Article

Characterization of wild and cultivated chia populations

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Abstract

It is an annual crop of temperate and semi-warm environments with clay and sandy soils. There are wild populations in Mexico that in pre-Columbian times allowed the selection of plants with larger fruit that did not disperse the seed. Domesticated varieties, compared to wild ones, have larger seeds, more compact inflorescence, closed calyces, longer flower, apical dominance, uniformity in flowering and ripening periods. Chia contains between 9 and 23% protein, 26-41% carbohydrates and 30 to 33% oil, 40% dietary fiber and calcium and a high antioxidant content. It has acquired great importance because it is considered a functional food. There is consensus on the importance of the study and conservation of plant genetic resources. The objective of this research was to characterize the morphological diversity of 31 chia genotypes based on the variations identified between wild and domesticated populations. It was observed that the presence of anthocyanins is characteristic of wild plants, as well as the presence of open calyx, which is related to the dispersal of the seeds; these were smaller and darker, and their calyces were short and opened when ripe. The size of the seed and the weight of a thousand seeds are highly correlated with the yield per plant. Domesticated plants presented closed calyx, without anthocyanin coloration, reduction of pubescence in most of the plant, larger inflorescence, greater number of florets, greater seed weight, higher yield. Domesticated, semi-domesticated and wild populations were characterized and grouped. The wild ones have an open calyx. The semi-domesticated ones are similar to the cultivated ones but have an open calyx. The domesticated ones had apical dominance, greater size of spike and closed calyx.

Keywords: cultivated, dendrogram, principal components, Salvia, wild chia.

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Introduction

Chia was used by pre-Columbian civilizations of Mesoamerica as food and medicine (Ayerza and Coates, 2004), it is the most important source, of non-marine origin, of polyunsaturated fatty acids (PUFAs) Omegas-3 and 6, proteins, oil and fiber, for man (Baginsky *et al*., 2016; De Souza *et al*., 2015; Ullah *et al*., 2017). The seed contains between 9 and 23% protein, 26-41% non-fibrous carbohydrates and 30 to 33% of the total weight of the seed is oil. At present, it has acquired great importance because it is considered as a functional food (Olivos-Lugo *et al*., 2010; Oliveros and Paredes, 2013). Cahill (2005) states that the ability to disperse the seed, sizes and colors differ depending on the level of domestication.

This author mentions that there is great genetic diversity among wild chia populations; the divergences in the degree of domestication are probably because, in the pre-Columbian times, unique plants with larger and more productive fruits were selected. Mao *et al*. (2000) mention that the loss of dispersal mechanisms occurs when the area of abscission of the spike or part of anthropocentric interest is lost. For their part, Cong *et al*. (2002) point out that the effect of gigantism in plants is usually a marked character in hand-harvested plants, but there are often correlated effects in other parts of the plant.

Domesticated peppers not only have larger fruits than wild ones, but also larger leaves, flowers, and seeds (Medina-Santos *et al*., 2019). In relation to the species *S. hispanica*, Cahill (2005) describes that the pollination system is mainly autogamous in wild ones with a high heritability of phenotypic characteristics. Regarding the morphological and phenological characteristics that have been identified in domesticated varieties compared to wild ones are: larger seeds, more compact inflorescence, closed calyces, longer flower and apical dominance, uniformity in the periods of flowering and ripening. Hernández and Miranda (2008) found as differences between domesticated and wild populations: larger flowers, corollas protruding from the calyx, more compact inflorescences due to the greater number of whorls and a smaller distance between them, later biological cycle and larger seed size.

