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Full Length Research Paper

Assessment of genetic diversity in French bean (*Phaseolus vulgaris* L) based on RAPD marker

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RAPD molecular markers were used to evaluate the genetic diversity in the fourteen varieties of French bean (*Phaseolus vulgaris*) of three eco-geographical regions of Bangladesh. Out of the 20 primers only, 6 yielded polymorphic banding patterns. In total, 40 different DNA bands were reproducibly obtained, out of which 28 (70%) were polymorphic. The polymorphisms were scored and used in band-sharing analysis to identify genetic relationships. Cluster analysis based on Jaccard's similarity coefficient using UPGMA grouped all the 14 genotypes into two major groups. Pair-wise variety comparison of the varieties showed that inter-variety similarity indices for PV004 versus PV005 (98.51) was higher than all other varietals pairs. On the other hand, inter-cultivar similarity index for BARI 01 versus PV009 (59.58) cultivar was lower than all other cultivar pairs. Therefore, the inherent simplicity and efficiency of RAPD analyses will be to facilitate the construction of RAPD-based genetic linkage maps in French bean.

Key words: RAPD, French bean, polymorphism.

INTRODUCTION

The French bean (*Phaseolus vulgaris* L., syn. Kidney bean, haricot bean, snap bean, navy bean, string bean) is one of the most important leguminous vegetables, which is grown for fresh pod consumption and for processing as a frozen vegetable in many countries. In Bangladesh, it is grown for tender vegetables. This bean has wide genetic variation as regarding the edible parts and growing habits. Variation is also observed in the seed weight, volume, density, hydration capacity, hydration index, swelling capacity, cooking time and amylose content (Kaura et al., 2009). Information concerning the extent and nature of genetic diversity within a crop species is essential for an effective breeding programme.

The immense genetic diversity of landraces of crops is the most directly useful and economically valuable part of biodiversity. Unlike high yielding varieties, the landraces maintained by farmers are endowed with tremendous genetic variability, as they are not subjected to subtle selection over a long period. Because of the limitations of morphological and biochemical markers, efforts are being directed to use molecular markers for characterizing germplasm diversity. Molecular markers have demonstrated a potential to detect genetic diversity and to aid in the management of plant genetic resources (Virk et al., 2000; Song et al., 2003). In contrast to morphological traits, molecular markers can reveal differences among genotypes at the DNA level, providing a more direct, reliable and efficient tool for germplasm characterization, conservation and management. Several types of molecular markers are available, including those based on restriction fragment length polymorphism (RFLP, Botstein et al., 1980), random amplified polymorphic DNA (RAPD, Welsh and McClelland, 1990; Williams et al., 1990), amplified fragment length polymorphism (AFLP, Vos et al., 1995) and simple sequence repeats (SSRs, Singh, 1999).

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Abbreviations: DNA, Deoxyribonucleic acid; **RAPD,** random amplified polymorphic DNA; **RFLP,** restriction fragment length polymorphism; **AFLP,** amplified fragment length polymorphism; **PCR,** polymerase chain reaction; **CTAB,** cetyl trimethyl ammonium bromide; **EDTA,** ethylene diamine tetraacetic acid; **TAE,** tris-acetate-EDTA; **dNTPs,** deoxynucleoside 5'-triphosphates; **UPGMA,** unweighted pair group method of arithmetic means; **SI,** similarity index.

Table 1. Name of varieties and their sources using RAPD analysis.

S/N	Accession no.	Place of collection	S/N	Accession no.	Place of collection
1	BARI 01	BARI, Gazipur	8	PV 008	Raikahli, Chittagong
2	BARI 02	BARI, Gazipur	9	PV 009	Raikahli, Chittagong
3	PV 003	Raikahli, Chittagong	10	SL 01	Sylhet
4	PV 004	Raikahli, Chittagong	11	SL 02	Sylhet
5	PV 005	Raikahli, Chittagong	12	SL 03	Sylhet
6	PV 006	Raikahli, Chittagong	13	SL 04	Sylhet
7	PV 007	Raikahli, Chittagong	14	SL 05	Sylhet

Table 2. Polymorphism detected by the use of 6 random primers on 14 French bean individuals.

