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Genetic diversity analysis of exotic and Indian accessions of common bean (*Phaseolus vulgaris* L.) using RAPD markers

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Abstract

Genetic diversity of forty-six common bean (*Phaseolus vulgaris* L.) accessions of diverse geographical origin was studied using RAPD markers. Four out of the twenty primers screened showed polymorphism across present set of genotypes. A total of 43 DNA amplicons were scored using these 4 primers. Seventy-seven per cent of the amplification products showed polymorphism, indicating fair amount of variation at the DNA level among these accessions. The genotypes shared 59% genetic similarity among themselves. Cluster analysis delineated the genotypes into four groups. Twenty accessions from United States clustered in two separate groups, which possibly correspond to two well established phaseolus gene pools. Some of the accessions showed promise against the widely prevalent races of *Colletotrichum lindemuthianum*. Morphological variation for seed colour, shape and size showed no correspondence with molecular diversity.

Key words: Common bean, genetic diversity, RAPD markers, anthracnose

Introduction

In India, common bean (*Phaseolus vulgaris* L.), locally called as 'rajmash' is mainly consumed as a grain legume. Genetic improvement of common bean in India has been accomplished primarily by conservative breeding strategies aimed at satisfying the consumers' preferences for seed size, shape and color. The crop is, however, characterized by an extensive range of variability for various agro-morphological traits. Several morphological traits [1], phaseolin and total seed protein profiles [2] and allozymes [3] have been used for the evaluation of genetic relatedness among cultivars and between cultivars and landraces of common bean. Identification of races and origin of diversification, and the characterization of cultivar identity have also been carried out with these markers. Morphological traits, especially, quantitative are inherently subjected to environmental influences and intense selection pressure during domestication and improvement. A great diversity for agro-morphological traits like, seed size and colour also exists in *Phaseolus* germplasm of north-western Himalayan region comprising the parts of Himachal Pradesh and Jammu & Kashmir. However, it is not known whether this diversity of morphological traits also reflects a greater overall diversity that could be exploited in crop improvement or is merely a reflection of superfluous phenotypic diversity in an otherwise homogenous gene pool.

DNA markers offer precise means to measure genetic diversity and affinity among germplasm collections than the morphological and biochemical markers due to their environmental insensitivity and abundance in genome. The advent of molecular markers has revolutionized phylogenetic studies and characterization of germplasm. Assessments of genetic diversity in *Phaseolus* using restriction fragment length polymorphisms (RFLPs), [4] for single copy genomic sequences, random amplified polymorphic DNAs, RAPDs [5, 6], amplified fragment length polymorphism, AFLPs [7], and more recently the use of chloroplast DNA sequences [8] have been reported. Information regarding genetic variation in the available germplasm is essential for any crop improvement programme. Therefore, in the present study RAPD analysis was conducted to assess genetic diversity in local land races and a collection of exotic accessions representing lines of diverse origin. The germplasm was also evaluated for resistance to anthracnose (*Colletotrichum lindemuthianum*), which is a major constraint in the commercial cultivation of common bean in Himachal Pradesh.

Materials and methods

Plant material: Forty-six genotypes comprising seven local landraces of Himachal Pradesh, India and thirty-nine exotic lines received from International Centre for Tropical Agriculture (CIAT), Cali, Columbia were analyzed for RAPD diversity (Table 1).

Isolation of genomic DNA and PCR amplification: Genomic DNA was extracted from leaf tissue using CTAB (Cetyl trimethyl ammonium bromide) method of Doyle and Doyle [9]. After initial screening (20 primers), four 10-mer random primers (Operon Technologies) *viz.*, OPD 5, OPP 15, OPP 16 and OPJ 20 were selected to study genetic diversity among the genotypes. DNA

