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Molecular characterization of intrapopulation genetic diversity in *Chenopodium quinoa* (Chenopodiaceae)

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ABSTRACT. *Chenopodium quinoa* is a species native to South America. It continues to be cultivated in various regions of that continent, especially in Colombia, Chile, Bolivia, Ecuador and Peru, with wide adaptability to different agroecological conditions and great nutritional value. It is a food security crop in the Andean region. It presents great variability in its characteristics; however, genetic studies in Colombia are scarce. Though the Boyacá is one of the main quinoa producing departments in Colombia, there have been few studies on the characterization of genetic diversity in cultivated materials in this region. There are no certified planting materials, and various morphotypes are observed in the field within the same crop. We examined the genetic diversity, using microsatellites, of 54 Piartal and Blanca de Jericó morphotypes, selected for their different phenotypic characteristics on producer farms in seven municipalities in the department of Boyacá. The cluster analyses showed similarity values greater than 0.70, with a laxa distribution of individuals in each of the populations. The observed heterozygosity values were lower than the expected heterozygosity values, between 0.4 and 0.6,

demonstrating a greater frequency of homozygous individuals. The analysis of genetic diversity showed high genetic differentiation between the individuals in these populations. The analysis of molecular variance showed that 82% of the observed genetic variability in the evaluated quinoa materials was due to components within the groups and that the remaining 18% was attributed to differences between the groups, which indicated that microgeographic studies at the intra-group level are warranted. The results revealed intra-population diversity, which suggests that farmers within their farms should undergo a more rigorous seed selection process.

Key words: Genetic diversity; Microsatellites; Intra-population differentiation

INTRODUCTION

Quinoa (*Chenopodium quinoa*) an annual, dicotyledonous species that belongs to the Chenopodiaceae family, is cultivated from sea level to 4,000 m, with a broad agro-ecological adaptation to different types of soils (Hong et al., 2017). It is an Andean crop that originated around Lake Titicaca in Peru and Bolivia, the area with greatest diversity and genetic variation. Archaeological evidence shows that the cultivation of quinoa began in Peru 7,000 years ago, where it was cultivated in association with corn, while in Chile cultivation began 5,000 years ago. It is the most relevant food in the Inca culture, where it is called atacameño dahue. In Colombia it is believed that the Chibchas, like other tribes of the Cundiboyacense plateau, intensively cultivated quinoa, and that the ancient inhabitants of Cuyumbe (current ruins of San Agustín, Huila) who had relationships with the inhabitants of the Sabana de Bogotá, helped in its dispersion, towards the south of Colombia and later to Ecuador (Mujica and Jacobsen, 2006). This is how the quinoa was distributed throughout the Andean region, from Colombia to northern Argentina and Chile (Zurita et al., 2014). It has great nutritional value because of its high content of essential amino acids, vitamins and secundary metabolites, with possible beneficial health effects (Pereira et al., 2019; Rodríguez et al., 2020). It has high genetic diversity in its phenotypic and genotypic characteristics, providing wide adaptability to stressed agroclimatological conditions (Hinojosa et al., 2018; Ali et al., 2019).

In Colombia, quinoa is cultivated in the Departments of Nariño, Cauca, Cundinamarca and Boyacá; in the latter, it is found in the municipalities of Cucaita, Duitama, Tibasosa, Siachoque, Tunja, Soracá, Oicatá, Cerinza and Pachavita (Veloza et al., 2016; Yang et al., 2016). One of the main problems in cultivation is the lack of identification of planting materials since farmers select seeds from their own crops, cycle after cycle (Morillo et al., 2020).

The development of molecular biology and bioinformatic tools has facilitated the genetic study of various species in the *Chenopodium* genus, including the mapping and characterization of thousands of microsatellites, polymorphism markers in a single nucleotide (SNP), creation of expressed sequence libraries, BAC libraries, and as linkage map, which should facilitate the introgression of new alleles in the quinoa genome (Rojas *et al.,* 2015; Vía and Fernández, 2015; Jarvis et al., 2017).

