Article

Characterization of chia collections from the western region of Mexico

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Abstract

Chia is a very valuable resource since it is the main source of unsaturated fatty acids, mainly α-linolenic, in addition to protein and fiber. In this investigation, seven collections were made in seven locations for study and comparison. Seed size were measured, thousand seed weight, volumetric weight, color, germination percentage and oil extraction for chromatography analysis. The collection of cultivated chia from Guanajuato showed greater variability in seed size and that of Roque la minor. The wild collections had the smallest seed size. The cultivated one of Guanajuato has the greater weight in seed and the wild ones the minor, the latter did not germinate, but the domesticated ones did. There were no differences in volumetric weight; however, there were differences in chromatograms (HPLC) and in physical characteristics and color variation in domesticated ones, including white seeds. This prospective research shows that the variability present in wild and cultivated chia can be used for plant breeding.

Keywords: *Hirsuta*, *Salvia hispanica*, *Salvia tiliifolia*, diversity, fatty acid.

Introduction

Domestication is the result of a selection process that leads plants to a better adaptation to human management, although these adaptations may compromise their survival in nature (Brown 2010). In Mexico, despite being the center of domination of chia (*Salvia hispanica*), there are few studies of genetic variability; in 2008, a morphological characterization of 22 samples of chia collected in different sites in central and South America was carried out, as well as several states in Mexico including: Sonora, Chihuahua, Jalisco, Morelos and Guerrero (Palma-Rojas *et al*., 2017).

Wild and domesticated varieties were contemplated, most of these samples had very marked morphological differences. It is known that there are wild populations throughout the country, however, there are no indicators outside their geographical origin that help to differentiate them and the biochemical properties of most of these varieties are unknown. It is possible that by not having this information important productive genotypes are being wasted in the genetic improvement of chia; since variability is essential for sustainability (Brown, 2010).

Chia is a diagnostic species that defines the borders of Mesoamerica (Cahill, 2004), it is an annual crop that is distributed in semi-warm and temperate environments, adapts to clay and sandy soils that are well drained; however, it requires adequate levels of nitrogen to provide good yields (Herman *et al*., 2016). It is a short-day plant, does not tolerate frost and requires abundant sun to produce; in the first world countries, long-day varieties have been generated and capable of producing in different climatic conditions (Jamboonsri *et al*., 2012; Grimes *et al*., 2018). Chia is currently grown in several Latin American countries obtaining yields of up to 3 t ha^{-1} (Baginsky *et al.*, 2016; Muñoz-Maximo *et al.*, 2017).

In recent decades it has resurfaced, nutraceutical properties and their attractive nutritional benefits have increased their consumption (Xingu *et al*., 2017). The distribution of this crop grows rapidly, however, there are few sources of information that can provide updated data regarding its production and worldwide distribution (Jamboonsri *et al*., 2012; Baginsky *et al*., 2016; Grimes *et al*., 2018; Win *et al*., 2018), *S. hispanica* and *S. tiliifolia* are the two species that are used for seed production.

The usefulness of the collection and study of genetic resources is exemplified in the tomato or tomato (*S. Lycopersicum*) in which aspects related to morphology, physiology and yield have been investigated, both of the cultivated species and its wild relatives (Ichihashi and Sinha, 2014). Chia seed contains 32% oil and 20% protein; it contains essential amino acids such as lysine and sulfur amino acids (methionine and cysteine). It has a high antioxidant content (Oliveira-Alves *et al*., 2017), and has compounds with biological activity in different parts of the plant (Marineli *et al*., 2015; Fan *et al*., 2019; Kobus-Cisowska *et al*., 2019; da Silva, 2015).

Chia is the plant with the highest content of α-linolenic fatty acid, a precursor of eicosapentaenoic and docosahexaenoic fatty acids, important fatty acids for the development of cognition, vision and that reduce the risk of coronary heart disease. The seed also contains 40% of dietary fiber and calcium (Sreedhar *et al*., 2015; Medina-Santos *et al*., 2017). The planting location has an effect on

the content of α-linolenic, linoeic and oleic and on the omega6/omega3 ratio (Ayerza and Coates, 2009a). The objective of this research was to characterize and compare collections of wild chia (*S. tiliifolia* and *S. hirsutum*) and cultivated (*S. hispanica*) in order to contribute to the knowledge of the diversity of this important plant genetic resource.

