Article

Morphological characterization of *Simmondsia chinensis* (Link).C.K. Schneid. under irrigation conditions

Rigoberto Meza Sánchez[1*]

Carlos Alberto Núñez Colín[2]

Carlos Ariel Cabada Tavares[1]

[1]Campo Experimental Todos Santos, Centro de Investigación Regional Noroeste, INIFAP. México.

[2]Programa de Biotecnología, Universidad de Guanajuato. México.

[*]Autor por correspondencia, email: meza.rigoberto@inifap.gob.mx.

Abstract:

Jojoba is a shrub native to the Sonoran Desert, introduced in other countries because the seed contains a liquid wax for use in the cosmetics, lubricants, pharmaceutical industries and with potential in medicine. The purpose of this work was to know the variability of female jojoba plants native to *Baja California Sur*, Mexico, through morphological characters of a plantation under drip irrigation conditions. In the study we considered 18 morphological variables of the plant, seed and leaves. We performed a descriptive analysis of each variable and a cluster analysis for the conformation of groups of variability and later a canonical discriminant analysis to determine the variables with the greatest influence on the variability between the groups. High variability was found among female jojoba plants, which were agglomerated in five groups; the characteristics of slenderness index and seed width, perimeter and Feret diameter of the leaves and the major diameter and average diameter of the crown of the plants were the most discriminant variables. Also, it was found that group III is the most promising for selection of outstanding genotypes.

Keywords: Multivariate analysis, germplasm, jojoba, morphology, plant genetics resources, variability.

Fecha de recepción/ Reception date: 8 de abril de 2017.
Fecha de aceptación/ Acceptance date: 18 de mayo de 2017.

Introduction

Simmondsia chinensis (Link).C.K. Schneid. (*jojoba*) is a small evergreen shrub native to the Sonoran Desert and the Mojave desert in the southwest of the United States of America and adjacent areas in the northwest of Mexico (Modise, 2007; Osman and Abohassan, 2013). However, it was introduced for commercial purposes into South America, Africa, India, the Middle East and Australia (Reddy and Chikara, 2010; Aly and Basarir, 2012).

The seeds of the plant contain a liquid wax known as oil, which is used in the cosmetics, lubricants and pharmaceutical industries and which has medicinal potential (Wagdy and Taha, 2012; Al-Qizwini *et al.*, 2014).The pulp of the seed is used for livestock feed and as a soil improver (Aly and Basarir, 2012; Abdel-Mageed *et al.*, 2014).

Because *jojoba* is a dioecious species, its progeny is highly heterozygous and produces plants of various sizes, shapes and yields, which complicates its cultivation for commercial purposes (Ash *et al.*, 2005; Reddy and Chikara, 2010). However, without the knowledge of the plant material and the necessary technological development, its cultivation will not be an option for producers, and dependence on the wild product (seed or wax) will increase; furthermore, the global market for the wax and the adaptation advantages of the taxon to thrive in harsh environments with high temperatures and extreme drought (Hamerlynck and Huxman, 2009), which are necessary to address the adverse effects of climate change, will all be wasted.

Studies on the morphological characterization of jojoba are scarce; while some analyze different variables of the plants based on univariate statistics, others only describe certain attributes of the components of the plants and their productive characteristics based on the estimation of population parameters (El-Baz *et al.*, 2009; Osman and Abohassan, 2013).

Thus, the purpose of this study was to determine the variability of the female jojoba plants depending on the morphological characteristics of a plantation under conditions of drip irrigation. According to the above, the suggested hypothesis is that, based on certain morphological attributes, it is possible to identify groups of jojoba plants with similar characteristics that may serve as criteria for the selection of outstanding individuals.

Materials and Methods

The sampled jojoba plantation is located on the grounds of the *Todos Santos* Experimental Field of the National Institute for Research on Forestry, Agriculture and Livestock *(Instituto Nacional de Investigaciones Forestales*, *Agrícolas y Pecuarias*), at 23°25'7.35" N and 110°9'16.53" W, in *Todos Santos*, B.C.S., Mexico. It was established in 1989 with seeds of other plantations in the Field collected from various wild jojoba populations in the state.

