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Intrapopulation phenotypic variation in Piartal (*Chenopodium quinoa* Willd.) from the Department of Boyacá, Colombia

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***Chenopodium quinoa* Willd., is an interesting plant with a great adaptation to adverse environmental factors and exceptional nutritional qualities. It shows great genetic variation, which organization remains poorly documented. In Boyacá, there are few studies on the morphological characterization of cultivated materials, and there is no certified planting material, resulting that the farmers are planting a mixture of materials. Qualitative and quantitative descriptors and principal component and cluster analyses were used to characterize the structure of the intra-population phenotypic variation in Piartal quinoa materials grown in the Department of Boyacá. It was observed that the first two components, CP1 and CP2, explained more than 70% of the total observed phenotypic variation, and there was a significant contribution from all variables to the two components, except those related to the lower leaves, where P2, P3 and P4 presented defoliation and DP (CP2 and P6). The cluster analysis showed that the individuals of the Piartal were grouped mainly by morphological characteristics associated with plant height, panicle length, pigmented axillae, and leaf characteristics. Results showed that the variance in morpho-phenological traits was concentrated at the intra-population, due the high variation at the inter-individual level. A more efficient selection process should be carried out to find "pure" varieties.**

Key words: Andean culture, genetic diversity, improvement, morphological descriptors.

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is an annual, dicotyledonous species that belongs to the Chenopodiaceae family, cultivated from sea level to

4,000 m, with wide agroecological adaptation and to different types of soils. Native to South America, it continues to be cultivated in different regions of that

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continent, especially in countries such as Colombia, Chile, Bolivia, Ecuador and Peru, being recently introduced in Europe, North America, Asia and Africa (Zurita et al., 2014). It is estimated that more than 80% of its world production is concentrated in countries such as Peru, Bolivia and Ecuador (Zurita et al., 2014). It also occurs in the coastal areas of southern Chile and the Andean valleys of southern Colombia, more precisely in the department of Nariño, Cauca, Boyacá and Cundinamarca (Agronet, 2020), where it has currently had a great boost due to its agronomic potential and different benefits derive from the production, industrialization and commercialization of its products (Chura et al., 2019). It is considered a cereal with excellent nutritional properties, among which its high protein content stands out, since it has all the amino acids, trace elements and significant amounts of vitamins C, E (tocopherols) and B (B1, B2 and B3); along with important minerals (Ca, K, Fe, Mg, Mn and P), and isoflavones that can contribute to its antioxidant properties. Quinoa is gluten-free and its high-quality fatty acids (Pereira et al., 2019). The seed coat contains saponins, previously considered an antinutrient due to its bitter taste, currently it is extracted for industrial and biomedical purposes (El Hazzam et al., 2020). It has a high adaptability, due to its domestication process and high genetic variability. It is a species that can tolerate different types of stress such as salinity, cold, high solar radiation, freezing night temperatures and phytosanitary tolerance factors (Ebrahim et al., 2018; Hinojosa et al., 2018; Ali et al., 2019). Due to its economic potential and because it represents a food security crop for Andean communities, in the last decade its production has been encouraged among farmers, agro-industrial companies and institutions (Rojas et al., 2015).

Morphological characterization, including SEM study on seed coat ultrastructure, helped in the taxonomic delineation while the size and morphology of fruit surface were found diagnostic for segregating the wild and cultivated species and influence the genetic variability (Mishra et al., 2017). The genetic bases of several quinoa traits was identified several decades ago, but the first true genetic descriptions more recently provided the starting point for improvement of quinoa. Several genetic tools have been developed, and today morphological and molecular markers are an effective way to enhance breeding efficiency (Ruíz et al., 2014). Quinoa is one of the Andean crops with little research in the area of genetics and plant breeding, although, it has a high variability in characteristics such as plant color, flowers, nutritional contents and metabolites of interest (Bazile et al., 2014). Collecting, conservation and characterization studies are necessary for the development of strategies to improve of this species.

In Colombia, quinoa, in the last decade it has had an important recognition by government organizations at the national and international levels, promoting actions aimed

to replant and shaping its supply chain (Delgado et al., 2009). It is a small-scale crop, where the indiscriminate combination of varieties, together with a low level of technology, reduces their quality and profitability (Morillo et al., 2017). Due to the lack of cultures with a single variety, technological problems arise such as heterogeneity in morphological characteristics and maturation times of individuals (Delatorre et al., 2013). Although preferably autogamous, quinoa shows notable inter- and intra-population genetic variation, easily observable in rural plots, and quantifiable by molecular markers (Del Castillo and Winkel, 2014). For morphological markers, global studies on quinoa diversity have shown variability in the phenotypic characteristics of the evaluated germplasm (Chura et al., 2019; Maliro and Njala, 2019).

