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Molecular Diversity Analysis of Lentil (*Lens culinaris* Medik.) through RAPD Markers

M.E. Hoque and M.M. Hasan[1]

Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh

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Abstract

Random Amplified Polymorphic DNA (RAPD) markers were used to study the molecular genetic diversity analysis among six BARI released lentil varieties *viz*. BARI masur-1, BARI masur-2, BARI masur-3, BARI masur-4, BARI masur-5 and BARI masur-6. PCR amplified products were visualized on 1.0% agarose gel and the band for each primer were scored. Ten RAPD markers were used in this study. Out of them 7 primers showed amplification of 53 DNA fragments with 60.37% of them being polymorphic. The highest number of polymorphic loci was noticed in the variety BARI masur-3. The same variety also showed maximum Nei's gene diversity value (0.0552). The highest Nei's genetic distance (0.5002) was observed in BARI masur-1 vs. BARI masur-5 whereas, the lowest genetic distance (0.0692) was found in BARI masur-1 vs. BARI masur-2. The unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei's genetic distance grouped the six cultivars into two main clusters. BARI masur-1, BARI masur-2 and BARI masur-3 were in cluster I and BARI masur-4, BARI masur-5 and BARI masur-6 were in cluster II. The cultivar BARI masur-4 was closest to the cultivar BARI masur-6 with the lowest genetic distance (0.0972) and the highest genetic distance (0.5002) was found between BARI masur-1 and BARI masur-5. The RAPD markers were found to be useful in molecular characterization of lentil varieties which could be utilized by the breeders for the improvement of lentil cultivars.

Introduction

Lentil (*Lens culinaris* Medik.) is a diploid and autogamous species which is the oldest pulse crop in the world. It is one of the important pulses in Bangladesh. Lentil is the best source of plant protein with an excellent source of vitamin A and iron. It has been reported that, the average yield of lentil is about 985 Kg/ha

[1]Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh.

which is very low compared to world statistics (BBS 2010). Many biotic and abiotic stresses are major constraints for lentil improvement. Plant breeders and geneticists have addressed these problems by identifying resistant/tolerant germplasm and determining the genetic map positions of resistant genes to develop tolerant varieties of lentil (Muehlbauer et al. 2006).

Marker Assisted Selection (MAS) breeding approaches are now used to enhance traditional breeding program to improve crop varieties. Among the different molecular markers, RAPD is user friendly oligomer which has the potentiality to amplify reproducible DNA fragments among different genotypes.

RAPD technique developed by (Williams et al. 1990) is reliable, faster and easier for exploiting genetic polymorphism within and among species or populations. RAPD markers have been widely used for the identification of genetic relationship among cultivars (Afzal et al. 2004, Tosti and Negri 2002), wild forms (Cattan-Tou-Pance et al. 1998) and between cultivars (Ba et al. 2004, Xu et al. 2000). The yield of lentil has remained very low across tropical and subtropical Asia; hence, it is important to estimate the actual molecular genetic diversity of the existing cultivars to identify whether the lack of genetic variability might be the major constraining factor. The aim of this work is to provide information on genetic variation and relatedness of lentil varieties of Bangladesh by PCR based RAPD technique. The research program will significantly help in varietal selection for breeding purposes as well as for molecular characterization of different released cultivars available in Bangladesh. Therefore, the present investigation was carried out with the objectives of studying polymorphism among the different released lentil varieties and diversity analysis of lentils at the DNA level.

Materials and Methods

The experiments were carried out at the Pulse Research Center, Bangladesh Agricultural Research Institute (BARI), Gazipur. Fresh leaf samples from 10-15 days old seedlings were used for DNA extraction. Modified SDS mini-prep method (Murray and Thompson 1980, Frey et al. 2004) was followed to extract DNA from lentil plants. Quantification of the DNA was done through electrophoresis on a 1% agarose gel. Ten RAPD primers were tested for their ability to amplify scorable and reproducible DNA fragments. Primers resulting in faint or irreproducible bands were excluded from subsequent analysis. Finally seven primers were selected for this study.

PCR reactions were performed in a 10 µl reaction mix containing 3.3 µl sterile deionized water, 1 µl Taq polymerase buffer, 1 µl dNTPs (1 mM) mixture, 0.2 µl Taq DNA polymerase, 2 µl (approx. 50 ng) genomic DNA and 2.5 µl (10

mM) primer. Samples were subjected to the following thermal profile for amplification in a thermocycler: 5 min at 95ºC for initial denaturation (pre-heat) followed by 39 cycles of 1 min denaturation at 94ºC, 0.45 min at 34ºC (annealing) and 2 min at 72ºC (extension) and a final extension step at 72ºC for 7 min. Electrophoresis was carried out in 1 X TBE buffer on a 1% agarose gel and amplified fragments were visualized by staining with ethidium bromide. The amplified bands were visually scored as present (1) and absent (0) separately for each individual primer. The scores of bands were pooled to create a single data matrix. This was used to estimate polymorphic loci. Nei's (1972) genetic distance and identity and a dendrogram based on an unpaired group method of arithmetic means (UPGMA), was constructed using POPGENE (version 1.31) (Yeh et al. 1999).