The site is characterized by an annual precipitation of 170.1 mm; a maximum temperature of 27.5 °C, a mean temperature of 21.6 °C and minimum temperature of 15.7 °C; 764.4; an annual evaporation of 1 mm, and only 16 days of rain (SMN, 2016). The soil has a sandy, crumblike texture, a density of 1.6 g cm^{-3}, a field capacity of 10.0 and a permanent wilting point of 5.8.

The plantation under study was intervened in 2011, with a rejuvenation pruning at 50 cm above the ground; ripping and raking of the soil; irrigation, weeding, and replacement of dead plants (45 %). It eventually consisted of 300 plants (50 % female and 50 % male) at an equidistance of 2.5 m. 4-5 irrigations were applied per year, with a volume of 81.5 liters of water per plant in each irrigation; weed control was carried out manually and formative prunings were implemented at the beginning of each year.

In order to determine the morphological variability of the plants, the dimensions of 56 female plants were measured in 2012 and 2013. 18 variables were considered: the height, the maximum, minimum and mean crown diameter; the aerial cover and the slenderness index of the plants; the weight, length, width, and slenderness index of the seeds; the length, width, area, perimeter, elongation indices, roundness, narrowness and Feret diameter of the leaves.

The plants were measured with a ruler and a measuring tape; the measurements taken were height, maximum crown diameter and maximum diameter perpendicular to the latter; the mean crown diameter, crown area and slenderness index were estimated according to the following formulas:

$$Mn\ CD = \frac{M\ CD + pCD}{2} \qquad CA = \frac{M\ CD * pCD * \pi}{4} \qquad SI = \frac{H}{Mn\ CD}$$

Where:

H = Height

M CD = Maximum crown diameter of the plants

pCD = Crown diameter perpendicular to the M CD

Mn CD = Mean crown diameter of the plants

CA = Crown area of the plants

SI = Slenderness index of the plants

The total production of seeds per plant was obtained by weighing with a Model ACB 3000 Adam Equipment^{TM} digital scale, with an approximation of 0.10 g; the dimensions were measured using a digital caliper with a 0.01 mm resolution; 10 to 15 seeds were measured per plant or the total number of seeds, if it was lower. A sample of 16 well developed leaves per plant was scanned using an HP ScanJet G3110 flatbed scanner at 300 pixels per inch. A digital ruler with the same resolution was superimposed on each image as a reference; the images were then processed using the UTHSCSA Image Tool, ver. 3.0 software in order to obtain the above measurements (Wilcox *et al.*, 2002).

The information was arranged and processed in MS Office Excel^{TM} spreadsheets in order to calculate the basic parameters of the 18 morphological variables considered in the study. The characterization was carried out using a multivariate statistical analysis to form similar groups and differentiate the variability (Núñez and Escobedo, 2011; Núñez and Escobedo, 2014). The SAS^{TM} ver. 9.3 software was used. A cluster analysis was performed using the Euclidean distance as a dissimilarity index and Ward’s clustering method (Hernández *et al.*, 2011; Núñez *et al.*, 2011). In the definition of the groups, Hotelling’s t^2 pseudostatistic and the cubic clustering criterion (CCC) were utilized to determine the number of morphologically homogeneous groups (Johnson, 2000). Likewise, canonical discriminant analysis, multivariate variance analysis and Tukey’s standardized mean test were used for the canonical variables (Johnson, 2000).

Results and Discussion

Dimensions of the plants

Table 1 shows the dimensions of the plants. The crown area was the attribute with the highest coefficient of variation: 32.2 %. The mean slenderness index was low, indicating that most of the plants (80 %) were wilder than they were tall, and only 20 % were almost spherical; this was due to the formative prunings and to the fact that the individuals were in full recovery from the pruning. However, it is advisable to modify the architecture of the plants, since the conical shape of the crown is considered to facilitate the harvesting of the seeds (Milthorpe, 2004).

Dimensions of the seeds

The seed production per plant was low and highly variable, ranging between 0.6 and 241.7 g per plant (Table 1). This variable exhibited the highest coefficient of variation (105 %). The average length reveals that jojoba seeds are longer than they are wide; therefore, their mean slenderness index was 1.67, and only two plants exhibited very elongated seeds with a SI above 2.0.

Dimensions of the leaves

Table 1 shows the dimensions of the leaves; the leaf area had the highest coefficient of variation (17.4 %). In general, the length of the jojoba leaves was twice their width; hence their average elongation index of 2.1. For the same reason, they exhibit low values for their roundness and narrowness indices (0.60 and 0.65, respectively), and a Feret diameter of 2.1 cm.

