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PG Devhade
Department of Seed Science and Technology, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

AK Basu
Department of Seed Science and Technology, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Nation Chamling
Department of Seed Science and Technology, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Correspondence
PG Devhade
Department of Seed Science and Technology, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Identification of some French bean (*Phaseolus vulgaris* L.) genotypes through SDS-PAGE analysis of seed storage protein

PG Devhade, AK Basu and Nation Chamling

Abstract
The present investigation was done to discriminate eight genotypes of French bean based on the nature of storage protein present in the seeds. Storage protein of harvest fresh seeds of eight genotypes *viz*., Sonali, Selection 9, Deepali, Abhay, Victoria, Arka Suvidha, Arka Anoop and Falguni were analysed through SDS-PAGE and the banding pattern for individual genotypes were utilized to discriminate one from the others. Total number of 11 protein bands could be identified with Rm values ranging from 0.11 to 0.97. All the 11 bands could be noticed for three genotypes only *viz*., Sonali, Arka Anoop and Falguni, 10 bands could be recognised for both Abhay and Arka Suvidha with absence of band number 8 and 7 respectively. While 3 bands each were absent for Selection 9 and Victoria differing in position of two bands, and Deepali was the single genotype for which 4 bands were found to be absent. Grouping of the genotypes initially into two as well as its sub-grouping indicate the relationship among themselves.

Keywords: French bean, Varietal identification, Storage protein, SDS-PAGE analysis.

Introduction
French bean (*Phaseolus vulgaris* L.) is an annual legume grown for its nutritional value. It is traditionally a basic food crop in many developing countries and serves as a major plant protein source for rural and urban areas (Atilla *et al*., 2010). It has very high nutritional value containing 25.81% crude protein, 72.42% carbohydrates and 5.83% mg of iron. Moreover, it has good amount of ash content, crude fibre, and total sugars. It is rich in amino acid like tryptophan, methionine, and some phenolic compounds like tannin and polyphenol oxide (Sood *et al*., 2003). The genetic purity is one of the most important aspects of quality control. Seed quality includes good germination, purity, and vigour and seed health. With the increase in seed industry, there has been a refinement in the techniques used for testing genetic purity. Methods for testing genetic purity include different morphological, chemical, biochemical and molecular markers (Venkata and Reddy, 2014). Electrophoretic analysis of proteins and isoenzyme offers an efficient and cost effective method towards cultivar identification and varietal purity testing of seeds lot (Sammour *et al*., 2007). The electrophoresis of proteins is a method to investigate genetic variation and to classify plant varieties (Isemura *et al.*, 2001). Seed protein is not sensitive to environmental fluctuations; its banding pattern is very stable which advocated for cultivars identification purpose in crop. It has been widely suggested that such banding patterns could be important supplemental method for cultivars identification, particularly when there are legal disputes over the identity of a cultivar or when cultivars are to be patented (Tanksley and Jones, 1981). PAGE of the soluble proteins and esterase were found to be more useful than that of catalase and peroxidase for differentiating the hybrids from their respective parents in cotton (Agrawal *et al*., 1988). Presence or absence of any particular band helps in demarcation and identification of variety (Singh *et al*., 2006). Therefore, the present study was to investigate the differentiation of eight genotypes of French bean analysed on the basis of banding pattern of seed protein.

Materials and Methods
In the present investigation on seeds of eight genotypes *viz*., Sonali, Selection, Deepali, Abhay, Victoria, Arka Suvidha, Arka Suvidha and Falguni during 2014 at Department of Seed Science and Technology, Bidhan Chandra Kishi Vishwavidyalaya, Mohanpur, Nadia, west Bengal were used for identification. Sodium Dodecyle Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of seed storage protein analysis was done for all the 8 genotypes which has been effectively used to identify the differences in protein banding pattern of different crop genotypes (Cooke, 1993). Sodium Dodecyle Sulphate Polyacrylamide Gel Electrophoresis

consists of three steps- i) Extraction of soluble protein. ii) Estimation of protein iii) Electrophoresis (gel casting, run and staining). SDS-PAGE was carried out according to the method proposed by Laemelli (1970).