Nowadays, molecular markers are an effective tool for improving the efficiency of genetic improvement, including simple sequence repeats (SSR) or microsatellites, which are among the more common markers for crop genotyping since they have co-dominant inheritance and good genome coverage and are multi-allelic in nature (Al-Naggar et al., 2015). Several research studies have shown that SSRs and ISSRs are efficient at detecting polymorphisms and genetic diversity in quinoa, as well as for creating genetic maps (Jarvis et al., 2008; Lu et al., 2015; Morillo et al., 2017a,b). Unfortunately, few resources are available to facilitate its genetic improvement. Jarvis et al. (2017) identified sequences of the quinoa genome that code for characteristics of interest as a useful strategy for the genetic improvement of this species.

Germplasm characterization studies in quinoa (*C. quinoa* and *C. petiolare*) from Bolivia, Peru and Turkey, among others, have shown high genetic diversity in the evaluated materials and populations and have demonstrated the high discriminatory power of microsatellite markers (Hossein et al., 2019; Romero et al., 2019). In Colombia, in the Department of Boyacá, research carried out by Morillo et al. (2017b) characterized the genetic diversity of a quinoa collection (*C. quinoa*), finding intraspecific genetic variability according to a Molecular Analysis of Variance (AMOVA) and based on the estimation of unbiased heterozygosity and the percentage of polymorphic loci and that the materials are very homogeneous, similar to results found in another study on *C. quinoa* molecular characterization with ISSR markers (Morillo et al., 2017a). These studies showed the need to carry out microgeographic and intrapopulation studies for a better estimation of the genetic diversity of quinoa materials in the Department of Boyacá.

None of these studies sought to explain the distribution of genetic variation between the different levels of organization in this species. The objective of the current research was to characterize the intrapopulation genetic diversity in quinoa materials cultivated in the Department of Boyacá using microsatellite markers in order to analyze the structure of variation and thus be able to establish strategies for the establishment of better selection processes geared towards obtaining “pure” planting materials that respond to the needs of farmers, producers and consumers.

MATERIAL AND METHODS

A total of 54 different morphotypes were observed in established crops of the quinoa materials Piartal and Blanca de Jericó in seven municipalities of the Department of Boyacá. For this study, nine populations were defined, corresponding to the place or locality of the sampling and the two materials evaluated, Piartal and Blanca de Jericó (Table 1). A completely randomized stratified simple sampling was used, which consisted of identifying the plants in the field that showed phenotypic differences in characteristics within panicle color, presence of pigmented axillae and colored striae (morphotypes); the number of repetitions depended on the presence of these characteristics in the crop. For this study, the materials and locations were established for a total of nine populations. The molecular characterization was carried out in the Laboratorio de investigaciones en Biología Molecular Vegetal at the Universidad Pedagógica y Tecnológica de Colombia, in Tunja at 2,820 msnm, with an average temperature of 13°C.

The DNA extraction was done with the protocol of Dellaporta et al. (1983), as modified by Muñoz et al. (2008). The total DNA was visualized with 0.8% agarose gels in a

Maxicell Primo EC-340 electrophoresis chamber. A Hoefer Dyna Quant 200 fluorometer was used to determine the concentration, with dilution using HPLC water for a total volume of 100ul at 10ng/ul, stored at -20°C. Ten microsatellite markers that have been useful in determining genetic diversity in quinoa were used (Mason et al., 2005; Christensen et al., 2007, Jarvis et al., 2008) (Table 2).

Table 1. Geographical location of quinoa (*Chenopodium quinoa*) materials collection sites.

Population	Municipality	Coordinates		Material	No. Morphotypes
1	Tuta	5°39′53.3′′N	73°10′13.9′′W	Blanca de Jericó	15
2	Siachoque	5°30′0.6′′N	73°29′52.6′′W	Piartal	3
3	Sogamoso	5° 40′41" N	72° 56′ 38"	Piartal	6
4	Monguí	5°43′21″N	72°50′57″W	Piartal	6
5	Tunja	5°33′16″N	73°21′09″W	Blanca de Jericó	6
6	Tunja	5°33′16″N	73°21′09″W	Piartal	6
7	Combita	5°38′02″N	73°19′23″W	Piartal	6
8	Siachoque	5°30′0.6′′N	73°29′52.6′′W	Blanca de Jericó	3
9	Porvenir	5°31′06.1′′N	73°23′47.1′′W	Blanca de Jericó	9

Table 2. Microsatellites used in the molecular characterization of quinoa materials (*Chenopodium quinoa*).

