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Genome analysis of amaranths: Determination of inter- and intra-species variations†

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Abstract. Amaranths are an important group of plants and include grain, vegetable and ornamental types. Despite the economic importance of the amaranths, there is very little information available about the extent and nature of genetic diversity present in the genus *Amaranthus* at molecular level. We now report the randomly amplified polymorphic DNA (RAPD) profiles of different species of *Amaranthus* as well as different accessions of the species. These RAPD analyses have been carried out using 65 arbitrary sequence decamer primers. From the RAPD data, an UPGMA dendrogram illustrating the inter-as well as intra-species relationships has been computed. The putative hybrid origin of *A. dubious* from *A. hybridus* and *A. spinosus* is also ruled out by the RAPD data. The trends of species relationships amongst the amaranths determined by RAPDs is consistent with their cytogenetic and evolutionary relationships that have alٌeady been determined.

Keywords. Amaranths; DNA variations; RAPD.

1. Introduction

Amaranths are an important group of plants and include grain, vegetable and ornamental types. Botanically, these belong to the genus *Amaranthus* which includes approximately 60 species. Of the three types of amaranths, it is the grain types which have the maximum economic value. The grain amaranths were important food crops for the ancient middle and south American civilizations. In fact even today these are important food crops in Latin America. Elsewhere in the world, these are grown abundantly in northern India, Manchuria, southeast Asia and Africa (Kauffman and Weber 1988; Irving *et al* 1981). In terms of nutrition, the grain amaranths are characterized by a rich fiber and mineral content and a balanced amino acid profile, which is better than most of the known and heavily utilized cereals and other food grains. Despite these advantages, grain amaranths have never been able to gain prominence in the global agriculture scenario. It is only in the recent years that these have received renewed attention as alternative field crops (Bressani 1989; Tucker 1986). Despite the increasing attention to amaranths, the molecular data are scarce. Furthermore, there is neither a genetic nor cytological linkage map available in case of amaranths. In fact, the origin and interrelationship of different species of amaranths has also not been determined, though several studies have been carried out on this aspect

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(Sauer 1967; Khoshoo and Pal 1972; Pal and Khoshoo 1972; Jain *et al* 1982). The germplasm of amaranths also has not been well characterized from the point of view of its exploitation for the improvement of amaranths in general, and grain amaranths in particular. A recent report (Transue *et al* 1994) on the use of randomly amplified polymorphic DNA (RAPD) patterns for the determination of species amongst the germplasm of 3 grain amaranth species, namely, *Amaranthus hypochondriacus, A. cruentus* and *A. caudatus,* is perhaps the only report known on the molecular genetics of amaranths.

Work was therefore, taken up on the analysis of species diversity in amaranths at the molecular level and involves the grain amaranths to start with. In this paper, we report the use of powerful RAPD approach to understand both inter- as well as intra-species relationship.

2. Materials and methods

2.1 *DNA isolation*

The total genomic DNA was isolated from the leaves of the different *Amaranthus* species and accessions (table 1) according to the method of de Kochko and Hamon (1990).

2.2 *Amplification reactions andanalysis of amplification products*

The amplification reactions in case of RAPD primers (20 primers each of kits 'A', ΄AP', 'T' and U14 from 'U' all from Operon, and RAPD 40, 501, 502, 503 synthesized at Centre for Biochemical Technology, New Delhi) were carried out essentially following the conditions of Williams *et al* (1990) and included 45 cycles of denaturation at 94° C for 1 min, annealing at 35°C for 1 5min and extension at 72°C for 1 5 min. The final

Table 1. The different amaranths used in the present studies as well as their origins wherever known are listed below.

