International Journal of Applied And Pure Science and Agriculture

www.ijapsa.com

DNA profiling and phylogenetic relationship among common bean (*Phaseolus Vulgaris* L.) Genotypes using RAPD markers

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Absract

Common bean (Phaseolus vulgaris L.; Fabaceae) is a worldwide interesting crop as both grain legume and fresh vegetable. An understanding of genetic diversity is essential for proper utilization of genotypes in the target oriented research programmes. 15 RAPD primers generated a total of 85 bands/alleles out of which 56 bands were polymorphic with an average of 4 bands per primer and documented the 58.24 % polymorphism. Calculated values for PIC ranged from 0.142 (OPB-09) to 0.874 (OPD-07) with an average of 0.698 and the average RAPD primer index value was per primer. The genotype identification through molecular marker resulted in developing highly diversified dendrogram of 12 common bean genotypes. The data revealed that molecular techniques are more precise and more accurate & can be used for genetic diversity analysis of common bean genotypes.

Keywords: RAPD, PIC, dendrogram

I. Introduction

Common Bean is an annual, herbaceous plant. It has a fibrous root system, and an erect, twining stem with small side branches. Leaves are large and trifoliate. They grow from successfully pollinated flowers.

It is a plant domesticated independently in ancient Mesoamerica and the Andes, and now grown worldwide for its edible bean, popular both as a dry and green bean. The leaf is occasionally used as a leaf vegetable, and the straw is used for fodder. Beans, squash and maize constitute the "**Three Sisters**" that provides the foundation of Native American agriculture.

Common bean (*Phaseolus vulgaris* L.) is an annual, diploid (2n = 2X = 22) species derived from wild ancestors distributed from Northern Mexico to Northwestern Argentina. Mesoamerica and the Andes (Gepts, 1988) and possibly in a third minor centre in the northern Andes (Islam *et al*., 2002). The crop is consumed principally for its dry (mature) beans, shell beans (seeds at physiological maturity), and green pods.(Tiwari *et al*,2005)

Commonbean (*Phaseolus vulgaris* L.) is the primary source of protein in the human diet in some countries, such as Brazil. It consists of two major gene pools, a Mesoamerican and an Andean one, as determined by morphological and phaseolin seed protein attributes (Gepts 1988).

The Andean form has much larger seeds (>40 gm/100 seed weight), with a different type phaseolin. From a genomic perspective, Indian bean has a small genome comparable to rice, estimated to be about 450–650 million base pairs (Mb)/haploid (McClean *et al*. 2004).

The common bean is characterized by an extensive range of genetic variation, Biochemical, physiological, and morphological evidence strongly suggests that this variation is not distributed at random but is associated with two distinct centers of diversity commonly known as the Andean and Middle American gene pools (Gepts and Bliss, 1985; Gepts et al., 1986). Each of these gene pools has been subdivided into races based primarily on comprehensive analyses of germplasm within the Andean and Middle American centers (Singh et al., 1991a). Beau breeders have often confined

hybridization within specific races or seed types (Adams, 1972; Zaumeyer, 1972). Long-term genetic improvement of common bean will likely require methods that allow for more efficient use of the vast array of genetic resources available. Constructing comprehensive genetic linkage maps using DNA markers may promote the use of-genetic variation in breeding programs (Paterson et al., 1991).

II. Materials and Method

Plant materials

The leaves of Indian bean Genotypes used for the present study were obtained from Vegetable Research Station. A total of 12 hybrid varieties of common bean were analyzed in this present study. The 12 breeding lines, OPA-01, OPA-12, OPA-16, OPA-17, OPB-08, OPB-09, OPB-11, OPC-04, OPC-06, OPC-11, 0PC-13, OPD-07, OPD-08 & OPF-09 are good genetic sources for high yield potential, as a medicinal use & as a dietary source.

The experimental material comprised of 20 Genotypes of indianbean which are listed below.