Materials and methods

Chia cultivars, spikes in physiological maturity, were made in seven locations: 1) Roque (20° 35' 00.00'' north latitude 100° 50' 11.54'' west longitude), Celaya, Guanajuato (wild and domesticated); 2) Guanajuato (21° 00' 02.88'' north latitude 101° 14' 24.56'' west longitude), Guanajuato (domesticated); 3) Ballesteros, Salvatierra (20° 13' 33.99'' north latitude 100° 48' 38.42'' west longitude), Guanajuato (wild); 4) Comala (19° 19' 09.09'' north latitude 103° 46' 18.08'' west longitude), Colima (domesticated); 5) Irapuato (20° 16' 09.61'' north latitude 10° 13' 448.31'' west longitude), Guanajuato (wild and domesticated); 6) Uruapan (19° 26' 34.09'' north latitude 10° 20' 141.01'' west longitude), Michoacán (wild); 7)

La Calera, Sixto Verduzco (20° 16' 09.61'' north latitude 101° 34' 48.31'' west longitude), Michoacán (wild). The domesticated ones correspond to the species *S. hispanica* and that of La Calera and Irapuato to the species *S. tiliifolia*; that of Salvatierra to the species *S. hirsuta*. (Calderón de Rzedoswski and Rzedowski, 2004). To obtain the seed, the inflorescences were dried at room temperature and subsequently shaken in order to release the seeds, which were stored in plastic bags with their respective identification at room temperature in a cool and dry place.

To measure the seeds, these were photographed on millimeter paper and subsequently the pixels were quantified by means of the Golden Ratio measurement software, the length and thickness of the seeds in millimeters were calculated by means of the following formula.

$$\text{Length in mm} = \frac{\text{seed size in pixels}}{\text{pixels per mm}}$$

To determine the seed weight, three tests were carried out: individual seed weight, weight of 1 000 seeds and volumetric weight. The individual weight was obtained by weighing 50 seeds by means of an analytical balance. For the weight determination of 1000 seeds, 4 repetitions of 200 seeds were performed, weighed and the average was obtained, this average was multiplied by 5 according to the ISTA manual (ISTA, 2005).

The volumetric weight was carried out by weighing the seed that fits in a container of known volume, which was filled by dropping the seed from a funnel from an approximate distance of 5 cm over the central part of the container for a uniform distribution until the container is filled, the excess seed removed by passing a ruler over the edge of it. After obtaining the weight, the conversion to kg hL^{-1} was performed.

The seed color identification was made by taking samples of 50 seeds from each cultivar that were visually inspected and photographed to know the uniformity of the color, in the cultivars in which more than one seed color was presented, these were counted and determined the percentage of

seeds of each color. In order to obtain the germination percentage, three repetitions of 100 seeds were carried out in wet germination paper at a temperature of 25 °C for 8 days in the absence of light, at the end of this period, normal, abnormal and non-germinated seedlings were counted, and an average of the percentage of normal seedlings was obtained (ISTA, 2005).

The modified Folch method (1975) was used for oil extraction; the sample is ground and incubated in a 50:50 (v/v) chloroform-methanol solution under constant stirring at room temperature; the ratio of the solution to the flour for maceration was 4:1 (v/w). After maceration, manual pressing and filtering was done in 8 layers of gauze cloth. The liquid obtained in the filtration was allowed to stand at room temperature for 2-3 days until the solvent mixture was volatized obtaining the oil in the bottle (Puttini *et al*., 2005). The oil was transesterified in a constant temperature water bath (65-68 °C), the reaction was carried out by the method proposed by Torossi (2006).

For the analysis, a chromatograph (Thermo Scientific) for HPLC equipped with a C18 hydrophobic column and a UV absorbency detector was used. The fatty acid esters were diluted in HPLC grade methanol (1:1 000) as recommended in the Kromidas HPLC manual (2006), the method used for separation was methanol-water injection with an initial methanol concentration 100% and a final concentration 60% Kromidas (2006) As a comparison commercial chia oil (extracted and made in Jalisco) was used. The data was analyzed using a completely randomized design using the SAS 9.1 program.

Results and discussion

The accessions of cultivated chia evaluated showed highly significant differences in seed size and weight (Table 1), which allows us to assume that they have phenotypic and genotypic differences. The average of the seed length of the Colima collection was 1.95 mm, the Guanajuato had a range of 1.67-2.3 with an average of 2 mm. The Irapuato collection showed values between 1.69 and 3.32 mm. The Guanajuato collection showed greater variability in seed length and Roque's Chia presented the least variability in this characteristic.

Table 1. Mean squares, degrees of freedom and statistical significance of the analysis of variance of the variable length and weight of seed in chia collections. Celaya, S-S 2016.

