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SPECIES AFFINITIES BETWEEN *CAJANUS CAJAN* AND SOME *ATYLOSIA* SPECIES BASED ON ESTERASE ISOENZYMES

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INDEX WORDS

Cajanus cajan, pigeon pea, *Atylosia* species, esterase isozymes, species affinity.

SUMMARY

Esterase isozymes were studied in seed extracts of *Cajanus cajan* and six *Atylosia* species by polyacrylamide gel electrophoresis and isoelectrofocusing. The isozyme patterns were stable and accession specific. Within the accessions of the *Atylosia* species, *A. albicans* and *A. scarabaeoides* showed three common bands indicating that they are more closely related to each other than to the other species. Of the accessions of *Atylosia* only *A. cajanifolia* shares the esterase isozyme of *C. cajan* and hence seems to be the closest wild relative of *C. cajan*.

INTRODUCTION

Pigeon pea (*Cajanus cajan* (L.) MILLSP.) is a major pulse crop in tropical and subtropical regions. It is extensively grown for its protein rich seeds, which form an important constituent in predominantly vegetarian diets. *Cajanus cajan* and *Atylosia* species are self fertile. The genus *Atylosia* is closely related to *C. cajan* and is separated from the latter on the basis of presence of strophiole on the seeds (BAKER, 1876). Most *Atylosia* species readily cross with *C. cajan* and normal fertile hybrids can be obtained (DEODIKAR & THAKAR, 1956; SIKDAR & DE, 1967; REDDY, 1973, REDDY et al., 1980). Some of the *Atylosia* species possess agronomically desirable characters such as disease and insect resistance, high seed protein content (REDDY et al., 1979) and drought resistance (AKINOLA et al., 1975).

Comparative electrophoresis of general proteins and isozyme analysis have been successfully used to ascertain genetic homologies and in understanding the phylogenetic relationships among the related taxa. These studies have been particularly useful in clarifying systematic relationships in groups where morphological and cytological data were inconclusive. Electrophoretic studies by LADIZINSKY & HAMEL (1980) on the seed proteins of pigeon pea and four *Atylosia* species indicated a very close affinity among these species and they inferred a polyphyletic origin of *C. cajan* from several *Atylosia* species. SINGH et al. (1981) concluded from their studies on the salt soluble proteins of pigeon pea and eight wild relatives – including six *Atylosia* species – that there is a great similarity in the major protein subunits of these species. The present

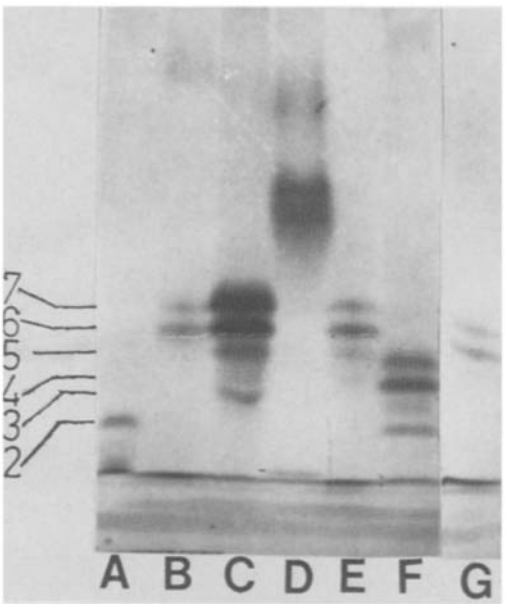

Fig. 1. Esterase zymograms on $7\frac{1}{2}\%$ polyacrylamide gel in (A) *Cajanus cajan*, (B) *Atylosia sericea*, (C) *A. scarabaeoides*, (D) *A. platycarpa*, (E) *A. albicans*, (F) *A. cajanifolia* and (G) *A. volubilis*. Origin is at the top and the migration was towards the anode at the bottom.

paper deals with the variation of esterase isozymes in *Cajanus cajan* and six *Atylosia* species.

MATERIALS AND METHODS

One cultivated variety of *Cajanus cajan* (cv. T-21) and one accession of six *Atylosia* species each were used. The *Atylosia* species studied include two erect shrubs, *A. sericea* BENTH. ex BAK. and *A. cajanifolia* HAINES, two herbaceous climbers, *A. scarabaeoides* (L.) BENTH. and *A. playcarpa* BENTH., and two perennial climbers, *A. volubilis* (Blanco) GAMBLE and *A. albicans* (W. & A.) BENTH.

Dry dehusked seeds (500 mg) were extracted in 2 ml of 100 mM tris-HCl buffer, pH 7.1 containing 100 mM sucrose at 4°C. The slurry was centrifuged at 12000 *g* for 30 minutes in cold. 100 μl of clear supernatant was used for disc electrophoresis following essentially the method of DAVIS (1964). 10 μl of the supernatant was used for isoelectric focusing which was performed in a LKB Multiphor apparatus using pH 3.5–10 ampholines. The procedure given in the LKB application note 250 was followed for isoelectric focusing.

