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Collection and morphological characterization of 149 accessions of achiote (*Bixa orellana* L.) from seven departments in Perú

Colecta y caracterización morfológica de 149 accesiones de achiote (*Bixa orellana* L.) provenientes de siete departamentos del Perú

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Abstract

The aim of this study was to characterize and identify groups of achiote (*Bixa orellana* L.) with characteristics related to increased yield and bixin content. A total of 149 achiote accessions were collected from the departments of Loreto, San Martin, Junín, Pasco, Huánuco, Ucayali, and Cusco, in Perú. These were then evaluated using ten quantitative and three qualitative capsule and seed descriptors. Undesirable characteristics such as high spinosity and dehiscence predominated, while none of the quantitative descriptors correlated significantly with bixin content. Principal component analysis indicates that the quantitative descriptors (except for bixin content) are statistically significant, forming four clusters. Notably, one of the clusters included accessions characterized by heavy seeds, and another cluster included accessions with high number of seeds and bixin content.

Keywords: *achiote, annatto, bixin, morphological characterization, Perú*

Resumen

El objetivo de este estudio fue caracterizar e identificar grupos de achiote (*Bixa orellana* L.) con características relacionadas con mayor rendimiento y contenido de bixina. Se recolectaron un total de 149 accesiones de achiote de los departamentos de Loreto, San Martín, Junín, Pasco, Huánuco, Ucayali y Cusco, en Perú. Luego se evaluaron utilizando diez descriptores de cápsulas y semillas cuantitativos y tres cualitativos. Predominaron características indeseables como alta espinosidad y dehiscencia, mientras que ninguno de los descriptores cuantitativos se correlacionó significativamente con el contenido de bixina. El análisis de componentes principales indica que los descriptores cuantitativos (excepto el contenido de bixina) son estadísticamente significativos, formando cuatro grupos. Notablemente, uno de los grupos incluye accesiones caracterizadas por semillas pesadas, y otro grupo incluye accesiones con alto número de semillas y contenido de bixina.

Palabras clave: *achiote, annatto, bixina, descriptores morfológicos, Perú.*

Introduction

Achiote (*Bixa orellana* L.), a shrubby plant standing from 3 to 10 m tall, is native to the American tropics, and bears globose to ovoid fruits with colors such as red, green, yellow, or shades of these. It has historically been used as a food, medicine, and colorant (Camacaro et al., 2018; Raddatz-Mota et al., 2017; Valério et al., 2015).

The use of achiote as a colorant in the food, textile, pharmaceutical, and cosmetic industries is due to the carotenoid compounds present in the seeds, especially the apocarotenoid bixin (Alcázar et al., 2017; Habibi-Najafi et al., 2018; Viuda-Martos et al., 2012). Bixin is the second most economically important natural colorant in the world and 60% of the world's supply comes from Latin America, mainly Perú, Brazil, and Mexico (Raddatz-Mota

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et al., 2017; Rajagopal et al., 2016; Yolmeh et al., 2015). Moreover, Perú is recognized as one of the main achiote seed exporters in the world (Stringheta et al., 2018).

According to the Peruvian Ministerio de agricultura y riego [MINAGRI] (2019), in 2018, achiote was cultivated in 11,635 ha of land. The departments with the largest areas of achiote cultivation were Cusco (6,205 ha), Pasco (4,390 ha), Huánuco (414 ha), Junín (203 ha), and Ucayali (185 ha). The national average yield was 601 kg ha^{-1}, producing 6,988 t of grain. Other countries such as Mexico and Colombia had annual yields of 1.2 and 1.13 t ha^{-1}, respectively, for the year 2017 (Servicio de Información Agroalimentaria y Pesquera [SIAP], 2019; Minagricultura, 2020).

Currently, the bixin content of achiote seeds varies from 1% to 6%, with Brazil reporting a national average of 3.5% and a maximum of 5% in the state of Sao Paulo (Albuquerque & Meireles, 2011). Brazilian varieties such as Embrapa 37 have bixin contents that exceed 5%, which is relevant because the market requires contents greater than 4% for export (Dias et al., 2017). In Perú, promising accessions with bixin contents of 3.55% to 4.55% have been reported in the national germplasm bank (Instituto Nacional de Investigación Agraria [INIA], 2009). Aside from this report, there is no official report or census on the bixin contents of Peruvian achiote, although several companies own accessions with bixin contents lower than the minimum percentage required for export in Brazil.

Despite being an important agro-industrial crop in Perú, local achiote production is characterized by low yields, with lower bixin contents compared to those of other countries. This may be partly attributed to the absence of an established variety or ecotype. Little is known about the characteristics of local achiote plants and there are also very few published studies on the characterization of Peruvian material. Such information is necessary to improve this crop genetically.

