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GENETIC DIVERSITY OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) GERMPLASM BASED ON RANDOM AMPLIFIED POLYMORPHIC DNA MARKERS

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ABSTRACT

Genetic diversity of 30 bean germplasm (*Phaseolus vulgaris* L.) was investigated using Random Amplified Polymorphic DNA (RAPD) markers. A total of 5 random primers (OPA1, OPA8, OPD3, OPB17 and OPG5) identified 18 polymorphic bands, having an average of 3.6 bands/primer. With cluster analysis, germplasm were grouped into two main clusters on the basis of the Jaccard coefficient and Unweighted Pair Group Method and Arithmetic Average (UPGMA). The average genetic distances between germplasm were calculated on the basis of marker frequencies. These distances were used for grouping the germplasm by UPGMA resulting in two main groups in dendogram. These two groups may have a relationship with two centres of origin of bean namely Middle American and Andian. It was concluded that RAPD markers could be used as an alternative or supplementary tools to prevailing methods for the evaluation and classification of bean genetic resources in Sri Lanka.

KEYWORDS: Common bean, Genetic diversity, *Phaseolus vulgaris*, RAPD markers.

INTRODUCTION

In conventional plant breeding programs, plants are selected in terms of their phenotypic characters and the evaluation procedures are time consuming and relatively expensive. A more efficient procedure for germplasm evaluation has been sought by employing biochemical markers such as isozymes (Nielsen, 1985). However, isozyme expression is also dependent on environment, development and tissue type (Tsaftaris, 1987). DNA-based markers such as RFLPs, RAPDs, AFLPs and SSRs have been successfully used for germplasm identification and characterization. These types of markers are phenotypically neutral, environmentally independent and not influenced by epistatic interactions (Koutita *et al.*, 2005).

Polymerase chain reaction (PCR) technology has led to the development of simple and quick techniques called RAPD (Williams *et al.*, 1990) which detects PCR fragment polymorphisms, using a single primer of arbitrary nucleotide sequence. Due to their inexpensiveness and simplicity they have been widely used for a number of

applications in plant breeding and genetic studies. In common bean, RAPD markers have been used to estimate intra-specific genetic relationships between genotypes (Alvarez *et al.*, 1998; Galvan *et al.*, 2006; Marotti *et al.*, 2007, Filimon *et al.*, 2011) and inter-specific genetic relationships between cultivars, accessions and landraces (Skröch and Nienhuis, 1995; Briand *et al.*, 1998; Duarte *et al.*, 1999; Ocampo *et al.*, 2005; Tiwari *et al.*, 2005).

The common bean was introduced to Sri Lanka in the 17th century by Dutch invaders and its' long-term cultivation at distinct micro-environments, combined with the extensive genetic heterogeneity, led to various landraces with particular genetic and morphological traits. Evidence based on allozymes (Singh *et al.*, 1991b), seed proteins (Gepts and Bliss, 1986), morphological traits (Nodari *et al.*, 1992) indicate that two major genepools exist in cultivated common bean, as Middle American (MA) and Andean South American (AA). These two genepools have been subdivided into races with comprehensive analysis of germplasm by Singh *et al.* (1991a). Beebe *et al.* (2001) stated that most of the bean types cultivated in Europe belong to Andean genetic pool (large-seeded race Nueva Granada). Majority of the landraces in Nilagiris, India belong to Andian genepool (Jose *et al.*, 2009) In Sri Lanka, selections have been made from landraces cultivated by farmers and have been developed as commercial varieties. Such local germplasm constitute valuable genetic resources that could be commercially exploited following appropriate evaluation and selection and can be used in hybridization programmes. The present investigation was aimed at assessing the genetic diversity of local germplasm and exotic common bean materials, based on RAPD molecular markers.

MATERIALS AND METHODS

Isolation of plant DNA

This study was conducted in Horticultural Crops Research and Development Institute, Gannoruwa, Peradeniya, using 11 local landraces, 3 locally released varieties and 16 exotic varieties (Table 1). The immature trifoliate leaves from each germplasm were used for extracting DNA. Total genomic DNA was isolated from each individual plant by CTAB method of Rogers and Bendich (1988).

RAPD markers

Twenty RAPD primers (Operon Thechnologies) were selected from the information gathered previously in common bean genetic evaluation (Jose *et al.*, 2009). The genomic DNA isolated from the varieties/landraces was used for RAPD analysis. Polymerase chain reaction was performed in a 15 µl reaction volume containing 1.25 unit *Taq* DNA polymerase (Promega, GoTag DNA polymerase 5 u/µl), $MgCl_2$ (25mM,

Promega), 10mM dNTP's (Promega), 0.2 μM primer and 50ng template DNA. Amplification was performed in Multi Gene Thermal Cycler (Applied Biosystems, USA).

