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ELECTROPHORETIC STUDIES ON SEED PROTEINS OF VIABLE MUTANTS IN *CAJANUS CAJAN* (L.) MILLSP.

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ABSTRACT

In present investigation, the protein and polypeptide profiles of some pigeonpea mutants have been analyzed on Native and SDS-PAGE to understand genetic variability .Native PAGE gel analysis of viable mutants in variety BDN 708 showed considerable variations in protein profiling. SDS-PAGE analysis of viable mutants of pigeonpea revealed a wide range of protein polymorphism and variability with respect to number and mobility of polypeptide bands. Maximum bands could be found in early maturing mutant. Highest bands could be observed in *xantha* mutant followed by three seeded mutants in BSMR 853.

KEY WORDS: **Native, SDS-PAGE, proteins, polymorphism.**

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INTRODUCTION

Pigeonpea or red gram is an important crop in India next only to chickpea. Pigeonpea (Arhar / Tuar) is a major source of vegetable protein and it can be grown successfully by resource-poor farmers under rain fed dry lands. The seed proteins of wild species have a poor solubility than that of cultivated pigeonpea, and this indicates an increase in solubility under domestication, and perhaps improved nutritional quality in this grain legume [1]. A wide variability exists in chemical composition of pigeonpea seeds due to genotype, growth conditions, storage condition, and duration. [2 and 3] The protein content of commonly grown pigeonpea cultivars ranges between 17.9 and 24.3 g/100 g for whole grain samples. [4] Mutation breeding has been used for improving both oligogenic as well as polygenic characters. It has been employed to improve morphological and physiological characters, disease resistance and quantitative characters including yielding ability. It is a rapid method of developing new varieties.

MATERIALS AND METHODS

Three hundred uniform, dry and healthy seeds of two pigeonpea varieties Amol (BDN-708) and Vaishali (BSMR- 853) were surface sterilized with 0.1 % mercuric chloride solution for about one minute and washed meticulously with distilled water. Seeds were presoaked in distilled water for 6 hours and later immersed in the mutagenic solution for 5 hours with regular shaking. Seeds soaked in distilled water for 12 hours served as control. The presoaking enhances the rate of uptake of the mutagen by increasing– cell permeability and also initiates metabolism in the seeds for treatments. The different concentrations used for chemical mutagenic treatment were 0.05%, 0.10%, 0.15% for EMS and 0.010%, 0.015%, 0.020% for SA, respectively. Immediately after the completion of treatment, the seeds were washed under running tap water and subjected to post-soaking in distilled water for one hour. Healthy and dry seeds of aforesaid varieties were packed in small polythene covers (moisture level of 10%) and seed samples were exposed to 05kR, 10kR and 15kR doses of Gamma rays were applied from Co^{60}, in Department of Biophysics, Government Institute of Science, Aurangabad. (M.S.), India. The dose rate was 24,578 rads per hour. The seeds of each treatment were sown in the field following randomized block design (RBD) with three replications along with control for rising the M_1 generation. The viable mutants of M_2 generation were harvested separately and raised again for M_3 generation. The various types of viable mutants comprised high yielding, tall, dwarf, branched, early flowering, early maturing, *xantha,* light green pod, small pod, two seeded pod, five seeded pod types. Three plants from each mutant line were selected and biochemically analyzed.

1. Extraction of water soluble seed proteins

Mature seeds were washed with water, dried and grounded to make fine powder. It was defatted with hexane and 100 mg of the powder was kept for extraction in 1 ml of 1:6 proportion of 10 mM Tris buffer P^H 8.0 with 1 % PVP (Polyvinyl pyrolidone). The suspension was centrifuged at 12,000 rpm at 4 ^{0}C for 20 minutes and clear supernatant was used for protein estimation and Native /SDS-PAGE.