FV	GL	Mean squares	
		Seed length	Seed weight
Cultivate	7	5.48726[**]	0.7266[**]
Error	32	0.01006	0.0011
Total	39		

[**]= indicates statistical significance at the 0.01 level of probability.

The wild Chia seed of La Calera presented values between 1.26 and 1.47 mm with an average of 1.36 mm. The seed of the Irapuato collection had an average of 1.35 mm. The seed of the Roque collection presented an average of 1.36 mm. The wild chia of Salvatierra had an average of 1.43 mm. Under natural conditions, the large seed has a higher germination percentage and shows greater vigor, compared to the small ones.

Tenorio-Galindo *et al*. (2008) point out that the variation in size and color of the seed can be related to germination capacity under different environmental conditions. Jamboonsri *et al*. (2012), mention that the increase in the size of the part of anthropocentric interest is one of the characteristics of the cultivated plants, in this case the seed of the cultivated forms was twice as large as the wild ones (Table 2).

The cultivated Roque collection had a statistically larger seed size than the rest of the collections; Roque wild seed had the highest absorbency at 4 minutes, the next group regarding this variable was made up of cultivated Roque and wild Calera (Table 2). The wild collections of La Calera, Roque and Irapuato, as expected, had the statistically smaller seed size. Hoyle *et al*. (2008) found in *Goodenia fascicularis*, that when they grow in a warm environment, they accelerate the reproductive phase and produce more seed, while those that grow in a fresh environment, accumulate more biomass and produce less seed, but this seed is larger and has more viability.

Table 2. Comparison of means using the Tukey test of the variables individual weight, seed length and absorbency (0.4 minute) of eight chia collections. Celaya, Guanajuato. S-S 2016.

Cultivate	Weight mg	Length (mm)	Absorbency %
C. Guanajuato	1.285 a	2 b	0.696 c
C. Irapuato	1.135 b	1.98 b	0.731 c
C. Colima	1.125 bc	1.94 c	0.496 d
C. Roque	0.977 c	2.05 a	0.752 bc
S. Salvatierra	0.475 d	1.43 d	0.647 c
S. Irapuato	0.43 d	1.35 e	0.651 c
S. Roque	0.43 d	1.36 e	0.876 a
S. La Calera	0.406 d	1.36 e	0.848 b

Means with the same letter within each variable are statistically equal (Tukey, $p < 0.05$).

Ahmed and Fayyaz-ul-Hassan *et al*. (2015) found that in wheat, water stress increases the protein content in the grain and reduces its size. Ayerza and Coates (2009a) mention that the protein content in chia is influenced by the environment. In wheat, the highest yield is achieved with greater grain weight, which would be worth considering in the selection of chia cultivars (Sun *et al*., 2017).

The low yields in Chia may be due to the low weight of the seed and the low oil content. Ayerza (2010) mentions that as the altitude decreases the temperature increases and the oil content decreases, although at 1621 meters he found that the ratio ω6-ω3 decreases, which is good for the health of consumers. Celaya is 1750 meters above sea level, very close to the value reported by Ayerza (2010).

C: cultivated; S: wild. In the analysis of variance of the volumetric weight of the samples, no significant difference was found between the cultivars; values of 78.5 (kg hL^{-1}) were found for the Irapuato wild collection and 66.17 (kg hL^{-1}) for the Irapuato cultivated collection. Vázquez-Carrillo *et al*. (2012) found that in corn the size of the grain did not influence the hectolysis weight, but

environmental factors such as precipitation could affect it. Since we worked with wild collections, it could not be ensured if the plants had enough water, as well as domesticated ones, which could explain why there were no differences in this characteristic.

In the Table 3 shows the results of standard germination tests, it can be seen that wild seeds did not show germination; these seeds are dormant because they are wild, since in nature this characteristic is necessary to germinate at different times and when the conditions are optimal to ensure the survival of the species. The seeds of domesticated cultivars showed germination percentages of 78%, relatively low compared to highly domesticated species.

Table 3. Germination percentage of chia, wild and cultivated collections, Celaya S-S 2016.

Cultivate	(%) untreated seed germination	(%) seed germination treated with gibberellic acid
C. Colima	87	-
C. Guanajuato	80	-
C. Irapuato	71	-
C. Roque	74	-
S. La Calera	0	5
S. Irapuato	0	7
S. Salvatierra	0	2
S. Roque	0	9

This result is consistent with Lobo *et al*. (2007) who point out that the seeds may have morphophysiological latency, with embryos not fully developed and with physiological mechanisms that inhibit germination; the waterproofness of the testa could be what may be preventing germination. Canola studies show that germination depends on the content of fatty acids and other reserves; in corn, a positive correlation was found between lipid content and germination percentage (Seyyedi *et al*., 2018).

