Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

AK Hembram
Department of Seed Science and Technology, Institute of Agricultural Science, University of Calcutta, Hazra Road, Kolkata, West Bengal, India

Venkata Satish Kuchi
Department of Postharvest Technology, College of Horticulture, Dr. YSRHU, Anantharajupeta, Andhra Pradesh, India

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

Varietal identification in French bean through banding pattern of seed storage protein and its change due to storage

AK Hembram, Venkata Satish Kuchi and AK Basu

Abstract
Seed storage protein of five French bean varieties viz. Sonali, Abhay, Falguni, Mandulal and Selection-9 were extracted from both harvest fresh and one year old seed stored in desiccator. SDS-PAGE of storage protein of all the varieties were analyzed critically and tried to study the variation in banding pattern due to storage. A total number of 32 bands with different R_m values were noticed for harvest fresh seeds of five varieties out of which a minimum of 10 bands were noticed for Selection-9, 11 for Sonali, 12 for Falguni, 14 for Abhay and 16 for Mandulal. Comparison of banding pattern between fresh and old seeds of individual varieties indicate a varying scenario: for Sonali, band number 4, 15, 18 and 28 were disappeared in old seeds and instead 3 different bands newly appeared with R_m values 0.18, 0.56 and 0.85; for Abhay 2 bands with R_m values 0.23 and 0.94 were disappeared from old seeds; for Falguni also 2 bands with different R_m values (0.65 and 0.81) could not be identified in old seeds; for Mandulal, total 14 bands were noted for old seeds, of which 4 bands from fresh seed (R_m values 0.43, 0.48, 0.65 and 0.82) disappeared in old seeds and additionally 2 bands with R_m values 0.66 and 0.81 newly appeared in old seeds, and it is interesting to note that band numbers remain same in both fresh and old seeds of Selection-9 with a very minute change in width of band number 8 (R_m value 0.34). Therefore band number, their position, thickness as well as disappearance/ new appearance clearly help in identification of those five varieties under consideration.

Keywords: Change, French bean, Protein band, Storage, Variety identification

1. Introduction
Identification of varieties/cultivars is very essential for quality seed production through seed certification as well as granting and protecting both the farmers' and plant breeders' right. Among the various techniques available for assessment of genetic variability and relatedness among individuals, most commonly followed seed storage protein analysis represents a valid alternative to varietal identification [1]. Numerous electrophoretic methods are there to identify varieties/cultivars/genotypes by protein banding patterns of which sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) provides the best resolution [2] and SDS-PAGE analysis of seed storage proteins is the reliable method because these proteins are not influenced by environmental conditions and are highly polymorphic [3, 4]. The seed storage protein analyses help in identification and characterization of diversity in crop varieties, cultivars and their wild varieties and provides information on phylogenetic relationship of the accessions [5]. Hence, present study was undertaken to find out the varietal variation of five French bean varieties on the basis of banding pattern of seed storage protein and its change due to storage.

2. Material and methods
Five different varieties of French bean viz. Sonali, Abhay, Falguni, Mandulal and Selection-9 were grown in field in two consecutive years i.e. 2012-13 and 2013-14, seeds were collected very carefully, dried thoroughly upto 8-9 % of its moisture content. Seeds produced during 2012-13 were stored in desiccator using self-indicating coarse silica gel at around 25 -30 °C and preserved till harvesting of the crop of 2013-14.

Soluble protein of both harvest fresh and one year old seeds of all the five varieties was extracted using phosphate buffer (pH 8.0). 500 mg of pre-soaked seeds of all the five varieties, both fresh and one year old, were grinded thoroughly followed by addition of 8 ml phosphate buffer. Homogenates were centrifuged at 10,000 rpm for 30 minutes at 4 °C. Supernatant of each sample was collected individually and kept in refrigerator for analysis.

The SDS solubilized protein sample were subjected to vertical SDS-PAGE following Laemmli [6] with 12.5 % separating and 4 % stacking gels using Tris-glycine electrode buffer.

Correspondence
AK Hembram
Department of Seed Science and Technology, Institute of Agricultural Science, University of Calcutta, Hazra Road, Kolkata, West Bengal, India

The samples were electrophoresed at a constant voltage of 100 V. The run was stopped when dye was almost reached the bottom of the gel, approximately 0.5 cm apart. The gels were dipped overnight in staining solution (0.25 g Comassie Brilliant Blue R-250, 60 g TCA, 180 ml methanol and 60 ml glacial acetic acid). The staining solution was replaced with destaining solution of 3 % NaCl on the next day. The gels were intermittently shaken and destaining solution was changed till the blue colour of the background of the bands disappeared. The gels were visualized for documentation and photographed.

