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Assessment of the morphological and molecular diversity in *Amaranthus* spp.

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Genetic diversity and relationships of 15 *Amaranthus* species were examined by using both morphological traits and random amplification of polymorphic DNA (RAPD) markers. Ten morphological observations were recorded out of which seven were subjected to analysis of variance. Mean squares due to genotypes were highly significant. Wide mean range performance was observed for number of effective tillers per plant (9 to 12.3), spike length (16.7 to 42.4 cm), spike mass (16.2 to 24.5 g), seed yield per plant (6.4 to 16.6 g), biological yield per plant (19.8 to 28.6 g), harvest index (32.9 to 57.1%) and seed protein content (15.75 to 16.49). RAPD analysis has been carried out using 12 arbitrary sequence decamer primers. Seventy four amplicons were obtained out of which 58 were polymorphic and the level of polymorphism was 78.3%. The average number of polymorphic bands per primer was 4.8. From the RAPD data, an unweighted pair-group method arithmetic (UPGMA) dendrogram illustrating the genetic relationship among fifteen genotypes were computed. The trends of genotypes relationship amongst the *Amaranths* spp. determined by RAPDs are consistent with their morphological traits.

Key words: *Amaranthus*, random amplification of polymorphic DNA (RAPD), genetic relationship, polymorphism.

INTRODUCTION

Amaranthus is a cosmopolitan genus of herbs of the Amaranthaceae family. Approximately 60 species of the genus *Amaranthus* have been recognized. The word Amaranthus has its origin from the Greek word *amarantos,* meaning “one that does not wither” or the “never fading”. *Amaranthus* shows a wide variety of morphological diversity among and even within certain species. The chromosome number of *Amaranthus* is 2n=34. Several species are grown for grain in Asia and America. It is scattered throughout Asia, India, Africa and the Caribbean. The production levels of amaranth are not known. However, recent research indicates that under cultivated conditions, *Amaranth* produces fresh leaf yields of up to 40 t/ha. The yield of grain amaranth is highly variable with 1000 kg/ha considered a good yield.

Amaranthus is a very good source of vitamins, fiber, dietary minerals and balanced amino acids. The protein content of its seeds (15 to 18%) are greater than that of wheat (Dodok et al., 1996). *Amaranthus* seeds or oil is beneficial for those suffering from hypertension and cardiovascular disease. Its regular consumption reduces blood pressure and cholesterol levels, while improving antioxidant status and some immunological parameters (Gonor et al., 2006). *Amaranths* seeds have a unique quality in that the nutrients are concentrated in a natural “nutrient ring” that surrounds the centre, which is the starch section. For this reason, the nutrients are protected during processing. It is an excellent substitute for those who are allergic to grains. The plants of *Amaranthus* are highly valued, so breeders are desirable

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Table 1. Quality traits observed in *Amaranthus* genotypes.

S/N	Variety	Plant color	Spike color	Grain color
1	IC 35430	Green	Green	Tan
2	IC 519548 A	Green	Green	Tan
3	BGA 26	Green	Green	Tan
4	EC 519548 B	Purple	Purple	Tan
5	GA 2	Light purple	Light Purple	Tan
6	BGA 19	Dark purple	Dark purple	Tan
7	BGA 12	Green	Green	Tan
8	GA1	Green	Green	Tan
9	IC 35407	Green with purple shades	Purple with white shades	Tan
10	BGA 2	Green	Green	Tan
11	SUVARNA	Green	Green	Tan
12	IC 519526	Green	Green	Tan and shiny black
13	IC 382750	Orangish yellow	Orangish yellow	Pinkish
14	RMA 4	Green	Green	Tan
15	EC 519527	Green	Green	Tan

traits to increase the economic yield. However, conventional breeding techniques proved to be time consuming. Therefore, in the present investigation, molecular techniques for detecting differences in the DNA of individual plants were used to examine variability in cultivars.

MATERIALS AND METHODS

Plant materials

Experimental material consisted of 15 genotypes of *Amaranthus* spp. They were obtained from the Agriculture Research Station, Mandor, Jodhpur (India). Seeds were sown at the experimental farm of Rajasthan College of Agriculture, Udaipur (India).

Morphological traits

The crop was grown in random block design with 3 replicates. During maturity period, the plant colour, spike colour, grain colour, number of effective tillers per plant and spike length, were measured. After harvest, spike mass, seed yield per plant, biological yield per plant and harvest index were determined. Seed protein content was determined with the Colorimetric method (Snell and Snell, 1955). The analysis of variance for random block design was carried out for the data recorded for each parameter of yield, yield attributes and protein content as per standard procedure (Panse and Sukhatme, 1985).

