapas; Misantla, Tezonapa, Tierra Blanca, Tinaja, Papantla, and Los Tuxtlas, Veracruz; Lombardo and Tuxtepec, Oaxaca; Yucatán; Zona Maya, Unknown; Non-specific México and Guatemala*), determined by means of progeny-provenance tests carried out by INIFAP. The two clonal trials were established in 2012 in a randomized complete block experimental design with 90 clones and six replications; the clonal bank was established in 2013 with 25 replications per clone.

Assessed variables

The morphological descriptors described by Méndez and Sánchez (2012) were used. Four complete leaves were taken from the middle part of the crown. A sample for each cardinal point of the tree was evaluated and the data was averaged. The growth variables and morphological characters of the stem and crown were measured in the two experiments, but not in the clonal bank, since the trees are periodically pruned to obtain cuttings.

Quantitative characters

The total height (m) was measured with a Haga altimeter and the normal diameter (1.3 m high) of the stem (cm) with a Jackson MS 283D/10M diametric tape. The volume of the stem (dm³) was estimated with the Sánchez and García's (2009) equation:

$$VOL = (0.000065659) * (DIAM)^{1.768431077} * (ALT)^{1.137733502}$$

The straightness of the stem (degrees) was evaluated from the deviation of the stem with respect to the vertical line that it should follow, for which the Spirit level application of a mobile phone was used, placed at 1.5 m high from the tree and the observation it was expressed in degrees. The length of the leaf (cm), its width (cm) and the length of the petiole (cm) were measured with a Truper TFC-30ME graduated tape, Petiole length (mm), leaflet length (mm) and secondary leaflet width (mm) were measured with a Digimatic D 6 P Vernier caliper. In addition, the number of pairs of secondary leaflets and the shape index of the secondary leaflet (ratio between length / width) were determined.

Qualitative characters

The shape of the stem at a height of 1.30 m from the ground was categorized into: semicircular (1), rhomboid (2), deltoid (3), ellipsoid (4), ovate (5), pentagonal (6) and circular (7). The type of branching was classified as monopodial (1), sympodial (2), dichotomous (3) and polycotomic (4). The orientation of secondary leaflets was upwards (1), outwards (2) and downwards (3). The arrangement of secondary leaflets was evaluated as alternate (1), opposite (2) and alternate-opposite (3). The secondary leaflet shapes were falcate (1), lanceolate (2), ovate (3), elliptical (4) and oblong (5). The shape of the leaflet base was categorized as oblique (1), rounded (2) and attenuated (3). Finally, the shape of the apex of the secondary leaflet was evaluated as acute (1), muchronous (2), cuspid (3), caudate (4) and awned (5).

Statistical analyses

Statistical analyzes were performed for each test, for the data as a whole from the two experiments and for the clonal bank with the SAS statistical package (SAS, 2002). In order to evaluate the repeatability of morphological characters, that is, that they are homogeneous in size and shape between branches of a clone and different between clones, the following analyzes were carried out: for the values of the seven qualitative descriptors, the frequency distribution, for the 12 quantitative characters the mean, maximum, minimum value and coefficient of variation were obtained. For the 19 descriptors as a whole, the Principal Components analysis (PCA) was performed to determine which ones contribute the greatest weight to the variance. Once the PCA was performed, the descriptors that contributed low values to the components were excluded and the analysis was made again with the seven quantitative descriptors that contributed the most to the components in the clonal tests and four for the clonal bank. With the seven descriptors with the highest weight in the PCA, a clustering analysis was carried out with the average link method (AVERAGE). To avoid multicollinearity problems in the PCA, the volume variable was excluded since it presented a high Pearson correlation with diameter and height; likewise, the length of secondary leaflets was excluded because it showed a high correlation with leaf width.

Results and Discussion

The mean and maximum values and the coefficients of variation (CV) were higher in trial 1, except for the inclination that was greater in trial 2, which indicates that the environmental conditions were more favorable in the first, and therefore, the individuals showed greater growth. Under suitable conditions of humidity and light, the seedlings have a rapid initial development (Hernández-Zaragoza *et al.*, 2019). In general, the values of the morphological characters were higher in the clonal bank and lower in clonal experiment 2 (Table 1). High CVs suggest that test 1 is

more heterogeneous in all variables, except petiole length, which was greater in the clonal bank. No repeatability was observed in the morphological assessed characters within the ramets of each clone in such a way that it was not possible to distinguish a clone by means of the descriptors.

