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Research Article

Protein profile studies in French bean mutants

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Abstract

French bean botanically described as *Phaseolus vulgaris* L. is a protein rich crop. It is grown throughout the world both for its immature pods and mature seeds. In present investigation healthy seeds of french bean variety Varun (ACPR-94040) were treated with various doses/concentrations of physical mutagen gamma rays and chemical mutagens ethyl methanesulphonate and sodium azide. Treated seeds were sown and M_1 generation was raised. Various biological parameters were studied from M_1 generation. Seeds of M_1 generation were collected separately and sown to rise M_2 generation. M_2 generation was screened thoroughly and various morphological mutants were isolated. The true breeding nature of all these mutants was assessed in M_3 generation. All the true breeding mutants along with control were analyzed for water soluble seed protein content. As well as the protein profiles of seed storage proteins (water soluble proteins) of control and all viable mutants were analyzed using Native and SDS-PAGE. Variabilities were recorded for protein content of control and induced mutants. Protein profile studies demonstrated marked differences in number, mobility and presence or absence of bands in the viable mutants as compared to control.

INTRODUCTION

French bean botanically described as *Phaseolus vulgaris* L. is a protein rich crop. It is grown throughout the world both for its immature pods and mature seeds. It belongs to family Fabaceae and also known as haricot bean, salad bean, runner bean, snap bean, string bean, frijoles, kidney bean, common bean and navy bean (Singh, 1999; Pandey, 2003). Among food legumes the french bean has been the third most important worldwide famous crop. It is a source of low cost protein rich crop in many countries. It is recognized as an excellent source of protein also containing large quantities of complex carbohydrates, fibers and isoflavonoids (Anderson *et al.*, 1999). It is an important source of iron, phosphorous, magnesium and manganese and to a lesser extent of zinc and calcium (Broughton *et al.*, 2003). The present work was started with the objective of inducing genetic diversity in existing variety of french bean Varun (ACPR-94040). The induced diversity was studied with the help of biochemical parameters like protein content and protein profile. The outcome of the present Khillare and Kamble (2016). Studied that seed samples show potent insect amylase inhibitors which block the activity of *T. castaneum* amylase with varying percentage. Mogle and Maske (2012) found that *Cassia* leaf extracts were effective against seed borne pathogenic fungi and also used as a ethnoverterinary medicine. Kamaleswari and Nandagopalan, (2016) concluded that the extract of

P. auricularis possess a good source of essential oil, alkaloids, flavonol glycosides followed by phenolic flavonoids, gardiac glycosides and phytosterols. Shunmuga *et al.* (2015**)** identifies presence of twenty eight phytocomponents in the *Senna italica* ssp *italica* with the retention time ranging from 2.20 to 33.41 in profile of leaf samples.

MATERIALS AND METHODS

Seeds of French bean variety Varun (ACPR-94040) were collected from National Agricultural Research Project, Ganeshkhind-7, Pune (M.S.) India. The healthy seeds were treated with physical mutagen gamma rays (5kR, 10kR and 15kR) and chemical mutagens EMS (0.05%, 0.10% and 0.15%) and SA (0.01%, 0.015% and 0.02%). M_1 generation was raised and studied for different biological parameters. Seeds of M_1 generation were harvested separately and sown to raise M_2 generation. M_1 generation was thoroughly screened and various morphological mutants were isolated. True breeding nature of all these morphological mutants was assessed in M_3 generation. Three plants from each true breeding mutant line of M_3 generation were selected for water soluble seed protein content. Single plant was used for protein profile of each mutant along with control. The standard biochemical methods were employed for protein estimation and protein profile.

Estimation of water soluble seed proteins

Water soluble seed proteins were first extracted and then estimation was carried out by Lowry's method (Lowry *et al.*, 1951).

Protein Profile

In present study water soluble proteins of viable mutants and their controls were analyzed by Native and SDS-PAGE by using the vertical slab polyacrylamide gel electrophoresis apparatus. 12 % polyacrlamide gels were used for separation of proteins and polypeptides. Both Native-PAGE and SDS-PAGE gels were documented in Ingenious LHR (Gel Doc.) and banding pattern of proteins and molecular weight of polypeptides was determined in mutants and their controls.

I. Non-denaturing discontinuous (Native) PAGE

It was performed by using Davis (1964) system. In Native-PAGE separation of proteins relies on both the charge and size of proteins.

II. SDS-PAGE

In SDS-PAGE, polypeptides were separated according to their molecular weight, not by intrinsic electrical charge. Sodium dodecyl sulphate (SDS) is an anionic detergent that denatures proteins by wrapping around the polypeptide backbone.

