Genetic Diversity Analysis of Field Bean (*Lablab purpureus* L.) Sweet Through RAPD Markers

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RAPD analysis based on six random primers detected a high level of genetic variation among the 15 field bean genotypes of southern India. A total of 46 bands were generated of which 42 were polymorphic. A high degree of polymorphism was obtained with the primers OPM 20 (11 bands), OPM 1 (9 bands) and OPM 3 (9 bands). The similarity index values ranged from 0.286 to 0.897 indicating a wide range of genetic diversity. The dendrogram consisted of four close-knit groups having 6, 5, 2 and 2 genotypes in groups 1, 2, 3 and 4, respectively. Based on RAPD marker analysis, MAC 8, GA 100 and GA 74-4 were found to be diverse when compared to other genotypes.

Key words: Field bean, *Lablab purpureus* (L.) Sweet, Clustering, Diversity, RAPD markers

In India to date, field bean remains an important but minor pulse crop. Field bean is used as a vegetable and pulse crop for human consumption, as fodder for cattle and as cover crop. The species is extremely diverse (Verdcourt, 1970 and Duke *et al.*, 1981) with two subspecies namely *typicus,* the perennial garden type and *lignosus,* the annual field type. Genetic diversity is an important factor for heritable improvement in any crop and knowledge of genetic diversity, its nature and degree of variability would be useful for selecting desirable parents from available germplasm for a successful breeding programme. The efforts of improving the field bean utilizing indigenous germplasm have been useful in breaking the yield barriers resulting in compact plant type, reduced duration and photo-insensitive types. The present study was undertaken to elicit information on nature and extent of genetic diversity among promising field bean genotypes using Random Amplified Polymorphic DNA (RAPD) markers.

Materials and Methods

Fifteen genotypes from different districts of Karnataka, Andhra Pradesh and Tamil Nadu, were drawn from germplasm collection of field bean being maintained at UAS, GKVK, Bangalore. 18-days old healthy leaf samples from the plants grown in green house were used for DNA extraction as per modified CTAB (Cetyl Trimethyl Ammonium Bromide) method given by Murray and Thompson (1980). Healthy leaves were taken, weighed and cut into small pieces and were ground in liquid nitrogen with pre-chilled pestle and mortar and the powder was transferred into eppendorf tubes and suspended in a pre-heated (65°C) 900 μ l of extraction buffer containing (2% CTAB, 100 mM Tris-HCl, 20mM EDTA, 1.4M NaCl and 1% PVP). The tubes were incubated at 65°C for an hour followed by addition of chloroform: isoamyl alcohol (24:1) and spunned at 14000 rpm for 10 minutes. Upper aqueous phase was precipitated with 200 l of 95% ethanol and 10 l of 3M sodium acetate (pH 5.2) and centrifuged at 14,000 rpm for 10 minutes. DNA recovered as pellet was washed with 70% ethanol. The concentration and quality of DNA was estimated using spectrophotometer at 260nm and verified by running sample on one per cent agarose gel along with 1 Kb DNA ladder.

Amplification reaction was carried out in a 25 l reaction volume containing 25ng of template DNA, 20ng of random primer (Operon, USA), 0.1mM dNTPs, 0.5U of Taq polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India) and 2.5 l of 1 x PCR buffer (10mM Tris-HCl, pH 8.0; 50mM KCl, 1.8mM $MgCl_2$ and 0.01 mg/ml gelatin). Amplification was carried out on Corbett Research Thermocycler following one cycle of initial denaturation at 94°C for 2 min; 45 cycles of 92°C for 1 min, 37°C for 1 min and 72°C for 2 min then closed by 72°C for 5 min, for post extension. The PCR products were separated on 1% agarose gel stained with ethidium bromide. The gels were visualized with UV transilluminator and photographed. Only reproducible and clear RAPD bands were scored as present (1) or absent (0). The sizes of different DNA fragments were revealed with reference to 1 Kb DNA ladder. The estimates of genetic similarity were calculated following Jaccard (1908). The similarity coefficients were subjected to un weighted pair-group method on arithmetic average (UPGMA) method of cluster analysis to group the genotypes based on their overall similarities. SPSS

package was used for the cluster analysis and subsequent dendrogram preparation.

