Phenotypic intrapopulation variation in quinoa from the department of Boyacá, Colombia

Variación fenotípica intrapoblacional en quinua en el departamento de Boyacá, Colombia

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ABSTRACT

Quinoa (Chenopodium quinoa Wild.) is an Andean crop that originated from the Andes of South America, with great agronomic, industrial, pharmaceutical potential and also a great capacity to tolerate adverse environmental factors. In Colombia, more accurately in the Department of Nariño, Cauca, Cundinamarca and Boyacá. Shows great genetic variation, both molecular and morphological, 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, with farmers 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 Blanca de Jericó quinoa materials grown in the Department of Boyacá. The principal component analysis explained more than 70 % of the observed variation, with the AP, LP, DP, LHS, and AHS characteristics being more variable. The cluster analysis showed grouping by characteristics, such as AP, panicle color, and the presence of pigmented axillae. Results show that the variance in morpho-phenological traits was concentrated at the intra-population, due to high variation at the inter-individual level. A more efficient selection process should be carried out to find materials or “pure” varieties with higher yields, resistance to biotic and abiotic factors, and adaptation to local conditions, which make quinoa an economically profitable crop in the Boyacá department.

Keywords: Andean culture, Genetic diversity, Plant breeding, Morphological characteristics; Pseudocereal.

RESUMEN

La quinua (Chenopodium quinoa Wild.) es un cultivo andino, originario de los Andes Suramericanos, con gran potencial agronómico, industrial y farmacéutico y también con una gran capacidad para tolerar factores ambientales adversos. En Colombia, actualmente, se cultiva en los departamentos de Nariño, Cauca, Cundinamarca y Boyacá. Presenta una gran variación genética,
tanto a nivel molecular como morfológica, la cual, ha sido poco documentada. En Boyacá son pocos los estudios de caracterización morfológica de materiales cultivados y no hay material de siembra certificado, por lo que los agricultores siembran una mezcla de materiales. Descriptores cualitativos y cuantitativos y un análisis de componentes principales y de agrupamiento fueron usados para caracterizar la estructura de la variación fenotípica intrapoblacional de los materiales de quinua Blanca Jericó, que son cultivados en el departamento de Boyacá. El análisis de componentes principales explicó más del 70 % de la variación observada, siendo las características más variables AP, LP, DP, LHS y AHS. El análisis clúster mostró un agrupamiento por características, tales como AP, color de la panicula y presencia de axilas pigmentadas. Los resultados mostraron que la variación en las características morfológicas estaba concentrada dentro de la población, debido a la alta variación, a nivel inter-individual. Se deben llevar a cabo procesos de selección más eficientes para encontrar materiales “puros” o variedades con más altos rendimientos, con resistencia a factores bióticos y abióticos y adaptados a las condiciones locales, para así hacer de la quinua un cultivo económicamente rentable para el departamento de Boyacá.

Palabras clave: Cultivo andino; Descriptores morfológicos; Diversidad genética; Mejoramiento vegetal; Pseudocereal.

INTRODUCTION

Quinoa (*Chenopodium quinoa* Wild.) is native to the Andean region, it has a high nutritional value because of its high content of essential amino acids, with possible beneficial health effects. Quinoa has broad genetic diversity for qualitative and quantitative characteristics, which allows for wide adaptability to agroclimatic stress conditions (Hussain *et al.* 2020; Matías *et al.* 2021). As a result, global consumer demand has increased, and cultivation has spread throughout the world in recent decades. In Colombia, it is cultivated in the departments Nariño, Cauca, Cundinamarca and Boyacá (Veloza Ramírez *et al.* 2016). One of the main domestic problems, in the quinoa production system, is the lack of identification of planting materials since farmers select seeds from their cycle crops. This has led to a varietal mixture in the field as a result of this selection process (Infante *et al.* 2018; Morillo Coronado *et al.* 2020).

In the last two decades, intraspecific diversity has been shown to represent a non-negligible part of the total biodiversity measured in plants and animals (Siefert *et al.* 2015). Although preferably autogamous, quinoa shows notable inter and intra-population genetic variation, easily observable in rural plots, and quantifiable by molecular markers (Del Castillo Gutiérrez & Winkel, 2014). For morphological markers, global studies on quinoa diversity have shown variability in the phenotypic characteristics of the evaluated germplasm (Malro & Njala, 2019).

