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Detection of species specific protein markers for *Cajanus cajan* and *C. cajanifolius*

J. Panigrahi, S. N. Patnaik[1] and C. Kole[2]

Laboratory of Molecular Biology and Biotechnology, Orissa University of Agriculture and Technology, Bhubaneswar 751 003

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Abstract

Electrophoretic analysis of seed albumin of six varieties of *Cajanus cajan*, its two local land races (LLRs) and its putative progenitor *C. cajanifolius* resulted in detection of 16 distinct polypeptide bands of molecular weights ranging from 10.0 to 54.1 kd. Of these, four albumin bands of 10.0, 14.7, 39.8 and 54.1 kd were unique to *C. cajanifolius*, while two bands of 13.6 and 29.2 kd were specific to *C. cajan*. The four *C. cajanifolius* specific bands could be used as reliable markers to verify hybridity in wide hybridization for introgression of stress resistance genes into *C. cajan* using the former as the male donor parent.

Key words : *Cajanus cajan, Cajanus cajanifolius*, seed albumin, species specific marker

Introduction

Cajanus cajan (L.) Millsp., commonly called as pigeonpea, contains about 24% protein in seeds and is grown widely in the tropical and subtropical regions as a grain legume crop. Several studies including cyto-morphological [1, 2, 3] and RFLP marker analysis [4] revealed *C. cajanifolius* (Haines) van der Maeson to be the closest wild species and its putative progenitor. *C. cajanifolius* could be used as donor of drought tolerance [5] and insect resistance [6, 7] for genetic improvement in *C. cajan*. Use of molecular markers could facilitate the inter-specific back cross breeding involving these two species.

Markers detected from seed protein electrophoresis of are known to be quite stable and have been effectively employed for variety and hybrid identification in several crops. Detection of seed protein markers involves relatively simple and inexpensive techniques as compared to DNA markers. In *Cajanus*, electrophoretic banding patterns of crude seed proteins have been used to elucidate phylogenetic relationship and evolution [8]. *Cajanus* seed contains 54-60% globulin, 15-20% albumin, 15-20% glutelin and 4-5% prolamin [9]. Literature, however, reveals no report on electrophoretic analysis of these seed protein fractions and its use in verification of hybridity in inter-specific or intra-specific crosses.

We report here on the detection of polypeptide bands specific to *C. cajan* and *C. cajanifolius* from electrophoretic analysis of seed albumins.

Materials and methods

This study included six varieties of *C. cajan*: ICPL 87, BDN 2, UPAS 120, AKPH 1156, AKPH 6190 and AKT 9013; its two local land races (LLRs): *Kandula* and *Rahada* collected from the districts of Phulbani and Keonjhar respectively, of Orissa [3]; and its putative progenitor *C. cajanifolius*.

Albumins were extracted by suspending seed flour in pre-chilled distilled water for 4 hr at 4°C and centrifugation at 12000 × g at 0°C for 5 minutes Electrophoresis was done in 12% Sodium dodecyl sulphate-polyacrylamide gel (SDS-PAG) following Laemmli [10]. Gel staining was done in 0.05% neutral Silver Nitrate [11].

Results and discussion

Seed protein expression is controlled by multigene families. Deletion or mutation in these structural genes or their regulatory loci results in the inhibition of transcription or translation of polypeptides. Expression of these proteins is governed monogenically, presence being completely dominant over absence. Polypeptides varying for presence or absence could be used as markers. Such marker(s) unique to a male parent can be used as a useful parameter for confirmation of hybridity in crossing programmes. Introgression of biotic and abiotic stress tolerance from *C. cajanifolius* into

[1]PG Dept. of Botany, Utkal University, Bhubaneswar 751 004; [2]College of Agriculture, Allahabad Agricultural Institute, Allahabad 211 007

Table 1. Electrophoretic banding patterns of six varieties and two LLRs of *Cajanus cajan*, and *C. cajanifolius* derived from SDS-PAGE of seed albumins

