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A comparative study of Inter Simple Sequence Repeat (ISSR), Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeat (SSR) loci in assessing genetic diversity in *Amaranthus*

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Abstract

Genetic diversity in a set of 31 *Amaranthus* accessions was analysed using RAPD, ISSR and SSR markers. Polymorphic Information Content (PIC) values of the polymorphic RAPD, SSR and ISSR markers ranged from 0.067 to 0.471, 0.178 to 0.452 and 0.11 to 0.362 with an average of 0.229, 0.371 and 0.441 per primer, respectively. Gene diversity for RAPD, SSR and ISSR markers respectively ranged from 0.032 to 0.487, 0.11 to 0.362 and 0.104 to 0.384. The similarity metrics values for the accessions ranged from 0.58 to 0.98 with RAPD, 0.70 to 1.00 with SSR and from 0.44 to 0.88 with ISSR markers. The dendrogram generated by the markers had five major clusters in case of RAPD markers, two distinct clusters in case of SSR markers and six clusters in case of ISSR markers. Amongst all, ISSR markers provided greater confidence for the assessment of genetic diversity in comparison to SSR and RAPD markers. All the accessions studied were found genetically diverse which may be useful in breeding for improving the genotypes of *Amaranthus*.

Key words: *Amaranthus,* genetic diversity, molecular markers, principal coordinate analysis

Introduction

The genus *Amaranthus* (family Amaranthaceae), consists of about 60 species, which include mostly wild or weedy species [1]. Only few of these are abundantly cultivated in Northern India, Manchuria, Southeast Asia and Africa [2]. Amaranths have high nutritional value due to higher amount of protein (14-18%) with balanced essential amino acid content. It has high photosynthetic efficiency, low input requirements and high yield potential for grain, vegetable and fodder production [3]. In addition to the nutritional value of both seeds and leaves, it is an excellent source of various vitamins and trace elements [4]. Further, amaranths offer low demand for climate and soil, and also renders as a major food source to fight malnutrition and underfeeding in developing areas. Besides, it also exhibits a tremendous amount of morphological diversity and a wider adaptability to different eco-geographical situations [5]. With all the pleasing traits, amaranths has, of late, received attention from plant breeders and agriculture scientists worldwide [6].

Genetic erosion and habitat destruction by modern agriculture has increased the significance of collection and preservation of plant germplasm. Despite increasing attention to amaranths, the germplasm of amaranths has not been well characterized. However, there is an increased interest for a meaningful understanding of the genome of the amaranths and the extent of its genetic diversity. Though morphological observations have been made, but these are inherently weak identifiers as they are influenced by the environmental factors [7].

DNA markers are most suitable for genetic diversity estimates and their use as a means to assay diversity at the locus, chromosome and whole genome level is now well established. The use of random amplified polymorphic DNA (RAPD) [8] markers for

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species determination amongst the germplasm of three grain amaranth species, namely, *Amaranthus hypochondriacus*, *A. cruentus* and *A. caudatus*, is perhaps one of the few reports known on the molecular genetics of amaranths [9]. Inter-simple sequence repeats (ISSRs) described by Zietkievicz *et al*. [10] have been widely used in genomic fingerprinting studies, genetic diversity and phylogenetic analyses [11], evolutionary biology [12], and in several other fields. However, genus *Amaranthus* was rarely characterized by ISSR markers, only been used by Roy and Ray [13], to study the phylogenetic relationships between amaranthaceae and chenopodiaceae. Therefore, in order to assess utility of PCR based molecular markers (RAPD, ISSR, SSR) in deciphering genetic diversity as well as to assess the genetic relationship among major cultivated amaranth species the present study was undertaken. The results provided an evidence for the use of a suitable marker system for characterization of this precious but orphan crop.

Materials and methods

Amaranthus accessions

A total of 31 cultivated amaranth accessions, collected from farmers' fields of Tehri Garhwal region of Uttarakhand, India, were obtained from Pantnagar Centre for Plant Genetic Resources, Pantnagar, and used for the present investigation (Table 1).