Table 1. Dimensions of female *Simmondsia chinensis* (Link) C.K. Schneid. in a plantation under conditions of drip irrigation, *Todos Santos*, BCS.

Variables	**Mean**	**C.V. (%)**	**Max**	**Min**
H	80.04 ± 4.3*	20.09	110	45
M CD	122.91 ± 4.81	14.65	160	84
m CD	111.91 ± 5.38	17.98	156	62
Mn CD	117.14 ± 4.94	15.78	157.99	77.55
AC	1.12 ± 0.1	32.22	1.96	0.47
SI	0.69 ± 0.04	18.66	1.04	0.4
SW	54.29 ± 15.25	105.13	241.7	0.6
SL	15.54 ± 0.39	9.36	19.75	12.26
SW	9.36 ± 0.2	8	10.82	7.91
SIS	1.67 ± 0.06	12.81	2.5	1.25
LL	32.21 ± 0.76	8.87	38.28	25.05
LW	15.48 ± 0.46	11.01	19.43	11.6
LA	351.68 ± 16.4	17.45	510.89	210.52
LP	85.13 ± 1.94	8.53	99.63	64.97
LEI	2.10 ± 0.05	9.57	2.62	1.7
LRI	0.60 ± 0.01	7.09	0.67	0.49
LFD	20.97 ± 0.5	8.91	25.39	16.26
LNI	0.65 ± 0.01	5.25	0.73	0.57

* = Error in the estimation; CV(%) =Coefficient of variation; Max = Maximum, Min = Minimum, H = Height (cm); M CD = Maximum crown diameter (cm); m CD = Minimum crown diameter (cm); Mn CD = Mean crown diameter (cm); AC = Aerial cover (m^2); SI = Slenderness index; SW = Seed weight (g); SL = Seed length (mm); SW = Seed width (mm); SIS = Slenderness index of the seeds (SIS = SL / SW); LL = Leaf length (mm); LW = Leaf width (mm); LA = Leaf area (mm^2); LP = Leaf perimeter (mm); LEI = Leaf elongation index; LRI = Leaf roundness index; LFD = Leaf Feret diameter (mm); LNI = Leaf narrowness index.

Formation of groups

The results (Figure 1) showed the grouping of the population into four (cubic clustering criterion) or five groups, according to Hotelling's t^2 pseudostatistic; hence, the formation of five groups was considered, and the resubstitution analysis proved that the groups exhibit no atypical individuals.

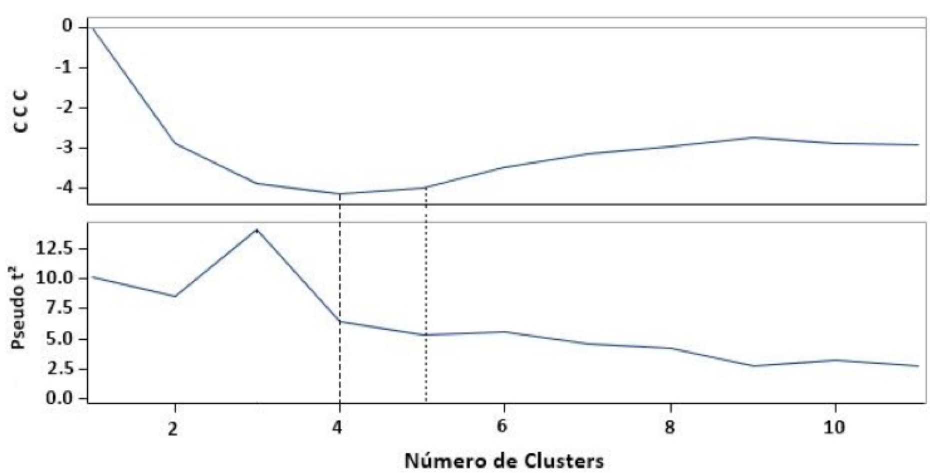

Figure1. Pseudostatistical tests to determine the number of groups of *Simmondsia chinensis* (Link) C.K. Schneid plants.

Therefore, the 56 jojoba plants formed five working groups, which ranged between two individuals in group V and 21 plants in group II. Furthermore, the Mahalanobis distance test (Table 2) confirmed that all the plant groups are different ($P < 0.0001$).

