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Genetic variability in *Amaranthus* based on biochemical traits

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ABSTRACT

Amaranthus species are important crops that resist environment stresses (heat, draught, disease and pests). Some species of this genus are cultivated for their grains or leaves. Some others are useful as colorful ornamentals. In addition, it is used in folk medicine, thousands of years ago. Information on genetic variability and relationships within and among *Amaranthus* species and their wild relatives is essential for the efficient utilization of their plant genetic resource collections for propagation, domestication, and breeding programs as well as conservation of genetic resources. Therefore, this review is focused on evaluating the genetic variability between wild and cultivated species and assessing the evolutionary relationships between the cultivated species and their putative species using biochemical markers. The protein content varied between the different collections of Amaranthus species. It is ranged from 12.43 to 18.20%. There is also a great variability in protein content within the accession of the same species. For example the variation in protein content between the accessions of *A. cruentus* extends from 13.2 – 18.2%. 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 *Amaranthus* species were successfully used in the discrimination between *Amaranthus* species. Isozymes, as the second tool of the biochemical 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 inter-specific and intra-specific variations were found between *Amaranthus* spp. using isozyme markers. Biochemical data supported a monophyletic origin of grain amaranths, with *A. hybridus* as the common ancestor. They also showed genetic variation among and within the populations of *Amaranthus* spp. and indicated that genetic variability within wild was lower than grain species.

KEYWORDS

Protein content;
Genetic diversity;
SDS-PAGE;
Isozymes;
Electrophoretic pattern.

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INTRODUCTION

Amaranthus commonly known as "chaulai", *belongs to family Amaranthaceae,* order Caryophyllales. It includes about 87 species[1-3]. Some of the *Amaranthus* spp. are cultivated for their grains (*A.caudatus, A.cruentus* and *A. hypochondriacus*) or leaves (*A.blitum,A.dubius* and *A.tricolor*)[4]. The cultivated *Amaranthus* is commercial crops in many countries of the world, especially Mexico and 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 coloring, 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.

There are different centers of domestication and origin for *Amaranthus* species. These centers are widely distributed in North America, Central America, and the South American[9-10]. "It is estimated that there are 87 species of *Amaranthus*: 17 in Europe, 14 in Australia, and 56 in America"[10]. However, the number of species is still tentative because of the paucity of studies on *Amaranthus* systematics,. Some species, such as *A. retroflexus, A. hybridus, A. powellii,* and *A. viridis,* are cosmopolitan; being both introduced and naturalized plants, with a weed-like behavior[11-12]. The cultivated species, *A. cruentus, A. hypochondriacus,* and *A. caudatus* are considered as pseudocereals, with a high seed protein content, a balanced amino acid composition, and a high lysine content[13,10]. They are cultivated in different regions of South and Central America, India, and Nepal[14-15].

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[16-19]. *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[20]. 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. Traditionally, *Amaranthus* has been divided into two sections: *Amaranthus* and *Blitopsis* Dumort[21-22]. Carretero[23] divided the genus into three sections. Later, based on inflorescence and flower characters, Mosyakin & Robertson[24] suggested a new classification in which the genus is divided into three subgenera and nine sections. Many systematic revisions have been developed to clarify the taxonomy of *Amaranthus.* Some were based on leaf anatomy and morphology[25], pericarp structure[26], and stem morphology and anatomy[27]. Some other systematic studies have been carried out on the use of molecular markers[28, 29, 14, 30, 9].

Germplasm collections of amaranths will play a key role for genetic improvement. However, only limited information is available on intra- and interspecific genetic diversity and relationships within *Amaranthus* germplasm collections[29]. The genus *Amaranthus* is still poorly understood, being widely considered as a "difficult" genus[32]. Because of the widespread nomenclatural disorder caused chiefly by repeated misapplication of names[33], the taxonomic problems are far from being clarified. Due to variation of morphological characters, accurate classification of *Amaranthus* genetic resources is not always possible[34].

The objectives of this review were to evaluate the inter- and intra-specific variability of *Amaranthus* species, and their taxonomic and evolutionary relationships using biochemical markers.

GENETIC VARIABLITY

Genetic diversity

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Genetic diversity is defined as the variation of individual genotypes within and among species[35-37]. It is important for two reasons. First of all, when a population of an organism contains a large gene pool—that is, if the genetic blueprints of individuals in the population vary significantly—the group has a greater chance of surviving and flourishing than a population with limited genetic diversity, because some of the individuals may have inherited traits making them particularly resistant to disease or tolerant of cold, for example[38-40]. Or they may possess other traits that increase their chance for survival. Plant breeders take advantage of this genetic diversity to improve existing plants and create new varieties. Genetic diversity also reduces the incidence of unfavorable inherited traits. In a small, isolated population of organisms, individuals may be forced to breed with close relatives[41-44]. When this happens, the genetic makeup of the individuals becomes more and more uniform, and genetic flaws become increasingly more common. In summary, genetic diversity strengthens a population by increasing the likelihood that at least some individuals will be able to survive major disturbances, and by making the group less susceptible to inherited disorders.

