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Genetic diversity in genus *Amaranthus*: From morphology to genomic DNA

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ABSTRACT

Some species of the genus *Amaranthus* are cultivated for their grains or leaves. Some others are useful as colorful ornamentals. The crops are very promising food crop in arid region, due to its resistance to heat, draught, disease and pests. Genetic diversity studies for this genus are essential for providing information for propagation, domestication, and breeding programs as well as conservation of genetic resources. Therefore, this review are devoted for evaluating the genetic diversity between wild and cultivated species and assessing the evolutionary relationships between the cultivated species and their putative species using wide array of available markers.. A wide morphological variability between *Amaranthus* species and different accessions of vegetable *Amaranthus* was reported. This variability was useful in cultivar improvement for agronomic traits. The chromosome number for *Amaranthus* species is normally 2n=32 (n=16), but occasionally it is 34 (n=17). It has been suggested that the gametic number n=17 has originated from n=16 through trisomy. Karyotypes are mainly comprised of many metacentric chromosomes and few submetacentric ones. There is a variation in chromosome size between *Amaranthus* spp. and the accessions of each species. Based on cytological data, it was proposed that *A. hybridus is the* putative ancestors of the cultivated amaranths. Buffer extracts of seed storage proteins of taxa of *Amaranthus spp*. analyzed on SDS-PAGE under reducing conditions divided *Amaranthus* taxa into two groups; group with n=17 and the other group with n=16, indicating the relation between the chromosome number and the electrophoretic pattern. The electrophorectic patterns of the seed proteins of amaranth species can be used to discriminate between *Amaranthus* species. Isozymes markers showed low heterozygosity in the New World populations of *Amaranthus.* A wide genetic distance was detected between crop and weed species. Alleles at several loci proved to be diagnostic of the crop and weed groups. High levels of interspecific and intraspecific variation were found between Amaranthus spp using isozyme marker. Biochemical and molecular data sets supported a monophyletic origin of grain amaranths, with *A. hybridus* as the common ancestor. The molecular data showed genetic variation among and within the populations of *Amaranthus* spp. and indicated that genetic diveristy within wild was lower than grain species.

KEYWORDS

Morphological traits;
Cytological traits;
Isozyme;
Electrophoretic pattern;
RAPD;
ISSRs;
ITS;
AFLP.

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INTRODUCTION

The genus *Amaranthus belongs to family Amaranthaceae,* includes about 75 species[1], some of them being cultivated for more than 5000 years for their grains (*A.caudatus, A.cruentus* and *A.hypochondriacus*) or leaves (*A.blitum, A.dubius* and *A.tricolor*). Some species are useful as colorful ornamentals[2]. *Amaranthus* species have different centres of domestication and origin, being widely distributed in North America (Canada, United States), Central America (Mexico, Guatemala), and the South American Andes (Peru, Bolivia, Ecuador), where also the greatest genetic diversity is found.

The taxonomy of the genus *Amaranthus* has been confused by the extremely used range of phenotypic plasticity among species and the possible introgression and hybridization involving weedy and crop species[3]. *Amaranthus* is often difficult to characterize taxonomically, due to the similarity between the large number of species and difficulty to see diagnostic parts, intermediate (hybrid) forms and the broad geographical distribution, which is the reason for many synonyms[4]. The difficulty in distinguishing *Amaranthus* hybrids from non-hybrids based on morphological feature has contributed to the lack of information in this area. Hybridization among weedy *Amaranthus* is hypothesized to adapt more quickly to cropping system. Little is known about the genetic or evolutionary origin of grain *Amaranthus*, and without such knowledge scientific breeding, especially making use biotechnological methods, is not possible.

