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Phenotypic diversity of agromorphological characteristics of quinoa (*Chenopodium quinoa* Willd.) germplasm in Colombia

Ana Cruz Morillo Coronado[1]*, Elsa Helena Manjarres Hernández[1], Yacenia Morillo Coronado[2]

[1]Universidad Pedagógica y Tecnológica de Colombia/ Facultad de Ciencias Agropecuarias, Avenida Central Norte # 39-115 – Tunja – Colombia.
[2]Corporación Colombiana de Investigación Agropecuaria/ AGROSAVIA, Cra 36a–23, Palmira – Valle del Cauca – Colombia.
*Corresponding author <ana.morillo@uptc.edu.co>

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ABSTRACT: *Chenopodium quinoa* Willd. is an Andean crop with great nutritional value, economic potential, and a broad genetic and phenotypic diversity with adaptation to different conditions. In Colombia, *C. quinoa* is cultivated mainly in Cundinamarca, Nariño and Boyacá, where studies have been conducted to characterize the germplasm and lack of seed materials, some challenges for the quinoa crop. This work assessed agromorphological characteristics of 50 quinoa genotypes from the germplasm collection of Boyacá the using a completely randomized design on the farm in Tunja. The multivariate analysis followed by a clustering approach were conducted on agromorphological descriptors, in which 16 were qualitative descriptors (e.g, panicle shape, episperm color, leaf shape) and five quantitative descriptors (e.g, plant height, panicle number). The results showed that higher coefficients of variation were found in characteristics associated to yield. The principal component analysis (PCA) of the quantitative variables showed that the first two components explained 88 % of the total variation with the characteristics of plant height, length, diameter, and panicle number showing the highest variability. The quantitative characteristic clusters comprised length and diameter panicle, weight 1,000 seeds, and plant height, while the qualitative characteristic clusters comprised stem shape, branching habit, panicle shape, and color of the axils. The factorial analysis of mixed data discriminated the materials with outstanding morphoagronomic characteristics. Agromorphological characterization revealed a broad variability, which should be conserved and used in genetic improvement programs of *C. quinoa*.

Keywords: Andean cultivation, phenotypic variability, genetic diversity, yield

Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a native species to the Andes with great nutritional value because of its high protein content, essential amino acids, vitamins, minerals, isoflavones, carbohydrates and unsaturated fats (Jaikishun et al., 2019; Sampaio et al., 2020). It has efficient water use, tolerance to low soil moisture, wide adaptation to different climates, including deserts (Bazile et al., 2016). Therefore, quinoa can contribute to food security in various regions of the world (Pinto et al., 2020).

Quinoa has been cultivated in the Andes from Colombia to Chile. In Colombia, it is cultivated mainly in Cundinamarca, Boyacá, Cauca and Nariño (Jager, 2015). However, Colombia does not have certified seeds or commercial varieties, which has led to a mixture of varieties in the fields (Morillo et al., 2020). Therefore, characterization, conservation, and use of this phytogenetic resource is of great strategic importance for Colombia. This crop has great genetic potential thus providing a solution to the lack of seed materials, an issue in the productive chain in Colombia. In Colombia, few studies have investigated the morphological characterization of quinoa genotypes. The first studies were carried out in Bogotá and Nariño.

In Boyacá, few studies have estimated the agromorphological parameters of the cultivated materials. Infante et al. (2018) evaluated the physiological parameters in the vegetative and reproductive phase in quinoa varieties from of Boyacá, considering different variables in certain stages of plant development and establishing a taxonomic key for the identification of varieties. Morillo et al. (2020) studied the intrapopulation phenotypic variation of Piartal material and found that phenotypic variation is concentrated within populations because of high variations at the inter-individual level. Studies have reported a mixture of materials in the fields (Salazar et al., 2019), leading to high phenotypic variability with no certified cultivated material thus requiring more efficient seed selection processes to obtain quinoa genotypes or varieties (Morillo et al., 2020).