Name of primers	Sequence of primer	Total number of bands	Number of polymorphic bands	% of polymorphic bands
OPA 04	AATCGGGCTG	6	3	50
OPB 17	AGGGAACGAG	5	2	40
OPD 03	GTCGCCGTCA	9	9	100
OPF 01	ACGGATCCTG	8	4	50
OPZ 03	CAGCACCGCA	8	6	75
OPZ 13	ACTAAGCCC	4	3	75
Total		40	27	65
Average		6.67	4.5	

It is particularly useful for characterizing individual genotypes and selection of the parents for successful hybridization. Among the molecular markers, in the present study, RAPD method of DNA fingerprinting was used in the present study. This technique is widely used in conservation biology because of quick results, cost-effectiveness and reproducibility. The PCR-based RAPD approach using arbitrary primers requires only nanogram quantities of template DNA, no radioactive probes and is relatively simple compared to other techniques (Williams et al., 1993). However, morphological traits have certain limitations such as easy availability of scorable markers, difficulty in scoring homozygous from heterozygous individuals, influence of environment in equating phenol-types with genotypes, etc. On the other hand, molecular markers have many advantages such as abundance in polymorphism, no pleiotropic effect, less affected by environment and subjected to rapid detection (Singh et al., 2005). Therefore, RAPD has been used extensively for studying genetic diversity of French bean. However, no report on RAPD is available regarding the diversity of French bean in Bangladesh contrast.

MATERIALS AND METHODS

Plant material

Seeds from 14 French bean variety (Table 1) were collected from three locations of Bangladesh. Out of which two from BARI, seven form Raikhali and five from Sylhet region. The seeds were sown in polybag for getting young leaf for DNA extraction.

DNA extraction

Total genomic DNA from young leaves were isolated following Choloroform: Isoamyl alcohol purification and propanol precipitation method (Doyle and Doyle, 1987). Fresh young leaves were collected in alluminium foil and immediately stored at 4°C. Approximately 150 mg of leaf tissue was ground with 1 ml DNA extraction buffer (2% CTAB; 1.4M NaCl; 20 mM EDTA, pH 8.0; 100 mM Tris-HCl, pH 8.0; 0.2% ß-mercapto-ethanol) using mortar and pestle. The crushed was transferred to a 1.5 mL polypropylene centrifuge tube and incubated at 65°C for 45 min in hot water bath. After incubation, the contents were centrifuged at 14000 rpm for 15 min at room temperature. Then upper aqueous phase was transferred in another centrifuge tube and emulsified with an equal volume of chloroform: iso-amyl alcohol (24:1) by inversion and again the contents were centrifuged at 14000 rpm for 15 min at room temperature. Then the DNA was isolated from the aqueous phase following propanol precipitation. The isolated samples were stored at -20°C. DNA concentrations were determined at 260 nm with Spectrophotomer and the quality verified by electrophoresis on 1% agarose gel in TAE (Tris-acetate-EDTA) buffer.

DNA amplification

Several RAPD primers were used for PCR amplification. A list of the primers was given in Table 2. The PCR reaction mixtures were prepared in 25 µL volumes containing 10 µL of 5X Flexi Taq buffer, 4.0 µL $MgCl_2$ (25 mM) , 2.5 µL dNTPs (4 mM/ µL), 2.5 µL random primer (10 pmol/ µL), 0.25 µL Taq DNA polymerase (5 U/µL) and 4 µL of the extracted DNA (25 ng). The mixture was made up to 25 µL by addition of sterilized distilled water.