Wild chia populations are found in pine-oak forests towards western Mexico between the heights of 1 400 to 2 000 masl, in the Sierra Madre Occidental, in the Trans-Mexican volcanic belt and the Sierra Madre de Chiapas; they are also found in Guatemala (Cahill, 2004; Hernandez and Miranda, 2008). Ayerza and Coates (2004); Capitani *et al*., (2012); Loreto *et al*. (2013) point out that the origin of *S. hispanica* is Mesoamerica, the decline in the sowing and use of chia after the Spanish conquest undoubtedly led to the loss of knowledge of the crop and also of the genetic diversity of the cultivated populations. Even the varieties selected in various parts of the world come from a recovered population that is sown in Acatic, Jalisco (Orozco de Rosas, personal communication). The objective of this research was to characterize the morphological diversity of 31 chia genotypes based on the variations identified between wild and domesticated populations.

Materials and methods

The work was carried out in the Bajío Experimental Field (CEBAJ), belonging to the National Institute of Forestry, Agricultural and Livestock Research (INIFAP), located at km 6.5 of the road Celaya-San Miguel de Allende, Celaya, Guanajuato, at 20º 34’ 49’’ north latitude and 100º 49’ 31’’ west longitude, at an altitude of 1 768 m. The obtaining of the 31 populations (Acc) was through direct collection, acquisition from producers and institutional exchange.

The seeds of the populations studied with harvest dates 2014 and 2015 were increased in greenhouse in the 2015 spring-summer cycle. In order to ensure the establishment of the crop, the seedlings were obtained in greenhouse, peat-type substrate and vermicompost were used in a proportion of 50% (V/V) in expanded polystyrene trays of 200 cavities for germination. The transplantation of the materials (Acc) to the field was carried out on June 24, 2016, by means of a randomized complete block design with three repetitions, at a distance of 20 cm between plants, the experimental unit consisted of two furrows of 5 m long by 0.80 m wide. A foliar application (Bayer, Bayfolan®) was carried out in the vegetative stage (August 25, 2016).

The data collection was carried out from the appearance of the flower buds and the characterization of the 57 variables was carried out when there was 50% + 1 of flowering. A graduated ruler and a metal vernier were used for the quantitative variables. The qualitative ones were based on the UPOV descriptor, characterizing three plants in each repetition. The germination test was performed at a constant temperature of 25 ºC, on paper with four repetitions of 50 seeds (Rovati *et al*., 2009). To estimate the germination speed, the count was performed every 12 h, for 96 h. With the data obtained, an outlier test was carried out, where all those that registered the same value were eliminated, so it is understood that this evaluated character did not show differences between the characterized populations. The data were run in XLSTAT Software version 2017.02 in Excel Office package version 15.3.

Results

The percentage of germination at 96 hours in all populations was greater than 80%. These results are consistent with Cahill and Provance (2002), who point out that wild and domesticated populations have a good percentage of germination; in the wild ones, the germination period lasted up to ten days for the seed to germinate. The height variable is highly correlated with the density of shoots and the number of spikes. So, the higher the plant, the greater the density of shoots, as well as the number of spikes, which agrees with Sosa *et al*. (2016) (Table 1).

Table 1. Joint analysis of the correlations and principal components of the 57 variables evaluated and identification of outliers of the measured variables.

SOP	Variables	Correlations between variables and components			Cosine squared of variables		
		PC1	PC2	PC3	PC1	PC2	PC3
Plant	Growth habit	0	0	0	0	0	0
	Height (cm)	-0.212	0.113	0.583	0.045	0.013	0.34
	Width (cm)	0.106	-0.182	0.215	0.011	0.033	0.046
Stem	Density of shoots	-0.046	0.074	0.482	0.002	0.005	0.232
	Anthocyanin coloration	-0.544	0.068	0.129	0.296	0.005	0.017
	Pubescence	-0.699	0.142	-0.04	0.489	0.02	0.002
Leaf	Petiole length (mm)	0.555	0.042	0.031	0.308	0.002	0.001
	Lamina length (mm)	0.523	-0.139	-0.015	0.273	0.019	0
	Lamina width	0.570	-0.451	-0.267	0.324	0.203	0.071

