Genetic diversity in Indian common bean (*Phaseolus vulgaris* L.) using random amplified polymorphic DNA markers

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ABSTRACT

Genetic diversity of twenty-six common bean (*Phaseolus vulgaris* L.) accessions of diverse geographical origin was studied using Random Amplified Polymorphic DNA (RAPD) markers. Fifteen out of forty four primers screened showed polymorphism across present set of genotypes. A total of 124 amplicons were scored using these 15 primers. Ninety five percent of the amplified products showed polymorphism, indicating fair amount of variation at the DNA level among these accessions. Cluster analysis delineated the genotypes in to four groups. [Physiol. Mol. Biol. Plants 2008; 14(4) : 383-387] E-mail : Vipinch1@yahoo.co.in

Key words : Genetic diversity, Common bean, RAPD, UPGMA

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.; $2n=2x=22$) is a predominantly self-pollinated crop originated mainly in Latin America. From Latin America, it spread to other parts of world and now it is widely cultivated in the tropics and subtropics as well as in temperate regions of the world (Gepts and Bliss 1988; Zeven 1997, 1999).

In India, common bean is known by the names like 'Rajmash' and 'Frash bean (green bean)' and grows in certain parts of the country. Indian farmers are also growing it due to its good market price and it is mainly consumed as grain legume. Common bean production in India is 4,340 million tones as compared 18,943 million tones in the world. (Anonymous, 2003). In due course of time many common bean varieties have been released in India by different research institutes. A great diversity for agro-morphological traits like, seed size and colour also exists in *Phaseolus* germplasm of northwestern Himalayan region comprising the parts of Himachal

Pradesh and Jammu and Kashmir State of India. But still there is need to select genetically diverse.

Parental lines to obtain superior recombinants. Conventionally, the identification of genetically distinct genotypes is accomplished by using morphological characteristics, which is later supplemented with molecular markers for reliable results. In the present study an effort was made to assess the genetic diversity among released varieties from India and abroad using RAPD markers. Random amplified polymorphic DNA (RAPDs) markers have been utilized successfully by many workers for genetic diversity analysis in common bean (Beebe *et al.* 2000; Metais *et al.*, 2002; Sharma *et al.* 2006). These markers are selected because they are technically simple, rapid, comparatively cheap, does not require the use of radio activity and very small quantity of DNA is required for RAPD analysis.

MATERIALS AND METHODS

Plant materials

A total of 26 accessions including high yielding released

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Table 1. Common bean accessions subjected to RAPD analysis to determine genetic diversity