The cultivated *Amaranthus* is not present in Egypt, though they are commercial crops in many countries of the world. It is an important crop especially among the Aztecs of Mexico and the Incas of Peru[5]. *Amaranthus cruentus* is a widespread traditional vegetable in all countries of tropical Africa. Grain *Amaranthus* is produced commercially in hot and dry areas of the United States, Argentina and China. Ornamental types of *Amaranthus cruentus* characterized by big bright-red inflorescences can be frequently found in tropical and subtropical countries[6]. *Amaranthus* spp. can be used as commercial food colouring, as an alternative for the pigments from red beet (*Beta vulgaris* L.)[7,8]. The crops are very promising food crop in arid region, due its resistance to heat, draught, disease and pests. In addition, the nutritional value of both the seeds and leaves is excellent. The importance of the crop to Egypt is twofolds, due to the shortage of grain production and suffering the majority of Egyptian territory drought and heat.

The objectives of this review are evaluating the genetic diversity between wild and cultivated species and assessing the evolutionary relationships between the cultivated species and their putative species using wide array of available markers.

Genetic diversity

Genetic diversity is defined as the variation of individual genotypes within and among species. It is important trait for long-term survival of species and enables a population to adapt to new conditions brought by environmental change[9]. It is the raw material permitting species to adjust to a changing world, whether these changes are due to natural or human factors. The genetic profile of whole populations typically varies from place to place across a species range. These differences may arise as the result of chance occurrences, such as the genetic composition of dispersing individuals that create a new population (founder effect), or changes in allele frequencies that result from chance matings in very small populations (genetic drift)[10]. Differences among populations can also arise systematically, especially if the environment in various places exposes individuals to different optima for survival and reproduction (fitness). For these and other reasons, populations often diverge from each other in their genetic composition. Such divergence is especially strong and rapid when there is little gene flow among populations (e.g., limited dispersal of seeds or pollen, or limited movement of animals across physiographic barriers)[10]. Over evolutionary time, such among-population genetic differences can accumulate and result in the development of a new species (allopatric speciation). Knowledge of the amount and distribution of genetic variability within a species is vital to plant breeders because it is an important consideration when selecting germplasm to be included in a breeding program. Also, it is helpful to geneticists managing plant genetic resources and provides information for designing sampling protocols[11]. So, genetic diversity studies are essential for providing information for propagation, domestication, and breeding pro-

grams as well as conservation of genetic resources for plant species.

Genetic diversity can be analyzed within population (intra population= among individuals), within species (intra-specific= among populations) and among species (inter-specific) levels. Measuring genetic diversity aims to reveal potentially useful variability by screening a fraction of all possible loci of the genome[12,13]. There are numerous methods available to achieve such aim. Their employment depends on the type of information required.

Estimations of genetic variability are based on morphological, cytological, biochemical and molecular traits. However, the estimation of genetic variability based on morphological and cytological traits has the disadvantages of being influenced by both environmental and genetic factors and may therefore not provide an accurate measure[14].

Morphological traits

Morphological variation in populations has been described for characters controlled by a single or multiple gene systems. The greater of gene loci numbers that determine a trait, the more continuous the variation will be[15]. The expression of quantitative traits is influenced by the environment and the variation pattern in these traits is generally considered to be the result of both genetic and environmental attributes.

The morphological traits have been used by geneticists and evolutionists to describe genetic variation within and among populations of the same species, for example, sessile oak[16], *Hordeum vulgare*[17], *Lathyrus sativus*[18-20], *Lespedeza*[21], *Lactuca*[22,23] and *Amaranthus*[24-28].

Wu *et al.*[25] in a study of 229 genotypes from 20 *Amaranthus* species observed wide variability which was useful in cultivar improvement for agronomic traits, such as plant height, seed, stem and leaf color among genotypes within the same species and among different *Amaranthus* species. Similar results were also observed by Xiao *et al.*[26,27], in the evaluation of different accessions of vegetable *Amaranthus*. Qualitative characters have been used for plant description and mainly influenced by the consumers' preferences, socio-economic scenario and natural selection[29]. They are also useful in separating varieties especially when the range of quantitative characters is limited[30].

Cytological study

It is well documented that each species has its own specific and constant diploid chromosome complement. Consequently, it is considered a basic taxonomic parameter for fitting individuals into a logical hierarchy of species, genera and higher categories, and of identifying them according to their position in this hierarchy[30].