Results and Discussions

Electrophoregram of SDS-PAGE of the genotypes (Figure 1) revealed that a total number of 11 protein bands could be identified with Rm values ranging from 0.11 to 0.97. Three genotypes *viz*., Sonali, Arka Anoop and Falguni exhibited all the 11 bands indicating a close relationship amongst these three, but they differed among themselves with regard to the thickness and intensity of a few bands which could be utilized for discrimination of one genotype from the other two. A total number of 10 protein bands could be recognised for both Abhay and Arka Suvidha, but they were different from each other with regard to absence of a single band having different Rm values: band number 8 with Rm value 0.78 was absent in Abhay, while it was band number 7 with Rm value 0.71 for Arka Suvidha. Similarly three protein bands were absent for both Selection 9 and Victoria, though the position of the bands and its Rm values differed: band numbers 7, 8 and 9 with Rm values 0.71, 0.78 and 0.92 were absent in Selection 9, while band number 2, 4 and 7 with Rm values 0.26, 0.35 and 0.71 were absent in Victoria, indicating that band number 7 could be identified as the common which was absent in both of these two genotypes; the other two absentee bands could be utilized in identification of two genotypes. Deepali is the only genotype for which highest number of absentee bands could be visualised *viz*., band number 4 (Rm 0.35), 7, 8 and 9; it almost resembled to Selection 9 excepting band number 4 for Deepali, which could be utilized for its identification. The total scenario could be made clear from the concerned Dendrogram (Figure 2), in where all the eight genotypes were initially grouped in of two- Selection 9 and Deepali were constituents of the first group, Victoria was the single constituent of subgroup 1 of group two and among the five constituents of subgroup 2 of group two Sonali, Arka Anoop and Falguni were very closely related, Abhay was to some extent distantly related with these three and Arka Suvidha was comparatively distantly related with the other four constituents of the same subgroup. Characterization of French bean genotypes through seed protein profile obtained by SDS-PAGE analysis have also been made by Ram *et al.* (2005) and Berber and Yasar (2011)

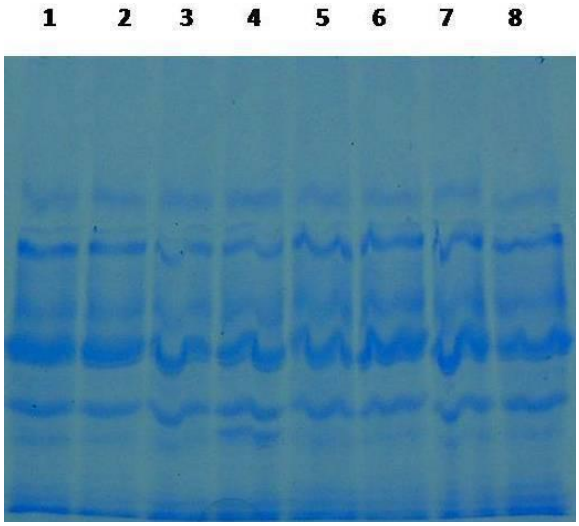

Fig 1: Electrophoregram showing banding patterns of eight French bean genotypes

Table 1: Presence /absence of bands for eight genotypes of French bean

Band No.	Rm values	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8
1	0.11	+	+	+	+	+	+	+	+
2	0.26	+	+	+	+	-	+	+	+
3	0.31	+	+	+	+	+	+	+	+
4	0.35	+	+	-	+	-	+	+	+
5	0.57	+	+	+	+	+	+	+	+
6	0.60	+	+	+	+	+	+	+	+
7	0.71	+	-	-	+	-	-	+	+
8	0.78	+	-	-	-	+	+	+	+
9	0.92	+	-	-	+	+	+	+	+
10	0.95	+	+	+	+	+	+	+	+
11	0.97	+	+	+	+	+	+	+	+

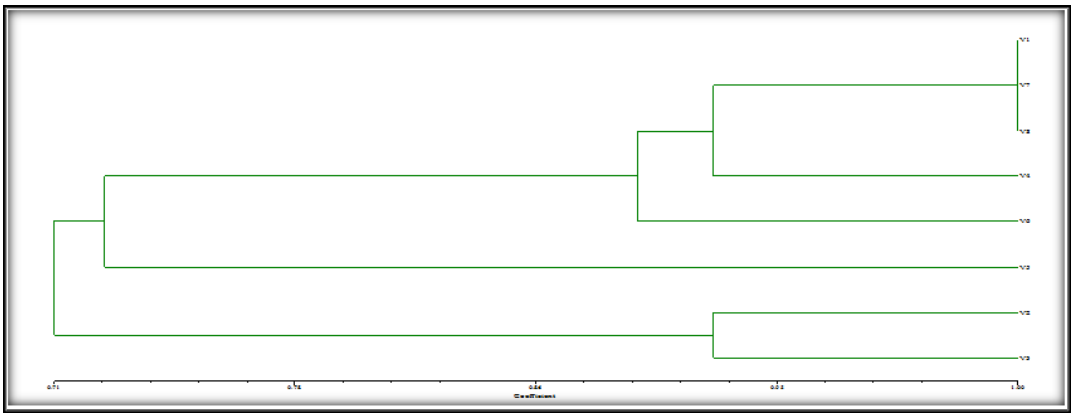

Fig 2: Dendrogram of eight genotypes of French bean

Conclusions

The results of present investigation suggested that SDS-PAGE provided a useful tool in genotypic identification and discrimination based on variation in seed protein banding pattern of French bean genotypes.

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