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Genetic diversity and relationships detected by isozyme and RAPD analysis of crop and wild species of *Amaranthus*

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Abstract Genetic diversity and relationships of 23 cultivated and wild *Amaranthus* species were examined using both isozyme and RAPD markers. A total of 30 loci encoding 15 enzymes were resolved, and all were polymorphic at the interspecific level. High levels of inter-accessional genetic diversity were found within species, but genetic uniformity was observed within most accessions. In the cultivated grain amaranths (*A. caudatus*, *A. cruentus*, and *A. hypochondriacus*), the mean value of H_T was 0.094, H_S was 0.003, and G_{ST} was 0.977 at the species level. The corresponding values in their putative wild progenitors (*A. hybridus*, *A. powellii*, and *A. quitensis*) were 0.135, 0.004, and 0.963, respectively. More than 600 RAPD fragments were generated with 27 arbitrary 10-base primers. On average, 39.9% of the RAPD fragments were polymorphic among accessions within each crop species; a similar level of polymorphism (42.8%) was present in the putative progenitors, but much higher levels of polymorphism were found in vegetable (51%) and other wild species (69.5%). The evolutionary relationships between grain amaranths and their putative ancestors were investigated, and both the RAPD and isozyme data sets supported a monophyletic origin of grain amaranths, with *A. hybridus* as the common ancestor. A complementary approach using information from both isozymes and RAPDs was shown to generate more accurate estimates of genetic diversity, and of relationships within and among crop species and their wild relatives, than either data set alone.

Key words *Amaranthus* · Crop evolution · Isozyme · Genetic diversity · RAPD

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Introduction

Information on genetic diversity and relationships within and among crop species and their wild relatives is essential for the efficient utilization of plant genetic-resource collections. Several molecular approaches have been employed to assess genetic diversity and relationships, but isozyme or random amplified polymorphic DNA (RAPD) data can be generated faster and with less labor than other methods, such as RFLP (restriction fragment length polymorphism) and the use of microsatellites. However, isozyme studies are limited by the numbers of enzymes and loci that can be resolved, and reveal only genetic changes in coding regions of the genome that have resulted in an altered amino-acid sequence. In many cases, lack of allozyme polymorphism may further restrict its application in plant germplasm classification. In contrast, RAPD analysis enables the detection of informative genetic markers at a large number of loci in both coding and non-coding regions of the genome (Williams et al. 1990). However, the dominant nature of RAPD markers requires that more individuals and loci be sampled, compared to co-dominant markers such as RFLP and isozymes (Lynch and Milligan 1994), and the low homology between co-migrating fragments from different species may limit its application in phylogenetic studies at the interspecific level (Thormann et al. 1994; Rieseberg 1996). Thus, for comparative studies of crops and their wild relatives in plant germplasm collections, a complementary approach using both isozymes and RAPDs may be more appropriate for generating accurate estimates of genetic diversity and relationships than either method used alone.

The genus *Amaranthus* consists of about 60 species distributed throughout the world, including cultivated grain and vegetable crops as well as wild species. The three grain amaranths, *A. caudatus*, *A. cruentus*, and

A. hypochondriacus, are among the earliest New World domesticates as grain crops and have been cultivated in Mexico, Central America, and the Andean highlands of South America for several thousand years (Sauer 1950, 1967, 1976). Grain amaranths are considered a promising potential alternative grain crop (Tucker 1986). For genetic improvement of *Amaranthus*, germplasm collections will play a key role. However, only limited information is available on intra- and inter-specific genetic diversity and relationships within *Amaranthus* germplasm collections.

Sauer (1967, 1976) proposed two alternative hypotheses for the origin of grain amaranths, primarily on the basis of morphology and geography. One hypothesis is that the three cultivated grain amaranths may have been domesticated independently in different areas from different progenitors, such as *A. hybridus*, *A. powellii*, and *A. quitensis*. According to this hypothesis, *A. caudatus* was most likely domesticated from *A. quitensis* in South America, *A. cruentus* from *A. hybridus* in Central America, and *A. hypochondriacus* from *A. powellii* in Mexico. The alternative hypothesis postulates a single primary domestication of *A. cruentus* from *A. hybridus*, presumably in Central America, with the other two domesticates, *A. hypochondriacus* and *A. caudatus*, evolving secondarily by repeated crossing of *A. cruentus* with weedy *A. powellii* in the north and *A. quitensis* in the south, as the original domesticate spread into their respective territories. Despite several studies of the relationships between grain amaranths and their putative progenitors, no conclusive evidence for either hypothesis has yet been produced (e.g. Pal and Khoshoo 1972, 1973, 1974; Hauptli and Jain 1984; Lanoue et al. 1996).

The objective of the present study was to investigate intra- and inter-specific genetic diversity and relationships in 23 crop and wild species of *Amaranthus* using both isozyme and RAPD approaches, with emphasis on testing the two hypotheses on the origins of the grain amaranth species. Results obtained using isozyme versus RAPD data sets were compared to determine a suitable strategy for characterizing *Amaranthus* germplasm collections.