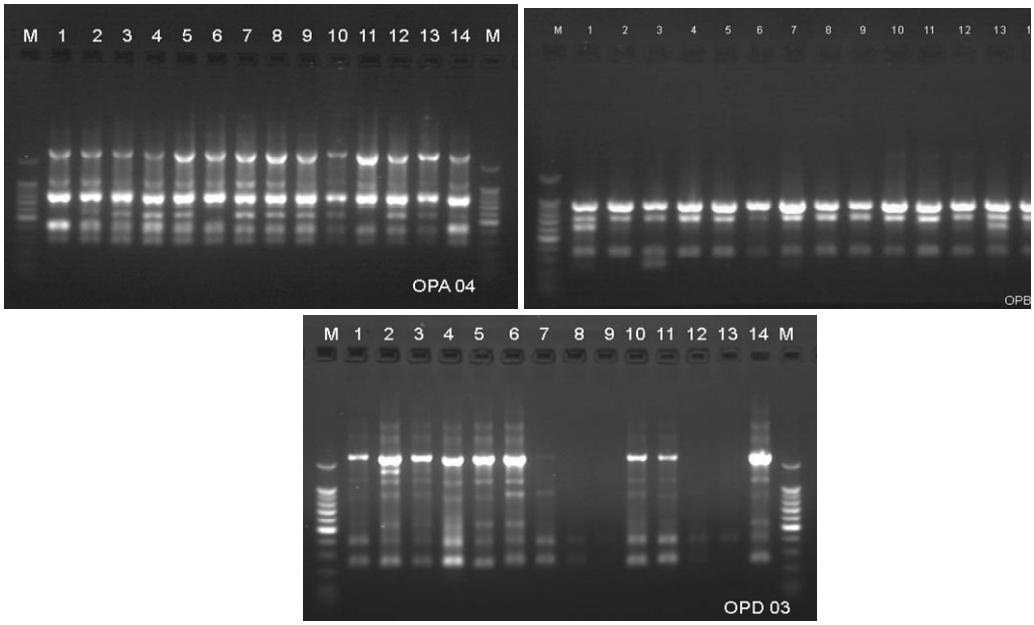

Figure 1. RAPD profile of 14 French Bean varieties (*Phaseolus vulgaris* L.) produced using the random decamer primers OPA04, OPB17 and OPD03. M, 100 bp DNA ladder. Lane numbers correspond to the varieties BARI 01 to SL05 given in Table 1.

RAPD/PCR reactions were initiated and optimized using an Applied Biometra thermal cycler programmed to repeat the thermal profile. Setting of the PCR program based on three steps. Step one, was an initial denaturation step at 94°C for 1 min. Step two, was run for 45 cycles, each starting with denaturation at 94°C for 1 min, followed by annealing 42°C for 1 min and ended by extension at 72°C for 1 min. Step three, was a final extension cycle performed at 72°C for 7 min. The PCR machine was adjusted to hold the product at 4°C. The PCR product was mixed with 6 µL of loading dye (0.25% bromophenol blue, 0.25% Xylene Cyanol and 40% Sucrose, w/v) and spun briefly in a micro centrifuge before loading. The PCR products and 100 bp DNA ladder were electrophoresed using 1% agarose gel at 80 volts followed by staining with ethidium bromide then separated fragments and were visualized with an ultraviolet (UV) transilluminator (Biometra gel documentation system).

Data analysis

Since RAPD markers are dominant, it was assumed that each band represented the phenotype at a single allelic locus (Williams et al., 1990). For each primer, the number of polymorphic and monomorphic bands was determined. Bands clearly visible in at least one genotype were scored (1) for present and (0) for absent and entered into a data matrix. Fragment size was estimated by interpolation from the migration distance of marker fragments. Percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of bands. The scores obtained using all the primers in the RAPD analysis were then pooled to create a single data matrix, 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 weight on the data matrix. Similarity index (SI) was calculated from $2n_{xy}/n_x+n_y$, when n_{xy} is the number of the common DNA bands in x and y plants, n_x and n_y are the total DNA bands of X and Y plant respectively (Hill et al., 1996).

