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Characterization of the genetic diversity of *Chenopodium quinoa* from the Department of Boyacá-Colombia using microsatellite markers

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Abstract. *Manjarres-Hernández EH, Morillo-Coronado AC, Morillo-Coronado Y. 2025. Characterization of the genetic diversity of* Chenopodium quinoa *from the Department of Boyacá-Colombia using microsatellite markers. Biodiversitas 26: 1239-1246. Chenopodium quinoa* is an Andean pseudocereal with excellent nutritional, pharmaceutical and industrial properties. In Colombia, this crop is considered marginal, and genetic studies are limited. The objective of this study was to characterize the genetic diversity present in 81 quinoa accessions from the Department of Boyacá using 13 microsatellite markers. Through the analysis of population structure, three populations developed, with an average expected heterozygosity of 0.472. The fixation indices in these three populations were less than 0.233, showing that there is no differentiation between the general population and the subpopulations. Analysis of molecular variance (AMOVA) showed that most of the genetic variation is found within populations (87%). The 13 microsatellites evaluated were highly informative, producing 42 alleles, which ranged from 2 to 4 per locus with an average of 3.051 alleles per locus. The microsatellites with the highest numbers of alleles were the KAAT037 and KGA03 loci (A=4,333). The values of expected heterozygosity (He) were lower than that observed (Ho), with an average of He=0.472. Both Fis and Fit confirmed a high number of heterozygotes compared to Hardy-Weinberg equilibrium conditions. Thus, microsatellite markers allowed genotypes to be differentiated according to morphological characteristics more than their origin. The findings of this study highlight the existence of significant genetic diversity in the quinoa accessions studied, which could be used in conservation and genetic improvement programs of this species.

Keywords: *Chenopodium quinoa*, genetic diversity, molecular characterization, population structure, SSR

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal that belongs to the Amarantaceae family, native to the Andean region of South America and has been widely cultivated in Bolivia, Peru, Chile and Colombia (Ain et al. 2023). It is traditionally used in the Andean region for human and animal consumption due to its excellent nutritional properties (Trotsenko et al. 2020). This species is allotetraploid (2n=4x=36) and its genome size is estimated to be 1448 megabases (Mb) (Zhang et al. 2017). Furthermore, quinoa exhibits wide intraspecific variability and plasticity in its phenotypic characteristics (Manjarres-Hernández et al. 2021), which gives it great adaptability to adverse climatic conditions (Morillo-Coronado et al. 2021; Pulvento and Bazile 2023) and tolerance to various abiotic stresses such as frost, salinity and drought (Ain et al. 2023). As a result, global demand for this grain has increased significantly, and its cultivation has expanded throughout the world in recent decades.

In Colombia, quinoa is grown in the departments of Cundinamarca, Nariño, Boyacá and Cauca, with an average yield of 2.17 t/ha (García-Parra et al. 2020), where it has competitive advantages, such as the potential to obtain two harvests per year with good agronomic management. In Boyacá, it is grown at an altitude between 2538 and 3031 m in the Centro, Tundama and Sugamuxi Provinces (Veloza et al. 2016), and presents great genetic diversity, represented in a wide range of phenotypic characters, due mainly to unique morphological characteristics given by the agroclimatological conditions and the coevolution processes of the species in the department (Manjarres-Hernández et al. 2021). Hence, this species requires collection, conservation, and molecular characterization studies for improvement (Morillo-Coronado et al. 2022).

Colombia lacks studies on quinoa conservation, management, and genetic improvement due to its status as a food security crop and the absence of improved materials. Although several tools have been developed for genetic characterization, currently, molecular markers are the most effective way to improve plant breeding efficiency (Flórez-Martínez et al. 2024). Genetic characterization is essential for the improvement of crops, generating valuable information for the conservation, selection and improvement of species. Moreover, contributes to the obtaining of new and better cultivars with desirable agronomic characteristics, including yield, quality and adaptation to adverse environmental conditions (El-Harty et al. 2021). In this

sense, genetic markers have been widely used for conservation studies and development of core collections, avoiding the influence of the environment (Abd El-Moneim et al. 2021). Several molecular markers have been used in determining the genetic variability, and phylogenetic relationships between different quinoa genotypes (Abd El-Moneim et al. 2021; El-Harty et al. 2021; Habib et al. 2024).