Materials and methods

Plant materials

Seeds of 60 cultivars/accessions representing 23 crop and wild species of *Amaranthus*, including an unknown hybrid taxon, were obtained from the USDA/ARS Plant Introduction Station at Ames, Iowa, USA (Table 1). On average, five accessions were studied for intra- and inter-specific comparisons of the three grain amaranth species and their three putative progenitor species. One to three accessions were randomly chosen to represent each of all the other species studied.

Table 1 Species and accessions of *Amaranthus* studied

No.[a]	Species	Accession	Origin
1	*A. acutilobus*	Ames 13787	Germany
3	*A. albus*	Ames 13788	Canada
5	*A. australis*	PI 553076	USA-Florida
10	*A. caudatus*	PI 511679	Argentina
11		PI 568132	Bolivia
13		PI 490609	Ecuador
14		PI 166045	India
19		PI 553073	United States
20	*A. crassipes*	Ames 10339	Czechoslovakia
21	*A. cruentus*	Ames 5142	Guatemala
22		PI 566897	India
23		PI 511731	Mexico
24		Ames 5638	Mexico
25		PI 482049	Zimbabwe
30		PI 566896	USA-Arizona
31		Ames 5369	Zaire
32	*A. deflexus*	Ames 13785	France
34	*A. dubius*	PI 572254	India
35		Ames 5326	Jamaica
36		Ames 5105	Seychelles
37		PI 482047	Zimbawe
38	*A. fimbriatus*	Ames 15304	Mexico
39	*A. floridanus*	PI 553078	USA-Florida
41	*A. hybrid*[b]	PI 572255	USA-California
42	*A. hybridus*	Ames 5331	Argentina
43		Ames 5684	USA-Delaware
44		Ames 2026	USA-Indiana
45		Ames 14358	USA-Virginia
46		PI 482049	Zimbabwe
47	*A. hypochondriacus*	PI 477915	India
48		PI 477916	Mexico
53		Ames 2178	Nepal
54		PI 540446	Pakistan
55		Ames 10847	USA-California
59		PI 568125	USA-Iowa
66	*A. lividus*	PI 288277	India
69	*A. palmeri*	Ames 5665	Mexico
70		Ames 5305	Senegal
74	*A. powellii*	PI 572259	Czechoslovakia
75		PI 572260	France
76		PI 572261	Germany
77		PI 538793	Russian Federation
78		PI 572257	Rwanda
83	*A. quitensis*	PI 511734	Bolivia
84		PI 568154	Bolivia
85		PI 511745	Eucador
86		PI 511733	Peru
87	*A. retroflexus*	Ames 2050	Indonesia
93	*A. spinosus*	Ames 2200	Taiwan
94		PI 500235	Zambia
95		PI 482057	Zimbabwe
as1		–	Hong Kong[d]
96	*A. species*[c]	Ames 5366	Bangladesh
100	*A. standleyanus*	Ames 15312	Argentina
101	*A. tricolor*	PI 419057	China
102		PI 566899	India
103		Ames 5147	India
105	*A. viridis*	PI 540445	Indonesia
106		PI 536442	Maldives
107		Ames 5583	Philippines

[a] Accession number used in this study
[b] Unknown hybrid
[c] Unidentified species
[d] The seeds of as1 were collected in Hong Kong from a single individual of *A. spinosus*

Isozyme electrophoresis

Enzyme extraction was carried out by grinding young seedlings using the buffer described by Sun and Ganders (1990). Five seedlings from each accession were surveyed individually. Enzyme electrophoresis was conducted using two buffer systems. The "L" buffer system (Shields et al. 1983) was used to resolve aconitase (ACO, E.C. 4.2.1.3), alkaline phosphatase (ALP, E.C. 3.1.3.1), diaphorase (DIA, E.C. 1.8.1.4), esterase (EST, E.C. 3.1.1.1), glutamate dehydrogenase (GDH, E.C. 1.4.1.3), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), leucine aminopeptidase (LAP, E.C. 3.4.11.1), malic enzyme (ME, E.C. 1.1.1.40), phosphoglucose isomerase (PGI, E.C. 5.3.1.9), shikimate dehydrogenase (SKDH, E.C. 1.1.1.25), and triose-phosphate isomerase (TPI, E.C. 5.3.1.1). The histidine buffer system (Sun and Corke 1992) was used to resolve aspartate aminotransferase (AAT, E.C. 2.6.1.1), acid phosphatase (ACP, E.C. 3.1.3.2), glucokinase (GK, E.C. 2.7.1.2), glucose-6-phosphate dehydrogenase (G6PD, E.C. 1.1.1.49), malate dehydrogenase (MDH, E.C. 1.1.1.37), 6-phosphogluconate dehydrogenase (6PGD, E.C. 1.1.1.44), and phosphoglucomutase (PGM, E.C. 5.4.2.2).

