Electrophoretic Characterization of *Cajanus cajan* x *C. cajanifolius* Hybrids

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Electrophoretic analysis of seed protein of nine F_4 progenies of three interspecific hybrids and their parents was carried out to study the variability in protein band expression. In all, 23 protein bands were identified ranging with molecular weight of 98 to 16 kD. Of these, three protein bands of 95.0, 71.5 and 60.0 kD were unique to *Cajanus cajan*, while four bands of 77.5, 67.5, 29.5 and 22.5 kD were specific to hybrids only. On the other hand *C. cajanifolius* has no unique protein band. Parents showed variability in protein band expression ranging from 7 in ICPL 84023 to 9 in UPAS 120 and *Cajanus cajanifolius*. The inter-progeny differences were observed for number, length and intensity of polypeptide bands of the protein. The similarity index of hybrids with *Cajanus cajanifolius* ranged from 35.29 to 66.66%. In general, bands at 87.5, 74.0, 46.0 and 34.5 kD were common in parents as well as hybrids.

Key Words: *Cajanus cajan*, *Cajanus cajanifolius*, Electrophoresis, Interspecific hybrids, Pigeonpea, Seed protein

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is an important grain legume crop in the semi-arid tropics and contains about 24% protein in seeds. Breeding tools employed for genetic improvement in this crop are limited to selection and intervarietal hybridization. Conventional intervarietal hybridization has not met with much success in developing high seed protein cultivars. Seed size and quality are important attributes in pigeonpea.

It is imperative to introduce alien variation by transferring desirable protein trait from wild species into cultivated ones. *C. cajanifolius* (Haines) Van der Maesen, the putative progenitor of pigeonpea, can be used as a potential donor for genes conferring high protein (Panigrahi *et al.*, 2001). These genes could be effectively introgressed into pigeonpea genotypes by interpecific hybridization. Seed protein markers have been effectively employed for varietal differentiation in several crop plants (Mohanty *et al.*, 2001). Electrophoretic banding patterns of seed proteins helps in differentiating similarities and differences between genotypes and hybrids derived among them. For most seeds, except cereals, storage proteins are predominantly globulins (Krishna and Bhatia, 1985). The mature seed provides a stable and convenient system for biochemical analysis to establish relationship in parents and hybrids. *C. cajanifolius*, a wild species of pigeonpea, has specific features such as high seed protein content, mechanical resistance to pod borer and high pod set, etc. Of the techniques available, analysis of seed protein using electrophoresis is widely used because of their reliability, rapidity and cost effectiveness. Gel electrophoresis of seed protein act as a function of genotypic fingerprinting for distinguishing plant varieties and allied aspects. Since genetic differences are reflected in shifts of seeds protein patterns, the present study was undertaken on biochemical characterization of seed storage protein in order to assess variation for seed protein in F_4 populations of *C. cajan* x *C. cajanifolius* by using sodium dodecyl sulphate-polyacrylamide slab gel electrophoresis (SDS-PAGE).

Materials and Methods

Nine F_3 progenies of three interspecific hybrids derived from hybridization of three cultivated lines, *viz.*, Pant A 134, UPAS 120 and ICPL 84023 with *C. cajanifolius* were raised in Randomized Complete Block Design with three replications at Crop Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar during *Kharif* 2003-04. The F_4 seeds obtained in hybrid population were grouped in three seed types, *viz.*, cultivated, intermediate and wild types on the basis of seed shape appearance. Protein separation in duplicate sets was carried out by polyacrylamide slab gel electrophoresis (SDS-PAGE) according to standard method of (Laemmli, 1970) using 12% acrylamide separating gel with a top layer of 6% acrylamide stacking gel. The protein sample of 10-12 µl was loaded on slab gel wells. Initially the electrophoresis was conducted at 10 mA at normal room temperature and than raised to 30 mA sometime after the dye front crossed the stacking and separating gel interface. The run was

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continued till the tracking dye front migrated upto about 1-1.5 cm from the bottom of the separating gel. The staining was done overnight in Coomassie Brilliant blue solution. Then the gel was destained by repeatedly washing with methanol: acetic acid: water (50:70:880, V/V/V, respectively). The prepared electrophoregrams was photographed by gel documentation. The protein bands were numbered from cathode end and their relative mobilities were calculated. The degree of electrophoretic similarity was calculated by pair wise comparison of the parents and hybrids. Similarity Index (SI) was worked out as per the following method suggested by Mishra *et al.* (1996):

$$SI = \frac{\text{Number of similar bands in a sample}}{\text{Total number of bands for the two samples}} \times 100$$