Table 1. Mean, minimum, maximum values and coefficients of variation for the quantitative characteristics in two tests and a clonal bank of *Cedrela odorata* L.

Character	Clonal test 1				Clonal test 2				Clonal bank			
	Mean	Min.	Max.	CV	Mean	Min.	Max.	CV	Mean	Min.	Max.	CV
DIAMETER	16.0	6.7	26.3	23.6	11.1	3.3	18.4	22.6	-	-	-	-
HEIGHT	8.5	4.0	12.0	19.1	6.3	3.0	10.0	17.9	-	-	-	-
VOLUME	110.1	10.1	320.6	55.7	40.4	1.9	236.6	55.1	-	-	-	-
STRAIGHTNESS	86.5	59.6	89.5	70.9	80.3	51.5	89.5	8.3	-	-	-	-
LLEAF	35.3	21.5	51.1	15.2	31.1	18.9	51.8	15.9	34.8	19.4	54.0	15.4
WLEAF	16.5	11.5	22.6	12.4	16.9	10.6	23.5	11.6	18.4	12.8	26.3	11.8
LPET	6.2	3.2	9.8	20.5	6.4	3.8	9.5	16.4	6.7	3.3	11.7	19.2
LPU	8.3	2.7	18.4	32.7	7.5	2.5	16.1	30.0	5.6	1.4	12.4	34.6
NPFS	8.1	5.0	12.0	13.4	8.4	5.8	12.5	12.6	8.6	5.5	11.8	12.1
LFS	87.5	58.2	115.7	12.2	82.1	52.1	157.6	12.7	92.2	62.1	178.4	12.2
WFS	31.7	20.1	57.7	15.1	30.9	17.2	46.3	14.6	31.5	17.4	52.0	15.1
ISSL	2.8	1.5	3.8	12.7	2.7	1.7	4.0	13.2	3.0	1.8	5.1	13.0

Diameter (cm), Height (m), Volume (dm³), Straightness (degrees); LLEAF and WLEAF = Length and width of the leaf (cm); LPEC and LPU = Length of the petiole (cm) and petiolule (mm); NPFS, LFS, WFS and ISSL = Number of pairs, length, width and shape index of the secondary leaflet (mm).

Qualitative descriptor frequencies

The predominant descriptors in clonal trials 1 and 2 were the circular stem shape, the most desirable shape (42 and 41 %), and the type of sympodic branching (63 and 72 %). The type of desirable branching is monopodic, which registered a low frequency in both evaluation sites (<19 %).

The morphological descriptors with the highest frequency in the three evaluation sites (test 1, 2 and clonal bank) were: the arrangement of alternate secondary leaflets (61.6, 65 and 69 %), the lanceolate form of the secondary leaflet (95, 98 and 94 %), the oblique shape of the base of the secondary leaflet (75, 67 and 70 %) and the mucronate shape of the apex of the secondary leaflet (75, 69 and 69 %). The orientation of secondary leaflets was predominantly outward in trial 1 and in the clonal bank (38 and 56 %), while in experiment 2 the downward orientation predominated (54 %) (Table 2).



Table 2. Frequencies percentage of qualitative characters evaluated in two tests and a clonal bank of *Cedrela odorata* L.

Character	Category	Level of expression	Frequencies (%)		
			Test 1	Test 2	Clonal Bank
Stem shape	1	Semicircular	23.6	27.1	-
	2	Rhomboid	4.2	0.9	-
	3	Deltoid	13.4	5.7	-
	4	Ellipsoid	9.0	13.1	-
	5	Ovate	7.5	11.9	-
	6	Pentagonal	0.3	0.0	-
	7	Circular	42.0	41.2	-
Type of ramification	1	Monopodial	18.7	12.5	-
	2	Sympodial	62.9	72.4	-
	3	Dichotomic	1.5	0.0	-
	4	Polycotomic	16.9	15.1	-
Orientation of secondary leaflets	1	Upwards	33.5	9.4	15.4
	2	Outwards	37.6	37.1	55.9
	3	Downwards	28.9	53.5	28.7
Arrangement of secondary leaflets	1	Alternate	61.6	65.2	69.2
	2	Opposite	31.2	23.2	19.7
	3	Alternate-opposite	7.2	11.6	11.1
Shape of the secondary leaflet	1	Falcate	1.8	0.9	0.4
	2	Lanceolate	95.2	97.7	94.3
	3	Ovate	0.2	0.1	0.1
	4	Elliptical	0.8	0.3	0.1
	5	Oblong	2.0	1.0	5.1
Shape of the leaflet base	1	Oblique	75.3	67.4	70.1
	2	Rounded	13.4	19.3	11.0
	3	Attenuated	11.3	13.3	18.9
Shape of the apex of the secondary leaflet	1	Acute	14.6	22.3	10.0
	2	Muchronous	75.3	69.2	68.7
	3	Cuspid	7.7	5.8	13.1
	4	Caudate	1.1	0.6	1.7
	5	Awne	1.3	2.1	6.5