Esterase activity on the gels was visualised by incubating the gels in 50 mM sodium phosphate buffer, pH 7.1 containing α-naphthyl acetate (final concentration 0.03%) and 0.1% fast blue RR at room temperature. The control gels incubated without the substrate α-naphthyl acetate, did not show any enzymatic activity. The isozymes were numbered according to the standard procedure, that is the fastest (anodic) moving band being number one. The ampholines were obtained from LKB-Produkter A.B.,

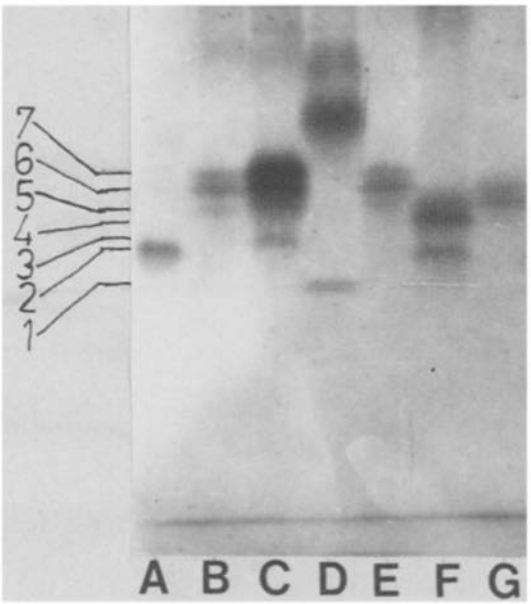

Fig. 2. Esterase zymograms on 10% polyacrylamide gel. Other details as in Fig. 1.

Bromma, Sweden. All other chemicals used in the study were of analytical grade and were mostly obtained from Sigma Chemical Company, Saint Louis, USA.

RESULTS AND DISCUSSION

The esterase isozymes in *C. cajan* and *Atylosia* species were resolved into seven clear bands and a zone of activity (Fig. 1). *C. cajan* and the *Atylosia* species studied showed species specific esterase zymograms. While *C. cajan* (cv. T-21) showed a single band of esterase activity (band number 2), in the *Atylosia* species the number varied. *A. volubilis* (band nos. 5 and 6), *A. sericea* (band nos. 6 and 7) showed two esterase isozymes. In *A. albicans* (band nos. 5, 6 and 7) and *A. cajanifolia* (band nos. 2, 4 and 5) there were three esterase isozymes. Four esterase isozymes (band nos. 3, 5, 6 and 7) were observed in *A. scarabaeoides*. *A. platycarpa* showed a distinct zone of esterase activity and the number of distinct isozyme bands could not be determined. This broad zone of activity in *A. platycarpa* could not be resolved even by increasing the acrylamide concentration to 10 percent. However, a discrete band (number 1) moving with the bromophenol blue marker dye in 7.5 percent acrylamide gel could be clearly seen after electrophoresis in 10 percent polyacrylamide gels (Fig. 2). This zone of esterase activity in *A. platycarpa* could be separated into four distinct bands by isoelectric focusing (Fig. 3). The presence of a distinct zone of esterase activity in *A. platycarpa* suggests that it is distinctly different from the other species. These observations corroborate those at ICRISAT where it has been found that *A. platycarpa* is cross incompatible with any of the other *Atylosia* species (REDDY, unpublished observation) and with the pigeon pea cultivars (REDDY et al., 1980).

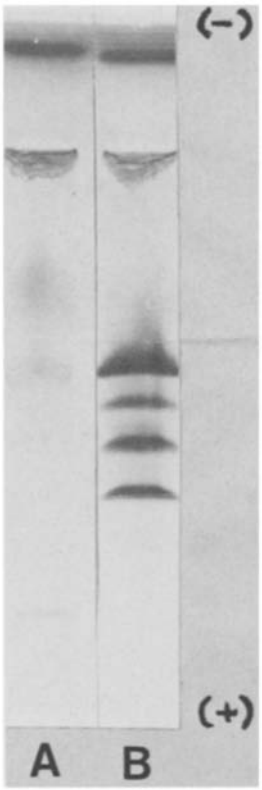

Fig. 3. Isoelectrofocusing pattern of esterase isoenzymes in (A) *C. cajan* and (B) *A. platycarpa.*

Table 1. The index of similarity* between *C. cajan* and *Atylosia* species.

	C. cajan	*A. sericea*	*A. scarabaeoides*	*A. platycarpa*	*A. albicans*	*A. cajanifolia*
A. sericea	0					
A. scrabaeoides	0	50				
A. platycarpa	0	0	0			
A. albicans	0	66	75	0		
A. cajanifolia	33	0	16	0	20	
A. volubilis	0	33	50	0	66	25

* Index of similarity $= \frac{\text{Number of pairs of similar bands}}{\text{Number of different bands} + \text{Number of pairs of similar bands}} \times 100.$

The index of similarity among the accessions of the species is given in Table 1. The fast moving band of *A. cajanifolia* (Fig. 1F) corresponds in mobility with the *C. cajan* esterase band. Coincidence in electrophoretic mobility of homologous proteins is indicative of genetic homology. The esterase zymogram pattern suggests that *C. cajan* is phylogenetically nearer to *A. cajanifolia* than the other *Atylosia* species. This further supports the contention – based on the morphological data – of VAN DER MAESEN

(1980) that *A. cajanifolia* is the putative progenitor of pigeon pea. The high cross compatibility of *C. cajan* and *A. cajanifolia* and similarity in the esterase zymogram would indicate that they have diverged from each other in the recent past or that both have originated from a common ancestral stock. *A. albicans* and *A. scarabaeoides* with three common bands (nos. 5, 6 and 7) appear to be more closely related to each other than to any of the other species.

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