Sample no.	Systematic name	Remarks
1	*A. caudatus*	–
2	*A. caudatus*	(4X colchicine)
3	*A. caudatus*	–
4	*A. edulis*	From *A. caudatus* × *A. edulis*
5	*A. hypochondriacus*	Mexican
6	*A. hypochondriacus*	S. Indian
7	*A. hybridus*	African
8	*A. hybridus*	Mexican
9	*A. hypochondriacus*	Gujarat
10	*A. hypochondriacus*	From *A. hybridus* × *A. hypochondriacus*
11	*A. hypochondriacus*	–
12	*A. lividus*	–
13	*A. dubious*	–
14	*A. spinosus*	–

Sample no. refers to numbers assigned to the DNAs prepared for all further analysis. The DNAs are listed according to the numbers. —, Indicates local accessions or accessions evolved at NBRI.*

cycle included extension at 72° C for an additional 5 min duration. All reactions were carried out in 25 µl volumes and contained 50 ng oftemplate DNA (quantitation was done by Dyna Qyant™200 fluorometer), 10 pmol of the primer, 200 mM each dNTP, 3·5 mM Mg^{2+} ion concentration in suitable IX assay buffers supplied along with the enzyme and 0·5 to 1 unit of the thermostable DNA polymerase. After overlaying the reaction mixes with 1 drop of mineral oil in each, the tubes were placed on the robotic arm of the thermocycler (Robocycler, Stratagene GmbH, Germany) for the PCR. After the PCR, to each reaction tube, 2·5 µl of 10X tracking dye mix were added and 25 µl aliquots of the PCR products were analysed by electrophoresis through 1·5% agarose gels.

2.3 *Analysis of the RAPD results*

The fragment sizes of amplification products were estimated from the gel by comparison with standard molecular weight marker (λ-DNA double digested with *Hind*III and *Eco*RI, Bangalore Genei, India). For each primer, a matrix of all the bands present in the different DNAs was generated using '1' when the band was present and '0' when the band was absent. Similarities of profiles were determined using algorithm of Jaccard (1901) using the RAPDistance package ver 1·03 (Armstrong *et al* 1994). UPGMA dendrogram was generated from the similarity data following the method of Sokal and Sneath (1963).

3. Results

The genomic variability can be studied using several different approaches. We have used the PCR-based DNA fingerprinting, namely, RAPD analysis, to differentiate between the different species and accessions of the same species of *Amaranthus*. For this purpose, 65 different arbitrary sequence decamer primers were used. These included 20 primers of "A", "T" and "AP" kit each as well as OP-U14 from Operon Technology, Inc. USA, and 4 others (RAPD 401, 501, 502, 503) that were synthesized at Centre for Biochemical Technology, New Delhi. Of these primers, OP-U14 was selected because it had already given good profiles in case of amaranth DNAs as reported recently (Transue *et al* 1994). Out of the 65 primers tested, 40 primers produced polymorphic patterns. However, only 24 primers of these gave consistent RAPD profiles with all the genotypes tested; 16 primers resulted in inconsistent profiles. The remaining 25 primers entirely failed to give any amplification in more than half of the genotypes. The profiles of 14 amaranth genotypes with 6 primers OP-T20, OP-AP06, OP-AP07, OP-T13, OP-T01and OP-T05 are shown in figure 1. From the similarity data, an UPGMA dendrogram was generated (figure 2).

4. Discussion

RAPD analysis is a powerful tool for determining inter- as well as intra-species genetic relationships (Ranade and Sane 1995). Such studies have been carried out amongst wild and cultivated species (Joshi and Nguyen 1993; Dunemann *et al* 1994; Sharma *et al* 1995), among self and cross-pollinated species (Jain *et al* 1994; Pammi *et al* 1994) and even within germplasm of a single species (Connolly *et al* 1994; Corniquel and Mercier