Band #	MW (kd)	*Kandul*	*Rahada*	BDN-2	ICPL 87	UPAS 120	AKPH 6190	AKPH 1156	AKT 9013	*C. cajanifolius*
1	54.1	-	-	-	-	-	-	-	-	+
2	53.1	+	+	+	+	+	+	+	+	+
3	42.9	-	-	-	+	+	-	-	-	+
4	39.8	-	-	-	-	-	-	-	-	+
5	36.9	+	+	+	+	+	+	-	-	+
6	34.1	+	+	+	+	+	+	-	-	-
7	29.2	+	+	+	+	+	+	+	+	-
8	26.6	+	+	+	+	+	+	+	+	+
9	25.1	+	+	+	+	+	+	+	+	+
10	19.9	+	+	+	-	-	+	-	+	-
11	18.5	+	+	-	-	-	-	-	-	-
12	16.2	+	+	+	-	-	+	-	-	-
13	14.7	-	-	-	-	-	-	-	-	+
14	13.6	+	+	+	+	+	+	+	+	-
15	11.7	-	-	+	+	+	+	-	+	-
16	10.0	-	-	-	-	-	-	-	-	+

C. cajan could be facilitated by using such markers specific to *C. cajanifolius* which serves as the male parent in successful wide hybridization involving these two species [3].

Albeit wide use of seed protein markers in elucidation of phylogenetic relationship and evolution, their use is limited to a few instances for detection of hybridity in intervarietal [12, 13] or interspecific crosses [14].

Seed albumins are mostly enzymatic and were, therefore, preferred for analysis in the present study. SDS-PAGE of seed albumins of eight genotypes of *C. cajan*, and *C. cajanifolius* led to the detection of 16 clearly distinguishable bands of heterogeneous molecular weights (MW) ranging from 10.0 to 54.1 kd (Table 1). Out of these, three bands were monomorphic and might be genus specific. Four bands of 10.0, 14.7, 39.8 and 54.1 kd were present only in *C. cajanifolius* (Fig. 1). The *C. cajan* genotypes had two such unique bands of 13.6 and 29.2 kd. These polypeptides might be species specific. The four bands specific to *C. cajanifolius* can be used for verification of hybridity of putative F_1s derived from wide crosses involving *C. cajan* and *C. cajanifolius*, latter as the male parent. The band of 10.0 kd was strikingly conspicuous and can be tacitly detected in electrophoregrams. A band of 18.5 kd was present only in the two LLRs, *Kandula* and *Rahada* among the *C. cajan* genotypes. These two LLRs are also known as donor source for drought tolerance and insect resistance [3]. The LLR specific band could, therefore, be useful in distinguishing hybrids of intra-specific crosses involving any of them as male parent and any of the six varieties as recipient female parent.

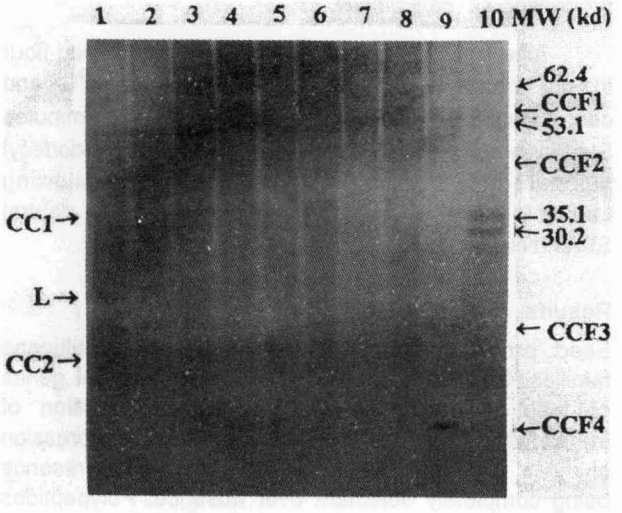

Fig. 1. Electrophoregram of polypeptide banding patterns of eight genotypes of *Cajanus cajan* and *C. cajanifolius* derived from SDS-PAGE of seed albumins; Lane 1-8: *C. cajan* genotypes: Kandula, Rahada, BDN 2, UPAS 120, ICPL 87, AKPH 6190, AKPH 1156, AKT 9013; Lane 9: *C. cajanifolius*; Lane 10: Molecular weight (kd) marker; unique bands for *C. cajan*, LLRs and *C. cajanifolius* and marked CC, L and CCF respectively; Molecular weights (kd) of the on the marker lane on the right.

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