SSR	Principal Sequence	Primer Forward	Primer Reverse
QAAT106	(ACTACC)8ACTGTT(ATTGTT)23(ATT)5 GTT(ATT)2(ACT)11	tcagtaagataatacccatcagtaag	aaaatcccctctataattaccaa
QAAT50	QAAT50	atggcgaatggttaatttgc	ggcacgtgctgctactcata
QAAT70	(AAT)15	tgaacaggatcgtcatagtcaa	gcttctcgttcatcatctgacccaat
QAAT112	(ATT)13	cccgatccaccataagagaa	tgaagtgtaagattggagaatgaca
QAAT022	(TTA)29	tggtcgatatagatgaaccaaa	ggagcccagattgtatctca
KGA020	(CT)22	ggagcagatgatgaacatgg	gcttcttcacctacctcggtaaaggaaa
QCA088	(TG)10	cagtcccggaatcgtaactc	gcttcttctggctgcttccacctaat
KAAT007	(AAT)30	aggtacaggcgcaaggatac	cggtagcatagcacagaacg
KCAA106	(CAA)20	gcatgctcatcatttgttgc	atatggaagtcggccaacag
QCA005	(CA)16	cttgccatcagggcatatct	gtggttcatggctgatcctt

The amplification reaction was prepared in a final volume of 25 µL. The reaction mixture included Buffer 1X, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1U Taq Polymerase, 2 µM primer and 10 ng genomic DNA. The amplification was carried out in a PTC 100 Programmable Thermal Controller thermocycler (M.J. Research, Inc.). The initial denaturation was 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 2 min and final extension at 72°C for 5 min. The amplified products were separated into high resolution 2% agarose gels at 150 volts for one h and stained with Z-Vision.

The band pattern data were recorded in a numeric matrix where a consecutive was assigned for each of the alleles found per locus, and each individual was assigned a maximum of two values per locus, depending on the genotype (homozygous-heterozygous). For the selection of polymorphic bands, polymorphic loci were determined as ones in which the frequency of the most common allele was less than 95%. Statistical analyses were performed starting from this matrix and using NTSYS-PC (*Numerical Taxonomy System for Personal Computer*), TFPGA (*Tool for Population Genetic Analysis*) and Arlequin version 3.11.

The dendrogram was constructed from the similarity matrix by grouping the data with the UPGMA method (*Unweighted Pair-Group Method Arithmetic Average*) by means of the TREE program of NTSYS-PC (version 2.02®). The coefficient of cophenetic correlation was calculated, which is a measure between the similarity values of the dendrogram and those of the original similarity matrix, through the COPH and MXCOMP programs of the NTSYS-PC package. A multiple correspondence analysis (MCA) was performed to associate columns and rows of the binary matrix by determining the level of association or determining proximity.

The parameters of expected average heterozygosity (He) and the percentage of polymorphic *loci* (P) were used to estimate the genetic diversity, which will be estimated over all the *loci* and their average according to Nei's (1979) unbiased formula:

$$H = 1 - \sum f\,(i)^2 \qquad \text{(Eq. 1)}$$

Where H: Probability that two individuals randomly taken have different alleles. H is the population.

f (i): Frequency of the allele *i* in the population.

Σ *f(i)* 2: Probability that two individuals randomly chosen have the *i* allele.

Molecular variance analysis (AMOVA) was performed with the GenAlex V. 6.41 program. Population structure parameters were estimated taking into account Wright's (1978) statistics:

a) 0-0.05 = Little genetic differentiation

b) 0.05-0.15 = Moderate genetic differentiation

c) Greater 0.25 = Great genetic differentiation.

Finally, the polymorphism information content (PIC) was calculated for the 10 loci analyzed and the corresponding population structure was subsequently determined.

RESULTS

In the intra-population molecular characterization of the materials Piartal and Blanca de Jericó using 10 microsatellite markers in five municipalities of the Department of Boyacá, it was found that the expected heterozygosity values (He) were lower than the observed values (Ho) (Table 3). The KAAT006 marker presented the highest values, except in population nine. The general He average per population and per marker was moderate (0.5), except with markers KGA020, QCA020, QAA005, QAAT70 and QAAT50, in which most populations were low. None of the evaluated markers had Hardy Weinberg equilibrium (Table 3).

