STUDIES ON GENETIC DIVERSITY IN *Amaranthus* SPECIES USING THE RAPD MARKERS

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Abstract. Here in this work we studied the genetic diversity and phylogenetic relationships among six popular species of *Amaranthus*: *A.cruentus* (Alegria) V1-R1, *A.cruentus* (Amont) V3-R1, *A.hypocondriacus* V2-R1, *Amaranthus sp. 13*, *A.cruentus* (MT3) V11-R1 and *A.chlorostachys* (powellii) from different geographic regions using the RAPD markers. Molecular methods used to characterize the amaranths plants showed that among the studied species there is a slightly intra- and inter-species polymorphism. Using ITS primers type that amplified characteristic fragments from rDNA region (relevant area for 5.8 S rRNA gene) after amplification a single product was obtained for all *Amaranthus* samples. Introns of tubulin genes are also useful tools for genotyping plant species and varieties, for parental assessment and for assisting breeding programs. Introns of plant tubulin genes are important regulatory elements for supporting gene expression. In our experiments applying the TBP (tubulin-based polymorphism) method revealed an intraspecific polymorphism in *A. cruentus* (Alegria) V1-R1, while no polymorphism was detected among the other studied species. From the RAPD data, an UPGMA dendrogram illustrating a difference between wild species of *Amaranthus (A. chlorostacys powellii)* and the other two species examined. In addition, between *A. hyponchondriacus* and *A. cruentus* there is a relationship, the genetic distance between them being ~18-20%.

Keywords: *A. cruentus*, *A. hypocondriacus, A. chlorostachys* (*powellii*), genetic diversity, RAPD analysis, plant β-tubulin genes

INTRODUCTION

The genus *Amaranthus* L. (Amaranthaceae) includes 60-70 species, comprised of cultivated grain (*A. caudatus, A. cruentus* and *A. hypochondriacus*) and vegetables crops (*A. hybridus*) as well as wild species [10]. About 60 *Amaranthus* species are native to the America while and the rest to Australia, Asia, Africa and Europe [30].

The cultivated amaranths are utilized as food grains, leafy vegetables, and forage crops in diverse geographic areas [31]. Compared with traditional crops, amaranth seeds are rich in protein (17–19% of dry weight) with double the amount of essential amino acids than wheat grain protein [5, 7]. Amaranths species also exhibit tremendous diversity related to their wide adaptability to different eco-geographic situations [21]. Three species of cultivated grain amaranths were recognized (*A. caudatus, A. cruentus* and *A. hypochondriacus*). Associated with these are three parental wild species: *A. hybridus, A. quitensis* and *A. powellii* [11]. Correct genotype identification is important to evaluate the genetic diversity of local amaranths. Identification and preservation of germplasm are necessary for maintaining genetic diversity, studying local genetic material in order to choose ecotypes having high nutritional interest in their place of origin [24].

The present data demonstrate taxonomic ambiguity at the basic morphologic level. The main cause is the enormous morphological and genetic variation exhibited by the species in the genus. Correct genotype identification is important to evaluate the genetic diversity of local amaranths. Identification and preservation of germplasm are necessary for maintaining genetic diversity, studying local genetic material, and even to choose ecotypes having high nutritional interest in their place of origin [24]. Considering the demand of *Amaranthus* genotypes with favorable agronomic traits, a field evaluation was performed to enhance the pool of agronomical useful *Amaranthus* resources [36].

Correct identification of *Amaranthus* species is often difficult and misidentification is common [17]. Ahrens et al. (1981) found that 13 of 14 accessions that were identified as redroot pigweed were actually smooth pigweed (*Amaranthus hybridus* L.) or Powell amaranth (*A. powellii* S. Wats.) [1]. The RAPD markers have considerable appeal for surveying genomic variation because it is relatively inexpensive, utilizes arbitrary primers, and randomly samples a potentially large number of loci in a less complex pattern than other polymerase chain reaction (PCR)-based markers [12, 16, 35]. Wetzel et al. (1999a) noted, based on molecular marker analysis of internal transcribed spacer (ITS) regions of nuclear ribosomal DNA (rDNA) that 12 of 92 *Amaranthus* accessions collected and identified by weed scientists were misidentified [33].

