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Morphological Characterization and Evaluation of Agroeconomic Traits of Annatto (*Bixa orellana* L.) Crops from the Social Program Sembrando Vida in Campeche, Mexico

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Abstract. **Our study aims to characterize morphologically the annatto crops from the social program Sembrando Vida in the State of Campeche based on qualitative and quantitative descriptors. The goal is to identify plants with superior agroeconomic traits related specifically to seed yields and bixin content. We evaluated 6 qualitative and 10 quantitative descriptors, focusing primarily on the flowers, fruit, and seeds from annatto plants of three different plantations: Calkini, Becal, and Bacabchen. The qualitative descriptors grouped the plants into four varieties: Criolla, Criolla roja, and Peruana roja morphotypes 1 and 2. The cluster analysis of the quantitative descriptors supported this classification. Cluster 3 presents the highest average seed yield per tree (0.61 kg) and bixin content (4.49%), and consists of Peruana roja morphotype 2. In contrast, clusters 1 and 4 exhibit the lowest seed yield (0.29 kg) and bixin content (2.41%), and are comprised of the Criolla varieties. A second cluster analysis identified five individual Peruana roja morphotype 2 from the Bacabchen locality, with an average bixin content of 6%, suggesting that these individuals have superior agroeconomic potential. In addition, we found a positive correlation between bixin content and seed yield ($r = 0.58$, $P \leq 0.001$). Both variables correlated positively with fruit height, seed width, seed number per fruit, and seed weight per fruit, suggesting that these traits could serve as criteria for selecting plants in the field. Our work facilitates the accurate identification and selection of annatto plants with high agroeconomic potential, paving the way for a future genetic improvement program.**

Bixa orellana L., annatto, or achiote, as it is known in Mexico, is native to the American continent and is widely distributed in tropical and subtropical regions (Avendaño-Arrazate et al. 2012). The agroeconomic value of annatto lies in bixin ($C_{25}H_{30}O_4$), an orange-red pigment in its seeds. The ability to produce bixin in high quantities is unique to annatto; it represents ∼80% of the total content of the resinous aril of the seeds, reinforcing its agronomic and commercial value (Ashraf et al. 2023; Giuliano et al. 2003). Bixin is considered one of the primary natural dyes worldwide, with demand extending to the food, textile, pharmaceutical, and cosmetic industries (Rivera-Madrid et al. 2016; Rivero-Manzanilla et al. 2023).

Latin America contributes 61% of the world's annual annatto production, with Peru, Brazil, and Mexico being the primary producers. Africa (Ivory Coast and Ghana) contributes 27% of the world's production, and Asia (India), 12% (Raddatz-Mota et al. 2017). The leading importers are the United States, Canada, western Europe, and Japan (Kapoor and Ramamoorthy 2021). In 2024, the global market for annatto-derived compounds was valued at US$226.5 million and is estimated to reach US$340.94 million in 2032, with a growth rate of 5.2% from 2024 to 2032 (Bindyalaxmi et al. 2024; Polaris Market Research 2023). The increased demand for natural colorants over synthetic ones has made the global annatto market a promising one. Its use has been approved by the US Food and Drug Administration. Between 2013 and 2018, more than 6600 products labeled with the pigment E160b were registered, with the vast majority belonging to the food category (Raddatz-Mota et al. 2017; Rivera-Madrid et al. 2016). In 2017, 532 ha of annatto were cultivated in Mexico, generating 570 t of seeds. The states of Tabasco, Quintana Roo, and Yucatan were the primary producers (Instituto Nacional de Investigacions Forestales, Agrícolas y Pecuarias 2022). In recent years, annatto has gained relevance in Mexico, primarily because of the implementation of the Sembrando Vida program. This program promotes food self-sufficiency, economic development, and environmental recovery by cultivating commercially valuable plants for personal consumption and sale. Nowadays, annatto is concentrated in the states of Tabasco, Chiapas, Campeche, Quintana Roo, and Yucatan, where environmental conditions favor its growth (Secretaria del Bienestar 2025).

In Campeche, the program has promoted the planting of annatto in 9 of 13 municipalities (Candelaria, El Carmén, Hopelchen, Champoton, Calakmul, Escarcega, Hecelchakan, Calkini, and Dzitbalche). More than 2,000,000 annatto plants have been cultivated in Campeche, benefiting more than 13,000 producers (69% men, 31% women) (Secretaria del Bienestar 2025). Interestingly, morphological variability has been observed in the flowers, fruit, and seeds of the annatto plantations of the Sembrando Vida Program. Furthermore, the number of plants has saturated the local and regional markets, making seed sales prices unprofitable (Rivera-Madrid 2021). This has generated a growing interest among farmers seeking national and/or international markets to sell seeds with high bixin content. Commercial seed exports require 2.5% to 4% bixin (Bindyalaxmi et al. 2024; Dias et al. 2017). This creates the need to identify and select potential varieties with high agroeconomic potential, thereby generating more homogeneous crops that can compete in national and/or international markets. Bindyalaxmi et al. (2024) indicate that two of the most important agroeconomic variables for the selection of superior annatto varieties are seed yield and bixin production.

