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Seed protein studies on *Cajanus acutifolius* derived interspecific hybrids in pigeonpea

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ABSTRACT

Storage seed protein of Pant A 134, UPAS 120, ICPL 84023 and of *C. acutifolius* wild species and their hybrids were analyzed by SDS-PAGE. Wide variability between parents and hybrids revealed a wide variability of electrophoretic pattern of seed protein. SDS-PAGE analysis showed the absence of 38 kD band in hybrid and presence of additional bands (47.5, 32.5, 17.0, 15.0 kD) in hybrids. A specific band at 71.5 kD was common among parents as well as hybrids.

Key words: *Cajanus acutifolius*, Electrophoresis, Pigeonpea, SDS-PAGE, Seed Protein.

Protein markers and electrophoretic banding patterns have successfully been used for varietal identification in several crops. Electrophoretic patterns of seed protein helps in differentiating similarities and differences between genotypes and hybrids derived among them. For most seeds, except cereals, storage proteins are predominantely globulins (Krishna and Bhatia 1985). The mature seed provides a stable and convenient system for biochemical analysis to establish relationship in parents and hybrids. The present study aims to compare seed protein banding pattern of *C. cajan* and *C. acutifolius* and their hybrids by studying protein variation in F_4 seeds by SDS-PAGE.

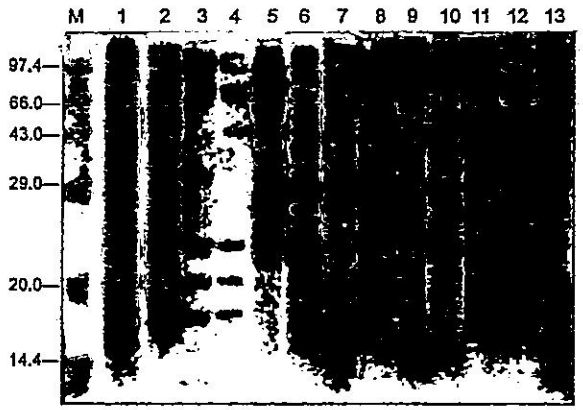
MATERIALS AND METHODS

The cultivated genotypes, Pant A 134, UPAS 120 and ICPL 84023 were crossed with wild species, *Cajanus acutifolius* as male parent. The F_4 generation seeds of these hybrids were grouped into cultivated type (C), intermediate type (I) and wild type (W) in each hybrid population on the basis of seed shape characteristics for the purpose of SDS-PAGE analysis. Hybrids of F_3 generation and their parents were raised at Crop Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand) during *kharif* 2003-2004. The electrophoretic procedure described by Laemmli *et al.* (1970) was adopted, 10-12 l of supernatant was loaded in each sample well. Initially, the electrophoresis was carried out at 10 mA at normal room temperature. Current was changed to 30 mA after the dye front crossed the stacking and separating gel interface. The gel was stained and destained to visualize as many band as possible. The relative migration (Rf) of each band was calculated and compared for molecular weight (kD). The degree of electrophoretic similarity was calculated by pair wise comparison of the parents and hybrids. Similarity index (SI) was worked out following Mishra *et al.* (1996) *i.e.*,

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

RESULTS AND DISCUSSION

The Gel of seed protein banding patterns of cultivated parents, Pant A 134, UPAS 120 and ICPL 84023 and of wild species *Cajanus acutifolius* and hybrids derived between them are presented in Fig. 1. The Rf values and molecular weight for protein bands were used for comparison (Table 1). UPAS 120 had maximum band (10) followed by Pant A 134 (9) and ICPL 84023 (7). *C. acutifolius* wild male parent showed nine bands. The similarity in protein bands of 87.5, 71.5 and 60 kD was noted among all three *C. cajan* parents. A band at 39 kD was common between UPAS 120 and Pant A 134 parents. It was interesting to observe that a band at 71.5 kD was common



Gel

1. Pant A 134 2. Pant A 134 x *C. acutifolius* (C) 3. Pant A 134 x *C. acutifolius* (I) 4. Pant A 134 x *C. acutifolius* (W) 5. *C. acutifolius* 6. UPAS 120 7. UPAS 120 x *C. acutifolius* (C) 8. UPAS 120 x *C. acutifolius* (I) 9. UPAS 120 x *C. acutifolius* (W) 10. ICPL 84023 11. ICPL 84023 x *C. acutifolius* (C) 12. ICPL 84023 x *C. acutifolius* (I) 13. ICPL 84023 x *C. acutifolius* (W)

