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Genetic diversity analysis among local bean (*Phaseolus vulgaris* L.) cultivars using RAPD markers

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ABSTRACT

Random amplified polymorphic markers were used to evaluate genetic variations among nine bean cultivars (*Phaseolus vulgaris* L.) of Kinnaur district, Himachal Pradesh. Thirty five polymorphic RAPD markers generated 230 bands. Among these, 209 bands were polymorphic (90.86%) and 45 were unique. The primer OPA09 gave highest polymorphic information content (PIC) value of 0.90 while primer OPA 16 had lowest PIC value (0.58). Resolving power was 20 for OPA-11 and 106 for OPP-16. Jaccard's coefficients based genetic similarity varied from 0.32-0.65. UPGMA based clustering patterns divided cultivars into two main groups and subgroups.

Key words: Common bean, Polymorphism, RAPD markers

The common bean is one of the most important legume crop worldwide (Anonymous 2009). It is major source of micronutrients e.g., iron, zinc, thiamin and folic acid (Pennington and Young 1990, Broughton *et al.* 2003). The annual global bean production is approximately 12 million metric tons, with 5.5 and 2.5 million metric tons alone in Latin America Caribbean (LAC) and Africa, respectively (Broughton *et al.* 2003, Anonymous 2012a). The highest producer is India at more than 4 million metric tons per year (Anonymous 2012b). Thus development of improved cultivars is one of the important objectives of Indian breeding program. However, due to lack of proper characterization of genetic diversity, it could not be used systematically in breeding programs.

Classical methods based on morpho-agronomic traits have been used to characterize genetic diversity among commercial cultivars, landraces and wild cultivars. Although, morphological data are easy to use for identification of genotypes, the number of distinctive characteristics is limited. Moreover, agronomic characteristics are often multi-genic and their expression is easily influenced by the environment. DNA markers developed to study genetic diversity and crop evolution are considered to be better as compared to morphologic markers (Burle *et al.* 2010). They can reveal differences among genotypes at DNA level and provides direct, reliable and efficient tool for germplasm characterization, conservation and management. The molecular markers, including RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA) and AFLP (amplified fragment length polymorphism) have been explored in common bean diversity studies. RAPD approach using arbitrary primers requires only nanogram quantities of template DNA, no radioactive probes and is relatively simple as compared to other techniques. RAPD and AFLP markers have been used to investigate genetic variability within landraces of common bean (Ojuederie *et al.* 2014, Ariyarathna *et al.* 2013). Microsatellite markers have been used to study the genetic variability and gene pool identity among common bean genotypes (Zhang *et al.* 2008). Newly discovered techniques like Td-DAMD-PCR, Td-SSR and CAPS-microsatellite have also been used to study bean diversity (Ince and Karaca 2011). Information on biodiversity studies using DNA markers in local common bean cultivars of Kinnaur district of Himachal Pradesh is lacking. Therefore aim of present study was to assess the genetic diversity among the local common bean cultivars using RAPD markers.

MATERIALS AND METHODS

Plant material: Local bean cultivars *viz.,* Luxmi, Contender, Kanchan, Kaju, Triloki, Baspa, Jawala, Kailash and Capsule were used to study the genetic diversity. The seeds of these cultivars were procured from Vegetable Research Station, Kalpa, Kinnaur district, Himachal Pradesh.

Isolation of genomic DNA: Genomic DNA was isolated from young leaves of plants grown in pots [by adding insoluble polyvinylpyrollidone (PVP) in tender leaves (10% (w/w)] by the method of Doyle and Doyle (1987). DNA was purified by successive RNase treatment followed by phenol: chloroform extraction. The DNA pellet was washed with 70% ethanol, vacuum dried and dissolved in of TE buffer 200 µl depending upon its yield. These aliquots of DNA were stored at -20^{0}C for further use. The quality of DNA was checked by calculating the ratio of absorbance at 260 nm and 280 nm, using a UV/VIS Spectrophotometer. To determine DNA concentration, an aliquot of DNA samples were suitably diluted and absorbance (A) was determined at 260 nm.