Table 1. ***Pheseolus vulgaris*** **germplasm used for the evaluation.**

Source	Origin of seeds	Growth Habit	Seed Size	Seed color	Flower color	Pod color
Karunarathna TB (1)	SL	Vine	Medium	Brown	White	Green
Karunarathna Dhanapala (2)	SL	Vine	Small	Brown	White	Green
Kaha Ata Role (3)	SL	Vine	Medium	Light Brown	White	Green
Kapri AB (4)	SL	Vine	Medium	Light Brown	White	Green
Karunarathna Mand.	SL	Vine	Medium	Dark Brown	White	Green
CBIII (6)	China	Vine	Medium	Black	Purple	Green
CB II (7)	China	Vine	Medium	Brown	White	Light Green
BIL (8)	SL	Vine	Medium	Brown	White	Light Green
AC#01398 (9)	SL	Vine	Medium	Brown	White	Light Green
KWG (10)	USA	Vine	Medium	Chocolate brown	White	Green
Kapri Wlimada	SL	Vine	Medium	Light Brown	White	Light Green
PBII (12)	SL	Vine	Medium	Light Brown	White	Green
Lanka Butter (13)	SL	Vine	Small	Black	Purple	Yellow
PC50 (14)	USA	Bush	Large	Coffee brown	White	Green
Aurora (15)	USA	Bush	Small	Black	White	Green
Contender (16)	USA	Bush	Small	Light Brown	White	Green
A55 (17)	USA	Bush	Small	Black	White	Green
Kappatipolanill	SL	Vine	Medium	Dark Purple	Purple	Green
CSW (19)	USA	Vine	Small	White	White	Green
Wade (20)	USA	Bush	Medium	Violet Brown	Purple	Green
Top Crop (21)	USA	Bush	Medium	Coffee Brown	Light	Green
Sitti Murunga (22)	Sri	Vine	Medium	Black	Purple	Green
CNC (23)	USA	Vine	Small	White	purple	Green
Cheroki wax (24)	USA	Bush	Medium	Black	Purple	Light
BN 16 (25)	SL	Vine	Medium	Black	Purple	Green
UB (26)	Sri	Vine	Medium	Brown	White	Green
CIAT (27)	Colom	Bush	Large	Coffee brown	White	Green
PB-8-18 (28)	Sri	Vine	Medium	Brown	White	Green
CB- I (29)	China	Vine	Medium	Brown	White	Green
PB- 1 (30)	Sri	Vine	Small	Black	Purple	Green

After initial denaturation at 94 °C for 4 minutes, PCR was run for 40 cycles consisting of a denaturation step at 94 °C for 30 seconds, primer annealing step at 36 °C for 1 minute and extension step at 72 °C for 2 minutes. At the end of the PCR-programme, the final extension period was appended for 10 minutes at 72 °C. Amplification products were separated in 2 % agarose gels using TAE buffer, stained

with ethidium bromide, visualized under an UV illuminator, and photographed. The primers which showed polymorphic bands were tested more than twice with germplasm to confirm the reproducibility of RAPD products.

Statistical analysis

Each amplified RAPD marker was treated as a unit character and was scored as present (1) and absent (0) for all genotypes and a data matrix was created. DNA bands of identical gel migration were assumed to represent the same allele at a locus. Only reproducible and clearly visible bands in gel were scored. The bands detected in all plant samples at the same position as well as bands not directly comparable between individuals were excluded from the analysis. Genetic similarities between germplasm were calculated using Jaccard coefficient (Sij) based on Rohlf (2000):

$$Sij = a / (a + b + c + d)$$

Where,

Sij is the similarity between two individuals i and j,
a is the number of bands scored in the both individuals,
b is the number of bands detected only in the individual i,
c is the number of bands detected only in the individual j, and
d is the number of bands absent in the both individuals.

The pair-wise similarity matrices produced were used to construct dendrograms based on the Unweighted Pair Group Method and Arithmetic average (UPGMA) method using the software SAS 9.1. The hierarchical dendrogram was used to determine the extent of distinct clusters from different germplasm.