This research studied the morphological and agronomic characteristics of 50 quinoa genotypes from the germplasm collection of Boyacá to improve varietal identification of cultivated germplasm quinoa and contribute to its breeding and conservation in Colombia.

Materials and Methods

Plant Material

We evaluated 50 quinoa (*C. quinoa*) cultivated materials from Boyacá under a completely randomized design (CRD) with three repetitions, where the experiment unit comprised 30 m by 30 m with 28 rows. The materials were sown during the first and second semesters of 2018 on a farm in Tunja, at 2,820 m above sea level, with an average annual temperature 13 °C, relative humidity 78 %, photoperiod 12:12, and rainfall 1,216 mm. The soils in the experiment site were loamy-clay with good drainage. The agronomic management was conventional, with organic fertilization, biological management of pests and diseases, and manual control of weeds. The harvest was carried out manually when the plants reached physiological maturity (Table 1).

Morphoagronomic characterization

We evaluated 16 qualitative and five quantitative descriptors (Table 2). Measurements were made on four individuals from each material. The FAO for quinoa and its wild relatives (Morillo et al., 2020) defined the descriptors evaluated.

Statistical analysis

The statistical analyses were carried out in three steps: (1) descriptive, (2) multivariate, and (3) clustering. First, data from morphoagronomic characterization was used in the descriptive analysis for the quantitative and qualitative variables in the statistical software InfoStat 2020 (Di Rienzo et al., 2020). We computed the Pearson's correlation for the quantitative variables and its significance was assessed using the t-test (null hyphothesis is H0: r = 0, with 5 % of significance). Second, the principal component analysis (PCA) was performed on the quantitative variables using the correlation matrix between the characteristics, which were graphed on a two-dimensional plane to group the accessions with the R Core Team Software (2020). For the qualitative variables, we carried out the multiple correspondence analysis (MCA) and the correlation analysis. Third, the distance matrix was performed using the Euclidean distance for the cluster analysis. We used the minimum Ward's distance as the grouping method where each cluster was given by the smallest increase in the total sum value of the squares of existing differences within each cluster of each observation concerning the cluster centroid. The analyses were carried out with the algorithms included in the extra-factor package of the R program version 1.07 for the cluster (Kassambara and Mundt, 2020). For the joint analysis of the quantitative and qualitative variables, a factorial analysis of mixed data was carried out with the factoextra package in the R program. Additionally, a dendrogram was generated using the Euclidean distance and hierarchical grouping method of Ward's minimum variance with the FactoMineR package (Le et al., 2008).

Results and Discussion

In Colombia, quinoa is produced with unidentified genetic materials because of varietal mixtures with morphological, phenological, and physiological changes that depend on edaphoclimatic conditions (Morillo et al., 2020). According to the qualitative and quantitative variables evaluated in this study, a broad phenotypic variability was observed in the quinoa materials from Boyacá, which has a direct effect on yield (Hancco, 2003). Understanding the evolutionary processes that determined the genetic diversity of this species allow management, conservation, and efficient use within a sustainable and economically profitable production scheme (Morillo et al., 2020).

For the evaluation of the quantitative characteristics, the number of panicles was the most variable descriptor, with a coefficient of variation of 39.43 % and a range from 2 to 32 panicles per plant. The least variable characteristic was plant height, with a coefficient of 27.1 % and a range from 43.3 to 131.5 cm (Table 3). Plant height is highly influenced by the environment variables; thus, G × E should be also observed for this characteristic. Lopez et al. (2012) reported that ecotypes cultivated in valleys are higher than on higher ground. Studies have shown that quinoa heights in Colombia range from 70 to 180 cm, consistent with our results (Morillo et al., 2020).