Molecular markers, especially simple repeat sequences or microsatellites (SSR), have become a fundamental tool for the identification of regions of the genome associated with traits of interest, through indirect selection or the marker-assisted selection method (Souri-Laki et al. 2024). Microsatellite markers have been one of the most used molecular markers in the identification of genetic diversity between and intra-species (El-Harty et al. 2021). Given their co-dominant inheritance, their wide distribution throughout the genome, the high level of polymorphism, their reproducibility, simplicity and effectiveness, make them useful for many applications (Habib et al. 2024). This genetic diversity reflects the wide range of quinoa ecotypes adapted to different agroecological zones in the Andean area (Flórez-Martínez et al. 2024), and also helps to establish the factors that influence population dynamics in the regions, and modify patterns of genetic diversity such as seed exchange or selection (Morillo-Coronado et al. 2022).

Taking into account that knowledge of the genetic structure of quinoa germplasm in Colombia is limited, and that molecular markers such as simple repeated sequences have been effective in identifying genetic variability in quinoa (Manjarres-Hernández and Morillo-Coronado 2022); The objective of this research was to characterize 81 quinoa accessions using microsatellite molecular markers, with a view to establishing strategies that allow the conservation and genetic improvement of the species.

MATERIALS AND METHODS

Vegetal material

Eighty-one quinoa accessions from the germplasm collection of the Boyacá Governor's Office, Colombia, originating from the municipalities of Cucaita, Duitama, Tibasosa, Siachoque, Tunja, Soracá, Oicaitá, Cerinza, and Motavita, were used for molecular characterization.

Molecular characterization

For molecular characterization, the seeds of each accession were germinated in greenhouse conditions at the Universidad Pedagógica y Tecnológica de Colombia using germination trays and substrate (soil+sand 2:1); After 4 weeks, 3 to 4 young leaves were collected, wrapped in aluminum foil and stored in a thermos with liquid nitrogen until use.

For DNA extraction, the Dellaporta protocol was modified by Muñoz et al. (2008) (the extraction buffer incubation time was extended from 20 to 30 min. After adding potassium acetate, the samples were incubated at -20°C for 40 min, without shaking, and then DNA was precipitated with isopropanol) was used. Total DNA was visualized on 0.8% agarose gels, in a Maxicell Primo EC-340 Electrophoresis Gel System chamber. To determine the DNA concentration, an EPOCH at 10 ng/µL, stored at -20°C.

Thirteen microsatellites (Table 1) were selected that were highly polymorphic, with heterozygosity (HE>0.7) and a high number of alleles per locus (>6), used in the genotyping of quinoa germplasm by Mason et al. (2005), Jarvis et al. (2008) and Manjarres-Hernández and Morillo-Coronado (2022). The SSR amplification reaction was prepared at a final volume of 25 µL. The reaction mixture included 1. buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 1U Taq polymerase, 2 µM primer, and 10 ng of genomic DNA. The amplification was carried out in a PTC 100 programmable thermal controller thermocycler (M.J. Research, Inc.). The amplification conditions included initial denaturation at 95°C for 1 min, followed by 35 denaturation cycles at 94°C for 30 s. For the hybridization, the temperatures depended on the primers (Table 1), with extension at 72°C for 1.5 min. The amplified products were separated on high resolution 2.5% agarose gels at 200 V for 2 h in a Maxicell Primo EC-340 Electrophoresis Gel System chamber and stained with GelRed.

Statistic analysis

The information on the banding patterns obtained in the evaluation of the 13 microsatellites, in the 81 quinoa accessions was recorded in a numerical 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, the locus in which the frequency of the most common allele was less than 95% was considered a polymorphic locus. From this matrix and using the NTSYS-PC (Numerical Taxonomy System for Personal Computer) and TFPGA (Tool for Population Genetic Analysis) programs, the statistical analyzes were carried out. The Nei-Li similarity index, which emphasizes the presence of alleles at each locus, was used to analyze the generated data.