Results and Discussion

Out of the 20 random primers tested, six primers were selected ultimately to detect the variation among the genotypes, which gave on an average 6-7 clear bands with fragment size varying from 100bp to 3000bp. A total of 46 bands were obtained among which 42 bands were polymorphic (91.30%) in nature (Table1). A high degree of polymorphism was obtained with the primers OPM 20 (11 bands), OPM 1 (9 bands) and OPM 3 (9 bands) indicating a wide range of diversity (Fig.1). The similarity index values ranged from 0.286 to 0.897, indicating the presence of wide range of genetic diversity at molecular level among the 15 genotypes (Table 2). Similarly, Renu and Mishra (2003) noticed a wide range of genetic diversity by RAPD markers in germplasm lines of pea.

The clustering pattern using RAPD data resulted in four major groups (Fig.2). The dendrogram of similarity coefficients indicated that genotypes GA 100 of the group-1 and genotype GA 40-2 (white) of the group-4 were clustered at the two extremes based on their similarity coefficients. Even though they belong to same ecological region (Mysore), they exhibited only 36.7 per cent of similarity. This clearly indicates diversity is mainly because of inherent genetic differences at DNA level. While genotype Co 1 from Tamil Nadu was genetically similar (88.0%) to GA 60-15, which is a local selection from Belur (Karnataka). Similarly RDT from Andhra Pradesh were closely associated (47.4%) to GA 2-27, which is a local selection from Bangalore (North). The tendency of accessions occurring in clusters irrespective of geographic boundaries demonstrates that geographical isolation is not the only factor causing genetic diversity. Liu (1996) also indicated in field bean that the existing variation in cultivated materials has no geographic basis and corroborated the results of the present study.

The first group comprising of 3 sub-clusters (1a, 1b, 1c), had a minimum similarity of around 57.1 per cent and maximum of 89.7 per cent. The genotypes GA 100 and GA 14-1 (black) of group 1a were closely related having 89.7 per cent similarity, while group 1b consisted of GA 60-15 and Co 1, which were genetically closer with 88.0 per cent similarity. Group 1c consisted only single genotype GA 20-6. Group 2 included 2 sub-clusters within which the maximum similarity is 80.0 per cent and minimum is 50.0 per cent. Group 3 included only 2 genotypes *viz.* GA 2-27 and RDT having similarity of 47.4 per cent indicating that they are moderately related. While group 4 included 2 genotypes i.e., MAC 8 and 40-2 (white) with only GA 42.9 per cent similarity. Sub-clustering pattern indicated

Table 1. List of Primers selected for DNA amplifications in Field bean

Primer code	Nucleotide Sequence	Total bands	Polymorphic bands	% Polymorphism
OPM-01	5'-GTTGGTGGCT-3'	11	9	81.8
OPM-03	5'-GGGGGATGAG-3'	9	9	100.0
OPM-05	5'-GGGAACGTGT-3'	3	2	66.6
OPM-12	5'-GGGACGTTGG-3'	6	5	83.3
OPM-18	5'-CACCATCCGT-3'	6	6	100.0
OPM-20	5'-AGGTCTTGGG-3'	11	11	100.0
	Total bands	46	42	91.3

Table 2. Similarity matrix of 15 field bean genotypes using Jaccard's coefficient of similarity