The length of panicles in quinoa plants depends on the growth and differentiation processes that occur in the apical meristem (Garcia et al., 2019). These processes are determined by the genotype, inflorescence type, and fertility conditions in the soil, among others (Temel and Keskin, 2020). Panicle lengths between 22 and 40 cm have been reported (Lopez et al., 2012) and, in this study, we found panicles between 12 and 45.6 cm in length. Studies have shown a strong correlation between the panicle diameter and yield (Hussain et al., 2020). Panicle diameters in this study ranged from 7.5 to 38.2 cm, which corroborates Morillo et al. (2020) in the study on the intrapopulation phenotypic variation in the material cultivated of Boyacá, Piartal, where a high diversity was found at the interindividual level, mainly in length, diameter, and color of the panicle.

Table 1 – Origin Sites of the evaluated quinoa (*C. quinoa*) materials.

Origen	Quantity	Altitude	North latitude	West Longitude
Nariño (Gen1, Gen3, Gen4, Gen5, Gen6, Gen7, Gen8, Gen9, Gen11, Gen13, Gen14, Gen16, Gen22, Gen25, Gen26, Gen27, Gen32, Gen34, Gen35, Gen39, Gen40)	21	2,817	3°17´20"	77°21´28"
Perú (Gen45)	1	3,259	12°04´15"	75°12´24"
Boyacá (Gen38, Gen41, Gen43, Gen44, Gen46, Gen47, Gen48, Gen50, Gen51, Gen52, Gen53, Gen54, Gen55, Gen56, Gen57, Gen63, Gen65, Gen66, Gen69, Gen71, Gen72, Gen73, Gen77, Gen80, Gen86, Gen87, Gen106, Gen107)	28	2,942	5°30´02"	73°19´59"

Table 4 presents the Pearson correlation matrix between each pair of variables, which shows three highly significant coefficients ($p < 0.05$). The associated characteristics included plant height and panicle length (r = 0.94), panicle diameter and length (r = 0.97) and plant height and panicle diameter (r = 0.9). These results agree with Spehar and Barros (2005) who evaluated quinoa varieties from Peru and Bolivia and reported a strong correlation between plant height, length, and diameter. Similarly, Morillo et al. (2020) evaluated quinoa materials from Boyacá and found a positive correlation between plant height and panicle length (r = 0.91) and plant height and panicle diameter (r = 0.78). Montes et al. (2018) also observed a positive correlation between plant height and panicle length, which means that taller plants also have a greater panicle length.

The PCA of the quantitative variables showed that 88 % of the total variation was explained by the first two components. In PC1, variables with greater contribution included plant height, length, diameter, and number of the panicles. In PC2, weight of 1,000 seeds was the most representative variable (Table 5). In general, quantitative variables that contribute more to the percentage of variance are related to yield (Morillo et al., 2020).

Table 2 – Morphoagronomic descriptors evaluated for the characterization of quinoa material from Boyacá.

Qualitative	Initials	Quantitative	Initials	Units of measurement
Growth habit	(H)	Panicle length	(PL)	cm
Presence of striae	(PS)	Panicle diameter	(PDI)	cm
Strie color	(SC)	N ° plant panicles	(NP)	#
Stem shape	(SS)	Weight of 1,000 seeds	(WS)	g
Stem color	(SCO)	Plant height	(PH)	cm
Pigmented axil	(PA)			
Axil color	(AC)			
Branch	(B)			
Leaf shape	(LS)			
Leaf margin	(LM)			
Panicle shape	(PSA)			
Panicle density	(PD)			
Panicle color at flowering	(PCF)			
Panicle color at physiological maturity	(PCM)			
Grain shape	(GS)			
Episperm color	(EC)			

Table 3 – Descriptive statistics for the quantitative variables.

Variable	Average	*D.E.	**CV	Mínimum	Maximum
PL	28.93	9.15	31.64	12.00	45.60
PDI	22.57	8.50	37.66	7.50	38.20
NP	13.65	5.38	39.43	2.30	31.70
WS	2.10	0.17	8.30	1.72	2.63
PH	79.64	21.62	27.15	43.30	131.50

*D.E.=Standard deviation; **CV=coefficients of variation; PL= Panicle length; PDI = Panicle diameter; NP = Number panicle; WS = Weight of 100 seeds and PH = Plant height.

