Genetic Variation for Seed Proteins in Cultivated and Wild Species of Pigeonpea

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Electrophoretic analysis of seed protein of three cultivated varieties of *Cajanus cajan* and its three wild relatives were carried out to study variability in protein band expression. In all, 22 protein bands were identified ranging with molecular weight of 97.0 to 16.5 kD. Cultivated and wild species showed variability in protein band expression ranging from 6 in ICPL 84023 to 8 in UPAS 120 and Pant A 134 whereas in wild species 9 bands in *C.cajanifolius* and *C.acutifolius* and 8 bands in *C.scarabaeoides*. The differences were also observed for number, length and intensity of polypeptide bands of the protein. Band at 87.5 kD was common in all the cultivated and in wild species. In the cultivated lines, bands at 92.5 and 37.0 kD were common, whereas two bands at 87.5 and 74.0 kD were common among wild species.

Key Words : *Cajanus cajan*, Electrophoresis, Pigeonpea, Seed protein, Wild species of *Cajanus*

Introduction

Pigeonpea [*Cajanus cajan* (L) Millspaugh] is the second most important pulse crop of India. It is rich in protein which constitutes an important component of human diet in developing countries like India. Besides its main use as *dhal* (dehulled split seed), its immature green seeds and pods are consumed as vegetable. Pigeonpea seed is a rich source of protein, carbohydrates, vitamins and certain minerals (Srivastava and Srivastava, 2006). Varietal differences exist for the nutritional composition in pigeonpea seeds (Yadava, 1984). *C.cajanifolius* and *C.scarabaeoides* has high seed protein. Since genetic differences are reflected in shifts of seed protein patterns, the present study was undertaken on biochemical characterization of seed storage protein in order to assess variation for seed protein in cultivated lines *viz*., Pant A 134, UPAS 120 and ICPL 84023 and wild species *viz*., *C.cajanifolius*, *C.acutifolius* and *C.scarabaeoides.*

Materials and Methods

Three cultivated lines of pigeonpea *viz*., Pant A 134, UPAS 120 and ICPL 84023 along with three wild species *viz*., *C. cajanifolius, C. acutifolius* and *C.scarabaeoides* were raised at Crop Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar during *kharif* 2003-04. Protein separation was carried out by SDS-PAGE according to the method of Laemmli (1970). The degree of electrophoretic similarity was calculated by pair wise comparison of the cultivated with wild species, cultivated lines with cultivated lines and wild species with wild species. Similarity index (SI) was also worked out following Mishra *et al*. (1996) *i.e.*

$$\text{Similarity Index (\%)} = \frac{\text{Number of similar bands in a sample}}{\text{Total number of bands for the two sample}} \times 100$$

Results and Discussion

The relative mobility (Rm) values for various seed protein bands of cultivated and wild species of pigeonpea used in the present study ranged from 0.03 to 0.74 suggesting a wide range of variability in protein band expression (Table 1). Among *Cajanus cajan* genotypes, Pant A 134 and UPAS 120 depicted highest number of bands (8) followed by 6 in ICPL 84023. Nine protein bands were observed in *C. cajanifolius* and *C. acutifolius* followed by 8 in *C. scarabaeoides*. One polypeptide band at 87.5 kD was noted in all the three cultivated lines *viz*., Pant A 134, UPAS 120 and ICPL 84023 and two wild species *viz*., *C. scarabaeoides* and *C. acutifolius,* which might be due to genetic relationship among them. The similarity in two polypeptide bands at 92.5 and 37.0 kD was noted in all the cultivated lines *viz*., Pant A 134, UPAS 120 and ICPL 84023, whereas one band at 74.0 kD was present in all the three wild species *viz*., *C. cajanifolius, C. acutifolius* and *C. scarabaeoides.* There was homogeneity of bands at 92.5, 87.5, 80.0, 71.5, 60.0, 49.5, 45.0, 35.0, 24.0 and 16.0 kD between cultivated lines and some wild species used in the present study.