SOP	Variables	Correlations between variables and components			Cosine squared of variables		
		PC1	PC2	PC3	PC1	PC2	PC3
	Length/width ratio of the lamina	0.054	0.621	0.471	0.003	0.385	0.222
	Position of the widest part of the lamina	0.256	-0.168	0.073	0.066	0.028	0.005
	Shape of the apex of the lamina	-0.377	-0.552	-0.21	0.142	0.305	0.044
	Shape of the base of the lamina	0.018	-0.178	-0.433	0	0.032	0.187
	Main color of the top of the lamina	-0.089	-0.257	-0.243	0.008	0.066	0.059
	Color variegation in the lamina	0	0	0	0	0	0
	Distribution of the variegation of the lamina	0.26	-0.484	-0.473	0.068	0.234	0.224
	Coloration of the variegation in the lamina	0.26	-0.484	-0.473	0.068	0.234	0.224
	Pubescence at the top of the lamina	-0.546	-0.146	0.151	0.298	0.021	0.023
	Roughness in the lamina	-0.217	-0.278	-0.003	0.047	0.077	0
	Brightness of the lamina	-0.506	-0.071	-0.017	0.256	0.005	0
	Incisions at the margin of the lamina	-0.154	0.171	-0.227	0.024	0.029	0.051
	Undulation at the margin of the lamina	0.365	0.481	0.396	0.133	0.231	0.156
Inflorescence	Inflorescence length (mm)	0.672	-0.16	0.06	0.452	0.026	0.004
	Internode length (mm)	0.721	0.181	0.123	0.520	0.033	0.015
	Number of florets	0.428	-0.146	0.186	0.184	0.021	0.035
	Number of lateral branches	0.361	-0.279	-0.236	0.13	0.078	0.056
	Tip position	-0.342	0.23	-0.002	0.117	0.053	0
Bract	Persistence	0.47	0.057	0.241	0.22	0.003	0.058
	Length (mm)	0.596	-0.192	-0.133	0.355	0.037	0.018
	Main color of the outer side	-0.33	-0.02	0.022	0.109	0	0
Calyx	Calyx length (mm)	0.557	-0.265	-0.133	0.311	0.07	0.018
	Main color of the outer side	0.304	-0.037	0.268	0.092	0.001	0.072
	Pubescence on the outer side	-0.477	0.495	-0.037	0.228	0.245	0.001
Corolla	Corolla length	0.851	-0.169	-0.269	0.724	0.029	0.072
	Length of the corolla tube (mm)	0.786	-0.093	0.079	0.618	0.009	0.006
	Main color of the outer side of the corolla tube	0.086	-0.364	0.443	0.007	0.133	0.196
Upper lip	Main color of the outer side	0.026	-0.553	0.653	0.001	0.306	0.426
	Secondary color of the outer side	-0.469	-0.437	-0.298	0.22	0.191	0.089
	Pubescence on the outer side	-0.364	0.44	-0.131	0.133	0.194	0.017