DNA extraction and quantification

DNA was extracted from young leaves using the CTAB method (Doyle and Doyle, 1990). DNA quantity and quality were measured with a UV spectrophotometer.

Random amplification of polymorphic DNA (RAPD) genotyping

RAPD assay was performed essentially following the conditions of Williams et al. (1990) using 20 random decamer oligonucleotide primers at Department of Molecular Biology and Biotechnology, Rajasthan college of Agriculture Udaipur India. All these primers were obtained from Bangalore Genei Pvt. Ltd (India). Polymerase chain reaction (PCR) amplification was performed in a 20 µl reaction mixture containing 200 µM of dNTP mix, 3U *Taq* polymerase, 1X reaction buffer (pH-7.0), 0.5 µM primers and 50 ng of template DNA. Amplification was performed in a thermo cycler with the following program: 1 initial denaturation step at 94°C for 4 min., followed by 44 cycles at 94°C for 1 min, 35°C for 1 min and 72°C for 2 min and a final cycle of 72°C for 7 min. The amplified products were separated by agarose gel electrophoresis. The gels were photographed under UV light and images transferred to a computer for further analysis. A 100 bp DNA ladder and lamda phase/EcoR1/Double digest ladder was included in the gel as a standard molecular weight marker.

Statistical analysis

Different genotypes of *Amaranths* spp. were compared on the basis of the presence or absence of amplified fragments produced by a series of random primers. Positions of unequivocally scorable RAPD bands were transformed into a binary character matrix ('1' for the presence and '0' for the absence of a band at a particular position). Likewise, data in the form of binary code was prepared as worksheet in MS-excel and pair wise distance matricx were compiled by the NTSYS-pc 2.0 software (Rohlf, 1997) using Jaccard's coefficient of similarity. A phylogenetic tree was created by the unweighted pair-group method arithmetic (UPGMA) average cluster analysis (Sneath and Sokal, 1973).

RESULTS

Morphological characterisation

An investigation was conducted to determine the extent of diversity and relationships among the *Amaranthus* genotypes based on morphological characteristics. Table 1 shows the qualitative trait observations, and Table 2

Table 2. Mean values of seven morphological characteristics.

S/N	Variety	Code No.	No. of effective tillers per plant	Spike length (cm)	Spike mass (g)	Seed yield/plant (g)	Biological yield/plant (g)	Harvest index (%)	Seed protein content (%)
1	IC 35430	C1	11.3	28.6	24.5	7.2	20.5	35.3	16
2	IC 519548 A	C2	12	28.4	19.6	11.6	24.9	46.7	16.04
3	BGA 26	C3	12.3	42.4	20	9.3	22.6	41.4	16
4	EC519548 B	C4	9.7	33.2	18.6	9.7	23	42.1	15.80
5	GA 2	C5	11.3	32.6	16.2	7.9	21.2	37.5	15.81
6	BGA 19	C6	11.3	25.5	23.9	7.8	21.1	37.3	15.81
7	BGA 12	C7	10.7	36.3	18.3	15.2	28.6	53.7	16
8	GA1	C8	10	33.6	19.1	6.4	19.8	32.9	16.37
9	IC 35407	C9	10.3	32.8	20.9	9.4	22.8	41.7	16.40
10	BGA 2	C10	9	38.6	20.5	12.6	25.9	48.5	16.40
11	SUVARNA	C11	10.3	35.4	21	8.6	21.9	39.5	16.40
12	IC 519526	C12	11.7	41	23.2	9	22.4	40.8	16.38
13	IC 382750	C13	11.3	41.9	21.7	16.6	29	57.1	15.75
14	RMA 4	C14	11.3	16.7	18.5	8.8	22	40	16.49
15	EC 519527	C15	10.7	19.5	19	7.1	20.8	34.4	16.49
	Mean		10.9	32.4	20.3	9.8	23.1	42	16.13
	Range		9-12.3	16.7-42.4	16.2-24.5	6.4-16.6	19.8-28.6	32.9-57.1	15.75-16.49
	CV		7.2	5.1	5.3	7.1	7.2	8.2	2
	CD		1.3	2.7	1.8	1.2	2.8	5.7	0.53
	SEm±		0.45	0.94	.62	0.40	1	1.98	0.18

shows the mean values of seven characteristics. The analysis of variance was done for all the seven characteristics. The mean squares due to genotype were highly significant for all the characteristics.