All microsatellites successfully amplified and were polymorphic. The highest average number of alleles per locus and per population was 2.3, found in populations six, eight and nine; the lower values were found in the remaining populations with a range between 1.9 and 1.7, respectively (Table 4).

The PIC values ranged from 0.44 (QAAT022) to 0.20 (QAAT70); the lowest value was observed with KGA020, while QAAT106 did not present information. The results were consistent with previously evaluated parameters (Table 4).

For most of the microsatellite loci, the F_{IS} values were significant with negative values, which meant that the populations presented an excess of heterozygotes. The populations were considered to be in Hardy Weinberg equilibrium when they had a $F_{IS} = 0$;

therefore, these populations did not meet this criterion and were not at WH equilibrium. For the Fst value, high levels of genetic structuring or cohesion were observed for all microsatellites, except QAAT70 (Table 5).

Table 3. Values obtained for genetic diversity, Ho: Observed heterozygosity, He: expected heterozygosity, for all populations evaluated of *Chenopodium quinoa*.; Na: Number of alleles, Ne: effective number of alleles.

	Population 1				Population 2				Population 3				Population 4				Population 5				Population 6				Population 7				Population 8				Population 9			
SSR	**Ho**	**He**	**Na**	**Ne**	**Ho**	**He**	**Na**	**Ne**	**Ho**	**He**	**Na**	**Ne**	**Ho**	**He**	**Na**	**Ne**	**Ho**	**He**	**Na**	**Ne**	**Ho**	**He**	**Na**	**Ne**	**Ho**	**He**	**Na**	**Ne**	**Ho**	**He**	**Na**	**Ne**	**Ho**	**He**	**Na**	**Ne**
QAAT106	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1	1
QAAT50	0	0	1	1	0	0	1	1	0.5	0.4	2	1.6	1	0.5	2	2	0.8	0.5	2	1.9	0.8	0.5	2	1.9	1	0.5	2	2	0.8	0.5	3	2.3	0.8	0.6	4	2.8
QAAT70	0	0	1	1	0	0	1	1	0	0	1	1	0	0.3	2	1.4	0	0	1	1	0.6	0.5	3	1.9	0	0.2	2	1.4	0.4	0.5	3	2	0.6	0.5	3	2.3
QAAT112	0.8	0.5	2	1.9	0.3	0.5	2	2	1	0.5	2	2	1	0.5	2	2	0.8	0.5	2	1.9	0.8	0.5	2	1.9	0.8	0.5	2	1.9	0.7	0.4	2	1.8	0.9	0.5	2	1.9
QAAT022	0.8	0.6	3	2.9	0.5	0.4	2	1.6	0.6	0.6	3	3	0	0.5	2	1.9	0.3	0.6	3	2.6	0.3	0.5	3	1.9	0.8	0.5	2	1.9	0.1	0.3	3	1.5	0.5	0.5	3	2.2
KGA020	0.2	0.2	2	1.2	0.6	0.4	2	1.8	0	0	1	1	0	0	1	1	0	0	1	1	0.2	0.2	2	1.2	0	0	1	1	0	0	1	1	0.3	0.3	2	1.4
QCA088	0.8	0.5	2	1.9	1	0.5	2	2	1	0.5	2	2	1	0.5	2	2	1	0.5	2	2	1	0.5	2	2	1	0.5	2	2	1	0.5	2	2	1	0.5	2	2
KCAA106	0	0	1	1	0	0	1	1	1	0.5	2	2	1	0.5	2	2	1	0.5	2	2	0.8	0.5	2	1.9	1	0.5	2	2	0.6	0.4	2	1.7	0.9	0.5	2	2
QCA005	1	0.5	2	2	0.3	0.2	2	1.3	0.8	0.5	2	1.9	0.7	0.4	2	1.8	0	0	1	1	0.3	0.3	2	1.4	0.3	0.3	2	1.4	0.9	0.5	2	1.9	0.4	0.3	2	1.5
KAAT006	0.8	0.5	2	1.9	1	0.5	2	2	1	0.5	2	2	1	0.5	2	2	1	0.5	2	2	0.5	0.5	2	1.9	1	0.5	2	2	0.9	0.5	2	1.9	0.2	0.3	2	1.5
Average	0.4	0.3	1.7	1.6	0.4	0.3	1.6	1.5	0.6	0.3	1.8	1.7	0.6	0.4	1.8	1.7	0.5	0.3	1.7	1.6	0.5	0.4	2.1	1.7	0.6	0.3	1.8	1.6	0.5	0.4	2.1	1.7	0.6	0.4	2.3	1.9
SD	0.3	0.1	0.2	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.06	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.1

Table 4. Estimated polymorphic information content (PIC) for the 10 microsatellite markers evaluated in *Chenopodium quinoa*.

