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Analysis of genetic diversity among Indian garlic (*Allium sativum* L.) cultivars and breeding lines using RAPD markers

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Abstract

Randomly amplified polymorphic DNA markers were used to assess the genetic diversity among 23 commercially released varieties and breeding lines of garlic along with onion as an out-group. A total of 100 operon decamer primers were screened in duplicate and fourteen of these primers were selected for further testing. The number of bands per primer varied from 3 to 19, with an average of 11.5. Based on RAPD data, similarity values among garlic genotypes ranged from 0.97 to 0.47 with a mean value of 0.72. A low degree of similarity (0.10-0.15) was observed with onion. The UPGMA dendrogram constructed using Jaccard's similarity matrix discriminated all the genotypes into four clusters. Cluster I comprised of 14 genotypes with a similarity index of about 89%. Cluster II comprised of only one line, SKAUG 151 that had a similarity index of 62% with Cluster I. Cluster III comprised of 8 garlic genotypes at a distance of about 52%. Variation amongst the garlic accessions was about 48%, whereas, with onion it showed a dissimilarity of 90%. The clustering pattern of garlic genotypes in the present study discriminated them as per the vernalization requirement for bulb formation.

Key words: Garlic, *Allium sativum*, genetic diversity, RAPD

Introduction

Garlic (*Allium sativum* L.) is one of the most important crops grown throughout the world for culinary purpose and for its medicinal values. Cultivated garlic is known for at least 5000 years ago in India and Egypt and is believed to have originated in Central Asia where garlic grows wild. Despite its sexually sterile nature, garlic displays an amazing variation in morphological diversity [1] and it has been presumed that the vast diversity observed in cultivated garlic may be due to variation generated from sexual reproduction in the wild crop [2]. India is one of the largest producers of garlic and is ranked next to China in area (120 thousand hectare) with a total production of 5 million tonnes [3]. Like other food crops, farmers are adopting cultivation of high yielding varieties neglecting valuable landraces and old varieties of garlic. Therefore, it is important to characterize and conserve genetic variation in the local landraces/varieties for future breeding programme.

Molecular markers have been applied to assess genetic diversity in many crops because they are unlimited in number, not affected by the environment and can be organized into linkage maps. There are a number of reports on characterization of garlic germplasm based on isozyme and RAPD markers [4], AFLP [5], comparison of different molecular markers [6] and some flower related morphological traits [7] also. Among these, RAPD markers have been frequently used for analyzing genetic relationships among garlic germplasm [1]. Inheritance studies of fertility [8], detection of putative duplicates [6] and markers linked to bolting, bulb color and clove adherent type [9], resistance to white rot disease [10], development of genetic maps [11] are also documented in garlic using molecular markers. However, most of the above work has been done in temperate and long day garlic type and is limited in Indian garlic, which are short day and tropical type. Hence, the present study was designed to assess the genetic diversity among 24 accessions including ten released varieties of garlic grown in different agro-ecological regions of India.

Materials and methods

Plant material

Twenty-two Indian garlic accessions (Ten released varieties; twelve clonal selections from indigenous material) obtained through All India Coordinated

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Vegetable Improvement Project (AICVIP); one exotic garlic collection (EC 286083 from NBPGR, New Delhi, location unknown) and one onion cultivar (GWO1 used as an out-group) were used for this study (Fig. 1). These lines were maintained at the National Research Centre for Onion and Garlic, Pune.

Fig. 1. Source of garlic genotypes used in the study

DNA extraction

Ten cloves per accession were planted and DNA was extracted from 1-2 g of immature leaves (bulk of ten plants per accession). DNA extraction was carried out using modified CTAB method [12]. DNA quantification was done on 1 % agarose gel with λ-DNA and subjected to further PCR analysis.