A treatment with gibberellic acid can be applied to wild seeds, since it is capable of inducing germination by helping to break dormancy. With the perception of gibberellins, many enzymes are activated and begin the process of degradation of the endosperm.

Figure 1 shows the representative chromatogram of the domesticated chia of Colima, in which we can observe the appearance of a 0.828 minute peak that does not appear in the commercial oil chromatogram. The highest peak represents α-linolenic acid, however, the percentage of absorbency is greater than the percentage of linolenic acid in commercial chia reported by Gutiérrez (2014). Chia contains between 5 and 16 fatty acids, depending on the growth conditions; the most common are palmitic, stearic, oleic, linoleic and α-linolenic. Ayerza and Coates (2009a) found no differences in four genotypes and reported a linoleic acid content of 20% and α-linolenic acid of 61.7% in the oil composition.

These researchers (2009) reported a selection of chia called Tliltic that has a lower content of linoleic acid (ω6), which could be used to achieve varieties with a good ratio ω3:ω6. The peaks that occur in different cultivars vary in size and are presented or not according to the oil composition of each cultivar. The most outstanding peaks that appear in the three repetitions of each collection were chosen. It is possible to appreciate that in the chromatograms there are three common peaks for all materials; however, the appearance profile of the peaks is different for all collections (Figures 1, 2 and 3). Table 2 shows the percentages of absorbance of the esters of the wild chia oil of La Calera, it can be seen that the percentage of absorbance of the first peak (minute 0.466) is quite high, after this peak, the most visible are those of minute 1.4 and 1.8.
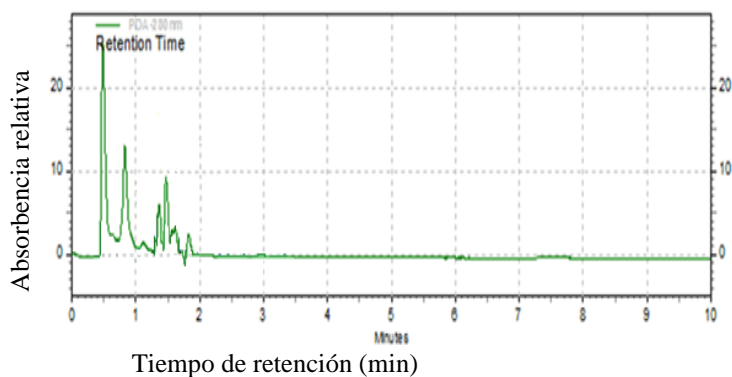

Figure 1. Chromatogram of the oil peaks of chia de Colima (cultivated).
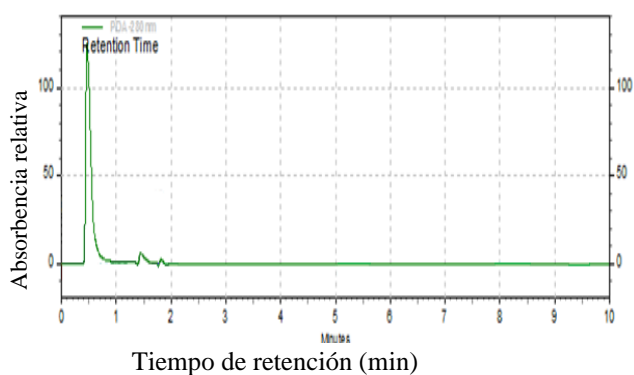

Figure 2. Chromatogram of peaks of oil extracted from chia de la Calera seed (*S. tiliifolia*, wild).
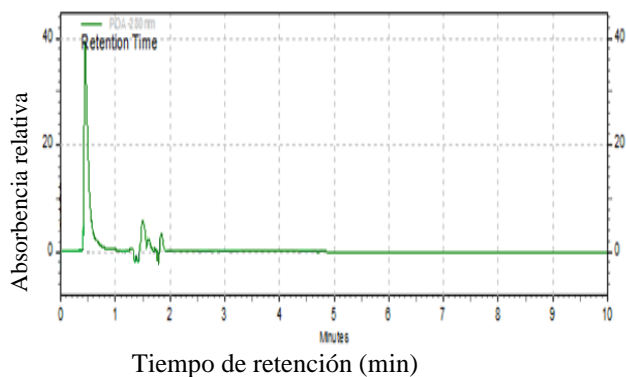

Figure 3. Chromatogram of the peaks of oil extracted from chia de Salvatierra seed (*S. hirsutum*).