RESULTS AND DISCUSSION

For the isolation of good quality DNA, a CTAB-based procedure was optimized in the present study that yields high quality DNA free of phenols. In this study, 20 primers were tested with the 14 genotypes of French bean. The results indicate that 6 primers show at least one consistent polymorphic band. The six informative primers were selected and used to evaluate the degree of polymorphism and genetic relationships within and between all genotypes under study. A total of 40 amplified fragments were distinguished across the selected primers and the statistical analysis showed 28 (70%) polymorphic bands among the 14 genotypes with an average of 4.5 polymorphic bands per primer. Monaj et al. (2005), found that the primer OPF-17 to be most powerful and efficient as it generated a total of 17 bands of which 15 were polymorphic in common bean. The maximum numbers of fragment bands were produced by the primer OPD03 (9 bands) with 100.0% polymorphism while the minimum numbers of fragments were produced by the primer OPZ13 (4 bands) with 75.0% polymorphism. Scott et al. (1994) detected high (39.2%) level of polymorphism between the navy-1 and navy-2 bean lines. The pattern of RAPD fragments produced by the 6-mer primer OPA04, OPB17, OPD03, OPZ03 and OPZ 13 is as shown in Table 2 and Figure 1. The inter-variety similarity indices within the four-

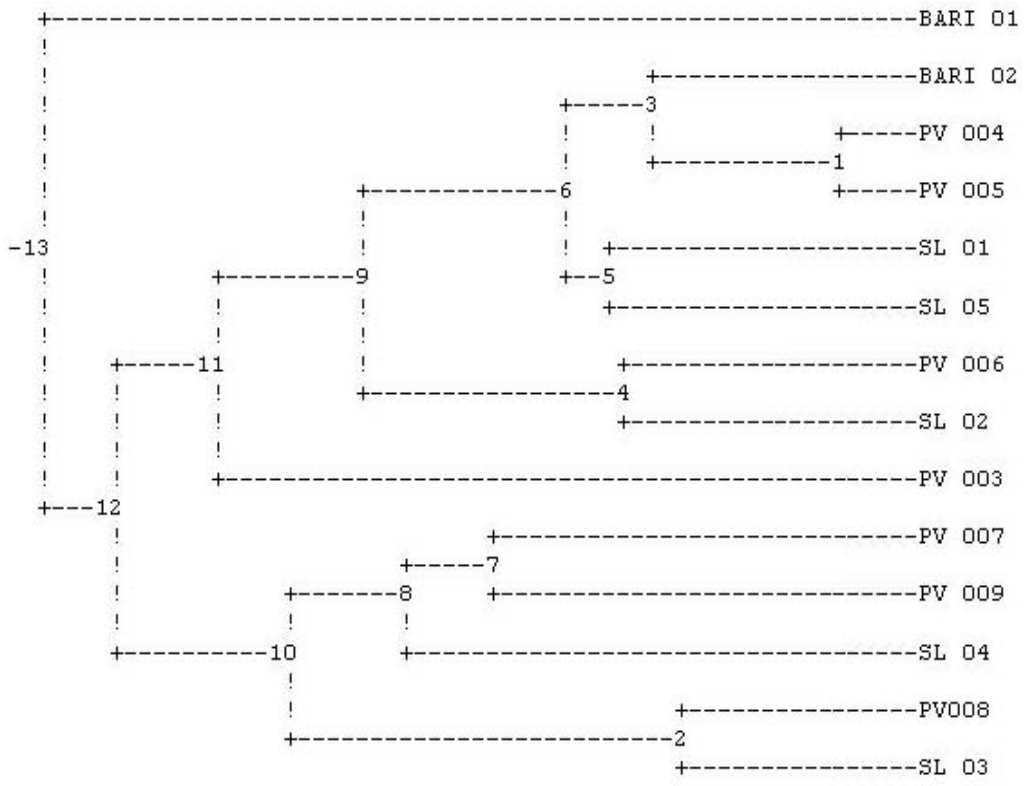

Figure 2. UPGMA cluster analysis-based dendrogram showing genetic relationship among fourteen varieties of French bean.