RAPD analysis

DNA was extracted from fresh young leaves of five individuals to represent each accession for the RAPD assay. The protocol for DNA extraction was based on Doyle (1991). DNA quantification was done by fluorometric analysis, and the extracted DNA was dissolved in a TE buffer (pH 7.4) with a final concentration of 6 ng/μl. The protocol for RAPD-PCR was based on Williams et al. (1990) with minor modifications: 3 mM $MgCl_2$, 0.1% Triton X-100, 75 ng primer, and 30 ng genomic DNA were used in each 25-μl reaction mix. The reactants were contained in a 0.5-ml Eppendorf tube and overlaid with 20 μl of mineral oil. PCR amplification was performed in a Stratgene RoboCycler™ 40 thermocycler (400832-X1) using the following cycle profile: 1 cycle at 94°C for 5 min followed by 45 cycles at 94°C for 1 min, 35°C for 4 min, and 72°C for 2 min; and the reaction was terminated with a 7-min DNA extension step at 72°C. This cycle profile was developed specifically for the RoboCycler™ 40 used, which requires a much longer annealing time than other thermocycler models. One-hundred oligonucleotide primers (10-mers) of arbitrary sequence (Set #1 Lot #1) were obtained from the Nucleic Acid-Protein Service Unit, Biotechnology Laboratory, University of British Columbia. Twenty seven primers out of the one-hundred screened gave reproducible bands and were selected for use (Table 2). The amplification products were electrophoresed on 1.4% agarose gels and visualized using the ethidium bromide staining method.

The repeatability of RAPD banding patterns was examined, and factors affecting the number of bands generated per primer were tested. In a range of template DNA concentrations, i.e. 5, 30, 70, 100, 150, 200, 250, and 300 ng/ul, concentrations $\geq$200 ng/μl resulted in a low number of amplified bands, while 5, 30, or 70 ng/μl of DNA gave the same and highest number of reproducible bands. Thus, 30 ng/μl of template DNA was selected for all PCR amplifications. Negative controls were routinely used to check for possible contamination. The banding pattern of bulked DNA samples of five individuals was found to be the same as that of individual DNA samples from the same accession due to genetic uniformity within most accessions. Both a DNA ladder and Accession 1011 (*A. cruentus*) were used as markers and as control DNA on the gels, since all individuals of Accession 1011 showed the same multilocus RAPD banding pattern.

Data analysis

For isozyme analysis, the number of loci and alleles were inferred from the banding pattern and enzyme structure following standard principles (Weeden and Wendel 1989; Wendel and Weeden 1989). Allele frequencies were calculated and analyzed by the SIMGEND (similarity for genetic data) program of NTSYS-pc, version 1.8 (Numerical Taxonomy and Multivariate Analysis System for Personal Computers, Exeter Software, Setauket, N.Y.) to calculate the distance index between any two accessions or species. Dendrograms were produced from the distance matrices using the unweighted pair group method with arithmetic averages (UPGMA). Additionally, the data matrices of allele frequencies were used to analyze gene diversity, as too was Nei's (1972) genetic distance using the GDD Program (Ritland 1990). In order to compare the levels of genetic diversity between the two groups of species (grain amaranths versus their putative ancestors), both intra- and inter-specific accessions were analyzed as conspecific populations for each group using the GDD program.

For RAPD analysis, the banding patterns were recorded using a gel documentation system (BIO-RAD Gel Doc 1000), and the image profiles and molecular weight of each band were determined by Molecular Analyst/pc (Version 1.2) software. The fragment size scored ranged from 300 to 1500 bp. Both weak bands with negligible intensity and smearing bands were excluded from final data analysis. The bands with the same molecular weight and mobility were treated as identical fragments. In the data matrices, the presence of a band was coded as 1, whereas the absence of the band was coded as 0. Amplification failure of a sample or missing data was coded as 9. The data matrices were analyzed by the SIMQUAL program of NTSYS-pc (Version 1.8), and similarities between accessions were estimated using the Jaccard coefficient, calculated as $J = a/(n–d)$ where a is the number of positive matches (i.e. the presence of a band in both samples), d is the number of negative matches (i.e. the absence of a band in both samples), and n is the total sample size including both the numbers of matches and "unmatches" (Rohlf 1994). Dendrograms were produced from the resultant similarity matrices using the UPGMA method.