DNA extraction and PCR conditions

The genomic DNA of different amaranth accessions was extracted by the standard method of Murray and Thompson [14] and quantified and analyzed on agarose gel electrophoresis. The DNA pellet was dried overnight and dissolved in 200 µl of TE buffer (pH 8.0). Quantification of DNA was performed on 0.8% agarose gel and diluted with sterile distilled water to a concentration of 40 ng per reaction for PCR analysis. The reaction mixture (20 µl) consisted of 12.8 µl of sterile distilled water, 2 µl 10x PCR assay buffer (50 mM KCl, 10 mM Tris-Cl, 1.5 mM $MgCl_2$), 0.5 µl dNTP mix (0.2 mM each of dATP, dTTP, dGTP and dCTP), 0.2 µl *Taq* DNA polymerase (1 unit), 0.5 µl primer each (0.5 µM) and 40 ng template DNA. The PCR amplification was carried out in a thermocycler (Eppendorff) and was set in accordance with marker type. The amplified products were resolved by electrophoresis in agarose gel at 1.5% for ISSR, 2.5% for RAPD and 3% for SSR in 0.50 TBE buffer (pH 8.0) at constant voltage at 80 V for 3 h. The DNA profiles were visualized on a UV transilluminator and photographed by using Gel Documentation System (Alpha Innotech, USA). The PCR reactions were repeated thrice for each primer to verify the reproducibility of the results. Only highly reproducible and polymorphic primers were included in the study.

Primer selection

A total of 60 ISSR primers, both anchored and non-anchored, having different repetitive units were screened. All the selected ISSR primers listed in table 2 were selected on the basis of their degree of polymorphism and reproducibility. Structurally, 16 primers were trinucleotide repeats with four mono and two di-nucleotide repeats and ten primers were dinucleotide repeat with four mono and six dinucleotide anchors. Four primers were tetra-nucleotide. Twelve SSR primer pairs were adopted from Ro-Lee *et al*. [15] and 20 RAPD primers, as listed in Table 3, were also included in this study.

Statistical analysis

DNA bands were scored for the presence (1) or absence (0) and used to form a binary matrix. The distance matrices were used to generate dendrogram with the UPGMA method of SAHN clustering using NTSYS-pc for Windows, version 2.11 [16]. Data were analyzed to obtain Jaccard's similarity coefficients among the genotype. The SIMQUAL program was used to calculate the Jaccard's similarity coefficients for all the markers used. Correlation between the two matrices obtained with molecular marker and disease data was scored and estimated by means of Mantel matrix correspondence test [17]. Similarity matrices were utilized to construct the UPGMA (unweighted pair-group method with arithmetic average) dendrograms. Principal coordinate analysis was performed in order to highlight the resolving power of the ordination. In order to estimate the congruence among dendrograms, cophenetic matrices for each marker and index type were computed and compared using Mantle test. Polymorphic information content (PIC) was calculated as per the formula:

$$\text{PICi} = 1 - \sum_{j=1}^{n} P_{ij}^2$$

Where, Pij is the frequency of the jth allele for marker i and the summation extended over n alleles [18]. Mean gene diversity (He) was estimated as $n(1-\Sigma Pi^2)/(n-1)$, where pi is the estimated allele frequency of the allele i and n give the size of the

sample [19]. To determine robustness of the dendrogram, the data were bootstrapped with 2000 replications along with Jaccard's coefficient using the computer programme WINBOOT [20].

Results and discussion

Genotypic knowledge and genetic relatedness in a crop species is important for efficient utilization of plant genetic resources**.** Molecular markers such as isozymes, RFLPs, RAPDs, AFLPs, SSRs, ISSRs, SNPs, etc. are efficiently used for germplasm characterization. The assessment of genetic diversity, efficiency of a molecular marker technique depends upon the amount of polymorphism it can detect among the set of accessions under investigation. These methods work on any organism without any prior knowledge of genomic sequences. Considering the problems associated with available genomic information, the researchers involved in diversity analysis have searched for alternative tools. Among the tools identified, usage of microsattelites, RAPD and ISSR markers attained a wider range for the characterization of the organisms. All the markers are known for their technical simplicity and high throughput [8].