2. *Protein estimation*

The protein estimation was carried out using Lowry's method .[5]

3. Electrophoresis

Electrophoresis is widely used to separate and characterize proteins by applying electric current. Analysis and comparison of protein in a large number of samples is easily made on polyacrylamide gel slabs. Polyacrylamide gel was formed by polymerizing acrylamide with a cross-linking in the presence of a catalyst (APS) and chain initiator (TEMED). Polyacrylamide Gel Electrophoresis (Native and SDS-PAGE):The water soluble proteins were analyzed using the vertical slab polyacrylamide gel electrophoresis apparatus.

4. Non-denaturing discontinuous (Native) PAGE

It was performed by using Davis system. [6]

5. SDS-PAGE

SDS-PAGE (Sodium dodecyl sulphate polyacrylamide gel) electrophoresis of proteins was carried out in 12 % polyacrylamide gels, under condition that ensured dissociation of proteins into their individual polypeptide subunits and that minimized aggregation. Here, discontinuous PAGE system was used as described by Laemmli.[7] Electrophoresis was performed at constant voltage (120) for 4.5 hours. Then the gels were stained with staining solution for 5 hours and washed briefly in destaining solution till the background became clear. The gels were photographed and molecular weight of every band was determined using gel documentation system. The Gene snap software was used for Rf value molecular weight determination.

RESULTS

Water soluble protein content: (Table 1)

Water soluble seed protein content (%) present in M_3 viable mutants of both varieties of pigeonpea has been shown in Tables 1. The viable mutants in both the varieties of pigeonpea showed variability in water soluble protein content. In variety BDN 708 highest value (21.15%) for soluble protein was observed in early maturing mutant, while the lowest (17.00%) was in dwarf mutant. In variety BSMR 853, the highest soluble seed protein value (22.20%) was noticed in three seeded mutant while lowest (17.89%) could be found in branched and five seeded mutant.

Protein profile: Plate -1 (Table 2- 3)

The protein polymorphism of different viable mutants revealed a wide variability with respect to the number and mobility of bands. Molecular characterization of all the viable mutants of pigeonpea varieties BDN 708 and BSMR 853 obtained in the present investigation was carried out by their protein profiles employing Native-PAGE and SDS-PAGE. The characterization studies were carried out on seed storage proteins (Water soluble proteins). Native PAGE gel analysis of viable mutants of pigeonpea variety BDN 708 showed considerable variation in protein profiling. In control plant, tall, branched and early flowering mutants a total of 09 protein bands were recorded. Maximum 12 bands could be found in early maturing mutant. Native PAGE gel analysis of viable mutants of pigeonpea variety BSMR 853 showed considerable variation in protein profiling. In control total 08 protein bands were recorded. Highest 13 bands could be observed in *xantha* mutant followed by 10 bands in three seeded mutants. In present analysis the seed protein banding pattern of viable mutants of both the varieties showed differences in respect to presence or absence of bands, besides theirs number and mobility. SDS-PAGE analysis of viable mutants of pigeonpea variety BDN 708 ranged from 12 to 19 bands as compared to14 bands in control (Plate-1). Maximum number of polypeptide bands were found in the high yielding (19) mutant having the molecular weight of 111.12 kD. Highest molecular weight (111.66 kD) was observed in small compact leaves mutant, which had 17 protein bands. While minimum number of polypeptide bands could be noted in tall and early flowering mutants. Lower molecular weight of protein bands could be found in tall mutant (91.90kD). The polypeptide bands of the viable mutants of the variety ranged from 09 to 13 as compared to 12 bands in control. Maximum number of polypeptide bands 13 was found in the three seeded and 12 bands of protein could be observed in the tall, branched, *xantha* and early flowering with branched mutant while minimum number of polypeptide bands (09) could be recorded in five seeded mutants. Highest molecular weight was observable in three seeded and *xantha* mutants (114.40 kD).While the least molecular weight could be found in small pod mutant (75.40 kD).