SOP	Variables	Correlations between variables and components			Cosine squared of variables		
		PC1	PC2	PC3	PC1	PC2	PC3
Lower lip	Width of the lower lip (mm)	0.683	0.231	0.363	0.467	0.053	0.132
	Position (tube corolla ratio)	-0.056	-0.615	0.163	0.003	0.378	0.027
	Main color of the inner side	-0.165	-0.22	0.876	0.027	0.048	0.767
	Secondary color of the inner side	-0.128	-0.597	0.632	0.016	0.356	0.399
	Distribution of the secondary color of the inner side	-0.128	-0.597	0.632	0.016	0.356	0.399
	Undulation at the margin	0.305	-0.118	0.078	0.093	0.014	0.006
Seed	Size	0.279	-0.474	-0.234	0.078	0.224	0.055
	Number of colors	-0.41	-0.592	0.189	0.168	0.351	0.036
	Main color (largest surface)	-0.324	-0.528	0.023	0.105	0.279	0.001
	Secondary color	0.096	0.048	-0.037	0.009	0.002	0.001
	Distribution of the secondary color	-0.33	-0.592	0.218	0.109	0.351	0.047
	Weight of 1 000 seeds	0.317	-0.48	-0.277	0.101	0.231	0.077
Other	Type of calyx	-0.642	0.17	0.127	0.412	0.029	0.016
	Colors of seeds in accession	-0.55	-0.365	0.127	0.303	0.133	0.016
Agronomic characters	Yield per plant	0.613	-0.041	0.326	0.376	0.002	0.107
	Number of spikes	0.22	-0.129	0.462	0.049	0.017	0.214
	Flower bud (days)	-0.792	-0.251	-0.163	0.628	0.063	0.027
	Flowering (days)	-0.794	-0.25	-0.168	0.631	0.063	0.028
Principal component analysis	Eigenvalue	10.872	6.327	5.34			
	Variability (%)	19.768	11.503	9.709			
	(%) accumulated	19.768	31.271	40.98			

The values in bold for each variable correspond to the principal component for which the cosine squared is the largest and, therefore, they are the ones with the highest negative or positive correlation; SOP= structure of the plant.

The presence of anthocyanins is correlated with the type of calyx (open) and the time it took to reach flowering (late), plants with the presence of anthocyanins are characteristic of wild plants, as well as the presence of open calyx, which is related to the dispersal of seeds (Cahill, 2005). The pubescence of the stem was correlated with the adaxial pubescence of the leaf, this characteristic was reported by Fernald (1907) in a description of chia varieties, the pubescence was also correlated with the type of open calyx and the delay in the beginning of the emission of the flower bud, so that plants with these characteristics were later (Table 1).

The length of the inflorescence was highly correlated with the length of internodes, the number of florets, number of lateral branches, bract length, corolla length, length of the corolla tube, width of the upper lip and the yield per plant, which agrees with the results of Hernández and Miranda (2008); Sosa *et al*. (2016) (Table 1).

Seed size and the weight of a thousand seeds are highly correlated with yield per plant (Cahill and Provance, 2002; Cahill, 2005; Cahill and Ehdaie, 2005; Hernández and Miranda, 2008; Sosa *et al*., 2016b). The characteristics that differed between wild and domesticated populations were: open calyx, anthocyanin coloration, pubescence in most of the plant, reduction of the inflorescence, fewer florets, lower seed weight, lower yield. Domesticated plants presented closed calyx, without anthocyanin coloration, reduction of pubescence in most of the plant, larger inflorescence, greater number of florets, greater seed weight, higher yield (Table 2).

Table 2. Characteristics that differed between wild and domesticated populations.

SOP	Variable	Minimum	Maximum	Mean	SD
Plant	Height (cm)	86	151	128.8	16.44
	Width (cm)	40	121.85	62.91	14.91
	Density of shoots	20	30.6	24.62	2.55
Stem	Anthocyanin coloration	1	4	1.47	0.86
	Pubescence	1	4	1.97	0.96
Leaf	Pubescence at the top of the lamina	1	3	1.73	0.64
Inflorescence	Inflorescence length (mm)	6.66	13.6	9.55	1.79
	Internode length (mm)	5.69	10.9	7.74	1.37
	Number of florets	9.4	16.67	12.35	1.64
	Number of lateral branches	14.67	26.5	19.5	2.62
Bract	Length (mm)	7.85	11.85	9.79	0.98
Calyx	Calyx length (mm)	7.13	9.62	8.17	0.63
	Pubescence on the outer side	2	4	3.17	0.46
Corolla	Corolla length	8.86	15.27	11.13	1.35
	Length of the corolla tube (mm)	7.29	11.05	8.67	0.78
Lower lip	Width of the lower lip (mm)	3.95	6.55	5.1	0.69
Seeds	Size	3	7	4.73	1.72
	Number of colors	5	7	6.73	0.69
	Main color (largest surface)	1	5	2.83	1.31
	Secondary color	1	5	3.13	1.14
	Distribution of the secondary color	3	7	5.73	1.43
	Weight of 1 000 seeds	0.79	1.83	1.21	0.23
Other	Type of calyx	1	9	3.4	3.73
	Colors of seeds in accession	1	9	6.73	2.72
Agronomic characteristics	Yield per plant (g)	8.2	47.13	26.48	11.45
	Number of spikes	29.5	147	75.16	25.5
	Flower bud (days)	123	171	145.53	12.18
	Flowering (days)	141	186	160.67	11.99

