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ASSESSMENT OF GENETIC VARIATION IN LOCAL GENOTYPES OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) USING MOLECULAR MARKERS

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ABSTRACT

Information about genetic diversity of any crop is important for successful employment of breeding programme and is of great significance to attain sustainability in crop production. Here we used 10 RAPD markers to elucidate diversity among 14 common bean genotypes collected from the various regions of Jammu province. The discriminatory power of these markers was determined using a parameter like: percent polymorphism, number of bands and GC content. 10 RAPDs produced 34 polymorphic bands. The dendrograms generated with hierarchical UPGMA cluster analysis grouped genotypes into two main clusters with various degrees of sub clustering within the cluster. Jaccard's similarity coefficient ranged from 0.13(between BR 301 and Bhaderwah Pole 301) to 0.60 [between Kishtwar Local 1 (23) and Bhaderwah Pole 301]. An adequate level of genetic diversity was observed within the genotypes. The results obtained in the present study have further implications in common bean breeding as well as conservation programs.

INTRODUCTION

Phaseolus vulgaris L. also known as common bean is an annual legume. Common bean, "poor man's meat" as it is sometimes called is an important source of dietary protein for millions of heads across the globe and hence is of great importance to the developing world (Broughton *et al.,* 2003; Biswas *et al.,* 2010). Apart from being the store house of the dietary protein, beans are an excellent reservoir of carbohydrates, vitamins, minerals, antioxidants, soluble fiber and low fat content (Svetleva *et al.,* 2006; Beebe *et al.,* 2000).Common bean is a crucial food legume consumed worldwide and accounts for approximately 15% of total daily calories and greater than 30% of daily protein intake (Hanai *et al.,* 2010; Khaidizar *et al.,* 2012). *Phaseolus vulgaris* shows wide variations in the growth habitat, pigmentation, pod, seed and phenology (Singh *et al.*, 1991) reflecting the wide range of ecological and human environments under which the crop has evolved over millennia. The wide variety of environments under which this species is grown has led to substantial phenotypic variation, especially for growth habit, seed type, phenology, and photoperiod sensitivity (Wallace *et al.,* 1985; Debouck 1991; Voysest and Dessert, 1991). Different molecular markers like RFLPs, RAPDs and SSRs have been used to study genetic diversity in common bean. RFLP markers were used to construct the first genetic linkage map of common bean (Adam-Blondon *et al.,* 1994; Nodari *et al.,* 1993; Vallejos *et al.,* 1992) but their application to breeders, however, was restricted by the costly and sophisticated techniques required(Grisi *et al.,* 2007). Later random amplified polymorphic DNA (RAPD) markers evolved as a rapid, cost effective tool for the indirect selection of economic traits. Apart from being useful in the genetic mapping (Grisi *et al.,* 2007) and 'gene tagging studies', RAPDs have been extensively used in different plant species for germplasm classification, genetic variation classification of germplasm accessions studies (Ender *et al.,* 2008; Tiwari *et al.,* 2005). In recent years, molecular techniques including RAPD markers have been used to elucidate the genetic variability in *Phaseolus* spp. (Williams *et al.,* 1990; Welsh and McClelland, 1990; Martins *et al.,* 2006; Marotti *et al.,* 2007), chilli (Bhaurupe *et al.,* 2013), rice (Kumar, 2015; Singh *et al.,* 2015). For a successful breeding program, knowledge about the existing genetic variation among the crops is a prerequisite. Studying the genetic variation within crop species will reveal some important information that can be used for mapping and crop improvement programme. In view of this, the present study was undertaken with the aim of unravelling the genetic diversity in common bean genotypes using molecular markers.

MATERIALS AND METHODS

Genotypes

Fourteen genotypes of *Phaseolus vulgaris* collected from

various regions of Jammu and Kashmir, India (Table 1) were used in this study.