In this country, there have been morphoagronomic characterization studies on quinoa materials cultivated on the Bogotá savanna and in Nariño (Veloza et al., 2016; García et al., 2018). In Boyacá, Infante et al. (2018) carried out a morphological characterization of quinoa varieties grown in that department; Morillo et al. (2020) evaluated 19 quinoa materials in the Department of Boyacá with 27 morphological descriptors. The results of these studies showed that the evaluated materials present great variability in both qualitative and quantitative characteristics, which can be used for the selection of materials.

None of these studies attempted to explain the distribution of genetic variation between the different levels of organization of the species, especially in commercial materials like Piartal. The objective of this research was to determine the intrapopulation phenotypic variation in the quinoa materials Piartal in the Department of Boyacá in order to analyze the structure of the variation of the morphological markers and, thus, be able to establish strategies for obtaining "pure" planting materials with high yield and adaptation to local conditions, that respond to the needs of producer and consumer.

MATERIALS AND METHODS

Location

This study was carried out in the Department of Boyacá in the municipalities: Sichoque, Sogamoso, Monguí, Tunja y Combita on farms where cultivation was already established, and different morphotypes were selected, with a total of 27 samples Piartal materials (Table 1).

Morphoagronomic characterization

For the morphoagronomic characterization, a completely randomized stratified simple sampling was used, which consisted of identifying the plants in the field that showed phenotypic differences in characteristics such as panicle color, presence of pigmented axillae and colored striae (morphotypes); the number of repetitions

Table 1. Geographical location of Piartal collection sites.

Municipality	Coordinates	Population (N)
Siachoque	5°30'0.6"N 73°29'52.6"W	P1 (3)
Sogamoso	5° 40'41" N 72° 56' 38"	P2 (6)
Monguí	5°43'21"N 72°50'57"W	P3 (6)
Tunja	5°33'16"N 73°21'09"W	P4 (6)
Combita	5°38'02"N 73°19'23"W	P5 (6)

Table 2. Morphological descriptors used for the morphological characterization of Piartal materials.

Qualitative	Quantitative
Panicle color at physiological maturity	Panicle length (LP) (cm)
Panicle shape	Panicle diameter (DP) (cm)
Stem color	Plant height (AP) (cm)
Upper and lower leaf color	Number of teeth upper leaf (DHS)
Upper and lower leaf shape	Number of teeth lower leaf (DHI)
Upper and lower leaf edge	Main stem diameter (DT)
Presence of teeth on upper and lower leaves	Upper leaf length (LHS) and width (AHS)
Presence of pigmented axillae	Length (LHI) and width of lower leaves (AHI)
Axilla color	
Plant size (growth habit)	
Presence of stria marks	
Stria color	
Stem shape	

depended on the presence of these characteristics in the culture. The morphological characteristics proposed by Bioversity International were evaluated *in situ* at physiological maturity (Morillo et al., 2020) (Table 2).

Data analysis

A multivariate analysis was carried out with the data obtained from the morphoagronomic characterization using the statistical programs NTSYSpC® and InfoStat. A principal component analysis was used with a correlation matrix between the characteristics, performing a linear transformation of the original data, which generated a new set of independent variables. With the NTSYSpC® statistical package, a hierarchical cluster analysis was performed using the mean taxonomic distance matrix between the qualitative and quantitative characteristics and the hierarchical grouping algorithm (UPGMA), for which the squared Euclidean distance and the full link algorithm were applied (Figure 1).

RESULTS AND DISCUSSION

Regarding the evaluation of the qualitative characteristics of the Piartal quinoa material in the populations P1 (Siachoque), P2 (Sogamoso), P3 (Monguí), P4 (Tunja) and P5 (Cómbita), it was found that the most variable characteristic was panicle color, where P2, P3 and P5 showed a 1:1 segregation between green and purple, all

P1 individuals exhibited a purple panicle color and P4 had a green color. The shape of the panicle for P1 was 100% glomerulated, in P5 intermediate and amarantiform and simple for the rest of the populations evaluated (P2, P3 and P4). It was observed that the edge of the leaf was serrated and with teeth for the populations P2, P4 and P5, and 100% serrated for P1 and 50% whole and 50% serrated for P3. For the characteristic presence of pigmentation in the axillae, it was found that the individuals in the population P1 had no axillae, P5 did not have pigmented axillae, P2 was 50% purple color and 50% no axillae, and P4 had 50% purple and 50% pigment free. The stem color was green for P1, P4 and P5 and yellow for P2 and P3. The color of the upper and lower leaves in P1 and P4 was green. In populations P2, P3 and P5, they showed defoliation of the lower leaves during the evaluation and a green-yellow color in the upper leaves. It was observed that most of the Piartal individuals had a rhomboidal and triangular leaf shape, presence of teeth on upper and lower leaves, angled stem, simple growth habit, and green striations, except for P5, which present red striae.

