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Intra- and Interspecific Genetic Diversity in Grain Amaranthus Using Ramdom Amplified Polymorphic DNA Markers

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Abstract

Random amplified polymorphic DNA (RAPD) analysis was performed to study the genetic diversity in three grain amaranths - *Amaranthus hypo-chondriacus, A. caudatus, A. cruentus* comprising a total of 17 accessions. As many as 13 bands were identified and the extent of polymorphism was highest in *cruentus* with 69.2% followed by *caudatus* 38.5% and *hypochondriacus* having 15.4 %. Three *caudatus* accessions recorded 60 - 80% similarity followed by 50% between two accessions of *cruentus*, while 12 accessions of *hypochondriacus* displayed a similarity pattern ranging from 10 - 100%. The clustering pattern of gel fragments through dendrogram analysis revealed that *cruentus* stood apart while *hypochondricus* and *caudatus* overlapped. The study demonstrated much higher level of genetic similarity between *hypochon-driacus* and *caudatus*. The RAPD profile developed from primer 1 and 2 indicated a strong possibility of a single common progenitor of these three grain amaranth species.

Introduction

Of about 60 species distributed throughout the world, the three grain amaranths viz. *Amaranthus caudatus, A. cruentus* and *A. hypochondriacus* have been cultivated in Mexico, Central America and the Andean highlands of Southern America for several thousand years (Sauer 1976). The species have gained high popularity in the Indian subcontinent as well. These grain amaranths are highly rich in protein and the essential amino acid lysine (Tucker 1986, Bressani 1989). In recent years these have received global attention being a quality protein crop that can stand remarkable well against

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abiotic stress under marginal management practices. There is an increased interest for a meaningful understanding of the genomes of the amaranths and the extent of their genetic diversity. Morphological obsrvations have been made, but these are inherently weak identifiers as they are influenced by environmental factors (Dey 1997). The biochemical characterization through the pattern analysis of isozymes and seed protein gel electrophoretic bands has also been done (Douchers and Ladlam 1991). This is, however, not adequate enough to distinguish closely related genotypes/species due to small number of bands produced by such techniques. In recent times DNA based procedures have been proposed for evaluating genetic diversity within and between species. The randomly amplified polymorphic DNA (RAPD) analysis is attractive because of its simplicity, low labour intensity and capacity to generate quick data. The study of genetic variation in the cultivated grain amaranths at the DNA level is very limited (Chan and Sun 1997). The present study concerns application of RAPD is three grain amaranths, *A. hypochondriacus, A. caudatus* and *A. cruentus* comprising a number of accessions. RAPD analysis has also been used in other crops, e.g. *Brassica* (Hu and Quiros 1991), *Oryza* (Fukuoka et al. 1992) and *Solanum* (Mori et al. 1993).

Materials and Methods

Experimental material consists of 17 genotypes, three from *A. caudatus*, two from *A. cruentus* and 12 from *A. hypochondriacus* strains. They were obtained from National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India and National Botanical Research Institute (NBRI), Lucknow, India (Table 1).

Isolation of DNA: The DNA was isolated by the method of Doyle and Doyle (1987) with slight modifications. Approximately 250 mg of primary leaves were collected from ten one-week-old seedlings, ground in liquid nitrogen, and suspended in 1 ml of 2x CTAB buffer containing 1% sodium metabisulphite. Plant DNA concentration was visually assessed in comparison with known concentration of lamda DNA on a mini gel.

DNA amplification: PCR was performed in a 0.5 ml tube 10 µl volume consisting of 0.2 ng genomic DNA, 0.2 µM primer, 10 mM Tris-HCl, buffer (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.001% gelatin, 0.2 mM each of dATP, dCTP and dGTP, and 0.2 unit Taq DNA polymerase (Genei, Bangalore, India). Eight decamer primers (sequence 5´ to 3´, used were - (1) TGCGGCTGAG, (2) TTGGCACGGG, (3) GTGATCTCAG, (4) TATCAGTGCT, (5) TTGACAAGTC,

(6) GGGTTGACGA, (7) GATATCAAGC, (8) TGGTACACGC) purchased from Genei, Bangalore, India. The reaction mixture was overlaid with a drop of mineral oil. Amplifications were carried out in a thermocycler (Perkin Elmer) programmed for 45 cycles of 30 seconds at 93_C, 1 minute at 36_C and 2 minutes at 72_C.