Principal Component Analysis

In the Principal Component Analysis based on the correlation matrix of seven quantitative characters in the clonal tests and four in the clonal bank, the first three components explained 77 % of the variance in test 1, 85 % in test 2 , 82 % in the joint analysis of the two clonal trials, and 95 % in the clonal bank (Table 3).



Table 3. Proportion and accumulated variance in the Principal Component Analysis of morphological characters evaluated in two tests and a clonal bank of *Cedrela odorata* L.

Site	Principal Component	Variance proportion	Accumulated Variance
Clonal test 1	1	0.3646	0.3646
	2	0.2263	0.5909
	3	0.1840	0.7750
Clonal test 2	1	0.4569	0.4569
	2	0.2714	0.7284
	3	0.1284	0.8567
Joint analysis (clonal tests)	1	0.4291	0.4291
	2	0.2521	0.6813
	3	0.1437	0.8250
Clonal bank	1	0.6130	0.6130
	2	0.2709	0.8840
	3	0.0736	0.9576

Component one contributed 36.4 % of the total variance in test 1. The characters that contribute significantly were leaf length, secondary leaflet width, leaf width, petiole length; this component is associated with secondary leaves and leaflets. Component two contributed 22.6 % to the variance and the characters with the most weight were the straightness of the stem, the diameter, the length of the leaf and the height of the tree; this component is associated with growth. Component three contributed 18.4 %, and the characters with the greatest weight were the length of the leaf and the width of the secondary leaflets (Table 4).



Table 4. Eigenvectors of morphological characters in the first two components for two clonal tests, their joint analysis and a clonal bank of *Cedrela odorata* L.

Character	Test 1			Test 2			Joint analysis			Clonal Bank		
	Prin1	Prin 2	Prin3	Prin1	Prin 2	Prin3	Prin1	Prin 2	Prin3	Prin1	Prin 2	Prin3
DIAMETER	-0.05	0.49	-0.05	-0.04	0.19	-0.28	-0.08	0.29	-0.20	-	-	-
HEIGHT	0.01	0.20	0.04	-0.02	0.09	-0.05	-0.04	0.14	-0.07	-	-	-
STRAIGHTNESS	-0.28	0.79	-0.11	-0.53	0.80	0.23	-0.46	0.78	0.32	-	-	-
LLEAF	0.71	0.25	0.61	0.40	0.42	-0.71	0.43	0.42	-0.70	0.79	-0.59	-0.14
WLEAF	0.24	0.09	-0.01	0.20	0.13	-0.08	0.20	0.15	-0.08	0.23	0.14	0.62
LPU	0.14	0.15	-0.22	0.16	0.17	-0.03	0.17	0.22	0.07	0.15	0.05	0.73
WFS	0.58	0.03	-0.75	0.70	0.31	0.60	0.73	0.19	0.59	0.54	0.79	-0.26

LLEAF = Leaf length, WLEAF = Leaf width, LPU = Petiole length, WFS = Width of the secondary leaflet.