SSR	PIC
QAAT106	0
QAAT50	0.32
QAAT70	0.20
QAAT112	0.37
QAAT022	0.44
KGA020	0.10
QCA088	0.37
KCAA106	0.29
QCA005	0.27
KAAT006	0.36
Average	0.2722

Table 5. Inbreeding coefficient values (FIS), genetic differentiation coefficient (FST) and total inbreeding coefficient (FIT), obtained for each of the 10 microsatellite markers and the probability value obtained in the Hardy equilibrium test. Weinberg (P-value) in *Chenopodium quinoa*.

SSR	F_{IS}	F_{ST}	F_{IT}
QAAT106	N/A	N/A	N/A
QAAT50	-0.631	0.217	-0.277
QAAT70	0.207	0.590	0.675
QAAT112	-0.643	0.188	-0.334
QAAT022	-0.069	0.266	0.215
KGA020	-0.295	0.165	-0.082
QCA088	-0.964	0.138	-0.693
KCAA106	-0.858	0.231	-0.428
QCA005	-0.549	0.35	0.005
KAAT006	-0.715	0.28	-0.227
Average	-0.502	0.27	-0.127
SD	0.120	0.04	0.126

The analysis of molecular variance (AMOVA) showed that 18% of the total variation was due to the difference between populations, with a Fst of 0.25 which was highly significant. However, the highest percentage of variation was in the analysis within the populations, 80% (Table 6).

Table 6. Hierarchical analysis of molecular variance (AMOVA) of the genetic variation of nine quinoa (*Chenopodium quinoa*) populations.

Source of variation	GL	Suma of squares	Components of variance	Percentage de variance	Fixation index
Between locations	8	54.8	0.42	17.93	F_{ST}: 0.18***
Within locations	99	188.6	1.90	82.07	F_{IT}: -0.17***
Total	107	243.5	2.32	100	------

The cluster analyses generated dendrograms (Figure 1) that were unique for each population. Population one (Tuta - Blanca de Jericó) had two clusters (Dendrogram. A) with a similarity of 0.90: the first group contained individuals 1PA, 2 PA, 3 PA and 5 PA, where 2 PA, 3 PA, and 5 PA were identical individuals even though their morphotype was different. With a similarity coefficient of 0.78, individual 4 PA was farthest from the rest because of the morphological characteristics of this material.