Results and Discussion

Ten primers were initially screened on six lentil varieties for their ability to amplify polymorphic DNA. Out of them seven primers *viz*. OPA01, OPA18, OPC08, OPD02, OPD03, OPD18 and OPW01 showed reproducible and distinct polymorphic amplified products. A total of 53 bands were scored of which 32 (60.37%) were polymorphic. The size of the amplification products ranged from 120 - 1000 bp (Table 1). The seven selected primers produced comparatively the maximum number of high intensity bands with minimal smearing, good technical resolution and sufficient variation among different cultivars. The highest number of bands was generated by primer OPD 03 whereas; the least number of bands were produced by primer OPD02. In addition to that, the primer OPA18, OPW01 and OPD18 generated the maximum number of polymorphic bands (19%) while the primer OPA08 generated the least (7%) polymorphic bands. The number of amplified polymorphic bands ranged from 2 - 5 with an average of 4 bands. The reproducibility of the RAPD banding pattern was confirmed by 3 replicated reaction with same primer. The banding pattern of six lentil varieties using OPD02 is shown in Fig. 1. Rana et al. (2007) reported that 29 lentil (*Lens culinaris*) cultivars and landraces produced 42.3% polymorphic bands using 97 markers (72 RAPD and 25 STMS). Using RAPD markers on lentils, Tayyeba (2003) found the markers to be 50% polymorphic. Hence it reveals that differences can be attributed to the primers used and the genotypes evaluated.

The present work produced 7.5 scorable bands per primer and 4 polymorphic bands per primer. The reasons beyond the considerable number of average scorable and polymorphic bands might be the amount of GC content (60 - 70%) of the primers used in this study. Fukuoka et al. (1992) observed an

increase in the number of bands with increasing GC content of the primer. The explanation for this correlation between the GC content of the primer and the number of bands is that, the stability of base complementation is higher when G pairs with C than that of the complementation of A with T. The DNA polymorphisms were detected according to the presence and absence of bands. Absence of bands may be caused by failure of primers to anneal at a site in some individuals due to nucleotide sequence differences or by insertions or deletions between primer sites (Clark and Lanigan 1993).

Table 1. RAPD primers with corresponding bands score and their size range together with polymorphic bands observed in six lentil varieties.

Primer code	Total number of bands scored	Size ranges (bp)	Number of polymorphic bands
OPA01	8	380 - 1440	5
OPA18	9	380 - 920	6
OPC08	6	580 - 980	2
OPD02	5	400 - 1000	3
OPD03	10	400 - 1000	4
OPD18	8	350 - 1000	6
OPW01	7	320 - 980	6
Total	**53**		**32**

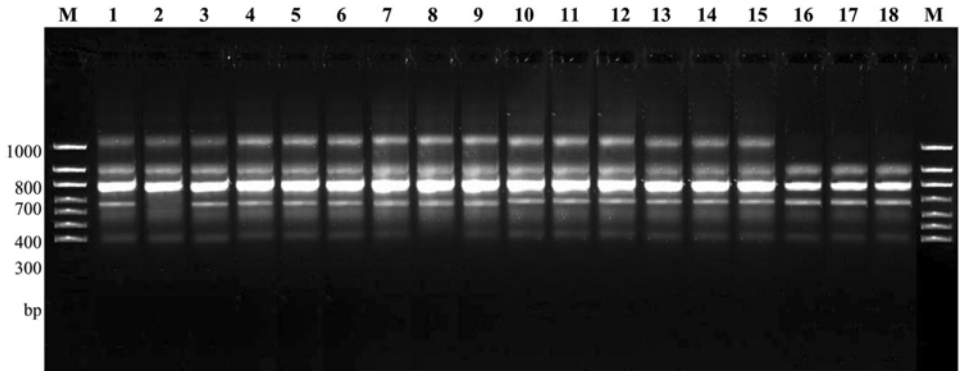

Fig. 1. RAPD profiles of 6 lentil germplasm using primer OPD02. Lane 1 - 3: BARI musur-1; Lane 4 - 6: BARI musur-2; Lane 7 - 9: BARI musur-3; Lane 10-12: BARI musur-4; Lane 13 - 15: BARI musur-5 and Lane 16 - 18: BARI musur-6. M: Molecular weight marker (100 bp DNA ladder in left side and PUC in right side).

