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Article

Molecular characterization of wild and cultivated *Chenopodium berlandieri* (Chenopodiaceae) from central Mexico

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Abstract

The genus *Chenopodium* contains two species of importance in the diet of Mesoamerica and South America, namely *Chenopodium quinoa* Willd. (Quinoa) and *Chenopodium berlandieri* subsp. *nuttalliae*, the genetic resources of which have not been characterized despite their great nutritional potential and adaptability. In order to molecularly characterize germplasm of red chia, huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*) and quinoa (*Chenopodium quinoa* Willd.), we molecularly studied 48 genotypes from the Germplasm Banks of the National Institute of Nuclear Research and the Plant Genetic Resources Laboratory of Brigham Young University. To determine the genetic variability, 14 microsatellite markers (SSRs), specific for *Chenopodium*, were used. Genetic affinity was assessed using the Jaccard similarity coefficient and the analysis of results was performed using the UPGMA method. The results indicate that, within the studied genotypes of both species, 175 alleles were produced, ranging from 8 (KGA16, QCA88) to 16 (QCA37, QAAT74, QCA57), these being the ones that obtained the most alleles per locus. The dendrogram showed that, at a coefficient of 0.9, four main groups were formed, where groups 1 and 2 join advanced lines of quinoa and red chia, mutants of red chia and huauzontle, groups 3 and 4 joins chia and huauzontle, and group five includes all the germplasm of the Plant Genetic Resources Laboratory of BYU, mostly made up of subspecies of *Chenopodium zsachei*, *boscianum* and *zinatum*. It was concluded that there is a great genetic affinity between quinoa, huauzontle and red chia, which opens the possibility of inter- and intraspecific crosses for the genetic improvement of both species.

Keywords:

Chenopodium berlandieri subsp. nuttalliae, Chenopodium quinua, molecular markers, SSR.

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Introduction

In recent years, there has been a growing interest in recovering and valuing crops with high protein content and nutritional value, which have a promising potential for exploitation and that contribute to reducing malnutrition, an example of which is red chia (*Chenopodium berlandieri* subsp. *nuttalliae*), huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*) and quinoa (*Chenopodium quinoa* Willd), edible grain pseudocereals (García, 2017).

Pseudocereals are of great relevance since they are a biological resource of high nutritional value and great hardiness since they tolerate cold climates, drought, salinity, and poor soils (Eisa *et al*., 2012; Jacobsen *et al*., 2012). Due to these characteristics, they constitute a cultivation alternative for the marginal regions of the country (De la Cruz *et al*., 2010).

Within this group are quinoa, red chia, huauzontle, and species of the genus *Amaranthus* (Xingú-López, 2010). These crops had great food, economic and religious importance among pre-Hispanic civilizations since they constituted the basis of the diet, like corn (*Zea mays* L.) and beans (*Phaseolus vulgaris* L.); however, upon the arrival of the Spaniards, their cultivation and consumption were left behind and even prohibited, surviving in very remote areas (Ramírez *et al*., 2011). Red chia, huauzontle, and quinoa have exceptional nutritional qualities (12.5 to 16.7% protein, 5% lipids, and 58 to 76.2% carbohydrates) (Yasui *et al*., 2016).

The study of genetic diversity is very important for the conservation, evaluation and use of genetic resources for plant breeding and to determine the authenticity of cultivars or varieties, facilitating sustainable agriculture practices, which can lead to food sovereignty (Xingú, 2010).

There are currently several molecular techniques that allow us to know the genetic variability in natural populations. Thus, there are several types of molecular markers that are used in genetic improvement to obtain estimates of genetic distances between populations, varieties, lines or hybrids, as well as to establish kinship relationships between lines or varieties by detecting polymorphisms in single or multiple loci of dominant or co-dominant type (Xingú, 2010).

Molecular markers are also used for the genetic characterization of *Chenopodium* germplasm since they have been employed to differentiate genotypes under environmental conditions that confused their phenotypes (Nolasco *et al*., 2013). Simple sequence repeats (SSRs) are one of the frequently used molecular markers for genotyping crops (Jarvis *et al*., 2008).

