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Article

Genetic variability in some cultivated and wild amaranth species

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Abstract

The genus Amaranthus is widely distributed in America. The study of genetic diversity within and between populations and species of Amaranthus is important to plan strategies for their conservation and continuity. In the present study, 2 species of cultivated species (*A. hypochondriacus* and *A. cruentus*) and 5 wild species (*A. hybridus*, *A. retroflexus*, *A. powellii*, *A. palmeri* and *A. spinosus*) were evaluated using ISSR-type molecular markers. The 154 *loci* were analyzed, finding that the average polymorphism percentage for the initiators was 97.9%. Cultivated amaranths were genetically closely related to each other and to their possible wild progenitors (*A. hybridus* and *A. powellii*). Among the wild materials those that were closest were *A. hybridus*, *A. powellii* and *A. retroflexus*, while *A. spinosus* and *A. palmeri* were the most distant. Most of the detectable genetic diversity was found between species and populations, while the least part was within them.

Keywords: *Amaranthus* spp., genetic differentiation, genetic relationships, ISSR.

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Introduction

Amaranth is a predominantly tropical annual herbaceous plant that belongs to the genus *Amaranthus*. This genus contains more than 70 species of which the majority are native to America and only 15 come from Europe, Asia, Africa or Australia. In the USA and Mexico have approximately 29 species. The genus *Amaranthus* is widely distributed in America, showing great genetic variability, which is observed when observing plant characteristics such as inflorescence type, seed color, precocity, protein content of the seed and resistance to pests and diseases (Akaneme and Ani, 2013; Erum *et al*., 2012; Mwase *et al*., 2014). It is an easy crop to establish since it thrives in rainfed regions where basic crops have little success, adapts to various types of soil, altitudes, temperatures and photoperiod, as well as various pH and precipitation requirements (Espitia *et al*., 2010).

Within the genus are the species *A. cruentus, A. hypochondriacus* and *A. caudatus* which are the most important for the production of amaranth grain. The first two are widely distributed in Mexico, forming the most important center of diversity (Espitia *et al*., 2010; Ruiz *et al*., 2013).

Mexico is considered a mega-diverse country because of its high number of species, but also because of its richness of endemisms (exclusive species of Mexico), of ecosystems and because of the great genetic variability shown in many taxonomic groups, the result of evolution or natural diversification and cultural in the country, in its territory are between 4 and 8% of the total number of plant species in the world, of which 51% are endemic. For this reason, there is great concern about the conservation of mexican flora, since several activities and factors that threaten it have been detected (Sosa and De-Nova, 2012).

Genetic variability in the broad sense is the most basic component of biodiversity and is defined as the heritable variations that occur in each organism, between individuals in a population and between populations within a species. In the characterization of a species, the variability existing in the genome of the population of individuals that comprise it is estimated (Piñero, 2008).

The study of genetic diversity within and between populations and species of *Amaranthus* is important to plan conservation strategies for the continuity of such species and populations, as well as to meet the demands of changing environments through the genetic improvement of amaranth and this It will depend to a large extent on the magnitude of the variability available in your gene pool. Therefore, the objective of the present investigation was to analyze the genetic diversity within and between the populations and species of *Amaranthus*, as well as to compare genotypic identities among the accessions of the seven mexican species under study.

Materials and methods

Vegetal material

Two cultivated species and five wild amaranth species were studied, represented by 13 national amaranth accessions, previously identified morphologically and coming from the INIFAP Genetic Resources program, Valley of Mexico Experimental Field located in Coatlinchan, Texcoco,

México (Table 1). The collections and the sampling strategies that were followed for them were determined based on the mandate of the Germplasm Bank and the objectives of the collection. From each accession, 5 individuals were evaluated for the genetic diversity analysis.

Table 1. Accessions used for the analysis of genetic diversity in Amaranthus populations.