Relative mobility (R_m) of the protein bands was determined using the formula:

$$R_m = \frac{\text{Distance travelled by individual protein band (cm)}}{\text{Total distance run by protein sample (cm)}}$$

3. Results and discussion
Appearance and disappearance of protein bands
The protein banding pattern of five French bean varieties are given in Table 1 and depicted in Figure 1. A total number of 32 bands with different R_m values were noticed from harvest fresh seeds of the varieties, out of which maximum of 16 bands were noticed for Mandulal followed by 14 for Abhay, 12 for Falguni, 11 for Sonali and it was as minimum of 10 for Selection-9. Band number 32 with R_m value 0.99 can be singled out for representing as common irrespective of varieties, both for harvest fresh and old seeds. Band number 9 (R_m value 0.35) was common for both fresh and old seeds excepting Selection-9. Similar nature of band number 16 (R_m value 0.59) could be noticed wherein Sonali could be identified as the exception one. Band number 1 (R_m value 0.15), Band number 17 (R_m value 0.60) and band number 25 (R_m value 0.78) were noticed in protein profile of both harvest fresh and old seeds of Falguni only, which can be utilized for identification of this variety. Similarly band number 6 (R_m value 0.28) and band number 10 (R_m value 0.37) can be utilized for identification of both fresh and old seeds of Mandulal, at the same time band number 12 and 13 (Rm value 0.43 and 0.48) were present in fresh seeds of Mandulal only.

Table 1: Presence or absence of band

| Band No. | Rm Value | Varietal seeds | | | | | | | | | |
| | | Sonali | | Abhay | | Falguni | | Mandulal | | Selection - 9 | |
		Fresh	Old	Fresh	Old	Fresh	Old	Fresh	Old	Fresh	Old
1	0.15	-	-	-	-	+	+	-	-	-	-
2	0.16	-	-	+	+	-	-	+	+	-	-
3	0.23	-	-	+	-	-	-	+	+	-	-
4	0.25	+	-	-	+	-	-	-	-	-	-
5	0.26	-	+	-	-	+	+	-	-	+	+
6	0.28	-	-	-	-	-	-	+	+	-	-
7	0.31	-	-	-	-	-	-	-	-	+	+
8	0.34	+	+	-	-	+	+	-	-	+	+
9	0.35	+	+	+	+	+	+	+	+	-	-
10	0.37	-	-	-	-	-	-	+	+	-	-
11	0.38	-	-	+	+	-	-	-	-	-	-
12	0.43	-	-	-	-	-	-	+	-	-	-
13	0.48	-	-	-	-	-	-	+	-	-	-
14	0.54	+	+	-	-	-	-	-	-	-	-
15	0.57	+	-	+	+	-	-	+	+	+	+
16	0.59	-	-	+	+	+	+	+	+	+	+
17	0.60	-	-	-	-	+	+	-	-	-	-
18	0.62	+	-	-	-	-	-	+	+	-	-
19	0.63	-	+	+	+	-	-	-	-	-	-
20	0.65	+	+	+	+	+	-	+	-	-	-
21	0.72	-	-	-	-	+	+	-	-	+	+
22	0.74	+	+	-	-	-	-	+	+	-	-
23	0.75	+	+	+	+	-	-	+	+	-	-
24	0.76	-	-	-	-	-	-	-	-	+	+
25	0.78	-	-	-	-	+	+	-	-	-	-
26	0.81	-	-	+	+	+	-	-	+	-	-
27	0.82	-	-	+	+	+	+	+	-	-	-
28	0.84	+	-	-	-	-	-	-	-	-	-
29	0.87	-	-	-	-	-	-	+	+	+	+
30	0.94	-	-	+	-	-	-	-	-	-	-
31	0.97	-	-	-	-	-	-	-	-	+	+
32	0.99	+	+	+	+	+	+	+	+	+	+