RAPD analysis

All fifteen genotypes of *Amaranths* spp. were examined for RAPD genetic marker with 20 decamer primers. Out of twenty primers, only twelve primers caused template DNA to amplify. Only those fragments which consistently amplified were considered for analysis. Electrophoresis pattern of RAPD profile on 1.2% agarose gel is illustrated in Plate 1 with three specific primers. Primer S-70, S-30 and OPC-09 gave bands in range of 200 to 1000, 300 to 800 and 200 to 800 with 100, 75 and 100% polymorphism, respectively.

Table 3 illustrates the total number of amplified products whether polymorphic or monomorphic with all primers. The maximum number of scorable bands is found in primer S-70 which gave 10. The minimum number of bands is obtained with primer S-30 which gave 4 scorable bands. The primers showing amplification were repeated twice to confirm the reproducibility and polymorphism. Six hundred and eighty two fragments were amplified in all genotypes. Seventy four scorable amplified fragments were obtained out of which 58 bands were polymorphic and hence the level of average polymorphism was 78.3% and an average of 6.2 bands per primer was observed. A dendrogram (Figure 1) was constructed using similar matrix values as determined from RAPD data for 15 genotypes using unweighted pair group method of arithmetic averages subprogramme of NTSYSpc programme. The dendrogram generated on the basis of Jaccard's similarity coefficient clearly indicated four main clusters. The major cluster included 9 genotypes viz. IC 35430, IC 519548 B, BGA 26, EC 519548 B, GA 2, BGA 19, BGA 12, GA 1 and IC 519526. Cluster1 can be divided into subgroup A and subgroup B. Subgroup A includes IC 35430 and IC 519548 A. Subgroup B includes BGA 26, EC 519548 B, GA 2, BGA 19, BGA 12, GA 1, and IC 519526. BGA 19 and BGA 12 showed maximum similarity. The other two pairs of genotypes which were closely related were GA 1 and IC 519526. Cluster II included two genotypes that is, RMA 4 and EC 519527. Cluster III included genotypes IC 35407, BGA 2 and SUVARNA. Cluster IV included only one genotype, that is, IC 382750 whose maximum similarity was with IC 35407.

DISCUSSION

Molecular marker data in conjugation to morphological data may be highly useful in precise differentiation and relatedness among the genotypes. The polymorphism (78.3%) observed during the present study is in

Table 3. Details of random primers used for amplification of genomic DNA of *Amaranthus* spp.

Total primers	20
Primers which showed amplification	12
Primers which not showed amplification	8
Primers which are polymorphic	12
Number of monomorphic bands	16
Total number of scorable bands	74
Total number of polymorphic bands	58
Average number of polymorphic bands	4.8
Total number of alleles produced	682
Percent polymorphism	78.3