Colombia does not have certified seeds or commercial varieties, which has led to a mixture of varieties in the fields (Manjarres-Hernández *et al.* 2021a; b). Therefore, characterization, conservation, and use of this phylogenetic resource is of great strategic importance for Colombia (Morillo Coronado *et al.* 2020). In Colombia, there have been morphoagronomic characterization studies on quinoa materials cultivated in the Bogotá savanna and in Nariño (Veloza Ramírez *et al.* 2016). In Boyacá, Infante *et al.* (2018) carried out a morphological characterization of quinoa varieties grown in that department; Morillo Coronado *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.

Manjarres-Hernández *et al.* (2021a) evaluated the phenotypic characteristics of thirty genetic *C. quinoa* accessions for the selection of outstanding accessions in terms of yield and grain quality, the results that the proposed selection index, based on yield components and morphological descriptors indicated four accessions as potential for quinoa breeding programs in Colombia.

None of these studies attempted to explain the distribution of genetic variation between the different levels of organization of the species. Thus, the objective of this research was to evaluate the phenotypic intrapopulation variation in the quinoa materials Blanca de Jericó in the Department of Boyacá 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 and determine their usefulness to increasing the efficiency of quinoa improved programs.

MATERIALS AND METHODS

For The morphological characterization *in situ*, the sampling of the individuals from Blanca de Jericó was carried out in the main producing municipalities in the Department of Boyacá: Tuta (P1), Tunja (P5), Siachoque (P8), and Porvenir (P9). In parentheses, is the name of each of the populations considered in this study. In total, four municipalities and 15 farms were sampled, and a total of 27 different morphotypes in the already established Blanca de Jericó quinoa crops. The type of sampling used was a completely randomized stratified, in which the morphotypes were selected according to clearly distinguishable phenotypic differences in characteristics such as panicle color, presence of pigmented axillae, and colored striae (morphotypes); the number of replicates depended on the frequency in which the morphotype was present in the crop samples.

The FAO for quinoa and its wild relatives defined the descriptors evaluated (Manjarres-Hernández *et al.* 2021a). Measurements *in situ* were made on each of the selected morphotypes at physiological maturity (Morillo Coronado *et al.* 2020). Qualitative descriptors: Panicle color at physiological maturity, panicle shape, stem color, upper and lower leaf color, upper and lower leaf shape, upper and lower leaf edge, and presence of teeth on upper and lower leaves. Quantitative descriptors: Panicle length (LP) (cm), panicle diameter.
(DP) (cm), plant height (AP) (cm), number of teeth upper leaf (DHS), number of teeth lower leaf (DHI), main stem diameter (DT) and upper leaf length (LHS) and width (AHS).

For the quantitative variables, a descriptive analysis was made. Then, the assumptions for the parametric analyzes were verified and the analysis of variance (ANOVA) was carried out. To determine the significant differences between treatments, Tukey multiple comparison test was performed with p < 0.05. These analyses were performed using InfoStat. For the multivariate analysis, a hierarchical grouping with principal components (HCPC) 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.

**RESULTS AND DISCUSSION**

The climatic conditions of the study region were typical of tropical zones, characterized by few fluctuations in the photoperiod and average temperatures. The minimum temperature during this study ranged between 7 and 12 °C, the maximum was between 14 and 18 °C, and the average relative humidity was 76 %. The daily illumination that the accessions received during the experiment were approximately 12 h.

When evaluating the qualitative variables of the Blanca de Jericó quinoa material in the populations of Tuta (P1), Tunja (P5), Siachoque (P8), and El Porvenir (P9), Department of Boyacá. In general, it was observed that the most variable qualitative characteristics were the panicle color, which, for populations 1 and 9 showed segregation between green, light purple, and purple, in population 8,57 % of the individuals have green panicles and 43 % purple. In P5 all morphotypes had green panicles Regarding the stem color of the P5 and P9 populations, it was only green, while in P1 and P8 the morphotypes presented stem colorations between yellow, green, and purple. For the P1 and P8 populations, phenotypic variation was observed in terms of the characteristic edges of the leaves, which were serrated, teethed, and entire, while the morphotypes in P5 only presented two types of serrated and teethed edges and in P9 the upper and lower leaves ones all exhibited serrated edges. Regarding the color of the lower leaves and the striae, these characteristics varied in all evaluated populations and showed colorations between green-yellow, yellow and green. The axilla color was purple for all populations except P5, where 50 % of the individuals had purple axillae and the other 50 % were pigmented. The less variable qualitative characteristics included stem shape, which was angular in all populations; the color of the upper leaves was green for P1, P5, and P9 but was green-yellow for P8; the shape of the upper or lower leaves was rhomboidal or triangular, along with a simple growth habit and green stria marks.