teen French bean varieties ranged from 59.58 to 98.51% (Table 2). Monaj et al. (2005) observed that the similarity coefficient values varied from 0.19 to 0.91 germplasm which indicates a wide and diverse base among 99 common bean. Pair-wise variety comparison of the varieties showed that inter-variety similarity indices for PV004 versus PV005 (98.51) was higher than all other varietals pairs. On the other hand, inter-cultivar similarity index for BARI 01 versus PV009 (59.58) cultivar was lower than all other cultivar pairs. Franklin et al. (2009) observed Jaccard's pair-wise similarity coefficient of 0.50 to 0.95, indicating that an intra-specific genetic variation prevails in landraces of common bean in the Nilgiris biosphere reserve.

Dendrogram based on Nei's (1972) genetic distance UPGMA indicated segregation of the fourteen French bean varieties into two main clusters. BARI 01 individually grouped in cluster I whereas rest 13 varieties together grouped in cluster II. In cluster II, BARI 02, PV 004, PV005, SL01, SL05, PV006, SL02 and PV003 formed sub cluster I and PV007, PV009, SL04, PV008 and SL03 formed together sub cluster II. In sub cluster I, PV003 singly formed sub sub cluster I and the rest formed sub sub cluster II. In sub cluster II, SL03 and PV004 together formed sub sub cluster I and PV007, PV009 and SL 04 together grouped in sub sub cluster II. Sub sub cluster PV004-PV005 and PV008-SL03 were grouped separately with lower level of genetic distance 0.03 and 0.11, respectively. A highest level of genetic distance was observed between BARI01-PV009, PV006-PV009, and PV006-SL04, while the lowest level of genetic distance showed between PV004-PV005 with 0.64 and 0.03, respectively (Table 3). Observed and effective numbers of alleles of all the varieties were 1.7 and 1.45, respectively. Nei's genetic diversity of the varieties was 0.26. So, for the hybridization or varietal development BARI01-PV009, PV006-PV009, PV006-SL04 pairs may be a good combination based on the DNA variability using RAPD markers.

In conclusion, RAPD analysis revealed high levels of genetic variability, even with the use of limited set of primers. This high level of polymorphism among individuals suggests that RAPD techniques can be useful for French bean for the maintenance of germplasm banks and the efficient selection of parents for breeding.

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Table 3: Nei's genetic distance among fourteen varieties of French bean

population ID	*BARI 01	BARI 02	*PV 003	PV 004	PV 005	PV 006	PV 007	PV 008	PV 009	*SL 01	SL 02	SL 03	SL 04	SL 05
BARI 01	****													
BARI 02	0.598	****												
PV 003	0.357	0.431	****											
PV 004	0.470	0.134	0.322	****										
PV 005	0.511	0.105	0.357	0.025	****									
PV 006	0.288	0.358	0.357	0.255	0.288	****								
PV 007	0.431	0.224	0.431	0.193	0.163	0.511	****							
PV 008	0.288	0.598	0.357	0.393	0.431	0.357	0.357	****						
PV 009	0.644	0.394	0.470	0.288	0.255	0.644	0.192	0.322	****					
SL 01	0.470	0.192	0.322	0.163	0.134	0.322	0.255	0.470	0.288	****				
SL 02	0.255	0.322	0.255	0.163	0.192	0.134	0.322	0.255	0.431	0.288	****			
SL 03	0.357	0.511	0.288	0.322	0.357	0.357	0.288	0.105	0.192	0.393	0.192	****		
SL 04	0.470	0.470	0.470	0.511	0.470	0.644	0.255	0.322	0.223	0.288	0.598	0.322	****	
SL 05	0.322	0.192	0.322	0.163	0.134	0.255	0.255	0.393	0.431	0.163	0.223	0.393	0.357	****

*BARI=Bangladesh Agricultural Research Institute; *PV=*Phasiolus vulgaris*; SL=Sylhet local

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