DNA extraction

The young leaves of common bean genotypes were powdered in liquid nitrogen and the genomic DNA was extracted based on the procedure of Doyle and Doyle (1987), with little modifications The DNA quantity as well as quality was checked by UV-vis. spectrophotometer (mySPEC, Wilmington,USA). Isolated high quality DNA was diluted to a concentration of 25 ng/μl for further use.

Molecular analysis

10 RAPD primers synthesized at IDT (Integrated DNA Technologies, Coralville, Iowa, USA) were used for studying polymorphism among 14 common bean genotypes. 25 μl reaction mixture containing 3 μl of template DNA (25 ng/ μl), 1X PCR Buffer, 50 mM $MgCl_2$, 2.5 mM of each dNTPs (dTTPs, dGTPs, dCTPs, dATPs), 5 pico molar primer concentration, 1U Taq DNA polymerase (Sigma Aldrich, USA) was amplified in a 96 well Universal Gradient Thermal Cycler (PEQLAB, Sigma-Svi). Products were separated on low melting agarose gel along with standard molecular weight marker (100 bp ladder, Sigma Aldrich, USA). The gel was visualized under UV and documented using gel documentation system (MiniLumi, Sigma-Svi). The list of RAPD primers used is detailed in Table 2.

Data analysis

Amplified fragments were scored as '1' for the presence and '0' for the absence of band generating the 0 and 1 matrix and per cent polymorphism was calculated by using the following formula.

Scored data was used for the estimation of Jaccard's similarity coefficient using NTSYS-pc version 2.02e (Rohlf, 1998) package to compute pair-wise Jaccard's similarity coefficient (Jaccard, 1908) and this similarity matrix was used in cluster analysis using the unweighted pair-group method with arithmetic averages (UPGMA) clustering algorithm to obtain dendrogram. Genotypes were divided in various clusters, sub-cluster and sub-sub clusters based on genetic diversity among them and their similarity co-efficient was calculated.

$$\text{Percent polymorphism} = \frac{\text{Number of polmorphic}}{\text{Total Number of bands}} \times 100$$

RESULTS AND DISCUSSION

Genetic relationships among common bean genotypes

Ten RAPD primers (Table 2) were tested against the fourteen common bean genotypes (*Phaseolus vulgaris* L.) and out of which four primers showed polymorphism. The sequences, GC content, % polymorphism and number of bands obtained for these primers are listed in Table 3. Total 34 bands were observed in all the fourteen genotypes out of which 9, 9, 4 and 12 bands were observed in primer OPD17, OPA04, OPAE11 and OPAE 14, respectively. All the bands obtained were polymorphic while some unique bands were also detected. A maximum number of 12 bands were obtained for primer OPAE 14 (GC content 70%) and a minimum of 4 bands for primer OPAE 11 (GC content 60%). The banding pattern of the polymorphic RAPD primers is shown in Figure 1.

The banding pattern of the RAPD primers goes well with the results obtained by Karp *et al*., 1997; Dursun *et al*., 2010; Jose *et al*., 2009; Biswas *et al*., 2010; Szilagyi *et al*., 2011 while evaluating the genetic diversity in common bean. In

maize and wheat nearly similar banding pattern has been observed by (Liu *et al*., 1999; Sivolap *et al*., 1997) and (Bernado *et al*., 1997) respectively, indicating that RAPD markers have been effectively used for elucidating the genetic relationships among various cultivars.

The Jaccard's similarity coefficient for RAPD based diversity analysis of the 14 common bean genotypes showed that the highest similarity value *i*.e. 0.60 recorded between Kishtwar

Table 1: List of common bean genotypes

S.No.	Genotypes	Area of collection	S.No.	Genotypes	Area of collection
1.	BR 301	Chatroo Kishtwar.	8	Bhaderwah Pole 104	Bhaderwah.
2.	Rajouri Local1	Kotranka Rajouri.	9	Poonch Local2	Mandi Poonch.
3.	Poonch Local 1	Mandi Poonch.	10	Bhaderwah Pole 303	Bhaderwah.
4.	BR 305	Chatroo Kishtwar.	11	BR 312	Bhaderwah.
5.	BR 302	Bhaderwah.	12	BR 104	Bhaderwah.
6.	BR 309	Bhaderwah.	13	Kishtwar Local 2 (24)	Barhoti Kishtwar.
7.	Kishtwar Local 1 (23)	Kalchanda Kishtwar.	14	BR 308	Thathri.