To investigate genetic diversity in plant species (*Brassica napus, Coffea, Lotus*) Bardini et al. (2004) reported the development of a new technique called Tubulin-Based Polymorphism (TBP) that is based on the length of the first intron present in the coding region of plant β-tubulin genes [4]. Introns of plant tubulin genes are important regulatory elements for supporting gene expression. Introns can either increase the level of expression through intron-mediated enhancement or they can change the actual site of gene expression. Introns of tubulin genes are also useful tools for genotyping plant species and varieties, for parental assessment and for assisting breeding programs [6, 8, 9]. Molecular markers are now the tools most widely used to assess genetic diversity [18, 19].

The aim of our study was to evaluate the genetic diversity and phylogenetic relationships among six popular species of *Amaranthus*: *A. cruentus* (Alegria) V1-R1, *A. cruentus* (Amont) V3-R1, *A. hypocondriacus* V2-R1, *Amaranthus sp. 13*, *A.*

cruentus (MT3) V11-R1 and *A. chlorostachys* (powellii) from different phytogeographic regions using the RAPD marker. Another aspect investigated was that to emphasize a possible polymorphism at the β-tubulin gene level.

MATERIALS AND METHODS

Plant Materials

Plants of 6 *Amaranthus* species were collected from different phytogeographic regions and used in experiments (table 1).

Table 1. Biological material used in experiments.

Species	Notation
A.cruentus (Alegria) V1-R1	V1.1
A.hypocondriacus V2-R1	V1.2
A.cruentus (Amont) V3-R1	V1.3
Amaranthus sp. 13	V1.4
A.cruentus (MT3) V11-R1	V1.5
A.chlorostachys (powellii)	C (control)

DNA isolation

Genomic DNA was isolated from the young plants using Genelute Plant Genomic DNA Miniprep Kit (Sigma) after the protocol specified by the manufacturer.

RAPD Analysis

RAPD analyses were performing using different decamer primers like psg3, UBC (UBC51) and OP (OPA17, OPC19, 0PG5 and OPG6) of Operon kits (Operon Technologies, Alameda, CA) followed by gel electrophoresis. rDNA region was investigated using two pairs of primers: ITS1/ITS2 (TCC GTA GGT GAA CCT GCG G/GCT GCG TTC TTC ATC GAT GC) and ITS1/ITS4 (TCC GTA GGT GAA CCT GCG G/TCC TCC GCT TAT TGA TAT GC) which allow rRNA 5,8S gene amplification [34] (Fig.1).

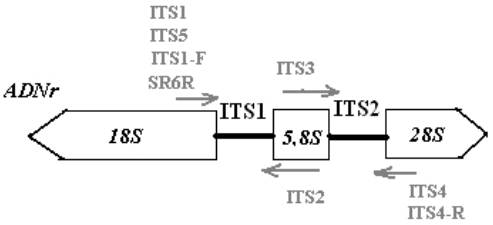

Figure 1. ITS primers used in the ITS-PCR amplification of the meaning of their action in rDNA.

DNA polymorphism analysis for β tubulin was carried out with 1x reaction buffer, 0.2 mM deoxinucleotide, 0.2 mM from each primer (Bt2a; Bt2b) -1 $MgCl_2$, 5 mm, 1U Taq polymerase enzyme (Promega) and 50-70 ng template DNA in 25 µl reaction volume. The amplification cycle consisted of an initial denaturation at 94 °C for 3 min. followed by 36 cycles of 1 min. at 94 °C, 1 min at 58 °C, 2 min. at 72 °C, and a final extension of 10 min. at 72 °C. Highlighting products was performed on agarose gel (2%), staining with ethidium bromide and visualization under UV light using UVP BioDocIt system.