RESULTS AND DISSCUSSION

Wide variations were observed among germplasm in seed size, seed colour, plant type, flower colour and pod colour (Table 1). A set of 20 random primers was tested with DNA samples and 15 of them were rejected from further analysis due to poor amplification and monomorphic or non-reproducible pattern. The five primers that successfully amplified were OPA1, OPA8, OPD3, OPB17 and OPG5. RAPD profile of 15 bean varieties/germplasm and 100 bp DNA ladder produced using the random decamer primers OPA1 is given in Figure 1. A total of 18 polymorpic bands ranging in size from 400 to 3,000 bp were detected in these 5 primers (Table 2).

Table 2. Polymorphism detected by five random primers on 30 bean germplasm.

RAPD primers	*Sequence*	*Number of bands*	*Number of polymorphic markers*	*Polymorphism (%)*
OPA-1	CAGGCCCTTC	6	6	100
OPA-8	ACTGGCTCTG	2	2	100
OPD-3	ACGGATCCTG	2	1	50
OPB-17	AGGGAACGAG	3	3	100
OPG-5	CTGAGACGGA	6	6	100
Total		19	18	

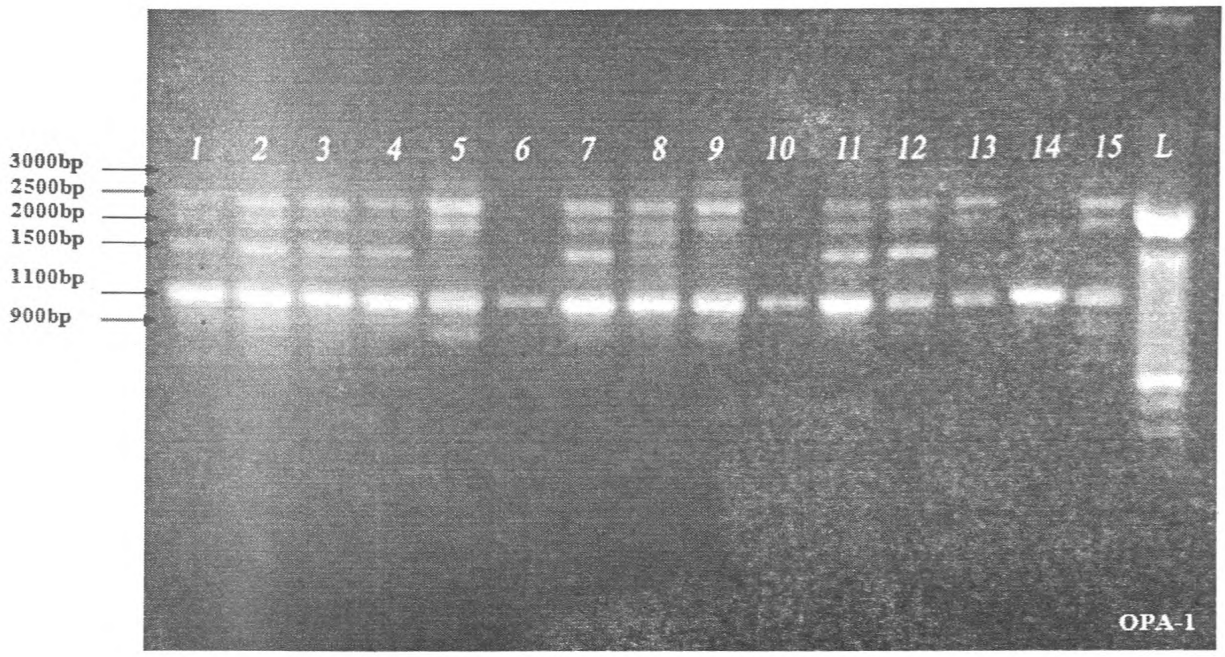

Figure 1. **RAPD profile of 15 bean varieties/germplasm and 100 bp DNA ladder produced using the random decamer primers OPA1.**
Note: Lane numbers are corresponding to the germplasm given in Table 1.

The number of bands obtained for each primer ranged from 2 to 6, with a mean of 3.6 bands/primer (Table 2). The level of polymorphism detected in present study was 20 % and it was low compared to the studies reported for bean (Skroch and Nienhuis, 1995; Alvarez *et al.*, 1998; Briand *et al.*, 1998; Duarte *et al.*, 1999; Tiwari *et al.*, 2005; Marotti *et al.*, 2007). The level of intra-species polymorphism depends on the level of divergence. Variety A55 is from Middle American origin (Ariyarathne *et al.*, 1999) and therefore, the germplasm in cluster B with this variety could be from Middle American origin. Similarly, the germplasm from cluster A could be Andean origin. Such type of clustering is indicative of a predominantly self-pollinating species (Debouck and Thome, 1988)