Sl.No.	Accessions	Name of variety	Source	State
1.	IC73559★ ¹	VL RAJMA 63	VPKAS, Almora	Uttanchal
2.	IC393852★ ¹	VARUN (ACPR-94040)	Regional fruit Station Pune	Maharastra
3.	IC296363★ ¹	IC296363	IIPR, Kanpur	Uttar Pradesh
4.	IC296095★ ¹	K-31 (F-BEAN)	MPKV, Rahuri	Maharastra
5.	IC393851★ ¹	MUTHA (HPR-35)	Regional fruit Station Pune	Maharastra
6.	IC326537★ ¹	KAILASH (SRC-74)	Ch. Swaran Kumar Krishi Vishwavidyalaya	HP
7.	IC296096★ ¹	VARUN	MPKV, Rahuri	Maharastra
8.	IC295928★ ¹	OOTY (FB-2)	HRS, Vijaynagaramooty	Tamilnadu
9.	IC296097★ ¹	BEAN-1 (GUJRAT BEAN)	GAU, VRC, Anand	Gujarat
10.	IC296098★ ¹	IIPR-96-4 (AMBER)	IIPR, Kanpur	Uttar Pradesh
11.	IC296331★ ¹	IIPR-96-3 (RUDRA)	IIPR, Kanpur	Uttar Pradesh
12.	IC393734★ ¹	IIHR- 909 (ARKA SUVIDHA)	IIHR, Bangalore	Karnataka
13.	IC75267★ ¹	ARKA KOMAL	IIHR, Bangalore	Karnataka
14.	IC73041★ ¹	HPR-12 (JWALA)	Ch. Swaran Kumar Krishi Vishwavidyalaya	HP
15.	IC73040★ ¹	HIM-1	Ch. Swaran Kumar Krishi Vishwavidyalaya	HP
16.	IC73039★ ¹	HPR-62 (HANS)	Ch. Swaran Kumar Krishi Vishwavidyalaya	HP
17.	IC296192★ ¹	OOTY-1	TN. Agr. Uni.	Tamilnadu
18.	IC121427★ ¹	IC121427	IARI, Katrain, H.P.	HP
19.	IC118935★ ¹	HUR-137 (MALVIYA RAJMA)	BHU, Varanasi	Uttar Pradesh
20.	IC118984★ ¹	HUR-15 (MALVIYA RAJMA 15)	BHU, Varanasi	Uttar Pradesh
21.	IC118933★ ¹	UDAY (PDF -14)	DPR, Kanpur	Uttar Pradesh
22.	IC79115★ ¹	PANT ANUTTAR PRADESHAMA	Agr. Uni. Pant Nagar	Uttanchal
23.	IC76299★ ¹	CONTANDER	IARI, Katrain, H.P.	HP
24.	IC76298★ ¹	PUSA PARVATI	IARI, Katrain, H.P.	HP
25.	IC76281★ ¹	KENTUKEY WONDER	IARI, Katrain, H.P.	HP
26.	IC384680★ ¹	SHALIMAR RAJMA (LAL RAJMA)	Share Kashmir Uni. of Agr. & Sciences	J& K

Table 2. List of primers along with their sequences and amplification details

Primer	Sequence (5'-3')	No. of amplified fragments	No. of polymorphic fragments	Polymorphism (%)	Mol. Wt range (kb)
OPA-08	GTGACGTAGG	8	8	100	0.2-2.5
OPA-11	CAATCGCCGT	7	7	100	0.2-2.0
OPA-12	TCGGCGATAG	4	4	100	0.3-1.5
OPA-13	CAGCACCCAC	5	4	80	0.25-1.7
OPA-14	TCTGTGCTGG	10	10	100	0.25-2.5
OPA-15	TTCCGAACCC	10	10	100	0.3-1.9
OPA-16	AGCCAGCGAA	4	4	100	0.6-1.5
OPA-17	GACCGCTTGT	6	6	100	0.2-2.5
OPA-18	AGGTGACCGT	17	16	94	0.2-2.0
OPB-01	GTTTCGCTCC	11	10	90	0.3-2.0
OPB-03	CATCCCCCTG	6	5	83	0.3-2.0
OPB-04	GGACTGGAGT	8	8	100	0.3-2.0
OPB-05	TGCGCCCTTC	9	9	100	0.5-2.0
OPB-07	GGTGACGCAG	12	12	100	0.25-2.5
OPB-08	GTCCACACGG	7	6	85	0.3-2.5

varieties of common bean were used in the present study (Table 1)

DNA isolation

DNA was extracted from one-month-old young furred leaves following modified CTAB method of Saghai - Maroof *et al.* (1984). DNA quality and quantity was checked on agarose gel with λ Hind III DNA marker.

RAPD analysis

PCR reactions were performed in 25 μ l reaction mix containing 10 mM Tris-HCl (pH 8.3), 50 mM NaCl, 2.5 mM MgCl₂, 200 μ M each of dATP, dTTP, dCTP and dGTP, 0.75 U of Ampli Taq DNA polymerase (all reagents from Perkin Elmer Cetus), 0.6 μ M of 10-mer random primer (Operon Technologies, Alameda, CA) (Table 2) and 20 ng of template DNA. Amplifications were performed in a 96-well thermocycler (Gene Amp 9600, Perkin Elmer Corporation, USA). PCR cycling conditions were as follows: 1 min denaturation at 93 °C, 1 min primer annealing at 35 °C and 1 min primer extension at

Fig. 1. PCR amplification patterns showing polymorphism detected by an RAPD primer OPA-15 (a) and OPB-4 (b) in different cultivars of common bean. M = 100 bp ladder. Numbers 1-26 represents different varieties as given in the Table 1.