Table 2. Mahalanobis test and significance between groups of female *Simmondsia chinensis* (Link). C.K. Schneid. plants.

Groups	**I**	**II**	**III**	**IV**	**V**
I		25.3189*	19.6288	47.0308	170.4671
II	<0.0001**		42.3432	57.1360	90.4055
III	0.0002	<0.0001		75.2420	199.7205
IV	<0.0001	<0.0001	<0.0001		161.0805
V	<0.0001	<0.0001	<0.0001	<0.0001	

* = Mahalanobis distance on the main diagonal; **= Significance under the main diagonal.

The dendrogram (Figure 2) shows the female jojoba plants, identified by line and plant numbers (L_{1-25}, P_{1-12}) in the plantation with similar morphological characteristics and located in each group, respectively; in this case, the cutting line has a semi-partial R^2 of almost 0.06.

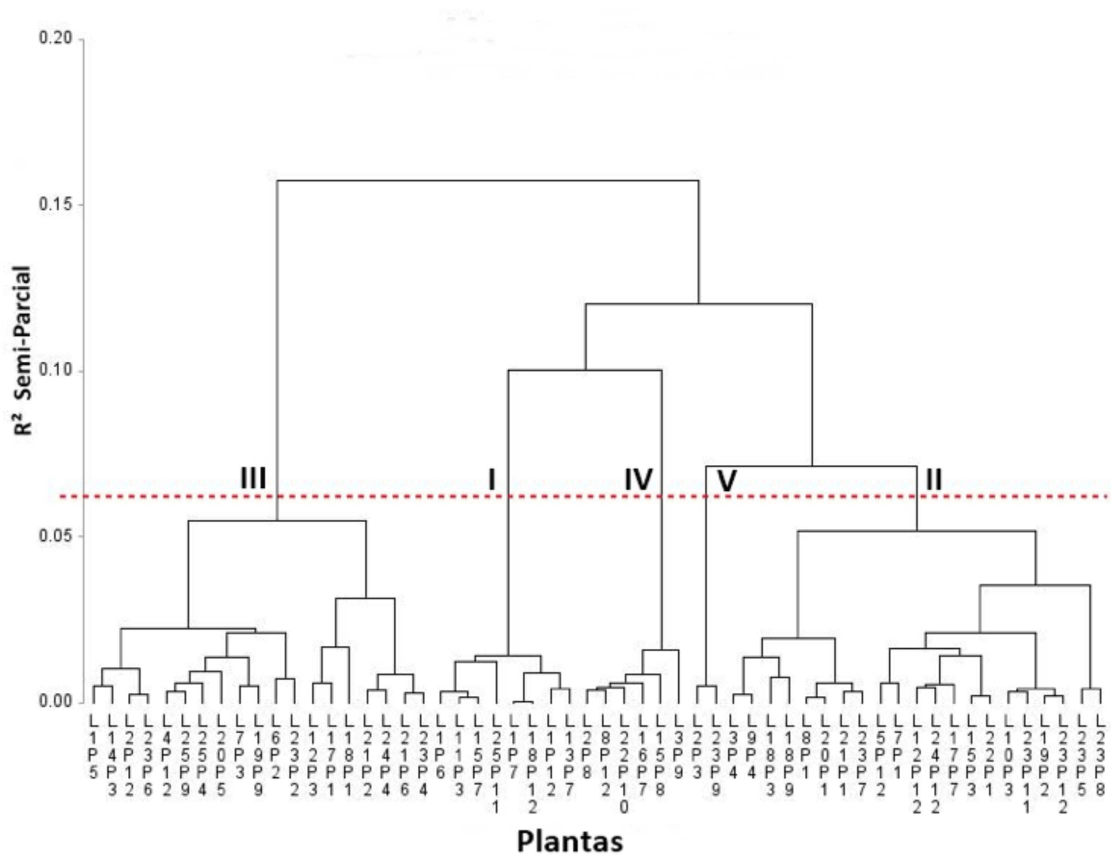

Figure 2. Dendrogram of 56 female *Simmondsia chinensis* (Link) C.K. Schneid plants. using morphometric data.

In the canonical discriminant analysis (Table 3), it was determined that the first three canonical functions (CF) accounted for 95.56 % of the morphological variability in female plants; the CF 1, for 58.3 %; the CF 2, for 27.3 %, and the CF 3, for 9.9 %.