RAPD analysis

Out of the 20 screened RAPD primers, only 11 showed polymorphism. The size of the amplified products ranged from 0.1-2.0kbp, with 3-5.1 bands per primer. Only four unique bands were revealed by all the polymorphic primers. A total of 103 of loci generated by RAPD primers were scored, of which 82 loci were found to be polymorphic and 21 were monomorphic. The number of amplified loci for the polymorphic markers varied from 3-13 with an average of 5.1 bands per primer. The PIC values of the polymorphic markers ranged from 0.067-0.471 with an average of 0.229 per primer. The total gene diversity ranged from 0.032 to 0.487.

The UPGMA based dendrogram obtained from the binary data deduced from the DNA profiles of the samples analysed revealed a total of five distinct clusters out of the pooled RAPD marker data. Principle coordinate analysis approved the SAHN clustering of UPGMA results. Matrix comparison was performed to calculate the cophenetic correlation between the similarity matrix and the cophenetic value matrix, and was found to be significant (r = 0.70), which indicated good fit of the clustering. Similarity matrix analysis revealed a high degree of similarity, to the extent of

Table 1. List of the selected accessions of *Amaranthus* used in the study

S.No.	Accession No.	Source (Village)	S.No.	Accession No.	Source (Village)
1.	HCGA3-02	Lohital	17.	HCGA76-02	Kethole
2.	HCGA4-02	Chaupariyal	18.	HCGA79-02	Sokarbhan
3.	HCGA7-02	Lohital	19.	HCGA82-02	Karras
4.	HCGA11-02	Mardhan	20.	HCGA93-02	Gowana
5.	HCGA12-02	Mardhan	21.	HCGA94-02	Saharsain
6.	HCGA16-02	Kurie	22.	HCGA98-02	Karras
7.	HCGA17-02	Kurie	23.	HCGA104-02	Karras
8.	HCGA19-02	Maun	24.	HCGA105-02	Karras
9.	HCGA47-02	Ghoghas	25.	HCGA106-02	Ghoghas
10.	HCGA51-02	Paitav	26.	HCGA107-02	Paitav
11.	HCGA59-02	Ghoghas	27.	HCGA108-02	Gowana
12.	HCGA60-02	Paitav	28.	HCGA109-02	Saharsain
13.	HCGA62-02	Ghoghas	29.	HCGA111-02	Pojar
14.	HCGA66-02	Bhavera	30	HCGA114-02	Ghoghas
15.	HCGA67-02	Kethole	31.	HCGA118-02	Karras
16.	HCGA71-02	Chauhdava			

(Source: Pantnagar Centre for Plant Genetic Resources, Pantnagar. All the collection sites were in Distt. Tehri Garhwal, Uttarakhand)