Fig. 2. Dendrogram showing clustering pattern of 26 common bean released cultivars based on genetic similarity values obtained from the RAPD data. Numbers 1-26 represents different varieties as given in Table 1.

72 °C for 40 cycles followed by a further extension at 72 °C for 8 min. Amplified products were mixed with loading buffer containing bromophenol blue, separated through gel electrophoresis on 1.4 % agarose (Bio-Rad, USA) gels in 1 x TAE buffer, stained with ethidium bromide and PCR products visualized on a UV transilluminator. The intensity of fluorescence of different ethidium bromide stained PCR products varied within and among the cultivars studied. For purposes of data analysis only amplicons that were reproduced over two amplifications were included.

Analysis of Data

Gels were scored in binary format with the presence of band scored as one and its absence scored as zero. The binary data were used to compute pair-wise similarity coefficients (Jaccard, 1908), and the similarity matrix thus obtained was subjected to cluster analysis using the UPGMA (unweighted pair-group method with arithmetic average) algorithm. Similarity coefficients were also subjected to Principal Coordinate (PCO) analysis. Data analyses were performed using NTSYS-PC version 1.9.

RESULTS AND DISCUSSION

Progress in crop breeding requires the exploitation of genetic variation among races and gene pools. Molecular

Principal Co-ordinate Analysis

Fig. 3. Patterns of relationships among 26 common bean varieties revealed by principal coordinate analysis based on RAPD data. Numbers 1-26 in figure represents different varieties as shown in Table 1.

markers are potentially useful in establishing genetic relationship among the wild species of common bean (Sharma 2006). Genetic diversity among twenty six varieties of common bean was investigated in the present study using 15 RAPD primers, which generated 124 bands/amplicons. The size of amplicons ranged from 200 bp to 2500 bp. The number of bands generated ranged from 4 to 17 with a mean of 8.26 bands per primer (Table 2). Out of 124 bands generated, 119 (95.96 %) were polymorphic and 5 (4.03 %) were monomorphic. Primer OPA-18 showed maximum number of polymorphic bands (17 bands). The percentage of polymorphic bands ranged from 83 to 100 % with an average of 95.46 %. Out of total 15 primers, 10 displayed 100 % polymorphism (Table 2). RAPD profiles obtained from primer OPA-15 and OPB-4 are shown in Fig. 1

Cluster analysis revealed that 26 varieties were grouped in two major clusters (Fig. 2). The clusters I and II contains 23 and three accessions, respectively. The genetic similarity coefficient (GS) values based on RAPDs for all the possible 325 pairs of genotypes ranged from 0.191 (between IC296363) and IC295928, Ooty FB-2) to 0.923 (between IC118935, Malviya rajma (HUR-137) and IC118984) with a mean of 0.646.

The results of principal coordinate analysis are shown in Fig. 3. The variation accounted in the first, second and third components accounted for 26.25 %, 11.41 % and 8.46 % of total variation, respectively while the

total variation was 46.12 %. The results of PCO corresponded well with cluster analysis obtained through UPGMA. The present results support the earlier views that RAPD analysis is a desirable technique for detection of genetic diversity in Indian common beans. Results of cluster analysis and PCO clearly showed the RAPD primers used in the present study can easily demarcates the accessions. The overall results indicated that a considerable diversity exists in a set of accessions analysed in the present investigation. This information can be used for selection of desirable parents for improvement of common beans.

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