Table 2 Nucleotide sequence of the 27 primers used in this study. Primer number follows that in UBC Set 1 (#1–100)

Primer	Sequence	Primer	Sequence
13	CCT GGG TGG A	77	GAG CAC CAG G
16	GGT GGC GGG A	78	GAG CAC TAG C
17	CCT GGG CCT C	81	GAG CAC GGG G
18	GGG CCG TTT A	83	GGG CTC GTG G
23	CCC GCC TCC C	85	GTG CTC GTG C
29	CCG GCC TTA C	86	GGG GGG AAG G
30	CCG GCC TTA G	88	CGG GGG ATG G
31	CCG GCC TTC C	89	GGG GGC TTG G
34	CCG GCC CCA A	90	GGG GGT TAG G
40	TTA CCT GGG C	91	GGG TGG TTG C
43	AAA ACC GGG C	95	GGG GGG TTG G
47	TTC CCC AAG C	97	ATC TGC GAG C
65	AGG GGC GGG A	98	ATC CTG CCA G
72	GAG CAC GGG A		

Results

High levels of isozyme diversity were found both within and between grain amaranths and their putative progenitors. Of the 30 isozyme loci resolved, all were polymorphic at the interspecific level. At the intraspecific level, however, no allozyme variation was

detected within 50 of the 60 accessions studied, and genetic diversity existed primarily among accessions within each species. Rare polymorphisms at 1 of the 30 loci surveyed were detected within four accessions of grain amaranths: one accession of *A. hybridus* (Ames 2026) and three accessions of *A. hypochondriacus* (PI 477915, PI 477916, and PI 540446). The three putative progenitor species also lacked intra-accessional variation, and rare allozyme polymorphism at one or two loci was detected in only three of the accessions studied: one accession of *A. powellii* (PI 572259) and two accessions of *A. quitensis* (PI 568154 and PI 511734). However, a higher level of allelic diversity was found in the putative progenitor species than in the three grain species. Comparing the two species groups, 25 unique alleles were found in the putative progenitors, and only six were present in grain amaranths. The major genetic differences between grain amaranths and their putative progenitors were largely due to the presence or absence of these unique alleles, rather than to a difference in the frequency of shared alleles between the two groups. Similar to grain amaranths and their putative progenitors, lack of allozyme variation was a striking feature within most accessions of vegetable and other wild species, except that Ames 5665 (*A. palmeri*) from Mexico exhibited polymorphism at eight of the loci studied. In addition, PI 288277 of *A. lividus* from India was polymorphic at two loci, and polymorphism at one locus was found in Ames 2050 of *A. retroflexus.*

The total gene diversity (H_T) in *A. caudatus*, *A. cruentus*, and *A. hypochondriacus* was 0.130, 0.038, and 0.115, respectively. The mean values of allozyme diversity at the species level were $H_T = 0.094$, $H_S = 0.003$ and $G_{ST} = 0.977$. Treating all accessions studied as conspecifics, the estimate of H_T was 0.202 in grain amaranths as a group. Higher values of H_T were obtained in the putative progenitor species, ranging from 0.061 in *A. hybridus*, 0.163 in *A. quitensis*, to 0.182 in *A. powellii.* The mean values of allozyme diversity were $H_T = 0.135$, $H_S = 0.004$, and $G_{ST} = 0.963$ at the species level. The estimate of H_T was 0.384 in the putative progenitors as a group.

Based on isozyme data, the average Nei's genetic identity (*I*) was $86 \pm 4\%$ between intraspecific accessions in grain amaranths and $81 \pm 8\%$ in the putative ancestors. Grouping both intra- and inter-specific accessions together, the average value of *I* was $77 \pm 2\%$ between accessions in the grain amaranth species group, and $55 \pm 5\%$ in the putative progenitor species group. Based on pairwise comparisons with each of the three putative progenitors, the grain amaranths as a group were closest to *A. hybridus* ($I = 78 \pm 2\%$), compared to $I = 71.6 \pm 2\%$ with *A. quitensis*, and $I = 56 \pm 5\%$ with *A. powellii.* These intra- and interspecific relationships are shown in Fig. 1. Interspecific relationships between grain amaranths and all other species were also examined based on Nei's genetic identities. No species was found to be genetically closer to the grain amaranth species than *A. hybridus* (Fig. 2).

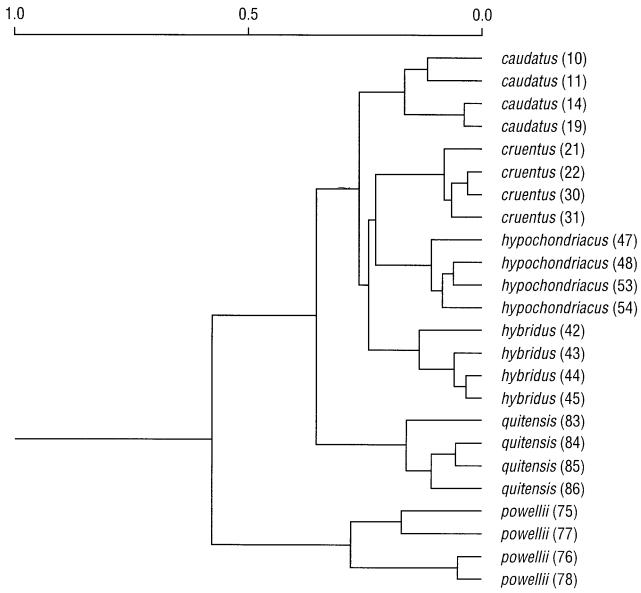

Fig. 1 UPGMA dendrogram of grain amaranths and their three putative progenitor species based on isozyme data using Nei's (1972) genetic distance metrix. *Numbers in parenthesis* represent the accession numbers used in this study (see Table 1)