Table 1. Common bean genotypes used in the study

Geno type No.	Accession No.	Country of origin	Geno-type No.	Accession No.	Country of origin
1	RKS-885	India	24	EC 385306	USA
2	TRS-1029	India	25	EC 398575-2	USA
3	AS/TRS-11	India	26	EC 398501	USA
4	AS/TRS-13	India	27	EC 400436	USA
5	RKS-385	India	28	EC 398547	USA
6	VJ-930	India	29	EC 398511	USA
7	AS/TRS-417	India	30	EC 405207	Brazil
8	EC 10057	Nepal	31	EC 77004	Brazil
9	EC 18137	Nepal	32	EC 405199	Brazil
10	EC 398574	USA	33	EC 405215	Brazil
11	EC 222737	USA	34	EC 271493	Columbia
12	EC 398556	USA	35	EC 400403	Columbia
13	EC 398575-1	USA	36	EC 348571	Columbia
14	EC 398490	USA	37	EC 400491	Columbia
15	EC 224997	USA	38	EC 385305	Belgium
16	EC 400451	USA	39	EC 78755	Belgium
17	EC 398534	USA	40	EC 132714	Hungry
18	EC 313489	USA	41	EC 397836	Slovakia
19	EC 400415	USA	42	EC 74509	South Africa
20	EC 400418	USA	43	EC 241425	South Africa
21	EC 41655	USA	44	EC 421102	Zambia
22	EC 398537	USA	45	EC 421101	Zambia
23	EC 398558	USA	46	EC 127581	Australia

amplification was performed in 25 µl volume consisting of 2 µl DNA template (25 ng/µl), 1 µl of 5 µM primer, 0.2 µl of Taq DNA polymerase, 17.3 µl of sterilized distilled water, 2.0 µl of dNTP mix (0.2 mM each of dATP, dCTP, dTTP and dGTP) and 2.5 µl of 10 × buffer. Amplification was carried out in a thermal cycler (MJ Research) programmed for 5 min at 94°C for initial denaturation and 40 cycles consisting of 1 min at 94°C, 1 min at 37°C and 2 min at 72°C with final 5 min extension at 72°C using fastest ramp time between the temperature transitions. After amplification, 10 µl of the amplified product from each sample was resolved by electrophoresis using 1.4% agarose gel in 0.5 × Tris Borate EDTA (TBE) buffer (0.05 M tris, 0.05 M boric acid, 1mM EDTA, pH 8.0). The gel was run at 120 V for 90 min. After electrophoresis, the gel was stained with ethidium bromide (0.5 rg/ml) for 10 min, followed by 30 min destaining in running tap water. The destained gel was viewed over an ultraviolet transilluminator and the image was stored in gel documentation system (Bio Rad, CA, USA).

RAPD analysis: A binary data matrix with '1' indicating the presence of a band and '0' indicating its absence was generated. The Numerical Taxonomy System of Multivariate Statistical Programme (NTSYS) software package 1.8 was used for analysis [10]. The Jaccard's similarity coefficient (F = 2Nxy/Nx + Ny, where Nxy is the number of bands either present or absent in both the isolates x and y and Nx + Ny is the total number of bands observed for that pair of isolates) within the SAHN programme was used for the construction of dendrogram by the unweighted pair group method using arithmetic averages (UPGMA) based on NTSYS software.

Evaluation for disease resistance: Two widely prevalent races of *C. lindemuthianum* were used for evaluation of genotypes for resistance to anthracnose. Inoculum was prepared by harvesting conidia from 7-day old cultures multiplied on potato dextrose agar medium in distilled water and spore count was adjusted to 2.1×10^6 spores/ml with the help of haemocytometer. The germinated seed dip method of inoculation was employed for evaluation [11]. The rolled towel method was employed for germination of seeds [12]. Surface sterilized seeds of each genotype were placed in a double layer of moistened germination paper and kept at 25 ± 1°C in a seed germinator with 12 h photoperiod for 3-days. Seed coat of germinating seeds was removed and seeds were dipped in standard spore suspension for 5-10 min. Thereafter the seeds were sown 3.0 cm deep in a plastic tray (30 × 5 cm) containing sterilized river sand. Ten seeds of each genotype were used for inoculation. The inoculated seedlings were kept in a growth room at 22 ± 1° C with more than 90% relative humidity and 12 h photoperiod for 4 days and then transferred to a screen house with a temperature ranging from 21 to 24° C and relative humidity of about 70%.

Disease reaction was recorded after 10-12 days by following 0-5 point scale [13], where 0 = no disease symptoms; 1 = pin point lesions; 2 = small lesions, not sunken; 3 = large sunken lesions; 4 = large deep lesions upto stem centre and 5 = seedling killed by the pathogen. Plants showing reaction types 0, 1 and 2 were categorized as resistant while those showing reaction types 3, 4 and 5 as susceptible.