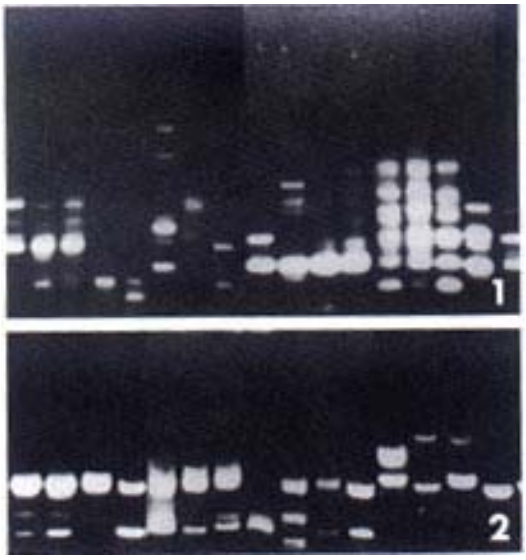
Materials	Place of cultivation	Source of collection	Yield/plant (g)	Protein (%)
A. caudatus				
AG302	South America	NBRI	0.931	15.60
AG302/2	South America	NBRI	1.280	16.63
AG323	South America	NBRI	1.200	16.40
A. cruentus				
AG122	South America	NBRI	11.440	16.80
EC150194	The Netherlands	NBRI	4.505	18.70
A. hypochodriacus				
AG284	Rajasthan	NBRI	4.781	17.33
AG67	Tamil Nadu	NBRI	8.815	15.80
AG66 (R-103)	South America	NBRI	4.310	15.70
IC37320	North Sikkim	NBPGR	5.446	17.41
AG291	Sikkim	NBRI	9.572	17.90
IC35633	Mehsana	NBPGR	2.901	17.30
IC35590	Mehsana	NBPGR	3.419	15.33
IC42258-2	Uttar Pradesh	NBPGR	5.037	14.50
AG114	Uttar Pradesh	NBRI	3.707	15.47
IC42258-1	Uttar Pradesh	NBPGR	1.667	16.53
IC38060	Himachal Pradesh	NBPGR	3.919	16.13
IC36834	Himachal Pradesh	NBPGR	2.957	17.40

Separation of amplified DNA : PCR products were separated on 1.5% agarose (BioRad) gels in TAE (Sambrook et al. 1989) buffer at 80 V constant for 3 hours, stained with ethidium bromide and photographed over a transilluminator.

Clustering : RAPD data were recorded as presence or absence of amplification fragments. Each polymorph fragment was treated as a unit character and compared between each pair of accessions. The percentage of difference in each pair of accessions was given by 100∇ the number of different fragments/the number of total fragements detected between the accessions. The cluster analysis was performed, based on the percentage of different fragments, using unweighted - pair-group method (Sneath and Sokal 1973).

Results and Discussion

The eight primers tested, had 40 - 70% G + C content. Only the two primers (TGCGGCTGAG and TTGGCACGGG) having 70% G + C content generated consistent profiles. A combination of RAPD profiles generated by the two primers was used to distinguish accessions both at intra- and interspecies. The RAPD data in the present study indicated that no two primers revealed identical profiles (Figs. 1, 2). As many as 13 bands were identified and maximum of nine bands were revealed in the three accessions (IC4258-2, AG114, IC42258-1) of *hypochondriacus*, while minimum of one appeared in *cruentus* accession AG122, whereas in *caudatus* it ranged four - five. The extent of polymorphism appeared to be highest in *cruentus* with 69.2%, followed by *caudatus* with 38.5% and *hypochondriacus* having 15.4%. RAPD profile (Fig. 1)



Figs. 1 - 2 : RAPD profile through primer (1) TGCGGCTGAG (Fig. 1) and primer (2) TTGGCACGGG (Fig. 2) showing conspicuous band amplification in different accessions of *Amaranthus*. In each case starting from left three lanes represent three accessions of *A. caudatus* (AG302, AG302/2, AG323), next two lanes record two *A. cruentus* accessions (AG122, EC150194) and last 12 lanes display *A. hypochondriacus* accessions (AG284, AG67, AG66, IC37320, AG291, IC35633, IC35590, IC42258-2, AG114, IC422581-1, IC38060 and IC36834).

was studied to record the similarity or dissimilarity between accessions within and between species as well. The significant points emerged from the dissimilarity per cent record (Table 1). Three *caudatus* accessions displayed 60 -

Table 1. Dissimilarity percentage as presence or absence of amplification fragments.

	AG302 (1)	AG302/2 (2)	AG323 (3)	AG122 (4)	EC150194 (5)	AG284 (6)	AG67 (7)	AG66 (8)	IC37320 (9)	AG291 (10)	IC35633 (11)	IC35590 (12)	IC42258-2 (13)	AG114 (14)	IC4Z258-2 (15)	IC38060 (16)	IC36834 (17)
1	0	50	40	100	100	50	60	85.7	80	75	83.3	57.1	55.6	55.6	55.6	66.7	66.7
2		0	20	80	83.3	70	66.7	50	83.3	75	60	57.1	44.4	44.4	44.4	50	50
3			0	75	80	62.5	75	60	80	75	83.3	75	55.6	55.6	55.6	60	60
4				0	50	100	100	60	100	100	100	100	88.9	88.9	88.9	100	100
5					0	100	100	80	100	100	100	100	90	90	90	100	100
6						0	62.5	80	75	37.5	77.8	60	45.4	45.4	45.4	66.7	66.7
7							0	60	33.3	71.4	50	50	66.7	66.7	66.7	60	60
8								0	50	88.9	25	57.1	55.6	55.6	55.6	40	40
9									0	85.7	25	66.7	77.8	77.8	77.8	50	50
10										0	87.5	80	63.6	63.6	63.6	66.7	66.7
11											0	50	66.7	66.7	66.7	25	25
12												0	33.3	33.3	33.3	57.1	57.1
13													0	0	0	55.6	55.6
14														0	0	55.6	55.6
15															0	55.6	55.6
16																0	0
17																	0