Simple sequence repeats (SSR) microsatellites, also known as short or simple sequences, are repeats of mono-, di-, tri- and tetranucleotides made up of 2 to 10 base pairs as a basic unit, and are found throughout the genome of eukaryotic organisms in both coding and non-coding regions. Their technique requires little DNA, without having a high quality of purity, and provides highly polymorphic results, with its interpretation being relatively simple (Allende, 2014).

In order to determine the genetic variability in the germplasm of red chia, huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*), and quinoa (*Chenopodium quinoa* Willd.) collected in producing areas of the State of Mexico and wild materials from the United States, 48 materials were molecularly characterized, which include the collection of the National Institute of Nuclear Research of Mexico and the Plant Genetic Resources Laboratory of Brigham Young University, USA, using 14 primers for microsatellites developed specifically for *Chenopodium* by Maughan *et al*. (2013).

This characterization made it possible to determine the degree of variability within species, as well as the affinity within quinoa, huauzontle and chia, to design genetic improvement strategies through hybridization that allow combining desirable traits. This study also corroborated evolutionary work since it is considered that *Chenopodium quinoa* Willd. and *Chenopodium berlandieri* were independently domesticated, the latter in Mesoamerica and North America, whereas the former in South America (Maughan *et al*., 2024).

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Materials and methods

A total of 48 genotypes were evaluated, including varieties (a group of plants resulting from breeding work) and collections (samples taken in the field from cultivated and wild specimens) from the Germplasm Bank of the National Institute of Nuclear Research (ININ, for its acronym in Spanish) and the Germplasm Bank of the Plant Genetic Resources Laboratory of Brigham Young University.

Seed of the genus *Chenopodium* was used: three collections of *Chenopodium berlandieri* subsp. *nuttalliae* var. huauzontle (H3, H16, and H18 from the Toluca Valley), five collections of *Chenopodium berlandieri* subsp. *nuttalliae* var. red chia (J. Silva, D. Oros, R. Rguez, P. Bravo, and Zumbaro from the shores of Lake Pátzcuaro, Michoacán), two advanced lines of *Chenopodium quinoa* donated by the National Germplasm Bank of the College of Postgraduates (640304 and 11L240), four lines of *Chenopodium quinoa* obtained by radiation mutagenesis (ININ136, ININ240, ININ311, and ININ333), one F_1 line from the cross (42AdeM x Red Chia), and 33 collections of *Chenopodium berlandieri* ssp. donated from the Germplasm Bank of the BYU Plant Genetic Resources Laboratory (Table 1 and 2).

Table 1. Genetic material and origin of *Chenopodium* used for genetic diversity assessment using simple sequence repeats (SSR) (part 1).

Num.	Genotype	Species	Variety	Locality	City	State	Country
1	H-3	*C. berlandieri* subsp. *nuttalliae*	Huauzontle	Atlacomulco	Toluca	Sta. of Mex.	Mexico
2	H-16	*C. berlandieri* subsp. *nuttalliae*	Huauzontle	San Andrés, Cuexcontitlán	Toluca	Sta. of Mex.	Mexico
3	H-18	*C. berlandieri* subsp. *nuttalliae*	Huauzontle	Valle de Toluca	Toluca	Sta. of Mex.	Mexico
4	J.Silva	*C. berlandieri* subsp. *nuttalliae*	Red chia	Opopeo	S. Escalante	Mich.	Mexico
5	D.Oros	*C. berlandieri* subsp. *nuttalliae*	Red chia	Opopeo	S. Escalante	Mich.	Mexico
6	R.Rguez	*C. berlandieri* subsp. *nuttalliae*	Red chia	Sta. Ma. Huiramangaro	Pátzcuaro	Mich.	Mexico
7	P.Bravo	*C. berlandieri* subsp. *nuttalliae*	Red chia	Opopeo	S. Escalante	Mich.	Mexico
8	Zumbaro	*C. berlandieri* subsp. *nuttalliae*	Red chia	Sta. Ma. Huiramangaro	Pátzcuaro	Mich.	Mexico
9	640304	*C. quinoa*	Quinoa	C.P.	Texcoco	Sta. of Mex.	Mexico
10	11L240	*C. quinoa*	Quinoa	C.P.	Texcoco	Sta. of Mex.	Mexico
11	ININ136	*C. quinoa*	Mutant quinoa	ININ	La Marquesa	Sta. of Mex.	Mexico
12	ININ240	*C. quinoa*	Mutant quinoa	ININ	La Marquesa	Sta. of Mex.	Mexico
13	42AdeM x CR	*C. quinoa* x *C. berlandieri* subsp. *nuttalliae*	cross F1	ININ	La Marquesa	Sta. of Mex.	Mexico
14	ININ311	*C. quinoa*	Mutant quinoa	ININ	La Marquesa	Sta. of Mex.	Mexico