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

Table 1. Microsatellites used in the molecular characterization of 81 accessions of *Chenopodium quinoa* from the department of Boyacá, Colombia

Identity	Primer forward	Primer reverse	Annealing temp. (°C)
KAAT037	TCAACCTCCGAATCCTATCAA	GGATGCTGATTGGTGGATAAA	57
KGA03	ATTGCCGACAATGAACGAAT	GCTTCTATGTAAATGGCATGTCCCAAC	58
QAAT024	GCTTCTGAACCTTTAATAGGTCTGTACCAAATC	AAGAAATGTCACAAGCAAGCA	56
QAAT50	GGCACGTGCTGCTACTCATA	ATGGCGAATGGTTAATTTGC	53
QAAT74	GCTTCTATGGAACACCCATCCGATAA	ATGCCTATCCTCATCCTCCA	56
QAAT022	TGGTCGATATAGATGAACCAAA	GGAGCCCAGATTGTATCTCA	58
KGA016	CCCTGCTTAATCTCCGTGAA	CCGAACCAAGACTACGAAACA	53
KGA20	GCTTCTTCACCTACCTCGGTAAAGGAAA	GGAGCAGATGATGAACATGG	58
QCA88	GCTTCTTCTGGCTGCTTCCACCTAAT	CAGTCCCGGAATCGTAACTC	53
QAAT78	AGCGAAGGAAATTTGGAACT	GCTTCTTAACGATACGCTCCAAGGAA	53
QGA032	GGTGAATTCGAATGGCAAAG	TGGCAGTTGGATCCACTATAAA	53
QCA096	CCATGTTATTGATGTCTTGTCCTC	CTGCTGGCTGTTAGCTGTTG	53
QCA005	GTGGTTCATGGCTGATCCTT	CTTGCCATCAGGGCATATCT	53

The diversity parameters were estimated for the three subgroups of the population detected as: number of alleles, private alleles, Shannon information index, % of polymorphic loci, expected heterozygosity and fixation index like Fst, the fixation index, is a measure of genetic differentiation that represents the proportion of total genetic variance attributable to differences among subpopulations (subscript S) relative to the total genetic variance (subscript T). Fst values range from 0 to 1, with higher values indicating greater population differentiation; Fis (inbreeding coefficient) measures the deficit of heterozygotes in each population and Fit (total inbreeding coefficient) calculates the overall heterozygous deficit. A molecular analysis of variance (AMOVA), was carried out to test if there were differences between and within the groups formed by the previous analyzes and thus determine where the greatest genetic diversity is found, using GenAlex 6.5 program.

The genetic diversity parameters were estimated for the three subgroups of the population detected as: number of alleles per locus (Na), number of effective number of alleles (Ne), private alleles, Shannon information index, % of polymorphic loci, fixation index, expected heterozygosity (He) and observed heterozygosity (Ho), using the POPGENE, GenAlex 6.5 and Microsatellite Tool Kit programs. An analysis of molecular variance (AMOVA) was performed to test if there were differences between and within the groups formed by the previous analyzes, and thus determine where the greatest genetic diversity is found. For this, the GenAlex 6.5 program was used.

To determine the existence of population structure of the 81 quinoa accessions, the grouping method based on a Bayesian model and a discriminant analysis of principal components (PCA) was used, using the STRUCTURE program version 2.3.4 (Pritchard et al. 2000). A mixed model of correlated allele frequencies was used, evaluating between 1 and 8 supopulations (K) with 10 repetitions and for each number of groups allowed in the analysis (K), 100.000 burn-in iterations and 1.000.000 steps of Markov chains Monte Carlo (MCMC). The parameter r was evaluated to know if the information about the localities was informative (Bolton et al. 2016). This optimal value was determined by observing the graphs of the log probability of the data (LnP(K)) and the ΔK values with the Pophelper program.