The cluster analysis (Figure 1) formed three clusters related to the quantitative variables. The first (a) twenty materials were grouped (46, 56, 48, 65, 27, 32, 53, 50, 51, 6, 9, 4, 5, 38, 43, 8, 25, 47, 57 and 77), characterized by panicle length between 28.9 and 45.6 cm, panicle diameter from 22.5 to 38.2 cm, number of panicles from 11 to 32, plant height between 73.3 and 131.5 cm and weight of 1,000 seeds from 1.9 to 2.4 g. The second group (b) comprised eleven materials (52, 71, 72, 44, 55, 106, 69, 107, 80, 86, 87), which were characterized by a panicle length between 22.7 and 37.7 cm, panicle diameter from 17 to 28.6 cm, number of panicles between 7 to 20, plant height between 59 and 108.3 cm and weight of 1,000 seeds from 1.7 to 2, 1 g. The third group (c) comprised materials (11, 26, 13, 1, 22, 14, 16, 3, 7, 63, 66, 41, 39, 40, 45, 34, 35, 54, 73) with a panicle length between 12 and 26.3 cm, panicle

Table 4 – Simple correlation matrix between quantitative variables.

Variable	PL	PDI	NP	WS	PH
Panicule lenght	1				
Panicule diameter	0.97**	1			
N° panicule plants	0.61	0.63	1		
Weight of 100 seeds	–0.09	–0.08	–0.17	1	
Plant height	0.94**	0.90**	0.64	–0.05	1
	PL	PDI	NP	WS	PH

**Highly significant values ($p < 0.05$). Variables evaluated: PL = Panicle length; PDI = Panicle diameter; NP = Number of panicles; WS = Weight of the 1,000 seeds; PH = Plant height.

Table 5 – Principal components and percentages of total variance explained.

Component	Eigenvalues		
	Total	% de variance	% acumulate
1	3.38	68.00	68.00
2	1.00	20.00	88.00
3	0.48	10.00	97.00
4	0.12	2.00	1.00
5	0.02	0.00	1.00

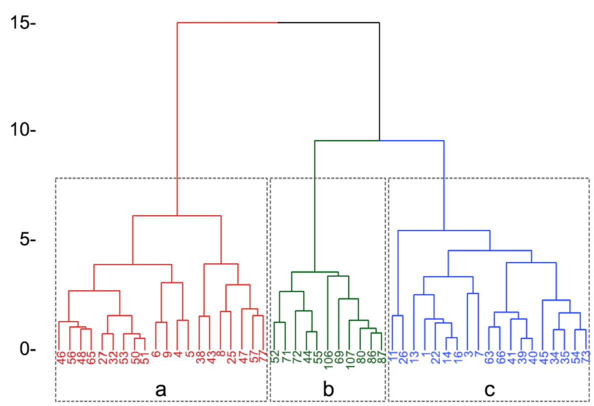

Figure 1 – The cluster analysis of the quinoa materials evaluated for quantitative traits. The letters correspond to each group formed according to the characteristics evaluated.

diameter from 7.5 to 20.3 cm, number of panicles from 2 to 16, plant height between 43.3 and 74.7 cm and weight of 1,000 seeds from 1.7 to 2.6 g. In general, the groupings were conditioned by the characteristics associated to the panicle, seeds, and plant height.

In this study, the morphological characteristics showed a wide range of variation. The evaluated germplasm was very diverse, possibly because of its coevolution process with the environment. The frequency analysis showed that the panicle color had most variability, evaluated twice during the phenological cycle. The results showed that 46 % of the materials had purple panicles, 28 % had green ones, and 14 % had a mixture of green and yellow panicles (Figure 2). Panicle colors changed during physiological maturity. Hence, other studies on quinoa reported significant decreases in plant height, stem thickness, number of branches, and panicle ratios, as the sowing time progressed (Hirich et al., 2014; Temel and Keskin, 2020). Montes et al. (2018) reported that the wide range of colors in the materials evaluated is because the panicle is covered by granular vesicular pubescence rich in calcium oxalate with white, pink, and purple tones that contribute to panicle coloration of each variety.

