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# Study of Seed Protein Profiling in Kashmiri Common Bean cultivars.

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***Abstract***
*The present study was undertaken to evaluate the genetic diversity among common bean cultivars from Kashmir on the basis of seed storage proteins as these are rich in proteins. Eight cultivars i.e Brown Bean (B1), Surbhi (B2), Contender (B3), Beans French Yellow (B4), Kentucky Wonder (B5), Laffa (B6), Early Master Beans (B7) and Painted Lady (B8) of Common beans were characterized using SDS-PAGE. Cultivars showed differences in the banding pattern and staining intensities. Seven bands were observed in Brown Beans (B1) and three minimum number of protein bands in Beans French Yellow (B4) and Kentucky Wonder (B5). The regions were divided as per band intensity. The genetic diversity observed among the cultivars could help in breeding programmes in near future.*

***Keywords: -*** *Morphological characters, Phaseolus vulgaris. L., Seed Proteins (SDS-PAGE).*

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## I. Introduction

*Phaseolus vulgaris* L. Common bean is the most important edible food in the world, representing 50% of the grain legumes for direct human consumption. *Phaseolus vulgaris* L. of family Leguminosae originated in Latin America (Southern Mexico and Central America) is an important legume providing major protein source for humans worldwide. The crop is consumed principally for its dry (Mature) beans, shell beans (seeds at physiological maturity) and Green pods (Gepts,2001).In India it is traditionally grown in Hilly trails of Jammu and Kashmir,Himachal Pradesh,Uttar Pradesh etc and some parts of Maharashtra state in kharif season.

The largest producers of dry beans are Brazil, Mexico, China and the USA. *Phaseolus vulgaris* L. being an important vegetable crop of the genus Phaseolus.Phaseolus is a well-known genus of the family fabaceae with more than 150 species. The plant varies from being a climbing, viny or bushy, slightly annual herb ranging in height from 40cm to 3m. The root system has a pronounced tap root with well-developedlateral and adventitious roots. Stems are angular or nearly cylindrical, leaves alternate, trifoliate and pulvinous at the base.Inflorescence may be axillary or terminal racemes with several to many white, pink or purple papilinaceous flowers. Flowers are zygomorphic with a bipetalled keel, two lateral wing petals and a large outwardly displayed standard petal.Seed may be rounded, elliptical, somewhat flattened or rounded elongate in shape and a rich assortment of coatcolours and patterns exists. The most frequently used organ for conducting biochemical research is the seed.In bean seeds, Globulins account for the largest proportion followed by Albumins and the lowest are Glutelins. The cultivars cannot be characterized based on the number ofbands, but they could be differentiated clearly by bandingintensity and relative mobility (Rm).Variety development is an important part of the plant breeding and the identification of these varieties by different parameters plays an important role in seed industry and seed trade.

However, with the increase in the number of varieties of each crop, it was difficult to distinguish the varieties on the basis of morphological characters alone. This had led to the development of the new stable parameters such as use of their genetic material (Nucleic acid and Proteins) as a tool for varietal identification. The results of SDS-PAGEhave been used correctly to evaluate genetic diversity. SDS-PAGE of proteins is the most commonly used method to discriminate the varieties (Duran *et al*., 2005). The banding patterns produced by seed protein electrophoresis have been used to effectively characterize cultivars of pasture grasses and legumes (Sheidai*et al*.,2000).

The main objective of the study was to characterize bean variety on the basis of morphological characters and SDS-PAGE. SDS-PAGE was used to study varietyinter-relationship, cultivar identification, domestication and genetic differentiation.To study variation incultivars morphology and electrophoretic profiles of seed storage proteins for conservation of common bean.Gene pool in India and for future improvement of crop characters.

## II. Materials and Methods

### A) Collection of Plant Material

Eight cultivars of *Phaseolus vulgaris* L. namely Brown bean (B1), Surbhi (B2), Contender (B3), Beans French Yellow (B4), Kentucky wonder (B5), Laffa (B6), Early master bean (B7) and Painted lady (B8) were obtained from the Sher-e-Kashmir University of Agricultural Sciences and Technology(SKUAST-K),Division of Plant Breeding and Genetics, Jammu and Kashmir (J&K) and maintained in the Botanical Garden, Department of Botany, SantGadge Baba Amravati University, Amravati (MS).