In general, in the evaluation of the qualitative characteristics of the Blanca de Jericó material, it was observed that the color of the panicle had the greatest variation, as reported in characterization studies or evaluations of the Chenopodium germplasm (Del Castillo Gutiérrez & Winkel, 2014; Afiah et al. 2018; Al-Naggar et al. 2018).

A study carried out by Infante et al. (2018), with a comparison of morphological characteristics, identified varieties in quinoa from Boyacá in the vegetative and reproductive phases and reported that the color of the stem and panicle in Blanca de Jericó varied from green to light green and those characteristics such as stem and axilla color were also variable. The color characteristic in different plant structures was variable, and this variation is subject to the phenological stage of the crop, as reported in other studies (Noulas et al. 2018; De Santis et al. 2018).

In this research, the morphological characteristic showed a wide range of variations. The evaluated morphotypes were very diverse, possibly because of their coevolution process with the environment. The frequency analysis showed that the panicle color had the most variability, results similar to those reported by Morillo Coronado et al. (2022) who found that 46 % of the genetic materials had purple panicles, 28 % had green ones, and 14 % had a mixture of green and yellow panicles and panicle color changed during physiological maturity. Montes Rojas et al. (2018) reported that the wide range of colors in quinoa evaluated is because the panicle is covered by granular vesicular pubescence rich in calcium oxalate with white, pink, and purple tones that contribute to the panicle coloration of each variety.

In quinoa, characteristics associated with the stem, such as color, striae, and/or axils, can be used to identify varieties (Kir & Temel, 2016), since combinations of these characteristics differentiate the materials. Manjarres-Hernández et al. (2021a) reported although qualitative variables constitute a fundamental tool to determine the adaptation strategies of plants and are used as varietal descriptors (Katwal & Bazile, 2020), in their study, these traits had broad genetic variability, as is shown in the different colorations of the striae, axillae, panicles, and seeds. In addition, these traits were highly variable associated with variables. This behavior was also observed in quinoa materials evaluated in the Rio Grande do Sul region of Brazil (Vergara et al. 2020) and in cultivars of Quinoa Blanca de Jericó, expressed in different pigmentation within individuals in structures such as panicles and stems. These variations allow plants to adapt more quickly to environmental conditions (Alandia et al. 2020). However, these variables are the basis for genetic improvement programs because, if there is no variability, no selection can be made since all individuals respond in the same way to the evaluated conditions.
In the evaluation of the quantitative characteristics for the Blanca de Jericó material (Table 1), it was found that the more variable characteristics were DHI (28.27 % - 103.96 % for P5 and P7, respectively), DHS (22.36 % -59 % for P1 and P8, respectively), AHS (20.27 % -26.98 % for P1 and P8, respectively), DP (25.96 % for P9), LP (24.25 % for P5), AP (23 % P8), and AHI (21.90 % P1). In the quantitative characteristics, such as AR values similar to those reported by Delgado et al. (2009) in the morphoagronomic evaluation of Blanca de Jericó from the Department of Nariño were recorded. The values found in the other quantitative variables were similar to those reported in other genetic diversity studies on quinoa (Afah et al. 2018; Ebrahim et al. 2018; Infante et al. 2018; Morillo Coronado et al. 2020). The results obtained of variance detected statistically significant differences (p <0.05) between the evaluated morphotypes for the characteristics of plant height, stem diameter, length and diameter of the panicle, number of panicles, number of teeth on the leaf, yield, and seed weight and diameter.

Table 1. Descriptive statistics and analysis components of the quantitative morphological variables and showing the contribution variables, ordering the morphotypes according the quantitative variables in Blanca Jericó.

