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Genetic diversity analysis among garlic (*Allium sativum* L.) genotypes through PCR based molecular markers

D.A.Muthamizh[1]
[1] PG Scholar, Department of Biotechnology

Abstract

Garlic is one of the most important spices and condiments for home consumption. It is chiefly used for flavouring and seasoning vegetable dishes. It is commonly termed as "Lasan" and botanically known as Allium sativum L. Sixteen RAPD primers were used to characterize polymorphismand generated a total of 87 bands/alleles out of which 82 bands were polymorphic with an average of 5.43 bands per primer and documented the 92.85 % polymorphism. Calculated values for PIC ranged from 0.473 (OPD-05) to 0.858 (OPG-11) with an average of 0.731 and the average RAPD primer index value was 4.24 per primer. The genotype identification through molecular markers resulted in developing highly diversified map of 16 garlic genotypes. The data revealed that molecular techniques are more accurate than biochemical markers, and can be used for characterization of garlic genotypes, seed purity and for diversity analysis.

Keywords: ***Rapd,PIC***

I. INTRODUCTION

Garlic (*Allium sativum* L.) belongs to the genus *Allium*, family *Alliaceae*, and consists of approximately 700 species. Several of them are important vegetables, spices and medicinal plants, including important vegetable crops such as onion (*Allium cepa*), leek (*Allium ampeloprasum*), shallots (*Allium ascalonicum*) and garlic (*Allium sativum* L.) (Keller, 2002).

Garlic (*Allium sativum* L.) belongs to the family *alliaceae*. The chromosome number is 2n = 16. It is probably native of central Asia and southern Europe, especially Mediterranean region. It is one of the important underground bulb crops of tropical and subtropical countries (Thompson and Kelly, 1957).

Garlic is one of the most important spices and condiments for home consumption. It is chiefly used for flavouring and seasoning vegetable dishes. It is commonly termed as "Lasan" and botanically known as *Allium sativum* L. This crop is one of the important foreign exchange earner crops of India because of good quality and quantity of garlic is exported every year.

The value of garlic as a crop has been recognized from very ancient times; it is estimated that it has been cultivated for over 5000 years. It is rich in proteins (1.5-2.1%), minerals (0.7%) (like phosphorus, calcium, magnesium) and carbohydrates (26-30%), lipid (0.1-0.2%), fiber (1-5%), nitrogen (0.6-1.3%), total oil soluble compounds (0.15%). It also contains fat, vitamin C (0.015%) and sulphur (0.23-0.37%). According to the Unani and Ayurvedic systems as practiced in India, garlic has important medicinal values against digestive disorders, eye sore and earache and also for managing high cholesterol levels. (Billore *et al.,* 2004).

II. Materials and Method

Plant materials and DNA extraction

The cloves (seeds) of garlic genotypes used for the present study were obtained from Vegetable Research Station, Raipur. The experimental material comprised of 11 genotypes of garlic which are listed in below Table.

No.	Name of genotypes	No.	Name of genotypes
1.	IC-3287	**7.**	M-287
2.	IC-372900	**8.**	M-337
3.	IC-372904	**9.**	RG-82
4.	IC-372939	**10.**	WG-23
5.	IC-49066	**11.**	WG-71
6.	M-220		

RAPD analysis

Various molecular marker techniques such as Randomoly Amplified Polymorphic DNA (RAPD) were used. Primers required for the above techniques were synthesized from Bangalore Genei Pvt Ltd., Bangalore. Chemicals for molecular studies were received from Bangalore Genei unless otherwise stated. All the primers for RAPD were diluted by adding equal amount of deionized sterile distilled water equal to its concentration. e.g. If the concentration of RAPD primer OPE-03 is 81.0 nmoles then adding 81.0 µl of deionized water made a concentration of 1 nmole.μl^{-1}=1000 pmoles.μl^{-1}. This is kept as a stock solution of primer. By taking 5 µl of stock (1000 pmoles.μl^{-1}) and 195 µl of deionized sterile distilled water gave a final concentration of 25 pmoles.μl^{-1}. This working solution is used for PCR amplification for various molecular techniques.

RAPD-PCR Components

The reagents used for RAPD-PCR amplification of DNA were as follows.

(a) PCR buffer (10X) – Bangalore Genei
(b) Taq DNA polymerase – Bangalore Genei
(c) dNTPs (dATP, dCTP, dGTP and dTTP) - Bangalore Genei
(d) Primer – Bangalore Genei

List of RAPD primers

The genomic DNA was amplified using random primers of OPA, OPD, OPE and OPG series.