There should be sufficient information to allow other researchers to repeat the experiment by clearly defining the experimental design. A clear description or a specific reference to all biological, analytical, and statistical procedures is required. All procedure modifications must be explained. Field experiments that are sensitive to interactions and where the crop environment cannot be rigorously controlled, such as crop production and yield component assays, must be repeated for time and/or space, in order to ensure representative results.

RESULTS AND DISCUSSION

The molecular characterization of the 81 quinoa accessions showed through the Nei-Li coefficient that with a similarity level of 0.65 three groups were formed (Figure 1). These groupings were not established according to the collection area or place of origin, but rather according to the morphological characteristics of the accessions. These can be discriminated by the color and diameter of the inflorescence, plant height, number of inflorescences per plant, glomerulated panicle shape, grain diameter and weight of 1000 seeds (Manjarres-Hernández et al. 2021). A cophenetic correlation coefficient of 0.82 was found, and a similarity index between 85 and 97%, thus indicating a high degree of similarity between the evaluated quinoa accessions.

The formation of the three population groups was corroborated by the analysis of population structure using the STRUCTURE program (Pritchard et al. 2000) which showed the peak in delta K at K=3, suggesting the presence of three main populations (Figure 2.A). Thus, the quinoa accessions evaluated formed three genetic groups (Cluster 1, 2 and 3) (Figure 2.B), according to the phenotypic variation observed, however, no defined grouping pattern was observed, which may be associated with the allogamous nature of the species and the coevolution and domestication processes to which it is subjected in its natural environment. The average expected heterozygosity for the three groups was 0.472, with the highest value for population 3

(He=0.510), followed by population 1 with a He value of 0.497 and finally population 2 with He=0.409.

The genetic diversity values among the population groups identified both in STRUCTURE, and by the UPGMA classification method are presented in Table 2. The results showed intermediate genotypic diversity and variability within each population analyzed. The fixation indices in the three populations are less than 0.233, which shows that there is no significant differentiation between the general population and the subpopulations. The percentage of polymorphic loci was greater than 92%.

Pairwise comparisons of the Nei identity coefficient demonstrated that three populations are significantly interrelated with each other. Thus, population 2 and 3 appeared more related (NeiGI=0.892), population 1 and 2 present a genetic distance of identity of 0.827 (Table 2).