Results and discussions

Progress in crop breeding requires the exploitation of genetic variation among races and gene pools. Genetic diversity among thirty-seven exotic and nine local accessions of common bean was investigated by RAPD analysis. RAPD profiles of forty-six genotypes generated with primers OPP 15 and OPD 5 are shown in Fig. 1. Number of RAPD bands produced per primer ranged from 9 to 13. In total, 43 scorable bands were obtained, of which 33 were polymorphic. Dendrogram generated through cluster analysis of RAPD data showed a high level of genetic diversity among the genotypes under study. All the genotypes shared 59% of the RAPD amplicons among themselves. Cluster analysis of these genotypes delineated them into 4 groups. Group I represented the genotypes from India, Nepal, Belgium, Brazil, Columbia, Zambia, South Africa and Hungary,

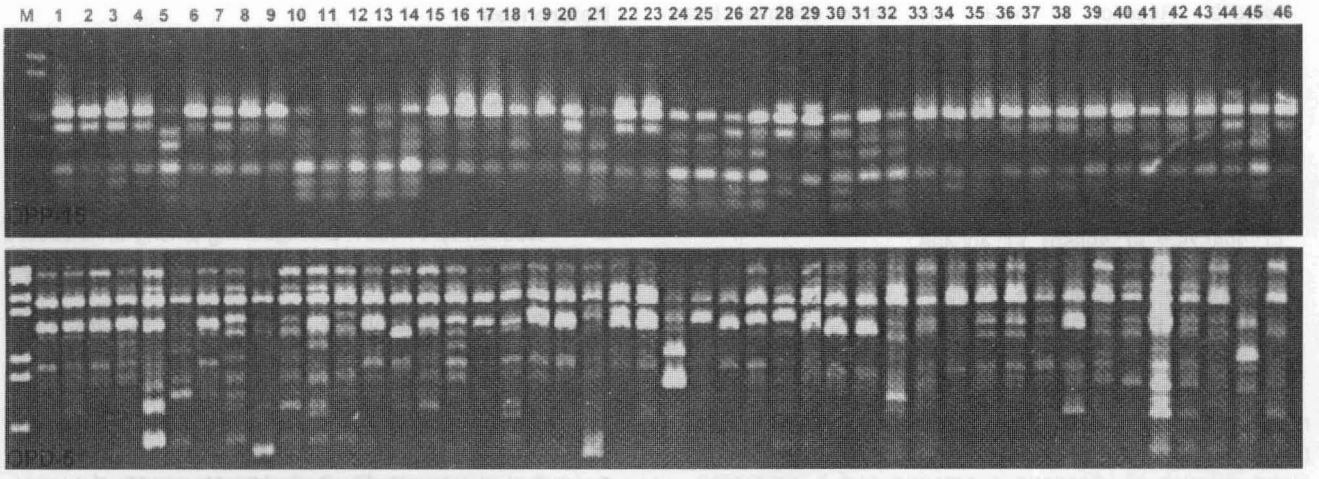

Fig. 1. Random amplified DNA polymorphisms of 46 bean accessions of Indian and exotic origin obtained with primer OPP 15 and OPD 5. Lanes 1 to 48 correspond to the numbers assigned to bean accessions as per table 1. Lanes M : Molecular weight size marker (λ DNA EcoR1/HindIII double digest)

which shared 82% to 100% similarity among themselves. Of the seven local land races, five were included in group I and three of them namely RKS-885, TRS-1029 and AS/TRS-11 were 100% identical. All the accessions from South Africa, Belgium, and Zambia were clustered in group I. Twenty accessions from the USA differentiated into two separate groups. Eleven accessions from the USA sharing 82% genetic similarity were clustered in group II. One accession (EC 10057) from Nepal was also included in group II. Group III was constituted by nine genotypes from USA, two from Brazil and one from India that showed 76% genetic similarity among themselves. Two accessions from Brazil (EC 77004), and Columbia (EC 400491) constituted the group IV with 69% genetic identity. The genotypes representing an accession each from Slovakia (EC 397836), Australia (EC 127581) and India (VJ 930) did not cluster into any of the groups.

The dendrogram (Fig. 2) depicted a high degree of genetic diversity as revealed by the presence of 43 distinct multilocus genotypes among 46 accessions studied. In two instances more than one genotype shared identical multilocus genotype; genotypes RKS-885, TRS 1029 and AS/TRS 11 from India (group I) and genotype EC 398537 and EC 398558 from USA (group II) exhibited identical genotypes at all the RAPD loci.