### B) Seed Storage Protien Extraction

A single seed was grounded with a mortar and pestle and 100 mg of this seed flour was taken into 1.5ml micro tube. 400 µ*l* of the protein extraction buffer (0.036MTB) pH 8, 0.5M NaCl (pH 2.4),2% SDS,10% glycerol,5% β-Mercaptoethanol,0.1% Bromophenol blue dye was added and mixed well by vortexing. The crude homogenate were then centrifuged at 14000rpm for 10 minutes. The supernatant was used for loading on to the gel. Thereafter 15µ*l* of extract was directly analyzed by SDS-Polyacrylamide gel electrophoresis using 12.5% resolving gel and 5% stacking gel according the method ofLaemmeli, (1970).

A current of 1.5 mA per well with a voltage of 80 V was applied until the tracking dye crossed the stacking gel. Laterthe current was increased to 2 mA per well and voltage upto 100 V. The electrophoresis was stopped when the tracking dye reached the bottom of the resolving gel. Then the gel was stained using CBB G-250 solution overnight and destained until clear bands were visible.

### C) Evaluation and Documentation

The gel was documented and photographed using gel documentation system 'Uvitech'. Determination of molecular weight of proteiniscalculated by using medium range molecular weight protein marker i.e(14.4 KDa-97.7 KDa) and their relative mobility (Rf), Banding pattern, banding intensity were also studied and recorded to discriminate the cultivars.

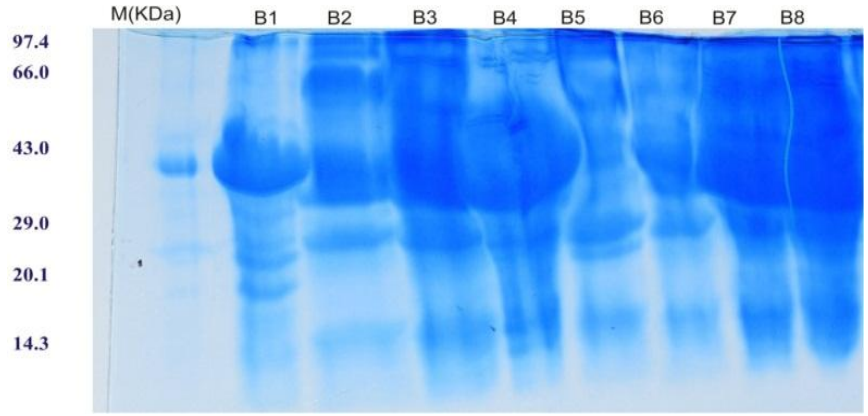


Fig.1 Electrophorogram showing banding pattern of common bean proteins and molecular weight marker. Where,B1 = Brown Bean,B2 =Surbhi,B3 = Contender, B4 = Beans French Yellow,B5 = Kentucky Wonder, B6 = Laffa, B7 = Early master Beans,B8 = Painted Lady.

**Table No.1 Morphological Characters of *Phaseolus vulgaris* L.**

| Name of Cultivars | Plant Height (cm) | Plant growth habit. | State or origin of seeds | Seed coat color | Hilum color | Seed size | No. of seed/Pod | Seed Brilliance | Seed shape | Color of flower | Pod color |
|---|---|---|---|---|---|---|---|---|---|---|---|
| B1 | **50-70 cm** | Determinate Bush type | J&K | Dark brown | Off-white | Small | 6/7 | Dull | Kidney shape | White | Brown |
| B2 | **30-50 cm** | Determinate Bush type | J&K | White | White | Medium | 5/6 | Shinny | Straight | Yellow | Shinny White |
| B3 | **60-80 cm** | Indeterminate Bush type | J&K | Light brown | Off-white and brown outline | Long | 6/8 | Dull | Kidney shape | Pink | Brown |
| B4 | **60-70 cm** | Indeterminate Bush type | J&K | Yellow | Off-white and brown outline | Medium | 4/5 | Shinny | Kidney shape | White | Brown |
| B5 | **80-90 cm** | Pole type | J&K | Chocolaty brown | Off-white | Medium | 7/8 | Dull | Straight | Purple | Reddish spot on brown pod |
| B6 | **600-800 cm** | Pole type | J&K | Dark brown | Off-white& black border | Long | 19/25 | Dull | Kidney shape | White and Pink Shades | Off-white color |
| B7 | **45-60 cm** | Indeterminate Bush type | J&K | Brown &off-white spots. | white outline brown | Medium | 9/10 | Dull | Kidney shape | White | Brown |
| B8 | **56-70 cm** | Indeterminate Bush type | J&K | Pink and off white spot | White | Medium | 6/8 | Shinny | Kidney shape | Pink | Purple spot on offwhite |