***Table 2**. Different polymorphic parameters of ISSR markers in a set of accessions of *Amaranthus*

Primer	Sequences	TA (No.)	PB (No.)	Polymorphism (%)	UB (No.)	He	PIC
Di-nucleotide							
LC-51	$(GA)_8$	7	6	85.71	0	0.3434	0.405
Di-nucleotide, 3' mono-nucleotide anchored							
LC-7	$(AG)_8G$	10	8	80	1	0.289	0.313
LC-46	$(AG)_8T$	17	13	76.47	1	0.229	0.428
LC-48	$(AG)_8C$	6	4	66.67	0	0.235	0.301
LC-49	$(GA)_8T$	10	9	90	0	0.205	0.574
Di-nucleotide, 3' di-nucleotide anchored							
LC-413	$(AG)_8TA$	11	9	81.81	2	0.189	0.581
LC-414	$(AG)_8TC$	4	3	75	0	0.247	0.419
LC-415	$(CT)_8AC$	6	3	50	0	0.390	0.435
LC-421	$(GT)_6CC$	10	7	70	2	0.160	0.503
LC-411	$(CA)_6GC$	6	4	66.67	0	0.253	0.274
LC-59	$(GA)_8YT$	10	7	70	3	0.127	0.539
Tri-nucleotide							
LC-6	$(GAC)_5$	15	12	80	2	0.220	0.501
LC-9	$(GTG)_5$	13	11	84.61	1	0.257	0.399
LC-17	$(GTC)_5$	7	7	100	2	0.212	0.668
LC-20	$(GCA)_5$	12	8	66.67	1	0.144	0.339
LC-419	$(ATG)_6$	6	6	100	2	0.228	0.742
LC-16	$(AGC)_5$	12	7	58.33	3	0.112	0.441
LC-15	$(CAG)_5$	12	8	66.67	1	0.191	0.306
LC-424	$(CTC)_6$	8	8	100	0	0.258	0.540
LC-12	$(CTC)_5$	5	3	60	2	0.160	0.335
Tri-nucleotide 3' mono-nucleotide anchored							
LC-34	$(GAC)_5A$	10	8	80	2	0.275	0.203
LC-38	$(ACG)_5C$	9	9	100	0	0.217	0.674
LC-403	$(TCG)_5G$	10	7	70	1	0.197	0.570
LC-404	$(TCG)_5T$	10	6	60	1	0.104	0.483
Tri-nucleotide 3' di-nucleotide anchored							
LC-420	$(GAG)_4GC$	7	5	71.43	1	0.256	0.304
LC-412	$(CAC)_4GC$	16	15	93.75	3	0.215	0.605
Di-nucleotide, 5' anchored							
LC-63	$BHB(GA)_7$	9	7	77.78	2	0.239	0.466
LC-64	$VHV(GT)_7$	11	9	81.81	0	0.312	0.284
LC-65	$HVH(TG)_6G$	11	9	81.81	2	0.325	0.274
LC-418	HVH(TG)6	2	1	50	0	0.166	0.226
LC-409	VHV(GT)7	5	4	80	0	0.284	0.290
Tetra-nucleotide							
LC-13	$(GTCT)_3$	11	9	81.81	3	0.258	0.519
LC-14	$(AGAC)_3$	18	15	83.33	3	0.276	0.498
LC-29	$(TCAC)_3$	5	4	80	1	0.187	0.419
LC-32	$(ACGC)_3$	11	9	81.81	1	0.384	0.575
LC-425	TGT GGG CGT AAG CGC AT	7	7	100	2	0.283	0.456

TA: Total bands amplified; PB: Polymorphic bands; UB: Unique band; He = Mean gene diversity

98.73 per cent, existing between accessions HC71-02 and HC76-02 and a minimum of 58.44 per cent similarity between accessions HC108-02 and HC7-02. The bootstrap values were found to be significant for all the clusters. By RAPD marker, moderate or low degree of variability were found among all the

***Table 3**. Different polymorphic parameters of RAPD markers in a set of *Amaranthus* accessions

Primer name	Primer Sequence	TB (No.)	PB (No.)	Polymorphism (%)	UB (No.)	He	PIC
LC-70	CAGGCCCTTA	3	1	0.33	0	0.032	0.312
LC-75	GAAACGGGTG	6	4	0.67	1	0.253	0.069
LC-76	GTGACGTAGG	8	5	0.625	0	0.127	0.471
LC-77	GGGTAACGCC	9	6	0.67	1	0.225	0.208
LC-84	AGGTGACCTG	4	3	0.75	0	0.487	0.266
LC-86	GTTGCGATCC	12	6	0.5	0	0.124	0.089
LC-87	AGGTGACCGT	11	5	0.45	0	0.161	0.158
LC-88	CAGGCCCTTC	6	3	0.5	0	0.248	0.129
LC-89	AGTCAGCCAC	9	1	0.111	0	0.156	0.057
LC-90	GTGAGGCGTC	13	9	0.69	1	0.186	0.419
LC-91	GACGGATCAG	10	8	0.8	0	0.331	0.342

TA: Total bands amplified; PB: Polymorphic bands; UB: Unique band; He = Mean gene diversity

accessions because of either lesser number of markers screened or accession collected from same locality or both. The lesser number of variability among intraspecific accessions of amaranthus also reported using RAPD markers [21].