RAPD analysis

PCR amplifications were performed with 100 random decamer primers (OPA01-20, OPC01-20, OPD01-20, OPE01-20, and OPG01-20) obtained from Operon Technologies (Almeida, California, USA). Amplifications were carried out in 25 µl reaction volume containing 1U of Taq DNA polymerase (Biogene, USA), 10X PCR buffer containing 15 mM $MgCl_2$ (Biogene, USA), 1 mM primer, 0.25 mM of dNTP (Biogene, USA) and 20 ng of template genomic DNA. The conditions for PCR amplification (GeneAmp - 2400 thermocycler; Perkin Elmer, USA) were optimized for Taq DNA polymerase and DNA templates. Amplification conditions included a preliminary 5 min. denaturation at 95°C followed by 35 cycles of denaturation at 94°C for 1 min. annealing at 36°C for 1 min. and extension at 72°C for 2 min. and terminated with incubation at 72°C for 10 min. and finally held at 4°C. Final PCR products were resolved in 1.5 % agarose gel (SRL, India) stained with ethidium bromide along with ΦX 174 Hae III digest and Lambda DNA Hind III digest (Sigma-Aldrich, USA) as molecular markers. Gel was visualized by illumination under UV light and documented with GelDoc 2000 (Bio-Rad, CA, USA) using the software Quantity One.

Data analysis

RAPD bands were scored as present (1) or absent (0) and data were analysed using NTSYSpc ver. 2.1 [13]. Similarity matrices, generated according to the coefficient of Jaccard [14] were used to perform cluster analysis using the unweighted pair group method with arithmetic average (UPGMA) [15]. Dendrogram, indicating the estimated similarity among the garlic genotypes, was constructed with the TREE programme of NTSYSpc.

Cophenetic values were calculated to compare the dendrogram to the similarity matrix. This cophenetic matrix was then compared with the original similarity matrix from which the dendrogram was prepared and a correlation coefficient derived to determine the extent of distortion in converting the data into the dendrogram using the Mantel test of significance [16].

Results and discussion

Out of the 100 RAPD primers screened, 14 produced clear polymorphic bands in all the accessions on preliminary analysis and were selected for further analysis. Only clear and unambiguous bands were taken for scoring (Fig. 2). The number of bands for each primer varied from 3 to 19, with an average of 11.5 bands (Table 1) and the size of the amplicons generated from fourteen primers ranged from 280 to 4000 bp.

A total of 162 RAPD bands were generated of which 93 bands (57.4%) were polymorphic, with an average of 6.6 polymorphic bands per primer. The number of polymorphic bands varied from 1 (OPG 04) to 16 (OPA 07) (Table 1). Etoh and Hong [17] also observed a similar percentage of polymorphic bands (64 %). In their study with 60 arbitrary 10-mer primers, 625 discrete fragments were amplified and 397 (64 %) of them showed polymorphism.

Analysis of the RAPD data using Nei's [18] original measures of genetic identity and genetic distance showed that genetic similarity (GS) value ranged from 0.97 to 0.47 with a mean value of 0.72 (Table 2) among garlic accessions whereas a low degree of similarity

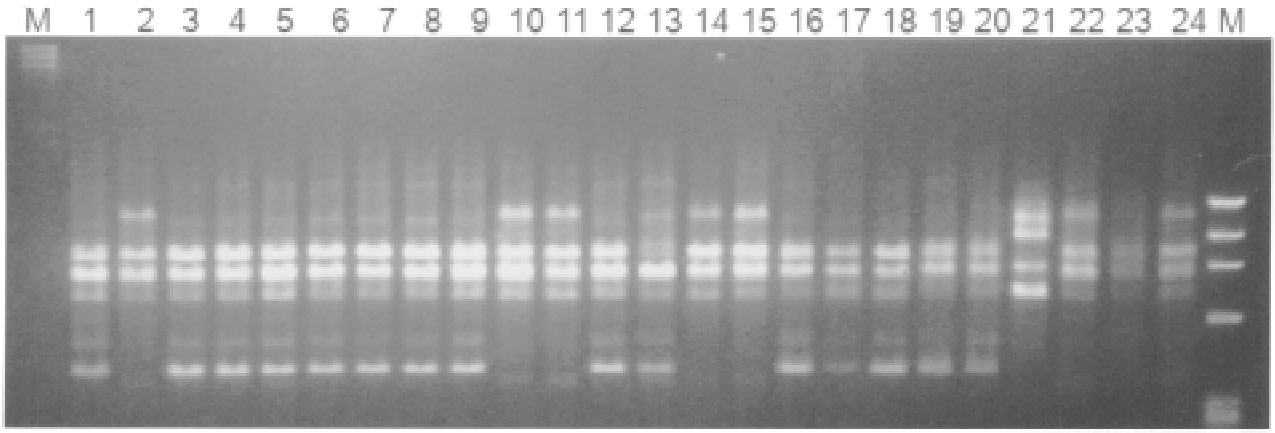

Fig. 2. Amplification products obtained from DNA extracted from 23 garlic and one onion accession using primer OPA-17. Lane M (Left) –Lambda DNA Hind III and M (right) size marker, M, is Phi X 174-Hae III digest