The objectives of this research are to analyze and classify, using morphological descriptors, achiote accessions from seven departments in Perú in order to identify accessions with great potential to increase yield and bixin content.

Methodology

Germplasm collection

Achiote capsules (fruits) were collected between May and November 2019. A total of 149 achiote accessions were collected from the departments of Loreto, San Martín, Junín, Pasco, Huánuco, Ucayali, and Cusco (25, 24, 25, 23, 4, 24, and 24 accessions, respectively). Each accession was provided by farmers, and was derived from plants that grew from seeds. The majority of plants were older than 3 years and less than 2.5 m tall. The location of each collection site was mapped by GPS (**Fig. 1** and mean GPS data on **Table 1**). Prior to sampling, we verified, together with the farmer that the plant was a different type. Samples consisted of two panicles collected at the stage of commercial maturity (capsules and seeds in good quality). The panicles were transported to the laboratory of the La Molina Experimental Center of the Instituto Nacional de Innovación Agraria (INIA), where the fruits were characterized.

Figure 1. Sites (red dots) where the 149 achiote accessions were collected.

Table 1. Average locations of collection points of achiote accessions by department.

Department	Number of accessions	UTM* latitude	UTM* length	Elevation (m.a.s.l.)
Loreto (L)	25	683192	18M 9563896	102
San Martín (SM)	24	275200	18M 9326970	708
Junín (J)	25	522614	18L 8780152	659
Pasco (P)	23	502876	18L 8864447	282
Huánuco (H)	4	474506	18L 8932754	288
Ucayali (U)	24	515248	18L 9039663	222
Cusco (C)	24	745212	18L 8609063	793

*Universal Transverse Mercator

The passport data for each sample were compiled in accordance with directive No. 001-2005-INIEA-DGIA-SUDIRGEB of INIA-Perú, specifically, within the "Norms that define the standardized use of formats for the documentation of the Data of Passport in the SUDIRGEB Ex Situ Germplasm Bank." (Instituto Nacional de Investigación y Extensión Agraria [INIEA], 2006). Each

Table 2. Quantitative descriptors used to characterize achiote (*Bixa orellana* L.) accessions.

Organ	Descriptor	Unit	Corresponding abbreviation used in text
Capsule	Length	cm	LC
	Width	cm	AC
	Thickness	cm	GC
	Length-width ratio	-	RLA
	Number of seeds per capsule	-	NS
	Seed weight per capsule	g	PSC
Seed	Weight of 100 seeds	g	PP100S
	Seed length	mm	LS
	Seed diameter	mm	DS
	Bixin content	g $100g^{-1}$	BIX

Table 3. Qualitative descriptors used to characterize achiote (*Bixa orellana* L.) fruits.

Descriptor	Score	Description	Corresponding abbreviation used in text
Capsule spinosity	0	No spines	EC
	1	Very low number	
	3	Low number	
	7	High number	
	9	Very high number	
Spine length	1	Very short	LE
	3	Short	
	7	Long	
	9	Very long	
Capsule dehiscence	0	Non-dehiscent	Deh
	1	Dehiscent	

of the samples was admitted into the National Achiote Germplasm Bank of the El Porvenir-San Martin Agrarian Experimental Station-INIA as new accessions.

The conditions of the plants from which samples were collected varied greatly. For example, they differed in management, age (from 3 years to older than 15 years), soil, and other characteristics. The data of yield per plant (which is linked to descriptors such as number of capsules per panicle, number of panicles per plant, among others) was not considered in our analysis. The accessions were coded with the initials of the department from where they were collected followed by a correlative number (for example, code L-020 corresponds to accession number 20 collected in Loreto).

Characterization of the fruits and seeds

The samples were cleaned, and then dried in an oven at 40°C for 2 days (Arce, 1999) prior to evaluating the morphological descriptors of the fruits. The seeds were extracted from each capsule and they were stored under controlled conditions (seeds were packed in trilaminate aluminum envelopes and placed in a sealed container without light, with a temperature of 2°C–8°C and relative humidity of 50%–60%) for a maximum of 3 days before determining the bixin content.

Quantitative (**Table 2**) and qualitative (**Table 3**) descriptors reported by Arce (1999) were used to characterize achiote fruit and seeds. These descriptors have been used previously in similar studies (Valdez-Ojeda et al., 2008). In addition, we also used other descriptors (López et al., 2018) such as capsule thickness, weight of seeds per capsule, seed length, and seed diameter.

The lengths, widths, and thicknesses of 20 randomly selected capsules were measured from each accession using a digital vernier according to the methodology of Arce (1999). Seed weight was determined with an analytical balance and the number of seeds per capsule was counted manually. The lengths and diameters of the seeds were measured using Dinocapture 2.0 (2019) software after photographing 20 randomly selected seeds from each accession using a digital microscope (Dino-Lite, New Taipei City, Taiwan). The qualitative characteristics (degree of spinosity, length of spines, and dehiscence), were scored by visual observation and compared to the graphs of Arce (1999).