Mulligan[31] reported a chromosome count of 2n = 32 for *A. albus* from Indian Head. This is identical to that reported in California by Heiser and Whitaker[32] and in other regions of the world. In contrast, Sharma and Banik[34] reported 2n = 34 from India.

The chromosome number for *A.caudatus, A.hipochondriacus, A.cruentus,* and *A.hybridus* is normally 2n=32, but occasionally it is 34[35], i.e. these species are diploids with a basic chromosome number of 16 or 17[4]. Among *Amaranthus* species and varieties studied *A. blitoides, A. cruentus, A. graecizans* and *A. albus* possessed 2n=2x=32 while the other species possessed 2n=2x=34, chromosome numbers supporting the previous reports on these species[36]. The occurrence of two basic chromosome number of x=16 and 17 in a single species and also the role of aneuploidy in chromosome evolution of the genus *Amaranthus* is a well-known fact. It has been suggested that the gametic number n=17 has originated from n=16 through trisomy[36]. The cytological investigation of 14 samples belong to 6 species of *Amaranthus* (*A.viridus L., A.sylvestris, A.graeccizans, A. hypochondriacus L., A. cruentus L., A.chlorostachys L.(hybridus*) revealed that the genus is diploid with 2n = 32 and 34. Karyotypes are mainly comprised of many metacentric chromosomes and few submetacentric ones. However, there has been a noticable variation among accessions in the number of chromosomes in each type. *A.viridus* could be the most advanced species amongst all the investigated taxa. It exhibits the diploid number 2n = 34 and the shortest haploid genome length with more Karyotypic activity, concerning chromosome length and centromeric position, recorded among its different accessions. This might have been produced as a result of differences in the degree of chromatin condensation and /or chromosomal changes such as translocations and pericentric inversions[37,38]. The small size of the chromosomes, together with their unclear centromeres, has hampered a detailed Karyotype analysis[37,39]. The chromosome size in

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A.viridis L. was ranged from 0.47 to 0.80, in *A.sylvestris* (Desf.)Vill was 0.84, in *A.graecizans* L. was ranged between 0.81 and 0.87, in *A. hypochondriacus* L. was extended from 0.89 to 0.92, in *A.cruentus* L. was between 0.78 and 1.04, and in *A.chlorostachys* L. was 1.03.

Cytogenetic analysis of 12 populations of 10 *Amaranthus* L. (2*n*=32 and 34) showed a normal meiosis forming manly bivalents in metaphase of meiosisI[40]. A post pachytene diffuse stage occurred in all the species possibly as a means of adaptation to adverse environmental conditions. ANOVA test revealed significant differences in relative cytogenetic characteristics including chiasma frequency and distribution as well as chromosome pairing among the studied species, indicating their genomic differences.

Some species of the genus *Amaranthus* are polyploids (basic number x=8) and the haploid chromosome number n=17 originated later by primary trisomy (2n+1)[41]. The evaluation of the chromosome analysis of *A. turicensis* hybrid (2n=34) showed that both parental species (*A. cruentus and A. retroflexus*) should hybridize relatively easily. However, most of the *Amaranthus* hybrids exhibit relatively high level of sterility which was already confirmed by Gupta and Gudu[42].

Most *Amaranthus* species have chromosome numbers n = 16 or n = 17, but *A. dubius* is unusual for having n = 32[43]. The grain *Amaranthus* are paleoallotetraploids, as indicated by observations of pairing in their hybrids[44,41]. However, it was found that the species *A. retroflexus, A. cruentus and A. turicensis* have the same chromosome number 34. No higher ploidy level was detected. The chromosomes of all species studied uniform, short, and monotypic. No marked differences in chromosome counts and visual aspects (length, centromere position) were observed.

Sauer[5] proposed the 3 weedy *Amaranthus*, namely, *A.powellii, A.hybridus and A.quitensis* as putative ancestors of the cultivated amaranths, namely, *A.hypochondriacus, A.cruentus* and *A.caudatus* respectively. This scheme has been refuted by Pal and Khoshoo[45] on the basis of cytogenetic studies on *A.powellii and A. hypochondriacus* since the 2 species have different basic chromosome numbers (n = 17 and n = 16 respectively) and since the hybrid between the two was sterile. Further, they have also suggested that *A. hybridus* is the more likely ancestral species for *A.hypochondriacus*.