1	**10/DOLPVAR-1**	7	**10/DOLPVAR-7**
2	**10/DOLPVAR-2**	8	**10/DOLPVAR-8**
3	**10/DOLPVAR-3**	9	**10/DOLPVAR-9**
4	**10/DOLPVAR-4**	10	**10/DOLPVAR-10**
5	**10/DOLPVAR-5**	11	**SWARNA UTKRISHTA (NC)**
6	**10/DOLPVAR-6**	12	**GJIB-11 (SC)**

DNA EXTRACTION:

Total genomic DNA was isolated from young leaves of different genotypes of Indianbean plants which were grown in field. The DNA extraction was carried out by CTAB method as described by Doyle and Doyle (1990) with minor modifications.

SR. NO.	RAPD Primer	Sequence (5`→3`)
1.	OPA-01	CAGGCCCTTC
2.	OPA-12	TCGGCGATAG
3.	OPA-16	AGCCAGCGAA
4.	OPA-17	GACCGCTTGT
5.	OPB-09	TGGGGGACTC
6.	OPB-11	GTAGACCCGT
7.	OPB-08	GTGACGTAGG
8.	OPC-04	CCGCATCTAC
9.	OPC-06	GAACGGACTC
10.	OPC-11	AAAGCTGCGG
11.	OPC-13	AAGCCTCGTC
12.	OPD-07	TTGGCACGGG
13.	OPD-08	GTGTGCCCCA
14.	OPF-09	CCAAGCTTCC

RAPD Marker Assay

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The strains were genotyped using 14 SSR primers. PCR was performed in a 25 µL reaction with 2.5 µL of 10X buffer, 2.0 µL of dNTPs, 1µL of primer (100ng/µL), 0.3 µL of taq polymerase and 1µL of template DNA (20ng/µL). The PCR protocol comprised of the initial denaturation of 94oC for 5 min. This was followed by repeat of 39 cycles of denaturation at 94oC for 1 min, annealing at 46-51oC for 2 min and extension at 72oC for 2 min followed by final extension at 72oC for 10 min and stored at 4oC. Amplification product andloading dye were mixed in 10:1 ratio and fractioned on 2.0% agarose gel. Electrophoresis was performed for 2.5h with constant voltage of 80 V.

RAPD Data Scoring and Analysis

In order to score and preserve banding pattern photograph of the gel was taken by a Gel Documentation System, under UV transilluminator. RAPD bands were designated on the basis of their molecular size (length of polynucleotide amplified). 100 bp DNA ladder for PCR product loaded simultaneously with primer products in the gel was used to estimate the molecular size. The distance run by amplified fragments from the well was translated to molecular size with reference to molecular weight of marker. The presence of each band was scored as '1' and its absence as '0'. Faintly visible bands were not scored. Clear and distinct bands amplified by RAPD primers were scored for the presence (1) and absence (0) for the corresponding band among the Genotypes. The data were entered in to MS-Excel data sheet and subsequently analyzed using NTSYSpc version 2.02 (Rohlf, 1994).

III. RESULTS

The highest numbers of 10 bands were produced by OPB-11 primer and the lowest number of 1 band by OPA-12 . The largest fragment of 8192 bp was amplified by OPA-16 and the smallest fragment of 104 bp was obtained with OPC-13. The polymorphic information content (**PIC**) was calculated for each primer and it varied between 0.142 (OPB-09) and 0.874 (OPD-07) with an average of 0.698 per primer. RAPD primer index (**RPI**) ranged from zero to 8.48 with an average of 4.78 per primer. The highest RPI value was obtained by OPB-11 and the lowest was obtained by OPB-09 and OPA-12

14 RAPD primers amplified a total of 85 bands/alleles out of which 56 bands were polymorphic with an average of 4 bands per primer while remaining 29 bands were monomorphic and out of 85 polymorphic bands 56 were polymorphic, 79 shared band & 12 unique. The percentage of polymorphic markers varied from 25 to 100% with an average of 58.24 % polymorphism per primer. 3 primers OPB-08, OPC-13 and OPF-09 resulted in 100 % polymorphism, while 8 primers OPA-01, OPB-09, OPB-11, OPC-04, OPC-06, OPC-11, OPD-07 and OPD-08 gave 60 %, 50.0 %, 90 %, 77.7%, 60%, 75%, 25% and 77.7 % polymorphism respectively & in 3 primer OPA-12, OPA-16, OPA-17 0% polymorphism was found.