Determination of bixin content

The bixin content of achiote seeds was determined using standard methods described by the Food and Agriculture Organization [FAO] (1982); and seeds were extracted using Peruvian standard methods for the extraction of bixin and norbixin, as reported in NTP 209.256: 1991 (Instituto de Investigación Tecnológica Industrial y de Normas Técnicas [INITEC], 1991).

The bixin extraction step was performed exhaustively on the seeds using chloroform until complete discoloration. The resulting extracts were further diluted with chloroform and the absorbance's of the diluted samples were measured at 470 nm using a UV-Vis spectrophotometer (Thermo Scientific, Genesys 10S). Absorbance values were converted to the equivalent g of bixin 100 g^{-1} sample, using a standard curve of known bixin (chemical purity 99.3%) concentrations (i.e., from 0.25 to 3 mg L^{-1}). Bixin concentrations were calculated using the following equation: $Y = 0.406x - 0.005$ ($r^2 = 0.9998$), where Y = bixin content in mg L^{-1}; and X = absorbance.

Table 4. Statistical values of ten quantitative descriptors used to describe 149 achiote accessions and the identities of the accessions having the minimum and maximum values.

		LC*	AC	GC	RLA	NS	PSC	PP100S	LS	DS	BIX
Population (N = 149)											
Average		4.72	2.97	2.15	1.64	39.55	1.15	2.93	4.87	3.97	2.27
s.d.		0.91	0.53	0.48	0.42	8.86	0.34	0.73	0.43	0.31	0.69
C.V.		19.29	17.78	22.38	25.76	22.39	29.86	24.98	8.73	7.87	30.54
Minimum	Value	2.54	1.75	1.15	0.86	18.60	0.50	1.16	3.43	3.23	0.895
	Accession	J-055	SM-047	J-051	U-107	U-125	U-123	U-123	U-123	L-024	J-061
Maximum	Value	7.17	4.81	3.48	2.74	69.90	2.42	5.14	6.09	4.67	3.997
	Accession	J-072	J-061	L-002	J-060	L-008	J-072	C-128	J-072	C-129	L-021

s.d.: Standard deviation; C.V.: Variability coefficient (%). *LC: Capsule length (cm); AC: Capsule width (cm); GC: Capsule thickness (cm); RLA: Capsule Length-width ratio; NS: Number of seeds per capsule; PSC: Seed weight per capsule (g); PP100S: weight of 100 seeds (g); LS: Seed length (mm); DS: Seed diameter (mm); BIX: Seed bixin content (g $100g^{-1}$)

Table 5. Frequencies of the grades of the qualitative descriptors used to characterize 149 achiote accessions.

Descriptor	Grade	Frequency	
		Number	Percentage
EC*	0	3	2.0
	1	11	7.4
	3	29	19.5
	7	60	40.3
	9	46	30.9
LE	1	15	10.1
	3	33	22.1
	7	48	32.2
	9	53	35.6
Deh	0	68	45.6
	1	81	54.4

EC: Capsule spinosity; LE: Spine length; Deh: Capsule dehiscence

Statistical analysis

Data were analyzed using SAS (2020) software. Pearson coefficients and the descriptive statistics were generated using the corr function. Principal component analysis (PCA) (only quantitative descriptors) and cluster analysis (quantitative and qualitative descriptors) were performed using RStudio (2020) software. Specifically, we implemented the prcomp function within the package factoextra (Kassambara & Mundt, 2020), and the functions daisy and hclust within the cluster package (Maechler et al., 2019), to perform PCA and cluster analysis, respectively. We determined the number of clusters using the function kgs within the package maptree (White & Gramacy, 2012).

PCA was performed by first standardizing the data using the scale function within R. For the analysis, we employed the criteria of Cliff and Kaiser as described by Franco & Hidalgo (2003). According to these criteria, the analysis should only consider the components that, when added together, contribute 70% or more of the variance and whose eigenvalues are greater than or equal to one.

In the cluster analysis, a dendrogram based on both quantitative and qualitative descriptors was constructed. The distance matrix was based on Gower distances, which is recommended for mixed data (Franco & Hidalgo, 2003). Groupings are based on Ward's hierarchical method, and the number of clusters was estimated using the Kelley-Gardner-Sutcliffe penalty function as described by Grum & Atieno (2007).

Results

Morphological variability

The means of the ten quantitative characteristics measured in the 149 achiote accessions, collected in seven departments of Perú, are shown in **Table 4**. The results indicate a moderate level of variation as evidenced by high coefficients of variability (7.87% to 30.54%), with the highest value observed for bixin content. This is reflected by the wide range of bixin contents among the accessions, with the highest and lowest values observed for accessions L-021(3.997 g 100 gr^{-1}) and J-061 (0.895 g 100 gr^{-1}).