Species	Accession	Abbreviation	Type of sex
A. hypochondriacus	Tlax-089	89HYP	Monoecious
	Pue-027	27HYP	
A. cruentus	Gto-092	92CRU	Monoecious
	Mor-042	42CRU	
A. hybridus	Silv-39	39HYB	Monoecious
	Silv-43	43HYB	
A. retroflexus	Silv-26	26RETR	Monoecious
	Silv-38	38RETR	
A. powellii	Silv-49	49POW	Monoecious
	Silv-50	50POW	
A. palmeri	Silv-27	27PALM	Dioic
	Silv-35	35PALM	
A. spinosus	Silv-47	47SPIN	Monoecious

DNA purification

The DNA extraction was done according to the protocol of De la Cruz *et al*. (1997), with some modifications. Young leaves (0.3 g) were taken and macerated in liquid nitrogen. The macerate was transferred to a 1.5 mL micro tube (Eppendorf) containing 600 µL of extraction buffer (20 mL 1 M Tris-HCL, pH 8, 20 mL 0.5 M EDTA, pH 8, 20 mL 5 M NaCl; µL β-Mercaptoethanol, 40 mL of sodium dodecyl sulphate 20%) and incubated at 65 °C for 10 min in a Standard Heatblock of VWR® Scientific Products, with occasional inversion of the tubes. Then, 200 µL of 5M potassium acetate was added, mixed by inversion and incubated for 30 min at 0 °C.

It was centrifuged at 15 000x g for 10 min at room temperature, and the supernatant was transferred to another tube containing 700 µL cold isopropanol (-20 °C). It was mixed by inversion and incubated at -20 °C for 30 min, then centrifuged for 5 min at 10 000 x g at room temperature. The supernatant was removed and the precipitate was dissolved in 200 µL of dilute solution (50 mM Tris-HCl, 10 mM EDTA-Na2, pH 8), stirred gently until the precipitate was well diluted. 2 µL of RNase A was added and incubated at 37 °C for 1 h in a Felisa® incubator.

Then 20 µL of 3M sodium acetate plus 200 µL of isopropanol was added, mixed by inversion and allowed to precipitate at -20 °C for 2 h. It was centrifuged at 10 000 × g for 5 min at room temperature. The supernatant was removed and the precipitate was washed with 300 µL of 70% ethanol. The pellet was dried and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA-Na2, pH 8) and finally stored at 4 °C. The concentration of the DNA was determined using a Genesys 10 UV Scanning® spectrophotometer (Thermo Scientific) and the quality thereof was observed by electrophoresis in a 0.8% agarose gel. The DNA was used in subsequent PCR reactions.

PCR reaction

The DNA concentration of each sample was adjusted to 10 ng·μL^{-1} and 10 primers previously selected were used for the amplification (Table 2). The amplification reactions were performed in a volume of 25 μL, which included 3.2 μL of sterile double distilled water, 10 μL of dNTPs (500 μM), 2.5 μL of 10X buffer (Tris-HCl 750 mM, pH 8.8; $(NH_4)_2S0_4$ 200 mM; Tween 20 at 1% (v/v)); 2 μL $MgCl_2$ (25 μM); 3 μL of initiator at a concentration of 10 pM; 0.3 μL of Taq DNA polymerase enzyme at a concentration of 5U μL^{-1} and 4 μL of genomic DNA at a concentration of 10 ng μL^{-1}. The PCR reactions were performed in a Techne® 28 TC-512 thermal cycler. The reaction conditions were: an initial denaturation step at 94 °C for 3 min, 30 cycles with denaturation at 94 °C for 1 min, alignment at 50 °C for 1 min, and extension at 72 °C for 2 min and finally a final extension cycle at 72 °C for 10 min.

The amplified fragments were separated by electrophoresis in agarose gels at a concentration of 1.2% (w/v) with TAE buffer (40 mM Tris-acetate, pH 7.6, 1 mM Na_2 EDTA), from 1 h to 120 V. For the sagging of the samples, 12 μL of the PCR reaction product were used adding 1 μL of charge buffer (0.1% bromophenol blue, 0.1% xylen cyanol, 30% Ficoll and 1% SDS). For reference, the 1kb DNA ladder plus marker (GeneRuler) was used. The gels were stained with ethidium bromide (0.5 mg mL^{-1}) for 5 min and the excess dye was eliminated by rinsing in water for 5 min and documented using the Kodak® High Perfomance Ultraviolet Transilluminator EDAS-290, under UV light.