Table 1. Primers producing polymorphic markers

Primer	Sequence (5'-3')	Total no. of bands	No. of poly-morphic	Polymor-phism (%)
OPA 07	GAAACGGGTG	17	16	94.12
OPA 09	GGGTAACGCC	7	2	28.57
OPA 10	GTGATCGCAG	10	5	50.00
OPA 11	CAATCGCCGT	14	9	64.29
OPA 14	TCTGTGCTGG	8	5	62.50
OPA 17	GACCGCTTGT	15	7	46.67
OPA 19	CAAACGTCGG	19	12	63.16
OPC 04	CCGCATCTAC	8	4	50.00
OPD 15	CATCCGTGCT	10	7	70.00
OPD 20	ACCCGGTCAC	10	3	30.00
OPG 04	AGCGTGTCTG	3	1	33.33
OPG 11	TGCCCGTCGT	17	10	58.82
OPG 12	CAGCTCACGA	14	9	64.29
OPG 16	AGCGTCCTCC	10	3	30.00
Total Score		162	93	57.41

(0.12) was found between onion and garlic genotypes. Bradley *et al*. [1] also obtained genetic diversity ranging from 58-97 % among twenty Australian garlic genotypes screened. These results indicate that despite the absence of sexual reproduction, there is a wide variation at the genetic level among different garlic cultivars.

The UPGMA dendrogram constructed using Jaccard's similarity matrix of RAPD data discriminated all the genotypes into four clusters (Fig. 3; Cluster I to IV; GS = 0.5-0.97). However, there was no association of banding patterns with geographical location, which is in accordance with the findings of Ipek and Simon [19] and Khar *et al*. [20]. Ipek *et al*. [6] attributed this non-association with geographical location to the fact that the garlic cultivars could have been passed between countries in the past that their true geographical origins were consequently lost.

Cluster I comprised of 14 garlic genotypes (Fig. 3; GS = 89%). A high degree of genetic similarity (GS = 95%) was observed between G1 and G50, G323 and VLG-7, GG3 and GG2 and JNDG 213 and PG 17. Among these accessions, G1 and G50 (tropical types) are commercially grown in the northern Indian plains of Haryana whereas G313 (temperate type; an introduction from Hong Kong) grown in the hills of Himachal Pradesh are included in the same cluster (Cluster 1). It is interesting to note that the accession G323 and VLG7 are also temperate type grouped in cluster I. It is known that the temperate types are big in size, with more average bulb weight (50-100 g) and fewer cloves (10-12) whereas the tropical types are small in size, less average bulb weight (10-15 g) and have more number of cloves (25-55) per bulb. Hence, the grouping based on molecular analysis neither differentiate these garlic accessions based on size and weight of the cloves nor based on climatic adaptation. The accessions, GG-3 and GG-2 (North western India) and JNDG 219, JGL-96-198, JNDG 213 and PG 17 (North western and Northern India) were also grouped in cluster I suggesting their common origin but adaptation to different environments after prolonged and continued cultivation. This can be explained by the fact that Gujarat is one of the largest producer and supplier of garlic (both domestic and international market) and farmers from other places may have selected the best adapted materials from Gujarat which in turn must have been evaluated and released