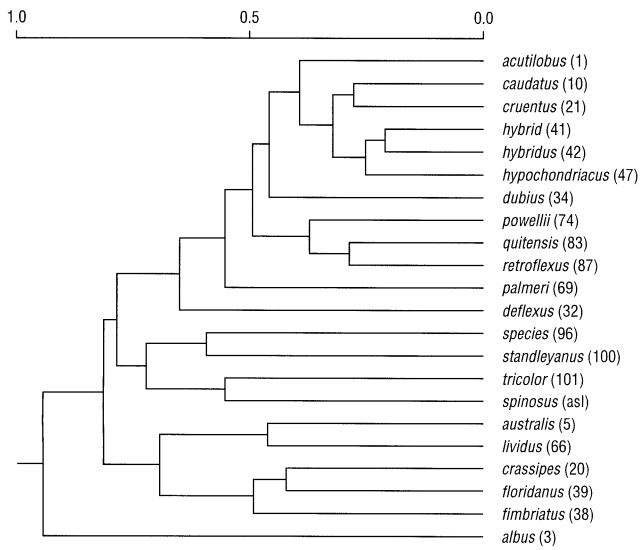

Fig. 2 UPGMA dendrogram of crop and wild species of *Amaranthus* based on isozyme data using Nei's genetic distance matrix. Of the four accessions sampled for each species of grain amaranths and their putative progenitors, only one was included in the cluster analysis

Using 27 primers, RAPD analysis generated a total of 600 bands (loci) in the 29 accessions surveyed for grain amaranths and their putative progenitors, and 900 loci for all 60 accessions studied. On average, 282

bands per species were amplified in the grain amaranths, 294 bands per species in the putative ancestors, and 238 per species in the vegetable and wild species. The differences between species in the number of amplified bands were primarily related to the number of intraspecific accessions assayed. Due to intraspecific polymorphism among accessions, more marker loci could be generated if more intraspecific accessions were assayed.

At the intraspecific level, RAPD polymorphism was measured as the proportion of polymorphic loci to the total number of loci scored in all accessions of the same species (Table 3). In the grain amaranth group, *A. cruentus* had the lowest level of RAPD polymorphism (31.5%), and the average RAPD polymorphism in the three species was 40 ± 7%. The three putative progenitor species showed different degrees of RAPD variation. The most polymorphic species was *A. hybridus* (P = 50%), whereas *A. powellii* and *A. quitensis* exhibited similar levels of polymorphism compared to the grain amaranths. The average RAPD polymorphism in the three putative progenitors was 43 ± 8%. Although only two to three accessions per species were surveyed, much higher levels of RAPD polymorphism were found in the vegetable and wild species (51 ± 2% and 70 ± 5%, respectively).

In all species with two or more accessions surveyed, intraspecific accessions exhibited much higher levels of genetic similarity than interspecific accessions. The intraspecific similarity index as measured by Jaccard's coefficient (Table 3) averaged 57.1 ± 10.8% in the grain amaranths, and 55.1 ± 1.2% in the putative progenitors. With one exception, all the accessions sampled for each species grouped together (dendrogram not shown).

Based on shared RAPD fragments, the average interspecific similarities were 35.7 ± 0.5% (Jaccard's coefficient) among grain amaranth species and 29.6 ± 5.5% among the putative progenitor species (Table 4). Interspecific relationships based on RAPD data are similar to those based on isozyme data between grain amaranths and their putative progenitors. Among the three putative progenitor species, *A. hybridus* was found to be most closely related to grain amaranths. The major difference between the RAPD and isozyme data sets was in the placement of *A. hypochondriacus* within the

Table 3 Intraspecific RAPD polymorphism and the average Jaccard similarity index between intraspecific accessions in grain amaranths, putative ancestors, vegetable and other wild species of *Amaranthus*

Species	No. of accessions	No. of RAPD markers		% Polymorphism of RAPD markers	Jaccard similarity (%)
		Total	Polymorphic		
Grain amaranths					
A. caudatus	5	276	122	44.2	55.7
A. cruentus	5	260	82	31.5	68.6
A. hypochondriacus	5	309	136	44.0	47.1
Putative progenitors					
A. hybridus	5	284	142	50.0	56.1
A. powellii	5	296	132	44.6	55.5
A. quitensis	4	303	102	33.7	53.7
Vegetable species					
A. dubius	4	269	142	52.8	47.2
A. tricolor	3	222	111	50.0	50.0
Wild species					
A. palmeri	2	261	181	69.3	30.5
A. spinosus	3	211	158	74.9	24.9
A. viridis	4	226	145	64.2	54.4