Biochemical traits

Proteins (SDS-PAGE)

Proteins are the post-transcriptional and translational products of an organism's DNA, and form structural and enzymatic components of cells. Their size and amino acids sequence are the direct results of transcription and translation of the nucleotide sequences of the genes[46]. Hence, any observed variation in protein systems is considered as a mirror for genetic variations, specifically seed proteins, they reflect the genetic history of the speciens and do not affect with the environmental fluctations[47].

Electrophoretic techniques have been widely used as a rapid and accurate test to identify and characterize different cultivars and genotypes of plants. Genotype identification by electrophoretic protein fingerprinting was used to assess the uniformity, purity and agronomic merits[48,49].

Sammour[31] reported that Polyacrylamide gel techniques allow us to; (1) identify variation among the taxa of each species, (2) screen the purity of the ever expanding number of cultivars, (3) verify whether or not two or more morphologically identical accession in the collection was also electrophoretically identical, (4) exploit the important traits of landraces and wild relatives to provide increasing crop production and stabilizing yield.

Electrophoretic analysis of native or denatured seed storage proteins was used to provide information concerning the genetic variability, which represent a source of information for assessing genetic and taxonomic relationships at the species level and below[19,23,51-53].

Buffer extracts of seed storage proteins of 44 taxa of *Amaranthus spp*. were analyzed on SDS-PAGE under reducing conditions in which *Amaranthus L.* taxa can be divided into two groups. Group one with basic chromosome number x=17 and the other group with basic chromosome number x=16[50]. This data undoubtly indicated the relation between the chromosome number and the electrophoretic pattern. The data also confirm the separation of *A.cruentus* from *A.hybridus and A.sylvestris and A.sylvestris from A.Graecizans*.

Zheleznov *et al.*[54] studied variation within genus *Amaranthus* using SDS-PAGE and reported that (1) the range of variation in protein content in seed both

among wild and cultivated forms of *Amaranthus* is rather wide, (2) *Amaranthus* seed proteins are highly nutritive and, on the whole, consist of easily digested albumins and globulins (more than 50% of total protein), 20.8% of alkali-soluble proteins-glutelins, which are close to albumins and globulins by their nutritive value, and only of 12% of alkali-soluble proteins prolamines that are poor in essential amino acids, (3) by means of polyacrylamide gel electrophoresis (buffer pH – 3.2) it was shown that the seed proteins of the studied amaranth species are heterogeneous and consist of 38 bands. By decreasing electrophoretic mobility these bands were conventionally assigned to 4 zones, (4) the study of electrophoretic patterns of seed proteins is very promising for establishment of phylogenetic relationship among the species of genus *Amaranthus*.

The SDS-PAGE of urea-soluble seed proteins is suitable for distinguishing both species and cultivars of *Amaranthus*. Samples of the seven species examined were divided into three groups. By protein patterns *A. tricolor* (leafy type of *Amaranthus*) clearly differs from other species. The study suggested a closer similarity between *A.caudatus* and *A.cruentus* species than between the pairs of species *A.hypochondriacus/ A.caudatus* and *A.hypochondriacus/A.cruentus*. Only slight differences were seen among cultivars, especially of grain amaranths. An evaluation of crossing rate on the basis of electrophoregrams of urea-soluble proteins, which were extracted from singular seeds is proposed by Drzewiecki[51].

The taxonomic complexity in the genus *Amaranthus* was studied based on the seed protein profiles[55]. A range of peptides varying from 64 to 12 kDa, with a larger number of protein bands observed between 25.1 and 12 kDa. The similarity analysis based on the SDS-PAGE profile was found to ba a useful character for the discrimination of species in *Amaranthus*, except for *A.cruentus* and *A.hypochondriacus*, for which a hybrid population was found.