Bixa orellana is characterized by high morphological and genetic variability, which is observable in the diversity of stem, fruit, and flower colors (Carballo-Uicab et al. 2019; Rivera-Madrid et al. 2006; Rodríguez-Ávila et al. 2011; Trujillo-Hernández et al. 2016; Valdez-Ojeda et al. 2008). In the case of annatto, qualitative and quantitative descriptors are used for its characterization, based mainly on distinctive characteristics of reproductive tissues such as flowers, fruit, and seeds. Some

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qualitative descriptors include flower color, fruit color, shape, and dehiscence. Quantitative descriptors include the number of seeds per fruit per tree, the number of panicles per tree, and seed size, among others (Arce Portuguez 1984; Hernández-Villareal 2013). Several studies highlight the use of descriptors for classifying annatto varieties (Arias-Pérez et al. 2017; Bindyalaxmi et al. 2024; Duque et al. 2022; Pech-Hoil et al. 2024). Interestingly, morphological variability observed in the color of the flowers and fruit of *B. orellana* has also been related to yield and the bixin content in the seeds (Arias-Pérez et al. 2017; Carballo-Uicab et al. 2019; Pech-Hoil et al. 2024; Rivera-Madrid et al. 2006; Rodríguez-Ávila et al. 2011; Trujillo-Hernández et al. 2016; Valdez-Ojeda et al. 2008).

The aim of our study was to characterize morphologically the annatto crops from the social program Sembrando Vida in the state of Campeche based on qualitative and quantitative descriptors to identify plants with superior agroeconomic characteristics, particularly concerning seed yield and bixin content. Understanding the agroeconomic characteristics of the annatto crops used in the program will enable the expansion of marketing opportunities for annatto plants in Mexican social programs. It will also enable more assertive identification and selection of annatto plants with agroeconomic potential and will help to develop future breeding improvement programs.

Table 1. Qualitative and quantitative descriptors evaluated in annatto cultivars of the Sembrando Vida program (*Bixa orellana* L.).

Descriptor type	Tissue	Descriptor	Unit of measurement/category
Qualitative	Stem	Stem color	Green, red
	Flower	Flower color	White, light pink, lilac
	Fruit	Dehiscence	Dehiscent, indehiscent
		Spinosity	Null, low, medium
		Color of thorns	Green, red
		Fruit shape	Round, lanceolate, oblong, elliptical
Quantitative	Fruit	Fruit height	mm
		Fruit width	mm
		Fruit thickness	mm
		Fruit per tree	n
	Seed	Seed height	mm
		Seed width	mm
		Seeds per fruit	n
		Seed weight per fruit	g
		Seed yield per tree	kg
		Bixin content	%

Materials and Methods

Study site and sample size. The study was conducted between Oct 2023 and Apr 2024 in three plantations of the Sembrando Vida program located in the localities of Becal (lat. 20°26′24.9″N, long. 90°00′16.8″W), Calkini (lat. 20°22′12.1″N, long. 90°04′34.0″W), and Bacabchen (lat. 20°13′28.4″N, long. 90°01′06.5″W). These plantations were chosen because they are large and close to downtown Calkini, facilitating crop monitoring and sample collection. Simple random sampling was applied, considering a total initial population of 150 plants across the three plantations. The sample size was estimated using the formula for finite populations:

$$n = \frac{NZ^2p(1-p)}{e^2(N-1)+Z^2p(1-p)},$$

where n is the sample size, N is the total population (150 plants), Z is the z-score for a 95% confidence level (1.96), p is the expected proportion (0.5), and e is maximum permissible error (0.1).

Although the initial estimated sample size was ~59 plants, only 50 were ultimately evaluated as a result of the natural loss of individual plants. The remaining plants were ~2.5 m tall and 2.5 years old, and were planted at a distance of 2.5 to 3 m. Each plantation had a mixture of plants with different morphological characteristics. The program provided all the annatto plants.

Qualitative and quantitative descriptors. Sixteen morphological descriptors of agroeconomic interest were considered and distributed among the stem, flower, fruit, and seed. Of these, 6 were qualitative and 10 were quantitative. The selection was based on previous reports of annatto descriptors (Arce Portuguez 1984; Manco Céspedes et al. 2022; Valdez-Ojeda et al. 2008). The qualitative and quantitative descriptors evaluated are presented in Table 1. For collection and sampling, each tree was assigned a unique numerical code. Fruit and seeds were monitored and collected 12 weeks postanthesis, after maturation. The color of the stem, flower, and thorns was determined using the Munsell color chart. Fruit shape, spinosity, and dehiscence were characterized according to Manco Céspedes et al. (2022) and by direct observation. For quantitative descriptors, fruit height, width, and thickness, as well as seed height and width, were measured after harvest using a digital vernier caliper. The number of fruit per tree and the number of seeds per fruit were obtained by manual counting. Seed weight per fruit and seed yield per tree were determined using an analytical balance.