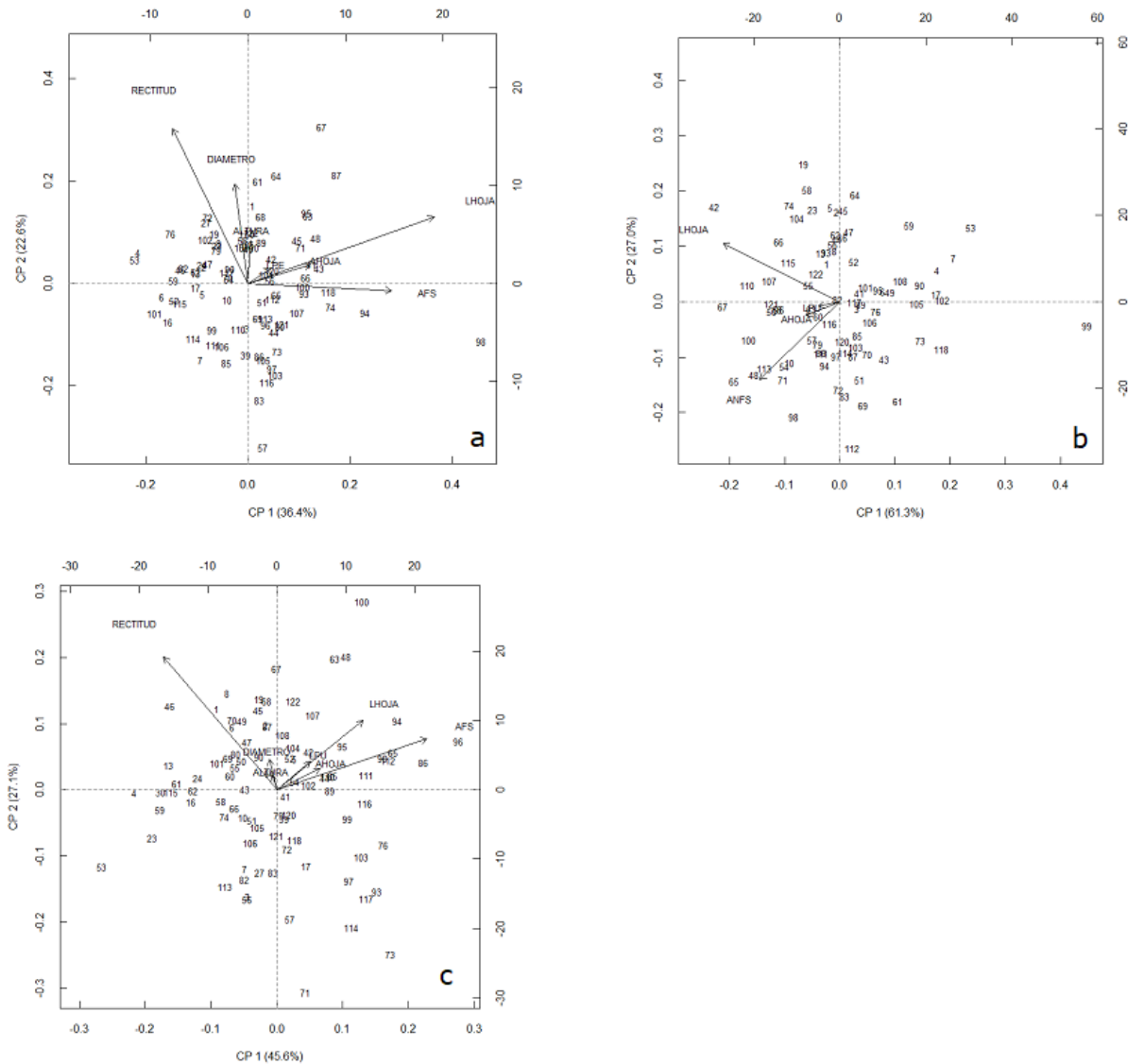
Component one contributed 45.6 % of the total variance in test 2. The characters with the most weight were the width of the secondary leaflets, the length of the leaf, the width of the leaf and the straightness of the stem. As in site 1, the component was associated with the leaf and secondary leaflets with negative weight in the straightness of the stem. Component two contributed 27.1 % to the variance, in which the straightness of the stem, the length of the leaf and the width of the secondary leaflets were the most determining characteristics; unlike site 1; in this case, diameter and height contributed less. Component three contributed 12.8 % to the variance and the characters with the greatest weight were the length of the leaf and the width of the secondary leaflets, as in test 1 (Table 4).

In the joint analysis of the two clonal tests, component one contributed 42.9 % of the variance, the width of the secondary leaflets, the length of the leaf and the width of the leaf and the straightness of the stem were the variables that most influenced. Component two contributed 25.2 % to the variance, the characters with the greatest weight were the straightness of the stem, the length of the leaf, the diameter, the length of the petiole and the height. Component three contributed 14.3 % to the variance in which, the most determining characters were also straightness, width of secondary leaflets and leaf length (Table 4).