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Table 2. Genetic material and origin of *Chenopodium* used for genetic diversity assessment using simple sequence repeats (SSR) (part 2).

Num.	Genotype	Species	Variety	Locality	City	State	Country
15	ININ333	*C. quinoa*	Mutant quinoa	ININ	La Marquesa	Sta. of Mex.	Mexico
16	HBYUMEX	*C. berlandieri*	Huauzontle	Provo	BYU	UT	USA
17	BYU 14108	*C. berlandieri*	Sinuatum	AZ Hwy 181	Cochise	UT	USA
18	402	*C. berlandieri*	-	Torrey Pines	San Diego	CA	USA
19	423	*C. berlandieri*	Zschackei	-	LA	CA	USA
20	447	*C. berlandieri*	Zschackei	Orem	Utah	UT	USA
21	457	*C. berlandieri*	Zschackei	-	Duchesne	UT	USA
22	505	*C. berlandieri*	Zschackei	-	Garfield	UT	USA
23	544	*C. berlandieri*	Zschackei	-	Yavapai	AZ	USA
24	629	*C. berlandieri*	Zschackei	S of Lusk	Niobrara	WY	USA
25	641	*C. berlandieri*	Zschackei	Pine Creek Ranch	Sanpete	UT	USA
26	642	*C. berlandieri*	Zschackei	1 mi S of Ephraim	Sanpete	UT	USA
27	880	*C. berlandieri*	Zschackei	Ramah	McKinley	NM	USA
28	881	*C. berlandieri*	Zschackei	Provo	Utah	UT	USA
29	882	*C. berlandieri*	Zschackei	Spanish Fork Cyn	Utah	UT	USA
30	902	*C. berlandieri*	Zschackei	Laguna Mts	San Diego	CA	USA
31	922	*C. berlandieri*	Zschackei	BYU	Provo	UT	USA
32	937	*C. berlandieri*	Boscianum	Galveston, Virginia Point	Brazoria	TX	USA
33	1007	*C. berlandieri*	Zschackei	Kyle Cyn. Rd., Spring Mts	Clark	NV	USA
34	1301	*C. berlandieri*	Boscianum	Eagle Point Marina, St. Leon	Galveston	TX	USA
35	1303	*C. berlandieri*	-	Kamas Valley	Summit	UT	USA
36	1306	*C. berlandieri*	-	N Amstrong Rd	Clark	NV	USA
37	1312	*C. berlandieri*	-	Cty Rd C	St. Charles	MO	USA
38	1316	*C. berlandieri*	-	N P. I-15 Frontage RD	Iron	UT	USA
39	1448	*C. berlandieri*	Zschackei	Sherman Oaks	LA	CA	USA
40	1449	*C. berlandieri* + *C. boscianum*	-	Sherman Oaks	LA	CA	USA
41	1452	*C. berlandieri*	Zschackei	Big Tujunga Cyn.	LA	CA	USA
42	1454	*C. album*	-	Big Tujunga Cyn.	LA	CA	USA
43	1455	*C. berlandieri*	Boscianum	Cypremort Point	St. Mary	LA	USA
44	1456	*C. berlandieri*	Boscianum	Cypremort Point	St. Mary	LA	USA
45	1457	*C. berlandieri*	Boscianum	Golden Meadow	Lafourche	LA	USA
46	1458	*C. berlandieri*	Boscianum	Golden Meadow	Lafourche	LA	USA
47	1459	*C. berlandieri*	Boscianum	Point Fourchon	Lafourche	LA	USA
48	1460	*C. berlandieri*	Boscianum	Grand Isle	Jefferson	LA	USA