Bixin extraction and quantification by high-performance liquid chromatography. Bixin extraction was performed in triplicate using seeds collected at week 12 postanthesis. Bixin extraction and quantification by high-performance liquid chromatography (HPLC) were based on the method reported by Chisté et al. (2011) with slight modifications. The seeds were lyophilized, and the extraction was performed using one seed with an approximate weight of 60 to 70 mg and 1 mL of a methanol:acetone (50:50) mixture. Bixin extraction was performed for 5 min using an ultrasonic bath (Creworks, USA) with a power of 120 W and a frequency of 40 kHz (Chisté et al. 2011). This procedure was performed five times to extract all the bixin present in the seed. Bixin quantification was performed by HPLC (Thermo Scientific Ultimate 3000; Thermo Scientific, Waltham, MA, USA). Twenty microliters of the extract were separated on a 15-cm × 4.6-mm Hypersil GOLD C18 column (Thermo Scientific) with a particle size of 5 μm. The mobile phase was maintained at a constant flow rate of 0.9 $mL \cdot min^{-1}$ and consisted of mobile phase A, water with 0.2% formic acid, and mobile phase B, methanol with 0.2% formic acid. A gradient elution system was used, starting with 30% mobile phase B and increasing to 60% in 15 min, then from 60% to 80% in 10 min, and finally to 95% in the last 10 min. The column temperature was maintained at 30 °C, and the bixin concentration was measured at 459 nm. A calibration curve was performed to quantify bixin, preparing standard solutions of known concentrations (20, 50, 100, 150, 180, and 200 $mg \cdot L^{-1}$).

Statistical analysis. Statistical analysis was conducted on 10 quantitative morphological agronomic descriptors evaluated from 50 plants. The descriptors included the number of fruit per plant, fruit length, fruit thickness, fruit width, seed height, seed width, number of seeds per fruit, seed weight per fruit, seed yield per tree, and bixin content (measured as a percentage). Descriptive statistical analysis [mean, minimum, maximum, standard deviation, and coefficient of variation (*CV*)] was performed to assess the variability of these descriptors. The mean and standard deviation were used in subsequent analyses. Two hierarchical cluster analyses were conducted using Ward's method and squared Euclidean distance. The first analysis included the 10 quantitative variables, whereas the second focused solely on bixin content. Last, Pearson correlation coefficients ($P \leq 0.05$) were calculated to assess the linear relationships between the quantitative descriptors and to identify significant associations between morphological and productive variables. All statistical analyses were performed using Statgraphics Centurion 19-X64 software (version 19.1.2; Statgraphics Technologies, Inc., The Plains, VA, USA).

Results

Qualitative descriptors enabled the classification of annatto plants into four varieties. As a result of its sexual reproduction, various authors have reported significant morphological diversity in annatto plantations (Akshatha et al. 2011; Arias-Pérez et al. 2017; Pech-Hoil et al. 2024; Valdez-Ojeda et al. 2008). In this context, visible morphological descriptors play a crucial role in the varietal classification of annatto (Arias-Pérez et al. 2017; Duque et al. 2022). In an initial effort to group annatto plants based on similar morphological characteristics, we focused solely on qualitative traits that are easily observed in three key organs: the flower, fruit, and stem. The classification was based on qualitative descriptors: flower color, stem color, fruit shape, dehiscence, fruit color, and spinosity. Plants from Calkini, Becal, and Bacabchen were categorized into four groups: Criolla, Criolla roja, Peruana roja morphotype 1, and Peruana roja morphotype 2. The names used were derived from how farmers named their annatto crops.

The Criolla variety is characterized by green stems, white flowers, and round, green fruit in their immature state. These fruit have a high spinosity and green thorns (Fig. 1A–C). In contrast, the Criolla roja variety features green stems and light-pink flowers. It has fruit similar in shape to the Criolla; they are green, but with red thorns, and present high spinosity (Fig. 1D–F). Notably, in the early stages of development, these fruit may appear predominantly reddish because of the color of the red thorns; however, as they mature, the green color of the fruit becomes more prominent, diminishing the visibility of the red color. Peruana roja morphotype 1 is identified by its red stems, lilac flowers, and lanceolate fruit, which exhibit null spinosity; the fruit and thorns are also red (Fig. 1G–I). Peruana roja morphotype 2 also features red stems and lilac flowers, elliptical-shaped fruit, and exhibits low spinosity, with red thorns and dehiscent fruit in the immature stage (Fig. 1J–L). These morphological traits facilitate the visual differentiation among the varieties and highlight the phenotypic diversity in the evaluated annatto populations. Representative differences in the flowers, fruit, and seeds of each variety are observable in Fig. 1. It is important to note that in the mature stage, the color of the fruit and thorns for all varieties turns dark brown and the fruit exhibits dehiscence.

The quantitative descriptors support the classification of plants into four distinct varieties. Significant variations were found in the 10 quantitative descriptors evaluated. The maximum *CV* was observed in bixin content (41.7%), with minimum values ranging from 0.5% to 9.02%, and the number of fruit per tree (40.06%), with minimum and maximum values of n = 500 and n = 1532, respectively. The lowest *CV* was observed in fruit width (13.84%), with minimum and maximum values of 2 and 39 mm, respectively (Table 2).

Subsequently, the 10 quantitative morphological descriptors were subjected to hierarchical cluster analysis to group the evaluated plants based on similar values. The resulting dendrogram displayed the formation of four distinct groups or clusters, each corresponding to a specific variety (Fig. 2).

These clusters represent particular combinations of the analyzed variables and highlight differences in the plant's important productive and morphological characteristics. The centroid or average values of all variables per cluster are indicated in Supplemental

Fig. 1. Qualitative morphological characteristics of the annatto varieties identified and used in the Sembrando Vida program. (**A–C**) The Criolla variety has white flowers; round, green fruit with green thorns; fruit seeds; and dehiscent fruit at the end of maturity. (**D–F**) The Criolla roja variety has light-pink flowers; round, green fruit with red thorns; fruit seeds; and dehiscent fruit at the end of maturity. (**G–I**) Peruana roja morphotype 1 has lilac flowers, lanceolate red fruit with no spinosity, fruit seeds, and dehiscent fruit at the end of maturity. (**J–L**) Peruana roja morphotype 2 has lilac flowers, elliptical red fruit, red thorns, low spinosity, fruit seeds, and dehiscent fruit at the end of maturity.