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Average</th>
<th>S.D</th>
<th>C.V (%)</th>
<th>Range</th>
<th>CP1</th>
<th>CP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP</td>
<td>5</td>
<td>114</td>
<td>12.60</td>
<td>11.04</td>
<td>27</td>
<td>0.89</td>
<td>-0.09</td>
</tr>
<tr>
<td>DT</td>
<td>5</td>
<td>3.60</td>
<td>0.47</td>
<td>13.18</td>
<td>1.1</td>
<td>0.89</td>
<td>-0.18</td>
</tr>
<tr>
<td>LP</td>
<td>5</td>
<td>25.80</td>
<td>4.15</td>
<td>13.6</td>
<td>1.6</td>
<td>0.41</td>
<td>-0.81</td>
</tr>
<tr>
<td>DP</td>
<td>5</td>
<td>27.43</td>
<td>4.97</td>
<td>15.1</td>
<td>1.3</td>
<td>-0.35</td>
<td>-0.89</td>
</tr>
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<td>DHS</td>
<td>5</td>
<td>23.46</td>
<td>13.89</td>
<td>19.1</td>
<td>34.3</td>
<td>0.05</td>
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<tr>
<td>DHI</td>
<td>5</td>
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<td>15.74</td>
<td>103.96</td>
<td>34.7</td>
<td>-0.24</td>
<td>0.96</td>
</tr>
<tr>
<td>LHI</td>
<td>5</td>
<td>4.76</td>
<td>0.79</td>
<td>16.6</td>
<td>1.59</td>
<td>0.84</td>
<td>0.46</td>
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<tr>
<td>AHI</td>
<td>5</td>
<td>4.34</td>
<td>0.95</td>
<td>21.90</td>
<td>2.0</td>
<td>0.97</td>
<td>0.20</td>
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<tr>
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<td>5.52</td>
<td>1.12</td>
<td>20.27</td>
<td>2.8</td>
<td>0.92</td>
<td>-0.28</td>
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<tr>
<td>P5</td>
<td>6</td>
<td>135.33</td>
<td>8.76</td>
<td>6.47</td>
<td>24</td>
<td>0.81</td>
<td>0.32</td>
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</table>

The Tukey test (p <0.05) for all the quantitative variables, shows differences between accessions evaluated, the most diverse characteristics being those associated with yield, such as the number and length of the panicle, height of the plant, number and diameter of seeds, similar results found in morphoagronomic characterization studies of quinoa in Boyacá (Morillo Coronado et al. 2020; Manjarres-Hernández et al. 2021a; b; Morillo Coronado et al. 2022).

The principal component analysis grouped the 10 quantitative variables into several components, with the first two (CP1 and CP2) accounting for more than 70 % of the total observed variation. All variables contributed significantly to each of the components, except DHS and AP (CP1, CP2, respectively) in P1 and DP (CP2) in P8 (Table 1). In the analysis of all evaluated quantitative descriptors in the nine populations from the seven municipalities, AP, LP, DP, LHS, and AHS were more discriminating variables for the evaluated quinoa materials, contrary to the findings of Morillo Coronado et al. (2020), who found that the presence and color of axillae, presence, and effusion of saponins, number of panicles and secondary branches, the yield of seeds/plant and grain diameter differentiated the evaluated quinoa materials, coinciding with evaluations of the Cochabamba germplasm in Bolivia (Del Castillo Gutiérrez & Winkel, 2014).

Manjarres-Hernández et al. (2021b) in the principal components analysis found that 67 % of the total variance was explained by the first two components, the variables that the greatest contribution was plant height, length, diameter, and the number of panicles, the weight of 1000 seeds, seed diameter and stem diameter contributed. On the other hand, the variables associated with weight and seed diameter correlated more with yield than the variables plant height, stem diameter, number of teeth on the leaves, and the variables associated with the panicle.

The correlation analyses showed very particular associations in each of the populations (Table 2); P1 had a total of 15 highly significant coefficients, with the highest correlation value (r = 0.98) between...
AP and DT. AP had a weak correlation with LHI (r = 0.54) but a significant one with LHS, AHI, and AHS (r = 0.81, r = 0.78, r = 0.75, respectively). The DT presented a high correlation with AHS, LHS and AHI, (r = 0.78, r = 0.77 r = 0.76, respectively). LP showed a significant and negative correlation with DHI (r = -0.82) and a moderate one with DP and LHS (r = 0.54, r = 0.61, respectively).

In P5, the highest correlation value was found between AP and AHS (r = 0.94). LP had a significant but negative correlation with DHI (r = -0.83), the same as with DP and DHI (r = -0.76).

### Table 2. Pearson correlation analysis for the quantitative variables (P ≥ 0.005) measured in each population from the Blanca Jericó material evaluated.

<table>
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<tr>
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<th>P1</th>
<th>P5</th>
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<th>P1</th>
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<tbody>
<tr>
<td>AP</td>
<td>DT</td>
<td>LP</td>
<td>DP</td>
<td>DHS</td>
<td>DHI</td>
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<tr>
<td>AP</td>
<td>0.98</td>
<td>0.74</td>
<td>0.67</td>
<td>0.68</td>
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<tr>
<td>DT</td>
<td>0.98</td>
<td>0.74</td>
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<tr>
<td>LP</td>
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<tr>
<td>DP</td>
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<td>0.73</td>
<td>0.64</td>
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Values in bold are not significant (P ≥ 0.005).