PCR Amplification and Gel Electrophoresis: The DNA samples were amplified using PCR as described by Williams *et al.* (1990). Forty arbitrary 10-mer primers were used for PCR amplification. The polymerase chain reaction was performed in a reaction volume of 20 µl contained 11.7 µl of sterile distilled water, 2 µl Taq DNA polymerase buffer (1X),

1 µl $MgCl_2$ (2 mM), 2 µl dNTPs (1 mM), 0.3 µl Taq DNA polymerase (1 U), 2 µl random primer (10 pmol) and 1 µl genomic DNA (50-100 ng). All the samples were given initial denaturation at 94ºC for 5 minutes in a thermal cycler. Forty five PCR cycles were carried out for OPA series primers and 42 cycles for OPP series primers. Each cycle consisted of 30 seconds of denaturation at 94ºC, 1 minute of annealing at 35ºC for OPP series primers and 37ºC for OPA series primers, 2 minutes of extension at 72ºC and a final extension of 7 minutes at 72ºC. PCR products were allowed to stand at 4ºC for 5 minutes. The amplification products were separated on 1.2% agarose gel. Gene Ruler TM, 100bp-3kb ladder was used as standard and the gel was run at 65 V. The image of the amplified DNA was using gel documentation system.

Data analysis: Each amplified product was scored for their presence or absence. Co-migrating bands were considered to represent the same locus and thus treated as the same band while scoring. Presence of an amplified product was designated as '1' and absence was marked as '0'. Intensity of the bands was not taken into consideration while scoring. A pair wise similarity index was constructed using Jaccard coefficients, it was subjected to UPGMA cluster analysis and a dendrogram was constructed using NTSYS-pc, version 2.02h software (Rohlf 1998). Polymorphic information content (PIC value) for each primer was calculated as described by Anderson *et al.* (1993). The power of each primer to distinguish among common bean cultivars was evaluated by resolving power (Rp) (Prevost and Wilkinson 1999).

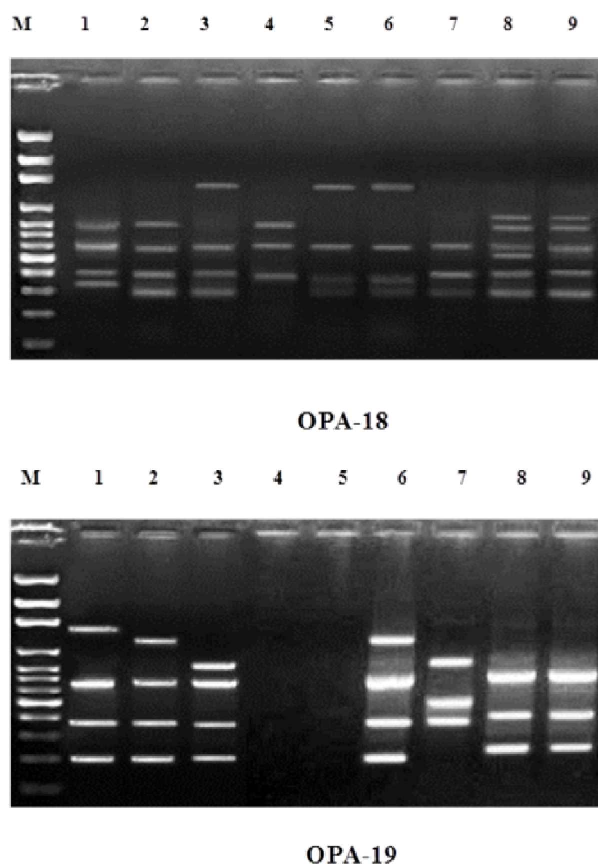

Figure 1. RAPD profile using primer OPA-18 and OPA-19 (M-marker, 1-Luxmi 2-Contender 3-Triloki 4-Capsule 5-Kanchan 6-Kaju 7-Baspa 8-Kailash 9-Jawala)