Microsatellite (SSR) analysis

The 12 microsatellite markers used in the study resulted into product size ranging from 0.15 kb to 0.4 kb with total alleles varying from one to five. Most of the markers were found to be monomorphic. Only two of the markers of the loci, GB-AMM-129 and GB-AMM-136 were biallelic, marker of the loci GB-AMM-078 and GB-AMM-099 were tri allelic and marker of the locus GB-AMM-051 had five alleles. Three markers of loci, GB-AMM-051, GB-AMM-099 and GB-AMM-136 were polymorphic with PIC values 0.452, 0.484 and 0.178, respectively (Table 4).

Jaccard's similarity co-efficient was used to cluster the data using the UPGMA algorithm. A total of two major clusters were obtained by SSR marker analysis. Principle coordinate analysis was also conducted to visualize genetic relationship among the genotypes. The results were similar to the UPGMA analysis. Similarity matrix analysis of the SSR data revealed high degree of similarity to the extent of 100 per cent existing among most of the accessions and a minimum of 70.95 per cent similarity between accessions HC105-02 and HC62-02. Matrix comparison was found to be significant (r = 0.81), which indicated good fit of the clustering.

ISSR analysis

Based on the difference in repetitive units, a total of 60 ISSR primers were selected and used. The repetitive unit was di-nucleotide, tri-nucleotide, tetra

Table 4. Different polymorphic parameters including, primer sequences, total bands, polymorphic bands, banding patterns, He and PIC among a set of accessions of Amaranthus using SSR primers

Primer name	Primer sequence product size (kb)	TB	PB	Polymorphism (%)	UBP	He	PIC
GB-AMM-051	F-GAGGAGACTTGGTGGCCT R- TCGGGAGCAATGTAGCAC	4	2	0.50	0	0.487	0.452
GB-AMM-099	F- AAATTGACAATGCGCAGC R- TTCCTCACCAAAATTGCC	3	3	1.00	0	0.445	0.484
GB-AMM-136	F- TCAGCAAAACATGATCAACAA R- GTTGCTGCATTGGTGGTT	2	1	0.50	0	0.335	0.177

TA: Total bands amplified, PB: Polymorphic bands, UB: Unique bands, PIC: Polymorphic information content. He: Mean Gene diversity
Ya = C, T; Db = non-C; Bc = non-A; Hd = non-G; Ve = non-T

nucleotide, etc. Out of 60 primers, 36 gave distinct polymorphic products (Fig. 1). A primer with dinucleotide repeat $(GA)_8$, showed 87.71 per cent polymorphism and a PIC value was 0.405. The four ISSR primers with di-nucleotide repeat and 3' mono-nucleotide anchored produced a total of 43 polymorphic bands with an average of 10.75 bands per primer and an average PIC value of 0.404. The di-nucleotide markers with 3' di-nucleotide anchored produced a total of 48 polymorphic bands with 10 bands per primer. The average PIC value for di-nucleotide with 3' mono-nucleotide anchored marker was 0.4585 and maximum per cent polymorphism (81.81%) was found for the marker $(AG)_8TA$. Among polymorphic nine tri-nucleotide markers, two markers *viz*., $(ATG)_6$ and $(CTC)_6$, showed 100 per cent polymorphism. The average PIC value for this group was 0.475. The tri-nucleotide 3' mono-nucleotide anchored with the average PIC 0.4825 showed the maximum (100 %) polymorphism for marker $(ACG)_5C$. The tri-nucleotide with 3' di-nucleotide anchored showed an average PIC value of 0.4545. The di-nucleotide, 5' anchored showed an average PIC value of 0.308 and the tetra-nucleotide repeat showed 0.503 PIC value. The size of the amplicons ranged from 0.1-1.8 kbp, with an average of 8.5 bands per primer. Among the studied ISSR primers percent polymorphism, gene diversity and PIC value were found to be higher in anchored primers and increased with repeating unit of the primers. The plausible explanation of these results is that the addition of one, two or three bases at three prime, selectively amplifies the product and with increase in repeating unit, it reduces the chance of finding particular repeat among different accessions. The dinucleotide microsatellites are prevalent in plants while mono-, tri- and tetranucleotide repeats are less common [22].

