Genetic Diversity in Common Bean (*Phaseolus vulgaris* L.) Germplasm to Establish a Breeding Program in the Mountainous Region of Jammu and Kashmir

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**ABSTRACT**

Common bean (*Phaseolus vulgaris* L.) is native to Middle America and Andean mountains of South America. Due to its nutritious value, it is one of the most important legume crops of the world. Evaluation of genetic diversity of a crop is extremely important for the establishment of a breeding program and selection of varieties for cultivation. We evaluated the genetic diversity and phylogenetic relationship of 40 *P. vulgaris* ecotypes from CIAT (International Center for Tropical Agriculture) germplasm using seed protein profiles produced by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), to screen the germplasm to be used in a breeding program. A total of 21 bands scored 14 were found polymorphic. A distance matrix was generated from the similarity matrix based on polymorphic bands. The UPGMA tree was established through cluster analysis performed on the distance matrix. Six major clusters were formed on the basis of SDS_PAGE analysis with one ecotype (E13-G16832) being the most diverse. This analysis provided the basis for selection of suitable ecotypes to be used in the establishment of a breeding program.

**Key words:** Biodiversity, Common bean, *Phaseolus vulgaris*, SDS-PAGE, Seed proteins.

**INTRODUCTION**

Common bean (*Phaseolus vulgaris* L.) is one of the most important food legumes. It is a self-pollinated leguminous crop (2n = 2x = 22) (Zezen, 1997). *P. vulgaris* originated in Latin America, from two major regions namely Middle America and Andean mountains (Gepts *et al* 1986). It was domesticated in the upland region of Latin America more than 7000 years ago (Kaplan, 1965). As an important food crop, it is widely grown in many countries in the world. This crop is mostly cultivated on P-deficient soils in the tropics (Beebe *et al* 2006). Due to its nutritive components, it is one of the 10 most important crop of the world. Different marker systems namely morphological (Galvan *et al* 2006) biochemical (Koenig and Gepts, 1989) and molecular markers (Metais *et al* 2000; Beebe *et al* 2000; Blair *et al* 2006; Tohme *et al* 1995) have been used for genetic diversity analysis of *P. vulgaris*. Various molecular marker techniques have been utilized to evaluate of genetic diversity in the different common bean germplasm collections, for example, special enzymes (Weeden, 1984; Koenig and Gepts, 1989; Belletti and Lottito, 1996; Lioli *et al* 2005), phaseolin diversity (Singh 1989; Gepts 1990; Duran *et al* 2005) and PCR-based techniques such as RAPD and AFLP (Tiwari *et al* 2005; Duran *et al* 2005).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using seed storage proteins is a very useful molecular technique, which has been used in the discrimination of various crop species, especially for the cultivars levels (Panda *et al* 1986; Koenig *et al* 1990; Karhaloo *et al* 2002; Lioli *et al* 2005; Yüzbaşoğlu *et al* 2008). The technique is rapid and generally free from environmental effects compared with the traditional morphological and other descriptive criteria derived from field trials (Cooke, 1984; Gepts, 1990; Panella *et al* 1993; Yüzbaşoğlu *et al* 2008). Moreover, it is reported that SDS-PAGE of seed proteins is potentially a useful identifier and descriptor for the purpose of seed certification and Plant Variety Rights (Gardiner *et al* 1986; Panella *et al* 1993; Duran *et al* 2005). Seed storage protein markers have been successfully used to resolve taxonomic relationships and characterize cultivated varieties in a number of crop plant species (Igrejas *et al* 1999; Vladova *et al* 2000; Jha and
Genetic Diversity in Common Bean (*Phaseolus vulgaris* L.) Germplasm to Establish a Breeding Program in the... Ohri, 2002; Kariharoo et al., 2002; Syros et al., 2003; Bhargava et al., 2005; Cherdouh et al., 2005; Alvarez et al., 2006; Stoiilova et al., 2006; Mirali et al., 2007; Rout and Chruntoo, 2007; Yuzbasioglu et al., 2008a) because their stability, uniformity, reliability, reproducibility and largely independence from environmental fluctuations (Ghafoor et al., 2002; Panigrahi et al., 2007). We evaluated 40 common bean ecotypes (*Phaseolus vulgaris* L.) based on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of seed proteins. The objective of the current study was the evaluation of germplasm for the selection of suitable varieties and ecotypes for the establishment of a *P. vulgaris* breeding program for the mountainous regions of Azad Jammu Kashmir.

**MATERIALS AND METHODS**

**Seed material**

Seeds of 40 Common beans (*Phaseolus vulgaris* L.) accessions examined in the present study were collected from gene bank of PGRP, Institute of Agri-Biotechnology and Genetic Resources (IABGR), NARC, Islamabad, Pakistan.

**SDS-PAGE**

Sodium Dodecyl Sulphate Poly-Acrylamide Gel Electrophoresis (SDS-PAGE) technique was used to identify diversity of available accessions of *Phaseolus vulgaris*.