Clustering pattern using RAPD data:

The RAPD data were subjected to statistical analysis for the calculation of Jaccard's similarity coefficient and cluster analysis by UPGMA (unweighted pair-group method with arithmetic averages) using NTSYSpc-2.02i software

The dendrogram constructed using UPGMA based on Jaccard's similarity coefficient for RAPD data of 12 commenbean genotypes is depicted in 12 commenbean genotypes were grouped into two main clusters viz. cluster-A and B. The cluster-B consisted of only one genotype viz.

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10/DOLPVAR-8. The cluster-A consisted of 11 genotypes and was divided into two main subcluster-A1 and A2. The subcluster-A1 consisted of 10 genotypes and segregate into two groups-A1(a) and A1(b). The group-A1(a) consisted of 4 genotypes and which are 10/DOLPVAR-1, 10/DOLPVAR-2, 10/DOLPVAR-3, and 10/DOLPVAR-10, group-A1(b) includes 6 genotypes which are 10/DOLPVAR-4, 10/DOLPVAR-5, 10/DOLPVAR-6, 10/DOLPVAR-7, 10/DOLPVAR-9, SWARNA-UTKRISHTA.

The subcluster-A2 consisted of only 1 genotype GJIB-11. The dendrogram constructed using the RAPD data clearly distinguished all genotypes. Genotypes 10/DOLPVAR-5 and 10/DOLPVAR-6 were found in one cluster with maximum 50% similarity. RAPD data revealed that two genotypes10/DOLPVAR-5 and 10/DOLPVAR-8 showed minimum similarity (13%).

To test the goodness of fit of the clustering of RAPD data, matrixes of cophenetic values were also computed using the program **COPH** In the present study also the Mantel test statistic-Z was normalized and degree of goodness of fit for a cluster analysis (Matrix correlation **r = 0.8**) was found to fall under the category of **"good fit"**, as categorized by Rohlf (2000).

IV. Conclusions

The genetic diversity observed at molecular level in 12 common bean genotypes were studied using RAPD marker. Total genomic DNA was isolated from leaf tissues of different Indian bean genotypes grown in field. DNA extraction was carried out by CTAB method as described by Doyle and Doyle (1987) with necessary modifications. In order to perform PCR based analysis, the DNA was quantified in PicroDrop Spectrophotometer to determine DNA concentration by calculating the absorbance ratio at A_{260}/A_{280} and it was ranged from 213.70 ng/µl to 11072 ng/µl with optical density near about 1.80 indicating that genomic DNA extracted in all the samples.

Out of 15 RAPD primers screened, 14 primers amplified a total of 85 bands/alleles out of which 56 bands were polymorphic with an average of 4 bands per primer, while remaining 29 bands were monomorphic. The average RAPD polymorphism was 58.24 %. The polymorphic information content (PIC) was varying from 0 to 0.874 with an average of 0.698 per primer. The phylogenetic tree constructed by UPGMA method generated two main clusters. Cluster B consisted of only one genotype 10/DOLPVAR-8, while cluster A consisted of rest of the genotypes grouped together in their respective sub-clusters.

The present study indicated that the RAPD markers resulted higher percentage of polymorphic loci and greater range of genetic distance (means lower genetic similarity) among genotypes. If the most important criteria for determining choice of assay are number of polymorphic alleles amplified per primer, polymorphism information content (PIC), primer index value.

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