The total numbers of loci generated by ISSR primers scored 306 bands, of which 224 bands were polymorphic and 72 monomorphic. The number of amplified loci for the polymorphic marker varied from 2-17 with an average of 8.5 bands per primer. The PIC values of the polymorphic markers ranged from 0.203-0.742 with an average of 0.229 per primer. The total gene diversity ranged from 0.112 to 0.384. Similarity matrix analysis of the ISSR data was calculated and the similarity ratio revealed high degree of similarity to the extent of 88.18 per cent between accessions HC71-02 and HC76-02 and the minimum of 43.87 per cent between accessions HC66-02 and HC7-02. The results of the principle coordinate analysis were similar to the UPGMA results. Matrix comparison was found to be significant (r = 0.60), which indicated good fit of the clustering.

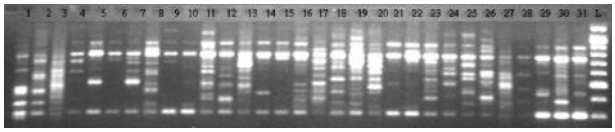

Fig. 1. The ISSR markers profile of 31 *Amaranthus* accessions with a marker Ic-59. Lanes 1 to 31 represent individual genotype per lane and correspond to the genotypes listed in table 1. L=100bp DNA marker

Combined analysis

The Mantel test for matrix comparison revealed a good correlation between RAPD-based and ISSR-based similarity matrices (r = 0.69), a moderate correlation between ISSR and SSR (r = 0.24) and the lowest value (r = 0.11) between RAPD and SSR. The individual data sets were combined to make comparison and examine dissimilarity among the results produced by RAPD, SSR and ISSR markers (Table 5). The combined cluster analysis revealed a total of seven clusters. Of these, three clusters had a single accession each. Similarity matrix analysis of all the markers data was calculated and the ratio revealed high degree of similarity, to the extent of 88.7 per cent between accessions HC71-02 and HC76-02, and the minimum 53.44 per cent between accessions HC66-02 and HC07-02.

The entire three marker systems used in this study detected different degree of polymorphism. The ISSR and RAPD markers generated more clusters with fewer genotypes in each cluster, while the SSR markers gave fewer clusters with more accessions in each and therefore, more revealed variation within each cluster. When more clusters are obtained with fewer accessions in each, it implies lower significance in clustering because of the smaller differences and smaller sample sizes for each cluster [23]. This indicates the differential power of DNA markers in grouping the genotypes according to their genomic constitution. Our results showed that ISSR markers were more polymorphic in comparison to other markers used. The variability among the selected ISSR primers had the advantage of being more polymorphic than other markers and differentiated the individuals more precisely. Similarity coefficient values of all the markers also approved the greater confidence of ISSR marker for the assessment of genetic diversity and relationships among the related cultivars.

Table 5. Matrix comparisons between similarity matrix, cophenetic value matrix and among the similarity matrices of different markers used

Markers/characters	SSR	ISSR	RAPD	ISSR and SSR	ISSR and RAPD	SSR and RAPD	Combining three markers
Correlation (r)	0.81	0.60	0.70	0.24	0.69	0.11	0.60
Mantle test (t)	21.43	20.30	21.48	2.56	6.05	1.29	20.34
Prob. random Z < obs. Z (P)	1.0	1.0	1.0	0.99	1.0	0.90	1.0
Eigen values	36.73	12.66	27.0	-	-	-	68.60
Similarity matrix	0.79-1.0	0.52-0.88	0.66-1.0	-	-	-	0.53-0.89