Table 3. Jaccard genetic distance among thirty germplasm of bean.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	.00																													
2	.15	.00																												
3	.31	.17	.00																											
4	.23	.08	.09	.00																										
5	.38	.27	.40	.33	.00																									
6	.54	.42	.45	.36	.50	.00																								
7	.15	.00	.17	.08	.27	.42	.00																							
8	.21	.08	.23	.15	.20	.46	.08	.00																						
9	.31	.17	.33	.25	.29	.30	.17	.23	.00																					
10	.50	.38	.42	.33	.36	.22	.39	.31	.27	.00																				
11	.21	.08	.23	.15	.20	.46	.08	.00	.23	.31	.00																			
12	.36	.23	.25	.17	.33	.50	.23	.15	.39	.33	.15	.00																		
13	.36	.23	.25	.17	.22	.36	.23	.15	.25	.18	.15	.17	.00																	
14	.50	.50	.43	.47	.23	.54	.50	.44	.53	.39	.44	.47	.36	.00																
15	.23	.23	.25	.17	.33	.50	.23	.15	.38	.33	.15	.17	.17	.47	.00															
16	.77	.86	.83	.85	.94	.90	.86	.87	.92	.92	.84	.85	.93	.71	.85	.00														
17	.62	.71	.77	.79	.75	.82	.71	.73	.67	.75	.73	.79	.79	.62	.79	.57	.00													
18	.71	.71	.67	.69	.75	.82	.71	.64	.77	.64	.64	.58	.69	.62	.69	.75	.67	.00												
19	.57	.67	.71	.73	.71	.85	.67	.69	.71	.79	.69	.73	.81	.67	.73	.67	.47	.60	.00											
20	.62	.71	.77	.89	.75	.82	.71	.73	.67	.75	.73	.89	.79	.62	.79	.57	.00	.67	.44	.00										
21	.77	.86	.83	.85	.88	.90	.86	.87	.83	.82	.87	.85	.85	.67	.85	.67	.33	.57	.50	.33	.00									
22	.53	.63	.67	.69	.59	.87	.63	.65	.75	.81	.65	.69	.77	.63	.69	.73	.55	.67	.20	.55	.60	.00								
23	.47	.56	.50	.53	.53	.80	.56	.59	.69	.75	.59	.53	.63	.56	.73	.75	.69	.69	.42	.69	.64	.25	.00							
24	.54	.64	.58	.62	.69	.83	.64	.67	.69	.77	.64	.71	.71	.73	.62	.78	.82	.70	.50	.82	.78	.58	.50	.00						
25	.50	.60	.54	.57	.65	.67	.60	.63	.64	.82	.63	.67	.67	.50	.57	.70	.64	.64	.30	.64	.56	.42	.33	.40	.00					
26	.69	.79	.75	.77	.88	.91	.79	.80	.75	.83	.70	.77	.86	.69	.77	.50	.43	.43	.38	.43	.20	.50	.55	.67	.44	.00				
27	.69	.79	.75	.77	.88	.80	.79	.80	.85	.83	.80	.86	.86	.69	.77	.50	.63	.63	.56	.63	.50	.64	.67	.67	.44	.33	.00			
28	.69	.79	.75	.77	.81	.80	.79	.80	.75	.73	.80	.77	.77	.58	.77	.41	.43	.63	.56	.43	.20	.64	.55	.80	.44	.33	.54	.00		
29	.67	.77	.73	.75	.88	.90	.77	.79	.83	.82	.79	.75	.85	.77	.75	.67	.57	.33	.50	.57	.40	.60	.64	.63	.56	.20	.50	.50	.00	
30	.75	.85	.82	.83	.87	.89	.85	.56	.82	.80	.86	.83	.83	.75	.83	.83	.50	.50	.63	.50	.25	.70	.73	.75	.67	.42	.64	.40	.25	.00

Average Jaccard distances, calculated on the basis of gene frequencies, varied from 0.33 between A55 (17) and Wade (20) to 0.94 between Bean Mandaram Nuwara (5) and Contender (16) (Table 3). These distances were used to construct a dendrogram based on the UPGMA method (Figure 2) which divided the populations into two major clusters. Jaccard Coefficient has been used to study genetic similarity. Alvarez *et al.*. (1998) and Tiwari *et al.* (2005) have successfully used Jaccard coefficient to estimate genetic similarity in bean genetic material.

