J. Agrofor. Environ. 6 (1): 63-66, 2012 ISSN 1995-6983

Molecular characterization of garlic germplasms using RAPD

M.S. Alam, M.A. Rahim[1] and P.W. Simon[2]
Germplasm Centre, [1]Department of Horticulture, Bangladesh Agricultural University, Mymensingh, [2]Department of Horticulture–Agricultural Research Service, Vegetable Crops Research Unit, 1575, Linden Drive, Madison, WI, 53706
E-mail: mithuhort@yahoo.com; marahim1956@yahoo.com

Abstract: Genetic variation and relationship among 25 garlic (*Allium sativum* L.) germplasm were analyzed using Random Amplified Polymorphic DNA (RAPD). Out of 25 primers screened, two were selected, which gave 10 clear bands, out of which 8 bands were considered polymorphic. The proportions of polymorphic loci were 79.16%. The UPGMA dendrogram based on genetic distance segregated the 25 garlic germplasm into three main clusters. Cluster II contained 17 germplasm, cluster II contained 5 germplasm and cluster III contained only 3 germplasm. The highest genetic distance was 1.60. The results of the present study indicated that the RAPD analysis could be utilized by breeders for further improvement of garlic varieties.
Key words: Germplasm, Characterization, Garlic and RAPD.

Introduction

Garlic (*Allium sativum* L.) is an important member of the family alliaceae and its primary centre of origin is Central Asia (Kazakhastan), and the secondary centre is the Mediterranean and Caucasus zones (Etoh and Simon, 2002). Garlic has an asexually propagated crop, and it displays great morphological diversity in bulb and leaf size, colour and shape, scape presence and height, flower colour, fertility, and bulbil (topset) development in inflorescence (Pooler and Simon, 1993). The characterization of garlic germplasm has been based on morphological data. However, morphological characters may differ under varying environmental conditions. This situation adds complexity to the characterization of garlic germplasm. To eliminate some of these limitations, biochemical and molecular markers have been used in genetic diversity studies of this germplasm (Al-Zahim *et al*., 1997; Bradley *et al*., 1996; Lallemand *et al*., 1997; Maass and Klaas, 1995; Pooler and Simon, 1993). Many of these complications of a phenotype-based assay can be overcome through direct identification of genotype with DNA based genetic markers. Polymerase Chain Reaction (PCR) technology had led to the development of several novel genetic assays based on selective DNA amplification. The protocol is also relatively quick and easy to perform. Because the RAPD technique is amplification –based assay, only nanogram quantities of DNA are required. One of the strength of these new assays is that they are more amenable to automation than convention al techniques .It is simple to perform and is preferable to experiments where the genotypes of a large number of individuals are to be determined at a few genetic loci. With this idea the experiment was undertaken to evaluate genetic variation and relationships of some garlic germplasm by RAPD (Random Amplified polymorphic DNA) technique.

Materials and Methods

Twenty five garlic germplasm were used in this study (Table 1). In order to carry out RAPD analysis, young leaves from each of the 25 germplasm were collected randomly from different parts of Bangladesh and out side the country. Total genomic DNA was isolated from garlic leaves following, phenol: chloroform: isoamyl alcohol purification and ethanol precipitation method. Finally, the DNA samples were stored at -20^{0}C, DNA concentrations were determined at 260 nm spectrophotometrically and the quality verified by electrophoresis on a 1% agarose gel.

DNA amplification was done using two arbitrary decamer primers (Operon Technologies, Inc., Alameda, California, USA OPC-12 and OPC-13) adopting the procedure of Williams *et al*., (1990) with some modification. PCR reactions were performed on each DNA sample in a 10µl reaction mix containing 1µl of 10x Ampli Taq polymerase buffer, 0.25 µl of 10µl mM primer, 1µl of 250µl mM dNTPs, 1 unit of Ampli Taq DNA polymerase (Bangalore Genei, India) and 4µl of 100ng genomic DNA (25ng/µl) and rest amount of sterile deionized water. DNA amplification was performed in an oil free thermal cycler (Master Cycler Gradient, Eppendorf). The reaction mix was preheated at 95^{0}C for 1 min. 48^{0}C for 1 min, 72^{0}C for 2 min, continuing with 40cycles at 94^{0}C for 30s, 48^{0}C for 40s, 72^{0}C for 2 min, and an extension period of 72^{0}C for 10 min. After completion of cycling programme, reactions were held at 4^{0}C. The amplified products were separated electrophoretically on a 1.5% agarose gel. One molecular weight marker, 100 bp DNA ladder were electrophoresed alongside the RAPD reactions. Electrophoresis was carried out at 120 V for 1 hour and 20 min. DNA bands were observed under UV light on a Transilluminator and photographed.