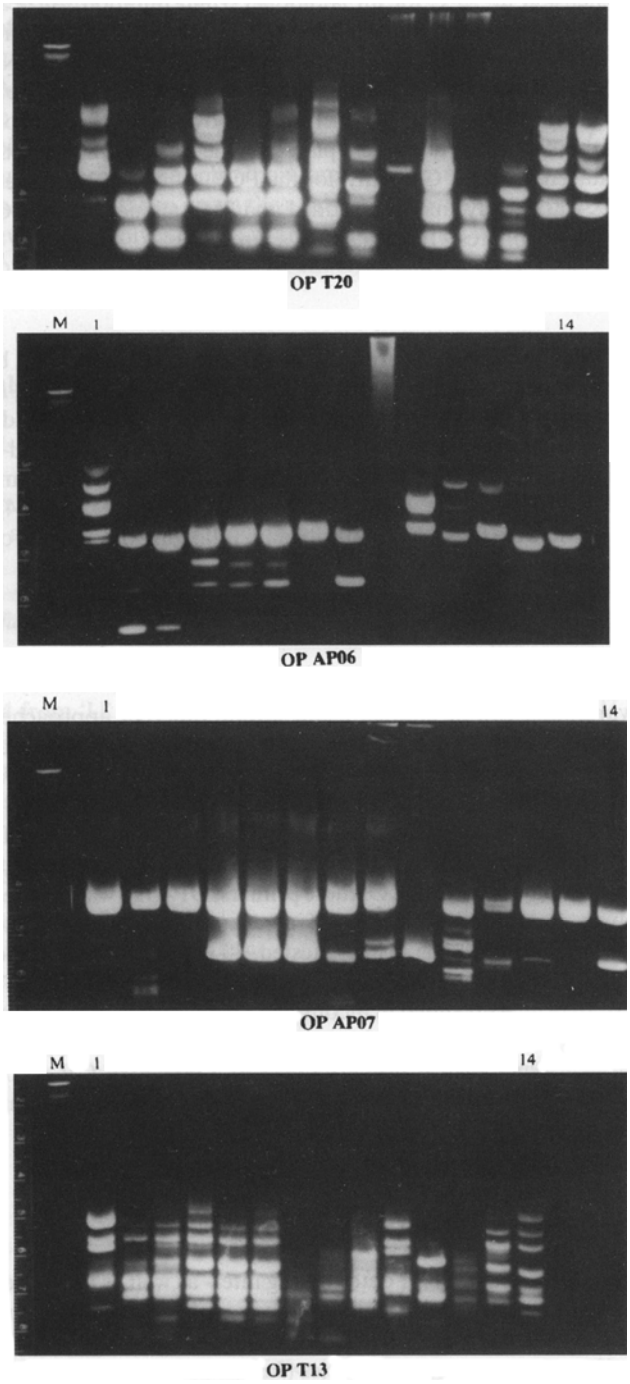

Figure 1. a – d.