Genetic differentiation

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 mating in very small populations (genetic drift)[45-49]. 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). Over evolutionary time, such among-population genetic differences can accumulate and result in the development of a new species (allopatric speciation).

Plant breeders and knowledge of genetic variability

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[50-53]. So, genetic diversity studies are essential for providing information for propagation, domestication, and breeding programs aswell as conservation of genetic resources for plant species.

Analysis of genetic variability

Genetic variability 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[54-58]. 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[59-61].

Biochemical traits

Protein content

The highest protein content in *Amaranthus* species was recorded for *A. cruentus* accessions (17.32 ± 0.82%) followed by *A. caudatus* (17.24±0.65%) and *A.hypochondriacus* (16.89±0.80%)[32, 62-63]. However, there is a variation in protein content between the different collections. Whereas, Segura-Nieto *et al.*[64] published, that the range of protein content is following: *A. cruentus* 13.2 – 18.2%, *A. hypochondriacus* 17.9% and *A. caudatus* 17.6 – 18.4%, the range of the total protein content into

Czech collection is from 12.43 to 17.33%[32]. The results of protein content in Czech collection were similar to the results of other authors investigating various *Amaranthus* genotypes[65].

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[66-69]. Hence, any observed variation in protein systems is considered as a mirror for genetic variations, specifically seed proteins which reflect the genetic history of the species and do not affect with the environmental fluctuations[70-72].

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[73-74]. Sammour[75] 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[76-80]. For example, it has been successfully used to clarify the taxonomy of Poaceae[81], Cucurbitaceae[82], and Fabaceae[83]. Gardiner *et al*.[84] and Gardiner & Forde[85-86] stated that electrophoresis can also be used to characterize the seed protein profiles of species and cultivars, compare cultivars of different geographical origin, and provide taxonomically useful descriptors that are substantially free from environmental influence. Hence, this method has been used to study cultivated plants, such as *Vitis vinifera*[87], *Lolium rigidum*[88], *Hevea brasiliensis*[89], and corn[90]. Moreover, 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 groupwith basic chromosome number x=16[91-92]. 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*.[93] 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 ratherwide, (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 seedproteins 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. Byprotein 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 amongcultivars, 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[94].

The taxonomic complexity in the genus

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Amaranthus was studied based on the seed protein profiles[95]. 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 SDSPAGE 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[96] 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 proteins used very efficiently to assess the genetic differences in two grain populations of *Amaranthus retroflexus* collected from field of the Maize Research Institute ZemunPolje, Serbia[97]. It was found that (1) two populations have different protein profile; (2) 18 protein fractionswere 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 a typical morphology.

Srivastava & Roy[98] examined the genetic diversity and relationships among 12 cultivated and wild *Amaranthus* species using protein markers. High level of genetic diversity was common within species contrary to genetic uniformity within most accessions. On average, the polymorphism reached 42.60% among the cultivars but rose to 46.88% in the wild counterpart. The seed protein content varied in the 11.80-17% range and seeds of amaranth proved highly nutritive. The SDS-PAGE analysis indicated that such proteins in case of amaranths were highly heterogeneous with 8-18 bands. Based on the electrophoretic mobility, such bands were assigned four zones (A, B, C and D).

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[99]. Data derived from electrophoretic gels consists of the number and relative nobilities of various enzyme forms, which with appropriate genetic analyses, become transformed into single or multi loci genotypes for each individual[100-102]. 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[100]. 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[103].

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[76, 77, 104]. It was also utilized in plant genetic resources management and plant breeding. Furthermore, isozymes analysis was used in control of breeding, estimation of out-crossing, testing purity and in species delimitation and conservation[105-107]. 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[108].

World *Amaranthus* along with 21weedyNewWorld populations were assayed using nine enzymatic systems. In the New World populations, heterozygosity was low, and polymorphic loci ranged from0 to 44%. Diversity index H2 was partitioned into the intra- and inter-population as well as the inter-specific components of variability.

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The crop versus weed genetic distances was the largest, whereas the intra- and inter-population 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 trans-domestication based on a weedy or semi-domesticated species having migrated from Meso- America to SouthAmerica. 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 homozygousweedyallele.

Genetic variation and genetic relationships of a total of 23 species and 60 populations of cultivated and wild amaranths were performed using isozyme marker[109]. High levels of inter-specific and intra-specific 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 encoding15 enzymes were resolved, and allwere polymorphic at the inter-specific level. High levels of inter-accessional genetic diversity were found within species, but genetic uniformity was observed within most accessions[110].

Iudina *et al*.[111] 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.

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