Table 5 shows the frequencies of the grades of each qualitative descriptor. High and very high grades (quantity of spines) of capsule spinosity (EC) were observed in 71.2% of all observations. Furthermore, long and very long spines (LE) were observed in 67.8% of the accessions. Three accessions (P-081, P-082, and P-083) have no spines. The distribution of dehiscent and non-dehiscent fruits among the accessions is roughly equal (54.4% and 45.6%, respectively).

Correlation between the descriptors

The pairwise Pearson correlation coefficients between the ten quantitative descriptors are shown in **Table 6**. Most of the correlations (36) between descriptors were significant ($p < 0.05$), and only nine were not significant.

Among the morphological descriptors of the capsule, significant correlations were found between capsule length (LC) and length-width ratio (RLA) (0.726), capsule width (AC) and capsule thickness (GC) (0.637), and capsule width (AC) and RLA (−0.655). This means that capsule length is directly correlated with the length/width ratio,

Table 6. Pearson correlation coefficient matrix of the quantitative descriptors used to characterize 149 achiote accessions.

	LC*	AC	GC	RLA	NS	PSC	PP100S	LS	DS
AC	0.012	-							
GC	−0.063	**0.637*** **	-						
RLA	**0.726*** **	**−0.655*** **	**−0.482*** **	-					
NS	0.059	**0.329*** **	**0.459*** **	−0.168*	-				
PSC	**0.474*** **	0.089	0.152	**0.270*** **	**0.506*** **	-			
PP100S	**0.501*** **	−0.153	−0.191*	**0.441*** **	−0.233**	**0.702*** **	-		
LS	**0.450*** **	**−0.280*** **	**−0.291*** **	**0.509*** **	−0.135	**0.480*** **	**0.620*** **	-	
DS	**0.407*** **	−0.213**	−0.254**	**0.422*** **	**−0.323*** **	**0.350*** **	**0.644*** **	**0.634*** **	-
BIX	−0.076	0.166*	0.193*	−0.186*	0.174*	−0.037	−0.202*	−0.178*	−0.206*

*, **, *** indicates statistical significance at p-value of 0.05, 0.01, and 0.001, respectively.

*LC: Capsule length (cm); AC: Capsule width (cm); GC: Capsule thickness (cm); RLA: Capsule Length-width ratio; NS: Number of seeds per capsule; PSC: Seed weight per capsule (g); PP100S: weight of 100 seeds (g); LS: Seed length (mm); DS: Seed diameter (mm); BIX: Seed bixin content (g $100g^{-1}$)

Table 7. PCA output of the quantitative descriptors used to characterize achiote accessions.

Component	1	2	3
Eigen value	3.9099	2.2292	1.0889
Variance percent	39.099	22.292	10.889
Cumulative variance percent	39.099	61.392	72.281
Descriptors	Correlation coefficients		
LC*	**−0.667**	0.383	−0.259
AC	0.498	**0.624**	0.395
GC	0.496	**0.666**	0.167
RLA	**−0.816**	−0.150	−0.478
NS	0.283	**0.712**	−0.459
PSC	**−0.515**	**0.761**	−0.070
PP100S	**−0.804**	0.286	0.298
LS	**−0.799**	0.139	0.119
DS	**−0.760**	0.049	0.391
BIX	0.313	0.191	−0.372

*LC: Capsule length (cm); AC: Capsule width (cm); GC: Capsule thickness (cm); RLA: Capsule Length-width ratio; NS: Number of seeds per capsule; PSC: Seed weight per capsule (g); PP100S: weight of 100 seeds (g); LS: Seed length (mm); DS: Seed diameter (mm); BIX: Seed bixin content (g $100g^{-1}$)

while width is negatively correlated to capsule length. RLA and GC are also negatively correlated (−0.482).

Among the seed-related descriptors, the number of seeds per capsule (NS) is correlated (0.459 and 0.506) to GC and seed weight per capsule (PSC), respectively. Moreover, the weight of the seeds (PSC) is significantly correlated with LC (0.474) and weight of 100 seeds (PP100S) (0.702). Lastly, PP100S is positively correlated to LC (0.501). Thus, accessions with high seed weight per capsule tend to have longer capsules and heavier individual seeds.

Capsule length is correlated with seed length (LS) (0.450), RLA (0.509), PSC (0.480), and PP100S (0.620), suggesting that accession with elongated seeds tend to have elongated capsules and heavier seeds. Furthermore, seed diameter (DS) is also positively correlated with PP100S (0.644) and LS (0.634).