Data Analysis

Data were analyzed using TREECON program for Windows, version 1.3b. Dendrogram was constructed using genetic distance calculation with the formulas of Nei and Li (1979) [22]. Binary matrix was constructed based on the presence ("1") or absence ("0") of RAPD bands and clustering was done with UPGMA cluster method (Unweighted Pair Group Mathematical Average).

RESULTS

Results obtained following DNA isolation showed that, after electrophoresis, samples contained DNA highly pure (not contaminated with RNA or protein) (Fig. 2).

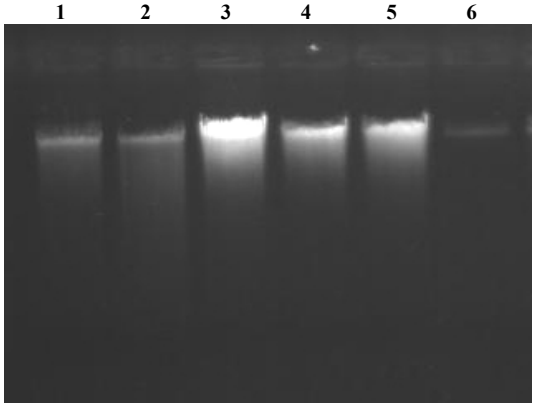

Figure 2. Electrophoretic DNA profiles from different species of *Amaranthus*. Samples order: 1 = V1.1; 2 =V1.2; 3 = V1.3; 4 = V1.4; 5 =V1.5; 6= Control.

Purified DNA samples were then subjected to amplification using various primers.

A first aspect followed was application of the ITS primers type that amplified characteristic fragments from rDNA region (relevant area for 5.8 S rRNA gene). Thus, by using ITS1/ITS2 primer pair for all *Amaranthus* samples tested a single product was obtained by amplification (Fig. 3).

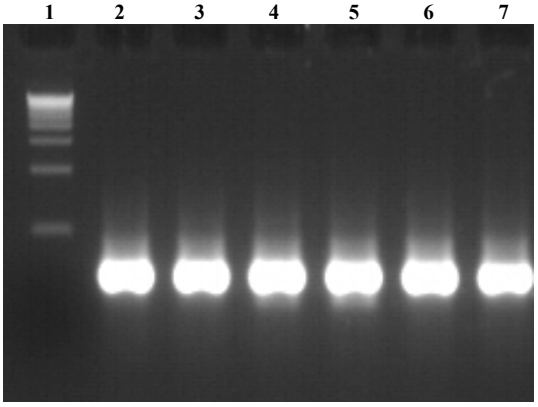

Figure 3. Results of amplification reaction using the ITS1/ITS2 primer pair. Order samples: 1 = ladder molecular weight marker (1 kb) 2 = V1.1; 3 =V1.2; 4 = V1.3; 5 = V1.4; 6 = V1.5; 7 = Control.

Applying ITS1/ITS4 pair of primers, both a major amplification product, in the size of ~220 bp and non-specific products were obtained. In both cases, all

plants variants tested showed uniformity in the resulting amplification products.

These amplification products cleaved with Msp I and Alu I restriction enzymes did not lead to a polymorphism of a restriction fragments. This can be explained by the fact that there were insufficient restriction enzymes used to reveal possible polymorphism or, between the variants tested is a high conservation of the restriction sites for enzymes and only nucleotide sequence determination would provide accurate data related the particular species.

Another aspect examined was that of highlighting a possible polymorphism in the gene for β-tubulin. In this case the Bt2a; Bt2b primer pairs was used. The results are presented in Fig. 4.

Analyzing the results obtained, we found that V1.2, V1.4 and V1.5 showed an identical profile (or very similar) of amplification products, even if the intensity of the bands varied. A similar aspect was also highlighted at V1.3 variant and control.

In V1.1 amplicons profile is different regarding the number of bands, although some of these are common with V1.2, V1.4 and V.1.5. The conclusion based on the results obtained is somewhat surprising: V1.2 and V1.5 could be to belong to different species. For further clarification of these observations, samples were subjected to RAPD analysis, primers used being: UBC51, OPA17, OPC19, 0PG5, OPG6 and psg3. Relatively low polymorphism was detected.