Data analysis

The materials were compared based on similarities and differences in banding patterns, assigning a value of 1 to the presence of a band and 0 to the absence of it, assuming that bands of equal molecular weight are identical between populations and species, building a Basic Data Matrix (MBD). With the use of the POPGENE 32 program (Yeh *et al*., 1999), the following parameters were estimated: percentage of polymorphic *loci* for the species and accessions considered as populations, the Nei genetic diversity coefficient, the Shannon information index, the G_{ST} parameter or coefficient of genetic differentiation between species and between populations, and the *Nm* or number of effective migrants, as well as the matrix of distances and genetic identities of Nei (1973).

In addition, with the software NTSYS-pc version 2.2 (Rohlf, 2000) the dendrogram generated in the POPGENE 32 program was visualized. Finally, the present variation among the species and populations was quantified by the molecular analysis of variance (Amova) using the GenAlEx 6.5 program (Peakall and Smouse, 2006) with 999 permutations.

Results

We studied 154 *loci* generated using ten ISSR-type primers to amplify the DNA of five individuals from each accession. In Figures 1A and 1B, an example of the patterns obtained using the primer P2 is shown. The average of bands generated per initiator was 15.4, with a range of 6 to 21. The average polymorphism percentage for the initiators was 97.9%, with a minimum of 91.6% and a maximum of 100% (Table 2).