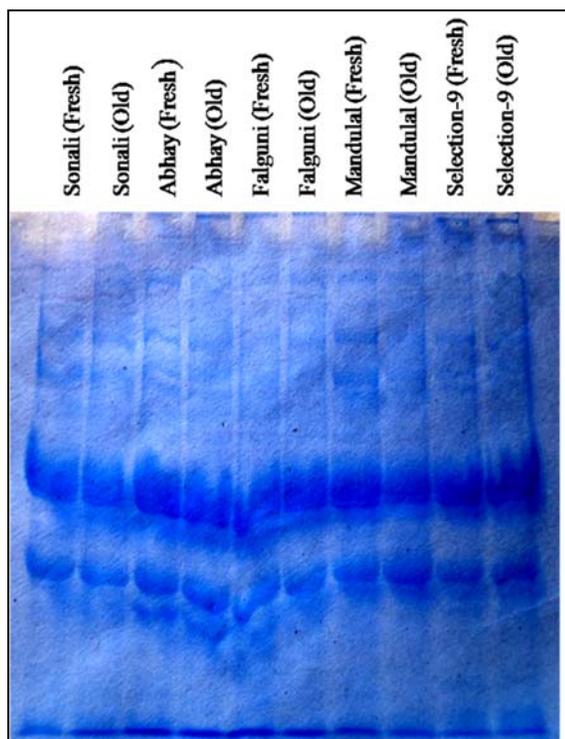The symbols (+) and (-) indicate the presence and absence of a band, respectively.

Fig 1: Seed protein profile of five French bean varieties through SDS-PAGE

Band number 7 (R_m value 0.31) and 31 (R_m value 0.97) for both types of seeds of Selection-9 as well as band number 11 (R_m value 0.38) and 19 (R_m value 0.63) for both harvest fresh and old seeds and band number 30 (R_m value 0.94) for fresh seeds only can be helpful in identification of Abhay. Band number 4 (R_m value 0.25) was present in fresh seeds of Sonali and old seeds of Abhay; which, therefore can be utilized for identification of harvest fresh seeds of Sonali only. Band

number 14 (R_m value 0.54) and band number 28 with R_m value of 0.84 were present in Sonali for both types of seeds and for fresh seeds only respectively. On the other hand it is to be noted that band number 4 was present in fresh seeds of Sonali which disappeared in old seeds and it was reverse for Abhay. Presence and absence of different bands in protein profile of individual varieties as well as disappearance/new appearance of any band can be utilized for identification of individual varieties.

Varietal similiarity through jacards similiarity coefficient
Jaccard's similarity coefficient values (Table 2) can be utilized in a better way for identification of the varieties through protein profile of its both old and fresh seeds. Selection-9 could be identified as the only variety for which no change in banding pattern occurred due to its seed storage for one year exhibiting highest norm of similarity. While similarity was as high as 0.9375 between fresh and old seeds of Falguni and gradually reducer for Abhay, Mandulal and Sonali, exhibiting more than 80% similarity in protein profile even after one year of storage.

Cluster analysis through UPGMA Dendrogram
Two clear groups were formed after UPGMA Dendrogram (Figure 2), of which group-I was constituted with both old and fresh seeds of Falguni and Selection-9. The second group was sub-grouped into two- first one constituted with Sonali and second one was with the remaining two Abhay and Mandulal, both harvest fresh and old seeds were closely related for every individual varieties, which help in identification of individual varieties.

Seed protein profiles have been successfully utilized in distinguishing varieties of French bean by Ram et al. [7], Berber and Yaşar [8]; of green gram by Tripathy et al. [9]; of black gram by Khan et al. [10]; and of Chickpea by Jahangir et al. [11], Ranjan et al. [12] and Khan et al. [13].

Table 2: Jaccard's similarity coefficient of harvest fresh and old seeds of five French bean varieties