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Molecular characterization

For DNA extraction, the tissue used was healthy leaf tissue from 10 individual plants 30 days after sowing (das) established under greenhouse conditions. The leaf tissue sample (four leaves) was introduced into Eppendorf microtubes to be placed in a LABIST FDL1R-1[a] freeze-drying chamber with a freezing dryer at 0.7 atm pressure for 24 h.

The freeze-dried leaf tissue was ground in a Retsch-Mill 200. DNA extraction was performed according to the procedures described by Maughan *et al*. (2013). The extracted DNA was quantified with a GBC Nanodrop spectrophotometer and diluted to 30 ng μl^{-1} in TE buffer solution (Tris 10 mM, EDTA 1 mM, pH 7.5).

All plants were grown in the greenhouse of the Plant Genetic Resources Laboratory of Brigham Young University in Provo, Utah, USA, in 15 cm pots at 25 °C under halogen lamps with a photoperiod of 12 h.

SSR primers used

Fourteen microsatellite primers (Table 3) developed by Mason *et al*. (2005), specific for *Chenopodium*, were used for the study of genetic diversity of the 48 genotypes, namely: QCA37, KGA20, QAAT74, QAAT50, QAAT70, QGA02, QCA14, KGA16, QCA57, QCA88, QAAT76, QAAT78, QCA38, and QAAT24.

Table 3. List of primers and sequences used in the study.

Num.	Primer	Forward sequence (5'- 3')	Reverse sequence (5'-3')	T (°C)
1	QCA37	gcttctccgttcttccagaccaattc	tcatgagccacttcatacacg	66
2	KGA20	gcttcttcacctacctcggtaaaggaaa	ggagcagatgatgaacatgg	64
3	QAAT74	gcttctatggaacacccatccgataa	atgcctatcctcatcctcca	66
4	QAAT50	ggcacgtgctgctactcata	gcttctatggcaatggttaaatttgc	68
5	QAAT70	tgaacaggatcgtcatagtcaa	gcttctcgttcatcatctgacccaat	64
6	QGA02	gcttctgaacctttaataggtcgtaccaaatc	aagaaatgtcacaagcaagca	64
7	QCA14	gcttctccctgagctgatttatcaaaggac	cctcttgcgagatttctgct	66
8	KGA16	ccctgcttaatctccgtgaa	gcttctccgaaccaagactacgaaaca	65
9	QCA57	gcttcttgcaaggaaaccatctttgg	tgcctcacagtcacacctaca	69
10	QCA88	gcttcttctggctgcttccacctaat	cagtcccggaatcgtaactc	66
11	QAAT76	gcttcatgtgttataaaatgccaat	gcttcttctcggcttcccactaatttt	63
12	QAAT78	agcgaaggaaatttggaact	gcttcttaacgatacgctccaaggaa	63
13	QCA38	gcttctcatttcccaaactgcatgaat	atgtgtgttgcgtgtgagtg	67
14	QAAT24	gcttctaccataacagcacccaccttt	agggatcaatcttgttcattca	62

SSR amplification by PCR

PCR amplifications were performed in 12 µl reactions consisting of 3 µl (30 ng μl^{-1}) of DNA, 0.5 µl of every 10 µm of forward and reverse primers, 6 µl of MyTaq HS Red Master Mix (Bioline, Taunton, Massachusetts, USA) and 2 µl of H_2O. PCR reactions were performed using a C1000 or T100 thermal cycler (Bio-Rad, Applied Biosystems, Foster City, California, USA) with the following parameters: 95 °C for 60 s, 35 cycles of 95 °C for 15 s, 60 °C for 15 s, 72 °C for 10 s, and a final extension cycle of 72 °C for 60 s.