Component one contributed 61.3 % of the total variance in the clonal bank. The characters with the most influence were the length of the leaf, the width of secondary leaflets, and the width of the leaf. Component two contributed 27 % to the variance and the characters with the most weight were the width of the secondary leaflets and the length of the leaf. Component three contributed 7 % to the variance and the width of the leaf and the length of the petiole are the determining characters (Table 4).

The previous results indicate that the contributions of the characters to the main components for each site (trials 1 and 2, their joint analysis and the clonal bank), from the first three components, the characters that coincide in contributing with the greatest weight are the straightness of the stem, the length of the leaf and the width of the secondary leaflets, for which there is stability in the characters of these components between the sites. The positive relationship between stem straightness and diameter also coincides, as well as between the length of the leaf and the width of the secondary leaflets, according to the Biplot diagram (Figure 1).





(a) clonal trial in *Santa Genoveva, Campeche*, (b) clonal trial in *Isla, Veracruz* and (c) in a clonal bank in *Palmar, Veracruz* according to the first two principal components.

Figure 1. Scatter plot of the 90 clones of *Cedrela odorata* L.

Other studies have also shown the importance of quantitative leaf variables, such as length, width and area (Said *et al.*, 2013; Santos *et al.*, 2012; Toili *et al.*, 2016). However, the results obtained differ from those recorded by Chimello *et al.* (2017), who described that the variables with the greatest variation were the shape of the

leaf, the shape of the leaf apex and the presence or absence of the petiole in *Tectona grandis* L.

Cluster analysis

The clones that make up each group do not present similar or distinctive morphological characteristics for a particular clone nor are they from the same provenances, but they do have similarities between groups, in terms of growth characteristics and there is a positive relationship between trees with good growth performance and straightness (Table 5).

Table 5. Clones that make up the groups formed by the dendrogram by similarity for two clonal tests, their joint analysis and a clonal bank of *Cedrela odorata* L.

Test 1	Test 2	Joint analysis	Clonal bank
1, 30, 42, 49, 50, 55, 58, 60, 61, 64, 68, 89, 108, 120, 122 (Group 1)	1, 2, 6, 8, 19, 45, 47, 49, 50, 55, 60, 64, 67, 68, 69, 70, 80, 87, 90, 101, 107, 108, 122 (Group 1)	1, 2, 8, 19, 30, 45, 47, 49, 50, 55, 58, 60, 61, 64, 68, 70, 122 (Group 1)	55, 57, 60, 63, 79, 82, 86, 94, 111, 116, 122 (Group 4)
2, 5, 8, 10, 13, 17, 19, 23, 24, 27, 47, 54, 62, 70, 72, 79, 82, 90, 102, 117 (Group 2)	5, 41, 42, 44, 52, 54, 85, 89, 95, 102, 104, 110 (Group 2)	5, 10, 27, 41, 42, 43, 51, 52, 54, 66, 69, 72, 74, 76, 79, 80, 89, 90, 102, 104, 107, 108, 120 (Group 2)	1, 2, 5, 8, 13, 19, 23, 44, 45, 46, 47, 52, 56, 58, 62, 64, 74, 93, 104 (Group 1)
3, 51, 56, 65, 69, 96, 104, 107, 110, 112, 113, 121 (Group 5)	3, 7, 27, 56, 57, 82, 83, 113 (Group 3)	3, 7, 17, 56, 105, 106, 113 (Group 3)	3, 6, 41, 49, 76, 89, 95, 101, 106, 108, 117 (Group 2)
6, 16, 46, 52, 59, 101, 115 (Group 4)	4, 13, 16, 23, 24, 30, 59, 61, 62, 115 (Group 5)	6, 13, 16, 24, 46, 53, 62, 82, 101, 115 (Group 7)	50, 66, 67, 68, 96, 107, 110, 115, 121 (Group 7)
57, 83 (Group 8)	73, 114 (Group 12)	57, 73, 83, 114 (Group 5)	
39, 44, 73, 80, 86, 97, 103, 105, 116 (Group 6)	76, 93, 97, 99, 103, 114, 116, 117 (Group 13)	86, 93, 97, 103, 116 (Group 12)	43, 51, 61, 69, 70, 72, 83, 85, 87, 97, 103, 114, 120 (Group 3)
4, 53, 76 (Group 3)	53 (Group 7)	4, 53, 59 (Group 6)	53 (Group 10)
41, 43, 45, 48, 63, 66, 71, 74, 93, 100, 118 (Group 9)	10, 17, 39, 43, 51, 58, 66, 72, 74, 79, 105, 106, 118, 120, 121 (Group 4)	39, 44, 71, 85, 99, 110, 111, 117, 121 (Group 4)	10, 48, 54, 65, 71, 98, 100, 113 (Group 6)
7, 85, 99, 106, 111, 114 (Group 7)	65, 94, 98, 111, 112 (Group 9)	64, 112, 118 (Group 10)	4, 7, 17, 73, 90, 102, 105, 118 (Group 8)
98 (Group 12)	86, 96 (Group 10)	98 (Group 13)	99 (Group 12)
94 (Group 10)	71 (Group 11)	94, 96 (Group 11)	112 (Group 5)
67, 87, 95 (Group 11)	48, 63, 100 (Group 8)	48, 95, 100 (Grupo 8)	59 (Group 9)
	46 (Group 6)	63, 67, 87 (Group 9)	42 (Group 11)