Results and Discussion

The relative mobility (Rm) values for various seed protein bands of inter-specific hybrids of *C. cajan* x *C. cajanifolius* ranged from 0.02 to 0.75, suggesting a wide range of variability in protein band expression (Table 1). In all, 23 protein bands of different Rm values were identified on the basis of electrophoretic mobility. Nine protein bands (98.0, 92.5, 87.5, 80.0, 74.0, 57.5, 46.0, 34.5, 23.0 kD) were observed in *C. cajanifolius* (Table 1). Among *C. cajan* genotypes, Pant A 134 depicted 9 bands followed by 8 in UPAS 120 and 7 in ICPL 84023. The similarities in polypeptide bands at 95.0, 87.5, 71.5 and 60.0 kD were noted in cultivated parents, *viz.*, Pant A 134, UPAS 120 and ICPL 84023, whereas bands at 34.5 and 26.0 kD were present only in two out of three female parents. One band of 87.5 kD was common in all the three cultivated lines as well as in *C. cajanifolius* which might be due to genetic relationship among them. Few polypeptide bands characterizing *C. cajanifolius* were also seen in the hybrids, *viz.*, Pant A 134 x *C. cajanifolius* (87.5, 80.0, 74.0, 57.5, 46.0, 34.5 and 26.0 kD), UPAS 120 X *C. cajanifolius* (98.0, 92.5, 87.5, 74.5, 46.0 and 34.5 kD) and ICPL 84023 x *C. cajanifolius* (98.0, 92.5, 87.5, 74.5, 46.0, 34.5 and 23.0 kD). It was interesting to note that one band (87.5 kD) was common in all the three cultivated parents, wild parent as well as in hybrids [Pant A 134 x *C. cajanifolius* (C), Pant A 134 x *C. cajanifolius* (W), UPAS 120 x *C. cajanifolius* (W), ICPL 84023 x *C. cajanifolius* (C) and ICPL 84023 x *C. cajanifolius* (I) and ICPL 84023 x *C. cajanifolius* (W)] and this might be due to genetic relationship among them. Bands of 46.0 kD was present in the UPAS 120, *C. cajanifolius* as well as in hybrids Pant A 134 x *C. cajanifolius* (I and W), UPAS 120 x *C. cajanifolius* (I), ICPL 84023 x *C.*

Table 1. Electrophoretic protein banding patterns of parents and interspecific hybrids of *C. cajan* x *C. cajanifolius* derived from SDS-PAGE of seed protein

Band No.	Rm Value	Mol. wt. kD	Pant A 134	UPAS 120	ICPL 84023	*C. cajanifolius*	Pant A 134 x *C. cajanifolius*			UPAS 120 x *C. cajanifolius*			ICPL 84023 x *C. cajanifolius*		
							C	I	W	C	I	W	C	I	W
1	0.02	98.0	-	+	-	+	-	-		-	+	-	+	-	-
2	0.04	95.0	+	+	+	-	-	-	-	-	-	-	-	-	-
3	0.05	92.5	-	-	-	+	-	-	-	+	+	+	+	-	-
4	0.08	87.5	+	+	+	+	+	-	+	-	-	+	+	+	+
5	0.11	80.0	-	-	-	+	+	-	-	-	-	-	-	-	-
6	0.12	77.5	-	-	-	-	-	-	-	-	-	+	+	+	-
7	0.14	74.0	-	-	-	+	+	+	+	-	-	-	-	+	+
8	0.15	71.5	+	+	+	-	-	-	-	-	-	-	-	-	-
9	0.17	67.0	-	-	-	-	-	-	-	-	-	+	-	-	-
10	0.20	60.0	+	+	+	-	-	-	-	-	-	-	-	-	-
11	0.21	57.5	-	-	-	+	-	-	+	-	-	-	-	-	-
12	0.25	51.0	-	-	-	-	+	-	-	-		-	-	-	-
13	0.28	46.0	-	+	-	+	-	+	+	-	+	-	-	+	-
14	0.30	44.0	-	-	+	-	+	-	-	-	-	-	+	+	+
15	0.34	39.0	+	-	-	-	-	-	-	-	-	+	-	-	-
16	0.39	34.5	+	+	-	+	+	-	+	+	+	+	-	+	-
17	0.41	33.0	+	-	-	-	-	+	+	-	-	-	+	-	-
18	0.44	31.5	-	-	+	-	-	+	+	-	-	-	-	+	-
19	0.48	29.5	-	-	-	-	-	-	-	-	-	+	-	-	-
20	0.55	26.0	+	+	-	-	+	+	+	-	-	+	-	-	-
21	0.61	23.0	-	-	+	+	-	+	+	-	-	-	+	+	+
22	0.62	22.5	-	-	-	-	-	+	-	-	-	-	-	-	-
23	0.75	16.0	+	-	-	-	-	+	-	-	-	-	-	-	-