Table 1
Water soluble seed protein content (%) in different viable mutants of pigeonpea variety BDN- 708 and variety BSMR -853.

variety BDN- 708				**variety BSMR -853**			
Sr. No.	Track No.	Name of mutant	Water soluble protein content (%) of defatted seed powder	Sr. No.	Track No.	Name of mutant	Water soluble protein content (%)of defatted seed powder
1	1	Control	19.55	1	1	Control	19.9
2	2	Tall	20.85	2	2	High yielding	19.62
3	3	Dwarf	17	3	3	Tall	18.08
4	4	Branched	20.2	4	4	Dwarf	19.06
5	5	Early flowering	20.96	5	5	Branched	17.89
6	6	Early maturing	21.15	6	6	Early flowering	18.66
7	7	Light green pod	21.11	7	7	Early flowering with branched	22.04
8	8	Dark black pod	19.76	8	8	Three seeded	22.2
9	9	Small compact leaves	19.93	9	9	*Xantha*	19.02
10	10	High yielding	19.76	10	10	Five seeded	17.89
11	11	Erect and high yielding	19.9	11	11	Small pod	19.48
12	M	Molecular Weight marker	-	12	M	Molecular weight marker	-
13	-	SD	1.1648	13	-	SD	1.4902
14	-	SE	0.3512	14	-	SE	0.4493
15	-	CD(p=0.05)	0.7832	15	-	CD(p=0.05)	1.0020
16	-	CD(p=0.01)	1.1133	16	-	CD(p=0.01)	1.4243

Table 2
Molecular weight of bands in SDS PAGE in variety BDN 708

Track 1		**Track 2**		**Track 3**		**Track 4**	
Band No.	**Mol. weight**	**Band No.**	**Mol. weight**	**Band No.**	**Mol. weight**	**Band No.**	**Mol. weight**
1	**98.87**	**1**	**91.90**	**1**	**103.29**	**1**	**93.71**
2	**91.46**	**2**	**87.96**	**2**	**94.63**	**2**	**72.40**
3	**73.82**	**3**	**82.17**	**3**	**61.05**	**3**	**61.35**
4	**69.63**	**4**	**79.80**	**4**	**42.11**	**4**	**42.55**
5	**59.30**	**5**	**59.01**	**5**	**39.89**	**5**	**40.23**
6	**42.11**	**6**	**42.03**	**6**	**39.56**	**6**	**36.09**
7	**39.56**	**7**	**39.48**	**7**	**35.57**	**7**	**33.70**
8	**35.72**	**8**	**35.35**	**8**	**35.20**	**8**	**31.65**
9	**33.42**	**9**	**33.28**	**9**	**33.21**	**9**	**27.27**
10	**31.32**	**10**	**27.04**	**10**	**26.71**	**10**	**18.99**
11	**26.82**	**11**	**18.68**	**11**	**18.83**	**11**	**17.21**
12	**18.68**	**12**	**13.33**	**12**	**16.18**	**12**	**16.72**
13	**15.65**	**-**	**-**	**13**	**13.61**	**13**	**12.38**
14	**13.01**	**-**	**-**	**-**	**-**	**-**	**-**

Track 5		Track 6		Track 7		Track 8	
Band No.	Mol. weight	Band No.	Mol. weight	Band No.	Mol. weight	Band No.	Mol. Weight
1	98.87	1	95.55	1	96.02	1	96.49
2	94.63	2	85.02	2	92.35	2	92.80
3	83.78	3	61.35	3	85.02	3	74.91
4	62.25	4	42.38	4	73.82	4	69.97
5	42.47	5	40.23	5	69.29	5	62.86
6	40.23	6	33.70	6	62.56	6	43.42
7	33.70	7	28.18	7	42.64	7	40.90
8	27.95	8	27.72	8	40.90	8	36.47
9	19.95	9	25.32	9	36.24	9	34.26
10	16.99	10	19.95	10	34.12	10	28.88
11	14.66	11	17.14	11	28.88	11	25.95
12	12.69	12	14.60	12	26.06	12	20.62
-	-	13	12.43	13	20.70	13	20.20
-	-	-	-	14	17.64	14	15.02
-	-	-	-	15	15.02	15	13.12