In test 1, group 11, composed of three clones, showed average values in volume of 170 dm³ and straightness of the stem of 86°; in this group are located two of the 18 clones with greater volume and greater straightness (67 and 95). Group 1 was the second with the highest average performance in volume, composed of 15 clones and was characterized by having clones of average volume of 128 dm³ and straightness of 85°; in this group clones 1, 60, 61, 64, 68, 89 and 108 are present, which are among the 18 with the highest average volume at the site.

Group 12, formed only by clone 98, registered the lowest average volume (70 dm³) and the lowest value of straightness of the stem (78°); this is one of the clones characterized by its lower performance. Other groups with a low average value of volume and straightness were group 8, with an average volume of 82 dm³ and straightness of 76°: in this group clones 57 and 83 meet; the first clone is one of the lowest average performance in volume and clone 6 that had an average volume value of 89 dm³ and a straightness value of 80°; this group has nine clones and eight of them are among the lowest values of the site's volume ranking (39, 73, 80, 86, 97, 103, 105, 116) (Table 6).

Table 6. Average values of discriminant morphological characters of the groups formed by the dendrograms by similarity of two tests and a clonal bank of *Cedrela odorata* L.



Site	Group	DIAM	ALT	VOL	STRAIG	LLEAF	WLEAF	LPU	WFS
Test 1	1	17.0	8.9	127.9	85.3	36.7	17.0	8.9	31.8
	2	16.4	8.5	116.2	85.2	33.9	15.9	8.5	30.7
	3	16.6	8.9	117.7	87.0	31.2	15.3	6.9	28.3
	4	15.2	8.3	101.2	84.4	32.0	15.2	7.5	28.3
	5	15.3	8.2	97.4	82.3	35.5	16.5	8.9	33.2
	6	14.8	7.9	88.7	79.5	35.5	16.3	7.2	32.0
	7	14.7	7.6	83.9	81.6	32.5	16.1	6.9	30.5
	8	14.2	7.6	82.1	75.8	34.7	16.0	9.6	30.2
	9	16.2	8.6	113.5	82.9	38.6	17.9	8.7	33.9
	10	14.4	8.6	85.4	79.9	40.9	18.6	10.3	34.5
	11	19.1	9.9	170.3	86.1	40.3	18.2	8.8	34.6
	12	12.9	8.3	70.2	77.7	44.6	19.5	7.6	41.9
Test 2	1	12.0	6.7	49.1	83.0	32.3	17.1	7.8	30.1
	2	11.0	6.3	39.7	80.0	32.1	17.6	8.0	32.6
	3	10.6	6.0	35.8	77.6	28.4	15.9	6.5	28.9
	4	10.5	6.1	36.4	79.6	30.0	16.5	7.4	29.8
	5	10.8	6.4	39.7	83.8	27.8	15.5	6.4	27.1
	6	13.3	6.8	57.2	86.0	31.6	16.3	9.0	25.8
	7	9.9	6.0	29.5	83.3	24.9	15.2	4.8	22.9
	8	12.0	6.8	49.1	83.9	34.4	19.1	8.9	36.9
	9	10.1	6.1	33.5	78.8	33.1	18.1	8.7	37.1
	10	11.4	6.3	41.3	76.4	36.0	18.4	9.5	38.1
	11	10.2	5.8	30.4	71.4	29.3	16.6	7.4	27.7
	12	9.9	5.4	27.4	71.5	31.6	18.1	8.0	31.3
	13	10.0	5.7	30.3	74.4	32.0	17.7	7.2	33.1
Clonal Bank	1	-	-	-	-	38.3	17.8	5.2	29.3
	2	-	-	-	-	33.3	17.9	5.5	30.2
	3	-	-	-	-	32.7	18.6	5.8	33.4
	4	-	-	-	-	35.7	19.8	5.9	33.3
	5	-	-	-	-	30.1	18.2	6.4	37.4
	6	-	-	-	-	37.5	19.8	5.3	37.9
	7	-	-	-	-	40.0	19.6	6.9	34.3
	8	-	-	-	-	29.5	17.0	4.3	27.7
	9	-	-	-	-	33.3	16.9	4.3	25.7
	10	-	-	-	-	29.5	16.1	2.7	23.2
	11	-	-	-	-	45.8	19.7	7.6	33.2
	12	-	-	-	-	19.6	12.6	1.5	22.9