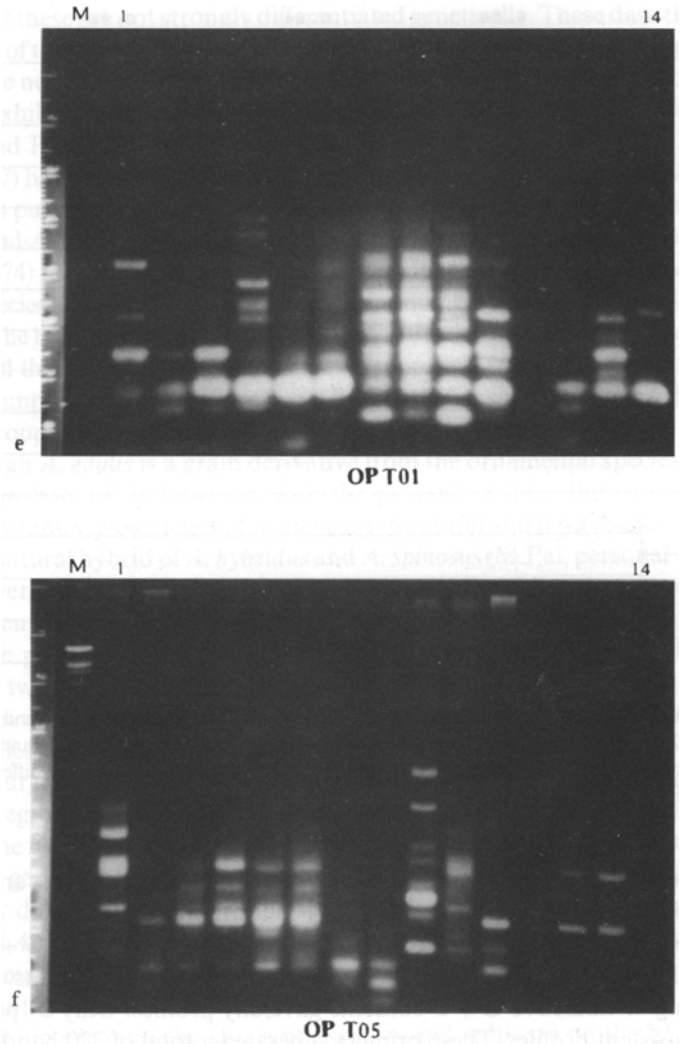

Figure 1. RAPD profiles of amaranths DNA using Operon primers. The gel lane marked with M is DNA double digested with *Eco*RI and *Hind*III used as a marker. Lanes 1-14 correspond to different amaranth DNAs as given in table 1.

1994; Virk *et al* 1995). In the earlier study on amaranths, the grain amaranth germplasm was analysed by RAPD (Transue *et al* 1994). However, this study did not assess inter-species relationship. Amaranths are an important group of plants and we, therefore, have attempted to determine inter- and intra-species variation amongst the amaranths.

The RAPD data in the present studies indicated that no two primers revealed identical profiles nor were any of the profiles detected by the same primer identical. Thus a combination of RAPD profiles generated by just 2 or 3 primers was adequate to distinguish the 14 genotypes (both inter- and intra-species). For example, primers OP-T01, OP-T05, OP-T13, OP-T20, OP-AP06 and OP-AP 07 as shown in figure 1,

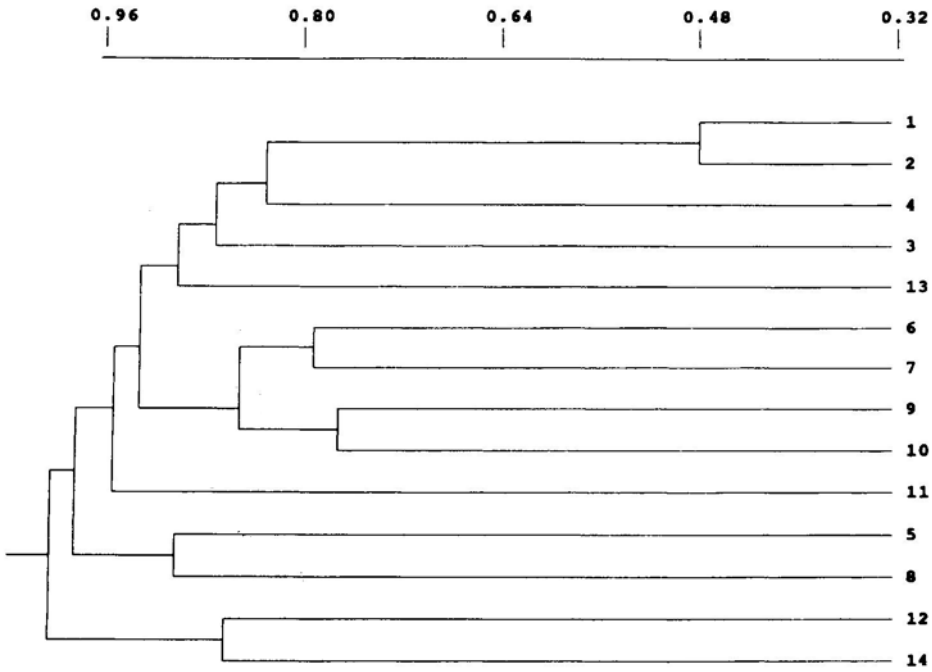

Figure 2. UPGMA dendrogram computed according to Sokal and Sneath (1963). The similarity matrix of RAPD data for 24 primers taken together was computed using the programme RAPDistance ver. 1·03 and from this similarity matrix, the dendrogram was computed. The numbers 1-14 are the different amaranth DNAs as given in table 1.

together can distinguish all 14 genotypes from each other. These results are consistent with the reported resolution power of the RAPD technology.