All types of protein band intensity i.e. low, medium and high were observed in various cultivated and wild species of pigeonpea. However, in some varieties the quantitatively similar numbers of total protein bands were noted, but differences in presence and / or absence of particular band at particular position and their Rm

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values as well as different protein band intensity for common bands showed diverse nature of these varieties to each other. Though there were total 9 protein bands in electrophoregram of species *C. cajanifolius* and *C. aculifolius*, but band position was different. The bands at 97.0, 80.0, 45.0 and 24.0 kD were characteristically present in *C.cajanifolius* while missing in *C. acutifolius*. Similarly, bands at 71.5, 42.5, 39.0, 26.0 and 21.5 kD were present in *C. acutifolius* and missing in *C. cajanifolius*. Eletrophoregrams of ICPL 84023 and *C. scarabaeoides* indicating quantitatively different protein bands 9, 6 and 8 respectively, and showing some similarities and some diverse banding pattern revealed clear-cut resemblances and differences among them. Similar to the present study Sharma and Maloo (2006) also reported quantitatively similar numbers of total protein bands but differences in presence and/or absence of particular bands at their particular position in soybean.

Highest similarity index (58.82%) for *C. cajanifolius* was seen with Pant A 134 followed by UPAS 120 with Pant A 134 (50.00%) and *C. scarabaeoides* with *C. cajanifolius* (47.05%) and *C. cajanifolius* with UPAS 120 (47.05%). High order of similarity within cultivated lines was observed in UPAS 120 with Pant A134 (50.00%) followed by ICPL 84023 with UPAS 120 (42.85%) and Pant A 134 with UPAS 120. All the three wild species also shared good similarity index ranging from 23.52 – 47.05% within the species. All types of protein band intensity were observed (Fig.1). Maximum 9 bands were noted in *C. cajanifolius* and *C. acutifolius* followed by 8 in Pant A 134, UPAS 120, and *C. scarabaeoides* and lowest 6 in ICPL 84023.

The cultivated lines and wild species of pigeonpea had protein bands of both high as well as low molecular weight ranging from 97.0 to 16.5 kD. Similarly, Naik and Kole (1992) observed bands ranging from 17.4 to 75.0 kD in mungbean. Singh *et al*. (1992) and Roy *et al.* (2001) also noted differences in band mobility, width and intensity in legumes. Proteins being the direct gene products reflect the genomic composition of lines accurately to some extent and, therefore, are ideal for genomic distinctiveness.

In all, 22 protein bands observed in all the cultivated as well as in wild species seem to be very informative and useful in deriving qualitative and quantitative differences in various protein fractions. It was noted that a protein band of 87.5 kD was present in all the three cultivated lines as well as in wild species indicating that the genes controlling expression of these proteins bands appeared to behave as a single block. The observed differences in protein band intensity in cultivated and wild species could be utilized in identification of high protein lines and these lines could be directly used or may be used as protein rich parents in hybridization programme to transfer high seed protein quality trait in to desirable lines. Such types of

Table 1. Relative mobility values for various protein bands electrophoressed in seed protein extract of cultivated and wild species of pigeonpea

Protein Band #	Rm value	Mol.wt. kD	Cultivated / Wild species					
			Pant A 134	UPAS 120	ICPL 84023	*C. cajanifolius*	*C. acutifolius*	*C. scarabaeoides*
1	0.03	97.0	—	—	—	+	—	+
2	0.05	92.5	+	+	+	+	—	—
3	0.08	87.5	+	+	—	+	+	+
4	0.09	85.0	—	+	—	—	—	—
5	0.11	80.0	+	—	+	+	—	—
6	0.14	74.0	—	—	—	+	+	+
7	0.15	71.5	+	+	—	—	+	—
8	0.20	60.0	—	+	+	+	+	—
9	0.26	49.5	+	—	—	—	—	+
10	0.29	45.0	—	+	—	+	—	—
11	0.31	42.5	—	—	—	—	+	—
12	0.34	39.0	—	—	—	—	+	—
13	0.36	37.0	+	+	+	—	—	—
14	0.38	35.0	+	—	—	+	+	—
15	0.45	31.0	—	+	—	—	—	—
16	0.47	30.0	—	—	+	—	—	—
17	0.55	26.0	—	—	—	—	+	—
18	0.59	24.0	+	—	—	+	—	+
19	0.64	21.5	—	—	—	—	+	—
20	0.66	20.5	—	—	—	—	—	+
21	0.67	20.0	—	—	—	—	—	+
22	0.74	16.5	—	—	+	—	—	+

Note : Protein band Absent, + : Protein band Present, Rm : Relative mobility

Table 2. Quantitative and qualitative differences among cultivated lines and wild species of pigeonpea