Where, J&K- Jammu and Kashmir,B1-Brown Beans,B2-Surbhi,B3-Contender,B4-Beans French Yellow, B5-Kentucky Wonder, B6-Laffa,B7-Early Master beans and B8-Painted Lady

**Table No.2 Banding Pattern and Molecular weight of SDS Protein for eight Cultivars of Common Bean.**

| Regions | M.Wt. (KDa) | Band No. | Rm Value | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A-3** | 97 | 1 | 0.08 | + | + | + | - | - | + | + | + |
| | 69 | 2 | 0.13 | + | + | - | - | - | + | + | + |
| | 66 | 3 | 0.16 | + | - | - | - | - | + | + | - |
| **B-2** | 50 | 4 | 0.33 | + | + | + | + | + | + | + | + |
| | 35 | 5 | 0.58 | + | + | + | + | + | + | + | + |
| **C-2** | 29 | 6 | 0.71 | + | - | + | + | + | + | + | + |
| | 14 | 7 | 0.80 | + | - | + | + | + | + | + | + |
| **Total No. of Bands.** | | | | **7** | **4** | **5** | **3** | **3** | **6** | **6** | **6** |

Note: (**–**) BandAbsent (**+**) Band Present

Where, B1 = Brown Bean, B2 =Surbhi, B3 = Contender, B4 = Beans FrenchYellow, B5 = Kentucky Wonder, B6 = Laffa,B7 = Early master Beans,B8 = Painted Lady.

**Table No.3 Intensity of protein bands for Common Bean using SDS-PAGE.**

| Band No. | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 |
|---|---|---|---|---|---|---|---|---|
| **1** | + | + | + | - | - | + | + | ++ |
| **2** | + | ++ | - | - | - | ++ | ++ | ++ |
| **3** | + | - | - | - | - | + | + | + |
| **4** | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| **5** | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| **6** | + | - | ++ | ++ | ++ | ++ | ++ | ++ |
| **7** | + | - | + | + | + | + | + | + |

Where, +- Low Intensity of Protein bands,++- Medium Intensity of Protein bands,+++ - High Intensity of Protein bands.B1 = Brown Bean, B2 =Surbhi, B3 = Contender, B4 = Beans French Yellow, B5 = Kentucky Wonder, B6 = Laffa,B7 = Early master Beans, B8 = Painted Lady.

## III. Results and Discussion

Great variation were observed in morphological characters among eight different experimental cultivars of *Phaseolus vulgaris L.*. Based on morphological characters alone, it is difficult to distinguish cultivars of common bean from each other because they have variations in terms of the major delimiting morphological and biological characters such as growth habit, stem length, thickness, number of internodes, leafletshape,size, color of flower, seed coat color,size etc. The molecular analysis through protein profiling help to find out the correlation between eight cultivars of common bean.The relative position of protein bands of different cultivars on 12.5% acrylamide gel is presented in Fig.1.The banding pattern intensity of protein represented by three different pattern or color i.e high intensity bands, medium intensity bands and low intensity bands. The entire protein was divided into three region start fromA to C. On the basis of intensity of protein bands and mobility the regions were divided.The difference in banding pattern was mainly confined only to A and C region (Table No.2),these regions of the total seed protein gel profile obviously indicated the usefulness of the high and low molecular proteins than the medium range molecular weight proteins for varietal discrimination of the common bean cultivars. Band number 2,6 and 7 shows medium intensity of protein bands which is present in region A and C (Table No. 3). Cultivar B1shows maximum number of bands i.e 7. B2 and B3 cultivar shows presence of 4 and 5 number of bands.B4 and B5 both cultivar shows same number of bands i.e. 3 and cultivars B6, B7 and B8 shows 6 same number of bands.

A total of 7 bands ranging in Rm values from 0.08 to 0.80 were observed in seed storage protein of common bean. In this study SDS-PAGE of seed storage proteins was performed in order to analyze molecular weight of crude seed protein and investigate genetic diversity among different common bean varieties. Theelectrogram showing proteins banding patterns of different common bean varieties are given in Fig.1.Electrogram show the variation in number of bands in which 35 KDa and 50 KDa were common in each cultivar. A total of 7 bands were obtained in B1 cultivar,among which band number4 and 5 were common in all varieties but the other bands show variation. Region A shows presence of 1,2,3 band with medium and very high intensity of Rf value0.08,0.13 and 0.16 at 66 KDa to 97.4 KDa.Region B (35 KDa-50 KDa) was characterized with two bands of low and medium intensity i.e 0.33 and 0.58.In region C, 14 KDa to 29 KDa shows two bands i.e 6 and 7 band with low and medium intensity was appeared with the Rf value of 0.71 and 0.80. Different kinds of electrophoretic methods based on cotyledon storage protein patterns have been used for the identification and the characterization of crop and herbage cultivars (Cooke, 1984; Panella *et al*.,1993; Karihaloo *et* al.,2002).