|	Sonali Fresh	Sonali Old	Abhay Fresh	Abhay Old	Falguni Fresh	Falguni Old	Mandulal Fresh	Mandulal Old	Selection9 Fresh	Selection9 Old
Sonali Fresh	1									
Sonali Old	0.8125	1								
Abhay Fresh	0.5625	0.625	1							
Abhay Old	0.6563	0.6563	0.9063	1						
Falguni Fresh	0.5313	0.6563	0.5938	0.625	1					
Falguni Old	0.5313	0.6563	0.5313	0.5625	0.9375	1				
Mandulal Fresh	0.5938	0.5313	0.6563	0.625	0.4375	0.4375	1			
Mandulal Old	0.625	0.5625	0.6875	0.6563	0.4688	0.4688	0.8438	1		
Selection9 Fresh	0.5313	0.5938	0.4688	0.5	0.625	0.6875	0.4375	0.5313	1	
Selection9 Old	0.5313	0.5938	0.4688	0.5	0.625	0.6875	0.4375	0.5313	1	1
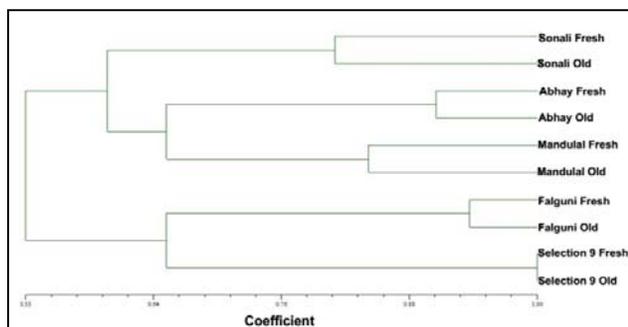
Fig 2: Dendrogram of harvest fresh and old seeds of five French bean varieties by UPGMA clustering method

4. Conclusion
SDS-PAGE analysis of storage protein of all the varieties of fresh and stored seeds varied in band number, their position, thickness as well as disappearance/ new appearance. Thus, this analysis clearly helps in identification of those varieties under consideration.

5. Acknowledgements

6. Authors' Contributions

"HAK'' designed the study, performed the statistical analysis and wrote the first draft of the manuscript. 'VSK' managed the analyses and the literature searches and 'BAK' designed the study, wrote the protocol and managed analyses. All authors read and approved the final manuscript.

7. References

1. Manella G, Onofaro SV, Tonini A, Mangifico V. Seed storage protein characterization of *Solanum* species and of cultivars and androgenic lines of *S. melongena* L. by SDS-PAGE. Seed Sci Technol. 1999; 27:23-35.
2. Smith DB, Simpson PA. Relationships of barley proteins soluble in sodium dodecyl sulphate to malting quality and varietal identification. J Cereal Sci. 1983; 1:185-197.
3. Sadia M, Malik SA, Rabbani MA, Peaece SR. Electrophoretic characterization and the relationship between some *Brassica* species. Electron J Biol. 2009; 5:1-4.
4. Zada M, Shinwari ZK, Zakir N, Rabbani MA. Study of total seed storage proteins in ethiopian mustard (*Brassica carinata* A. Braun) germplasm. Pak J Bot. 2013; 45:443-448.
5. Qari SH, Kamel EA, El Sayed H. Characterization and Identification of Seed Storage Protein of Twelve Lettuce Cultivars. Biosciences Biotechnology Research Asia. 2016; 13(4):1959-67.
6. Laemmli UK. Cleavage of proteins during the assembly head of bacteriophage T_4. Nature. 1970; 227:680-685.
7. Ram HH, Sharma K, Jaiswal HR. Distinguishing certain released varieties of Brinjal, French bean and Pea by SDS-PAGE. Veg Sci. 2005; 32(1):15-18.
8. Berber İ, Yaşar F. Characterization of bean (*Phaseolus vulgaris* L.) cultivars grown in turkey by SDS-page of seed proteins. Pak J Bot. 2011; 43(2):1085-1090.
9. Tripathy SK, Sardar SS, Mishra PK. Analysis of seed storage protein pattern: a method for studying genetic variation and diversity among Vigna genotypes. Indian J. Pl. Breed. & Genet. 2010; 70(2):140-144.
10. Khan J, Shah AH, Nisar M, Ali N, Khan U, Khan W *et al*. Evaluation of genetic diversity in Black gram through SDS-PAGE. Plant Cell Biotechnology and Molecular Biology. 2016; 17(3, 4):87-95.
11. Jahangir AD, Shahzada A, Kudesia R, Srivastava MK, Wani AA. Biochemical and cytological analysis of five cultivars of *Cicer* (Chickpea). Afr J Biotechnol. 2014; 13(11):1281-1286.
12. Ranjan S, Poosapati A, Harsha Vardhan BV, Matcha R. Seed storage protein profile of few leguminous grains grown in India using SDS-PAGE. Int J Adv Biotechnol Res. 2013; 4(4):505-510.
13. Khan SA, Shinwari ZK, Rabbani MA. Study of total seed protein pattern of rice (*Oryza sativa* L.) breeding lines of Pakistan through SDS-PAGE. Pak J Bot. 2013; 45(3):871-876.