Since RAPD markers are dominant, we assumed that each band represented the phenotype at a single allelic locus (Williams *et al*. 1990). All distinct bands or fragments (RAPD markers) were thereby given identification numbers according to their position on gel and scored visually on the basis of their presence (1) or absence (0), separately for each individual and each primer. The scores obtained using all the primers in the RAPD analysis were then pooled to create a single data matrix and used to estimate polymorphic loci, gene diversity, genetic distance (D) and to construct a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among populations using a computer program, POPGENE (Version 1.31) (Yeh *et al*. 1999).

Genetic similarity values defined as the fraction of shared bands between the RAPD profiles of any two individuals on the same gel were calculated manually from RAPD markers of the same molecular weight on the data matrix according to the formula: Similarity index (SI) = $2N_{xy}$ / $N_x + N_y$. Where, N_{xy} is the number of RAPD bands shared by individuals × and y respectively, and N_x and N_y are the number of bands in individual × and y, respectively (Chapco *et al*. 1992, Wilde *et al*. 1992, Lynch 1990).

Table 1. Particulars of twenty five germplasm of garlic used for RAPD analysis

Sl.No	Name of Germplasm	Origin	Collection site
01	G1	Bangladesh	BAU, Mymensingh
02	G2	Bangladesh	BAU, Mymensingh
03	G3	Bangladesh	Basherhat, Dinajpur
04	G4	Bangladesh	Lal Shi, Domar
05	G5	Bangladesh	Mahakunda, Ishurdi
06	G6	Bangladesh	Sada shil, Nilphamari
07	G7	Bangladesh	Lal Shi, Dinajpur
08	G10	Bangladesh	Rajshahi
09	G15	Bangladesh	Foridpur
10	G19	Bangladesh	Sada shil, Devigonj
11	G27	USA	Florida -1
12	G28	USA	Florida -2
13	G29	Turky	Turky-1
14	G30	Turky	Turky-2
15	G31	Tunisia	Tunisia-1
16	G32	Tunisia	Tunisia-2
17	G33	Tunisia	Tunisia-3
18	G35	China	China-1
19	G36	China	China-2
20	G37	China	China-3
21	G38	China	China-4
22	G50	Vietnam	Vietnam-2009
23	G51	Bangladesh	Putia-2010
24	G53	China	China-2010
25	G54	Vietnam	Vietnam-2010

Results and Discussion

Vegetative propagation of garlic has been used for many centuries; therefore the presence of genetically closely related germplasm and the presence of duplications in the germplasm banks are two facts that can be taken into account. A molecular marker such as RAPD, which does not require prior information from DNA sequences of the species, could be very useful to evaluate the genetic diversity of the garlic germplasm.

In this study, the analysis of the two primers generated 10 bands. Out of 10 bands, 8 scorable bands (79.16 %) were found to be polymorphic 2 bands (21.84%) were found to be monomorphic in Table 2. The primer OPC-13 contained 4 bands (Fig. 1) and the primer OPC-12 obtained 6 bands. Due to the large number of bands which detected polymorphism in this analysis, it can be said that the RAPD gave a good approximation of the genetic relationship existing among the evaluated germplasm. It is assumed that the amplified DNA fragments (amplicons) that co-migrate in the different accessions are similar in their sequences (Wilkie *et al.*, 1993). Isolated and analyzed several common RAPDs garlic bands and analyzed their homology by means of a DNA hybridization process under two types of astringency, low and medium-high. The results obtained indicated that all the common RAPDs bands isolated of similar intensity showed high homology. However, in the gel some of these common bands showed signals of hybridization of different strength, which would indicate the existence of some differences in sequences homology.