Bixin content (BIX) is significantly positively correlated (albeit at low coefficients) to AC, GC, and NS and negative correlated to RLA, PP100S, LS, and DS. These results indicate that, among accessions, bixin content tends to be higher with the more oval-shaped fruits and with fruits with high number of seeds (NS) per capsule.

Principal component analysis

The first three principal components (PC) contribute a total of 72.3% of the variance; and their corresponding eigenvalues are all greater than 1, as shown in **Table 7**.

Along the axis of Component 1, (which contributes 39.1% to the variance), the descriptors RLA, PP100S, LC, LS, DS stand out, followed by PSC, AC, and GC. NS and BIX are less relevant. Component 2 contributes 22.3% of the morphological variance, and along this axis, the descriptors NS, PSC, AC, and GC stand out. Component 3 contributes 10.9% of the variance, and along this axis, the descriptors RLA, NS, and AC stand out. The rest of the descriptors have low discriminatory power.

A graph of the first two PCs (**Fig. 2**) shows a somewhat positive correlation between NS, GC, and AC; these descriptors are correlated to a lesser extent with BIX. Conversely, the fruit descriptors RLA, LC, PSC, PP100S are more or less correlated to seed descriptors LS and DS due to the narrow angle between them. GC and AC are most closely related, as indicated by the smallest angle between the descriptors in this graphic. Whereas, BIX and RLA are furthest related, as indicated by the near horizontally opposite angle. Overall, the results show that

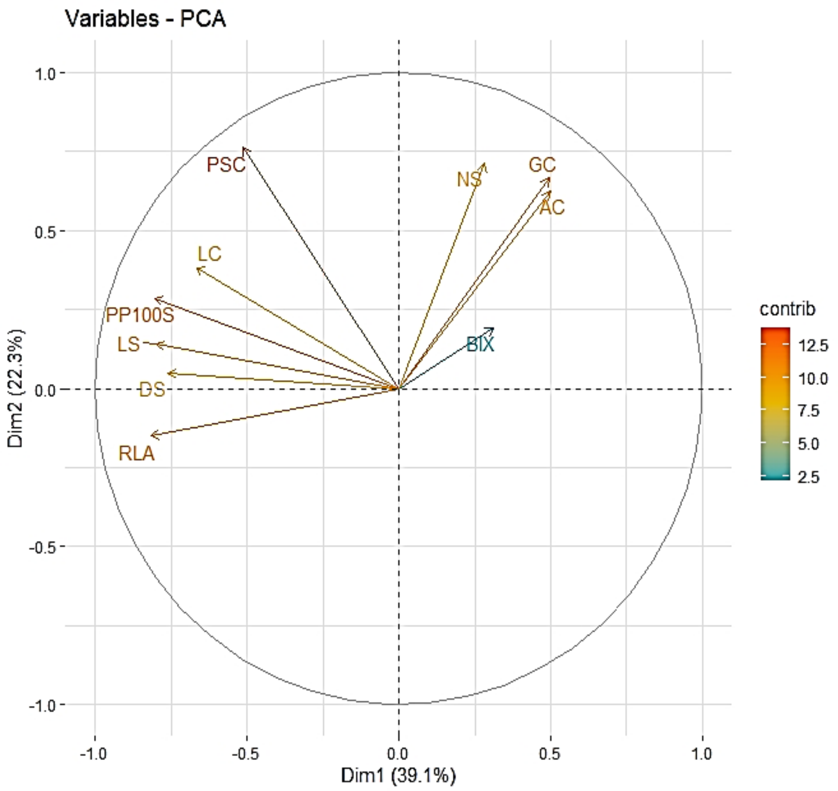

Figure 2. Contributions of the achiote descriptors* as determined by the two principal component axes.

*LC: Capsule length (cm); AC: Capsule width (cm); GC: Capsule thickness (cm); RLA: Capsule Length-width ratio; NS: Number of seeds per capsule; PSC: Seed weight per capsule (g); PP100S: weight of 100 seeds (g); LS: Seed length (mm); DS: Seed diameter (mm); BIX: Seed bixin content (g $100g^{-1}$)