In quinoa, characteristics associated to the stem, such as color, striae and/or axils, can be used to identify varieties (Kir and Temel, 2016), since combinations of these characteristics differentiate the materials. In this study, stem color was variable: 54 % yellow, 26 % purple, and 10 % red. Purple was the predominant color in 84 % of the plants had axils. Striae in 92 % of the plants varied in color according to the material: 56 % was yellow, 12 % was purple, and 12 % was dark green (Figure 2).

On the other hand, the branching habit of quinoa must be evaluated because mechanical harvesting tasks are difficult when the material presents certain types of branches. A simple branching habit has been observed in materials from highlands and salt flats (Kir and Temel, 2016). In this study, 10 % of the materials had a simple growth habit, while 84 % was branched in the lower third, which is common in valley quinoa. On the other hand, the branching habit is influenced by planting density, nutrients, and other environmental factors (Kir and Temel, 2016).

Similarly, leaf shape characteristic varies depending on the material. In the materials studied, 50 % of the plants had a rhomboidal leaf shape, 32 % was oval and 18 % was triangular. For the leaf margin, 68 % of the plants had an entire margin, while 32 % had a dentate margin. The panicle density is associated to the grain size and the smallest is formed in compact panicles (Gómez and Aguilar, 2016). In this study, 42 % of the quinoa evaluated had intermediate heads, between loose and compact, 40 % of the plants had compact heads, and 18 % had lax heads. The panicle shape in this study was mainly glomerular (80 %), 10 % was amarantiform, and the other 10 % was intermediate. Studies have shown an association between panicle density and its shape and genotype yield, since panicles with an intermediate shape tend to be compact with good yield (Pinto et al., 2020). This association with yield is because the flowers are sessile or pedicellate and are grouped in glomeruli. The glomerulus position in inflorescence and the position of flowers within glomerulus determine the size and number of grains or fruits (Chura et al., 2019).

An important aspect in quinoa commercialization is the grain color. In Colombia, white quinoa is preferred, but in countries such as Bolivia and Peru, black and brown varieties are desired in gourmet cuisine. In this study, grain color was variable: 48 % of the materials had beige grains, and 18 % of the plants had yellow grains, which is why they are acceptable materials for commercialization in Colombia.