SOP= structure of the plant; SD= standard deviation.

The PC1 contributed with 29.55% of the variation and among the variables that contributed with greater proportion are: anthocyanin coloration and pubescence of the stem, pubescence in the leaf, length of the inflorescence and its internode, the length of the bract and calyx, the length of the corolla tube, the width of the lower lip, type of calyx, colors of seeds in accession, yield per plant, days to the beginning of the flower bud and days to the beginning of flowering (Table 3).

Table 3. Eigenvalues of the principal components.

Components	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Eigenvalue	8.275	3.29	2.633	2.165	1.707	1.571	1.389	1.189
Variability (%)	29.552	11.75	9.402	7.732	6.095	5.61	4.961	4.248
(%) accumulated	29.552	41.302	50.704	58.436	64.532	70.141	75.102	79.35

The PC2 explained 11.75% and the variables that were attributed greater weight were: width of the plant, seed size, number of colors in the seed and main color and the pattern of the secondary color in the seed. The PC3 contributed 9.40% and the important variables for this component were the density of shoots and the weight of a thousand seeds. These three components explain 50.7% of the total variability obtained. The proportion of the variance explained by a low number of components allows us a better interpretation in this type of analysis (Table 3).

In the dendrogram (Figure 1), group A is given by the integration of populations (P, K, L, H, O, I, E, DD, D, FF, BB, GG, II, and B), in this group, populations with wild and domesticated-type characteristics were present and they were phenologically similar. Populations P and K showed open calyx and the entire plant presented abundant pubescence. The other populations presented anthocyanin coloration and a reduction in pubescence, until showing the total closure of calyx in populations B, DD, D, FF, GG. In this group, with the 28 variables evaluated, it could not be contrasted, so it was deduced that they are closely related (Figure 1).

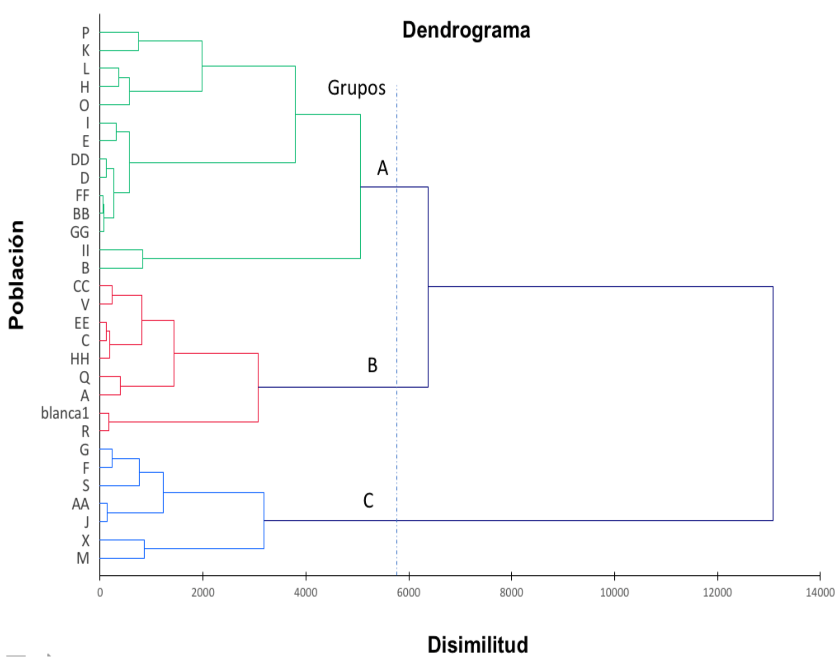

Figure 1. Dendrogram of 30 chia populations using 28 morphological characteristics.