Table 3. Canonical discriminant analysis of morphometric characteristics of *Simmondsia chinensis* (Link). C.K. Schneid. plants.

Canonical function	Intrinsic value	Explained variance	Accumulated variance	Approximate F value	Prob. > F
1	11.8893	0.5833	0.5833	7.23	<0.0001
2	5.5704	0.2733	0.8566	4.91	<0.0001
3	2.0185	0.099	0.9556	3.14	<0.0001
4	0.9046	0.0444	1.0	2.23	0.0238

The variables most correlated with the canonical root 1 (Can 1) were the slenderness index (X 10) and the width of the seeds (X 11) in relation to their size and shape; while in the canonical root 2 (Can 2) they were the leaf perimeter (X 12) and Feret diameter (X 17) in relation to the size and shape of the leaves; and in the canonical root 3 (Can 3), the largest crown diameter (X 2) and the mean crown diameter (X 4) of the plants (Table 4).

Table 4. Total canonical structure of the canonical discriminant analysis for the morphometric characteristics of female *Simmondsia chinensis* (Link). C.K. Schneid. plants.

Variables	Can1	Can2	Can3	Can4
X1 = Height of the plant	0.2718	-0.2619	-0.0791	0.5204
X2 = Maximum crown diameter	0.1398	-0.2969	-0.6289	0.6052
X3 = Minimum crown diameter	0.1623	-0.3561	-0.4987	0.4971
X4 = Mean crown diameter	0.1583	-0.3428	-0.5778	0.5679
X5 = Aerial cover	0.1910	-0.3109	-0.5289	0.6093
X6 = Slenderness index of the plant	0.1598	0.0126	0.4588	0.1747
X7 = Seed production	0.2450	0.0746	-0.0073	-0.0600
X8 = Seed length	-0.3669	-0.0175	0.4208	0.1320
X9 = Seed width	0.7276	-0.2813	0.1930	-0.0497
X10 = Slenderness index of the seeds	-0.7793	0.1863	0.2756	0.1709
X11 = Leaf area	0.4899	0.6592	-0.1315	0.0048
X12 = Leaf perimeter	0.2472	0.7028	-0.1301	0.1652
X13 = Leaf length	0.0777	0.6684	-0.1814	0.1521
X14 = Leaf width	0.6546	0.6119	-0.0523	-0.0076
X15 = Elongation index of the leaf	-0.6988	-0.0937	-0.0302	0.1542
X16 = Roundness index of the leaf	0.5941	0.0822	-0.0758	-0.3295
X17 = Feret diameter of the leaf	0.4830	0.7001	-0.1481	0.0175
X18 = Narrowness index of the leaf	0.6666	0.0859	0.0305	-0.2362

Figure 3 shows the distribution of the plant groups in terms of the canonical values; in Can 1, the most negative records corresponded to group V, followed by groups II and IV. The latter shows the most negative values in C 2. Positive values were observed in groups I and III. In Can 3, positive values were obtained by the plants of group V and by the majority of the plants of groups III and IV.

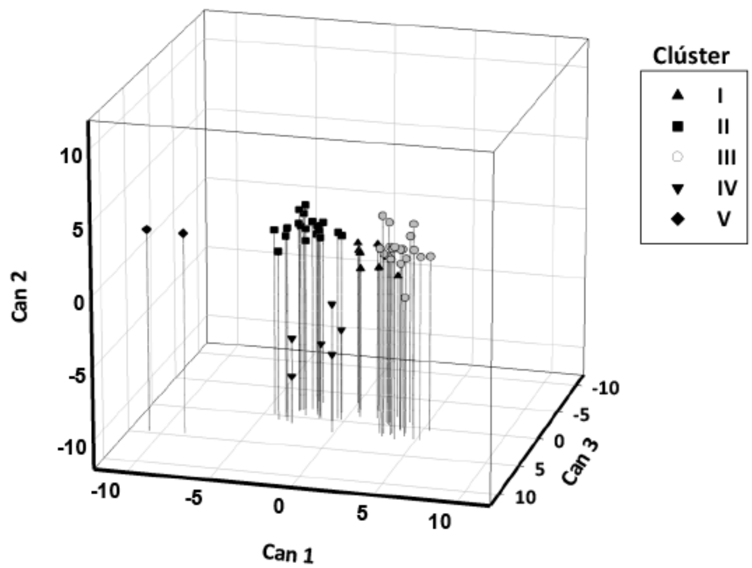

Figure 3. Distribution of female *Simmondsia chinensis* (Link). C.K. Schneid. plants in terms of the values of the canonical discriminant analysis of the morphometric values.