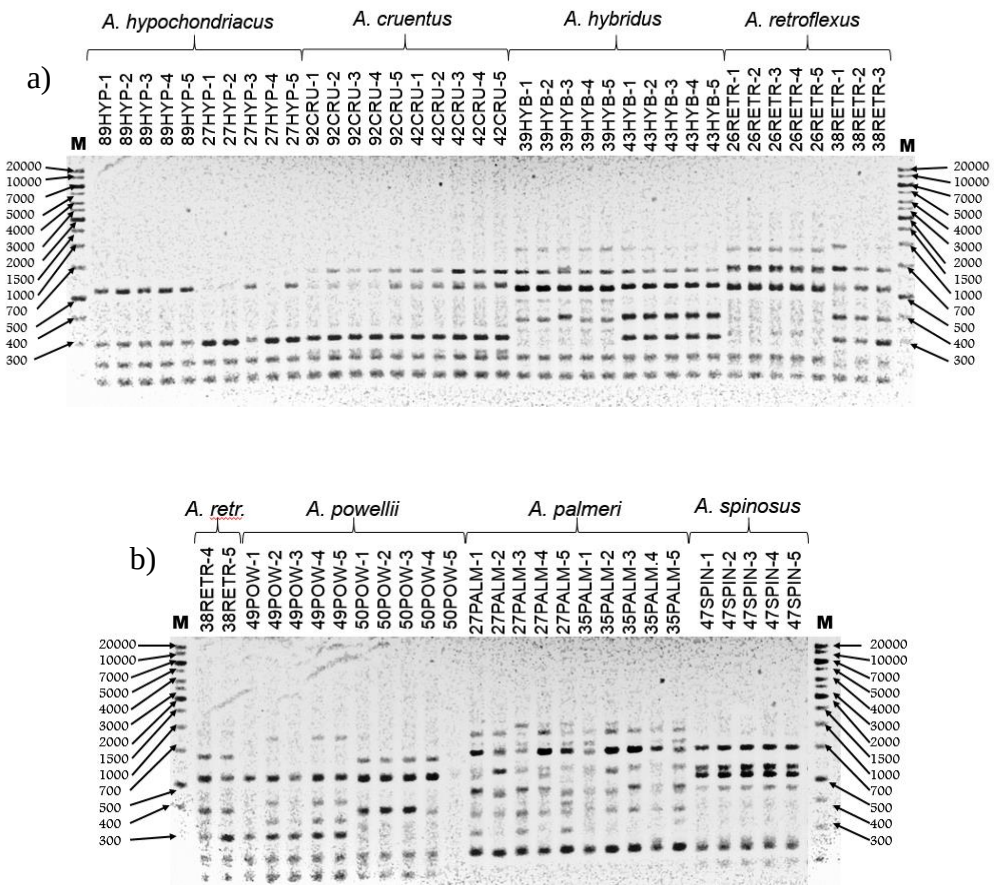

Figure 1. A. Banding patterns obtained with the P2 primer for a sample of 5 individuals from the populations of *A. hypochondriacus*, *A. cruentus*, *A. hybridus* and *A. retroflexus*. B. Banding patterns obtained with the P2 primer for a sample of 5 individuals from the populations of *A. retroflexus*, *A. powellii*, *A. palmeri* and *A. spinosus*. M, molecular weight marker, pb.

Table 2. List of initiators, their sequence, number of amplified products and percentage of polymorphism detected.

Initiator ISSR	Sequence	Products amplified	Polymorphism (%)
A3	5'-CTCCTCCTCCTCCTCCTC- 3'	17	100%
A8	5'-AGAGAGAGAGAGAGAGT- 3'	21	100%
A10	5'-GAGAGAGAGAGAGAGA- 3'	17	94.1%
P2	5'-CTGAGAGAGAGAGAGAGAG- 3'	16	93.7%
P3	5'-AGAGAGAGAGAGAGAGTG- 3'	13	100%
IAH-01	5'-ACACACACACACA- 3'	16	100%
IAH-02	5'-GTGTGTGTGTGTGG- 3'	16	100%
ISSR 01	5'-AGGAGGAGGAGGAGGAGG- 3'	12	91.6%
ISSR 04	5'-GAGAGAGAGAGAGAGAGAC- 3'	6	100%
(AG) 8YA	5'-AGAGAGAGAGAGAGAGYA- 3'	20	100%

Genetic diversity in 13 amaranth populations

In Figure 2, the identity dendrogram is shown where the genetic relationships between thirteen amaranth populations corresponding to seven species are established and using the Nei coefficient. At an identity coefficient of 0.718 the formation of two groups is observed.

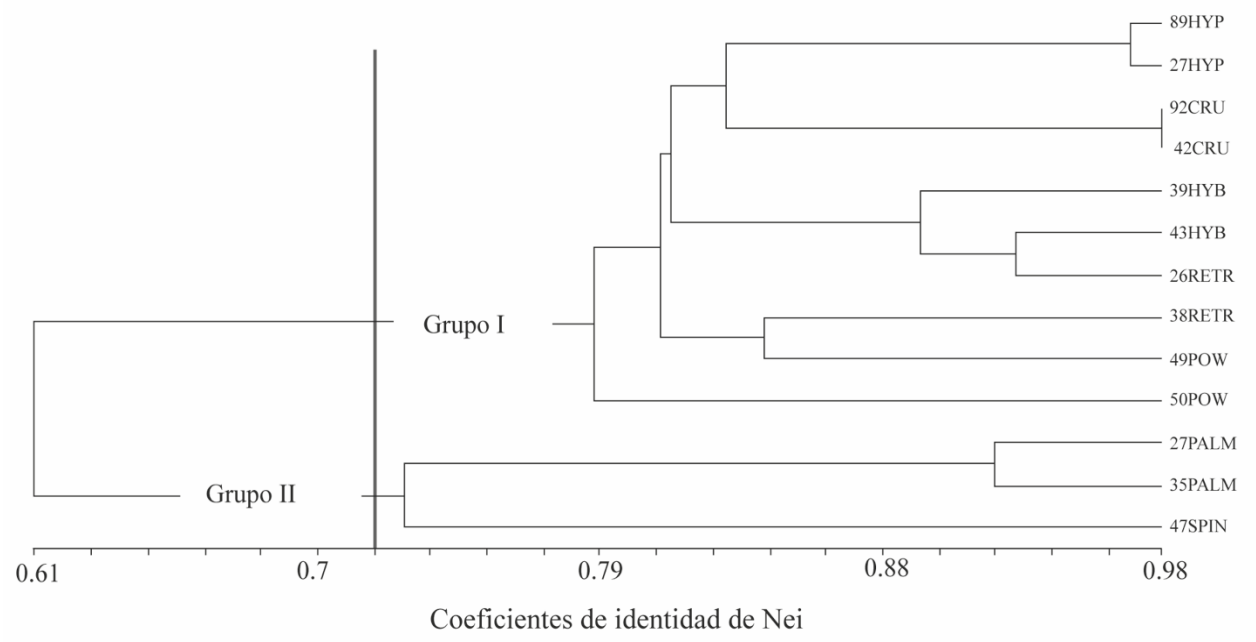

Figure 2. Dendrogram of genetic relationships between populations and amaranth species, constructed using the Nei identity coefficient.