Table 2. Similarity indices among different garlic accessions

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
G 1	1.00																							
G 41	0.52	1.00																						
G 50	0.95	0.50	1.00																					
G 282	0.88	0.52	0.85	1.00																				
G 313	0.94	0.54	0.92	0.90	1.00																			
G 323	0.93	0.56	0.90	0.91	0.95	1.00																		
GG 3	0.89	0.57	0.86	0.88	0.92	0.95	1.00																	
GG 2	0.92	0.54	0.89	0.90	0.94	0.95	0.95	1.00																
VLG 7	0.91	0.55	0.88	0.89	0.94	0.95	0.92	0.93	1.00															
Godavari	0.52	0.91	0.50	0.50	0.54	0.55	0.56	0.53	0.57	1.00														
RAUG 5	0.52	0.90	0.50	0.50	0.54	0.54	0.55	0.53	0.57	0.97	1.00													
KGS 2	0.91	0.54	0.88	0.88	0.93	0.91	0.87	0.90	0.92	0.55	0.56	1.00												
SKAUG 151	0.63	0.55	0.61	0.60	0.62	0.65	0.63	0.64	0.65	0.54	0.54	0.63	1.00											
DARL 52	0.50	0.82	0.49	0.52	0.53	0.52	0.53	0.52	0.52	0.87	0.86	0.54	0.52	1.00										
DG 1	0.53	0.86	0.51	0.51	0.53	0.52	0.53	0.53	0.54	0.87	0.88	0.54	0.53	0.89	1.00									
JNDG 70	0.90	0.52	0.89	0.87	0.90	0.90	0.86	0.91	0.90	0.52	0.52	0.91	0.63	0.52	0.54	1.00								
JNDG 219	0.82	0.49	0.83	0.79	0.80	0.80	0.78	0.80	0.80	0.47	0.48	0.81	0.57	0.48	0.50	0.84	1.00							
JGL-96-198	0.86	0.52	0.86	0.82	0.88	0.85	0.82	0.85	0.86	0.50	0.51	0.87	0.61	0.51	0.53	0.89	0.91	1.00						
JNDG 213	0.92	0.53	0.89	0.88	0.90	0.90	0.88	0.90	0.90	0.52	0.52	0.91	0.63	0.51	0.54	0.93	0.89	0.92	1.00					
PG 17	0.89	0.52	0.87	0.85	0.87	0.87	0.85	0.87	0.87	0.50	0.50	0.88	0.61	0.50	0.53	0.90	0.89	0.94	0.95	1.00				
GWO 1	0.11	0.15	0.10	0.12	0.12	0.12	0.13	0.12	0.12	0.14	0.14	0.13	0.10	0.13	0.12	0.11	0.11	0.10	0.11	0.10	1.00			
AC 50	0.52	0.92	0.51	0.50	0.53	0.55	0.56	0.54	0.55	0.93	0.91	0.54	0.55	0.85	0.88	0.52	0.47	0.50	0.51	0.51	0.13	1.00		
AC 200	0.53	0.90	0.52	0.52	0.54	0.56	0.55	0.54	0.56	0.92	0.91	0.55	0.55	0.84	0.87	0.54	0.49	0.52	0.53	0.52	0.14	0.94	1.00	
EC 286083	0.53	0.91	0.51	0.52	0.55	0.55	0.56	0.54	0.57	0.92	0.92	0.56	0.55	0.86	0.91	0.54	0.48	0.51	0.53	0.53	0.14	0.93	0.94	1.00