Table 5 shows the averages per group for the most discriminating morphometric variables; group V had the highest slenderness index of the seeds ––2.4, which are the longest and narrowest seeds––, with a difference of 58 % between the length and the width, while groups III, IV and I have the broadest seeds.

The plants of group III had the largest perimeter, followed by those of groups I and II, which coincide with the recorded Feret diameter of 21.0 to 22.2 mm; the plants of group IV had the smallest perimeter and Feret diameter.

The maximum and mean crown diameters are variable, with little contribution to the explained variance (9.9 %) and can be regarded as an effect of management, not as intrinsic traits of the plants, as these were exposed to formative prunings of various intensities at least once a year.

Table 5. Mean of the most discriminant morphometric variables in a cluster of *Simmondsia chinensis* (Link). C.K. Schneid. plants.

Groups	SIS	SW	LP	LFD	M CD	Mn C D
I	1.6 / 0.10*	9.5 / 0.51	86.7 / 6.05	21.2 / 1.01	152.3 / 9.6	145.8 / 10.1
II	1.8 / 0.1	8.8 / 0.5	86.1 / 5.0	21.0 / 1.0	119.9 / 13.7	113.2 / 13.4
III	1.5 / 0.1	10.0 / 0.5	88.1 / 5.9	22.2 / 1.4	115.7 / 12.8	110.4 / 15.5
IV	1.7 / 0.2	9.7 / 0.6	70.7 / 3.5	17.2 / 0.7	126.0 / 11.8	123.0 / 11.8
V	2.4 / 0.2	8.0 / 0.1	83.8 / 1.4	18.9 / 0.01	97.0 / 18.4	90.4 / 9.7

* = Mean / Standard deviation; SIS = Slenderness index of the seeds; SW = Seed width (mm); LP = Leaf perimeter; LFD = Leaf Feret diameter (mm); M CD = Maximum crown diameter (cm); Mn CD = Mean crown diameter (cm).

In the multivariate analysis of the canonical roots, a highly significant difference ($P < 0.0001$) was observed between the three canonical roots. When comparing the means (Table 6) in Can 1, differences were observed in almost all the groups except between groups II and IV, which exhibited negative values and differed from the rest of the groups; in Can 2, groups with positive values (II, III and V) exhibited differences in relation to the rest of the groups, but not between them; in Can 3, with the highest positive value, group V was the only one with differences with respect to the rest of the groups. There was similarity between group IV and groups III and II, and between the latter and group I.

Table 6. Tukey mean comparison test for the canonical functions of the canonical discriminant analysis of morphometric variables of *Simmondsia chinensis* (Link). C.K. Schneid.

Groups	n	Can 1 Mean*	Can 2 Mean	Can 3 Mean
I	8	1.3236 b	-0.8588 b	-1.67 d
II	21	-2.5294 c	1.1244 a	-0.8804 c d
III	19	3.6938 a	0.8429 a	0.9602 b
IV	6	-1.3837 c	-6.1764 c	0.7069 b c
V	2	-9.6759 d	2.1503 a	4.6818 a

* = In the columns, values with the same letter are statistically equal.

It is estimated that 6 % (Aryal and Ming, 2013) of the angiosperms species (14 620 out of 240 000) are dioecious (Renner and Ricklefs, 1995). This unisexuality of the plants stems from hermaphroditic ancestors and leads to sexual dimorphism between males and females, which is generally related to physiological and morphological characteristics of the life cycle (Kumar *et al.*, 2014).

Jojoba is a dioecious plant requiring cross-pollination; therefore, it exhibits a high variability in its phenology and in its morphological and performance parameters (Hassanein *et al.*, 2012); furthermore, according to the records, less than 1 % of the plants from seeds of wild populations have the potential to produce economically acceptable yields (Reddy and Chikara, 2010).

This supports the results obtained for female jojoba individuals under conditions of irrigation, since these exhibit morphological and productive differences when grouped together. The size and shape of the seeds, the sizes of the leaves and of the plant crowns are the most discriminant variables in the makeup of the groups.