The highest Nei's genetic identity (0.9331) on varietal pair was observed in BARI masur-2 vs. BARI musur-1 and the lowest Nei's genetic identity (0.6064) was observed in BARI musur-5 vs. BARI musur-1 (Tabel 2). The highest Nei's

(1972) genetic distance (0.5002) was observed in BARI musur-1 vs. BARI musur-5 whereas, the lowest genetic distance (0.0692) was found in BARI musur-2 vs. BARI musur-1 varietal pair (Table 2). Furthermore, high level of genetic distance was found in BARI musur-6 vs. BARI musur-1 (0.4908), BARI musur-5 vs. BARI musur-2 (0.4392) varietal pair and low level of genetic distance was observed in BARI musur-6 vs. BARI musur-4 (0.0972), BARI musur-3 vs. BARI musur-1 and BARI musur-2 (0.16988) (Table 2).

Table 2. Summary of Nei's genetic identity (above diagonal) and genetic distance (below diagonal) values between 6 lentil varieties.

Acc. No.	BARI musur-1	BARI musur-2	BARI musur-3	BARI musur-4	BARI musur-5	BARI musur-6
BARI musur-1	****	0.9331	0.8438	0.7094	0.6064	0.6122
BARI musur-2	0.0692	****	0.8438	0.7094	0.6445	0.6506
BARI musur-3	0.1698	0.1698	****	0.8093	0.7043	0.7109
BARI musur-4	0.3434	0.3434	0.2116	****	0.8231	0.9074
BARI musur-5	0.5002	0.4392	0.3506	0.1947	****	0.8044
BARI musur-6	0.4908	0.4299	0.3412	0.0972	0.2177	****

A dendrogram based on Nei's (1972) genetic distance using unweighted pair group method of arithmetic mean (UPGMA), indicates segregation of six varieties of lentil into two main clusters; BARI masur-1, BARI masur-2 and BARI masur-3 were grouped in cluster I and BARI masur-4; BARI masur-5 and BARI masur-6 were grouped in cluster II. In cluster I BARI masur-1 and BARI masur-2 formed sub-cluster I and BARI masur-3 formed sub-cluster II. In cluster II BARI masur-4 and BARI masur-6 were grouped as sub-cluster I and BARI masur-5 formed sub-cluster II. The results indicate that, low or high level genetic distance exists respectively between varieties with similar or different origins. BARI masur-1 vs. BARI masur-5 varietals pair showed the highest Nei's (1972) genetic distance (0.5002), as they were released from different parental origins. On the other hand, BARI masur-2 vs. BARI masur-1 varietal pair showed the lowest genetic distance (0.0692) as they were released from the same parental origin (Fig. 2). The present findings agree with the findings of Lakhanpaul *et al*. (2000) and Saini et al. (2004).

The present study indicates that the highest genetic variation between BARI masur-1 vs. BARI masur-5 and lowest genetic variation between BARI masur-1 vs. BARI masur-2 can be used in breeding programs to improve lentil varieties. The results also reveal that the genetic base among these lentil varieties is rather narrow. Collection of diverse germplasm from centers of diversity may broaden the genetic base. RAPD markers provide a fast, efficient technique for variability assessment that complements methods currently being used in genetic resource management.

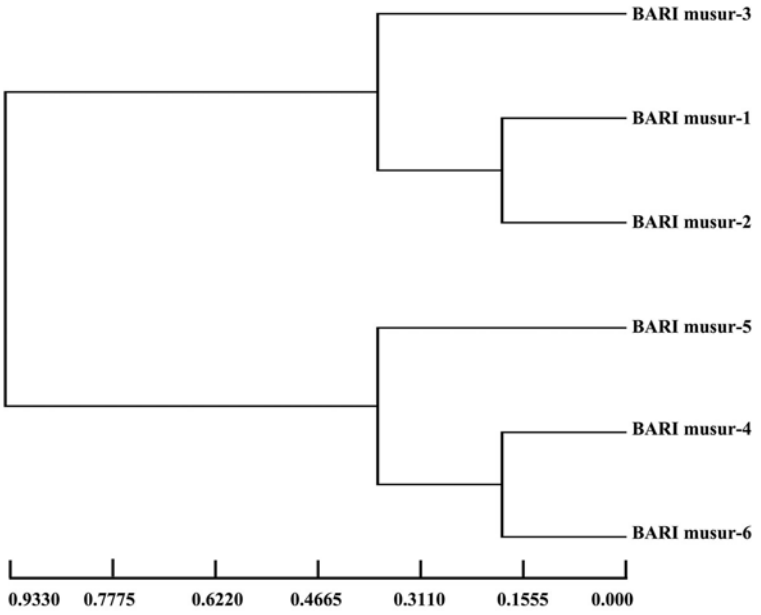

Fig. 2. Unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei's (1972) genetic distance, summarizing data on differentiation for 6 lentil accessions according to RAPD analyses.