**Preparation of seed samples**

For the extraction of total seed proteins, whole seeds were powdered with mortar and pestle. To extract proteins from flour 10mg flour was put into 1.5ml micro-tube; protein extraction buffer (400μl) was mixed thoroughly and vortexed. The extraction buffer contained 0.5M Tris-HCl (pH 8.0), 0.2% SDS, 5M Urea and 1% 2-mercaptoethanol. Bromophenol blue was added to extraction buffer as a dye to show the movement of protein in the gel. The homogenate samples centrifugation at 15,000 rpm for 10 minutes at room temperature (RT). Supernatant (10 μl) was used for protein separation.

**Gel electrophoresis**

SDS-PAGE of total seed protein was carried out in 11.25% polyacrylamide slab gels in discontinuous buffer system according to method of Laemmli (1970). Ten microliters of sample was loaded into the wells of stacking gel. Electrophoresis was carried out at 75V for 3 hours until bromophenol blue marker crossed bottom of the gel. Pre-stained protein marker, ranging from 10 to 180kDa (Fermentas Life Sciences) was run for reference to molecular

**Table 1:** Seed material of forty *P. vulgaris* ecotypes used for SDS-PAGE analysis.

| Ecotypes | Locality/CIAT Tag | Ecotypes | Locality/CIAT Tag |
|----------|------------------|----------|------------------|
| E1       | G16357           | E21      | G16881           |
| E2       | G16371           | E22      | G16885           |
| E3       | G16374           | E23      | G16906           |
| E4       | G16383           | E24      | G16907           |
| E5       | G16400           | E25      | G17068           |
| E6       | G16401           | E26      | G17070           |
| E7       | G16410           | E27      | G17085           |
| E8       | G16794           | E28      | G17100           |
| E9       | G16795           | E29      | G17117           |
| E10      | G16818           | E30      | G17161           |
| E11      | G16824           | E31      | G17162A          |
| E12      | G16829           | E32      | G17166           |
| E13      | G16832           | E33      | G17169           |
| E14      | G16835           | E34      | G17172           |
| E15      | G16840           | E35      | G17187           |
| E16      | G16843           | E36      | G17198           |
| E17      | G16845           | E37      | G17206           |
| E18      | G16849A          | E38      | G17211C          |
| E19      | G16856           | E39      | G17230           |
| E20      | G16863           | E40      | G17425           |

a) ecotypes 1-10, b) ecotypes 11-20, c) ecotypes 21-30, d) ecotypes 31-40.
weight of respective protein bands in kd. After electrophoresis, the gels were stained with 2% commassie blue solution for one hour and destained by solution containing 5% (v/v) acetic acid, 20% (v/v) methanol and distilled water in the ratio of 5:20:75 (v/v) for two hour.

**Drying of separation gel**

Wet filter paper was placed on the plate of gel dryer. Separation gel was carefully placed on the paper and covered with a wrap. It was dried in a drier for about 1.5 hours at 60°C. When gel sheet was completely dried, it was taken out while the pump was still running.

**Data analysis**

Depending upon the presence or absence of polypeptide bands and band migration, similarity index was designed. The score was 1 for the presence and 0 for absence of bands. Based on outcome of electrophoretic band spectra, similarity index (s) was deliberated for all possible pairs of protein type electropherograms by using the following equation: 

Table 2: Diagrametic sketch showing SDS-PAGE results of forty *P. vulgaris* ecotypes.

| Bands | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|-------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|
| E1    | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 0  | 1  | 1  |
| E2    | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1  | 1  | 0  | 1  | 1  |
| E3    | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1  | 1  | 1  | 0  | 1  |
| E4    | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1  | 1  | 1  | 0  | 1  |
| E5    | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0  | 1  | 1  | 1  | 1  |
| E6    | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1  | 1  | 1  | 1  | 1  |
| E7    | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1  | 1  | 1  | 0  | 1  |
| E8    | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1  | 1  | 1  | 0  | 1  |
| E9    | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0  | 1  | 1  | 1  | 1  |
| E10   | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0  | 1  | 1  | 1  | 1  |
| E11   | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0  | 0  | 0  | 1  | 1  |
| E12   | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0  | 0  | 0  | 1  | 1  |
| E13   | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 1  | 1  | 1  |
| E14   | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0  | 0  | 1  | 1  | 1  |
| E15   | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0  | 1  | 1  | 1  | 1  |
| E16   | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0  | 1  | 1  | 1  | 1  |
| E17   | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0  | 0  | 1  | 1  | 1  |
| E18   | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0  | 1  | 1  | 1  | 1  |
| E19   | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 1  | 1  | 1  |
| E20   | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 1  | 1  | 1  |
| E21   | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 1  | 1  | 1  |
| E22   | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 1  | 1  | 1  |
| E23   | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 1  | 1  | 1  |
| E24   | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 1  | 1  | 1  |
| E25   | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 1  | 1  | 1  |
| E26   | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 1  | 1  | 1  |
| E27   | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 1  | 1  | 1  |
| E28   | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 1  | 1  | 1  |
| E29   | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 1  | 1  | 1  |
| E30   | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 1  | 1  | 1  |
| E31   | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0  | 1  | 1  | 1  |
| E32   | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  | 1  | 1  |
| E33   | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  | 1  | 1  |
| E34   | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  | 1  | 1  |
| E35   | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  | 1  | 1  |
| E36   | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  | 1  | 1  |
| E37   | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  | 1  | 1  |
| E38   | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  | 1  | 1  |
| E39   | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  | 1  | 1  |
| E40   | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  | 1  | 1  |