RESULTS AND DISCUSSION

The CTAB method has been successfully used for DNA extraction from common bean leaves (Kumar *et al.* 2014, Cabral *et al.* 2011). However, insoluble PVP during DNA extraction from leaves of bean seedlings has not been used by other workers. Razvi *et al.* (2013) isolated genomic DNA from common bean leaves by using modified CTAB method. Sadeghi and Cheghamirza (2012) also used modified CTAB method for genomic DNA extraction from common bean leaves. Molecular characterization of local bean cultivars was investigated using 40 random decamer primers. DNA polymorphism was shown by 35 primers and the primer generated a unique set of amplification products ranging from 102 bp (OPA-02) to 1950 bp (OPP-04) (Fig.1 & Fig. 2). Number of bands for each primer ranged from 3 (OPA-10, OPA-13 and OPA-16) to 12 (OPA-19). Total numbers of bands amplified were 230, out of which 209 were polymorphic and 21 were monomorphic (Table 1). Percentage of polymorphic bands ranged from 76.4% to 82.4% and 45 unique bands were obtained. Specific RAPD markers obtained for cultivars could be used for their identification. Maximum number of unique bands was obtained for Triloki and Baspa cultivars and minimum number for Contender cultivar. Ariyarathna *et al.* (2013) used 5 RAPD primers (OPA-1, OPA-08, OPD-03, OPB-17 and OPG-05) to investigate genetic diversity among 30 bean genotypes, which generated 18 polymorphic bands and level of polymorphism was 20 %. Razvi *et al.* (2013) used 15 primers to discriminate 13 common bean genotypes, out of which 7 (OPA-01, OPA-02, OPA-03, OPA-10, OPA-11, OPA-13 and OPA-15) showed polymorphism. They reported 63 polymorphic bands with 96.62 % polymorphism. Filimon *et al.* (2011) used 8 RAPD primers to evaluate genetic diversity among 8 common bean (*Phaseolus vulgaris* L.) cultivars, out of which 4 primers (OPD-08, OPG-03, OPG-12 and OPY-20) showed polymorphism. They obtained 33 bands out of which 17 were polymorphic and 16 monomorphic, showing 48.84% polymorphism. Szilagyi *et al.* (2011) obtained 56 DNA bands using 4 primers (OPA-17, OPG-05, OPG-06 and OPG-14) from 20 common bean cultivars out of which 29 bands were polymorphic, showing 51.78% polymorphism. Mavromatis *et al.* (2010) obtained two poly morphic bands out of 160 bands using 20 random primers (OPB-07, OPB-10, OPB-16, OPB-17, OPC-01, OPC-03, OPC-04, OPC-05, OPC-06, OPC-07, OPC-08, OPC-09, OPC-10, OPD-11, OPD-20, OPE-14, OPE-06, OPF-04, OPF-11 and OPF-19) to assess genetic diversity among main local landraces and commercial cultivars of *Phaseolus vulgaris* L. cultivated in Greece. Jaccard's similarity

Table 1. Summary of RAPD amplified products from bean cultivars

Description	Luxmi	Contender	Triloki	Capsule	Kanchan	Kaju	Baspa	Kailash	Jawala
Number of bands scored	105	89	109	110	104	119	102	109	105
Number of monomorphic bands	21	21	21	21	21	21	21	21	21
Number of polymorphic bands	84	68	88	89	83	98	81	88	84
Average number of fragments per primer	3.0	2.54	3.11	3.14	2.97	3.4	2.91	3.11	3.0
Average number of polymorphic bands per primer	2.4	1.94	2.51	2.54	2.37	2.8	2.31	2.51	2.4
Percentage of total polymorphic bands	80.0	76.4	80.7	80.9	79.8	82.4	79.4	80.7	80.0
Number of unique bands	5	3	8	4	4	4	8	5	4

coefficient ranged from 0.32 to 0.65 indicating sufficient genetic diversity among bean cultivars used in present studies. Low similarity value was observed between Triloki and Jawala cultivars and highest between Kailash and Jawala cultivars. Razvi *et al.* (2013) reported similarity coefficient values of 27.72-82.35 in 13 bean genotypes by using Dice coefficient. Filimon *et al.* (2011) calculated genetic similarity by Nei and Li coefficient in 8 common bean cultivars and reported it to vary greatly (60% - 96%). Mavromatis *et al.* (2010) reported Jaccard's similarity values in common bean genotypes to range from 0.84-0.98. Jose *et al.* (2009) reported Jaccard's similarity coefficient in common bean to range from 0.50 to 0.95. In present studies, PIC value ranged from 0.58 (OPA-16) to 0.90 (OPA-09) and resolving power ranged from 20 (OPA-11) to 106 (OPP-16) were obtained. Sadeghi and Cheghamirza (2012) reported mean PIC value for RAPD primers in 21 common bean genotype to be 0.382. Blair *et al.* (2006) calculated PIC value for 129 microsatellite markers in 44 common bean genotypes and reported it to range from 0.446-0.594. Buso *et al.* (2006) also calculated PIC value in 85 representative accessions of bean from gene bank and it ranged from 0.23 to 0.80.