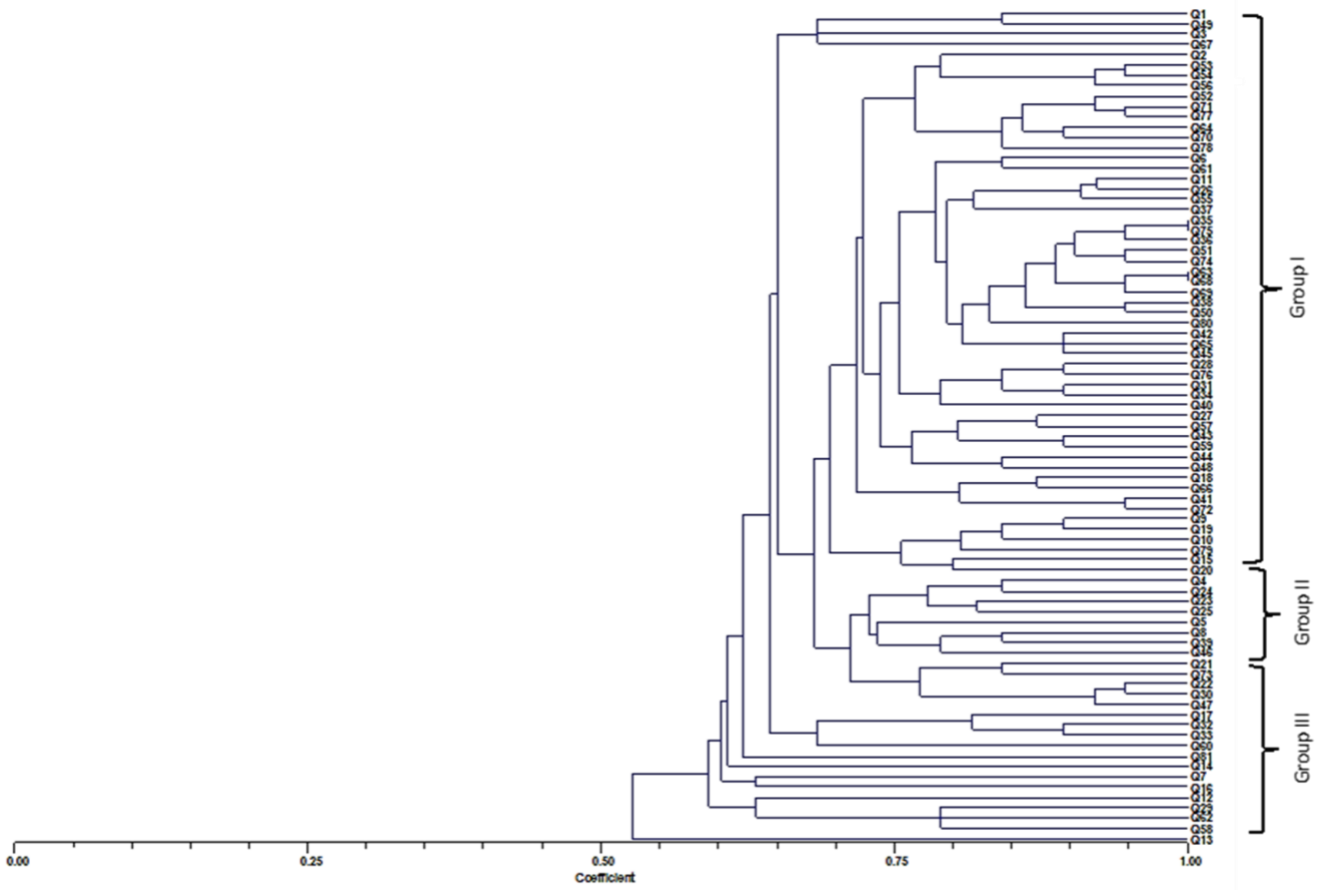
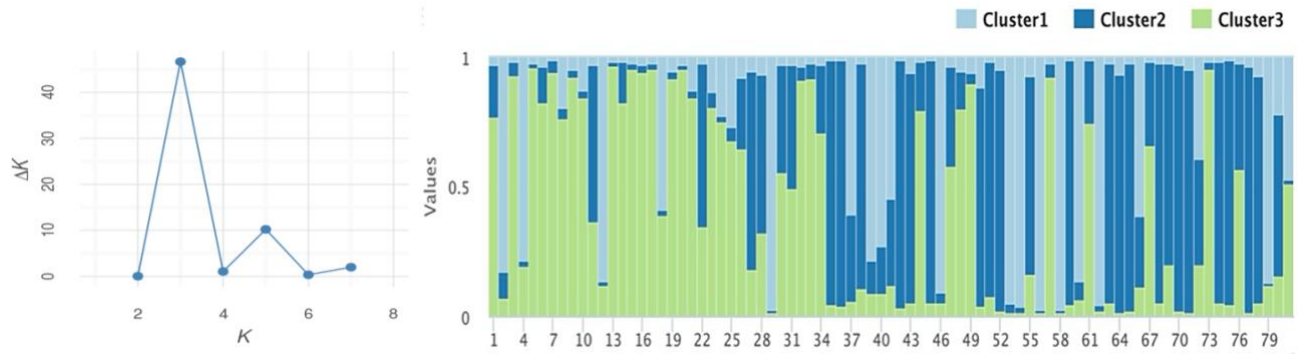


Figure 1. Dendrogram made with the UPGMA classification method, using data from 13 microsatellite markers in 81 *Chenopodium quinoa* accessions



A **B**

Figure 2. Population structure of 81 accessions of *Chenopodium quinoa*. A. Evanno method with an optimal model of K=3; B. Grouping of quinoa accessions, each vertical bar represents an individual sample and the color of the bar indicates the probability that an individual is assigned to one of the clusters

The analysis of molecular variance (AMOVA) showed that most of the genetic variation is in accordance with the differences observed within the populations (87%). In comparison, variability between populations was 13% and population differentiation was significant (PhipT=0.128, p<0.001) (Table 3).