Table 1. The identification number of each plant, the name of the variety, and the locality of origin are shown in Supplemental Table 2.

The first group represents 22% of the plants (n = 11). It is characterized by plants with an average of 31 seeds, small seeds (width, 1.24 mm; height, 2 mm), and round fruit (width, 34.64 mm; height, 30.64 mm). This group has the lowest seed yield per tree (0.37 kg) and the lowest percentage of bixin (2.41%), indicating low agroeconomic potential (Fig. 2, Supplemental Table 1). This group consists of plants from the Becal plantation, classified as Criolla roja (Supplemental Table 2). Morphologically, they exhibit pink flowers, green stems, green fruit with red thorns, and dehiscent fruit (Fig. 1D–F). These quantitative characteristics reflect poor productive performance and suggest that these cultivars are less suitable for commercial purposes.

The second group comprises 22% of the population (n = 11). It includes plants with an average of 34 seeds, larger seeds (width, 3.34 mm; height, 2.99 mm), and more elongated fruit (width, 24.49 mm; height, 53.36). This group has a moderate bixin content of 3.89% and an average yield of 0.39 kg of seeds per tree (Fig. 2, Supplemental Table 1).

Table 2. Results of 10 quantitative traits of the 50 plants of annatto (*Bixa orellana* L.).

Trait	*CV* (%)	Mean	*SD*	Min	Max
Seed weight/fruit (g)	29.57	1.63	0.48	0.69	2.5
No. of seeds/fruit	14.81	38.33	5.68	29	53
Fruit width (mm)	13.84	31.23	4.32	2	39
Fruit height (mm)	29.05	44.38	12.89	21	58
Fruit thickness (mm)	17.58	17.17	3.019	12	25
No. of fruit/tree	40.06	894.56	358.37	500	1532
Seed width (mm)	32.87	2.73	0.90	1	3.6
Seed height (mm)	14.91	2.77	0.41	1.9	3.1
Seed yield/tree (kg)	33.013	0.45	0.148	0.235	0.75
Bixin (%)	41.70	3.64	1.52	0.5	9.02

CV = coefficient of variation; Max = maximum; Min = minimum; *SD* = standard deviation.

These plants originate from the Becal plantation and belong to Peruana roja morphotype 1 (Supplemental Table 2). They are characterized by their lilac flowers, red stems, lanceolate and thornless red fruit, and dehiscent fruit (Fig. 1G–I). Although their production is low, the larger seed size could be a favorable trait in specific breeding contexts.

The third group represents 36% of the plants evaluated (n = 18) and is notable for containing plants with 43 seeds, elongated seeds (width, 3.33 mm; height, 2.99 mm), and fruit (width, 31.61 mm; height, 56.16 mm). This group features a very high average number of fruit per tree (n = 1472.78) and considerable fruit thickness (17.5 mm), with slightly higher values in seed size, resulting in an exceptional production profile. They have a high average bixin content of 4.49% and an average yield of 0.61 kg of seeds per tree (Fig. 2, Supplemental Table 1). The plants in this cluster are from Calkini (n = 5) and Bacabchen (n = 13) and belong to Peruana roja morphotype 2 (Supplemental Table 2). Lilac flowers, reddish stems, elliptical red fruit with low spinosity and red thorns, and dehiscent fruit identify them (Fig. 1J–L). These traits make this cluster particularly promising for selection and genetic improvement programs.

Last, cluster 4 comprises 20% of the plants (n = 10). It includes plants with an average of 40 seeds per fruit, thicker fruit (width, 34.2 mm; height, 28.2 mm), and shorter seeds (width, 2.364 mm; height, 2.99 mm). This group has an average seed yield of 0.287 kg per tree and a moderate bixin content of 3.15% (Fig. 2, Supplemental Table 1). The plants in this cluster belong to the Criolla variety and are found in Calkini (n = 8) and Bacabchen (n = 2) (Supplemental Table 2). Their distinguishing features include white flowers; green stems; round, green fruit with green thorns; and dehiscent fruit (Fig. 1A–C). Although they have robust vegetative structures, this does not increase productivity, suggesting that this group may represent less efficient plants from an agroeconomic perspective.

Individuals with agronomic potential were identified. Because bixin content is the primary variable of commercial interest in annatto, a second cluster analysis was performed that focused solely on the percentage of bixin. This approach enabled the targeted selection of specific individuals that could meet or exceed commercial standards. Commercial seed exports require 2.5% to 4% bixin (Bindyalaxmi et al. 2024; Dias et al. 2017). Our analysis grouped the plants into four clusters, revealing a heterogeneous distribution of varieties within each group (Fig. 3, Supplemental Table 2). The average values of bixin per cluster are indicated in Supplemental Table 3.