Track 9		Track 10		Track 11		Track 12	
Band No.	Mol. weight	Band No.	Mol. weight	Band No.	Mol. weight	Band No.	Mol. Weight
1	111.66	1	111.12	1	109.51	1	66.00
2	98.39	2	104.31	2	96.49	2	43.00
3	96.49	3	64.10	3	68.29	3	29.92
4	91.90	4	61.95	4	61.35	4	29.00
5	64.41	5	48.33	5	55.39	5	14.30
6	62.56	6	43.63	6	43.21	6	12.80
7	43.42	7	40.39	7	41.24	-	-
8	40.56	8	36.55	8	37.16	-	-
9	36.78	9	35.94	9	36.24	-	-
10	34.40	10	34.12	10	34.62	-	-
11	28.64	11	32.12	11	29.18	-	-
12	25.85	12	31.06	12	21.05	-	-
13	20.87	13	29.00	13	20.45	-	-
14	20.28	14	26.06	14	14.96	-	-
15	17.71	15	19.95	15	13.50	-	-
16	15.02	16	18.08	16	12.33	-	-
17	13.06	17	17.49	-	-	-	-
-	-	18	15.02	-	-	-	-
-	-	19	12.33	-	-	-	

Table 3
Molecular weight of bands in SDS PAGE in variety BSMR 853

Track 1		Track 2		Track 3		Track 4	
Band No.	Mol. weight	Band No.	Mol. Weight	Band No.	Mol. weight	Band No.	Mol. weight
1	106.10	1	110.49	1	110.49	1	110.49
2	71.99	2	73.68	2	74.10	2	79.43
3	38.68	3	66.38	3	70.34	3	66.77
4	31.67	4	39.37	4	38.46	4	37.78
5	24.92	5	24.70	5	32.24	5	30.94
6	21.82	6	18.32	6	24.02	6	23.48
7	18.07	7	10.61	7	21.32	7	17.34
8	10.51	8	8.17	8	18.16	8	9.72
9	8.17	9	7.22	9	10.23	9	7.80
10	8.06	10	5.63	10	7.98	10	6.86
11	7.22	-	-	11	7.05	11	5.61
12	5.68	-	-	12	5.58	-	-

Track 5		Track 6		Track 7		Track 8	
Band No.	Mol. weight	Band No.	Mol. weight	Band No.	Mol. weight	Band No.	Mol. weight
1	113.08	1	113.74	1	113.74	1	114.40
2	81.77	2	76.72	2	76.28	2	83.20
3	75.84	3	67.94	3	52.05	3	75.84
4	38.23	4	39.14	4	38.68	4	39.37
5	30.58	5	31.67	5	31.49	5	31.86
6	23.59	6	24.25	6	24.13	6	24.13
7	17.50	7	17.66	7	21.32	7	21.03
8	16.41	8	10.00	8	17.58	8	17.58
9	10.04	9	7.80	9	9.90	9	12.35
10	7.80	10	6.92	10	7.73	10	9.86
11	6.89	11	5.66	11	6.89	11	7.69
12	5.66	-	-	12	5.66	12	6.86
-	-	-	-	-	-	13	5.66

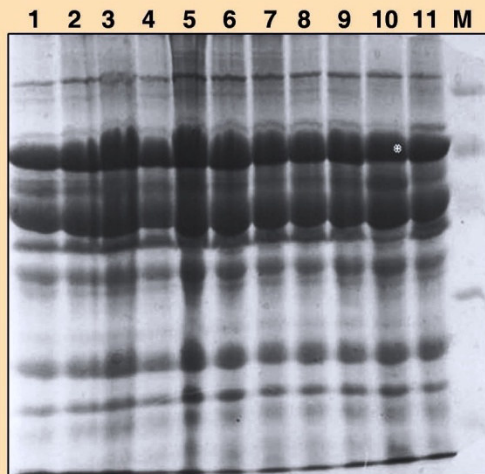
Track 9		Track 10		Track 11		Track 12	
Band No.	Mol. weight	Band No.	Mol. weight	Band No.	Mol. weight	Band No.	Mol. weight
1	114.40	1	75.84	1	75.40	1	104.28
2	76.72	2	38.23	2	40.31	2	66.00
3	66.77	3	31.30	3	32.43	3	43.00
4	39.14	4	23.91	4	25.74	4	29.00
5	31.30	5	17.50	5	23.16	5	14.30
6	24.25	6	10.04	6	18.49	6	6.99
7	21.22	7	7.77	7	10.51	7	5.79
8	17.58	8	6.80	8	8.09	-	-
9	10.04	9	5.74	9	7.08	-	-
10	7.66	-	-	10	5.84	-	-
11	6.73	-	-	-	-	-	-
12	5.71	-	-	-	-	-	-

No. = Number.
Mol .weight= Molecular weight.