The group I was divided into four subgroups. The first sub-group included the cultivated species *A. hypochondriacus* and *A. cruentus*, where the most identical materials were those belonging to the species of *A. cruentus* (92CRU, 42 CRU), followed by the materials of *A. hypochondriacus* (89HYP, 27HYP). The most distant materials within this sub-group were *A. hypochondriacus* (89HYP) and *A. cruentus* (42CRU). The second sub-group was formed with accessions of *A. hybridus* and *A. retroflexus*. The third sub-group included materials from *A. powellii* (49POW) and *A. retroflexus* (38RETR), while the fourth sub-group only integrated a material from *A. powellii* (50POW).

The group II included *A. palmeri* dioic amaranths and the monoecious material with the presence of spines of *A. spinosus*, where the most similar accessions are those belonging to *A. palmeri* (27PALM and 35PALM), while the most distant were 27PALM of *A. palmeri* and 47SPIN of *A. spinosus*. The above results suggest that the cultivated amaranths *A. hypochondriacus* and *A. cruentus* are closely related to each other and then moderately related to the wild amaranths *A. hybridus*, *A. retroflexus* and *A. powellii*, in the order indicated, and less related to the species *A. palmeri* and *A. spinosus*.

With regard to wild amaranths, it was observed that the genetically most related species were *A. hybridus* with *A. retroflexus* and *A. powellii*, and *A. palmeri* with *A. spinosus*. Among these, the least related were *A. palmeri* and *A. spinosus*.

In Table 3, the indices of diversity calculated within 13 amaranth populations are shown. It is observed that the majority of the populations have low polymorphic loci percentages, with an average of 13.18% and a range that goes from 3.25% for the accession 26RETR, belonging to the species *A. retroflexus* to 30.52% of the accession 27PALM belonging to the species *A. palmeri*.

Table 3. Indices of genetic diversity within 13 populations of the genus *Amaranthus*.

Species	Accession		NA	NE	H	I	NLP	LP (%)
A. hypochondriacus	89HYP	$\bar{x}$	1.1039	1.093	0.0487	0.0687	16	10.39
		σ	0.3061	0.278	0.1443	0.2029		
	27HYP	$\bar{x}$	1.1364	1.0997	0.0549	0.0799	21	13.64
		σ	0.3443	0.2737	0.1453	0.2076		
A. cruentus	92CRU	$\bar{x}$	1.1234	1.1144	0.0586	0.0822	19	12.34
		σ	0.3299	0.3116	0.1586	0.2214		
	42CRU	$\bar{x}$	1.0649	1.051	0.0274	0.0393	10	6.49
		σ	0.2472	0.207	0.1084	0.1536		
A. hybridus	39HYB	$\bar{x}$	1.0455	1.0334	0.0181	0.0263	7	4.55
		σ	0.209	0.168	0.0881	0.1253		
	43HYB	$\bar{x}$	1.0584	1.0446	0.024	0.0346	9	5.84
		σ	0.2353	0.1952	0.1013	0.1438		
A. retroflexus	26RETR	$\bar{x}$	1.0325	1.0192	0.0111	0.0168	5	3.25
		σ	0.1778	0.1213	0.0658	0.0961		
	38RETR	$\bar{x}$	1.1623	1.0964	0.0584	0.0877	25	16.23
		σ	0.37	0.2385	0.1382	0.2046		
A. powellii	49POW	$\bar{x}$	1.1104	1.0811	0.0451	0.0656	17	11.04
		σ	0.3144	0.2464	0.1328	0.1906		
	50POW	$\bar{x}$	1.1948	1.1573	0.085	0.1216	30	19.48
		σ	0.3973	0.3358	0.1774	0.2517		
A. palmeri	27PALM	$\bar{x}$	1.3052	1.2253	0.1245	0.1808	47	30.52
		σ	0.462	0.3727	0.1977	0.2819		
	35PALM	$\bar{x}$	1.2987	1.2014	0.1132	0.1667	46	29.87
		σ	0.4592	0.3536	0.1877	0.2686		
A. spinosus	47SPIN	$\bar{x}$	1.0779	1.0511	0.0287	0.0425	12	7.79
		σ	0.2689	0.2001	0.1062	0.153		