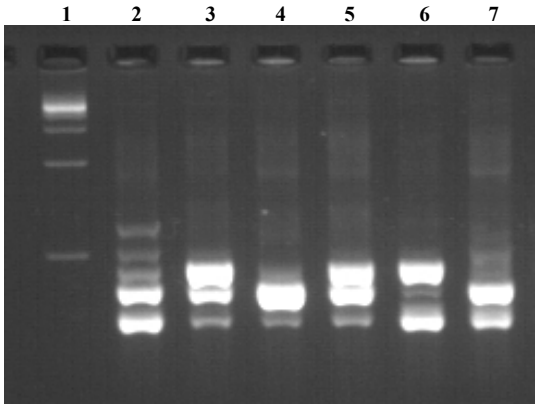

Figure 4. Results of DNA amplification from *Amaranthus* variants using specific primers for β-tubulin. Samples order is the same as in Fig. 3.

Based on the results with OPC-19 primer, two groups of samples could be associated: first included V1.2, V1.3 and V1.5, and V1.4 and control variants are grouped in the second one. Similar results were obtained with the primer UBC51: one group includes V1.1, V1.2, V1.3 and V1.5, and the other V1.4 and control (Fig. 5).

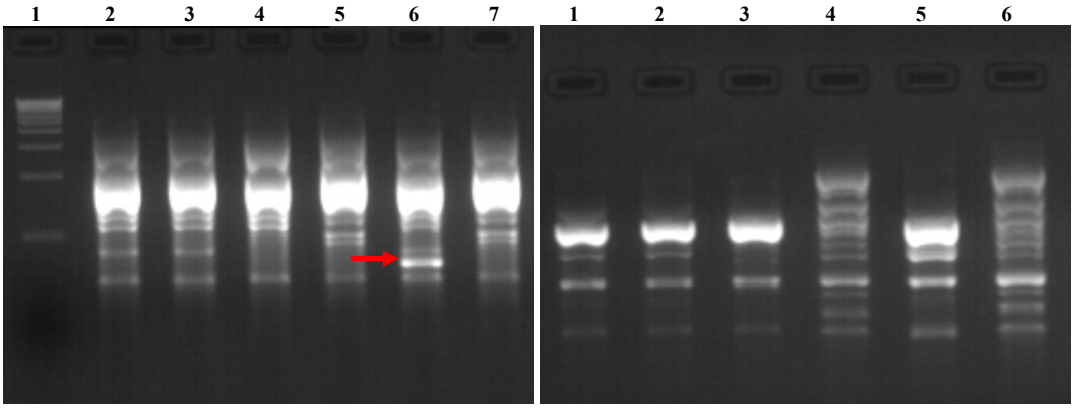

Figure 5. Electrophoretic profile of amplification products obtained with OPG 06 (left) and UBC 51 primers (right). Samples order is the same as in Fig. 3 and 2.

Interesting results were obtained with OPG 06 primer: four different electrophoretic patterns were detected. The first group included V1.1 and V1.2; the second - V1.4 and control; the third - V1.3 and the fourth - V1.5.

Differences in electrophoretic pattern of PCR products were also detected with the primers OPA 17 and OPG 05 (Fig. 6). It may be noted that by using OPA17 primer are three groups of electrophoretic profiles: the first group is composed of the following V1.1, V1.2 and V1.5, the second group includes V1.4 and control, and V1.3 variant shows a slight contrast to the other (by missing of two amplified products - one in the size of ~130 bp and another 350 bp (Fig. 6).

When using OPG 05 primer, clustering variants were similar, although in this case, the electrophoretic profile of the V1.3 variant was very close to that of variants V1.1, V1.2 and V1.5. Significant differences occurred when pgs3 primer was used (Fig. 7): V1.1 and V1.5 variants had identical profiles, as is the V1.4 variant and control. Regarding V1.2 variant, it deviates less in profile than V1.1, while in the V1.4. are higher.