C: cultivated, I: intermediate and W: wild

cajanifolius (I). Similarly, the band of 34.5 kD was also present in the Pant A 134 , UPAS 120, *C. cajanifolius* as well as in six hybrids, namely, Pant A 134 x *C. cajanifolius* (C and W), UPAS 120 x *C. cajanifolius* (C, I and W) and ICPL 84023 x *C. cajanifolius* (I). There was homogeneity of bands at 95.0, 71.5 and 60.0 kD among cultivated parents (Pant A 134, UPAS 120 and ICPL 84023). However, these bands were absent in the *C. cajanifolius* as well as from all the nine hybrids. Similarly, four polypeptide bands were expressed only in the two pigeonpea varieties and lacked expression in *C. cajanifolius* and the two putative hybrids (Panigrahi *et al.*, 2001). Deletion or mutation of structural genes coding for the polypeptides or their regulatory loci results in inhibition of transcription or translation of polypeptides leading to the lack of expression of the concerned polypeptides (Brown *et al.*, 1981).

Highest similarity index with wild parent, *C. cajanifolius* (Table 2) was seen in wild type seeds of hybrids Pant A 134 x *C. cajanifolius* (66.66%) followed

Table 2. Qualitative and quantitative differences among progenies of interspecific hybrids of *C. cajan* x *C. scarabaeoides*

Genotype	Qualitative differences		Quantitative differences				SI with *C. cajanifolius* (%)	SI with cultivated parents (%)
	Total no. of bands	Specific band number	Thick	Medium	Thin	Faint		
Pant A 134 x *C. cajanifolius*								
Male parent	9	98.0 (0.02), 92.5 (0.05), 57.5 (0.21), 46.0 (0.28), 23.0 (0.61)	1	0	0	8	-	-
Cultivated type seed	7	51.0 (0.25), 44.0 (0.30), 26.0 (0.55)	1	2	1	3	50.00	37.50
Male parent	9	98.0(0.02), 92.5 (0.05), 87.5 (0.08), 80.0 (0.11), 57.5 (0.21), 34.5 (0.39)	1	0	0	8	-	-
Intermediate type seed	8	33.0 (0.41), 31.5 (0.44), 26.0 (0.55), 22.5 (0.62), 16.0 (0.75)	1	1	4	2	35.29	35.29
Male parent	9	98.0 (0.02), 92.5 (0.05), 80.0 (0.11)	1	0	0	8	-	-
Wild type seed	9	33.0 (0.41), 31.5 (0.44), 26.0 (0.55)	2	1	2	4	66.66	44.44
UPAS 120 x *C. cajanifolius*								
Male parent	9	98.0 (0.02), 87.5 (0.08), 80.0 (0.11), 74.0 (0.14), 57.5 (0.21), 46.0 (0.28), 23.0 (0.61)	1	0	0	8	-	-
Cultivated type seed	2	Nil	0	1	0	1	36.36	20.00
Male parent	9	87.5 (0.08), 80.0 (0.11), 74.0 (0.14)), 57.5 (0.21), 23.0 (0.61)	1	0	0	8	-	-
Intermediate type seed	4	Nil	1	1	0	2	61.53	50.00
Male parent	9	98.0 (0.02), 80.0 (0.11), 74.0 (0.14), 57.5 (0.21), 46.0 (0.28), 23.0 (0.61)	1	0	0	8	-	-
Wild type seed	8	77.5 (0.12), 67.0 (0.17), 39.0 (0.34), 29.5 (0.48), 26.0 (0.55)	2	3	1	2	35.29	37.50
ICPL 84023 x *C. cajanifolius*								
Male parent	9	80.0 (0.11), 74.0 (0.14), 57.5 (0.21), 46.0 (0.28), 34.5 (0.39)	1	0	0	8	-	-
Cultivated type seed	7	77.5 (0.12), 44.0 (0.30), 33.0 (0.41)	2	0	2	3	50.00	42.85
Male parent	9	98.0 (0.02), 92.5 (0.05), 80.0 (0.11), 57.5 (0.21)	1	0	0	8	-	-
Intermediate type seed	8	77.5 (0.12), 44.0 (0.30), 31.5 (0.44)	0	1	1	6	58.82	53.33
Male parent	9	98.0 (0.02), 92.5 (0.05), 80.0 (0.11), 57.5 (0.21), 46.0 (0.28), 34.5 (0.39)	1	0	0	8	-	-
Wild type seed	4	44.0 (0.30)	0	1	0	3	46.15	54.54