Fig. 1. Electrophoretic banding pattern of seed protein of *C. acutifolius* derived interspecific hybrid in pigeonpea

Table 1. Relative mobility values for various bands electrophoressed in seed protein extracts of interspecific hybrid in pigeonpea

Band No.	Rf value	Mol.wt. (kD)	Pant A 134	UPAS 120	ICPL 84023	*C. acutifolius*	Pant A 134 x *C. acutifolius*			UPAS 120 x *C. acutifolius*			ICPL 84 x *C. acutifolius*		
							C	I	W	C	I	W	C	I	W
1.	0.04	95..0	+	—	+	—	+	+	—	—	—	—	—	+	—
2.	0.05	92.5	—	+	—	+	+	+	+	—	—	—	—	—	—
3.	0.08	87.5	+	+	+	—	—	—	—	—	—	+	—	+	—
4.	0.10	82.5	—	+	—	+	+	+	+	+	—	+	—	—	—
5.	0.15	71.5	+	+	+	+	+	—	+	+	+	+	+	+	+
6.	0.20	60.0	+	+	+	—	—	—	—	+	—	—	—	+	—
7.	0.27	47.5	—	—	—	—	—	—	—	+	—	—	—	—	—
8.	0.28	46.0	—	+	—	+	+	—	—	+	+	+	+	+	—
9	030	44.0	—	+	+	+	+	—	—	+	+	+	—	—	—
10.	0.34	39.0	+	+	—	+	+	—	—	+	—	—	—	—	—
11.	0.35	38.0	—	+	—	+	—	—	—	—	—	—	—	—	—
12.	0.37	36.0	+	—	—	—	—	—	—	—	—	+	—	—	—
13.	0.41	33.0	+	—	—	—	+	—	—	—	+	—	+	+	+
14.	0.42	32.5	—	—	—	—	—	—	—	—	+	—	—	—	—
15.	0.44	31.5	—	—	+	—	—	—	—	+	+	+	—	—	—
16.	0.48	29.5	—	+	—	+	+	—	—	—	+	+	—	—	—
17.	0.53	27.0	—	—	—	—	—	+	—	—	—	—	—	—	—
18.	0.55	26.0	+	—	—	—	—	+	+	—	—	—	—	—	—
19	0.57	25.0	—	—	—	—	—	+	—	—	+	+	—	—	—
20.	0.61	23.0	—	—	+	—	—	—	—	+	—	—	+	—	—
21.	0.65	21.0	—	—	—	+	+	—	+	—	—	—	—	—	—
22.	0.75	16.0	+	—	—	—	+	+	+	+	—	+	—	—	—

C - Cultivated type seed, I - Intermediate type seed, W - Wild type seed

Table 2. Qualitative and quantitative difference among progenies of interspecific hybrids derived with *C. acutifolius*

Genotype	Total no. of bands	Qualitative difference: Specific band number	Quantitative difference: Thick	Medium	Thin	Faint	SI with *Cajanus acutifolius*	SI with cultivated parents
Pant A134 x *C. acutifolius*								
Male parent	9	38(0.35)	2	3	4	-	-	-
Cultivated type seed	12	95(0.04). 33(0.41). 25(0.57). 16(0.75)	1	3	7	1	76.19	47.67
Male parent	9	71.5(0.15). 46(0.28). 44(0.30). 39(0.34). 38(0.35). 29.5(0.48). 21(0.65)	2	3	4	-	-	-
Intermediate type seed	6	95(0.04),27(0.53),26(0.55),16(0.75)	1	1	3	1	26.66	60.00
Male parent	9	46(0.28),44(0.30),39(0.34),38(0.35),29.5(0.48)	2	3	4	-	-	-
Wild type seed	6	26(0.55). 16(0.75)	2	1	3	0	53.33	60.00
UPAS 120 x *C. acutifolius*								
Male parent	9	92.5(0.05),38(0.35),29.5(0.48),21(0.65)	2	3	4	-	-	-
Cultivated type seed	10	60(0.20),47.5(0.27),31.5(0.44),23(0.61),16(0.75)	1	3	4	2	52.63	66.66
Male parent	9	92.5(0.05),82.5(0.10),39(0.34),38(0.35),21(0.65)	2	3	4	-	-	-
Intermediate type seed	8	33(0.41),32.5(0.42),31.5(0.44),25(0.57)	2	1	4	1	47.06	44.45
Male parent	9	92.5(0.05),39(0.34),38(0.35),21(0.65)	2	3	4	-	-	-
Wild type seed	10	87.5(0.08),36(0.37),31.5(0.44),25(0.57),16(0.75)	1	2	5	2	52.63	60.00
ICPL 84023 x *C. acutifolius*								
Male parent	9	92.5(0.05),82.5(0.10),44(0.30),39(0.34),38(0.35),29.5(0.48),21(0.65)	2	3	4	-	-	-
Cultivated type seed	4	33(0.41),23(0.61)	1	1	2	-	30.77	36.36
Male parent	9	92(0.05),82.5(0.10),44(0.30),39(0.34),38(0.35),29.5(0.48),21(0.65)	2	3	4	-	-	-
Intermediate type seed	6	95(0.04),87.5(0.08),60(0.20),33(0.41)	2	-	3	1	26.67	76.32
Male parent	9	92.5(0.05),82.5(0.10),46(0.28),44(0.30),39(0.34),38(0.35),29.5(0.48),21(0.65)	2	3	4	-	-	-
Wild type seed	2	33(0.41)	1	-	1	-	18.18	18.18