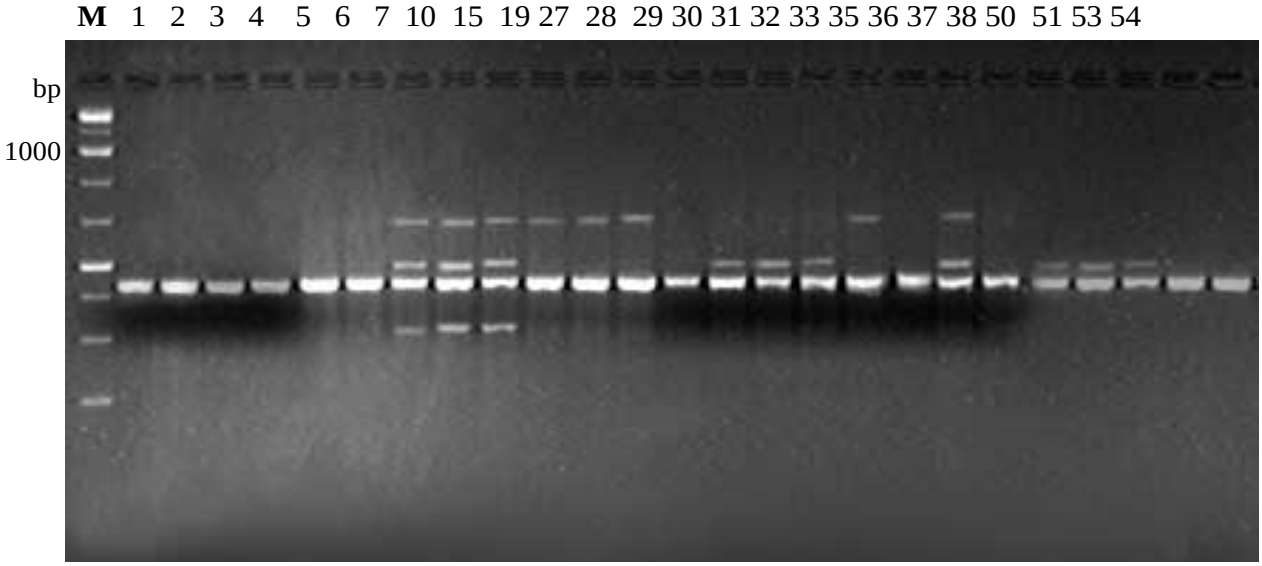

Fig. 1. RAPD profiles of 25 garlic germplasm using primer OPC-13. M;Molecular weight marker (100 bp DNA ladder left side)

Table 2. RAPD primers with corresponding bands scored and polymorphic bands observed in 25 garlic germplasm

Primer code	Sequences (5´-3´)	Total number of bands scored	Number of polymorphic bands	Proportion of polymorphic loci (%)
OPC-12	TGTCATCCCC	6	5	83.33
OPC-13	AAGCCTCGTC	4	3	75.00
Total		10	8	158.33
Average		5	4	79.16

Table 3. Estimation of genetic diversity using RAPD markers in 25 garlic germplasm

Loci	Nei's (1973) gene diversity (h)	Shanon information index (i)
OPC12-1	0.2688	0.4397
OPC12-2	0.4992	0.6923
OPC12-3	0.0768	0.1679
OPC12-4	0.2688	0.4397
OPC12-5	0.4352	0.6269
OPC12-6	0.4992	0.6923
OPC13-1	0.2112	0.3669
OPC13-2	0.4352	0.6269
OPC13-3	0.4928	0.6859
OPC13-4	0.2688	0.4397
Mean	0.3456	0.5178
Standard deviation	0.1464	0.1753

The highest genetic distance (1.60) was estimated in between the germplasm G1 and G37, G53, G54 whereas the lowest (0.00) was observed several pairs of germplasm. The Nei's (1973) mean gene diversity between all the germplasm were 0.345, and Shannon's information index was 0.517 (Table 3).

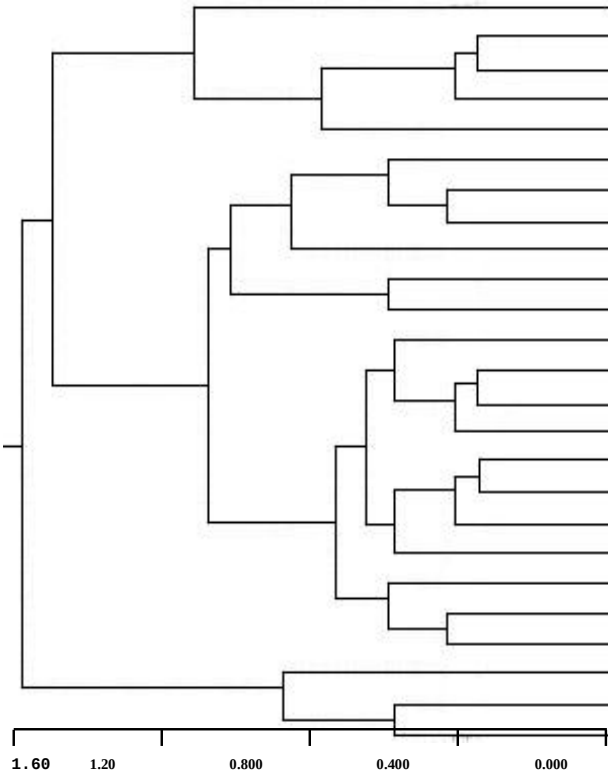

Fig. 2. Dendrogram showing genetic relationship among 25 garlic germplasm based on Nei's (1972) genetic distance, summarizing data on differentiation in 25 according to RAPD analysis.