PLATE - 1

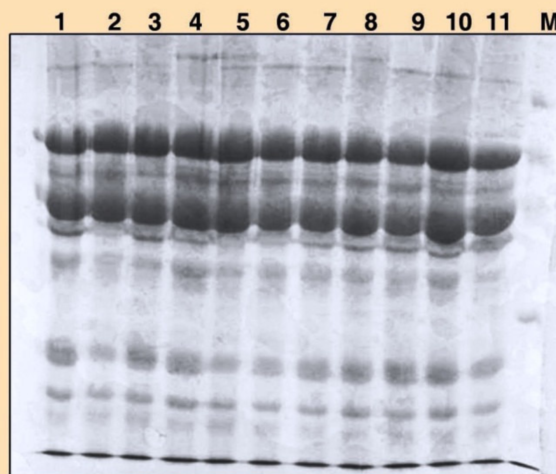
Documented SDS-PAGE and NATIVE-PAGE of pigeonpea [*Cajanus cajan* (L.) Millsp.]

1 2 3 4 5 6 7 8 9 10 11 M



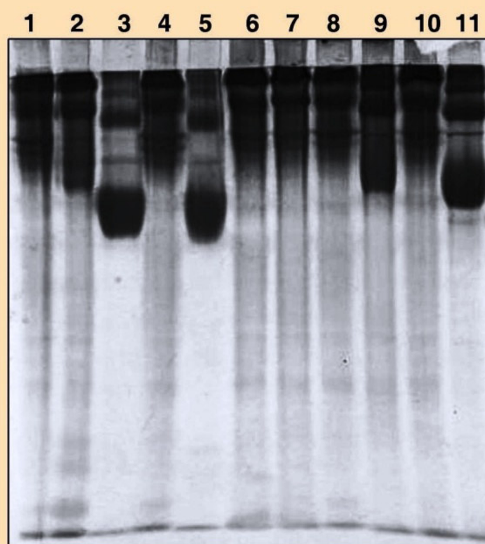
SDS-PAGE variety BDN-708

1 2 3 4 5 6 7 8 9 10 11 M



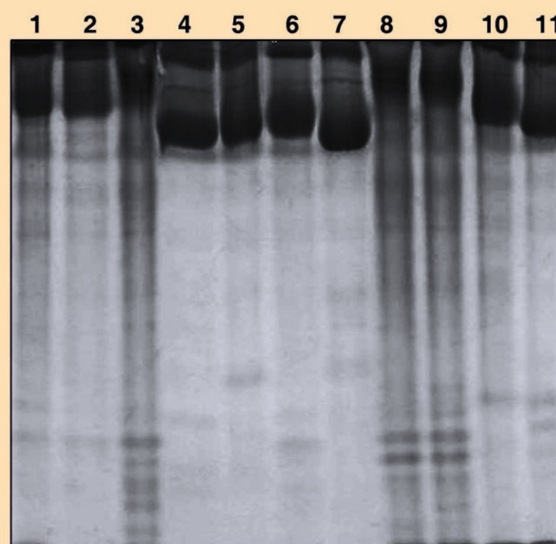
SDS-PAGE variety BSMR-853

1 2 3 4 5 6 7 8 9 10 11



NATIVE-PAGE variety BDN-708

1 2 3 4 5 6 7 8 9 10 11



NATIVE-PAGE variety BSMR-853

variety BDN- 708				variety BSMR -853			
Track No.	Name of mutant	Track No.	Name of mutant	Track No.	Name of mutant	Track No.	Name of mutant
1	Control	7	Light green pod	1	Control	7	Early flowering with branched
2	Tall	8	Dark black pod	2	High yielding	8	Three seeded
3	Dwarf	9	Small compact leaves	3	Tall	9	*Xantha*
4	Branched	10	High yielding	4	Dwarf	10	Five seeded
5	Early flowering	11	Erect and high yielding	5	Branched	11	Small pod
6	Early maturing	M	Molecular Weight marker	6	Early flowering	M	Molecular Weight marker