In this study, the genetic variation of six lentil varieties (BARI masur-1, BARI masur-2, BARI masur-3, BARI masur-4, BARI masur-5 and BARI masur-6,) were analyzed by polymerase chain reaction (PCR) using seven RAPD primers (OPA01, OPA18, OPC08, OPD02, OPD03, OPD18 and OPW01). RAPD markers have proven to be a powerful tool for molecular genetic analysis of lentil cultivars for plant breeding programs to assess genetic diversity for the development of improved varieties that are able to withstand biotic and a biotic stresses. Using a limited number of varieties and primers the present study is a preliminary research work to assess genetic variation of lentil varieties in Bangladesh. The results indicate that BARI masur-1 and BARI masur-5 have

higher genetic variation than others. Further research in this regard should be conducted with a broad spectrum.

References

Afzal MA, Haque MM and **Shanmugasundaram S** (2004) Random Amplified Polymorphic DNA (RAPD) analysis of selected mungbean *(Vigna radiaia* L. Wilczek) cultivars. Asian J. Plant Sci. **3**(1): 20-24.

Ba FS, Pasquet RS and **Gepts P** (2004) Genetic diversity in cowpea *(Vigna unguiculata* L. Walp.) as revealed by RAPD markers. Genet. Res. Crop. Evol. **51**: 539-550.

BBS (Bangladesh Bureau of Statistics Agriculture) (2010) Ministry of planning. Govt. Peoples Republic of Bangladesh, Dhaka.

Cattan-Tou-pance I, Michalakis Y and **Neema C** (1998) Genetic structure, of wild beari populations in their south-andean centre of origin. Theor; Appl. Genet. **96**: 844-851.

Clarck AG and **Lanigan CMS** (1993) Prospects for estimating nucleotide divergence with RAPDs. Mol. Biol. Evol. **10**: 1069-1111.

Fukuoka S, Hosaka K and **Kamijima O** (1992) Use of random amplified polymorphic DNAs (RAPDs) for identification of rice accessions. Jap. J. Genet. **67**: 243-252.

Frey, JE, Frey B, Sauer C and **Kellerhals M** (2004). Efficient low cost DNA extraction and multiplex fluorescent PCR method for marker assisted selection in breeding. Plant Breed. **123**: 554-557.

Lakhanpaul S, Chadha S and **Uliat, KV** (2000) Random amplified polymorphic DNA (RAPD) analysis in Indian mung bean *(Vigna radiaia* (L.J Wilczck) cultivars. Genetica **109**(3): 227-94.

Murray MG and **Thompson WF** (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. **8**: 4321-5.

Muehlbauer FJ., Cho S, Sarker A, McPhee, KEN and **Ford R** (2006) Application of biotechnology in breeding lentil for resistance to biotic arid abiotic stress. Euphytica, 147 (1-2): 149-165.

Nei M (1972) Genetic distance between populations. American Naturalist **106**: 283-292.

Rana MK, Ratna Kumari, Sonika S and **Bhat KV** (2007) Genetic analysis of Indian lentil (*Lens culinaris* Medikus) cultivars and landraces using RAPD and STMS markers. J. Plant. Biochem, Biotech. **16** (1): 53-57.

Saini A Reddy, SK and **Jawali N** (2004) Evaluation of long primers for AP-PCR analysis of mungbean *(Vigna radiata* L. Wilczek): Genetic relationships and fingerprinting of some genotypes. Indian J. Biotech. **3**:519-526.

Tayyaba S (2003) Geographical distribution of lentils in Pakistan as indicated by data on genetic markers, Biol. Sci. 234.

Tosti N and **Negri V** (2002) Efficency of three PCR-based markers in assessing genetic variation amon cowpea *(Vigna ungiculata spp unguiculata)* landraces. Genome **45**: 268-275.

Williams JGK, Kubelik AE, Levak KJ, Rafalski, JA and **Tingey SC** (1990) DNA polymorphisms anplified by arbitrary primers are useful as genetic markers. Nueleic. Acids Res. **28**: 6531-6535.

Xu R Q, Tomooka N, Vaughan DA and **Doi K** (2000) The *Vigna angulars complex:* genetic variation and relationships revealed by RAPD analysis, and the their implications for in situ conservation and domestication. Genet. Resour. Crop Evol. **47**: 123-134.

Yeh FC, Yang RC Boyle, TBJ Ye, ZH and **Mao JX** (1999) POPGENE, the user-friendly shareware for population genetic analysis.