The dendrogram based on Nei's (1972) genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) revealed segregation of the 25 garlic germplasm into three clusters (Fig.2). The cluster II constructed the highest number of germplasm (17) collected from different geographical locations. The source of collection of the germplasm has been shown in Table 4. The cluster I contained 5 germplasm and cluster III obtained 3 germplasm. Divergent genotypes may have good breeding value; genotypes in the same cluster may represent members of one heterotic group. The maximum variability for selection from segregation populations may be achieved by utilizing genotypes from different clusters as parents of crosses (Gwanama *et al*., 2000).

Table 4. Distribution of 25 garlic germplasm under different cluster based on RAPD polymorphism

Cluster	No. of germplasm in each cluster	Garlic germplasm	Place of collection
I	5	G1,G2,G3,G4, G19	BAU, Mymensigh
II	17	5,11,12,10,17,18,6,20, 24,25,14,15,16, 23,13, 21,22	Magura,Sirazgonj,Natore and Dinajpur
III	3	7,8,9	Taiwan,USA

There is a genetic diversity as detected by the RAPD, this molecular marker could be used to identify. This information could be quite useful to complement the morphologic and agronomic information for the registration of some of these germplasm, maintain the purity of the variety and protect the breeder's rights of some of the selections. However, to improve the identification process of these germplasm, it is necessary to incorporate another type of molecular markers which

would allow the greater genetic differences among germplasm.

Acknowledgement: Wish to acknowledge the authorities of the project "Collection, conservation, characterizations and varietals development of Garlic and onion through selection and molecular techniques" Funded by USDA for providing necessary facilities to conduct the present study as well as very much grateful to Philipp W. Simon, US Department of Agriculture –Agricultural Research Service, Vegetable Crops Research Unit, 1575, Linden Drive, Madison, WI, 53706 to give the opportunity for conducting research in his laboratory

References

Al-Zahim, M., H.J. Newbury, and B.V. Ford-Lloyd. 1997. Classification of genetic variation in garlic (*Allium sativu*, L.) revealed by RAPD. Hort. Sci.32:1102-1104.

Bradley, K.F., M.A. Rieger and G.G. Collins. 1996. Classification of Australian garlic cultivars by DNA fingerprinting. Austral. J. Expt. Agr. 36: 613-618.

Chapco, W., N.W. Ashton, R.K. Martel, N. Antonishishyn and W.L. Crosby .1992. A feasibility study on the use of random amplified polymorphic DNA in the population genetics and systematic of grasshoppers, Genome. 35: 569-574.

Etoh, T. and P.W. Simon. 2002. Diversity, fertility and seed production of garlic, pp.101-117. In: H.D. Rabinowitch and L. Currah (eds.) Allium crop science: Recent advances. CABI Intl., Oxon, U.K.

Gwanama, C. M. T. Labuschagne and A.M. Botha. 2000. Analysis of genetic variation in *Cucurbita moschata* by Random Amplified Polymorphic DNA (RAPD) markers. Euphytica, 113: 19-24.

Lallemand, J., C.M. Messian, F. Briad and T. Etoh. 1997. Delimitation of varietals groups in garlic (*Allium sativum* L.) by morp[hological, physiological and biochemical characters, pp. 123-132. In: J.L. Burba and C.R. Galmarini (eds.) Proc. 1 st Intl. Symp. Edible *Alliaceae.* Acta Hort.

Lynch, M. 1990. The similarity index and DNA fingerprinting. Mol. Biol. Evol. 7 (5): 478-484.

Lynch, M.1990. The similarity index and DNA fingerprinting. Mol. Biol. Evol. 7(5)

Mass, H.I. and M. Klass. 1995. Infraspecific differentiation of garlic (*Allium sativum* L.) by isozyme and RAPD markers. Theor. Appl. Genet. 91:89-97.

Nei, M. 1972. Genetic distance between population. Am. Nat., 196: 283-292.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Nat. Acad. Sci. USA, 70: 3321-3323.

Pooler, M.R. and P.W. Simon. 1993. Characterization and classification of isozyme and morphological variation in a diverse collection of garlic clones. Euphytica 98:121-130.

Popenoe, W. 1927. Manual of Tropical and Sub-tropical Fruits. Mc Millan, New York, USA.

Wilde, J., R. Waugh and W. Powell.1992. Genetic fingerprinting of Theobroma clones using randomly amplified polymorphic DNA markers. Theor. Appl. Genet. 83: 871-877.

Williams, J.G.K., A.R. Kubelic, K. J. Livak, J.A. Rafalski and S.V. Tingey .1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18(22): 6531-6535.

Yeh, F., C. Yang, T.B.J. Boyle, Z.H. Ye and J.X. Mao.1999. POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Albetra, Canada. (http:/ / ww. ualbetra.ca/~fyeh).