Amplified product electrophoresis

Electrophoresis of the amplified products was performed with 1.5% agarose gel (250 ml of TBE, 7.5 g of agarose, and 12.5 µl of midori green). DNA samples were run in an electrophoresis chamber with Bio-Rad power supply (Power-PAC 300, Berkely, Ca.) for 30 min. At the end of this time,

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the gel was washed with distilled water and placed in a Bio-Rad Universal Hood II ultraviolet (UV) light transilluminator and the gels were recorded and stored in a database using the Quantity one program.

Statistical analysis

For the statistical analysis, a binary matrix of absence (0) and presence (1) was generated. Diffuse bands were not considered, genetic similarity between individuals was assessed using the Jaccard similarity coefficient. The cluster analysis was performed using the UPGMA method. The corresponding dendrogram was generated using the statistical package of the numerical taxonomy system for personal computers (NTSYS), PC 2.02 version, to determine the similarities between the genotypes in question.

Results and discussion

The results indicate that, within the population of 48 genotypes from the Germplasm Banks of the National Institute of Nuclear Research and the Plant Genetic Resources Laboratory of BYU, they produced 175 alleles at 14 SSR loci. These alleles vary from 8 (KGA16, QCA88) to 16 (QCA37, QAAT74, QCA57), these being the loci with the highest number of observed alleles.

The primer QAAT74, according to Ormeño's (2015) work, is one of those with the highest number of observed alleles, whereas QCA88 presented the fewest alleles and was used in Donaire's (2018) work, where a high number of alleles was also recorded. This indicates that, in each job where they are used, they act differently.

Clustering analysis

The analysis was performed using data obtained from 14 loci recorded for 48 genotypes. From the results obtained, it is possible to have an approximate idea about the genetic diversity of the samples analyzed contained in the information from the microsatellites. The objective of cluster analysis is to form groups where the individuals in each group are more similar to each other than to the individuals of another group (Allende, 2017). To visualize the relationships between populations according to their distance, a hierarchical dendrogram was constructed.

The dendrogram in Figure 1 shows that, at a similarity coefficient of 0.9, five groups were formed; in contrast, in Ormeño (2015), where only 16 genotypes were evaluated, six groups were formed and in Xingú (2018), where 38 genotypes were evaluated, 10 fewer than in this study, six groups were formed.