In doing so, the SDS confers a negative charge to the polypeptide in proportion to its length. When proteins are treated with SDS and reducing agent 2ME (2-Mercaptoethanol), the polypeptides become rods of negative charges with equal charge unit per length. Here, denaturing discontinuous PAGE system was used as described by Laemmli (1970). This system is almost similar to the native PAGE (Davis, 1964) except for the presence of SDS.

RESULTS AND DISCUSSION

Water soluble protein content (Table 1)

All the viable mutants revealed differences in protein content. The water soluble seed protein content in control was 224 mg/g. Highest level of water soluble seed protein content (270 mg/g) was observed in short pod mutant while lowest content (194 mg/g) could be noted in blackish red seed coat mutant. An increase and decrease in protein content of viable mutants as compared to control was recorded. Similar results were obtained by Barshile and Apparao (2009), Pavadai *et al.,* (2010), Auti and Apparao (2009), Arulbalachandran and Mullainathan (2009), Bhalerao *et al.,* (2008) and Manjaya and Nandanwar (2007). Induced mutants with altered protein content have been reported by Gaikwad *et al.,* (2003) and Bhosle and Kothekar (2011). Hussein (1982) observed increased and decreased protein content in glabrous and indeterminate mutants as compared to control variety Contender of french bean.

Sangle (2015a) studied the crude protein content in the mutants ranged from 13.50% to 20.79% % as compared to control 14.61% in variety BDN 708. In variety BSMR 853, highest crude protein percentage could be recorded in small pod mutant (19.82%) and lowest in early flowering mutant (14.02%) as compared to control plant (15.13%) in legume pigeon pea. Nitrogen content was correlated with protein content of several legume crops.

Number of workers attributed increase in protein content at different concentrations/doses of mutagens to an increase in auxin level. Decrease in protein and amino acid content due to chemical mutagens was due to inhibition of auxin and DNA synthesis and degradation in protein and carbohydrate metabolism. The observation of present study indicated that the genetic improvement of protein quality and quantity is indeed possible through mutation breeding.

Protein profile: (Tables 2 and 3) (Plate 1)

Marked differences were observed in number, mobility and presence or absence of bands in the viable mutants as compared to control.

Native PAGE: Control plant exhibited 12 bands while 11 to 16 bands could be recorded in different viable mutants. Except compact leaf, violet flower and short pod mutant, all other mutants showed increased number of bands as compared to control. Maximum number of protein bands (16) could be recorded in early flowering mutant. Band number 04 from control was present in all the mutants. The lowest mobile band was present in short pod mutant. Highest mobile band (12) of control was present only in dwarf, large and linear leaf mutant. Protein polymorphism was observed in both the regions of low as well as high mobility.

SDS-PAGE: analysis of viable mutants of variety Varun indicated 10 to 17 bands as compared to 09 bands in control. Maximum number of polypeptide bands (17) was found in linear leaf and short pod mutants while minimum number of polypeptide bands (10) could be recorded in dwarf mutant. Some polypeptide bands present in control were absent in some viable mutants while polypeptide bands absent in control were seen to be present in the viable mutants. Along with the number, variability in molecular weight of polypeptide bands could also be observed in control and viable mutants. Molecular weight of bands in control ranged from 64.33 KD to 6.20 KD. Highest molecular weight (107.99 KD) of the polypeptide band was recorded in dwarf mutant while lowest molecular weight (4.31 KD) of the polypeptide band could be detected in large leaf mutant.

In present investigation, protein profiles and polypeptide profiles of mutants have been analyzed on Native and SDS-PAGE, respectively to detect genetic variability in french bean mutants. By using this technique the genetic variability among mutants of several crop plants has been studied by different workers in various crop systems. Such workers comprise Sangle (2015b), Dadke (1999), Kulthe (2003), Khadke (2005) and Bhalerao (2009). Hussein (1982) carried out electrophoretic studies in white seed coat mutant and parent variety, Fin de Villeneuve of French bean. He observed clear cut differences in the banding patterns of two genotypes. Auti and Apparao (2009) in mungbean, Barshile and Apparao (2009) in chickpea studied the diversity in viable macromutants by protein profiling. Tuber of *C. epigaeus* was analyzed using standard methods of food analysis. The result showed diverse level of total crude fiber, total, carbohydrate, moisture and ash content and enzymes. (Jayaseelan, 2016). Nandagoapalan and *et al.* (2016) qualitative phytochemical screening of the crude powder of 25 plants and assess the presence of bioactive components also determine presence of alkaloids, flavonoids, tannins, phenols, steroids, glycosides, terpenoids and saponins. Medicinal plants are of great importance in the field of biotechnology and most of the pharmaceutical industries depend on phytoconstituents for the productise of drug to cure many diseases. Plants contain a variety of phytocompounds which have found very important applications on the fields of agriculture, human and veterinary medicine (Sajal *et al.,* 2014).