A. caudatus - 1, 2, 3. *A. cruentus* - 4, 5. *A. hypochondriacus* - 6 to 17.

80% similarity amongst themselves followed by 50% between the two accessions of *cruentus*. On the other hand, 12 accessions of *hypochondriacus* recorded wide fluctuations in similarity pattern ranging from 10 - 100%. This means that a great deal of genetic diversity exists amongst themselves. In this context one cannot rule out the possible effect of sample size on the extent of genetic diversity at DNA level. The similarity analysis further revealed the closeness or differences between these three grain amaranth species. Similarity of 14 - 66% was observed between *caudatus* and *hypochondriacus*. This was 0 - 25% between *caudatus* and *cruentus* and 0 - 40% between *hypochondriacus* and *cruentus*. In other words, *caudatus* and *hypochondriacus* appeared to be closely related. This was further illustrated by constructing of a dendrogram (Fig. 3). Clustering pattern idicated that in the two primary clusters *cruentus* stood apart from others and *hypcohondriacus* and *caudatus* overlapped. Within *hypochondriacus* accessiosn, IC42258-2, AG114 and IC42258-1 recorded maximum divergence from accessions AG284 and AG291. Significantly accessions AG284 and AG291 displayed overlapped grouping with AG302, AG302/2 and AG323 of *caudatus* suggesting genetic similarity between them.

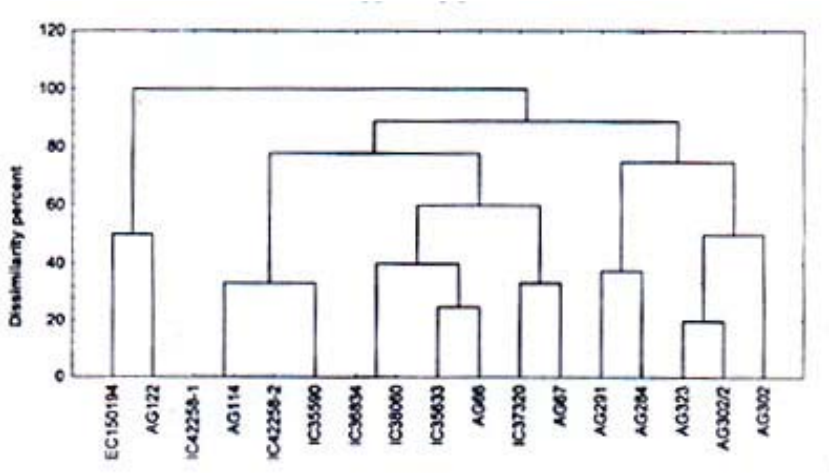


Fig. 3. Dendrogram of 17 accessions of three grain *Amaranthus* species based on dissimilarity per cent generated by RAPD profile analysis (Two from left *A. cruentus*, three from right *A. caudatus* and in between 12 accessions are *A. hypochondriacus*).

The study demonstrates a high level of genetic similarity between *hypochondriacus* and *caudatus* which supports earlier RAPD analytical observations of Transue et al. (1994) and Chan and Sun (1997). Significantly the present analysis is involved with diverse germplasms of different geographical origin ranging from Indian subcontinent to Europe and South

America. The experiment was conducted with eight decamer primers, which were very much different from those used by earlier workers. The striking similarity between the present observation and the previous findings did not seem to be a mere coincidence. 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 amaranth species group (Pal and Khoshoo 1974, Gupta and Guda 1991). Notwithstanding the limitations of the present study (few species studied and documentation of a small numbr of accessions) it is reasonable to suggest from the study of similarity/dissimilarity per cent and RAPD data clustering through dendrogram that at least *hypochondriacus* and *caudatus* are expected to have a common progenitor. Interestingly the RAPD profile developed from primer-2 (Fig. 2) indicates the strong possibility of a single common progenitor of all the three grain amaranth species because of the presence of very conspicuous common bands. In other words, our work supports the hypothesis proposed by Chan and Sun (1997) suggesting that these three grain species have been derived from a single progenitor. The wide genetic diversity among the accessions of *hypochondriacus* that was observed in the present study could be a reflection of selection procedure during domestication. Being a monoecious and wind-pollinated crop with the characteristic arrangement and sequence of anthesis of the unisexual flowers the grain amaranth as a rule favours self pollination (Sauer 1976). The observed loss of intra-accessional genetic variation (similarity % as high as 100 between some accessions) can be the effect of artificial selection in pure lines of cultivated amaranths.

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