Cluster 1 exhibited moderate-to-high bixin content at 3.46% (Fig. 3, Supplemental Table 3). This cluster included plants from the Criolla variety sourced from the localities of Calkini (n = 2) and Bacabchen (n = 2), Criolla roja (n = 1) from Becal, Peruana roja morphotype 1 (n = 5) from Becal, and Peruana roja morphotype 2 from Bacabchen (n = 1) and Calkini (n = 3) (Supplemental Table 2). Cluster 2 displayed the lowest bixin content at 2.40% (Fig. 3, Supplemental Table 3). It consisted primarily of the Criolla roja variety from Becal (n = 10), Criolla plants from Calkini (n = 5), and Peruana roja morphotype 2 from Calkini (n = 2) (Supplemental Table 2). Cluster 3 exceeded the accepted commercial standard of 4%, with a bixin content of 4.47% (Fig. 3, Supplemental Table 3). This cluster included mainly Peruana roja morphotype 2 plants from Bacabchen (n = 7), Peruana roja morphotype 1 from Becal (n = 6), and one Criolla (n = 1) from Calkini (Supplemental Table 2). Cluster 4 achieved the highest bixin value at 6% (Fig. 3, Supplemental Table 3). The plants in this cluster were exclusively Peruana roja morphotype 2 from Bacabchen (n = 5) (Supplemental Table 2). Notably, these individuals were also grouped within cluster 3 of the previous analysis, which comprises plants with the highest seed yield per tree (0.614 kg) (Fig. 2, Supplemental Table 1).

Correlation analysis revealed significant relationships between quantitative descriptors and agroeconomic traits. Correlation analysis identified the most significant relationships among the 10 quantitative descriptors and their influence on seed yield and seed bixin content. Pearson's correlation coefficient (r) was used to evaluate the associations of the quantitative variables ($P \leq 0.05$) (Table 3).

The percentage of bixin correlated positively with several morphological variables, including seed width (r = 0.67, $P \leq 0.001$) and seed height (r = 0.53, $P \leq 0.001$), fruit height (r = 0.57, $P \leq 0.001$), number of seeds per fruit (r = 0.51, $P \leq 0.001$), and seed weight per fruit (r = 0.56, $P \leq 0.0001$). This suggests that longer fruit with wider, heavier seeds produce higher bixin content, which is significant for product quality. Furthermore, seed yield was associated positively with the percentage of bixin (r = 0.58, $P \leq 0.001$), reinforcing the notion that yield is linked to the quantity of this pigment.

Seed yield per tree, a crucial productivity trait, also showed a strong positive correlation with seed weight per fruit (r = 0.84, $P \leq 0.001$), fruit height (r = 0.73, $P \leq 0.001$), and, to a lesser extent, with seed width (r = 0.59, $P \leq 0.0001$), number of fruit per tree (r = 0.57, $P \leq 0.001$), number of seeds per fruit (r = 0.51, $P \leq 0.001$), and seed thickness (r = 0.59, $P \leq 0.001$) (Table 3). This suggests that trees with longer fruit, wider seeds, and a significant number of fruit and seeds tend to have better yields.

One notable finding was the strong positive correlation between seed weight per fruit and fruit height (r = 0.82, $P \leq 0.001$), seed yield (r = 0.84, $P \leq 0.001$), and seed width (r = 0.65, $P \leq 0.001$), indicating that longer fruit tend to contain wider, heavier seeds, which in turn influences the seed yield per tree. In addition, it suggests that wider seeds contribute to a greater total weight per fruit.

The number of seeds per fruit also exhibited significant correlations with various important variables, such as fruit height (r = 0.39, $P \leq 0.01$), seed width (r = 0.57, $P \leq 0.001$), seed yield per tree (r = 0.51, $P \leq 0.001$), and bixin percentage (r = 0.51, $P \leq 0.001$) (Table 3). This suggests the number of seeds per tree is a variable that can be used to predict both yield and pigment content.

Fruit length has a high correlation with seed weight per fruit (r = 0.82, $P \leq 0.001$),

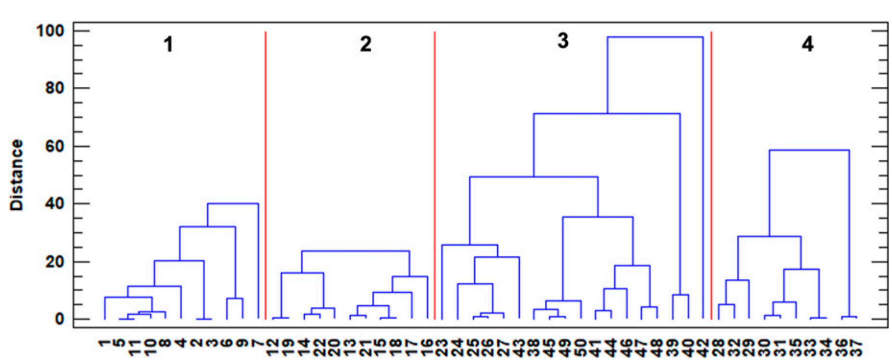

Fig. 2. Hierarchical cluster analysis of the 10 quantitative traits of the 50 annatto plants (*Bixa orellana* L.), using Ward's method and squared Euclidean distance.