In the results of the correlation analysis, a positive and significant correlation was observed for AP and LP in P7, which indicated that the increase in AP contributed to an increase in panicle length (Delgado et al. 2009; Ebrahim et al. 2018), contrary to the negative correlations between AP and LP in P5 and P8, which indicated that the higher the AP, the lower the LP. Also, in P1, the correlation of these variables was weak, as suggested by the existence of great variability for the management of these characteristics through genetic breeding (Al-Naggar et al. 2018; Afiah et al. 2018; Morillo Coronado et al. 2020).

For P1, P8 and P9, a positive correlation was observed between the characteristics associated with leaf morphology, such as AHS, LHS, AHI, and LHI, similar to that obtained. As was observed in the study carried out by Al-Naggar et al. (2018), a positive correlation was also found between the height of the plant and the length of the panicle, which showed that a high grain yield can be achieved by selecting the stem/inflorescence ratio. Afiah et al. (2018), in an evaluation of six quinoa genotypes by seed yield and attributes under the conditions of Toshka, found that the components of seed yield exhibited various associations. Highly positive and significant associations were observed between plant height and the number of secondary branches/plant (0.64) and the number of inflorescences/plant (0.59), revealing that the highest genotypes often had more branches and inflorescences. The path analysis showed that the maximum relative importance of the seed/plant was obtained with the height of the plant (14.36), followed by the number of secondary branches/plant (8.95) and the number of inflorescences/plant (20.66). Consequently, indirect selection of these three traits would improve seed/plant yield and would effectively contribute to the improvement of quinoa.

Morillo Coronado et al. (2020) found that, among plant architecture correlations, higher and more significant correlations were observed between plant height, grain diameter and yield/plant. Positive and significant correlations between phenological variables and plant architecture were also quantified. The variables grain diameter and weight of 1000 seeds had a high and positive correlation, which suggested that there was an important margin of variation with the possibility of selecting materials with a larger grain diameter without affecting the weight and carbon requirement to obtain it; similar results were reported by Del Castillo Gutiérrez & Winkel (2014).

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 (Al-Naggar et al. 2018; Ebrahim et al. 2018; Afiah et al. 2018).
The cluster analysis was used as an efficient procedure to show the structural relationships between the evaluated individuals and provided a hierarchical classification (Figure 1). In the present study, the distance between the individuals was estimated based on their morphological characteristics, where Euclidean distances greater than 7.0 were observed along with a distribution of the individuals according to mainly the characteristics of panicle color, plant height, and presence of pigmented axillae. In the study carried out by Morillo Coronado et al. (2020), the clusters showed a lax distribution of the materials with an association of the characteristics of 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 (Infante et al. 2018; Farooq Azhar et al. 2018).

In this research, as in the study by Morillo Coronado et al. (2020), no groupings were observed according to the site of origin of the materials, as observed when evaluating the intrapopulation and interpopulation 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 Gutiérrez & Winkel, 2014). These results are consistent with that reported by Farooq Azhar 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. (2018) 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.

Madrid et al. (2018), in a characterization of the phenotypic diversity of 12 coastal/lowland quinoa, used cluster analysis to show that the morphological variables of the plant were grouped independently of the grain yield components. In the studies carried out by Afiah et al. (2018), the grouping pattern of quinoa materials

Figure 1. Cluster analysis showing the groups formed in each population from the Blanca Jerícó material formed according to the qualitative and quantitative variables.
occurred according to the yield. De Santis et al. (2018) evaluated two groups of quinoa with different seed colors in Foggia, southern Italy, and found that the cluster analysis did not show a clear grouping of accessions based solely on seed color.

The morpchoagronomic characterization of Blanca de Jericó quinoa materials in the four evaluated municipalities showed high intrapopulation phenotypic variability that depended on the agroclimatological conditions of each site (Infante et al. 2018; Morillo Coronado et al. 2020), mainly as the result of the fact that quinoa is a rustic crop with a 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-Flores 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.

The evaluation of the intrapopulation diversity of Blanca de Jericó materials showed that there was segregation for most of the evaluated phenotypic characteristics. Differentiation of quinoa populations in the Department of Boyacá would be largely determined by phenotypic characteristics. Differentiation of quinoa populations can tolerate different conditions as has been done for cereals with uniform grain sizes and qualities.

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The evaluation of the intrapopulation diversity of Blanca de Jericó materials showed that there was segregation for most of the evaluated phenotypic characteristics. Differentiation of quinoa populations in the Department of Boyacá would be largely determined by phenotypic characteristics. Differentiation of quinoa populations can tolerate different conditions as has been done for cereals with uniform grain sizes and qualities.

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