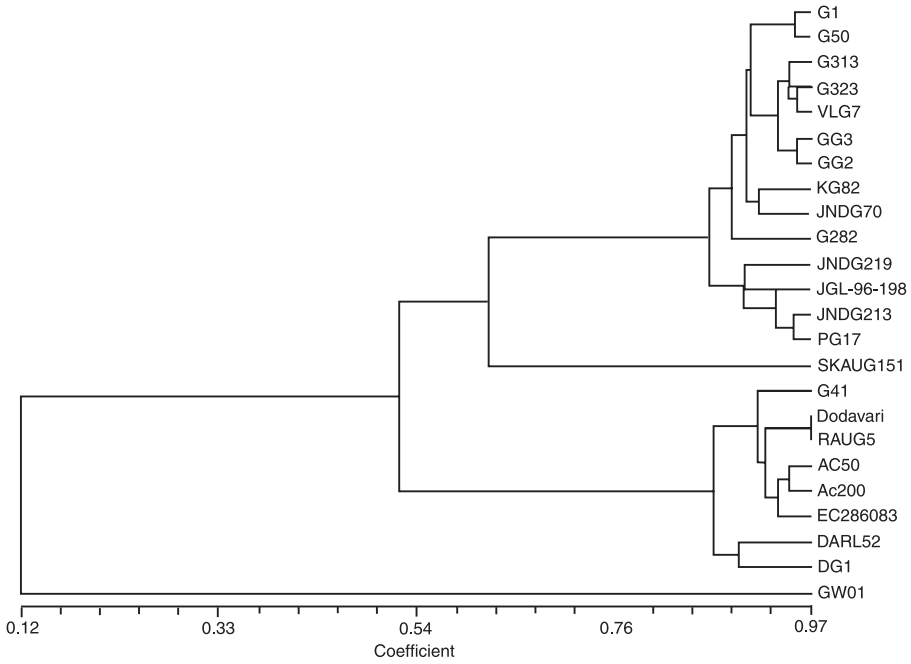

Fig. 3. Dendrogram showing the genetic relationships among garlic genotypes with GWO-1 (onion) as outgroup

as commercial release for that region also. Earlier reports on Australian garlic describe that environment conditions are known to cause large phenotypic variation in garlic and this can lead to the same cultivar being named differently in various regions [21]. In our case too, environmental conditions could have resulted in phenotypic variation within genetically related cultivars and hence the tropical and temperate accessions are grouped together in the same cluster.

The second cluster (Cluster II) consisted of only one genotype, SKAUG-151, obtained from Kashmir (North Indian hills), which is characterized by having wide leaves, big bulbs and 10-12 cloves. When this accession was tested at different locations under the multi-location trials through AICRP, it was not able to produce bulb anywhere except Kashmir and higher hills of Himachal Pradesh (personal communication). Cluster I was related to Cluster II at about 62% (Fig. 3).

Cluster III was the second biggest and comprised of 8 garlic accessions, at a similarity of about 52%; 'Godavari' and 'RAUG 5' showed the highest similarity value (GS = 97%). Other accessions namely, G41, AC50, AC200, EC286083 and DG1 clustered in this group, were good performers under the short day tropical conditions with the exception of 'DARL 52', which is released for the hilly region. Therefore, our study suggested that DARL 52 may have originated from tropical plains of India and during domestication it may have adapted the long day condition without changing the genetic makeup. The exotic line (EC286083) of unknown origin might be a collection from tropical region and was clustered with the rest of the tropical genotypes.

Cluster IV consisted of only one accession, which is classified under onion outgroup. The present study revealed that RAPDs are reliable genetic markers, which clearly discriminated between the garlic lines and the out-group. Variation within the garlic accessions was 48%, whereas, with onion it showed a dissimilarity of 90%. To check the fit of the dendrogram (Fig. 3) with the original similarity matrix, a cophenetic correlation coefficient was computed as described in materials and methods. The correlation coefficient was 0.99, which is described as a very good fit [13].

The explanation for the clustering of garlic lines from various locations can be better explained based on their light and temperature requirements for growth rather than geographical locations. For example, some varieties released for north India *i.e.*, G-41, DARL-52, RAUG 5 and DG-1 were clustered in group III, which also had tropical types namely, G41, AC50, AC200, EC286083 and DG1. It was observed that almost all the varieties falling in group 1 and group II did not grow well in Central Indian peninsula conditions and some lines (G 313, VLG 7 and SKAUG-151) only produced leaves and no bulbs because there is not much temperature variation in this region during garlic growing period (Sept.-March). However, when the same garlic

accessions were grown in the Northern plains, where there is variation in temperature and also the temperature falls below 7°C (Dec.-Feb.) coupled with intense fog and cold, garlic accessions are able to grow well and form bulbs except SKAUG 151. Therefore, we conclude that our classification was more consistent with the vernalization requirement for bulb development. Mota *et al*. [22] also reported clustering of garlic genotypes based on the vernalization requirement for bulb formation in Brazilian garlic.

In future studies, it is important to characterize Indian garlic germplasm using other markers *viz*., AFLP and SSR. Further research on estimating the relationships with the long day types from other countries will give an overview about the domestication and genetic relationships of garlic worldwide.

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