*1 representing the protein bands.
*0 representing absent.
formula (Sneath and Sokal, 1973):
\[ S = \frac{w}{(a + b - w)} \]

Where,
- \( S \) = similarity index, \( w \) = number of bands of common mobility,
- \( a \) = number of bands of protein a, \( b \) = number of bands in protein type. The similarity matrix thus generated was converted into a dissimilarity matrix (dissimilarity = 1 – similarity) and used to build dendrogram by unweighted pair-group method with arithmetic averages (Sneath and Sokal, 1973). All the analyses were carried out using statistical package NTSYSpc, version 2.1 (Applied Biostatistics Inc., USA).

**RESULTS AND DISCUSSION**

Seed storage proteins of the 40 *Phaseolus vulgaris* examined by SDS-PAGE in the present study produced reproducible and stable protein bands. (Fig 1 a, b, c and d). Total 21 polypeptide bands were scored, of which, 14 (66.66%) were polymorphic and 7 (33.33%) were monophorpic. The molecular weight of various bands ranged from 8 to 180 KDa.

The cluster analysis of the common bean ecotypes based on distance matrix has been represented by an UPGMA dendrogram in Fig 3. The banding pattern drawn from the photograph of gel, clearly indicated variation among ecotypes on molecular level, shown in (Table 2). Fourteen bands were visible at variable distance in the gel. In (Fig 2) at linkage, distance 2, the dandrogram divided 40 ecotypes in six main clusters on the basis of total proteins bands. Cluster I consisted of E13 (G16371) that formed an outlier and showed a unique pattern in this cluster, existing at 2.5 linkage distance. Cluster II also has an out liar named E28 (G16845). Cluster III divided into two sub-clusters III (a) and III (b). Sub-cluster III (a) consisted of only two ecotypes G16907 (E24), G16906 (E23) and sub-cluster III (b) comprised eight ecotypes, G17117 (E29), G17085 (E27), G17069 (E25), G17070 (E26), G16845 (E17), G16843 (E16), G16840 (E15) and E9. Among all ecotypes in this group, E9 and E15 are closely related to each other at same linkage distance 1.0. G16843 formed an outlier in this group, which shows maximum divergence in this group. Cluster IV also exhibited an outlier named E30, with a unique pattern in gel.

Cluster IV divided into two sub-clusters. IV (a) and IV (b). Sub-cluster IV (a) consist only one ecotype i.e. G16881 (E21) which show divergence at linkage distance 1.7. Sub-cluster IV (b) consisted of five ecotypes G16863 (E20), G16885 (E22), G16829 (E12), G16824 (E11) and G16818 (E10). This group indicated that ecotype E10 and E11 are similar to each other at linkage distance 1. G16863 formed an outlier in this group.

Cluster VI divided into two sub-clusters VI (a) and VI (b). Sub-cluster VI (a) further divided into two clusters VI (a) 1 and VI (b) 2. Sub-cluster VI (a) 1 have only one ecotype E31 and VI (a)2 comprised seven ecotypes G17220 (E39), G17211C (E38), G17206 (E37), G17425 (E40), G17166 (E32), G17198 (E36) and G17187 (E35). Among all ecotypes in this group ecotype E39, E36, E32, E40, E37, E38 expressed close genetic relationship to each other they are grouped together at same linkage distance 0. Sub-cluster VI (b) accumulated nine ecotypes E18, E14, E7, E5, E8, E4, E3, E2 and E1. Ecotype E7 formed an outlier between E4, E8 and E5, which show unique pattern in this cluster. Similar findings for common bean accessions were reported by (Kumar et al. 2008), who observed wide variations among

![Tree Diagram for 40 Common bean ecotypes](image)
all the accessions of common bean by using molecular marker (AFLPs). However, a considerable amount of variation was observed in peas based on SDS-PAGE (Ghafoor et al. 2008). In this dandrogram, the ecotypes E34, E33, E6 and E19 have more genetic similarity to each other, as they have an exclusive pattern of being in same cluster for molecular markers. Seed protein analysis by SDS-PAGE has proved to be an important way of revealing the differences and relationships between and within taxa and is mainly free of environmental variations (Javad et al. 2004; Iqbal et al. 2005). Common bean germplasm in Pakistan and Azad Jammu and Kashmir has not been explored for its genetic diversity. The broad gene pool with genetic variations is pre requisite for a successful breeding program. Common bean being the major crop of Azad Jammu and Kashmir has been neglected in research. The present research has opened new horizons of research and development on common bean in Jammu and Kashmir. Our results gave good insight into diversity of *P. vulgaris* L. germplasm and will be very helpful in selecting the right ecotypes for the establishment of a breeding program.

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