Although intra-and interspecific polymorphism of the resulting amplicons by using decamer primers was quite low, based on the results obtained was made a dendrogram showing genetic distance. The assessment data can be seen that control and V1.4 variant are identical, so they belong to *A. chlorostachys* species. In the other variants, V1.1 and V1.2 and V1.5, the genetic distance is between 0.3 and 0.6, and can be considered part of the same species, *A. cruentus*. In contrast V1.3 variant differs from all others, being at a distance of ~0.2 of the previous group, so it may correspond to other species. These results are somewhat surprising, at least in V1.2 and V1.3 variants have been considered previously that the species belong to *A. hypocondriacus* respectively *A. cruentus* (Fig. 8).

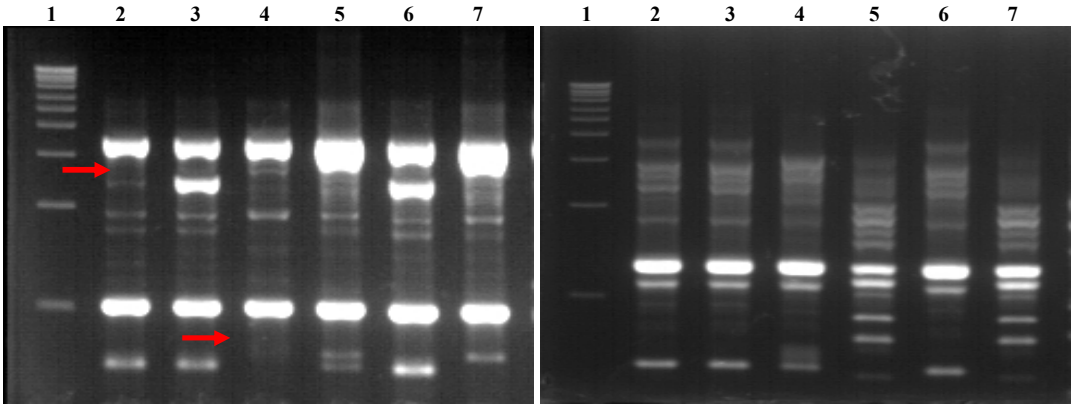

Figure 6. Electrophoretic profile of amplification products obtained by primers OPA 17 (left) and OPG 05 (right). Samples order is the same as in Figure 3.

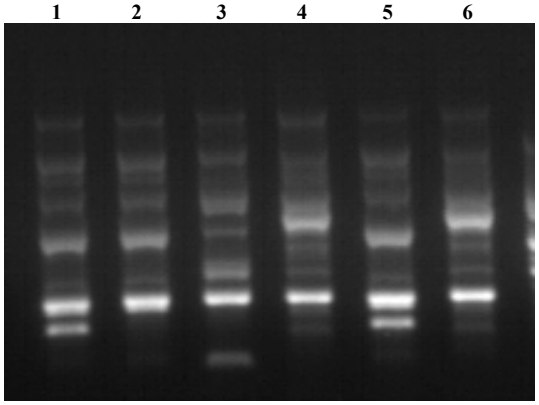

Figure 7. Electrophoretic profile of amplification products obtained with pgs 3 primer. Samples order is the same as in figure 2: 1 = V1.1; 2 =V1.2; 3 = V1.3; 4 = V1.4; 5 =V1.5; 6= Control.

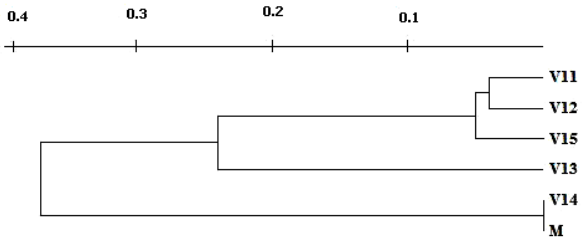

Figure 8. UPGMA - based dendrogram derived from RAPD analyses of *Amaranthus* species.