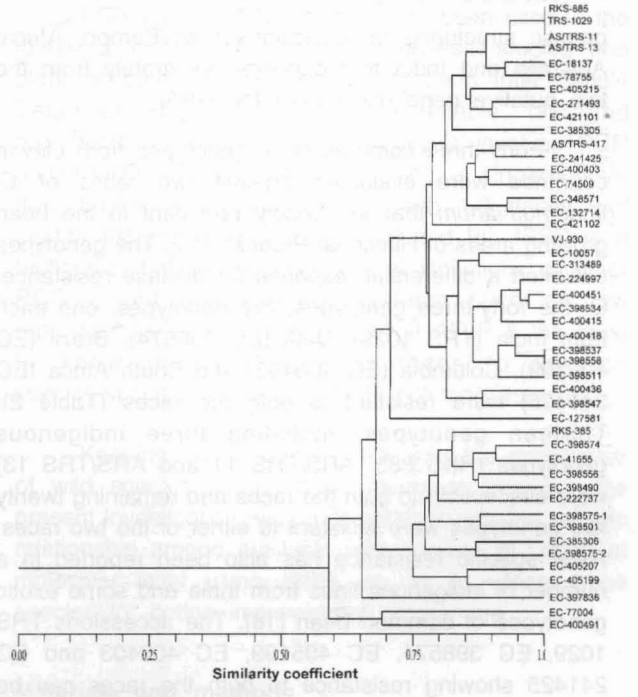

Fig. 2. Dengrogram of 46 accessions of *Phaseolus vulgaris* generated by UPGMA (unweighted pair group method arithmetic mean) analysis of RAPD bands obtained with four random primers (OPD 5, OPP 15, OPP 16 and OPJ 20). Scale at the bottom depicts the similarity values obtained using Jaccard's similarity coefficient

The common bean in its centre of domestication in the Americas has been shown to be organized into two major gene pools, the Meso-American and the Andean based on morphological traits, seed protein and DNA markers [14]. The two clusters (II and III) detected among the accessions from the USA in the present study possibly correspond to two well established gene pools of the species. Domestication of cultivated bean is thought to have occurred about 4000 years ago independently in the Mesoamerica and South America [15]. Following domestication, common bean spread between Mesoamerica and South America and, after European discovery of America, to Europe and Africa and thereafter to other countries. Prolonged cultivation of introduced germplasm under diverse environments coupled with human selection promotes genetic divergence in the introduced domesticates. The same evolutionary forces might have played role in

Table 2. Reaction of Indian and exotic common bean accessions to *Colletotrichum lindemuthianum*

Accession No.	Reaction to	
	Race 1	Race 2
TRS-1029, EC 398574, EC 405199, EC 400403, EC 241425	R	R
RKS-385, VJ-930, EC 18137, SE C222737, EC 398575-1, EC 400415, EC 398558, EC 398575-2, EC 398501, EC 400436, EC 398547, EC 421102, EC 421101	R	S
AS/TRS-417, EC 10057, EC 400451, EC 398534, EC 400418, EC 398511, EC 405207, EC 405215, EC 127581	S	R
RKS-885, AS/TRS-11, AS/TRS-13, EC 398490, EC 224997, EC 313489, EC 398537, EC 77004, EC 271493, EC 348571, EC 400491, EC 132714, EC 74509	S	S
EC 398556	NT	S
EC 385306, EC 385305	R	NT

R = resistant, S = susceptible, NT = not tested

genetic structuring of accessions from Europe, Africa, Australia and India that clustered separately from the two putative gene pools from the USA.

Forty-three common bean genotypes from eleven countries were evaluated against two races of *C. lindemuthianum* that are widely prevalent in the bean growing areas of Himachal Pradesh [16]. The genotypes exhibited a differential response for disease resistance. Of the forty-three genotypes, five genotypes, one each from India (TRS 1029), USA (EC 398574), Brazil (EC 405199), Columbia (EC 400403) and South Africa (EC 241425) were resistant to both the races (Table 2). Thirteen genotypes, including three indigenous genotypes (RKS 885, ARS/TRS 11 and ARS/TRS 13) were susceptible to both the races and remaining twenty five genotypes were resistant to either of the two races. Race specific resistance has also been reported in a number of indigenous lines from India and some exotic genotypes of common bean [16]. The accessions TRS 1029, EC 398574, EC 405199, EC 400403 and EC 241425 showing resistance to both the races can be exploited as resistance donors in local bean breeding programmes.

The overall results indicated that a considerable diversity exists in the set of accessions analyzed in this investigation. Considering the importance of diversity in germplasm improvement and that a greater combining ability is expected in crosses among genetically diverse parents, the genotypes belonging to different groups identified during the present study will constitute promising parents for hybridization in common bean improvement programme. However, the study revealed that the phenotypic manifestation of morphological traits is not always the true reflection of genetic differentiation among common bean germplasm.

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