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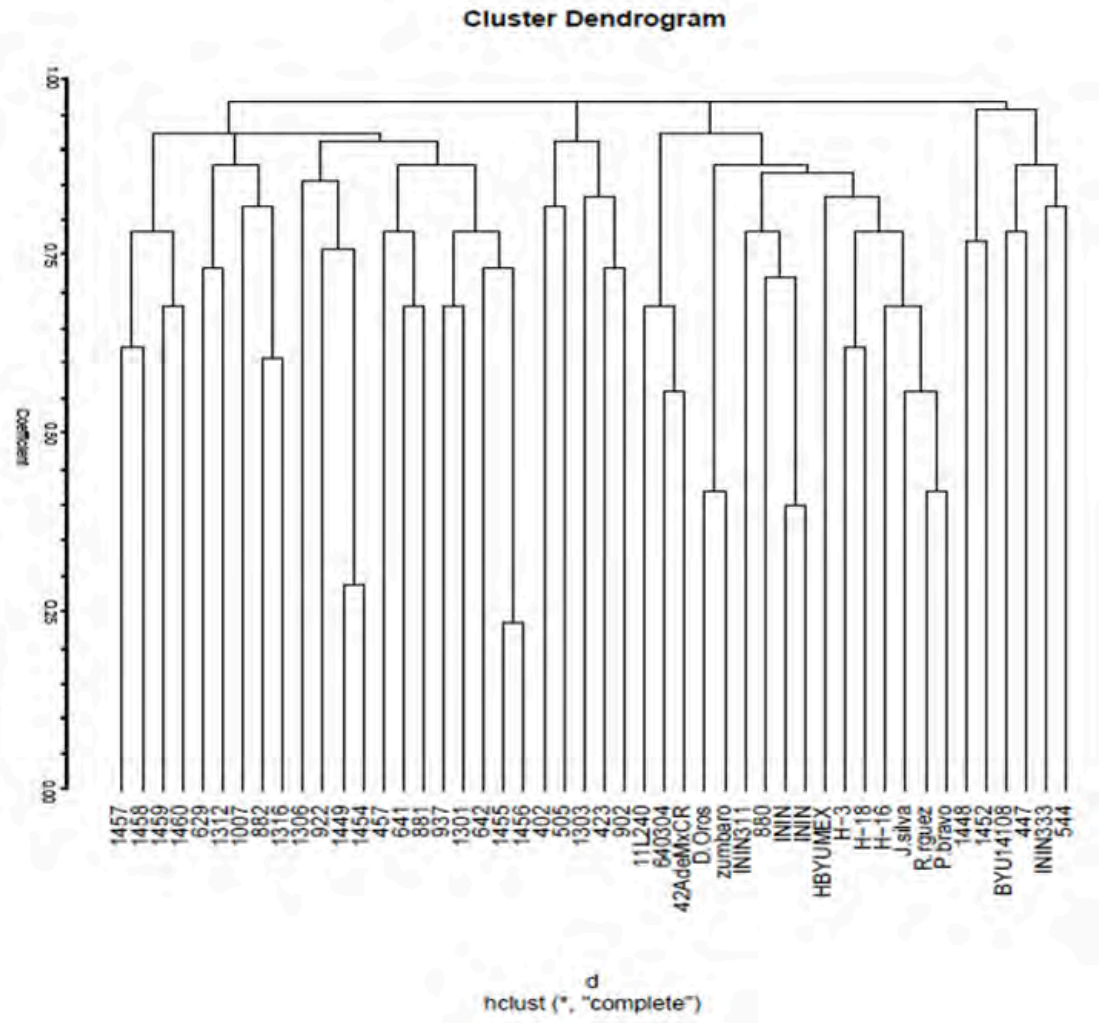
Figure 1. Dendrogram of 48 genotypes of *Chenopodium berlandieri* subsp. *nuttalliae* and *Chenopodium quinoa* from molecular data of SSR based on genetic distance by the UPGMA method.



Group 1 consisted of four genotypes, two advanced lines (11L240 and 640304) of quinoa, an F1 single cross between quinoa and red chia (42AdeMXCR), and a red chia (D. Oros).

Group 2 consisted of seven genotypes, one red chia (Zumbaro), three mutant quinoas (ININ311, ININ136, and ININ240), two huauzontle genotypes (H-3 and H-18), and a collection of BYU from *Chenopodium berlandieri* (HBYUMEX); in contrast to Allende (2014), there is a separation between the huauzontles and the chias.

Groups 3, 4 and 5 only had two genotypes each. Group 3 had a huauzontle (H-16) and a red chia (J. Silva). Group 4 had two genotypes of red chia (R. Rguez and P. Bravo). Group 5 was made up of a collection of BYU from *C. berlandieri* (BYU14108) and a mutant quinoa (ININ333).

Conclusions

The following conclusions were derived from the present research: Great genetic affinity was detected between the species *C. quinoa* Willd. and *C. berlandieri* since the primers designed for quinoa adequately amplified for huauzontle. A high genetic affinity was detected between the cultivated genotypes of C. quinoa and C. berlandieri subsp. nuttalliae local breeds red chia and huauzontle, which were only in groups 1, 2, 3 and 4.

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The genetic affinity between cultivated accessions allows us to predict favorable results in genetic improvement work by hybridization between *C. quinoa* Willd and *C. berlandieri* subsp. *nuttalliae*. The dendrogram shows two very interesting groups, such as groups 1 and 2, where the advanced quinoa lines join with red chia and mutant quinoas with red chia and huauzontle and groups 3 and 4 had all the germplasm from the Plant Genetic Resources Laboratory of BYU.

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