13 SSR markers used to characterize the 81 quinoa accessions were highly informative and produced 42 alleles, which ranged between 2 to 4 per locus and an average of 3.05 alleles per locus. The markers with the highest numbers of alleles were the KAAT037 loci. and KGA03 (A=4.33), while the marker with the lowest number of alleles was KGA20 with 1,667 alleles (Table 4). The evaluation of the effectiveness of the markers was also observed through the average value corresponding to the number of effective alleles per polymorphic locus (Ne). This value was 2.142, ranging between 2.853 (QAAT78) and 1.239 (QCA88) (Table 4).

The estimated genetic diversity parameters showed that the expected heterozygosity (He) was lower than that observed, the quinoa accessions evaluated presented values between 0.183 (QCA88) to 0.649 (QAAT78), with an average of 0.472 (Table 4). These results suggest an intermediate degree of genetic diversity for Colombian quinoa, which agrees with the genetic behavior expected for a predominantly autogamous species. The average value of the Shannon Index was 0.80, The average value of the Shannon Index was 0.80, showing that genetic and phenotypic segregation still exists in the sampled individuals.

Table 3. Analysis of molecular variance (AMOVA) and population differentiation result (PhiPT) for quinoa populations obtained through SSR allele analysis

Source	df	SS	MS	Est. var	%
Among pops	2	23.377	11.689	0.355	13%
Within indiv	78	189.006	2.423	2.423	87%
Total	80	212.383		2.778	100%
PhiPT	0.128	*p*<0.001			

Notes: df: Degrees of freedom, SS: Suma of squares, MS: Var components of variance, %: Percentage of variance, PhiPT: Genetic differentiation between populations

Table 2. Genetic diversity parameters for the three population subgroups formed by the clustering analyses

Population	N	Na	Ne	I	Ho	He	F	%P
1	18	2.846	2.149	0.825	0.466	0.497	0.233	100.00%
2	28	2.769	1.947	0.677	0.464	0.409	0.114	92.31%
3	35	3.538	2.331	0.901	0.473	0.510	0.193	92.31%

Pairwise population matrix of Nei genetic identity

	1	2	3
1	1.000		
2	0.827	1.000	
3	0.784	0.892	1.000

Notes: N: population size, Na: Number of alleles per locus, Ne: Number of effective alleles per locus, I: Shannon information index, Ho: Observed heterozygosity, He: Expected heterozygosity, F: fixation index, %P: Percentage of polymorphic loci

Table 4. Genetic diversity parameters, Fst, Fis and Fit, obtained from the 13 Microsatellites used for the genotyping of the evaluated *Chenopodium quinoa* accessions

SSR	Na	Ne	I	He	Fst	Fis	Fit
KAAT037	4.333	2.583	1.063	0.608	0.009	-0.644	-0.629
KGA03	4.333	2.714	1.114	0.624	0.022	-0.602	-0.566
QAAT024	2.333	1.764	0.556	0.340	0.208	1.000	1.000
QAAT50	3.000	2.283	0.856	0.502	0.195	0.844	0.875
QAAT74	3.667	2.648	1.076	0.621	0.002	-0.611	-0.608
QAAT022	3.000	2.596	1.008	0.608	0.048	1.000	1.000
KGA016	3.667	2.561	1.025	0.601	0.010	-0.663	-0.647
KGA20	1.667	1.358	0.350	0.229	0.511	1.000	1.000
QCA88	2.000	1.239	0.320	0.183	0.033	1.000	1.000
QAAT78	4.000	2.853	1.185	0.649	0.026	-0.540	-0.500
QGA032	2.333	1.517	0.512	0.323	0.379	1.000	1.000
QCA096	3.000	2.220	0.854	0.547	0.009	-0.829	-0.813
QCA005	2.333	1.513	0.494	0.299	0.095	1.000	1.000
Average	3.051	2.142	0.801	0.472	0.119	0.277	0.239