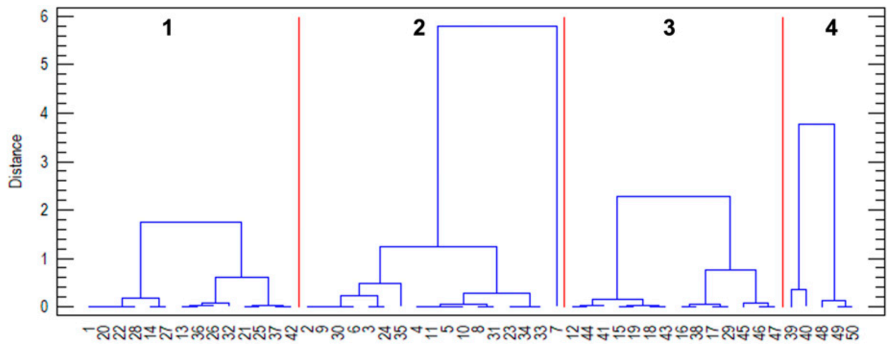

Fig. 3. Hierarchical clustering based on the bixin content of the 50 annatto plants (*Bixa orellana* L.), using Ward's method and squared Euclidean distance.

seed width (r = 0.87, $P \leq 0.001$), seed height (r = 0.57, $P \leq 0.001$), seed yield per tree (r = 0.73, $P \leq 0.001$), and even the percentage of bixin (r = 0.57, $P \leq 0.001$). Conversely, fruit width correlated negatively with several variables, including fruit height (r = –0.5667, $P \leq 0.001$), seed width (r = –0.59, $P \leq 0.001$), bixin percentage (r = –0.2815, $P \leq 0.05$), and yield per tree (r = –0.09) (Table 3). This suggests that longer, narrower fruit tend to have larger, heavier seeds, as well as a better yield and pigment content.

Discussion

The Criolla and Peruana roja varieties have distinctive features from those reported in the Yucatan Peninsula. Due to its sexual reproduction, various authors have reported the great morphological diversity in annatto plantations (Akshatha et al. 2011; Arias-Pérez et al. 2017; Pech-Hoil et al. 2024; Valdez-Ojeda et al. 2008). Morphological variants have been observed even within each annatto plantation, likely a result of cross-pollination occurring within the plantations (Pech-Hoil et al. 2017, 2024). In this context, visible morphological descriptors play a crucial role in the primary varietal classification of annatto (Arias-Pérez et al. 2017; Duque et al. 2022). Our initial analysis, based on qualitative morphological characteristics, suggests the presence of four annatto varieties, which we have named Criolla, Criolla roja, Peruana roja morphotype 1, and Peruana roja morphotype 2 (Fig. 1).

Duque et al. (2022) reported that during the characterization of 18 accessions, they found significant differences in the fruit, particularly in terms of color, shape, and spinosity. They also observed a relationship between the fruit and flower color. Similarly, diverse fruit shapes and colors have been identified in an analysis of 40 annatto accessions (Arias-Pérez et al. 2017). Rivera-Madrid et al. (2006) reported at least three varieties of annatto from the Yucatan Peninsula. The Criolla variety is characterized by flowers with white petals, green sepals, green fruit, and indehiscent fruit at the end of maturity. The second variety has purple petals, reddish sepals, fruit with high spinosity and red thorns, and is dehiscent at maturity. Last, the Peruana roja variety features pink petals, reddish sepals, and fruit with high spinosity, as well as red thorns. The fruit are dehiscent at maturity. In another study, 16 annatto accessions were classified into haplotypes A, B, and C based on morphological and genetic differences (Trujillo-Hernández et al. 2016). Haplotype A contains plants that are characterized by white flowers and green fruit with green thorns, and indehiscent fruit. Haplotype B presents pink flowers with green dehiscent fruit with red thorns, and haplotype C contains purple flowers and red or yellow dehiscent fruit with red thorns. Cárdenas-Conejo et al. (2015) reported a variety called Peruana roja that is characterized by having pink flowers, red flower buds, green fruit with elongated red thorns, dehiscent fruit, and a high bixin content, similar to that reported as haplotype B (Rivera-Madrid et al. 2006; Trujillo-Hernández et al. 2016).

Regarding the varieties reported in our study, some share morphological characteristics with those reported, such as flower color; however, they exhibit differences in shape, fruit, thorn color, and the level of spinosity (Fig. 1). For example, the Criolla variety exhibits morphological characteristics similar to those of Criolla and haplotype A, but differs in that its fruit dehisces at the end of the mature stage (Rivera-Madrid et al. 2006; Trujillo-Hernández et al. 2016). Similarly, Peruana roja morphotypes 1 and 2 exhibit differences from the Peruana roja variety in terms of flower color, fruit shape, spinosity level, and thorn size (Fig. 1) (Cárdenas-Conejo et al. 2015; Rivera-Madrid et al. 2006). The results suggest that the Criolla, Criolla roja, and Peruana roja morphotypes, specifically varieties 1 and 2 of the Sembrando Vida Program, exhibit distinctive qualitative morphological characteristics compared with those described previously in the Yucatan Peninsula (Carballo-Uicab et al. 2019; Rivera-Madrid et al. 2006; Rodríguez-Ávila et al. 2011; Trujillo-Hernández et al. 2016; Valdez-Ojeda et al. 2008).