NA= number of alleles per locus; NE= effective number of alleles per locus; H= genetic diversity of Nei's; I= Shannon index; NLP= number of polymorphic *loci*; % LP= percentage of polymorphic *loci*.

The Shannon indexes (I) and genetic diversity of Nei (H) (Table 3) were also low for the populations. The Shannon indexes had an average of 0.077, with a range of 0.0168 for the accession 26RETR of *A. retroflexus* and 0.1808 for the accession 27PALM of *A. palmeri*, while the index of diversity of Nei (H) showed an average of 0.054, with a range of 0.011 for the accession 26RETR of *A. retroflexus* and 0.124 for the accession 27PALM of *A. palmeri*.

For the number of alleles per locus (NA) and the effective number of alleles per locus (NE) the range of values between the populations was between 1 032 to 1 305 and 1 019 to 1 225, respectively. The analysis of genetic diversity among the thirteen populations showed that the percentage of polymorphic *loci* (LP %) was 99.35%. The coefficient of genetic differentiation between populations was high (G_{ST}= 0.82), and indicates that approximately 82% of the variation detected can be attributed to genetic differences between populations. The rest (18%) represents genetic diversity within the populations. Based on the coefficient of total genetic differentiation between populations (G_{ST}) the estimated gene flow level (Nm) was 0.11. This indicates that there is less than one migrant individual per generation among the populations, which also explains the high level of differentiation detected among them.

Genetic diversity in seven amaranth species

The genetically closest species were the wild *A. hybridus* and *A. retroflexus* with a coefficient of 0.93, followed by *A. powellii* with a coefficient of 0.87. The cultivated species were genetically close to each other, although related to the wild species mentioned above. *A. hypochondriacus* was related to *A. cruentus* to a coefficient of genetic identity of Nei equal to 0.86. In addition, the related *A. palmeri* and *A. spinosus* wild species were far removed from the cultivated amaranths *A. hypochondriacus* and *A. cruentus*, as well as the wild amaranths *A. hybridus*, *A. retroflexus* and *A. powellii*.

With regard to the genetic diversity indices calculated within species, Table 4 shows that the percentage of polymorphic loci was quite low for the seven species evaluated, with an average of 24.02% and a range of values that goes of 7.79% for *A. spinosus* and 44.16% for *A. palmeri*.

The Shannon (I) and genetic diversity indexes of Nei (H) were also low (Table 4), the Shannon indexes showed an average of 0.132, with a range of values ranging from 0.042 for the species *A. spinosus* and of 0.235 for the species *A. palmeri*, while the index of diversity of Nei (H) showed an average of 0.089, with a range of 0.029 and 0.157.

Table 4. Indices of genetic diversity within seven species of the genus *Amaranthus*.

Species		NA	NE	H	I	NLP	LP (%)
A. hypochondriacus	$\bar{x}$	1.1948	1.1254	0.071	0.1049	30	19.48
	Σ	0.3973	0.2916	0.1579	0.2271		
A. cruentus	$\bar{x}$	1.1429	1.102	0.0575	0.0837	22	14.29
	Σ	0.3511	0.2671	0.1464	0.2108		
A. hybridus	$\bar{x}$	1.1623	1.1139	0.0656	0.0959	25	16.23
	Σ	0.37	0.2695	0.1529	0.2218		
A. retroflexus	$\bar{x}$	1.2922	1.1732	0.1023	0.1534	45	29.22
	Σ	0.4563	0.3107	0.1738	0.2531		
A. powellii	$\bar{x}$	1.3701	1.2404	0.1409	0.2085	57	37.01
	Σ	0.4844	0.3433	0.1937	0.2817		
A. palmeri	$\bar{x}$	1.4416	1.2699	0.1574	0.2348	68	44.16
	Σ	0.4982	0.3607	0.1972	0.2842		
A. spinosus	$\bar{x}$	1.0779	1.0511	0.0287	0.0425	12	7.79
	Σ	0.2689	0.2001	0.1062	0.153		