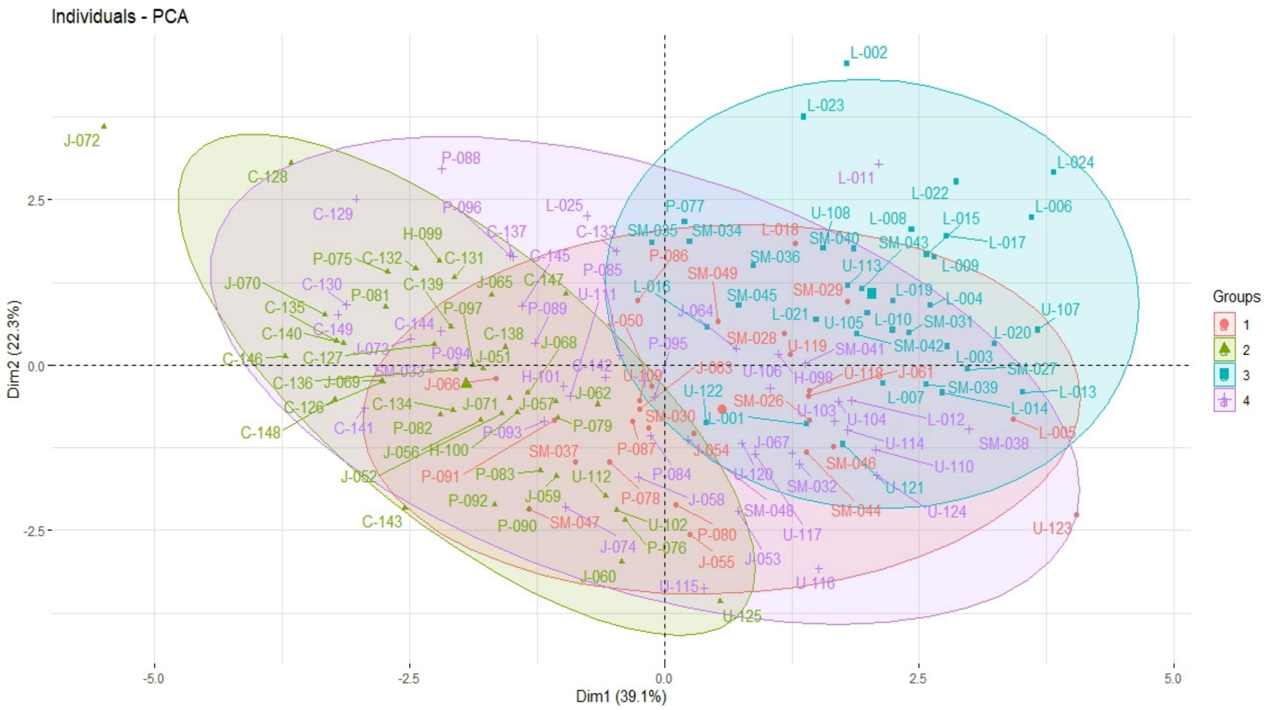

Figure 3. Distribution of the 149 achiote accessions along the two principal components. The different clusters (in graphic named groups) are indicated by a color and symbol (Cluster 1: red dot, Cluster 2: green triangle, Cluster 3: blue square, and Cluster 4: purple plus sign).