DISCUSSIONS

Studies on the evolution relationships of the genus *Amaranthus* species cultivated and theirs wild relatives have been made especially in the last 20 years, by applying various techniques. Studies on chromosome number and hybrid fertility [14, 23], random amplified polymorphic DNA (RAPD) [10, 32], restriction site variation of chloroplast and nuclear DNA [20] have clarified some aspects regarding genetic diversity and evolutionary relationships among grain amaranths and their wild relatives. These studies have allowed the development of some hypotheses about the geographical origin of species and establish phylogenetic links between them. Also, there has been a series of tests on the molecular characterization of germplasm in order to obtain useful results for breeders. Despite the progresses encountered, there is no single method of characterization to ensure differentiation between species or to allow obtaining of a particular molecular marker [28]. In recent years, special attention was given to examine the amplicons sequences variation obtained with ITS primers, AFLP analysis or ISSR sequences type [37]. Our report showed that the application of the ITS primers type for all *Amaranthus* samples we have tested a single product was obtained by amplification. These data confirm the results obtained by Xu and Sun (2001) [37]. Low ITS divergence in these taxa resulted in poorly resolved phylogeny [37]. ITS primers have proven to be a useful source of information for the resolution of phylogenetic relationships at the species level [2, 3].

Most of the current methods to determine genetic diversity are based on polymerase chain reaction (PCR) [25]. Compared with current methods, which are mostly based on anonymous DNA markers, the TBP (tubulin-based polymorphism) method is based on the genetic variation in the first intron of the coding sequence of members of the plant β-tubulin genes as source for DNA polymorphism [4]. TBP method was successfully applied to a wide range of species like oilseed rape, coffee, *Lotus* [4], *Brassica*, *Eleusine* and *Arachis* [8]. In our study we used TBP method on *Amaranthus* species for highlighting a possible polymorphism in the gene for β-tubulin. The results revealed an intraspecific polymorphism in *A.cruentus* (Alegria) V1-R1, while no polymorphism was detected among the other studied species. However, the low level of polymorphism in these species may reflect the high level of inbreeding in these *Amaranthus* species or the fact that the primers which we have used they were not successful in amplifying bands from members of the genus *Amaranthus* [4].

Analysis with the RAPD markers we used to clarify the phylogenetic relationships among the studied species revealed a relatively low polymorphism. It is possible that the primers we used in this study amplified mostly the conserved part of the genome, so they could not show any variation within a population. Cluster RAPD-based UPGMA analysis showed a difference between wild species of *Amaranthus (A. chlorostacys powellii)* and the other two species examined. In addition, between *A. hyponchondriacus* and *A. cruentus* there is a relationship, the genetic distance between them being ~18-20%. The data we

obtained are in according with those suggested by other researchers [26, 27, 32]. According to Sauer (1957) the amount of geographic advances has been very unequal, and in some cases the expansion was local causing an exceptional hybridization in amaranths [29].There is always a controversy on the relationship between genetic divergence and geographical origin of amarants. Some studies showed a low relationship [15], whereas others demonstrated a significant association between population characteristics and the environment in which they occur [13].

However, our study showed that applied molecular methods have proved useful in the characterization of biological material. Moreover, RAPD analysis using specific primers revealed some amplicons that, after confirmation, could be regarded as molecular markers for some of the species tested (*A. cruentus*, *A. chlorostachys*). This report will help us to find strategies for marker-assisted identification of amaranth genotypes in order to conservation of amaranth genetic resources or for different practical approaches.

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REFERENCES

[1] Ahrens, W.H., Wax, L.M., Stoller, E.W., (1981): Identification of triazine-resistant *Amaranthus* spp. Weed Science, 29: 345-348.

[2] Baldwin, B.G., (1992): Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the Compositae. Molecular Phylogenetics and Evolution. 1: 3-16.

[3] Baldwin, B.G., Sanderson, M.J., Porter, J.M., Wojciechowski, M.F., Campbell, C., Donoghue, M.J., (1995): The ITS region of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. Annals of the Missouri Botanical Garden, 82: 247-277.