Three large groups can be seen (Figure 1). Group B, made up of the populations CC, V, EE, C, HH, Q, A, blanca 1 and R presented characteristics typical of domesticated populations, such as calyx closure, reduction of pubescence and larger inflorescence. Group C made up of: G, F, S, AA, J, X and M, only populations G and X presented open calyx, and the rest presented characteristics of domesticated plants.

Discussion

Wild plants had small, dark seeds and their calyces were short and when ripened they opened for seed dispersal by movement because of the wind or animals, which agrees with Cahill (2005). Wild plants had fewer whorls when compared to cultivated ones; the separation of the florets is more conspicuous, resulting in little compact spikes and smaller flowers, as reported by Cahill and Provance (2002).

Wild populations showed a slight tone of anthocyanin coloration on stems and calyces; it has been observed that when a plant suffers stress from environmental damage, such as strong winds, it has intense coloration on the stems. Domesticated plants have closed calyx, large seed and little pubescence in the calyx. The closed calyx prevents the dispersal of the seed, so the survival of the species depends on the human being, a criterion that is identified as a characteristic of domestication in plants. In wild populations, the closed calyx was not present, something also observed by Harlan (1992). No doubt the closed calyx was selected during the domestication process.

According to Cahill and Provance (2002), the closed calyx is a recessive character and the gene or genes that control this trait possibly have expression patterns that are influenced by the environment. Domesticated chia from Guatemala, Nicaragua and El Salvador showed an increase in the density and length of calyx pubescence compared to wild ones (Cahill, 2005). The genotypes from Nicaragua expressed these traits notoriously and were one of the latest materials. The increase in length and density of calyx pubescence is associated with adaptation to an environment with higher humidity, since, in the absence of pubescence, dry (mature) calyxes absorb rainwater causing the seeds to hydrate and release mucilage (soluble fiber) resulting in a sticky coating that hardens affecting seed propagation.

In chia from Mexico, this problem does not commonly occur, but in areas with higher humidity, such as Nicaragua, protection against rainfall is required (Cahill, 2005). The domesticated varieties also presented a compact inflorescence, defined by a small space between the glomeruli and the number of flowers per whorl. Wild populations have great variation with respect to this trait. The longer inflorescence increases yield, but this character always appears along with apical dominance, so that only the inflorescence closest to the apex increases in length and the secondary branches with their respective inflorescences remain normal or with slight atrophies (Cahill, 2005).

Domesticated varieties found in the state of Jalisco and in the Central Valleys of Mexico produced more anthocyanin pigmentation on the stems and calyces. The stems had an interrupted purple pattern that extended to the calyx, some varieties have pure purple calyces, while in others, pigmentation only appeared on half of the outer side of each calyx. They ripen at the same time; the leaves senesce and fall off leaving only the stem and inflorescences. The lack of foliage facilitates harvesting, particularly if done mechanically (Cahill, 2005).

Conclusions

The characterization of chia populations made it possible to differentiate between wild, semi-domesticated and domesticated populations. The wild ones were characterized by open calyx, anthocyanin coloration on the stem, pubescence in the leaf, they did not present apical dominance. In the state of semi-domestication, the entire structure of the plant is similar to those cultivated and presented striated anthocyanin coloration on stems with an open calyx similar to wild ones. On the other hand, the domesticated populations presented apical dominance, greater spike size, closed calyx and stem coloration. The seed size of domesticated populations was larger than in wild ones, which confers greater productivity in the former.

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