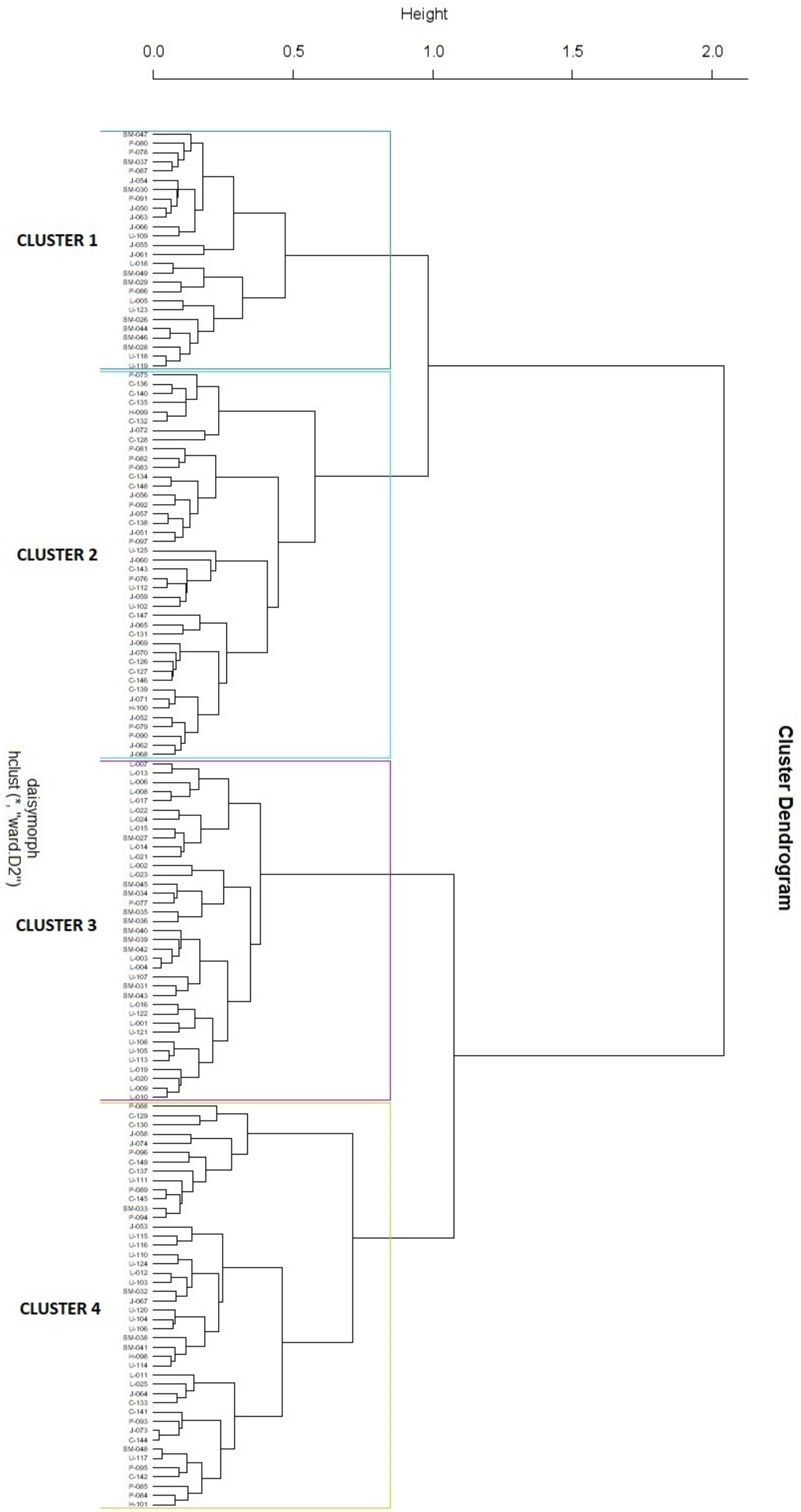

Figure 4. Dendrogram of 149 achiote accessions generated using cluster analysis by processing 13 quantitative and qualitative morphological descriptors.

accessions with higher bixin contents tend to have wide or rounded capsules and high numbers of seeds.

The PCA plot of all the accessions (**Fig. 3**) was constructed based on the first two components. While the plot shows no clear clusters, we nevertheless identify the clusters that were generated by cluster analysis.

Cluster analysis

Four clusters were identified at a Gower distance of 0.75 (**Fig. 4**). The features of each cluster are summarized below.

Cluster 1 contains 26 accessions from Loreto, San Martín, Junín, Pasco, and Ucayali departments. This group is characterized by the absence of capsule dehiscence and high spinosity (EC) and long spines (LE). The values for all descriptors (including BIX) were generally average, except for NS and LC, which were less than average.

Cluster 2 contains 42 accessions from Junín, Pasco, Huánuco, Ucayali, and Cusco departments. This group is characterized by non-dehiscent capsules, low degree of capsule spinosity, and short spines. In addition, the accessions in this cluster have the lowest AC, GC, and BIX values, while having the highest LC, RLA, PSC, PP100S, LS, and DS values. Furthermore, the highest seed weights were observed in this cluster, which suggests that it has good yield potential, however, such yields will have low bixin contents.

Cluster 3 is made up of 37 accessions from Loreto, San Martín, and Ucayali departments. It is characterized by dehiscent capsules with a very high degree of spinosity and very long spines. On average, this cluster has highest BIX, NS, AC, and GC values, while its RLA, PSC, PP100S, LS, and DS values are the lowest. Moreover, accessions in this cluster tend to have higher BIX values.

Cluster 4 is made up of 44 accessions from Loreto, San Martin, Junín, Pasco, Huánuco, Ucayali, and Cusco. It is characterized by dehiscent capsules with high degree of spinosity and long spines. Most of the descriptors (including BIX) tend to have average values, except for PSC values, which tend to be lower than the general average.

Figure 4 shows 2 major groups, which differ mainly by the non-dehiscent capsules of one group (which include clusters 1 and 2) and the dehiscent capsules of the other group (which contain clusters 3 and 4).

Discussion

The ranges of values observed for LC (7.17 to 2.54 cm), AC (4.81 to 1.75 cm) and GC (3.48 to 1.15 cm) (**Table 4**) agree to some extent with those obtained from India, Perú, and Venezuela by Akshatha et al. (2011), López et al. (2018), and Mazzani et al. (2000), respectively. These authors reported capsule dimensions ranging from 5.7 to 3.0 cm long and from 4.8 to 2.0 cm wide. We found RLA values ranging from 0.86 to 2.74 (average 1.64), which are higher than those (0.75 to 1.5) reported by Valdez-Ojeda et al. (2008) for Mexican accessions. RLA values can also be used to classify capsules as oval, elongated, or spherical (Akshatha et al., 2011). Our findings indicate that our accessions are predominantly elongated.