DISCUSSION

There was an increase as well as decrease in protein content. The amount and composition of seed proteins are widely influenced both by environmental and endogenous factors. Several attempts have been made to induce variations in protein quality and quantity using macromutations by researchers like. [8] Ahmed *et al*., [9] suggested that protein disappearance represents degradation of reserve proteins, while new proteins appearing at specific times during germination and seedling development have stage- specific developmental functions. Through this technique several researchers have proved the genetic variation in different mutants, cultivars and germplasm lines. Wide heterogeneity in legumin subunit pairs with respect to their molecular weight has been reported in *Vicia faba* [13] and in *Lathyrus sativus* .[14] It is believed that the SDS-PAGE analysis of seed storage proteins is suitable to identify plant genetic diversity and polymorphism. Protein variation among cultivars of cotton using PAGE and found that cultivars could be identified at 14% acrylamide monomer concentration with distinct and potential results. [15] Shah and Ratna Trivedi[16] reported in Aspergillus tamari the molecular weight of phytase enzyme was 85kDa. Fujita *et al.,* [17] reported phytase from Aspergillus oryzae under solid state fermentation conditions which showed an optimum pH of 5.0, and stability at 50oC and molecular weight 56 kDa. Thyagarajan *et al.,* [18] reported the molecular mass of purified phytase was found to be 56kDa, by SDS–PAGE analysis. The protein profiling of germplasm and use of genetic markers have been widely and effectively used to determine the taxonomic and evolutionary aspects of several crops[19]. Pugalenthi *et al.* [(20)] reported the protein content of *Mucuna* bean grown in different locations of the tropics and subtropics between 21.0% - 30.3%. The variation may be attributed to interaction between genetic makeup and the environment. Electrophoretic analysis of proteins revealed the presence of total 11 bands in the soaked seeds of *Mucuna.* After 24 hrs of germination, there are total 12 protein bands and intensity of the band 9 was increasing and was maximum at 48 hrs of germination. [(21)] Studies with seeds of *L. sativus*, *Dolichos lablab*, *Cicer arietinum*, *Vicia faba* and also *Gossypium hirsutum* revealed various protein fractions ranging from 92 to 12 kDa with a faster degradation of high-molecular weight proteins .[(22-23)] Protein electrophoresis is a better tool for the identification of genetic diversity and tracing evolutionary processes in plants than morphological markers. [(24-25)]

CONCLUSION

In present investigation the protein profiles and polypeptide profiles of some pigeonpea mutants have been analyzed on Native and SDS-PAGE to understand genetic variability prevailing in them. Native PAGE gel analysis of viable mutants in variety BDN 708 showed considerable variation in protein profiling. Maximum bands could be found in early maturing mutant. Highest bands could be observed in *xantha* mutant followed by three seeded mutants in BSMR 853. SDS-PAGE analysis of viable mutants of pigeonpea revealed a wide range of protein polymorphism and variability with respect to number and mobility of polypeptide bands. Maximum number of polypeptide bands was found in the high yielding mutant in BDN 708 and maximum number of polypeptide bands has been found in the three seeded, followed by tall, branched, *xantha* and early flowering with branched mutants while minimum number of polypeptide bands could be recorded in the five seeded mutant. As pigeonpea is a nutritious pulse in human diet, its mutants like tall, dwarf and nitrogen content carrying would assume substantial economic importance. From the foregoing it is concluded that the various mutagenic treatments tried in the present study have very much succeeded in generating genetic variability in pigeonpea. The results obtained decisively demonstrated the usefulness and the effective potential of the induced mutational approach in genetic improvement of pigeonpea for recovering superior mutant plant types having high yield, early maturity, besides high protein and carbohydrate content.

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