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
GA 74-4	1														
HA 3	.630	1													
MAC-8	.478	.500	1												
GA 49	.533	.800	.375	1											
GA 40-2 (White)	.455	.429	.429	.355	1										
Adagur-II	.593	.700	.464	.710	.444	1									
GA 16-3	.500	.686	.485	.743	.343	.657	1								
GA 2-27	.409	.345	.381	.323	.421	.407	.353	1							
GA 100	.364	.514	.303	.618	.367	.529	.622	.429	1						
GA 14-1 (Black)	.353	.459	.294	.556	.355	.559	.564	.414	.897	1					
RDT	.500	.367	.348	.344	.381	.481	.371	.474	.448	.483	1				
Devegowdana doddi I	.448	.515	.333	.529	.407	.581	.541	.542	.821	.733	.560	1			
GA 60-15	.448	.563	.333	.576	.462	.581	.500	.423	.759	.677	.500	.846	1		
CO-1 .414	.531	.345	.545	.480	.600	.514	.500	.786	.700	.520	.880	.880	1		
GA 20-6	.286	.514	.303	.447	.323	.405	.463	.379	.636	.571	.313	.645	.645	.613	1

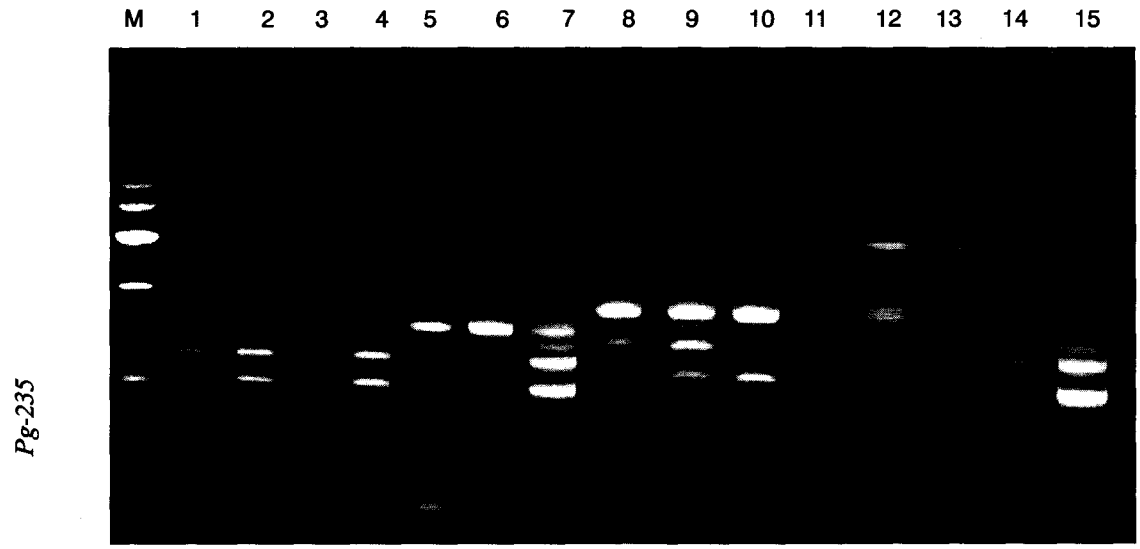

Fig. 1. Arepresentive RAPD banding patterns of 15 Field bean genotypes obtained with primer OPM-20. M=marker

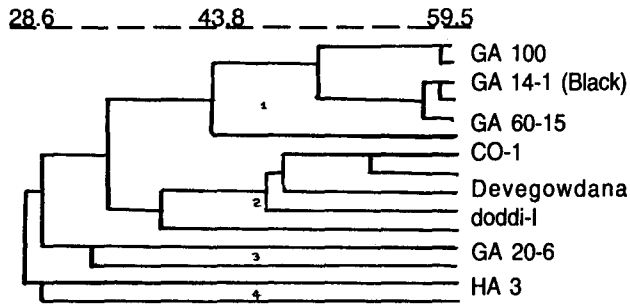

Fig. 2. Association among field bean genotypes revealed by UPGMA cluster analysis of Jaccard genetic similarity coefficients based on RAPD data

low variation within the cluster and high variation between the clusters.

In spite of more intensive cultivation and more concentrated breeding work in India, *Lablab purpureus* collections from Asia seem to possess a higher level of variation (Liu, 1996). Hence, the utilization of available local germplasm resources in hybridization programme is advocated to evolve high yielding and photo-insensitive cultivars of field bean.

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