Sr. No.	Primer Series	Sequence 5'- 3'	GC content (%)	Tm (^{o}C)
1.	OPA-01	CAGGCCCTTC	70	38.2
2.	OPA-02	TGCCGAGCTG	70	42.4
3.	OPA-07	GAAACGGGTG	60	34.5
4.	OPA-09	GGGTAACGCC	70	38.7
5.	OPA-11	CAATCGCCGT	60	40.8
6	OPA-15	TTCCGAACCC	60	38.8
7.	OPA-19	CAAACGTCGG	60	36.6
8.	OPD-01	ACCGCGAAGG	70	44.1
9.	OPD-03	GTCGCCGTCA	70	41.4
10	OPD-18	AGGTGACCGT	60	31.6
11.	OPD-20	ACCCGGTCAC	70	38.2
12	OPE-06	AAGACCCCTC	60	30.1
13.	OPE-11	GAGTCTCAGG	60	29.2
14.	OPE-14	TGCGGCTGAG	60	25.0
15.	OPE-18	GGACTGCAGA	70	42.4
16.	OPG-05	CTGAGACGGA	70	33.6
17.	OPG-11	TGCCCGTCGT	60	28.6
18.	OPG-12	CAGCTCACGA	70	45.6
19	OPJ-17	ACGCCAGTTC	60	33.7

Dendrogram Analysis

Clear and distinct bands amplified by RAPD primers were scored for the presence (1) and absence (0) for the corresponding band among the genotypes. The data were entered in to MS-Excel data sheet and subsequently analyzed using NTSYSpc version 2.02 (Rohlf, 1994).

The data matrix was read by NTSYS-pc version 2.2 (Numerical Taxonomy and Multivariate Analysis System for Personal Computers, Exeter Software) developed by F.J. Rohlf, and analyzed by the SIMQUAL (similarity for qualitative data) program with Jaccard's similarity coefficient. SIMQUAL

is a program for computing a variety of similarity and dissimilarity coefficients for qualitative data. The qualitative nature of the absence (0) or presence (1) state of a RAPD marker was used as the basis for similarity analysis among various garlic genotypes. A matrix of 0 and 1 act as the input, and the output is a matrix of similarity or dissimilarity coefficients.

III. RESULTS AND DISCUSSIONS

The study was encompassed of 11 garlic (*Allium sativum* L.) genotypes. The experiment was intended for genetic diversity analysis among garlic (*Allium sativum* L.*)* genotypes through molecular markers. The results are presented bellow.

Initially, screening of 19 random primers was carried out using genomic DNA of one genotype. These 19 primers were OPA-01, OPA-02, OPA-07, OPA-09, OPA-11, OPA-15, OPA-19, OPD-01, OPD-03, OPD-18, OPE-06, OPG-11, OPJ-17, OPG-01, OPG-05, OPE-14, OPE-18, and OPG-12. As a result, seven primers gave satisfactory results which were used for further amplification of genomic DNA of all garlic genotypes.

Clustering pattern using RAPD data

The RAPD data were subjected to statistical analysis for the calculation of Jaccard's similarity coefficient and cluster analysis by UPGMA (unweighted pair-group method with arithmetic averages) using NTSYSpc-2.02i software.

The dendrogram constructed using UPGMA based on Jaccard's similarity coefficient for RAPD data of 11 garlic genotypes is depicted in Fig. 4.2. Eleven garlic genotypes were grouped into two main clusters viz. cluster-A and B. The cluster-B consisted of only one genotype viz. IC-3287. The cluster-A consisted of ten genotypes and was divided into two main subcluster-A1 and A2. The subcluster-A1 consisted of 8 genotypes and segregate into two groups- A1(a) and A1(b). The group-A1(a) consisted of 7 genotypes and which are IC-372900, IC-372904, IC-372939, IC-49066, M-220, M-287 and M-337, group-A1(b) includes only RG-82. Jaccard's similarity coefficient ranging from 22% to 88%. Cluster A includes 10 genotypes and was further divided into two sub-clusters A1 and A2 while, B includes only one genotype which is IC-3287. The sub-cluster A1 further divided into A1(a) and A1(b). Sub-sub cluster A1(a) included 7 genotypes which are IC-372900, IC-372904, IC-372939, IC-49066, M-220, M-287 and M-337, while A1(b) included 1 genotype M-287.The dendrogram constructed using the RAPD data clearly distinguished all genotypes. Genotypes WG-71 and WG-23 were found in one cluster with maximum 88% similarity. RAPD data revealed that two genotypes M-287 and IC-3287 showed minimum similarity (22%).

Mario *et al.,* (2008) found little genetic diversity among the clones analyzed in spite of a high number of bands that detected polymorphism. The coefficient of similarity of the analyzed genotypes generated a dendrogram that grouped into two main clusters. The first group included a total of 44 clones. This group was homogeneous with 100% genetic similarity and second group clustered 21 clones 16.9% with relatively high genetic diversity as compared with first group.