NA= number of alleles per locus; NE= effective number of alleles per locus; H= genetic diversity of Nei; I= Shannon index; NLP= number of polymorphic *loci*; (%) LP= percentage of polymorphic *loci*.

For the number of alleles per locus (NA) and the effective number of alleles per locus (NE), the range of values for the evaluated species was between 1 078 to 1 442 and 1 051 to 1.27, respectively.

The analysis of genetic diversity among the species indicated a percentage of polymorphic loci (% LP) equal to 99.35%. The coefficient of genetic differentiation between the species was high (G_{ST}= 0.7), and indicates that approximately 70% of the genetic variation detected can be attributed to genetic differences between the species. The rest (30%) represents genetic diversity within the species. Based on the coefficient of total genetic differentiation between species (G_{ST}) the estimated gene flow level (Nm) was 0.21. This suggests that there is less than one individual migrant per generation among species, which also explains the high level of differentiation detected among them.

The genetically closest species were *A. hybridus* and *A. retroflexus* with an identity value of 0.927, while the most distant species were *A. spinosus* and *A. hybridus* with a value of 0.534.

Analysis of molecular variance (Amova)

The results of the molecular variance analysis (Amova) indicated significant genetic differences ($p \leq 0.001$) among the populations studied (Table 5). A genetic variability value equal to 79% was detected among the populations, while the remaining 21% is within the populations. When considering species, it was found that between them 68% of the total genetic variation is present and only 32% is within the species.

Table 5. Analysis of molecular variance (Amova) of amaranth species and accessions.

Source of variation	Analysis	Df	SS	MS	Est. var.	Vt (%)	PhiPT	Value Px
Between accessions	Between	12	1177.385	98.115	18.645	79	0.792	***
	Inside	52	254.4	4.892	4.892	21		
Between species	Between	6	970.485	161.747	16.661	68	0.677	***
	Inside	58	461.3	7.953	7.953	32		
Cultivated vs wild	Between	1	171.212	171.212	5.46	21	0.214	***
	Inside	63	1260.572	20.009	20.009	79		
Between cultivated	Between	1	119.85	119.85	11.531	72	0.718	***
	Inside	18	81.7	4.539	4.539	28		
Between wild	Between	4	679.422	169.856	18.041	66	0.655	***
	Inside	40	379.6	9.49	9.49	34		
Between dioicos *vs* monoicos	Between	1	284.948	284.948	15.762	46	0.464	***
	Inside	63	1146.836	18.204	18.204	54		

Df= degrees of freedom; SS= sum of squares; MS= sum of mean squares; vt (%)= percentage of variation; Px= probability, *= 0.05, **= 0.01, ***= 0.001.

When comparing the cultivated materials against the wild ones it was found that the genetic variation present within both types of materials is very low with a value of 21%, while the variation among them was very high (79%), which indicates the existence of genetic variation susceptible to use in breeding programs for cultivated amaranths.

Also, it was found that genetic variation among cultivated amaranth populations was 72%, while only 28% is within them, which also demonstrates the high degree of genetic differentiation present between *A. hypochondriacus* and *A. cruentus*

When only wild materials were compared, 66% of the genetic variation was found among the populations and only 34% within them. And finally, when making the comparison between the wild materials dioecious against the monoecious, the results indicated that the genetic variation present between the populations is 46% and the rest (54%) is within the populations.

Discussion

The results of the clustering analyze of this work indicate that the cultivated amaranths *A. hypochondriacus* and *A. cruentus* are closely related to each other and then moderately related to the wild amaranths *A. hybridus*, *A. retroflexus* and *A. powellii*, and less related with the species *A. palmeri* and *A. spinosus* (Figure 2). Pal and Khoshoo (1972) propose *A. hybridus* L. and *A. powellii* as the possible progenitors of cultivated amaranths, which may explain the genetic closeness of these species.