The study of Janovská[56] on the seed protein profiles of 15 *Amaranthus* accessions from the Czech Gene Bank using both SDS-PAGE and chip electrophoretic profiles exhibited that (1) chip electrophoretic technique is highly sensitive and produces wider range of bands; and (2) the obtained data confirmed the classification of Amaranthus species studied. The analysis of the total seed protteins used very efficiently to assess the genetic differences in two grain populations of *Amaranthus retroflexus* collected from field of the Maize Research Institute Zemun Polje, Serbia[57]. It was found that (1) two populations have different protein profile; (2) 18 protein fractions were obtained by protein analysis; (3) the populations differed in the four protein fractions of different molecular weight; and the seed protein electrophoresis are useful for genetic determination of *A.retroflexus* populations and identification of biotypes with atypical morphology.

Isozymes

Isozymes were defined as structurally different molecular forms of an enzyme with, qualitatively, the same catalytic function. Isozymes originate through amino acid alteration, which cause changes in net charge, or the spatial structure (conformation) of the enzyme molecules and also, therefore, their electrophoretic mobility. After specific staining the isozyme profile of individual samples can be observed[58]. Data derived from electrophoretic gels consists of the number and relative mobilities of various enzyme forms, which with appropriate genetic analyses, become transformed into single or multi loci genotypes for each individual[59]. Reasons are many for the popularity of electrophoretic data, but foremost among these is that isozymes provide a series of readily scored, single-gene markers[59]. Enzymes that are coded by different alleles of a distinct locus or those coded by separate loci frequently show different electrophoretic mobilities.

Allele frequency data are used to obtain a number of measures which include average level of heterozygosity (which estimates the probability that two alleles taken at random from the population are different), average level of polymorphism (which is the condition of polymorphic gene and characters, where the polymorphic gene has at least two alleles and polymorphic character has two or more qualitatively distinct morphs) and mean number of alleles per locus[60].

Isozyme analysis has been used for over 60 years for various purposes in biology, e.g., to delineate phylogenetic relationships, estimate genetic variability and taxonomy, identify cultivars and genes, and study population genetics and developmental biology[19,23,61]. It was also utilized in plant genetic resources management and plant breeding. Furthermore, isozymes analysis was used in control of breeding, estimation of outcrossing, testing purity and in species delimitation and conserva-

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tion[62,63,64]. Finally isozyme technique may be used by plant breeders to generate, evaluate, and select desired genotypes in early stage of the breeding program, which saves time, money and efforts of the breeders[65].

World amaranths along with 21 weedy New World populations were assayed using nine enzymatic systems[3]. In the New World populations, heterozygosity was low, and polymorphic loci ranged from 0 to 44%. Diversity index H2 was partitioned into the intra- and interpopulation as well as the interspecific components of variability. The crop versus weed genetic distances was the largest, whereas the intra- and interpopulation components of H2 were about equal. Genetic structure of all three species of the New World amaranths together can be described as a collection of distinct populations, each more or less a heterogeneous collection of highly homozygous individuals. The North Indian populations showed relatively less allozyme variability with the most common alleles same as those of Mexican landraces. Alleles at several loci proved to be diagnostic of the crop and weed groups, and of the three individual crop species. Genetic distances based on pooled gene frequencies showed the three crop species to be generally more closely related than they were to their putative weedy progenitor species, respectively (with the exception of the weed-crop pair *A.quitensis* and *A.caudatus*). This implies a single domestication event involving *A.hybridus* as the common ancestor rather than three separate domestication events. Close similarity between *A.caudatus* and *A.quitensis* might have resulted from transdomestication based on a weedy or semi-domesticated species having migrated from Meso-America to South America. Some evidence of recent introgression and/or segregation of crop-weed hybrids between *A.caudatus* and *A.retroflexus* is available in the form of rare individuals in crop populations with crop allozyme genotypes except for a single homozygous weedy allele.

Genetic variation and genetic relationships of a total of 23 species and 60 populations of cultivated and wild amaranths were performed using isozyme marker[66]. High levels of interspecific and intraspecific variation were found between the investigated species and populations. 132 alleles were detected for 15 enzymes. Total gene diversity for grain amaranths and wild species was 0.39 and 0.72 respectively. The polymorphism assays clarified the relationships of grain amaranths (*A.caudatus*, *A.cruentus*, *A.hypochondriacus*) and their putative ancestors (*A.hybridus*, *A.powellii*, and *A.quitensis*), and the results point toward a monophyletic origin of the grain amaranths. In addition, the genetic diversity and relationships of other species of amaranths were determined.