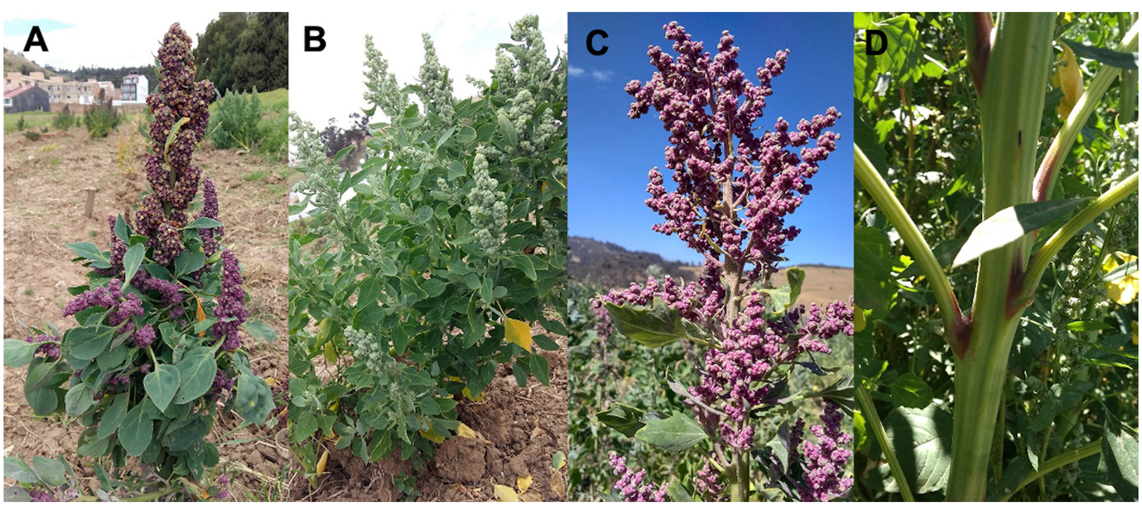

Figure 2 – Phenotypic diversity in panicle color (A). Quinoa material with compact panicles and purple color; (B) Quinoa material with intermediate panicles and green color; (C) Quinoa material with loose panicles and purple color; (D) Purple axils on the stem, yellow striae.

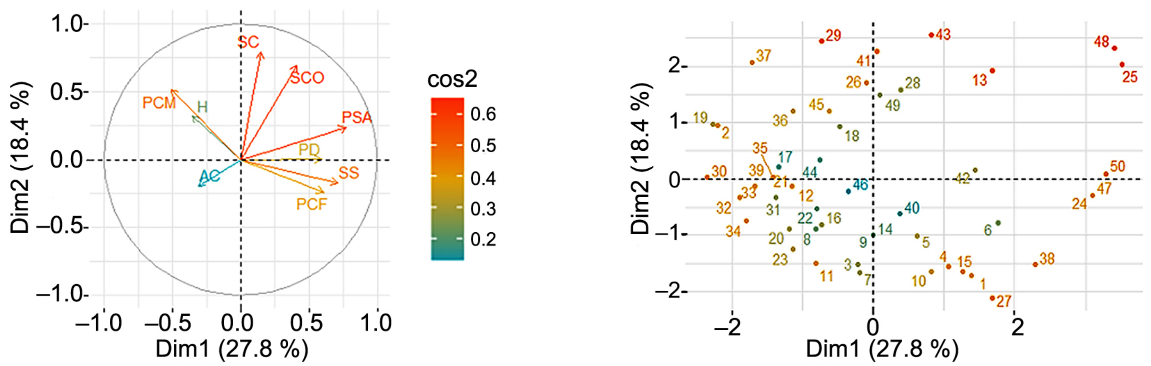

Figure 3 – Correlations for qualitative variables measured in materials of quinoa evaluated. H= Growth habit; PCM = Panicle color at physiological maturity; AC = Axil color; SC = Strie color; SCO = Stem color; PSA = Panicle shape; PD Panicle = density; SS = Stem shape; PCF = Panicle color at flowering.

The correlation analysis of qualitative variables was high and positive for characteristics, such as panicle shape, stem color, and striae color, while the axil color had little contribution to characterizing the materials, since 78 % of the evaluated plants had purple color (Figure 3). Correlation studies are important for quinoa improvement because of the selection response and allow significant genetic gains in less time (Al-Naggar et al., 2018).

In the dendrogram for qualitative characteristics, three clearly differentiated groups were shown (Figure 4). Group (a) had the materials (43, 86, 46 and 107) where most outstanding morphological characteristics were the shape of an angular stem, the color of the green axils, and the amaranthiform panicle shape. Group (b) had the materials (44, 87, 71, 69, 77, 22, 45, 66, 72, 73, 11, 5, 6, 26, 13, 63, 7, 27, 14, 16, 39, 34, 40, 8, 9, 1 and 4) with branching habits in the lower third, a glomerular panicle shape, and purple axils. Group (c) comprised materials (80, 57, 50, 51, 52, 41, 53, 3, 32, 35, 106, 47, 48, 56, 25, 38, 65, 54, and 55) that were characterized by a cylindrical stem, branching in the first third, glomerular panicle shape, and green axils. In general, the clusters were formed by characteristics, such as stem shape, branching habit, panicle shape, and axil color.