Similarity matrix correlation revealed that only significant correlation (0.69) was found between ISSR and RAPD (Table 4), which might be due to the fact that both the markers are multiple allelic revealing a good fit of matrices and robustness of these markers in comparison to the SSRs. Development of SSRs is expensive, but once the SSRs have been developed for a crop, they are cost- and time-effective in comparison to RAPD and ISSR markers. Unfortunately in amaranths, the developed SSR markers are quite low. Out of the adopted 12 SSRs in the present study, only three primers produced good diversity indices, compared to the 36 ISSR primers and 12 RAPD markers. The results indicated that ISSR and RAPD markers were highly discriminatory as compared to SSRs. This might be because of the lower number of SSR markers used in this study. Further, the ISSR markers detected relatively high polymorphism and reproducible results compared to the RAPD markers hence proved to be superior over RAPD markers.

Based on the Jaccard's similarity coefficient, most of the amaranth accessions showed less than 80 per cent genetic similarity. Only accessions HCGA16-02 and HCGA17-02 showed more than 80 per cent of the genetic similarity and were grouped together by each of the marker studied. Cluster analysis revealed minimum chance of duplication of the accessions among the selected germplasm and a highly diverse population of cultivated amaranths in Tehri Garhwal region. Thus, ISSR markers could be used as an alternative molecular marker to estimate the level of genetic diversity within and among amaranths to provide the necessary information needed for genetic conservation. In addition, genetic diversity information generated could be used in the amaranths breeding programs in order to cross

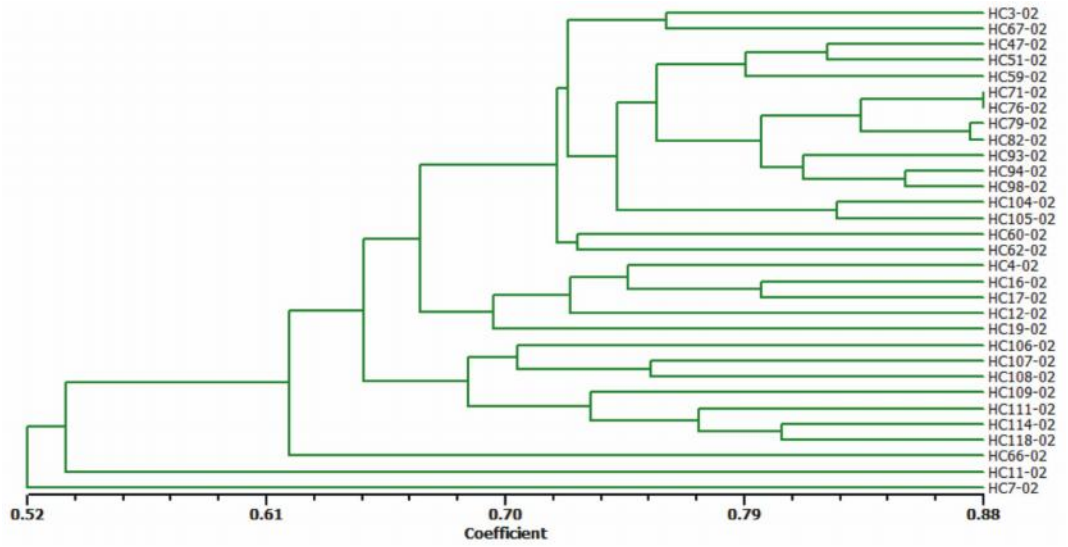

Fig. 2. Dendrogram constructed using UPGMA method depicting the classification of 31 Amaranthus accessions based on ISSR marker. The scale at the bottom is Jaccard's similarity coefficient. The numbers plotted represent the individual accession and correspond to the ones listed in Table 1

genetically diverse parents and maximize the level of variation present in segregating populations.