Figures in parenthesis are Rm values; SI = Similarity Index; Male parent = *C. cajanifolius*

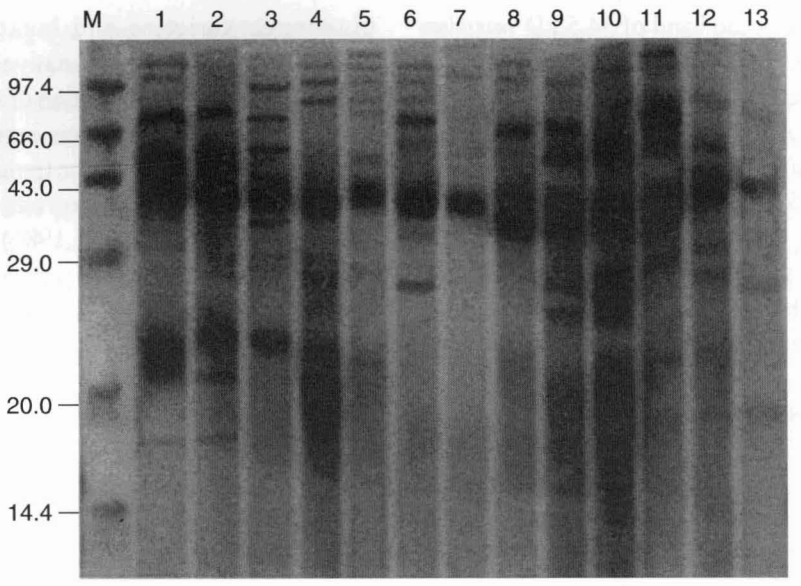

Fig. 1: Electrophoregram or polypeptide banding patterns of three genotypes of *Cajanus cajan*, nine interspecific hybrids and *C. cajanifolius* derived from SDS-PAGE of seed protein; Pant A 134 (Lane 1), UPAS 120 (Lane 2), ICPL 84023 (Lane 3), *C. cajanifolius* (Lane 4), Pant A 134 X *C. cajanifolius* (C) (Lane 5), Pant A 134 X *C. cajanifolius* (I) (Lane 6), Pant A 134 X *C. cajanifolius* (W) (Lane 7), UPAS 120 X *C. cajanifolius* (C) (Lane 8), UPAS 120 X *C. cajanifolius* (I) (Lane 9), UPAS 120 X *C. cajanifolius* (W) (Lane 10), ICPL 84023 X *C. cajanifolius* (C) (Lane 11), ICPL 84023 X *C. cajanifolius* (I) (Lane 12), ICPL 84023 X *C. cajanifolius* (W) (Lane 13)

by intermediate type seed of UPAS 120 x *C. cajanifolius* (61.53%) and ICPL 84023 x *C. cajanifolius* (58.82). High order of similarity with respect to cultivated parent was seen in wild type (54.54-37.50 %) in all the three hybrids. Cultivated seed type of two hybrids (ICPL 84023 x *C. cajanifolius* and Pant A 134 x *C. cajanifolius)* also shared fairly good similarity index, 42.85 and 37.50%, respectively with their respective cultivated parents. Gangwar and Bajpai (2007) also noted the similarity index of hybrids with *C. scarabaeoides* ranged from 33.33 to 75.75%. All types of protein band intensity were observed (Fig. 1). Maximum 9 bands were noted in wild seed types of hybrid derived with Pant A 134 followed by 8 bands in intermediate and wild seed types of all the three crosses.

The hybrids had protein bands of both high as well as low molecular weight ranging from 98.0 to 16 kD. Similarly, Naik and Kole (2001) observed bands from 17.4 to 75 kD in mungbean. Panigrahi *et al.* (2001) also noted polypeptide bands with Rm values ranging from 0.248 to 0.634 in five genotypes including two pigeonpea cultivars, C. *cajanifolius* and their two putative hybrids. 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 genotypes distinctiveness.

In all, 23 protein bands observed in the F_4 populations of *C. cajan* x *C. cajanifolius* hybrids 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 universally present in hybrid progenies as well as in parents indicating that the genes controlling expression of these protein bands appeared to behave as a single block. The observed differences in protein band intensity in cultivated, intermediate and wild type seeds could be utilized in identification of high protein rich progenies from successive generations. Such types of close relationships and/ or minor variations were also observed in chickpea (Kharkwal, 1999). The results also substantiated the conclusion that the interspecific hybridization is important for creating wide variability for seed protein in genus *Cajanus*. The present study is preliminary on interspecific hybrids of *C. cajan* x *C. cajanifolius* and further study of seed protein fractions would be required in advance generations of the hybrids.

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