Table 2: Details of RAPD primers used in the study

S.No.	Primers	Sequence
1.	OPD 17	5' TTT CCC ACG G 3'
2.	OPA 04	5' AAT CGG GCT G 3'
3.	OPAE 14	5' GAG AGG CTC C 3'
4.	OPAE 11	5' AAG ACC GGG A 3'
5.	OPD 03	5' GTC GCC GTC A 3'
6.	OPZ 13	5' ACT AAG CCC 3'
7.	OPB 17	5' AGG GAA CGA G 3'
8.	OPF 01	5' ACG GAT CCT G 3'
9.	OPAD 05	5' ACC GCA TGG G 3'
10.	OPAE 09	5' TGC CAC GAG G 3'

Table 3: Sequence, GC content, % polymorphism and number of bands of polymorphic markers

S.No.	Primer	Sequence	GC Content %	Polymorphic Percentage	No. of Bands
1	OPD 17	5' TTT CCC ACG G 3'	60%	100%	9
2	OPA 04	5' AAT CGG GCT G 3'	60%	100%	9
3	OPAE 11	5'AAG ACC GGG A 3'	60%	100%	4
4	OPAE 14	5' GAG AGG CTC C 3'	70%	100%	12

Table 4: Similarity coefficient common bean genotypes induced by RAPD primers

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1.00													
2	0.09	1.00												
3	0.38	0.30	1.00											
4	0.09	0.40	0.00	1.00										
5	0.13	0.38	0.11	0.22	1.00									
6	0.00	0.25	0.13	0.11	0.17	1.00								
7	0.09	0.18	0.14	0.08	0.10	0.10	1.00							
8	0.15	0.55	0.33	0.31	0.17	0.18	0.33	1.00						
9	0.29	0.38	0.11	0.22	0.33	0.00	0.10	0.17	1.00					
10	0.13	0.11	0.12	0.00	0.06	0.07	0.60	0.21	0.13	1.00				
11	0.29	0.38	0.25	0.22	0.33	0.17	0.15	0.40	0.14	0.06	1.00			
12	0.09	0.27	0.18	0.40	0.10	0.25	0.08	0.31	0.00	0.05	0.22	1.00		
13	0.25	0.33	0.22	0.20	0.50	0.14	0.09	0.25	0.13	0.06	0.50	0.33	1.00	
14	0.18	0.50	0.27	0.36	0.20	0.10	0.17	0.64	0.20	0.05	0.50	0.25	0.30	1.00