The Peruana roja morphotype 2 variety has superior agroeconomic potential. Because bixin is the primary product of interest, its analysis is crucial for understanding the morphological variability of annatto crops and their economic implications. Bindyalaxmi and Mohammed (2023) indicate that two of the most critical agroeconomic characteristics for selecting superior annatto plants are seed yield and bixin production. The cluster analysis of the 10 quantitative descriptors suggests that varieties with lilac flowers and red fruit grouped in clusters 2 and 3 (Peruana roja morphotypes 1 and 2) exhibit better agroeconomic characteristics, particularly in terms of bixin content and seed yield, compared with plants with white (Criolla) and pink (Criolla roja) flowers and green fruit grouped in clusters 4 and 1, respectively (Fig. 2, Supplemental Table 1). Other reports indicate that annatto varieties with pink or light-pink flowers and red fruit tend to have a higher bixin content than annatto varieties with white flowers and red fruit

Table 3. Pearson correlation coefficient (r) between ten quantitative morpho-agronomic descriptors of annatto plants (*Bixa orellana* L.).

	Descriptor									
Descriptor	SWF	SF	FW	FH	FT	FPT	SW	SH	SY	BX
SWF	—	0.51***	–0.22	**0.82*****[i]	–0.2	0.5***	0.66***	0.33*	**0.84*****	0.56***
SF	0.51***	—	0.21	0.39**	0.5***	0.32*	0.57***	0.63***	0.51***	0.51***
FW	–0.22	0.21	—	–0.57***	0.28*	0.09	–0.59***	–0.44**	–0.09	–0.28*
FH	**0.82*****	0.39**	–0.57***	—	–0.28*	0.45**	**0.87*****	0.57***	0.73***	0.57***
FT	–0.2	0.50***	0.28	–0.28*	—	0.03	0.13	0.48***	–0.09	0.08
FPT	0.50***	0.32*	0.09	0.45**	0.03	—	0.33*	0.17	0.57***	0.17
SW	0.66***	0.57***	–0.59***	**0.87*****	0.13	0.33*	—	**0.89*****	0.59***	0.67***
SH	0.33*	0.63***	–0.44**	0.57***	0.48***	0.17	**0.89*****	—	0.3*	0.53***
SY	**0.84*****	0.51***	–0.09	0.73***	–0.09	0.57***	0.59***	0.3*	—	0.58***
BX	0.56***	0.51***	–0.28*	0.57***	0.08	0.17	0.67***	0.53***	0.58***	—

[i] Values of $r \geq 0.80$ are in bold type.

*, **, *** Significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

BX = bixin (%); FH = fruit height (mm); FPT = fruit per tree (n); FT = fruit thickness (mm); FW = fruit width (mm); SF = seeds per fruit (n); SH = seed height (mm); SW = seed width (mm); SWF = seed weight per fruit (g); SY = seed yield per tree (kg).

(Akshatha et al. 2011; Carballo-Uicab et al. 2019; Cárdenas-Conejo et al. 2015; Pech-Hoil et al. 2024; Trujillo-Hernández et al. 2016).

The bixin content varied among the varieties, with a maximum average value of 4.49% (Peruana roja morphotype 2) and a minimum value of 2.41% (Criolla roja). In addition, it was found that the maximum *CV* was observed in bixin content (41.7%), with minimum and maximum values ranging from 0.5% to 9.02%, respectively (Table 2, Supplemental Table 1). Bindyalaxmi and Mohammed (2023) report a maximum bixin level of 2.37% and a minimum of 0.94% in annatto varieties from India. In Brazil, bixin levels range from 1% to 7% in annatto varieties (Carvalho et al. 2005; Dequigiovani et al. 2017; Dias et al. 2017). In the Yucatan Peninsula, varieties with values $\leq$ 4% are reported (Pech-Hoil et al. 2024). Regarding seed yield, we found a maximum average value of 0.61 kg (Peruana roja morphotype 2) and a minimum value of 0.29 kg (Criolla) (Supplemental Table 1). In addition, trees of annatto in India report yields of 0.210 to 1.50 kg (Bindyalaxmi et al. 2024). The average seed yield from a 3-year-old plant ranges from 500 g to 1 kg per tree per year (Math et al. 2016). Nolasco-Chumpitaz et al. (2020) reported an average seed yield value of 0.514 kg from annatto cultivars in Peru. The maximum yield of plantations is obtained between 4 and 10 years, after which there is a gradual decline in crop yield (Math et al. 2016). Cluster 3, formed by the red Peruvian morphotype 2, is notable because of its high seed yield per tree (0.61 kg) and an average bixin content of 4.49%, which exceeds the 4% required by both national and international markets (Dias et al. 2017; Pech-Hoil et al. 2024). At the international level, the commercial export of annatto seeds requires a minimum of 2.5% to 4% (Bindyalaxmi et al. 2024; Dias et al. 2017). Our results suggest that Peruana roja morphotype 2 has the potential to be an internationally marketable source of bixin.

Another outstanding characteristic of Peruana roja morphotype 2 is the highest average number of red elongate fruit (n = 1472) and seeds (n = 43) compared with the other varieties (Supplemental Table 1). These traits have been associated with superior varieties of annatto. Akshatha et al. (2011) reported 46 ± 4.7 seeds for red fruit and 37.2 ± 4.2 seeds for green fruit. In addition, they found that annatto plants with red fruit had a greater number of fruit per panicle and a higher bixin content. This finding is consistent with our results, which show that red fruit tend to have a greater number of fruit and seeds than green fruit (Supplemental Table 1). The Peruana roja varieties, morphotypes 1 and 2, were characterized by presenting red, elongated (lanceolate and elliptical-shaped) fruit compared with the round, green fruit of the Criolla varieties (Fig. 1). Taboada (1993) observed that larger varieties, such as those of Peruvian origin, tended to have a greater pigment content than native varieties. Although his work does not specify flower color or thorn type, Taboada (1993) associated the robust morphology and large fruit with high bixin content. Akshatha et al. (2011) found that ovate, red fruit were associated with better productive characteristics and higher bixin content, reinforcing the association between fruit morphology (shape, color, and spinosity) and pigment yield.