Table 4 Interspecific similarity (% Jaccard's coefficient) between grain amaranths and their putative progenitors based on RAPD data

Species	*A. caudatus*	*A. cruentus*	*A. hypochondriacus*	*A. hybridus*	*A. powellii*	*A. quitensis*
A. caudatus	–	36.2	35.2	33.0	27.2	34.6
A. cruentus		–	35.7	38.0	27.1	30.4
A. hypochondriacus			–	31.0	30.2	33.5
A. hybridus				–	24.8	28.3
A. powellii					–	35.6
A. quitensis						–

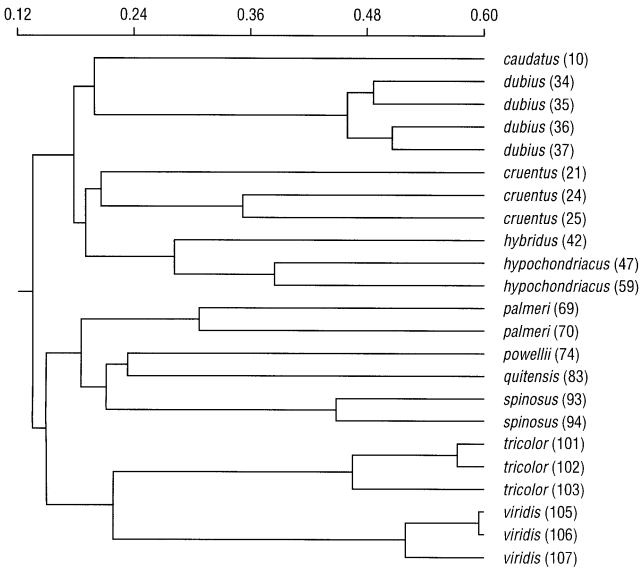

Fig. 3 UPGMA dendrogram of five vegetable and wild species of *Amaranthus* with two or more accessions studied, based on RAPD data using Jaccard's similarity coefficient matrix, showing that the RAPD markers separated the intraspecific accessions into distinctive species groups, corresponding to the original species identification in the *Amaranthus* germplasm collections. A few randomly chosen accessions of crop species and their putative progenitors were included in the dendrogram for reference

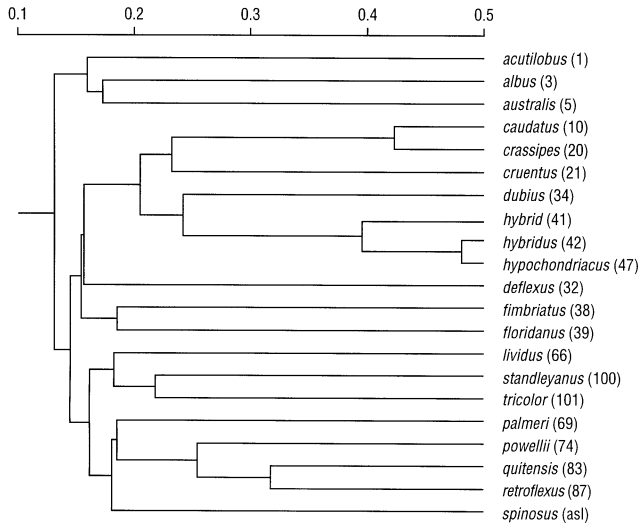

Fig. 4 UPGMA dendrogram of crop and wild species of *Amaranthus* based on RAPD data using Jaccard's similarity coefficient matrix

A. hybridus-grain amaranths species group. The value of interspecific similarity was much lower when averaged over all species studied (Jaccard's coefficient: $15.4 \pm 4.6\%$). The dendrograms based on RAPD data (Figs. 3, 4) showed major differences in the placement of several wild species in comparison with the dendrograms based on isozyme data (Figs. 1, 2).

Discussion

Genetic diversity in *Amaranthus* species

Intraspecific variation exists in *Amaranthus* species primarily among accessions at both the allozyme and RAPD levels. The generally lower values of isozyme and RAPD polymorphism of grain amaranths in comparison with the wild amaranth species studied may reflect a narrower range of intraspecific variation in the crop species. This pattern of genetic variation in grain amaranths suggests that they may have passed through genetic bottlenecks during the process of speciation and/or experienced strong directional selection under domestication. *A. cruentus* is considered the most important grain species in the genus (Grubben and Van Sloten 1981), and it probably experienced the highest selection pressure under domestication resulting in the lowest level of genetic variation as shown at both isozyme and RAPD levels. Domestication of a small subset of wild populations, or introduction of a few accessions to new geographic regions with subsequent inbreeding and artificial selection for favorable cultivars, could both reduce genetic variation (Hilu 1995).