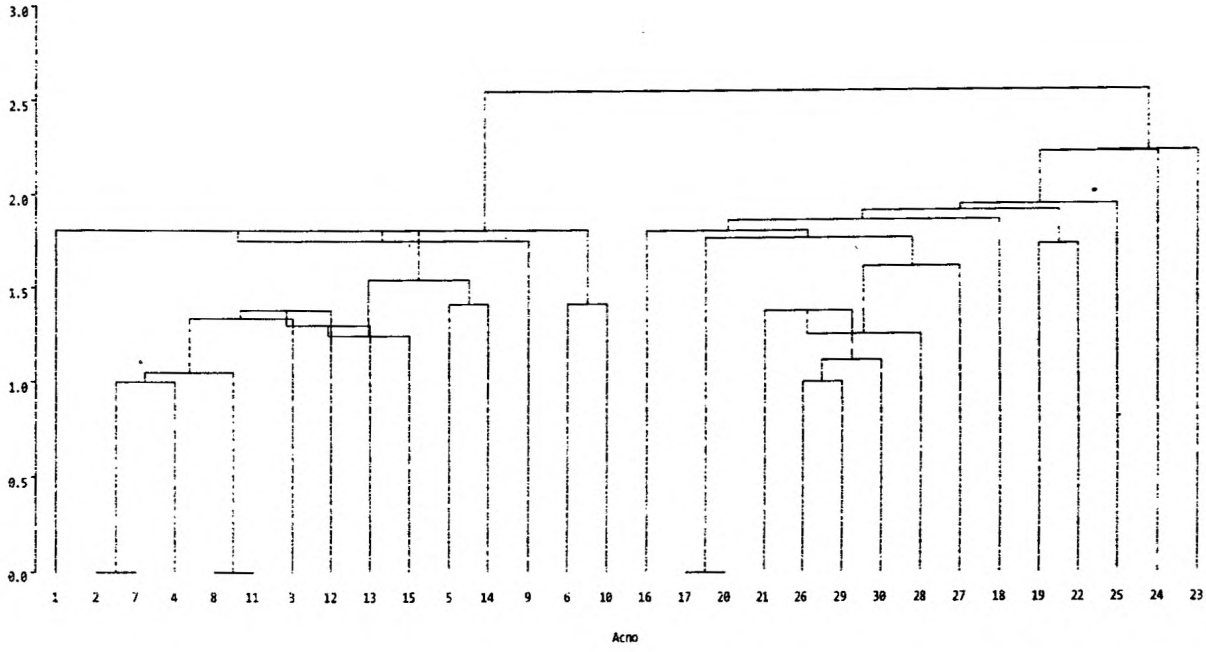

Figure 2. Dendrogram produced by UPGMA cluster analysis of 30 germplasm.

All 30 germplasm analyzed could be grouped in two main groups (Figure 2) and genetic maturity matrixes of the 30 germplasm are presented in Table 3. Figure 2 shows two distinct clusters; cluster A consisted of 15 germplasm including 10 germplasm from Sri Lanka, two from China and three from USA. In Cluster B there were 15 germplasm including five germplasm from Sri Lanka, nine from USA and one from China. Cluster A could be divided into four sub clusters while cluster B could be divided into five sub clusters.

Germplasm originated from Sri Lanka were found in both clusters (as 10 in cluster A and five in cluster B) indicating that Sri Lanka may have received bean germplasm form both centres of origin. Crosses between bean genotypes from these major groups might lead to high heterosis, despite the theory that in autogamous species that have undergone evolution under domestication the dominant and additive alleles

prevail, reducing the advances of F1 heterosis (Fasoula and Fasoula, 1997). The high heterosis shown crosses between okra cultivars indicate that heterosis in autogamous species is not rare (Koutsos *et al.*, 2000). Galvan *et al.* (2006) speculated that genetic diversity can be exploited through breeding programs to broaden the genetic base of commercial beans and develop high yielding varieties.

This study reveals that RAPD markers could successfully be used to study the diversity of germplasm. Therefore this simple and quick technique can productively be used to identify the genetic variance exits among different germplasm and to cluster them at the DNA level. Further studies are needed to link the RAPD markers detected in this study to breeding traits for cultivar development. In order to overcome the limitation of the RAPD analysis, utilization of genetically mapped markers, such as microsatellites or semi-random PCR is suggested (Marotti *et al.*, 2007).

CONCLUSIONS

The results of this study indicate that RAPD analysis could be successfully used for the estimation of genetic diversity among common bean germplasm. The distinguished two clusters showed that the Sri Lankan bean germplasm have two centres of origin. RAPD markers are important tools for a better understanding of genetic relationships among accessions and germplasm, and for accession selection and construction.

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