Our findings indicated that SDS-PAGE of seed protein supplied additional banding patterns for the discrimination of the bean genotypes, however; the differentiations were not sufficient in distinguishing among the cultivar. The results were in partial agreement with the findings of Balkaya and Yanmaz,(2002).

Protein electrophoresis is a powerful tool for population genetics .As storage proteins are not affected by environmental fluctuations, their profiling using SDS-PAGE technique is particularly considered as a reliable tool for economic characterization of germplasm.Seed protein patterns can also be used as a promising tool for distinguishing cultivars of particular crop species.Ladizinsky (1979) used morphological and seed protein comparisons but found no biological basis for separating closely related small and large seeded lentils.Cultivar identification is useful for describing a new cultivar, testing genotype purity and speeding up-distinctness uniformity stability (DUS) test for candidate cultivar.

The proteinprofiling of germplasm and use of genetic markers have been widely and effectively used to determine the taxonomic and evolutionary aspects of several crops Sodium dodecyl sulphate polyacrylamide gel

electrophoresis (SDS-PAGE) is most economical simple and extensively used biochemical technique for analysis of genetic structure of germplasm. As seed storage proteins are largely independent of environmental fluctuation, their profiling using SDS-PAGEtechnology is particularly considered as a consistent tool for economic characterization of germplasm (Javaid *et al*., 2004).The current increased interest in utilization of legume grains not only from the fact that they typically possess two to four times the quantity of protein than traditional cereal grains, but that their protein are typically of higher nutritional quality. It is an important technology for improving the nutritional quality of legumes and variably affects the proximate composition of seeds.

## IV. Conclusion

A total of 7 bands were obtained in B1 cultivar, among which band number 4 and 5 were common in all varieties but the other bands show variation .Region A shows presence of 1,2,3 band with medium and very high intensity of Rf value 0.08,0.13 and 0.16 at 66 KDa to 97.4 KDa.Region B (35 KDa-50 KDa) was characterized with two bands of low and medium intensity i.e0.33 and 0.58.In region C 14 KDa to 29 KDa shows two bands i.e6 and 7 band with low and medium intensity.The variation in number and intensity of the bands might be due to differential extraction or difference in solubility of protein or differential extraction or difference in solubility of protein or lack of separation of several proteins having similar migration rates. To find out closeness among them study at molecular level was done by using SDS-PAGE.

## Refrences

[1]. Balkaya, A. and Yanmaz.R. (2002). Morphological properties of cultivar nominate selected Black Sea Region bean populations and identification by protein markers. *Ankara Univ. J. Agric. Sci*., 9: 182-188.
[2]. Cooke, R.J. (1984). The characterization and identification of crop cultivars by electrophoresis.*Electrophoresis*, 5: 59-72.
[3]. Duran,M.W., M.C. Blair, R. Giraldo, E. Macchiavelli, J.C. Prophete, J.C. Nin and J.S. Beav,(2005) Morphological and Molecular Chacterization of Common Bean Landraces and Cultivar from the Caribbean. *Crop Science*, 45: 1320-1328.
[4]. Javaid A.A., Ghafoor and Anwar R.,(2004) Seed storage protein electrophoresis in groundnut for evaluating genetic diversity, *Pak. J. Bot*., 36**,** 87-96**.**
[5]. Karihaloo, J.M., M. Kaur and Singh. S. (2002). Seed protein diversity in *Solanummelongena* L., and its wild and weedy relatives. *Genet. Resources Crop. Evol*., 49: 533-539.
[6]. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T. *Nature*, 227**:** 680-685.
[7]. Panella, L., J. Kami and P. Gepts. (1993). Vignin Diversity in Wild and Cultivated Taxa of *Vignaunguiculata* (L.) Walp. (Fabaceae). *Econ. Botany*, 47: 371-386.
[8]. Sheidai, M., Hamta, A., Jaffari, A. and Noori-Daloli, M. R. (2000). Morphometric and seed protein study of Tripholium species and cultivars in Iran. *Plant Genetic Resources and News Letter*. 21: 83- 91.

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