In addition, results indicate that five individuals of the Peruana roja morphotype 2 variety from the Bacabchen locality are particularly relevant as a result of their high bixin content and seed yield (Bindyalaxmi et al. 2024; Dias et al. 2017) (Supplemental Tables 1 and 3). Furthermore, it meets the global requirements for the natural coloring industry, which typically requires cultivars with more than 5% bixin to ensure process profitability (Arias-Pérez et al. 2017). Individuals of Peruana roja morphotype 2 can be selected and used to develop genetic improvement programs. The genetic improvement process begins with the selection of morphotypes with high bixin content, and subsequently initiates appropriate ex vitro or in vitro vegetative propagation protocols (Rivera-Madrid et al. 2006).

Several quantitative morphological traits were associated with higher yield and bixin content. Correlation analysis allowed us to identify which quantitative traits influenced seed yield and bixin content. Our results indicate a positive correlation between bixin content and seed yield (r = 0.58, $P \leq 0.001$) (Table 3). Similarly, Joseph and Siril (2014) found a positive correlation in elite annatto plants between bixin percentage and seed yield (r = 0.788). In addition, we observed a strong positive correlation between both variables and fruit height and seed width. Fruit height and seed width also showed a strong correlation with each other (r = 0.87, $P \leq$ 0.001) (Table 3). Seed width also showed a high correlation with number of seeds (r = 0.89, $P \leq 0.001$). The results also indicate a positive correlation between seed number per fruit and yield (r = 0.51, $P \leq 0.001$) and bixin content (r = 0.51, $P \leq 0.001$) (Table 3). These results suggest that elongated fruit; wide, long seeds; and a greater number of seeds per fruit are associated with better yield and bixin content. These morphological characteristics are consistent with the fruit and seeds of Peruana roja morphotypes 1 and 2, but specifically Peruana roja morphotype 2, which presented the best results in terms of yield and bixin content. This behavior has been reported previously in varieties with more compact fruit that concentrate greater levels of pigment (Arias-Pérez et al. 2017). Akshatha et al. (2011) reported that conical-shaped fruit have a high bixin content. Arce Portuguez (1999) noted that plants with elongated fruit and a greater number of seeds yield better results and have a higher bixin content. Pech-Hoil et al. (2024) reported similar results; elongated fruit with a greater number of seeds showed a positive correlation with a higher bixin content.

Seed weight per fruit also showed a high correlation with fruit height (r = 0.82, $P \leq$ 0.001), seed width (r = 0.66, $P \leq 0.001$), number of fruit per tree (r = 0.50, $P \leq$ 0.001), seed yield (r = 0.84, $P \leq 0.001$), and bixin content (r = 0.56, $P \leq 0.001$) (Table 3). This finding is consistent with previous studies, which found a correlation between seed weight, color, and bixin concentration (Pech-Hoil et al. 2024). Bindyalaxmi et al. (2024) indicated that bixin content is directly influenced by the weight of 100 seeds and other variables, such as the number of primary and secondary branches, tree height, and basal tree diameter, emphasizing the importance of these descriptors in bixin production.

The study of morphological traits of fruit and seeds is a valuable tool for determining the yield and bixin content of annatto crops, as well as the genetic variations that may exist among plants (Bindyalaxmi et al. 2024; Joseph and Siril 2014; Pech-Hoil et al. 2024).

Conclusion

Morphological description is essential for accurately identifying and selecting annatto plants with agroeconomic potential. Our work allowed for the classification of the annatto crops of the Sembrando Vida social program in the state of Campeche into four main varieties: Criolla, Criolla roja, Peruana roja morphotype 1, and Peruana roja morphotype 2. Each variety has distinctive morphological characteristics, including flower color, fruit shape and color, and the level of spines and color of the thorns. Our hierarchical cluster analysis reveals that the Peruana roja morphotype 2 variety exhibits the best agroeconomic potential, with superior yield and bixin content, indicating its potential as a national and internationally marketable source of bixin. This variety is characterized by its lilac flowers, reddish stems, elliptical-shaped fruit, low-spinosity red fruit, and red thorns, as well as dehiscent fruit at the end of maturity. Furthermore, correlation analysis indicates that traits such as fruit height, seed width, seed height, and seed weight per fruit are associated positively with yield and bixin content, providing clear criteria for selecting superior genotypes. This association can be helpful for farmers for visual preselection under field conditions, especially in regions where laboratory tools are unavailable for bixin quantification.

These findings provide valuable information to help farmers identify, select, and use outstanding cultivars, especially those with high bixin content, thereby expanding national and international marketing opportunities. Also, this represents a valuable opportunity for selecting outstanding varieties and developing scientific breeding programs. Ultimately, our study underscores the significance of conserving and studying the genetic diversity of local annatto, thereby enhancing the crop's productivity and competitiveness in the region.

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