The NS values of our accessions range from 18.6 to 69.9 seeds per fruit, which are higher than those (30 to 60 seeds per fruit) reported by other authors (Akshatha et al., 2011; Moreira et al., 2015; Rivera-Madrid et al., 2006). The average PSC of our accessions (1.15 g) is lower than that reported by López et al. (2018) (1.55 ± 0.29 g).

The PP100S values of our accessions (5.14 to 1.16 g) are generally higher than the reference values of 3.8 to 1.10 g reported previously (Akshatha et al., 2011; Mantovani et al., 2013).

The LS (6.09 to 3.43 mm) and DS (4.67 to 3.23 mm) values of our accessions are slightly higher than the previously reported length (0.5 ± 0.02 cm) and width (0.4 ± 0.02 cm) of seeds in Perú (López et al., 2018). Comparing our results to previous studies, the extreme values we found for various descriptors suggest that the achiote populations are diverging in terms of capsule and seed characteristics.

Bixin contents in our accessions range from 0.895 (J-061) to 3.997 g 100 g^{-1} (L-021), which is consistent with previously reported values (0.49 to 2.65 g 100 g^{-1}) generated by similar extraction methods (Akshatha et al., 2011; Rivera-Madrid et al., 2006; Valdez-Ojeda et al., 2008; Viuda-Martos et al., 2012). Other authors, using exhaustive methods such as supercritical fluid extraction, have obtained higher BIX values, i.e., from 4.90 to 7.58 g 100 g^{-1} (Albuquerque & Meireles, 2012; Rodrigues et al., 2014). Meanwhile, extractions using alkaline solvents result in BIX contents ranging from 1.66 to 5.05 g 100 g^{-1} (Dias et al., 2017; Mantovani et al., 2013). In both the cases of exhaustive methods (super critical fluid extraction and alkaline solvents), Brazilian achiote seeds were used. We suggest that commonly used standard methods of bixin extraction often underestimate the bixin content of the accessions. Therefore, in the future, we intend to use exhaustive methods to better extract pure bixin. In our study, only two accessions have bixin contents that are close to the 4% bixin requirement for export; L-021 and L-024 produce achiote seeds with 3.997% and 3.934% bixin, respectively.

Bixin is degraded by light, high temperatures (Arce, 1999), and high environmental humidity (Biego et al., 2013). Dehiscent fruits tend to expose seeds to such unfavorable environmental conditions, generating low-quality seed (Medina et al., 2001). Furthermore, capsules with little or no spines are preferable because they make the fruit harvesting easy. The frequencies of these desirable characteristics (low spinosity and no dehiscence) in our accessions were low to medium (**Table 5**), indicating that selection may be an effective genetic improvement tool.

The low Pearson coefficients (**Table 6**) indicate little correlation between the descriptors, especially for BIX. This may be related to the differences in location, climate, age, and management among the accessions. Most likely, however, this is because the accessions are biologically independent from each other. Therefore, future characterization studies of achiote should focus on accessions of the same age, planted in the same plot, and managed under controlled conditions.

The PCA results (**Table 7**) indicate that most of the descriptors are relevant, that is, they are indeed important in describing the accessions. The exception is BIX, which is of little relevance in discriminating between accessions. This agrees with the Pearson correlation analysis and can be explained by the fact that BIX is unrelated to other traits studied. Analyzing achiote accessions using PCA and morphological descriptors such as those evaluated in the present study has been done previously (Mazzani et al., 2000). These authors obtained three main components that explained 83.8% of the variance, however, they did not evaluate bixin contents.

The results of correlation analysis and PCA are somewhat concordant with the clusters generated by cluster analysis (both graphically represented in **Fig. 3**). For example, Cluster 3 includes accessions with high values of NS, AC, and BIX. in Clusters 2 and 3 include accessions with high values in descriptors related to yield (PP100S and PSC) and quality (NS and BIX), respectively. Therefore, these clusters may identify potential candidate accessions for subsequent genetic improvement work. The results of cluster analysis show that the departmental origin of the accessions is not related to their clustering, which indicates that the achiote plants in each department are highly diverse, based on the descriptors used in this study.

Future work should include establishing the progeny of the accessions used in this study in a germplasm bank to monitor their morphological descriptors in order to confirm the stabilities of these characteristics and the clusters obtained in this study through time. We have also identified accessions with potential for genetically improving this crop; these accessions with the highest bixin contents (3.997 to 3.761 g 100 g^{-1} bixin, top 5th percentile) are L-021, L-024, C-130, L-27, and L-22.

Conclusion

We characterized 149 achiote (*Bixa orellana* L.) accessions from the departments of Loreto, San Martin, Junín, Pasco, Huánuco, Ucayali, and Cusco. Various fruit and seed traits are correlated. A few accessions have high values for characteristics related to crop yield and bixin content such accessions may have potentially important roles to play both commercially and as materials for future achiote breeding programs.

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