Genetic diversity and relationships of 23 cultivated and wild *Amaranthus* species were examined using isozyme marker. 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[67].

Iudina *et al*.[68] examined the electrophoretic patterns of five isozymes systems in total, 52 populations and two varieties (Cherginskii and Valentina). Allozyme variation of this material was low. Irrespective of species affiliation, 26 populations and two varieties were monomorphic for five enzymes; a slight polymorphism of three, two, and one enzymes was revealed in three, nine, and fourteen populations, respectively.

Molecular traits

During the last decades, molecular markers have proven to be powerful tools for assessing genetic variation within and among populations of plants. Several criteria should be considered in choosing molecular techniques for genetic diversity studies including the following: whether the techniques are highly reproducible between laboratories and whether the data that is generated can be reliably transferred; whether markers are dominant or codominant, allowing homozygotes and heterozygotes to be distinguished; the amount of genomic sequence information required; and whether the markers detect highly polymorphic loci (Osman *et al*., 2003). At present, various molecular techniques are available for assessing genetic diversity in plants including identification of random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), restricted fragment length polymorphism (RFLP), internal transcribed spacer (ITS-1), and microsatellites or inter simple sequence repeat (ISSR).

RAPD

RAPD is one of the molecular techniques used on a wide scale in genetic diversity. This technique is PCR-based technique that uses short primers of arbitrary sequences to produce random amplification of DNA

fragments from the genome being studied[69]. These fragments vary in migration on the gel when they vary in length. These arbitrary primers method yield at least several genetic markers, which are generally inherited as dominant alleles, whereas fragments absence generally is recessive. Amplification products separated on agarose gel in the presence of ethidium bromide and visualized under ultra- violet light[58]. The advantages of this method are many. Genetic analysis with RAPD markers is fast, less technical, less expensive and involves no radioactivity and hybridization. Also, RAPD markers are usually scored as dominant alleles, since the amplified DNA product is present in one parent but absent from the other. For repositories with large collections, this technique represents an important advance towards detailed characterization of individual accessions at the molecular level[70].

The RAPD technology find its greatest application in detecting polymorphisms in the closely related organisms (low divergence) such as those that compose a species complex, different populations of a single species or individuals within a population[69]. RAPD has proved to be a good genetic marker to assay and evaluate the genetic diversity among species and even among populations and individuals of the same species[23].

RAPD analysis is a powerful tool for determining inter- as well as intra-species genetic relationships[69]. Such studies have been carried out amongst wild and cultivated species[71], among self and cross-pollinated species[72] and even within germplasm of a single species[73]. In the earlier study on *Amaranthus*, the grain *Amaranthus* germplasm was analysed by RAPD[74]. However, this study did not assess inter-species relationship.

The RAPD technique has been successfully used for evaluating variation within plant accessions and to establish differences among lines of apparently closely related populations in germplasm collections, for example, American chestnut[75], barely[76], *Pinus longaeva*[77], strawberry[78], *Trigonella*[79], *Morus*[80], *Orobanche*[81], *Lactuca*[82], Curcuma species[83], White sapote[84] and *Amaranthus*[85].

Mandal and Das[86] demonstrated a high level of genetic similarity between *A. hypochondriacus* and *A. caudatus* which supports earlier RAPD analytical observations of Chan and Sun[87]. The experiment was conducted with eight decamer primers. Following interspecific hybridization analysis and the hybrid fertility data it was also concluded that these two are the most closely related pair in the grain *Amaranthus* species group[43]. It is reasonable to suggest from the study of similarity/dissimilarity percent and RAPD data clustering through dendrogram that at least *A.hypochondriacus* and *A.caudatus* are expected to have a common progenitor.