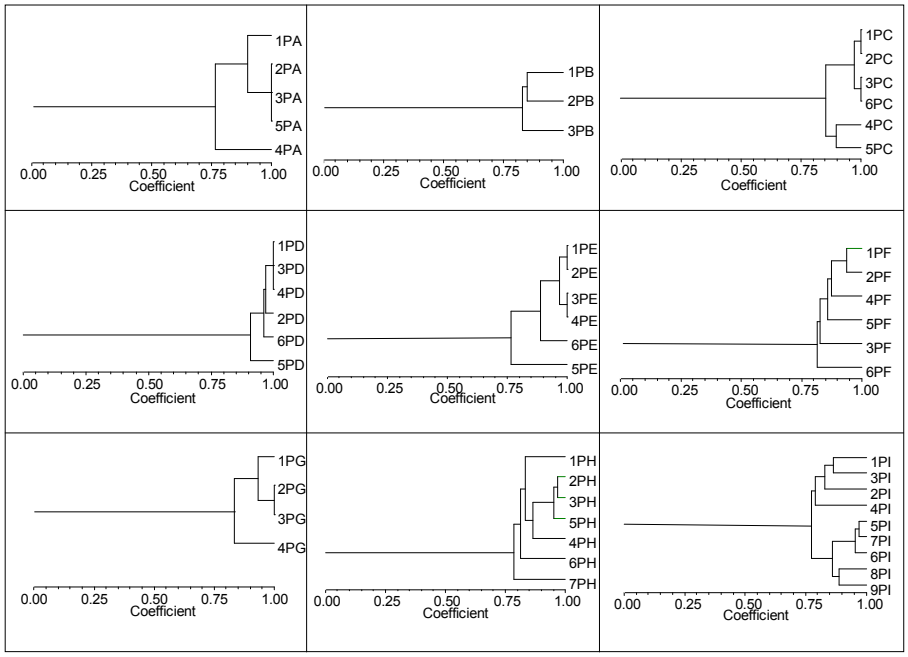

Figure 1. Dendrograms performed with the UPGMA classification method using data from ten microsatellite markers in 9 populations of *Chenopodium quinoa*. A: Population 1. B: Population 2. C: Population 3. D: Population 4. E: Population 5. F: Population 6. G: Population 7. H: Population 8. I: Population 9.

In dendrogram B, corresponding to population two (Siachoque - Piartal), two clearly differentiable groups were formed: the first group (1PB, 2PB) had a similarity of 0.90 and the s group (3PB) had a similarity of 0.77. In population three (Sogamoso -

Piartal), two defined groups were observed (C): the first consisted of the 1PC and 2PC materials, which, according to the similarity coefficient, were identical to 3PC and 6PC; the s group had 0.90 for the Piartal 4PC and 5PC individuals.

Dendrogram D represented the following populations (Monguí - Piartal): the first grouping had 1PD, 3PD, and 4PD, which were identical to individuals 2PD and 6PD (0.95); the third group contained 5PD (0.90). Population 8 (E), the material Blanca de Jericó in the municipality of Siachoque, presented two groups: the first one had genetically equal plants (1PE, 2PE and 3PE), grouped with 4PE and 6PE at a distance 0.95; the s one had the 5PE plant with a coefficient of 0.76.

The ninth population (F) formed two large groups: the first had a similarity coefficient of 0.80, made up of individuals (1PI, 3PI, 2PI and 4PI), where the greatest similarity was found at 1PI and 3PI (0.90). Group two was made up of two large subgroups, where the materials were 5PI, 7PI and 6PI at 0.95 and 8 PI and 9 PI at 0.90.

DISCUSSION

The results revealed intra-population genetic variability, showing that a rigorous selection process has not been carried out, which is why phenotypic variation can still be observed in in some plantingss (Infante et al., 2018; Madrid et al., 2018; Morillo et al., 2020). In this study, an average of 2.3 alleles/locus was found, which is an adequate value for the estimation of genetic parameters when compared to studies on genetic diversity in quinoa using microsatellite markers (Fuentes et al., 2009; Costa et al., 2012; Zhang et al., 2017).

Taking into account Ott's (1992) definition, which considers a marker as polymorphic if the heterozygosity (H) is greater than 0, equal to 0.1, and highly polymorphic if it is equal to or greater than 0.7, the microsatellite markers used in this study were polymorphic and informative (PIC = 0.20 QAAT70- PIC = 0.44 QAAT022), with the exception of the QAAT106 and KGA020 microsatellites (Table 3).

In the study carried out by Morillo et al. (2017a), where the genetic diversity of a quinoa collection was characterized in the Department of Boyacá with randomly amplified microsatellite markers (RAMs), it was found that the seven RAM primers generated a total of 178 bands, adequate for the estimation of genetic parameters (Vía and Férnandez, 2015; Oduwaye et al., 2014); the percentage of polymorphic loci ranged from 86.36% to 100%; therefore, the RAMs are polymorphic markers that can discriminate between closely related quinoa individuals (Vía and Fernández, 2015).

Different studies have shown that microsatellite markers have been successful in discriminating related materials, as well as in characterizing germplasm in different plant species (Jarvis et al., 2008; Costa et al., 2012; Fuentes et al., 2012; Suresh et al., 2014). Likewise, the more common sequences in the quinoa genome have been identified as CAA, GA and AT, which have been reported in genetic diversity studies and linkage mapping in quinoa using dominant and codominant markers (Mason et al., 2005; Jarvis et al., 2008; Al-Naggar et al., 2017; Morillo et al., 2017a).