The analysis of the qualitative characteristics showed that the variable of the panicle was the most diverse since, within the populations (Table 3), there was segregation between green and purple. Similar results

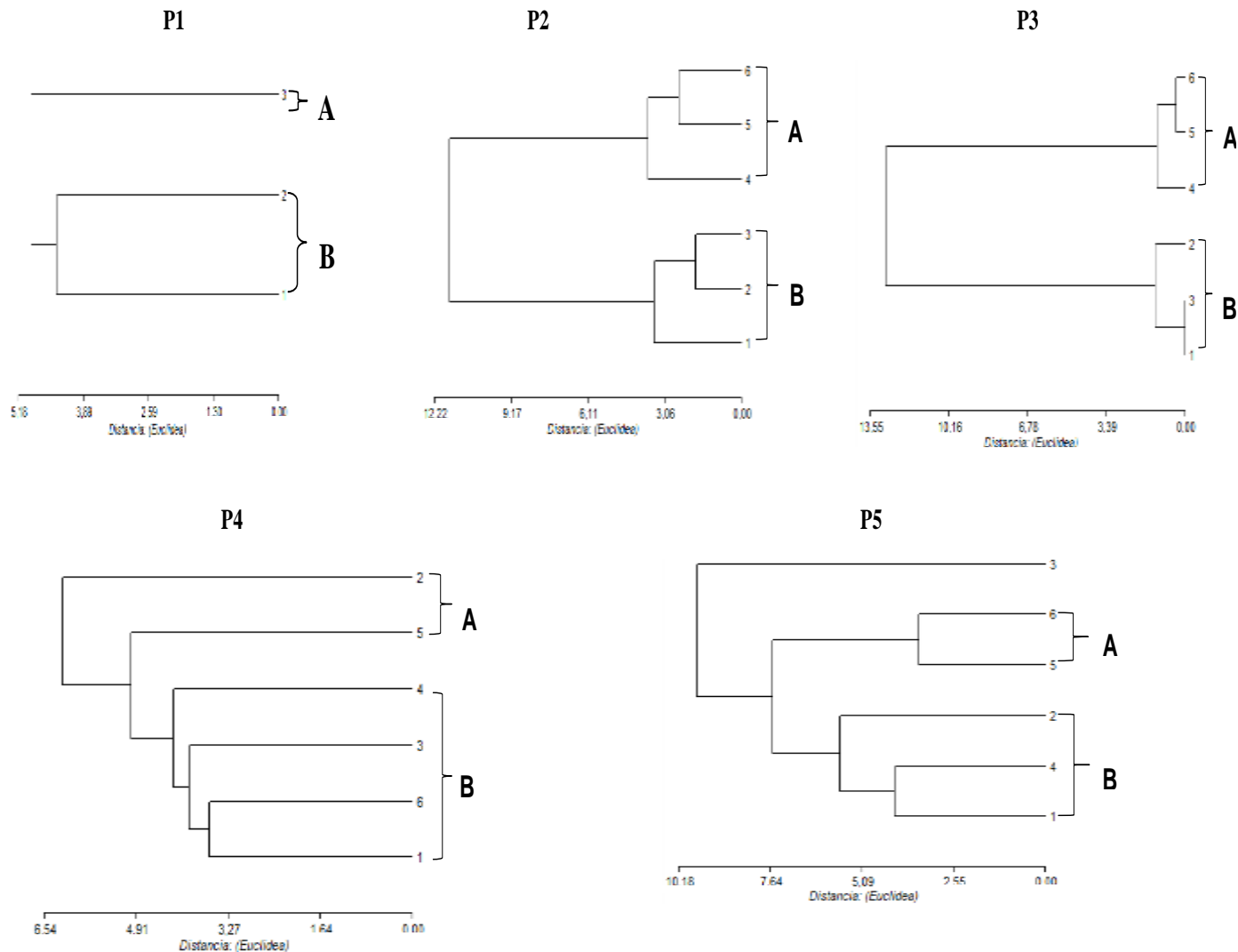


Figure 1. Dendrogram of the hierarchical classification analysis for the Piartal material.