Most of the vegetable species have been cultivated relatively recently compared with the ancient grain amaranths. In a review of vegetable amaranths, Kauffman and Gilbert (1981) stated that *A. tricolor* has only been selected and bred as a vegetable in southern China for a few centuries. The relatively high level of RAPD polymorphism in the vegetable species (*A. dubius* and *A. tricolor*) may be a result of their relatively short cultivation history or lack of selection pressure in cultivation. This is especially true for *A. dubius*, since it has been grown as a pot herb and minimal artificial selection is expected. Garden escapes of *A. dubius* can also be found in open fields (Behera and Patnaik 1982), and these escaped plants could be more polymorphic genetically than their cultivated counterparts. Greater RAPD polymorphism was found in all other wild amaranths with two or more accessions studied than in the cultivated species. High levels of genetic variability of the wild species are expected because they were not subject to any of the selection pressures of domestication, and the maintenance of high genetic variability would favor their survival under natural conditions. Other studies also found that wild species usually maintain higher level of polymorphism compared to cultivated species (e.g. Aldrich et al. 1992; Abo-elwafa et al. 1995).

The observed genetic uniformity within most accessions of both cultivated and wild amaranth species would not normally be expected in light of their

breeding systems. All grain amaranths and their putative ancestors are monoecious and characteristically wind pollinated. However, the arrangement and sequence of anthesis of the unisexual flowers favor a combination of self- and cross-pollination, with selfing likely to be more common than crossing (Sauer 1976). A full range of outcrossing rates (0–100%, averaging 31%) has been reported in a population of *A. cruentus* (Hauptli and Jain 1985). Although species with a mixed-mating system can maintain a higher level of genetic variation within populations than selfing species (Hamrick et al. 1991), the loss of intra-accessional genetic variation could have occurred in cultivated amaranths as a result of artificial selection for pure lines. On the other hand, the germplasm collections may represent only a limited portion of the wild gene pool, and subsequent maintenance procedures, such as those used in seed regeneration or multiplication, could further deplete genetic variation within accessions maintained in the USDA/ARS amaranth collections.

The evolutionary origin of grain amaranths

Based on geographical distribution and morphological characteristics, Sauer (1967, 1976) postulated two hypotheses for the origin of grain amaranths. The polyphyletic hypothesis proposes that the three grain amaranths originated independently from separate ancestors in different parts of the world. The alternative monophyletic hypothesis suggests that all three grain species were derived from a single progenitor through introgressive hybridizations with two other wild species. In both cases, the proposed progenitor species involved in the evolution of grain amaranths are the same, i.e. *A. hybridus*, *A. powellii*, and *A. quitensis*. Both isozyme and RAPD data showed that grain amaranths are more closely related to each other and to *A. hybridus* than to *A. powellii* or *A. quitensis*, suggesting that *A. hybridus* is most likely the common ancestor of the grain amaranths. The present data support Sauer's monophyletic hypothesis for the origin of grain amaranths. If the polyphyletic origin were true, the expected relationships would be that *A. caudatus* should more closely related to *A. quitensis*, *A. cruentus* to *A. hybridus*, and *A. hypochondriacus* to *A. powellii*. Although the dendrograms based on isozyme data differ in topology from those based on RAPD data in clustering species and/or accessions, the common feature of all the dendrograms was the clear separation of grain amaranths/*A. hybridus* from *A. powellii*/*A. quitensis* (Figs. 1–4). It would be more difficult to discriminate between the two competing hypotheses if the putative ancestors were closely related to each other. However, at both the RAPD and isozyme levels, the putative ancestors exhibited a higher level of interspecific divergence (Jaccard's similarity coefficient: 30%, and Nei's identity: 0.55) than grain amaranths (Jaccard's similarity coefficient: 36%; and Nei's identity: 0.77).

Other studies of the relationships among amaranth species have given varying results (e.g. Pal and Khoshoo 1972, 1974; Greizenstein and Poggio 1994; Transue et al. 1994; Lanoue et al. 1996). Studies of chromosome numbers and interspecific fertility among the species supported the hypothesis of independent domestication, and the hybrid fertility data indicated that *A. hypochondriacus* and *A. caudatus* are the most closely related pair in the grain amaranth species group (e.g. Pal and Khoshoo 1972, 1993, 1974; Gupta and Gudu 1991). RAPD data supported a close relationship between *A. hypochondriacus* and *A. caudatus* (Transue et al. 1994). Hauptli and Jain (1984) found that with the exception of the *A. caudatus* – *A. quitensis* pair, grain amaranths are more closely related to each other than to their respective putative progenitors on the basis of isozyme data. On the basis of restriction-site variation in chloroplast and nuclear DNA, Lanoue et al. (1996) found that *A. caudatus* and *A. cruentus* are more closely related to each other and to their respective putative progenitors *A. quitensis* and *A. hybridus* than either is to *A. hypochondriacus*. Some of the incongruence between these various studies is probably caused by intraspecific variation among the accessions used to represent each species. Due to both inter- and intraspecific variation, differences in the number of species compared and in the sample size of intraspecific accessions could also lead to different results among studies.