DIAM = Diameter (cm); ALT = Height (m); VOL = Volume (dm³); STRAIG = Straightness (degrees); LLEAF = Leaf length (cm); LPU = Petiole length (mm); WFS = Width of the secondary leaflet (mm).

In test 2, group 6 showed a greater volume (57 dm³) made up of a single clone (46), and was identified among the 18 clones with the largest volume with a stem straightness value of 84°. The second best performing group was number 8, formed by three clones, in which two of the clones (48 and 63) with the largest volume (average 49 dm³) and a straightness of the stem of 84° were together. Group 12 recorded the lowest volume (27 dm³) and straightness of the minor stem (72°), formed by two clones. Another group with low volume was 7 (also made up of a single clone), with low volume as well (30 dm³), but with a higher stem straightness value (83°) (Table 6).

In the clonal bank, group 7, made up of nine clones, had the longest leaf length (40 cm), but the group with the highest value for the width of the secondary leaflets was group 6 (38 mm). Group 12 was formed by a single clone, with a leaf length of 19.6 cm and width of secondary leaflets of 23 mm (Table 6).

No clustering was observed between clones from the same provenance. The results coincide with the lack of clustering of nine provenances of *Tectona grandis* L. in southern India, and unrelated to morphological variables in a multivariate analysis (Sreekanth *et al.*, 2014).

Groupings between individuals of the same provenance have been determined in wild and cultivated materials or accessions with specific characteristics (Bayramzadeh *et al.*, 2012; Said *et al.*, 2013; Baldanzi *et al.*, 2015; Toili *et al.*, 2016; Anushka *et al.*, 2017; Lo Bianco and Mirabella, 2018). Kjær (1996) recorded the grouping of *T. grandis* among provenances according to the geographical origin of the families. Likewise, the correlation between the grouping and the geographical origin of six populations has been confirmed with individuals sampled in natural populations of *Adansonia digitata* L., whose morphometric differences are correlated

with environmental factors (Assogbadjo *et al.*, 2006). In these studies, although such results have been recorded, only one site was analyzed, without considering the effect of the genotype x environment interaction.

Morphological variation is influenced by genetics and the environment that acts specifically on each genotype, which leads to adaptation and, with it, phenotypic plasticity (Galván *et al.*, 2018). The diameter, height and straightness of the stem, as well as the width and length of the leaf are under genetic control, but also by the local environment, so the effect of the site and its interaction with the genotype could explain the lack of clustering between clones or their provenances (Hovenden and Vander, 2003).

Cedrela odorata has high values of genetic differentiation between populations, as well as high morphological variability, due to the fact that its natural environmental distribution is diverse (Navarro *et al.*, 2004). On the other hand, fragmentation and reduction of natural populations due to overexploitation and dysgenic selection suffered by the species may be the reasons for the high differentiation between populations, an issue that prevents genetic flow between populations (De la Torre, 2013). Likewise, the species is capable of developing in environments with different environmental conditions (Pennington and Sarukhán, 2005), which produces differential adaptation of the clones to different environments. This may partially explain the lack of consistency in morphological characters within the clones tested in the present study.