Florence and *et, al.* (2015) performed phytochemical screening of the leaves of *L. reginae, L. microcarpa, L. inermis and P. granatum* which exhibited phytochemical constituents such as alkaloids, carbohydrates, flavonoids, saponins, coumarins, quinones, phytosterols, proteins, glycosides, steroids, terpenoids and phenols in varied compositions in various extracts which has great medicinal and pharmacological properties. Madhu and Anita Kochhar, (2014) were analyzed proximate composition, available carbohydrates, mineral content, dietary fiber and anti-nutritional factors from Broccoli (*Brassica oleracea* L. var. *italica plenck*) floret and leaf powder. Ghosh and *et, al.* (2015) state that direct relationship of protein yield with all the component characters including nitrogen harvest index indicates that with increase of NHI, the leaf protein can be improved. The present study demonstrated that induced mutations are useful in creating variability in biochemical constitution of french bean.

Mutation breeding is the best technique in inducing morphological and biochemical diversity in french bean. The present study revealed that the seed protein extracted from french bean mutants shows the electrophoretic variation in protein bands. Changes in banding pattern of french bean mutants, observed in present investigation might be due to change in the gene sequence or posttranslational modifications of proteins as a consequence of change in their genotype due to mutation breeding

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Table 1: Water soluble seed protein content in different viable mutants of French bean variety Varun

Sr. No.	Name of mutant	Water soluble protein content (mg/g) of defatted seed powder (Mean±SE)
1	Control	224±2.18
2	Tall	205±1.60
3	Dwarf	245±2.85
4	Large leaf	215±1.74
5	Linear leaf	237±1.98
6	Compact leaf	195±3.85
7	Early flowering	262±4.67
8	Violet flower	218±1.60
9	Short pod	270±3.85
10	High yielding	228±0.90
	Mean	**229.90**
	SD	**23.95**
	SE	**6.65**
	CD (p=0.05)	**14.50**
	CD (p=0.01)	**20.35**

Table 2: Protein profile of viable mutants in French bean variety Varun.

Sr. No.	Name of mutant	Total No. of polypeptide bands in SDS-PAGE	Total No. of protein bands in Native-PAGE
1	Control	09	12
2	Tall	15	13
3	Dwarf	10	14
4	Large leaf	12	13
5	Linear leaf	17	14
6	Compact leaf	12	12
7	Early flowering	13	16
8	Violet flower	15	11
9	High yielding	16	14
10	Short pod	17	12

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Table 3: Molecular weight (KD) of polypeptide bands in SDS PAGE of variety Varun.

Track 1 (Control)		Track 2 (Tall)		Track 3 (Dwarf)		Track 4 (Large leaf)		Track 5 (Linear leaf)	
Band No.	**Mol. Weight**	**Band No.**	**Mol. Weight**	**Band No.**	**Mol. Weight**	**Band No.**	**Mol. Weight**	**Band No.**	**Mol. Weight**
1	64.33	1	71.95	1	107.99	1	67.28	1	85.78
2	55.00	2	67.28	2	66.42	2	54.48	2	71.72
3	50.94	3	64.54	3	64.33	3	50.29	3	66.64
4	41.51	4	55.00	4	54.65	4	45.69	4	54.30
5	34.89	5	50.62	5	50.45	5	41.91	5	50.94
6	32.82	6	49.97	6	41.11	6	40.65	6	49.50
7	20.15	7	42.38	7	34.78	7	37.81	7	46.73
8	7.05	8	41.24	8	32.66	8	35.86	8	42.04
9	6.20	9	38.18	9	17.34	9	34.67	9	40.84
		10	34.95	**10**	5.11	10	32.61	10	38.30
		11	34.28			11	26.05	11	37.99
		12	32.93			**12**	4.31	12	37.27
		13	26.62					13	35.63
		14	18.10					14	34.72
		15	5.69					15	32.98
								16	27.78
								17	18.89

Track 6 (Compact leaf)		Track 7 (Early flowering)		Track 8 (Violet flowers)		Track 9 (High yielding)		Track 10 (Short pod)	
Band No.	**Mol. Weight**	**Band No.**	**Mol. Weight**	**Band No.**	**Mol. Weight**	**Band No.**	**Mol. Weight**	**Band No.**	**Mol. Weight**
1	76.46	1	106.62	1	65.58	1	98.43	1	102.61
2	66.42	2	64.33	2	63.11	2	86.34	2	86.34
3	63.31	3	62.51	3	53.44	3	75.24	3	66.85
4	53.96	4	53.10	4	48.87	4	66.42	4	63.31
5	48.71	5	49.34	5	45.26	5	62.51	5	55.00
6	45.55	6	41.44	6	41.64	6	54.13	6	50.13
7	41.57	7	40.13	7	40.32	7	48.87	7	44.40
8	37.51	8	37.63	8	38.12	8	45.99	8	42.38
9	35.68	9	36.67	9	37.69	9	41.77	9	39.42
10	34.33	10	34.28	10	35.63	10	41.04	10	38.48
11	32.72	11	33.95	11	34.00	11	37.99	11	37.93
12	5.22	12	32.35	12	32.98	12	35.74	12	35.11
		13	6.07	13	32.66	13	34.50	13	34.50
				14	26.05	14	33.57	14	30.29
				15	6.34	15	7.20	15	29.09
						16	6.61	16	16.97
								17	8.55