The observed heterozygosity values were lower than the expected heterozygosity, with values between 0.4 and 0.6 for the evaluated populations, demonstrating a greater presence of homozygous individuals. The results were contrary to those obtained by Balaguera and Beleño (2018), who characterized the genetic diversity present in 19 quinoa

materials in the Department of Boyacá, finding an average of 3.5 alleles; ICP values between 0.49-0.80 for the eight microsatellites and the observed heterozygosity (Ho = 0.67) were higher than expected (He = 0.62), evidencing a low presence of homozygotes. Therefore, the results of this study demonstrate that the Piartal and Blanca de Jericó materials are highly diverse.

On the other hand, the heterozygosity values were higher than those reported by Costa et al. (2012), who evaluated the genetic structure of quinoa materials in Argentina (He = 0.16-0.42); by Zhang et al. (2017), who obtained values of Ht = 0.33 for highland quinoa and Ht = 0.38 for coastal quinoa; and by Morillo et al. (2017b), who characterized the genetic diversity of 55 quinoa materials with seven RAMs and found an average heterozygosity value of 0.34, much lower than that found in the populations evaluated in this study where there was still segregation of the characteristics in the individuals (Zurita et al., 2014; Infante et al., 2018; Morillo et al., 2020).

According to Kolano et al. (2016), high percentages of polymorphic loci and high heterozygosity values can be attributed to the allotetraploid origin of *C. quinoa* and related species, within *C. berlandieri* (Amarantaceae), and to the gene flow naturally present in quinoa populations. Quinoa is an autogamous species with a percentage of allogamy from 2% to 17%, depending on the variety and the planting density, so low intra-population genetic variability is expected, contrary to that observed in this study (Costa, 2014).

The polymorphic information content (PIC) values found in this study demonstrated that the microsatellites differentiated the evaluated quinoa materials (Table 4) and can be used for parental selection strategies for obtaining higher yield and identifying markers associated with characteristics of interest, within yield and saponin content, among others (Al-Naggar et al., 2017; Jarvis et al., 2017; Mestanza et al., 2017; Zhang et al., 2017).

A decrease in heterozygosity because of subdivision within a population has generally been quantified using an index known as Wright's F statistic (1978) or the fixation index, which varies from zero (indicating that there is no differentiation between the general population and subpopulations) up to a theoretical maximum of one. The analysis of genetic diversity with the F_{IS}, F_{ST} and F_{IT} values showed a great genetic differentiation between the individuals evaluated in the nine populations (Table 5). The negative mean F_{IS} (-0.5) and F_{IT} (-0.13) values show an excess of heterozygotes, which may indicate a tendency for exogamy in each population, as reinforced by the differences observed between the Ho and He, leading to a loss of HW balance.

The genetic differentiation coefficient obtained (F_{ST}) when evaluating the nine populations in the five municipalities of the Department of Boyacá was 0.25; according to Wright (1978), values between 0.15 and 0.25 show high differentiation genetics that may be associated with the level of population structure, which tends to stabilize. The increase in the number of heterozygotes may have been the result of the species' reproduction system, also reflecting the influence of cultural practices since the materials selected for this study may have different origins and some may have been through the exchange of seeds between neighboring farmers or individuals selecting seeds from a crop cycle after cycle, both without pollination control (Costa et al., 2012; Morillo et al., 2017b; Morillo et al., 2020; Rodríguez et al., 2020).

In the studies carried out by Morillo et al. (2017a) with ISSR markers, the analysis of the genetic structure showed a degree of differentiation between the evaluated individuals, while a deficiency of heterozygotes was revealed as a result of space-temporal

dynamic processes in these materials, in their natural environment and in the complex mating system of the quinoa species (Fuentes et al., 2012; Costa et al., 2012; Bazile et al., 2014). The QAAT70 microsatellite made the greatest contribution to the genetic variation, with a Fst of 0.59, which means that it can be useful for the differentiation of materials of the *Chenopodium* genus in studies on intra- or interspecific genetic diversity.

In this study, the average value of gene flow or migrants per population was 0.75, possibly due to the intrinsic characteristics of the materials, the sampling and the number of morphotypes evaluated. No HW balance was found because of the mating nature of the individuals or mainly because of the population size (Cuajapé-Castelles, 2006). It is possible that, since the Blanca de Jericó and Piartal quinoa materials are the most cultivated in the Department of Boyacá, some individual efforts by quinoa farmers have been concentrated there from the genetic point of view, increasing the selection processes for obtaining individuals with higher yields and resistance to biotic and abiotic factors, thus causing changes in the allelic frequencies of certain genes and favoring some genotypic forms; therefore, the populations are not in balance (Costa et al., 2014; Rodríguez et al., 2020).