Notes: Na: Number of alleles per locus, Ne: Number of effective alleles per locus, I: Shannon information index, He: Expected heterozygosity, Fst: Coefficient of genetic differentiation, Fis: Coefficient of inbreeding within populations, Fit: Total inbreeding

The KGA20 marker was the one with the highest Fst value (Fst=0.511), which shows that it can be useful for the differentiation of genotypes of the genus *Chenopodium* in studies of intra- and interspecific genetic diversity. While the average Fst value was 0.119 (Table 4), indicating that there is a moderate genetic differentiation of the accessions evaluated. The average Fis value obtained in the evaluation of the 13 microsatellite markers was 0.277 and ranged from -0.602 (KGA03) to 1.000 for the markers QAAT024, QAAT022, KGA20, QCA88, QGA032 and QCA005, where six of the thirteen markers evaluated had negative values, which shows an excess of heterozygotes, compared to what was expected under Hardy Weinberg equilibrium conditions. The Fit value, which corresponds to total inbreeding, was 0.239. Therefore, the three previous parameters are related and explain the differentiation in the evaluated quinoa accessions.

Discussion

Knowledge of the genetic diversity of quinoa constitutes a fundamental tool for the genetic improvement of the species. This genetic wealth has been preserved over time thanks to the traditions and ancestral knowledge of peasant communities, turning quinoa into a family legacy crop (García-Parra et al. 2020). However, in recent years, the increase in demand in both national and international markets has generated a tendency to replace native varieties with those of commercial origin, which leads to a significant loss of genetic diversity (Alandia et al. 2020).

Therefore, the collection, characterization and evaluation of the genetic diversity present in quinoa germplasm are fundamental processes that allow identifying genotypes, determining their usefulness, establishing core collections, detecting duplicates and facilitating data exchange. In addition, they promote its application in genetic improvement programs (Stanschewski et al. 2021). The results obtained in this study revealed that there is significant genetic diversity in the quinoa accessions evaluated, which allows for in situ or ex situ conservation strategies to be proposed with a view to the implementation of genetic improvement programs in this species (Anuradha et al. 2023).

In this study, an average of 3.05 alleles per locus was observed, which is considered an adequate value for the estimation of genetic parameters, when compared to studies of genetic diversity in quinoa using SSR markers (Salazar et al. 2019; Manjarres-Hernández and Morillo-Coronado 2022). As a fundamental component to study the survival of a species, it is the kowledge of its genetic diversity, which is determined by the number of alleles, effective alleles, polymorphic loci, observed and expected heterozygosity, genetic structure and spatial distribution of allelic variants (Almeida-Rocha et al. 2020). The effective number of alleles refers to the ability of alleles to pass to the next generation, and is a good indicator of the markers that make important contributions in genetic diversity studies, thus finding that the value of the effective number of alleles is very close to the number of total alleles found (Romero et al. 2019).

The SSRs markers used were highly polymorphic and informative, as had already been previously reported in studies of genetic diversity in quinoa, such as the one carried out by Souri-Laki et al. (2024) who used SSR markers to determine their association with phenotypic characteristics of interest, in this study a total of 140 scorable alleles with an average of 3.5 alleles per marker were amplified by 40 SSR markers, of which 136 alleles (97%) were polymorph. The relatively high diversity was also observed for most of the studied markers, but the markers KAAT023, KAAT027, KAAT036, and KGA006 showed the highest values, for most of the diversity indices in this research and can be recommended as the appropriate and informative markers to evaluate the genetic diversity of quinoa. The genetic diversity found can be useful in genotype selection programs for high yield, disease resistance or adaptation to adverse conditions (Manjarres-Hernández et al. 2021).