Genetic diversity and relationships among six *Amaranthus* species from eight phytogeographic regions were analyzed using a random amplified polymorphic DNA (RAPD) marker. RAPD primers yielded a total of 262 amplicons, ranging from 250 to 3000 bp in size with an average of 13.1 amplicons per primer, of which 254 amplicons (96.94%) were polymorphic. The genetic similarity coefficient among all the *Amaranthus* species ranged from 0.16 to 0.97 with a mean similarity coefficient of 0.56, indicating that variation existed in the genetic diversity of different populations[85].

RAPD results tend to support a closer genetic relationship between *A.caudatus* and *A.hypochondriacus* species[88], the hybrid of *A.edulis* and *A.caudatus* is clustered together with *A.caudatus*, while the hybrid of *A.hybriuds* and *A.hypochondriacus* is in the cluster of the latter species. The low values of genetic distance between these hybrids and other accessions of *A.caudatus* and *A.hypochondriacus* respectively, indicated that these are not strongly differentiated genetically[89].

Genetic diversity and relationships of 23 cultivated and wild *Amaranthus* species were examined using both isozyme and RAPD markers. 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 *Amaranthus* 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 RAPD was shown to generate more accurate estimates of genetic diversity, and of relationships within and among crop species and their

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wild relatives, than either data set alone[88].

AFLP, ITS, ISSR

AFLP, ITS and ISSR markers were used very efficiently to study genetic diversity[90], phylogenetic relationships, to asses the putative wild progenitor of wild amaranthus[91,92] and to resolve taxonomic confusion between the taxa of *Amaranthus*[91].

Genetic diversity and the phylogenetic position of *A. pumilus* was measured using single primer ISSRs[90]. The obtained data showed genetic variation among and within *A. pumilus* populations, though variability was low. Fenwick populations exhibited the highest genetic variability (0.1016), while on Assateague the wild *A. pumilus* population had higher variability (0.0340) than the propagated population (0.0185). Comparing genetic diveristy within *A.pumilus* with those of grain varieties *A.hypochondriacus* L. and *A.cruentus* L. revealed that genetic diversity within *A.pumilus* was lower than either grain species sampled (0.2263 and 0.2947). Phylogenetic analyses included 41 accessions representing 33 *Amaranthus* species; detected considerable phylogenetic signal within the data matrix, though the phylogenetic resolution was low. In all consensus trees *Amaranthus pumilus* grouped with the coastal species *A. arenicola* I.M. Johnst, which is the first postulated relationship of this pair.

A comparative analysis of phylogenetic relationships among the 'Morelos' accessions of *Amaranthus* from Mexico using amplified fragment length polymorphism (AFLP) and micromorphology methods was conducted[91]. The data exhibited that all the controversial 'Morelos' accessions can be consistently placed into a single *A. cruentus* species clade, which is clearly separated from the *A.caudatus* species clade. The AFLP-based phylogenetic relationship of 'Morelos' and delimitation of *A.cruentus* and *A.caudatus* are further supported by micromorphology, showing that the combination of these techniques can provide more reliable data for germplasm identification than each method used alone.

Phylogenetic relationships of grain amaranths and their wild relatives, and taxonomic confusion exists among three cultivated grain amaranths, *A.cruentus*, *A.caudatus*, and *A.hypochondriacus*, and their putative wild progenitors, *A.hybridus*, *A.quitensis*, and *A.powellii* was reexamined using ITS, AFLP and ISSR[92]. Low ITS divergence in these taxa resulted in poorly resolved phylogeny. However, extensive polymorphisms exist at AFLP and ISSR loci both within and among species. In phylogenetic trees based on either AFLP or ISSR or the combined data sets, nearly all intraspecific accessions can be placed in their corresponding species clades, indicating that these taxa are well-separated species. The AFLP trees share many features in common with the ISSR trees, showing a close relationship between A. caudatus and A. quitensis, placing A. hybridus in the same clade as all grain amaranths, and indicating that *A. powellii* is the most divergent taxon in the A. hybridus species complex. This study has demonstrated that both AFLP and double-primer fluorescent ISSR have a great potential for generating a large number of informative characters for phylogenetic analysis of closely related species, especially when ITS diversity is insufficient.

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