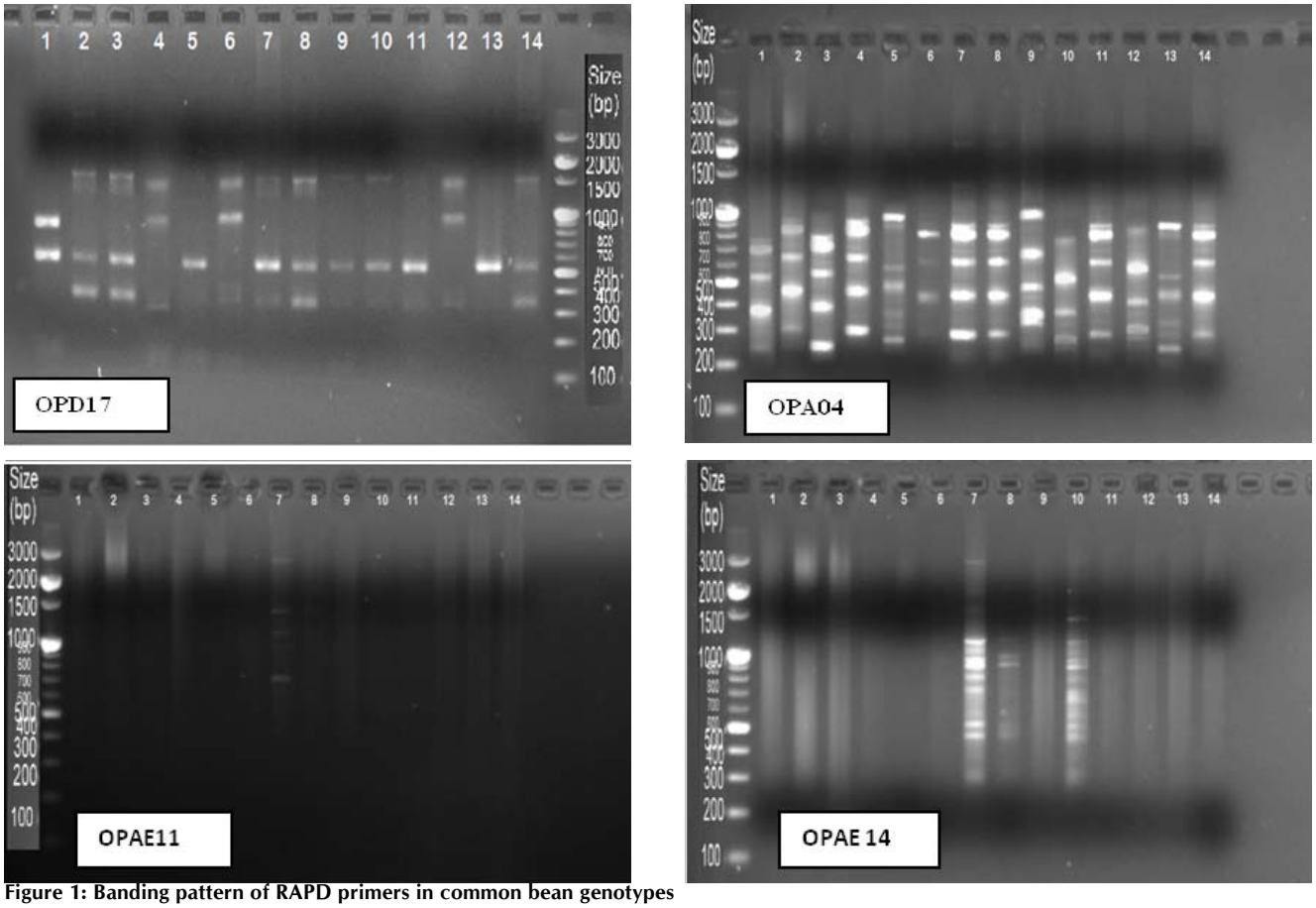

Figure 1: Banding pattern of RAPD primers in common bean genotypes

Local 1 (23) and Bhaderwah Pole 301, indicating the close relationship between these genotypes. While the lowest similarity value 0.13 was recorded between BR 301 and Bhaderwah Pole 301, indicating the presence of diversity

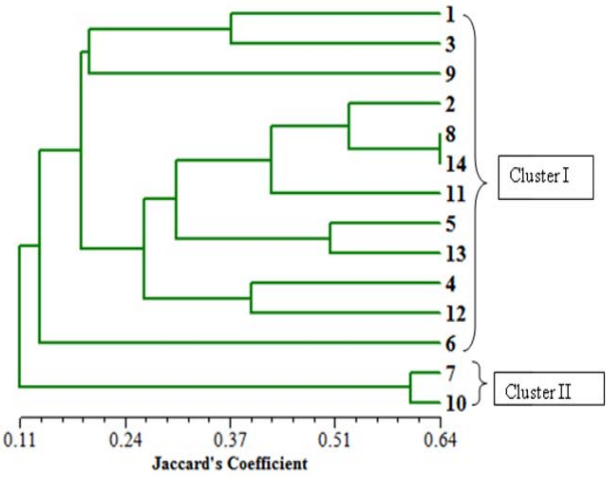

Figure 2: Dendrogram of fourteen common bean lines produced by UPGMA clustering method based on the genetic similarity.