were obtained by Morillo et al. (2020), when evaluating 19 quinoa materials in the Department of Boyacá. Alanoca and Machaca (2017) reported that the expression of this characteristic is greatly affected by the morphological changes that quinoa shows during its maturation. In general, the color characteristic, both in stems and in other structures, also presented variation between and within the evaluated populations, contrary to the results obtained by Infante et al. (2018), who described Piartal as a material with more stable morphological characteristics. However, other germplasm evaluation studies have also shown segregation in color and in other qualitative characteristics in quinoa (Del Castillo and Winkel, 2014; Alanoca and Machaca, 2017; Afiah et al., 2018; Al-Naggar et al., 2018; Morillo et al., 2020). The color characteristic in different plant structures was variable, and this variation is subject to the phonological stage of the crop, as reported in other

studies (Noulas et al., 2017; De Santis et al., 2018).

In the evaluation of the quantitative characteristics of the Piartal quinoa material in the five populations, according to the cv, the more variable characteristics were AHS (14.62-112.9%, P3, P2, respectively), DHS (23.53 - 110.4% for P1 and P3, respectively), DP (33.52%, P2), LP (24.25% -31.23%, P4 and P2, respectively), DHI (28.27%, P4), AHI (26.62%, P5), LHI (22.54%, P1) and AP (22.26%, P1) (Table 3). In the principal component analysis, it was observed that the first two components, CP1 and CP2, explained more than 70% of the total observed phenotypic variation, and there was a significant contribution from all variables to the two components, except those related to the lower leaves, where P2, P3 and P4 presented defoliation and DP (CP2, P4) (Table 3).

The AP and DT characteristics were between 16.5-177 and 1.33-435 cm, respectively, similar to that found by

Table 3. Descriptive statistics of the quantitative morphological variables and main components for the Piartal materials.

Variable	N	Average	S.D.	CV (%)	Range	CP1	CP2
P1							
AP	3	77.33	17.21	22.26	32	0.95	-0.31
DT	3	3.97	0.25	6.34	0.5	-1	0.05
LP	3	27.92	1.47	5.27	2.95	0.98	0.19
DP	3	24.44	4.46	18.25	8.91	1	0.03
DHS	3	15.53	3.66	23.53	7.3	0.4	0.92
DHI	3	23.30	4	17.17	8	0.45	0.89
LHI	3	4.47	1	22.54	2	-0.64	0.77
AHI	3	4	0.47	11.72	0.9	-0.97	0.25
LHS	3	7.27	0.40	5.56	0.7	0.83	0.55
AHS	3	5.37	0.76	14.23	1.5	-0.38	0.92
P2							
AP	6	166.50	20.42	12.27	55	-0.68	0.71
DT	6	4.15	0.72	17.29	1.7	-0.68	0.66
LP	6	29.27	9.14	31.23	27	-0.77	0.09
DP	6	28.98	9.72	33.52	27	-0.81	0.35
DHS	6	7.33	8	110	16	0.85	0.48
LHS	6	2	2.38	114	5	0.87	0.49
AHS	6	1.33	1.51	112.9	3	0.85	0.52
P3							
AP	6	160	21.84	13.65	60	0.89	0.21
DT	6	4.28	0.79	18.41	2.1	0.95	0.23
LP	6	28.13	3.85	13.70	9.7	0.13	0.98
DP	6	24.28	3	12.36	8.4	-0.29	0.95
DHS	6	9	9.94	110.44	20	0.96	-0.27
LHS	6	2.75	0.52	1.9	1.5	0.95	0.19
AHS	6	1.77	0.26	14.62	0.5	0.98	-0.19
P4							
AP	6	132	30.38	23	90	0.83	-0.35
DT	6	7	0.92	13	2.5	-0.66	0.47
LP	6	31.18	3.60	11.53	10	0.96	0.16
DP	6	30.55	5	16.41	13.6	-0.16	0.09
DHS	6	16	3.58	22.36	10	0.71	0.69
LHS	6	3.6	0.53	14.80	1.3	0.82	0.39
AHS	6	2.58	0.70	26.98	1.5	0.78	-0.32
P5							
AP	6	177.33	29.87	16.84	90	-0.75	0.28
DT	6	4.5	0.81	17.94	2.5	-0.77	0.34
LP	6	26.35	6.48	24.58	17.9	0.18	-0.76
DP	6	20	1.54	7.68	4.3	0.75	-0.22
DHS	6	10.33	6.38	61.71	18	0.21	0.72
DHI	6	11	6.16	56	16	0.76	0.44
LHI	6	5.55	1	18.68	2.5	0.87	0.42
AHI	6	4.78	1.27	26.62	2.8	0.90	0.31
LHS	6	5.95	1	16.73	2.2	-0.21	0.95
AHS	6	4.87	1	21.54	2.2	-0.16	0.98