Through cluster analysis and population structure analysis, the quinoa germplasm formed three population groups that present varying degrees of genetic exchange between them. In addition, an association is observed more by morphological characteristics than by its geographical origin as had been reported previously in other genetic diversity studies (Romero et al. 2019; Barut et al. 2020). El-Harty et al. (2021) reported that ecological diversification improves the diversity of quinoa and that the cluster analysis grouped the accessions into three groups, according to their origin Peru, Bolivia, the United States and Ecuador, however, they mention that some genotypes do not they were aligned strictly by their geographical origin, which could be due to the wide range of agroecological adaptability and constant seed exchange.

On the other hand, heterozygosity can be considered an indicator of genetic variability in plant species, and its high values can be attributed to the low homozygosity (Table 4) found in the different genotypes (Morillo-Coronado et al. 2023). According to Subedi et al. (2021), the high percentages of polymorphic loci and the high values of heterozygosity may be related to the allotetraploid origin of *C. quinoa* and related species such as *C. berlandieri* (Amarantaceae), as well as to the gene flow present in quinoa populations. Studies of genetic diversity in the *Chenopodium* genus using SSR markers have shown high values of heterozygosity, which may be due to the nature of the markers (codominant), their distribution in the genome, reproductive system of the species in their natural environment (self-pollination, cross-pollination, seed dispersal), gene flow between wild and ancestral relatives (Habib et al. 2024). Previously, several research studies have demonstrated the population structure and diversity of quinoa using genetic markers (Manjarres-Hernández and Morillo-Coronado 2022; Souri-Laki et al. 2024). The observed variation will allow selection within genetic improvement programs of the species that seek to identify superior genotypes.

Furthermore, it must be considered that genotypes that are very heterogeneous for the traits of interest are not suitable for genetic studies. Quinoa is a predominantly autogamous species but has a natural residual heterozygosity of 10-17%, which can be lower or higher depending on the spacing between plants, the flow of pollen through the wind or perhaps the presence of pollinators (Habib et al.

2024). Therefore, the genetic diversity of quinoa is wide, due to less intensive genetic improvement processes (fewer bottlenecks in the population), and several quinoa accessions are landraces that produce a heterogeneous phenotype (Stanschewski et al. 2021).

Analysis of molecular variance (AMOVA) revealed that the highest percentage of variation occurs within (87%) and not between groups (13%) (Table 3). This high variation (87%) could indicate the presence of higher levels of subdivision and hierarchy. This genetic variation among groups could be used for the conservation and breeding of this species (Morillo-Coronado et al. 2023). 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. Similar results have been reported in other studies of genetic diversity in the genus *Chenopodium* using microsatellite markers (Romero et al. 2019; Morillo-Coronado et al. 2020; El-Harty et al. 2021).

The average Shannon diversity index evaluated in 81 quinoa accessions using 13 SSRs was 0.80 (Table 4), indicating a high genetic diversity. These results are similar to those reported in quinoa (Souri-Laki et al. 2024; Habib et al. 2024) and in other cross-pollinated species (Castañeda et al. 2020). This represents an advantage for crop improvement because species with high genetic diversity allow the selection of genotypes with agronomically favorable alleles (Flórez-Martínez et al. 2024). Likewise, the values of Fst, Fis and Fit revealed differentiation between the accessions, evidencing an increase in the number of heterozygotes. This is probably attributed to the reproduction system of quinoa and also reflects the influence of cultural practices, and domestication processes to which the species has been subjected (Manjarres-Hernández et al. 2021).

Quinoa is a crop that has very important agronomic and quality characteristics within the scenarios of food security and climate change, which is why it is necessary to expand knowledge about this crop. This research showed that there is unexplored genetic diversity, and that microsatellite markers effectively identified this variation in the evaluated quinoa germplasm. It also highlights the need to carry out targeted selection processes that lead to obtaining certified planting material for quinoa producers the Quinoa in Colombia (Morillo-Coronado et al. 2023; Flórez-Martinez et al. 2024).

Morpho-agronomic and molecular characterization studies carried out by Manjarres-Hernández et al. (2021) have already allowed the identification of some promotive genotypes, which could initiate the processes of seed increase and agronomic evaluation tests before the ICA (Colombian Agricultural Institute) to obtain the first quinoa varieties in the country.

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