	IC-3287	IC-372900	IC-372904	IC-372939	IC-49066	M-220	M-287	M-337	RG-82	WG-23	WG-71
IC-3287	1.0000										
IC-372900	0.4516	1.0000									
IC-372904	0.4333	0.6970	1.0000								
IC-372939	0.3846	0.4848	0.5161	1.0000							
IC-49066	0.3846	0.4412	0.5161	0.8182	1.0000						
M-220	0.4063	0.8125	0.6970	0.4848	0.4412	1.0000					
M-287	0.2258	0.5000	0.5313	0.3125	0.3548	0.5000	1.0000				
M-337	0.3704	0.6129	0.6552	0.4138	0.4138	0.6667	0.5926	1.0000			
RG-82	0.3077	0.5161	0.5517	0.4074	0.4074	0.5161	0.5385	0.7727	1.0000		
WG-23	0.3438	0.6471	0.6364	0.4688	0.5667	0.6000	0.5806	0.6000	0.5517	1.0000	
WG-71	0.3333	0.6563	0.5455	0.5172	0.5172	0.6061	0.5333	0.6071	0.6154	0.8889	1.0000

Table1: Jaccard's similarity coefficient of 11 garlic genotypes based on RAPD data.

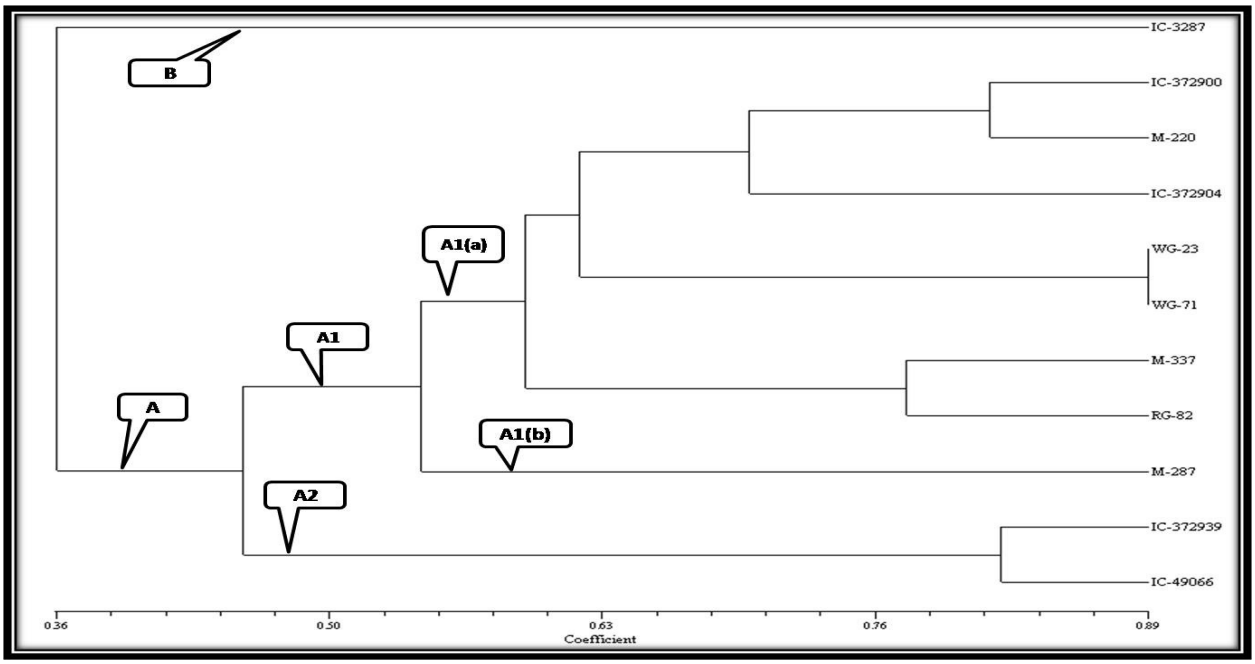

Fig.1:Dendrogram depicting the genetic relationship among 11 garlic genotypes based on the RAPD data.

IV. CONCLUSIONS

Seven RAPD primers generated total of 46 alleles in which 41 bands were polymorphic with an average 6.57 bands per primer and 89.13% polymorphism. Out of 46 bands, 41 bands were polymorphic and 5 bands were monomorphic. RAPD primers amplified the largest fragment of 1298bp and the smallest fragment of 193bp. The PIC values for RAPD markers ranged from 0.683 (OPJ-17) and 0.868 (OPD-18) with an average of 0.768 per primer.The phylogenetic tree constructed by UPGMA method generated two main clusters that consisted all the garlic genotypes grouped in their respective sub-cluster. The Jaccard's similarity index was varied from 22% to 88%.

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