Morillo et al. (2020). On the contrary, Infante et al. (2018) reported an AP of 135 to 170 cm and DT of 3 to 4 cm. These studies show that the phenotypic behavior of quinoa does not have particularly extreme values for any of the evaluated variables and that the variability of the morphological characters results from environmental factors that can be controlled by genes with pleiotropic or epistatic effects (Farooq et al., 2018). The values found in the other quantitative variables were similar to those reported in other genetic diversity studies on quinoa (Afiah et al., 2018; Ebrahim et al., 2018; Infante et al., 2018; Morillo et al., 2020).

The analysis of correlations showed that the highest value was obtained with the variable AP, which, in P1, had a perfect and negative correlation with AHI ($r = -1$), a negative correlation with DT ($r = -0.97$) and a positive, significant correlation with DP ($r = 0.94$). DP was negatively and significantly correlated with AHI ($r = -0.96$) and positively correlated with LP ($r = 0.99$). LP had negative correlation values with AHI ($r = -0.91$), and AHI was positively correlated with DT ($r = 0.98$) (Table 4). In P2, positive and significant correlations were found for DHS and AHS ($r = 0.98$), DHS and LHS ($r = 0.97$), AP and DT ($r = 0.87$), AP and DP ($r = 0.80$) and DP and DT ($r = 0.74$). P3 had the highest number of positive and significant correlations between the evaluated variables; the highest was AHS with DHS ($r = 0.98$). In P4, significant and positive correlation values were obtained for AP and LP ($r = 0.82$), LHS and DHS (0.82), DHS and LP ($r = 0.79$) and LHS and LP (0.78), and negative values were obtained for DT and AP ($r = -0.74$). In P5, the positive and more significant associations were for AHS and LHS ($r = 0.99$), AHI and LHI (0.99), AP and DT ($r = 0.91$), and DHI and DHS ($r = 0.78$), and there were significant negative associations for LHS and LP ($r = 0.81$) and AHS and LP ($r = 0.81$) (Table 4).

The results showed a significant correlation between AP and DT for all populations, as also been observed by Spehar and Santos (2005) when evaluating the agronomic performance of selected quinoa and by Madrid et al. (2018) in the evaluation of morphological characteristics related to the improvement of yield in quinoa. LP and DP were positively associated with AP in P1, P2 and P4, indicating that individuals with a greater height develop larger panicles, as found by Alanoca and Machaca (2017), Farooq et al. (2018) and Morillo et al. (2020).

Based on the correlations of all the variables and the evaluated populations, the phenotype of the material was highly influenced by the environment. Correlation studies are an important step in quinoa improvement programs since the information that is obtained is useful for estimating the correlated response to selection for the formulation of selection indices (Afiah et al., 2018; Al-Naggar et al., 2018; Ebrahim et al., 2018).

The cluster analysis showed that the individuals of the Piartal quinoa material were grouped mainly by

morphological characteristics associated with plant height, panicle length, pigmented axillae, and leaf characteristics, as reported by Morillo et al. (2020); the clusters showed a lax distribution of the materials with an association of the characteristics presence or absence of striae, growth habit, color, shape, length and diameter of the panicle, seed/plant yield and weight of 1000 grains, results that were consistent with morphological characterization studies on quinoa (Curti et al., 2014, Infante et al., 2018, Farooq et al., 2018).

In this study, as in study by Morillo et al. (2020), no groupings were observed according to the site of origin of the materials, as observed when evaluating the intra and inter-population phenotypic variation in seven quinoa populations from the Bolivian altiplano, in which the morpho-phenological markers separated the quinoa from the most limiting sectors for agriculture (southern plateau and cold zones of the northern plateau) from quinoa cultivated in more temperate zones (Del Castillo and Winkel, 2014). These results are consistent with that reported by Farooq et al. (2018) where all quinoa accessions showed good growth in subtropical and semi-arid climatic conditions in Pakistan. In addition, the studies carried out by Noulas et al. (2017) demonstrated not only the wide adaptation of quinoa materials to the agroclimatological conditions of Greece but also the variation of quinoa phenotypic characteristics according to the environment.