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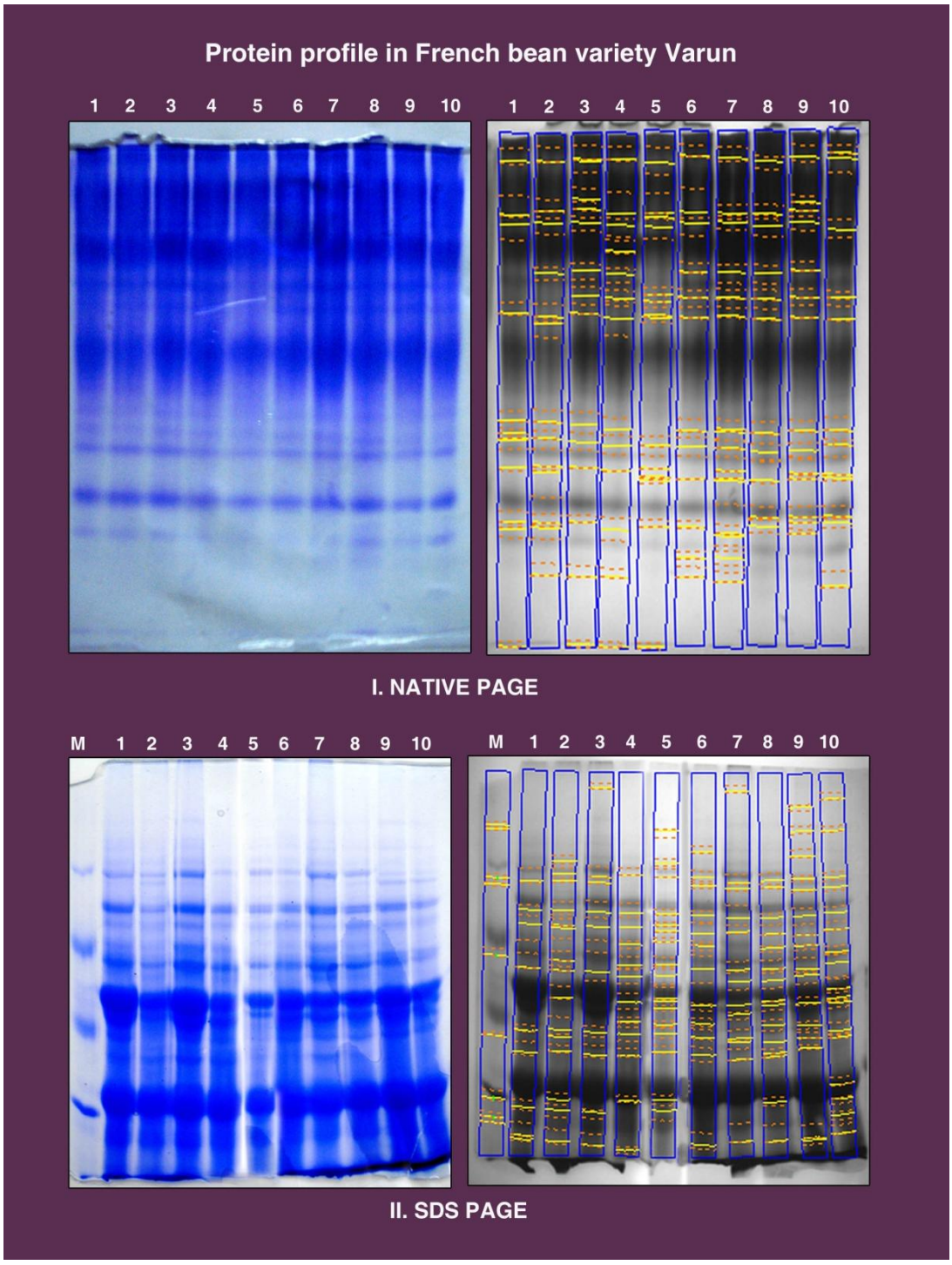
Plate 1: Protein profile of Morphological mutants of French bean variety Varun.



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