The analysis of molecular variance showed that 82% of the genetic variability observed in the evaluated quinoa materials was due to a component within the groups, and that the remaining 18% was attributed to differences between the groups (Table 6), which indicated microgeographic studies at the intra-group level, which is where individuals with the best allelic combinations for production, industrialization and market characteristics can be identified, are necessary. However, 18% variation between groups is significant and can also be incorporated into species improvement strategies. Similar results were also reported by Morillo et al. (2017a), who found 77% of the variation within groups and 23% between groups using ISSRs markers; and by Balaguera and Beleño (2018), who found that of the observed variation, 80% corresponded to variation within the group and 20% was attributed to variation between the groups with microsatellite markers.

On the contrary, in the studies carried out by Zhang et al. (2017) with the sequencing of 129 quinoa materials and the development of InDels markers, AMOVA established the genetic relationships between quinoa groups from coastal areas and high mountain areas. The results showed that approximately 70% of the total variation was due to differences between the groups and the remaining 30% was due to variation within the groups or subgroups. Costa (2014), in the evaluation of the Argentine germplasm, observed that 18% of the total variance was due to the differentiation between regions, 39% between populations, 27% between individual plants and the remaining 16% was intra-individual.

The cluster analyses showed similarity values greater than 0.70 for the majority of the evaluated individuals in each of the populations; a lax distribution of materials was observed, associated more with morphological characteristics than with geographic origin, as reported in other studies on genetic diversity in quinoa (Costa et al., 2012; Maughan et al., 2012; Ruíz et al., 2014; Al-Naggar et al., 2017; Morillo et al., 2017 a,b). The high values of similarity between the materials that made up each subgroup can be attributed to the domestication processes that this species has been subjected to, the constant exchange of seeds between farmers from the same area or other producing areas of the country, the mating system, and the bottleneck events through which the species has passed, which has led to the loss of genetic diversity (Zurita et al., 2014). The high similarity values can be

exploited by breeding programs that implement hybridization strategies and seek to exploit the heterosis of the parents (Vía and Fernández, 2015).

In general terms, the groupings did not correspond to the geographical site where the materials were evaluated. The low variability in the evaluated individuals had already been reported in studies carried out in other Andean countries, which was expected because of the systematic selection processes carried out by farmers or breeders (Bazile et al., 2014). The genetic diversity of quinoa (*C. quinoa*) has managed to be maintained over time thanks to the traditions and ancestral knowledge of rural communities, which is why it is considered a family heritage crop. However, in recent years, the increase in demand in both the domestic and international markets has led to a substitution of native varieties for commercial ones, generating a loss of genetic diversity and increased homozygosity in the populations, making them more vulnerable to phytosanitary problems and reducing their ability to adapt to changing environments (Fuentes et al., 2012). Therefore, small scale preservation of the genetic diversity of local quinoa materials, which is strongly associated mainly with its cultivation, is essential. Conserving agrobiodiversity also means preserving the cultivation associated with rural communities in the Andean region (Bazile, 2014; Rodríguez et al., 2020).

Colombia has the genetic and agro-ecological potential to increase the cultivation of quinoa, especially in the Department of Boyacá, where there are more than 11 producing municipalities and producer associations. It also has a collection of materials with genetic diversity that can be harnessed in obtaining new and better materials that respond to the needs of farmers, producers and consumers. In addition, the intra-population study of the materials Blanca de Jericó and Piartal showed that we must continue working on more efficient selection processes for individuals that avoid cross-pollination and that will produce a certified variety of quinoa with higher yields, resistance to biotic and abiotic factors and adaption to the agroclimatological conditions of the Department of Boyacá.

CONCLUSIONS

The evaluation of the intrapopulation genetic diversity of the quinoa materials showed that there is interindividual variation as a result of the segregation of some characteristics that is not desirable since it reduces the quality and profitability of this crop. The microsatellite markers discriminated the materials and determined differences at the intra-population level; therefore, they can be used for the evaluation and selection of quinoa germplasm.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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