Apparently, polymorphism in jojoba seeds is an important adaptive strategy against the heterogeneity and unpredictability of the desert environment, so that large and medium-sized seeds germinate first, after adequate rainfall, and small seeds remain dormant longer; in general, however, *jojoba* seeds are large and heavy, and therefore they are exposed to immediate consumption by rodents (Matthews, 1994).

According to the literature, the size of jojoba seeds varies according to the locality and the genotype; thus, the range of length is 0.8 to 2.33 cm, and the width is 0.5 to 1.33 cm. In addition, seed size has a positive correlation with seed weight, which ranges between 0.33 and 1.88 g seed^{-1}, with 660 to 3 300 seeds kg^{-1} (Meyer, 2008; Inoti *et al.*, 2015; Khan *et al.*, 2015a). During the present work this behavior was observed to occur, even under the same agronomic management; therefore, this variability is attributed to the genotype of the species and is consistent with a study according to which the vegetative morphology of *jojoba* is compatible with the idea of *intralocus* conflict, in which the same characteristic is expressed in a similar manner in both sexes and at least part of the observed variation is assumed to have a genetic basis (Prasad and Bedhomme, 2006).

Large jojoba seeds are preferred for establishing plantations, since they have a higher percentage of germination than medium-sized and small seeds; besides, they produce more vigorous seedlings in the first months of growth, and there is a positive correlation between the size and the wax content (Meyer, 2008; Hassanein *et al.*, 2012; Inoti *et al.*, 2015) ––the most important feature for the cultivation and exploitation of *jojoba*. In average, the plants of group III have the broadest (10.0 mm) and heaviest (71.0 g) seeds.

A strong sexual dimorphism of the leaves has been observed in certain wild *jojoba* populations; in these, the leaves of female plants are larger and have a greater variability than those of the male plants. However, the degree of sexual dimorphism in the size of the leaves is reduced with the increase in the annual precipitation (Kohorn, 1995). As for the leaf area, in the plants of group III it was 3.9 cm^2; in groups I and II, it was 3.5 cm^2; group IV exhibited the smallest leaf area. In all the groups, the leaf area of the plants was similar, as they were all under the same soil moisture regime.

Studies on the morphological characterization of jojoba are scarce, but they are necessary for determining the variability that exists in their area of origin or in plantations under agronomic management; most studies have focused on describing and comparing a single characteristic, such as the size of the plants, branches, leaves or seeds, or certain physical or chemical characteristics of the oil (Ayerza, 2001; El-Baz *et al*., 2009; Khan, *et al*, 2015 b).

Multivariate statistics are a suitable tool for assessing more than one attribute of the plant at the same time, and they are utilized to classify the plants according to their similarities or differences in all the measured characteristics, as well as to select those variables that contribute most to their characterization and to the detection of differences between and within groups (Núñez and Escobedo, 2014).

With this procedure, it was possible to group female *jojoba* plants with similar morphological characteristics, in addition to identifying the most relevant variables for the characterization of the plants ––related to the dimensions of the seeds and leaves––, which can be used as a basis for further studies in this and other locations. Furthermore, differences between the groups of female plants thus formed were successfully determined.

Conclusions

There is a high morphological and productive variability among female *jojoba* plants under conditions of drip irrigation. Five groups of plants were formed, each with similar morphological characteristics within the group, but with morphological differences in relation to those of other groups.

High values in the canonical structure of the discriminant analysis indicate that the morphological variables of slenderness index and seed width, leaf Feret diameter and perimeter, and maximum and mean crown diameters, are the most important variables for defining the groups of female *jojoba* plants.

The individuals in group III represent the most outstanding genotypes for starting the selection of jojoba plants with a good productive potential, as they have the highest seed weight per plant.

Acknowledgements

We wish to express our gratitude to SAGARPA, SNICS, SINAREFI and Cofupro for the financial support provided to the Jojoba Network for the development of this work, as well as to C. Pilar Cota Ruiz for her support in the field work.

Conflict of interest

The authors declare that they have no conflict of interest.

Contribution by autor

Rigoberto Meza Sánchez: drafting of the manuscript; Carlos Alberto Núñez Colín: support in the statistical analysis and revision of the manuscript; Carlos Ariel Cabada Tavares: support in the data collection in cabinet and revision of the manuscript.