The morphoagronomic characterization of the Piartal quinoa materials in the five evaluated municipalities showed high intrapopulation phenotypic variability that depended on the agroclimatological conditions of each site (Infante et al., 2018; Morillo et al., 2020), mainly as the result of the fact that quinoa is a rustic crop with broad agroecological adaptation that can tolerate different types of stress and that is a food security crop for the Andean community since with farmers who have maintained and selected seeds for generations (Alvarez et al., 2018). However, the presence of morphotypes in quinoa crops is not a desirable condition since it means that there are still no pure materials or local varieties but only materials in the process of domestication, which is a limitation for the implementation of cultivation technologies. For example, populations can have differences in the maturity stage of the plants, which can complicate uniform agronomic management. In addition, the size and color of the seeds are different between materials and within each material, which prevents the development of machinery for threshing processes as has been done for cereals with uniform grain sizes and diameters.

Conclusion

The morphoagronomic evaluation of the intraspecific variation of the Piartal quinoa material in the Department

Table 4. Pearson correlation analysis for the quantitative variables ($P \geq 0.001$) in the Piartal material.

P1										
	AP	DT	LP	DP	DHS	DHI	LHI	AHI	LHS	AHS
AP	1									
DT	-0.97	1								
LP	0.87	-0.97	1							
DP	0.94	-1	0.99	1						
DHS	0.09	-0.35	0.56	0.42	1					
DHI	0.15	-0.40	0.61	0.47	1	1				
LHI	-0.85	0.68	-0.49	-0.62	0.45	0.40	1			
AHI	-1	0.98	-0.91	-0.96	-0.16	-0.21	0.81	1		
LHS	0.62	-0.80	0.92	0.85	0.84	0.87	-0.11	-0.67	1	
AHS	-0.65	0.43	-0.20	-0.36	0.70	0.65	0.95	0.60	0.19	1

P2							
	AP	DT	LP	DP	DHS	LHS	AHS
AP	1						
DT	0.87	1					
LP	0.61	0.54	1				
DP	0.80	0.74	0.40	1			
DHS	-0.21	-0.32	-0.54	-0.53	1		
LHS	-0.25	-0.26	-0.62	-0.55	0.97	1	
AHS	-0.21	-0.22	-0.57	-0.54	0.98	1	1

P3							
	AP	DT	LP	DP	DHS	LHS	AHS
AP	1						
DT	0.84	1					
LP	0.33	0.33	1				
DP	-0.06	-0.04	0.88	1			
DHS	0.80	0.82	-0.12	-0.54	1		
LHS	0.79	0.96	0.32	-0.09	0.86	1	
AHS	0.84	0.89	-0.06	-0.46	0.98	0.89	1

P4								
	AP	DT	LP	DP	DHS	DHI	LHS	AHS
AP	1							
DT	-0.74	1						
LP	0.82	-0.60	1					
DP	0.21	0.33	-0.04	1				
DHS	0.32	-0.24	0.79	-0.17	1			
LHS	0.41	-0.18	0.78	-0.26	0.82	0.21	1	
AHS	0.70	-0.37	0.64	-0.06	0.26	-0.47	0.70	1

P5										
	AP	DT	LP	DP	DHS	DHI	LHI	AHI	LHS	AHS
AP	1									
DT	0.01	1								
LP	-0.36	0.44	1							
DP	0.04	-0.03	0.17	1						
DHS	0.46	-0.39	-0.87	0.12	1					
DHI	-0.02	-0.07	-0.09	0.58	-0.01	1				
LHI	0.05	0.50	-0.23	-0.53	-0.11	-0.13	1			

Table 4. Contd.

AHI	-0.17	0.49	0.10	-0.59	-0.37	-0.43	0.88	1		
LHS	0.10	0.52	-0.37	-0.51	0.33	-0.37	0.60	0.44	1	
AHS	0.20	0.34	-0.35	-0.73	0.29	-0.66	0.54	0.49	0.91	1

Values in bold are not significant

of Boyacá showed a wide segregation of the phenotypic characteristics especially related to the color of different plant structures. This variation that occurs at the inter-individual level in farmers' fields, in the materials that they plant and select cycle after cycle is not desirable, since it reduces the quality and profitability of the crop, and also suggests selection and purification processes efficient that lead to obtaining "pure" materials with better yields and adapted to local conditions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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