The general characteristics of chia seeds are presented in Tables 4 and 5 and photographs of some of them are shown (Figures 4 and 5). The great similarity between the seeds of the collections can be observed, all were oval and with smooth pericarp.
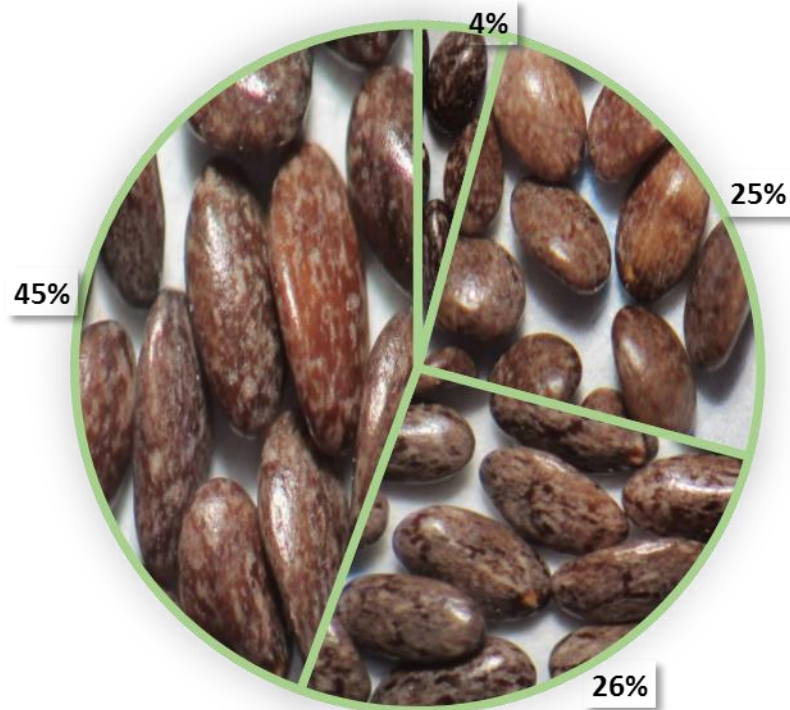

Figure 4. Percentage of colors found in chia Silvestre de La Calera, Michoacán.

Figure 5. Percentage of colors found in the cultivated chia seed crop of Colima.

Table 4. Characteristics of wild chia seeds.

General characteristics of wild seeds	Locations			
	Irapuato	La Calera	Roque	Salvatierra
Average size (mm)	1.36	1.36	1.43	1.43
Average weight per seed (mg)	1.8	1.5	1.7	1.8
Predominant color	Gray with black lines	Brown with white spots	Gray with black lines	Brown with white spots

Table 5. Characteristics of cultivated chia seeds.

General characteristics of the seeds	Locations			
	Irapuato	Colima	Guanajuato	Roque
Size (mm)	2.2	2	1.9	2
Average weight per seed (mg)	4.5	4.7	5	5.2
Predominant color	Gray with black lines	Light brown with dark brown lines	Dark gray with black lines	Light brown with dark brown lines

Cahill (2004) located, when studying 348 collections of wild and domesticated chia, greater diversity among wild varieties than among all domesticated ones; points out that there is little cross-linking between wild and domesticated, with differences between them at the level of anthers, calyx and seed morphology. In the varieties of Central America, he did not find gigantism in the seed, with respect to what was found in the Mexican varieties. Jamboonsri *et al*. (2012) point out that domesticated plants produce large seeds and have no dispersal mechanisms.

Among the wild plants no white seeds were found, up to 1% of white seeds are reported in the cultivated plants. Ayerza (2010) found no differences in the oil content between dark and white seeds, although given that a consumer preference is assumed for white ones, chia selections are already available with this seed color; the white color is governed by a recessive gene, called scc, which would partly explain the absence of seeds of this color in wild collections. In general, the seeds were very similar between the different collections, although they varied in size (Table 4 y 5).

Conclusions

Seeds from wild plant collections are smaller than those produced by cultivated plants. In weight the same thing happens, the seeds of domesticated plants were more than twice as heavy as the seeds of wild plants. The domesticated have white seeds. The wild chia, *S. tiliifolia* and *S. hirsuta* show a major peak, approximately 8 to 10 times in greater quantity than the other fatty acids present, which indicates a high content of α-linolenic acid, such as domesticated chia, *S. hispanica*, shows the peak, but is smaller and statistically different from the wild chia.

Acknowledgments

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