[4] Bardini, M., Lee, D., Donini, P., Mariani, A., Gianì, S., Toschi, M., Lowe, C., Breviario, D., (2004): Tubulin-based polymorphism (TBP): a new tool, based on functionally relevant sequences, to assess genetic diversity in plant species. Genome, 47(2): 281-291.

[5] Becker, R., Wheeler, E.L., Lorenz, K., Stafford, A.E., Grosjean, O.K., Betschart, A.A., Saunders, R.M., (1981): A compositional study of amaranth grain. Journal of Food Science, 46: 1175-1180.

[6] Braglia, L., Manca, A., Mastromauro, F., Breviario, D., (2010): cTBP: A successful Intron Lenght Polymorphism (ILP)-Based genotyping method targeted to well defined experimental need. Diversity, 2: 572-585.

[7] Bressani, R., Gonzales, J.M., Zuniga, J., Brauner, M., Elias, L.G., (1987): Yield, selected chemical composition and nutritive value of 14 selections of amaranth grain representing four species. Journal of the Science of Food and Agriculture, 38: 347-356.

[8] Breviario, D., Baird, W.V., Sangoi, S., Hilu, K., Blumetti, P., Gianì, S., (2007): High polymorphism and resolution in targeted fingerprinting with combined β-tubulin introns. Molecular Breeding, 20(3): 249-259.

[9] Breviario, D., Gianì, S., Ponzoni, E., Mastromauro, F., Morello, L., (2008): Plant tubulin intronics. Cell Biology International, 32(5): 571-573.

[10] Chan, K.F., Sun, M., (1997): Genetic resources and breeding of *Amaranthus*. Plant Breeding Reviews, 19: 227-285.

[11] Costea, M., Weaver, E.S., Tardif, J.F., (2004). The biology of Canadian weeds. 130. *Amaranthus retroflexus* L., *A. powellii* S. Waston and *A. hibridus* L. Canadian Journal of Plant Science, 84: 631-668.

[12] Das, M., Bhattacharya, S., Pal, A., (2005): Generation and characterization of SCARs by cloning and sequencing of RAPD products:a strategy for species-specific marker development in bamboo. Annals of Botany, 95: 835-841.

[13] Ge, X.J., Yu, Y., Zhao, N.X., Chen, H.S., Qi, W.Q., (2003): Genetic variation in the endangered Inner Mongolia endemic shrub *Tetraena mongolica* Maxim (Zygophyllaceae). Biological Conservation, 111: 427-434.

[14] Gupta, V.K., Gudu, S., (1991): Interspecific hybrids and possible phylogenetic relations in grain amaranths. Euphytica, 52: 33-38.

[15] Hadian, J., Tabatabaei, S.M.F., Naghavi, M.R., Jamzad, Z., Ramak-Masoumi, T., (2008): Genetic diversity of Iranian accessions of *Satureja hortensis* L. based on horticultural traits and RAPD markers. Scientia Horticulturae, 115: 196-202.

[16] Hadrys, H., Balick, M., Schierwater, B., (1992): Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. Molecular Ecology, 1: 53-63.

[17] Horak, M.J., Peterson, D.E, Chessman, D.J., Wax, L.M., (1994): Pigweed Identification: A Pictorial Guide to the Common Pigweeds of the Great Plains. Manhattan, KS: Kansas State University, p. 12.

[18] Karp, A., Isaac, P.G., Ingram, G.S., (1998): Molecular tools for screening biodiversity: Plant and Animals. Chapman & Hall, Thompson Sci. London, pp. 10-17.

[19] Koebner, R.M.D., Powell, W., Donini, P., (2001): Contribution of DNA molecular marker technologies to the genetics and breeding of wheat and barley. Plant Breeding Reviews, 21: 181-220.

[20] Lanoue, K.Z., Wolf, P.G., Browning, S., Hood, E.E., (1996): Phylogenetic analysis of restriction-site variation in wild and cultivated *Amaranthus* species (Amaranthaceae). Theoretical and Applied Genetics, 93: 722-732.