Of the 65 primers which were tested, all the OPERON primers had G + C contents of 60-70% while the synthesized primers had 40 and 50% G + C contents. None of the primers having 40 and 50% G + C contents gave any profiles. Only 24 primers have generated consistent profiles. These primers generated a total of 220 bands in the size range of 135-3100 bp. The pattern of bands generated by the primer OP-U14 were similar to those reported by Transue *et al* (1994) for the same species (data not shown).

Most of the species of *Amaranthus* are predominantly self-pollinated, although outcrossing is also possible (Jain *et al* 1982). The UPGMA dendrogram (figure 2) which is computed using data from the 24 primers results in grouping of the different DNAs studied in various clusters. Thus *A. caudatus* and *A. hypochondriacus* are genetically more similar as judged by the observation that they group together in the dendrogram. This genetic relatedness has also been observed by Gupta and Gudu (1991) by a series of crossing experiments and on the basis of inter-specific fertility. Similarly, recent RAPD results also tend to support a closer genetic relationship between the 2 species (Transue *et al* 1994), 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. These data thus conform to the results of the cytogenetic studies in *Amaranthus* wherein it has been reported that the species are not greatly distinct and that they show some differentiation only because the hybrids exhibit some virus-like syndrome and considerable seedling infertility in F2 (Khoshoo and Pal 1972).

Sauer (1967) has proposed the 3 weedy amaranths, namely, *A. powellii, A. hybridus* and *A. quitensis* as putative ancestors of the cultivated amaranths, namely, *A. hypochondriacus, A. cruentus* and *A. caudatus -A. edulis* respectively. This scheme has been refuted by Pal and Khoshoo (1974) on the basis of cytogenetic studies in *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, Pal and Khoshoo (1974) have also suggested that *A. hybridus* is the more likely ancestral species for *A. hypochondriacus,* a suggestion amply borne out by the present RAPD studies since it is observed that the two species are grouped in the same clusters (figure 2).

Even though *A. edulis* is a grain derivative from the ornamental species *A. caudatus* (Pal and Khoshoo 1972), however, with the passage of time, the two have become genetically distant. Cytogenetics of *A.dubious* is doubtful and it was believed that the *A. dubious* is a natural hybrid of *A. hybridus* and *A. spinosus* (M Pal, personal communication). However, Pal and Khoshoo (1965) have provided evidence that *A. spinosus* could not have been the progenitor species for *A. dubious*. This is well supported by the results in the present studies with RAPD primers, since on the basis of the RAPD profiles, the two putative progenitor species are found to be, clustered closer to *A. hypochondriacus* and *A. lividus* respectively. Furthermore, from the dendrogram in the present studies (figure 2), it is suggested that *A. dubious* itself may be closer to *A. caudatus*. Our results also further indicate that the same species from different geographic regions can also be distinguished from each other. In fact, Sauer (1967) has stated that the new species may arise from same species or wild species after adaptation to different geographic regions. Finally, *A. lividus* and *A. spinosus* appear to be considerably different since these cluster distinctly apart from *A. hypochondriacus, A. hybridus* and *A. caudatus*. In the case of the former *(A. lividus),* this is not surprising since it is a leafy amaranth with basic chromosome number *n* — 17, while the other three are grain amaranths with *n* = 16. The present studies have thus generated data on the interrelationship amongst the amaranth species and cultivars on the basis of RAPD profiles besides evaluating the other approaches for the determination of genetic variations amongst the amaranths.

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