Genotype	Qualitative differences Total no. of bands	Quantitative differences Specific band number	Thick	Medium	Thin	Faint (%)	Similarity Index(%)
Pant A134	8	71.5,49.5,37.0	2	1	—	5	—
C. cajanifolius	9	97.0,60.0,45.0	1	—	—	8	58.82
Pant A134	8	92.5,80.0,49.5,37.0,24.0	2	1	—	5	—
C. acutifolius	9	74.0,60.0,42.5,39.0,26.0,21.5	1	—	1	7	35.29
Pant A134	8	92.5,80.0,71.5,37.0,35.0	2	1	—	5	—
C. scarabaeoides	8	97.0,74.0,20.5,20.0,16.5	—	—	2	6	37.50
UPAS 120	8	85.0,71.5,37.0,31.0	1	1	—	6	—
C. cajanifolius	9	97.0,80.0,74.0,35.0,24.0	1	—	—	8	47.05
UPAS 120	8	92.5,85.0,45.0,37.0,31.0	1	1	—	6	—
C. acutifolius	9	74.0,42.5,39.0,35.0,26.0,21.5	1	—	1	7	35.29
UPAS 120	8	92.5,85.0,71.5,60.0,45.0,37.0,31.0	1	1	—	6	—
C. scarabaeoides	8	97.0,74.0,49.5,24.0,20.5,20.0,16.5	—	—	2	6	12.50
ICPL 84023	6	37.0,30.0,16.5	3	—	—	3	—
C. cajanifolius	9	97.0,80.0,74.0,35.0,24.0	1	—	—	8	40.00
ICPL 84023	6	92.5,80.0,37.0,30.0,16.5	3	—	—	3	—
C. acutifolius	9	87.5,74.0,71.5,42.5,39.0,35.0,26.0,21.5	1	—	1	7	13.33
ICPL 84023	6	92.5,80.0,60.0,37.0,30.0	3	—	—	3	—
C. scarabaeoides	8	97.0,87.5,74.0,49.5,24.0,20.5,20.0	—	—	2	6	14.28
Pant A134	8	80.0,49.5,35.0,24.0	2	1	—	5	—
UPAS 120	8	85.0,60.0,45.0,31.0	1	1	—	6	50.00
Pant A134	8	87.5,71.5,49.5,35.0,24.0	2	1	—	5	—
ICPL 84023	6	87.5,85.0,71.5,45.0,31.0	3	—	—	3	42.85
UPAS 120	8	80.0,30.0,16.5	1	1	—	6	—
ICPL 84023	6	37.0,30.0,16.5	3	—	—	3	42.85
C. cajanifolius	9	97.0,87.5,74.0,45.0,35.0,24.0	1	—	—	8	—
C. acutifolius	9	71.5,60.0,42.5,39.0,350.,26.0,21.5	1	—	1	7	44.44
C. scarabaeoides	9	97.0,49.5,24.0,20.5,20.0,16.5	1	—	—	8	—
C. cajanifolius	8	92.5,80.0,60.0,45.0,35.0	—	—	5	6	47.05
C. scarabaeoides	8	49.5,20.5,20.0,16.5	—	—	2	6	—
C. acutifolius	9	71.5,60.0,42.5,39.0,35.0,26.0,21.5	1	—	1	7	23.52

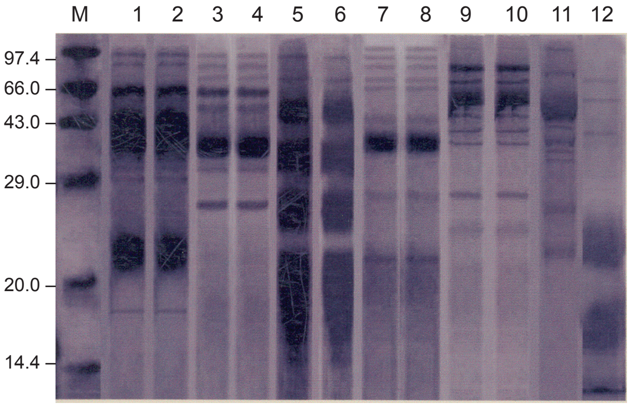

Fig. 1: Electrophoretic banding pattern of cultivated and wild species of pigeonpea
Lane No. 1 & 2 (Pant A 134), 3 & 4 (UPAS 120), 5 & 6 (ICPL 84023) 7 & 8 (*C. cajanifolius*), 9 & 10 (*C. acutifolius*) , 11 *C. scarabaeoides*

close relationships and / or minor variations were observed in chickpea also (Kharkwal, 1999). The present study is preliminary to assess the variation in seed protein banding patterns in order to identify the potential genotype(s) in pigeonpea and further study of seed protein fractions would be required for identifying the true high protein rich lines.

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