[21] Lee, J.R., Hong, G.Y., Dixit, A., Chung, J.W., Ma, K.H., Lee, J.H., Kang, H.K., Cho, Y.H., Gwag, J.G., Park, Y.J.., (2008): Characterization of microsatellite loci developed for *Amaranthus hypochondriacus* and their cross-amplification in wild species. Conservation Genetics, 9: 243-246.

[22] Nei, M., Li, W.H., (1979): Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences, 76: 5269-5273.

[23] Pal, M., Khoshoo, T.N., (1972): Evolution and improvement of cultivated amaranths. V. Inviability, weakness, and sterility in hybrids. Journal of Heredity, 63: 78-82.

[24] Perez-Gonzalez, S., (2001): The importance of germplasm preservation and use for temperate zone fruit production in the tropics and subtropics. In: Perez-Gonzalez S, Dennis F, Mondragon C, Byrne D, editors. VI International symposium on temperate fruit growing in the tropics and subtropics. Mexico: Acta Horticulturae, 565: 25-32.

[25] Powell, W., Orozoco-Castillo, C., Chalmers, K.J., Provan, J., Waugh, R., (1995). Polymerase chain reaction-based assays for characterization of plant genetic resources. Electrophoresis, 16: 1726-1730.

[26] Ranade, S.A., Kumar, A., Goswami, M., Sane, P.V., (1997): Genome analysis of amaranths: determination of

inter- and intraspecies variations. Journal of Biosciences, 22: 457-464.
[27] Ray, T., Roy, S.C., (2008): Genetic Diversity of *Amaranthus* Species from the Indo-Gangetic Plains Revealed by RAPD Analysis Leading to the Development of Ecotype-Specific SCAR Marker. Oxford Journals, 100 (3): 338-347.
[28] Rao, N.K., (2004): Plant genetic resources: advancing conservation and use through biotechnology. African Journal of Biotechnology, 3: 136-145.
[29] Sauer, J.D., (1957): Recent migration and evolution of the dioecious amaranths. Evolution, 11: 11-31.
[30] Sauer, J.D., (1967): The grain amaranths and their relatives: a revised taxonomic and geographic survey. Annals of the Missouri Botanical Garden, 54: 103-137.
[31] Sallknecht, G.F., Schulz-Schaeffer, J.R., (1993). Amaranth rediscovered. In: New crops. Editors: Janick J, Simon JE,. New York: Wiley, pp. 211-218
[32] Transue, D.K., Fairbanks, D.J., Robison, L.R., Andersen, W.R., (1994): Species identification by RAPD analysis of grain Amaranth genetic resources. Crop Science, 34: 1385-1389.
[33] Wetzel, D.K., Horak, M.J., Skinner, D.Z., (1999): Use of PCR-based molecular markers to identify weed *Amaranthus* species. Weed Science, 47: 518–523.
[34] White, T.J., Bruns, T., Lee, S., Taylor, J.W., (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. pp. 315-322. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., (eds.): PCR Protocols: A Guide to Methods and Applications. Academic Press, Inc., New York.
[35] Williams, J.G.K., Hanafey, M.K., Rafalski, J.A., Tingey, S.V., (1993): Genetic analysis using random amplified polymorphic DNA markers. Methods in Enzymology, 218: 704-740.
[36] Wu, H., Sun, M., Yue, S., Sun, H., Cai, Y., Huang, R., Brenner, D., Corke, H., (2000): Field evaluation of an *Amaranthus* genetic resource collection in China. Genetic Resources and Crop Evolution, 47: 43-53.
[37] Xu, F., Sun, M., (2001): Comparative analysis of phylogenetic relationships of grain amaranths and their wild relatives (*Amaranthus*; *Amaranthaceae*) using internal transcribed spacer, amplified fragment length polymorphism, and double-primer fluorescent intersimple sequence repeat markers. Molecular Phylogenetics and Evolution, 21: 372-387.

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