Cluster analysis of RAPD data obtained during present studies was used to construct dendrogram using UPGMA cluster analysis (Rohlf 1998). This resulted in two main clusters *viz.,* A and B. The cluster A was subdivided further into 2 sub-clusters C and D (Fig. 3). Sub cluster C was further divided into 2 sub-clusters E and F, which included Luxmi and Contender cultivars, showing 72 % similarity with each other. Sub cluster D included only Triloki cultivar, showing 66 % similarity with Luxmi and Contender. Cluster B was also divided further into 2 sub-clusters G and H. The sub-cluster G had further 2 sub-clusters I and J. Cluster I included Capsule and Kanchan cultivars showing 71 % similarity with each other. Cluster J included Kaju and Baspa cultivars, which showed 74 % similarity with each other. Kailash and Jawala were included in a separate cluster H and they showed maximum similarity of 79 %. Both clusters A and B merged into a single cluster at 39% similarity. Genetic similarity was also compared with morphological traits like seed colour and seed size but these characters did not seem to be related with molecular analysis. Mavromatis *et al.* (2010) also found seed morphological characteristics and agronomic performance in main local landraces and commercial cultivars of *Phaseolus vulgaris*

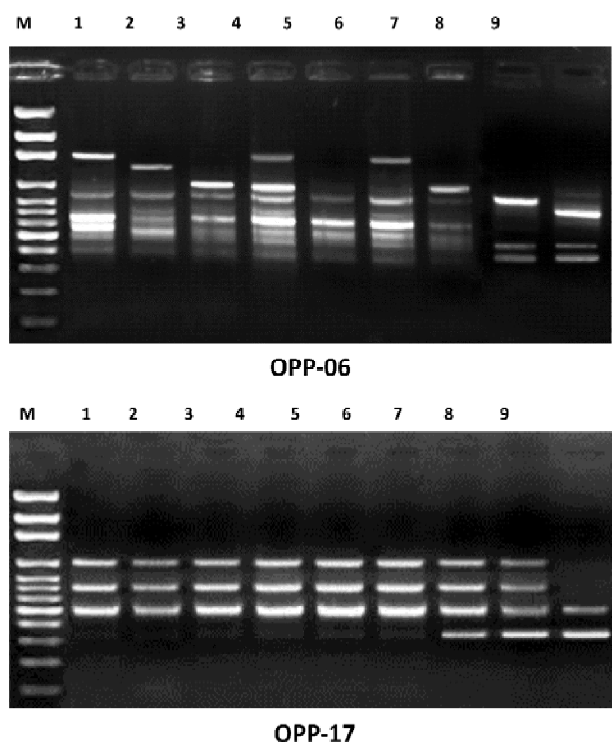

Figure 2. RAPD profile using primer OPP-06 and OPP-17 (M-marker, 1-Luxmi 2-Contender 3-Triloki 4-Capsule 5-Kanchan 6-Kaju 7-Baspa 8-Kailash 9-Jawala)

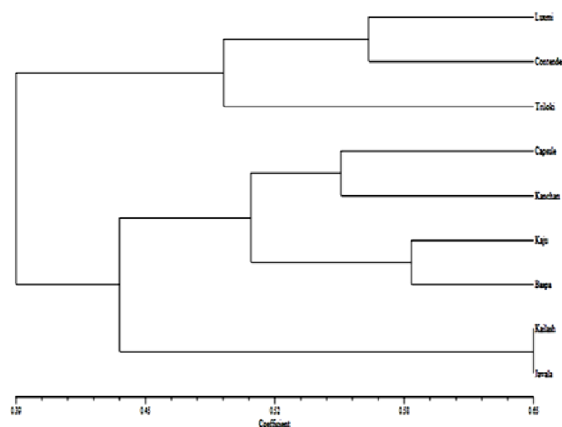

Figure 3. RAPD dendrogram using Jaccard's coefficient

L. of Greece not to be related with genetic similarity. Razvi *et al.* (2013) constructed dendrogram based on RAPD data using UPGMA (Dice coefficient) that divided common bean genotypes into 3 clusters in which cluster-1 contained maximum number of 7 genotypes, cluster-2 contained 5 genotypes and cluster-3 contained only 1 genotype. Filimon *et al.* (2011) constructed dendrogram based on Nei and Li (1979) that divided 8 common bean cultivars into two main clusters (A and B). Cluster A comprised of all Romanian bean cultivars while cluster B contained all Columbian cultivars. Biswas *et al.* (2010) constructed a dendrogram based on genetic distance in 14 French bean genotypes as described by Nei's (1972) and obtained 2 main clusters. The findings of the present investigations indicated high genetic diversity in local bean cultivars of Kinnaur district of Himachal Pradesh, which could be used in bean breeding programs. RAPD markers were found to be effective in assessing diversity. Specific RAPD markers obtained for cultivars could be used for their identification.

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