In the present study, *A. dubius* was shown to be the next most closely related to the grain amaranths after *A. hybridus*, based on both isozyme and RAPD data. However, the high genetic similarity of *A. dubius* to the grain amaranth species could be a result of its tetraploidy ($2n = 4x = 64$; Behera and Patnaik 1982; Chen and Sun, unpublished). If *A. dubius* is an allotetraploid, and has evolved from natural hybridization between two diploid species in the grain amaranths/*A. hybridus* group, the present clustering relationship can arise (Figs. 2–4). This hypothesis can be tested by further cytological and genetic studies of the related species. Chromosome numbers were investigated for 23 accessions representing 14 species of *Amaranthus*, including all grain amaranths and their putative progenitors (Chen and Sun, unpublished). Except for *A. dubius* ($2n = 64$), a mitotic chromosome number of $2n = 32$ was found in all species investigated. Occasional variant numbers were obtained within one accession of *A. hypochondriacus* (PI 477915, $2n = 32$ or 34) and one accession of *A. powellii* (PI 572257, $2n = 32$ or 33).

A. tricolor has also been suggested as one of the common progenitors of *A. cruentus* and *A. hypochondriacus* (Pal and Khoshoo 1973). However, the present study revealed low genetic similarities between *A. tricolor* and the grain amaranth species. Similarly, no close relationship between *A. tricolor* and grain

amaranths was indicated in the study by Lanoue et al. (1996) based on chloroplast- and nuclear-DNA data.

Comparison between isozyme and RAPD analysis

In the present study, nearly all accessions of the same species were grouped together in all dendrograms irrespective of whether the RAPD or isozyme data sets were used, indicating that, at the intraspecific level, the reliability of RAPD data is comparable to that of isozyme data. Comparative studies using RAPD, RFLP and/or allozyme markers have shown that RAPD is a valuable tool for assessing population genetic variation (e.g. Liu and Furnier 1993), and complete congruence has been found between gene diversity estimates derived from isozyme and RAPD data sets (Isabel et al. 1995). There are other studies showing agreement between RAPDs and RFLPs in identifying accessions or classifying genotypes of the same species (e.g. N'goran et al. 1994; Thormann et al. 1994).

Although the species grouping based on RAPD data differed from that based on isozyme data in the present study, there are some similarities between the dendrograms. The following clustering relationship can be observed whether based on RAPD or isozyme data: group 1 *A. caudatus*, *A. cruentus*, *A. dubius*, *A.* hybrid, *A. hybridus*, and *A. hypochondriacus*; group 2 *A. standleyanus* and *A. tricolor*; group 3 *A. powellii*, *A. quitensis*, and *A. retroflexus*; and group 4 *A. fimbriatus* and *A. floridanus* (Figs. 2, 4). One of the major differences between the two dendrograms is the placement of group-2 species relative to group 1 and others. The dendrogram based on the isozyme data set indicated a closer evolutionary relationship between grain amaranths and their putative progenitors. However, the relationship between grain amaranths and *A. powellii*/*A. quitensis* was not apparent from the dendrogram based on the RAPD data set, although, assuming a monophyletic origin of grain amaranths, the expected closer relationships between the *A. hybridus*–*A. cruentus*, *A. powellii*–*A. hypochondriacus*, and *A. quitensis*–*A. caudatus* species pairs were well supported by pairwise interspecific similarity coefficients based on RAPD data (Table 4).

At the interspecific level, RAPD markers are considered less suitable for studying phylogenetic relationships, compared to isozyme or RFLP markers, because some of the co-migrating RAPD bands from different species may not be homologous (Thormann et al. 1994; Rieseberg 1996). The differences in the placement of *A. acutilobus*, *A. albus*, *A. australis*, *A. crassipes*, and *A. lividus* between the dendrograms in the present study could, in part, be caused by a lack of homology among co-migrating RAPD fragments. Although it seems that complementary approaches, such as using both isozyme and RAPD data, may provide more accurate information on genetic diversity and relationships within the amaranth germplasm collections, the different data sets need to be analyzed separately. Due to the incongruence between the isozyme and RAPD data sets, the 50% majority rule consensus tree, constructed using both data sets combined, failed to cluster intraspecific accessions into distinctive species groups (dendrogram not shown), suggesting that the combination of the two data sets was not appropriate.

There is no doubt that when morphological variation causes confusion or mis-identification RAPD analysis alone can aid the correct identification of species in amaranth genetic resources (Transue et al. 1994; and the present study). RAPD analysis is especially valuable for classifying germplasm collections that lack sufficient isozyme variation (Sun et al., unpublished data). However, isozyme analysis appears to be more suitable for phylogenetic investigations of interspecific relationships and, in particular, for studying the evolutionary origin of crop species.

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