The factorial analysis of mixed data considered all quantitative and qualitative variables and discriminated the materials with outstanding morphoagronomic characteristics (Figure 5). The cluster analysis formed four groups. First, material was grouped (3), characterized by the panicle yellow color and the number of panicles of 23 per plant. The second group comprised 21 materials, which were characterized by an average of 14 panicles per plant, plant height 79.63 cm, panicle length 28.93 cm, and density of the compact panicle. Group 3 was characterized by glomerulate panicle shape, cylindrical stem shape, plant height between 74.3 and 132.0 cm, and weight of 1,000 seeds from 1.7 to 2.4 g. Group 3 was formed by materials with glomerulate panicle shape, cylindrical stem shape, plant height ranging from 74.3 to 132.0 cm and weight of 1,000 seeds from 1.7 to 2.4 g. The last group it is characterized by growth habit of branching to the lower third, panicle color at physiological maturity green and purple, panicle length from 12 to 35 cm, and plant height from 43.3 to 86 cm.

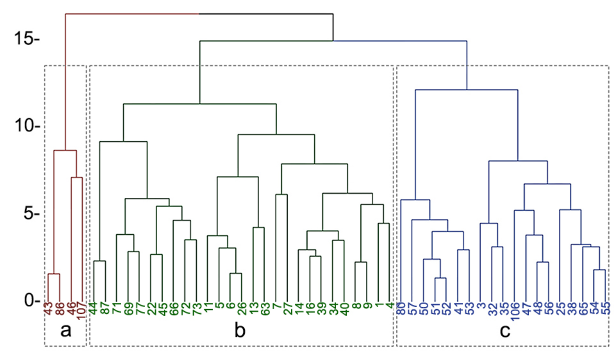

Figure 4 – The cluster analysis of quinoa materials evaluated for qualitative characteristics. The letters correspond to each group formed according to the characteristics evaluated.

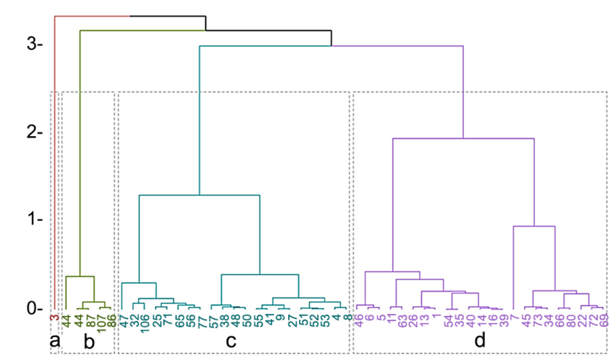

Figure 5 – The cluster analysis of quinoa materials evaluated for qualitative and quantitative characteristics. The letters correspond to each group formed according to the characteristics evaluated.

Studies on the morphoagronomic characterization of quinoa in Colombia have shown diverse quinoa germplasm with potential use. The studies report that producers require more stable materials in terms of phenotypic traits that allow optimal development of harvesting and postharvest processes and yields that are economically viable.

The phenotypic characterization carried out on the 50 quinoa materials suggest a broad genetic diversity, confirming that these materials result from mixtures that farmers keep crop after crop. However, it is necessary to consider the experimental conditions of the study, which was conducted in a single location and two semesters in a year. It is thus convenient to determine the genotype-by-environment interaction of the materials selected in the study for more accurate conclusions. Therefore, genetic improvement processes are needed to facilitate sustainable quinoa production in Colombia through the identification of elite materials that respond to the needs of farmers, producers and consumers.

Authors' Contributions

Conceptualization: Morillo Coronado, A.C.; Manjarres Hernández, E.H.; Morillo Coronado, Y. **Data acquisition:** Morillo Coronado, A.C.; Manjarres Hernández, E.H. **Design of methodology:** Morillo Coronado, A.C.; Manjarres Hernández, E.H. **Writing and editing:** Morillo Coronado, A.C; Manjarres Hernández, E.H.; Morillo Coronado, Y.

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