In wild amaranths it was observed that the genetically most related species were *A. hybridus* with *A. retroflexus* and *A. powellii*, and *A. palmeri* with *A. spinosus*. Among these, those with the lowest percentage of similarity were *A. palmeri* and *A. spinosus* (Figure 2).

The highest percentages of polymorphism were found in the wild materials of *A. powellii* (37.01%), *A. palmeri* (44.16%) and *A. retroflexus* (29.22%) (Table 4) because these materials may have been less exposed to a selection pressure either by biotic or abiotic environment factors or by man through genetic improvement (Maughan *et al*., 2011). Also, the type of sex or pollination of the species could be influencing the genetic variability present (Štefunova, 2014) since the dioic species *A. palmeri* presented the highest value of polymorphism in comparison with the rest of the species, which are monoecious (Tables 1 and 4).

The genetic diversity index of Nei (H) had an average of 0.089 within the populations, with a range of 0.029 to 0.157 (Table 4), which means low genetic variability in the species and populations studied (Nei, 1973). This low genetic variability present within the different populations and species of amaranth can be explained by the high percentage of self-pollination that occurs in the plant (Agong and Ayiecho, 1991). The low level of polymorphism detected in *Amaranthus* may reflect the high level of inbreeding that exists in the species (Htet and Park, 2013).

Interspecific hybridization works have been carried out in both wild materials and cultivated materials. When crossbreeding between *Amaranthus* species has been made, it has been difficult to obtain hybrids due to their low survival rate. Trucco *et al*. (2005) obtained only 5.9% hybridization between *A. hybridus* and *A. tuberculatus*, while Gaines *et al*. (2012) when crossing between *A. palmeri* and *A. spinosus* obtained 0.01% to 0.4% hybridization, between *A. palmeri* and *A. tuberculatus* less than 0.2% hybridization and between *A. palmeri* and *A. hybridus* less than 0.01% hybridization.

For cultivated species *A. hypochondriacus*, *A. cruentus* and *A. caudatus*, crossing percentages ranging from 5 to 30% have been reported. In this regard, Agong and Ayiecho (1991) estimate between 10.4% and 10.9% of hybridization between *A. hypochondriacus* and *A. cruentus*. With this low percentage of cross-links, it is possible to deduce the high rate of differentiation between the different species and the low genetic variability present within the populations (Table 5), because the gene flow between the species is very limited.

The coefficient of genetic differentiation (G_{ST}) among the populations was 0.82 and among the species 0.7. These values are high and resemble those reported by Chan and Sun (1997) for the cultivated grain amaranths (*A. caudatus*, *A. cruentus* and *A. hypochondriacus*), which indicate an average value of G_{ST} equal to 0.98 at the level of species. For wild species *A.* hybridus, *A. powellii* and *A. quitensis*, the same authors report a value of 0.96.

The number of migrating individuals (Nm), which gives an idea of the degree of gene flow, was 0.112 between the populations and 0.214 between the species. These values indicate that on average there is less than one migrant individual per generation between populations and between species, which confirms the low percentage of crossing and the high degree of differentiation between populations and species.

When comparing the cultivated materials against the wild ones, it was found that the genetic variation present within both types of materials is very low with a value of 21% (Table 5), while the variation between them was very high (79%). which indicates the existence of genetic variation susceptible to use in breeding programs for cultivated amaranths, as well as to define strategies for the conservation of the genus.

Conclusions

Cultivated grain amaranths are genetically closely related to each other and to their possible wild progenitor's *A. hybridus* and *A. powellii*. Among the wild materials those that were closest were *A. hybridus*, *A. powellii* and *A. retroflexus*, while *A. spinosus* and *A. palmeri* were the most distant. Most of the detectable genetic diversity was found between species and populations, while the least part was within them. The ISSR type molecular markers were useful for characterizing accessions and estimating genetic diversity in *Amaranthus* populations.

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