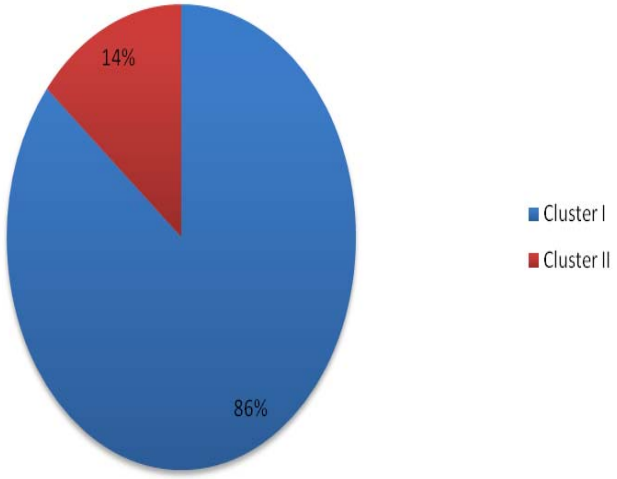

Figure 3: Grouping of common bean genotypes based on similarity coefficient

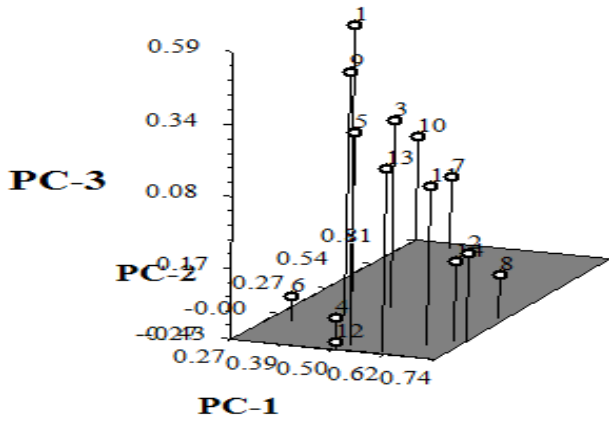

Figure 4: Principal Component Analysis of 14 common bean genotypes using 10 RAPD primers

within these genotypes (Table 4). Since the similarity coefficient is an index of the genetic diversity prevailing among the genotypes. It suggests that the genotypes showing the minimum value of similarity coefficient between them may be used in breeding programmes to obtain wide variation. Similarity coefficient matrix was used to generate a dendrogram of common bean genotypes based on UPGMA analysis (Fig. 2). Jose *et al.,* 2009, found that Jaccard's pair-wise similarity coefficient value of 0.5 to 0.95 indicated an intra-specific genetic variation prevalent in landraces of common bean. 14 common bean genotypes were divided into two distinct clusters *i.e.* Cluster I and Cluster II. The Cluster I comprised of 12 genotypes BR 301, Poonch Local1, Poonch Local2, Rajouri Local, Bhaderwah Pole 104, BR 308, BR 312, BR 301, Kishtwar Local 2, BR 305 and BR 104 and BR 309 while the remaining two genotypes Kishtwar Local 1 (23), Bhaderwah Pole 301 fell under cluster II (Fig. 3). Principal component analysis (PCA) of 14 common bean genotypes using 10 RAPD confirmed the results obtained by UPGMA based clustering (Fig. 4). Choudhary *et al.,* 2016 and Rai *et al.,* 2016 found similar results while carrying out the diversity analysis studies in *Brassica napus* and tomato respectively. Razvi *et al.,* 2013 also obtained similar results while studying the genetic diversity in the common bean.

The present study explained the utility of RAPD markers for carrying out DNA fingerprinting which revealed the presence of genetic diversity among the genotypes studied. In spite of the fact that common bean has been described as an autogamous plant, recent evidences obtained from the diversity studies have indicated the presence of some variability in the reproductive system of domesticated and wild varieties of common bean (Santalla *et al.,* 2002) making it suitable for various breeding and crop improvement programmes.

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