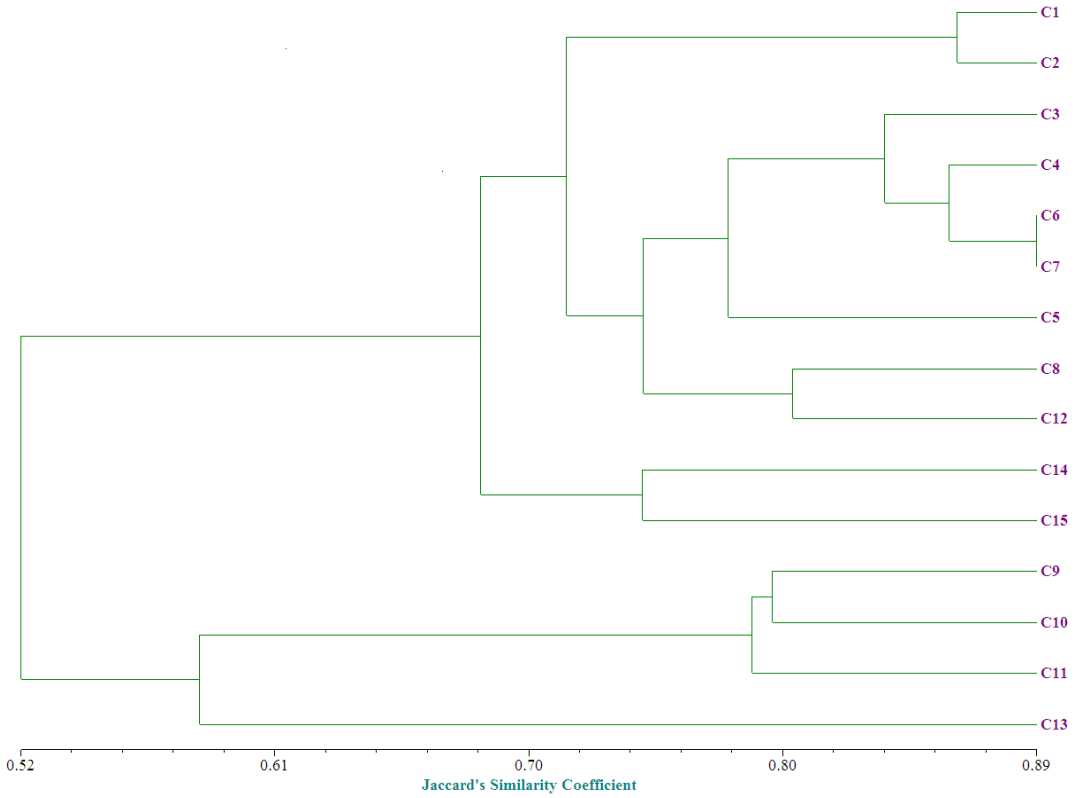

Figure 1. Dendrogram generated for fifteen Amaranthus genotypes using UPGMA cluster analysis based on Jaccard similarity coefficient.

agreement with results of Choudhury et al. (2008). who observed a total of 796 amplified products using RAPD, of which 587 showed polymorphism (73.7%) in pigeonpea [*Cajanus cajan* (L.)] cultivars. Similarly Mandal and Das (2002) studied the genetic diversity in three grain *Amaranths*, namely *Amaranths hypochondriacus, Amaranths caudatus* and *Amaranths cruentus* comprising a total of 17 accessions. Thirteen bands were identified and the extent of polymorphism was highest in A. *cruentus* with 69.2% followed by A. *caudatus* 38.5% and A. *hypochondriacus* having 15.4%. In the present study, all primers used were polymorphic but the extent of polymorphism was high in some and moderate in some others primers (Bhagwat et al., 1997).

Assessment of relationship between morphological and molecular characteristics based on RAPD

It is interesting to note that genotypes grouped in Cluster I and subgroup A (IC 35430 and IC 519548 A) were having green plant and spike pigmentation with grains of

a tan colour. The number of effective tillers and the spike length of both genotypes were almost similar. Furthermore, the protein content of these genotypes was very much the same. In subgroup B, plant and spike colour of three genotypes (EC 519548 B, GA 2 and BGA 19) were purple, while other genotypes (BGA 26, BGA 12, GA 1 and IC 519526) were green. Grain of all genotypes was tan in colour except IC 519526 which displayed some shiny black colour seeds also. The numbers of effective tillers of GA 2, BGA 19 and IC 519526 were similar, EC 519548 B and GA 1 were similar and BGA 26 and BGA 12 was-12.3 and 10.7, respectively. BGA 26, EC 519548 B, GA 2, BGA 19 and BGA12 were similar in protein content and GA 1 and IC 519526 had almost the same protein content.

Genotypes in Cluster II (RMA 4 and EC 519527) were green in plant and spike colour, and had grain which was tan in colour. Cluster III involved genotypes IC 35407, BGA 2 and SUVARNA. Plant and spike colour of BGA 2 and SUVARNA were green while these traits for IC 35407 were different. Plant colour of this genotype was green with purple shade and the spike colour was purple with white shade. The grain colour of all the varieties was tan. The number of effective tillers of IC 35407 and SUVARNA was 10.3 and of BGA 2 was 9. The spike mass and protein content of all varieties were almost the same.

Cluster IV involved only one genotype, namely IC 382750. Plant and spike colour of IC 382750 were orange-yellow, and grain color was pink. The number of effective tillers, spike length and protein content of this genotype were 11.3 and 41.9 cm and 15.75%, respectively. Hence, this study at the morphological and molecular level, comprising 15 *Amaranths* spp. cultivars, showed that variation at the morphological level was more. Some genotypes categorized in the same group showed different plant, spike and grain colours and the possible explanation for this result is that: genotypes sharing common parents tend to group together (Nicese et al., 1998). Except at some point there was significant associations between the dendrogram obtained by RAPD markers and morphological characteristics. This was in accordance with the results reported by Nebauer et al. (2000) on the genus *Digitalis* and Thakur et al. (2008) on maize. The notion is that the distribution of genetic variation using RAPD analysis will help in identification of superior genotype for cultivar upgrading as well as to evolve breeding strategies for genetic improvement in *Amaranthus* spp. Nevertheless, it could be concluded that RAPD profiles were more efficient in detecting polymorphism in Amaranths varieties.

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