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Reference

1. **Sauer J. D.** 1967. The grain amaranths and their relatives: a revised taxonomic and geographic survey. Annals of Missouri Botanical Garden, **54**: 103-137.
2. **Kauffmann C. S. and Weber L. Å.** 1988. Grain amaranth: in Advances in New Crops: Proceedings of the First National Symposium of New Crops: Research, Development and Economics (eds.). J. Janick and J. Å. Simon (Oregon: Timber Press) pp. 127-139.
3. **Transue D. K., Fairbanks D. J., Robison L. R. and Andersen W. R.** 1994. Species identification by RAPD analysis of grain amaranth genetic resources. Crop. Sci., **34**: 1385-1389.
4. **Sreelathakumary I. and Peter K. V.** 1993. Amaranth – *Amaranthus* spp. *In*: Genetic improvement of vegetable crops. Pergamon Press, pp. 315-331.
5. **Pedersen B., Bach Knudsen K. E. and Eggum B. O.** 1990. The nutritive value of amaranth grain (*Amaranthus caudatus*). Plant Foods Hum. Nutr., **40**: 61-71.
6. **Grubben G. J. H.** 1977. Tropical vegetables and their genetic resources. International Board for Plant Genetic Resources. Rome.
7. **Dey G.** 1997. Genetic divergence, developmental allometry and adaptibility for grain yield and protein content in grain amaranth. Ph. D. Thesis, BCKV, West Bengal, India.
8. **Williams J. G. K., Kubelik A. R., Livak K. J., Rafalski J. A. and Tingey S. V.** 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., **18**: 6531-6535.
9. **Tucker J. Â.** 1986. Amaranth: The once and future crop. Bio Science, **36**: 9-13.
10. **Zietkiewicz E., Rafalski A. and Labuda D.** 1994. Genomic fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, **20**: 176-183.
11. **Arif M., Zaidi N. W., Singh Y. P., Haq Q. M. R. and Singh U. S.** 2009. A comparative analysis of ISSR and RAPD markers for studying genetic diversity in shisham (*Dalbergia sissoo*). Plant Mol. Biol. Rep., **27**: 488-495.
12. **Wolfe A. D., Xiang Q. Y. and Kephart S. R.** 1998. Assessing hybridization in natural populations of *Enstemon* (*Scrophulariaceae*) using hypervariable inter simple sequence repeat markers. Mol. Ecol., **71**: 1107-1125.
13. **Ray T. and Roy S. C.** 2007. Phylogenetic relationships between members of Amaranthaceae and Chenopodiaceae of lower Gangetic plains using RAPD and ISSR markers. Bangladesh J. Bot., **36**: 21-28.
14. **Murray M. G. and Thompson W. F.** 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res., **8**: 4321-4325.
15. **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-amplifications in wild species. Conserv. Genet., **9**:243-246.
16. **Rohlf F. J.** 2000. NTSYS-pc Numerical Taxonomy and Multivariate Analysis System *Version 2.1.* Exeter Software, Setauket, New York, USA.
17. **Mantle N. A.** 1967. The detection of disease clustering and a generalized regression approach. Cancer Res., **27:** 209-220.
18. **Botstein D., White R. L., Skolnic M. and Davia R. W.** 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphism. Am. J. Hum. Genet., **32**: 314-331.
19. **Nei M.** 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA, **70**: 3321-3323.
20. **Yap I. V. and Nelson R. J.** 1996. WinBoot: A program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendrograms. IRRI Discussion Paper Series 14. International Rice Research Institute, Manila, Philippines.
21. **Popa G., Cornea C. P., Ciuca M., Babeanu N., Popa O. and Marin D.** 2010. Studies on genetic diversity in amaranthus species using the RAPD markers. Tom. XVII, Issue: 2, 2010, pp. 280-285.
22. **Wang Z., Weber J. L., Zhong G. and Tanksley S. D.** 1994. Survey of plant short-tandem DNA repeats. Theor. Appl. Genet., **88**: 1-6.
23. **Ravi M., Geethanjali S., Sameeyafarheen F. and Maheswaran M.** 2003. Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR markers. Euphytica, **133**: 243-252.