Among the reasons why ramets of the same clone presented differences in their morphological characteristics, it may be that the rootstock affects the development of the ramets, since each of these plants is a different genotype, which can generate a different response in each clone. (Schmutz and Lüdders, 1999). The rootstock exerts influence on the adaptability, vigor and growth in each ramet (Hernández *et al.*, 2014), since an interaction is established between two genetically different individuals, which may even be incompatible (Forner, 1984), which causes heterogeneity between ramets of the same clone (Darrouy *et al.*, 2010). On the

other hand, to date there are no studies in which the age-age correlation is estimated, so it is unknown if in any other stage of the evaluated individuals it is possible to identify repeatability in the evaluated characters. There is also the effect of culture (C effect), which causes members of a group of relatives, such as a clone or a family, to present differences between them. The C effects can result from the location of the crook cut in the ortet or the environmental situation at the time when it was grafted (Forner *et al.*, 1984).

For example, the analyzes of the morphological characterization were not useful to obtain variables that may be important in the definition of descriptors of *tejocote* (*Crataegus* spp.) In a germplasm bank whose genetic material was obtained from sources of the State of Mexico, *Puebla* and *Chiapas*, and were produced by grafting (López-Santiago *et al.*, 2008).

The reduction of variables with the Principal Component Analysis was convenient since the first two components allow understanding 59 and 72 % of the variation in each of the evaluated sites. The quantitative characteristics were more relevant than the qualitative ones for the Principal Component Analysis, as they contributed more weight to the first two components.

Despite the stability in the weight that they provide to the main components, the morphological characters evaluated did not show repeatability between the clones. Due to this lack of repetition of the values of the evaluated characters of the ramets of the same clone, it was not possible to distinguish by means of these morphological descriptors a particular clone, nor the superior clones that are the most interesting for its distinction. In this way, it is not sufficiently advisable to use the characters as descriptors to request the registration of the intellectual property of such clones.

It is worth mentioning that in future studies it will be necessary to carry out tests of distinction, homogeneity and stability (DUS) requested by SNICS (SAGARPA, 1996), only with outstanding clones that are those of interest for the registration and use

of their germplasm. It is also necessary to continue exploring other alternatives such as molecular markers (SNICS, 2014).

However, it must be considered that the assessed materials are wild, that they have been selected for their performance in other tests, but they are not materials that have entered an advanced domestication process as occurs in agricultural crops, and therefore, they have not fixed characters that can fully distinguish them. On the other hand, the rootstock must exert influence on the graft, because they are different genotypes and the ramet is the result of the interaction that occurs between both. Currently, the vegetative propagation of *C. odorata* is carried out by means of grafting, but, to obtain genetically equal clones and improve the evaluation of morphological characters, it will be necessary to carry out rooting of cuttings; this makes it possible to have only one genetic material per clone. Rooting cuttings of *C. odorata* show good results in small experiments (Sampayo *et al.*, 2019). However, operationally there are no large-scale works, so more research is required in this regard due to the importance of the species. The present study contributes to the formation of bases in the morphological characterization of the species and the registration of outstanding clones of *C. odorata* before the SNICS, which to date does not exist in the country.

Conclusions

The diameter, the height, the straightness of the stem, the length of the leaf, the width of the leaf and the width of the secondary leaflets were the variables that best characterize the red cedar clones produced by grafting, since they contributed greater weight to the principal components. Repeatability was not confirmed in the size and shape of the morphological characters evaluated, since they did not show consistency between ramets of the same clone nor were they clearly distinguished from other clones that grow in the same site or between different planting sites. Therefore, clones could not be identified in particular by the morphological

characters evaluated, although the formation of groups of clones with morphological or growth similarities was recognized and some maintained the same grouping between sites. despite the effects of interaction with the environment. Therefore, with these results it is not possible to submit them to intellectual property register with the SNICS.

There was little grouping of the genotypes evaluated with the geographical origins of the clones. The formation of clones by means of grafts does not seem to favor the existence of morphological characteristics that are repeatable between ramets and clones that develop in the same or in different sites.

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Conflict of interest

The authors declare no conflict of interest.

Contribution by author

Susana Olvera Moreno: data collection and analysis and writing of the manuscript; Javier López Upton: data analysis and review of the manuscript; Vicente Sánchez Monsalvo: establishment of the experiments, data analysis and review of the manuscript; Marcos Jiménez Casas: style correction and review of the manuscript.



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