Figures in parenthesis are Rf values; SI = Similarity index; Male parent = *C. acutifolius*

among wild and cultivated parents. The near similarity in protein banding pattern (92.5, 82.5, 71.5, 46.0, 44.0, 39.0, 29.5 and 21.0 kD) of *C. acutifolius* with either of cultivated lines indicated sharing of some common genomic relationship between wild and cultivated species in genus *Cajanus*. Roy (2001) observed variation in number, width and intensity of bands among genotypes of *Lathyrus* for seed protein. The unique bands at 92.5, 82.5 and 16.0 kD in all 3 seed types (C, I, W) of hybrid between Pant A 134 x *C. acutifolius* were useful to distinguish it from that of other hybrids. A band with 92.5 kD seems to be unique characteristic of *C. acutifolius* as was noted in hybrids also. The hybrids, Pant A 134 x *C. acutifolius* had 12 bands (cultivated type), UPAS 120 x *C. acutifolius* had 8 bands (Intermediate type), UPAS 120 x *C. acutifolius* had 10 bands (Wild type) and ICPL 84023 x *C. acutifolius* had 6 bands (Intermediate type), as expected, protein bands of both high as well as low molecular weight were present in their respective parent.

It was interesting to note that a specific band at 71.5 kD was common in all hybrids as well as parents except for intermediate seed types in Pant A 134 x *C. acutifolius* followed by bands at 82.5, 46.0 and 44.0 kD. Parallel to present finding (95.0 to 16.0 kD), Naik and Kole (2001) evidenced polypeptide bands dispersed over 17.4 to 75.0 kD in mungbean. It suggested interspecific and intraspecific variation in F_4 generation. Proteins are the primary product of genes, so the proteins observed in hybrids were encoded by parental genomes. Also, the hybrid showed some additional protein bands (47.5, 32.5, 27.0 and 25.0 kD) which may be the result of interaction between the nuclear and mitochondrial genome of female parent with that of the nuclear genome of male parent.

A protein band at 38.0 kD was missing in the hybrid when compared to parents which might be due to interaction between parents which might result in suppression of certain proteins. Some of the new protein bands were observed in hybrid, Pant A 134 x *C. acutifolius* (25.0 and 21.0 kD), UPAS 120 x *C. acutifolius* (47.5, 36.0, 33.0, 32.5, 31.5, 25.0, 23.0 and 16.0 kD) and ICPL 84023 x *C. acutifolius* (33.0 kD) may be due to the interaction of both the parental genome. Maximum similarity index (SI) with wild *C. acutifolius* (Table 2) was observed in hybrid with Pant A 134 cultivated type seed (76.19%) parent followed by hybrid with Pant A 134 -wild type seed (53.33) and hybrid with UPAS 120-cultivated as well as wild type seed (52.63). Whereas hybrid ICPL 84023 x *C. acutifolius* – intermediate type seed (76.32) showed highest similarity index with cultivated parent ICPL 84023, followed by hybrid UPAS 120 x *C. acutifolius* – cultivated hybrid type seed (66.66 and 60.00).

The present studies on *C. cajan* x *C. acutifolius* indicated that the electropheretic pattern of protein bands could provide quick identification of segregating breeding material. Present information can be used for characterizing breeding material and determine the extent of gene transfer from *C. acutifolius* to *C. cajan* by means of protein markers. Further, studies on the major seed protein fractions such as albumin and globulin could possibly generate more information on wild vs cultivated pigeonpea parents and their introgression in interspecific hybrids.

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