4-m border of sorghum (CSH 9). Plots (8 rows) of short-duration pigeonpea (ICPL 87) were intercropped with sorghum (4 rows) within this border. Glass tubes (3.4-cm diameter; 14.5-cm length) coated with transparent insect glue (Tanglefoot®, Tanglefoot Company, Michigan, USA) were used as sticky traps. White paper was rolled inside the glass tube as this color was found to be preferred by the parasitoids (Romeis, unpublished data). The surface area of the trap was approximately 132 cm^2 (12 × 11 cm). Five traps were placed in each crop: at panicle height in sorghum (1.5 m) and at canopy height in pigeonpea (0.7 m). Traps were left in the field for 2 or 3 days. Before removal they were covered with clear plastic for easier handling. The number of parasitoids caught per trap per day was counted under a dissecting microscope in the laboratory.

Up to 9 parasitoids day^{-1} were caught on traps placed in sorghum (Fig. 1). The maximum parasitoid density in sorghum was detected when *H. armigera* egg density was greatest (standard week 39). Regardless of the high parasitoid population in sorghum, the parasitoid population in pigeonpea remained low, even at the highest egg density (week 39). In contrast to findings by Duffield (1994), no movement of parasitoids between the two crops was detected. Sticky traps are a useful tool in monitoring the dynamics of natural occurring *Trichogramma* populations in the field.

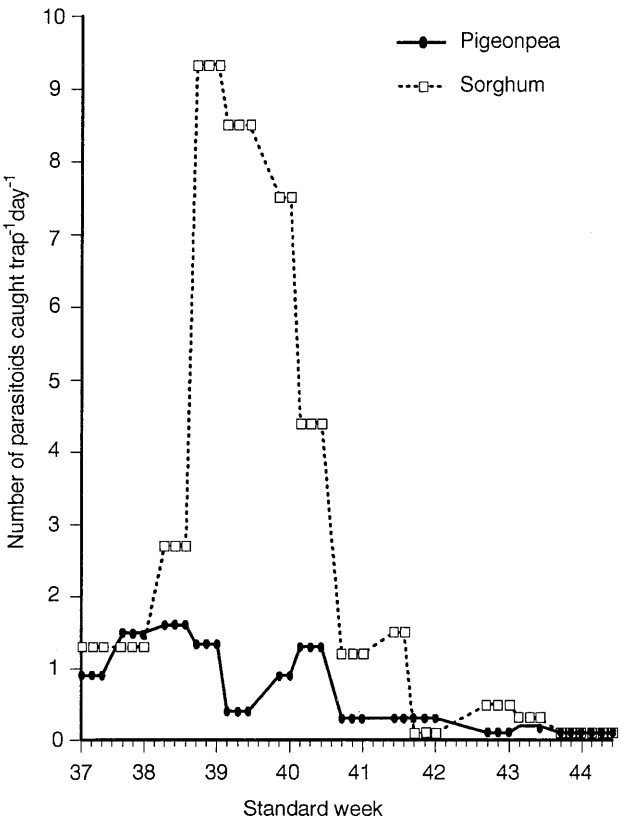

Figure 1. Number of *Trichogramma* parasitoids caught per sticky trap per day in sorghum and pigeonpea, IAC, rainy season 1995.

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Biotechnology

RFLP Analysis of Cytoplasmic-Genic Male-Sterile Lines of Pigeonpea Developed by Interspecific Crosses

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Pigeonpea is a partially cross-pollinated crop. The outcrossing nature of pigeonpea is being exploited for hybrid breeding using stable genetic male sterility discovered by Reddy et al. (1978). Commercial production of pigeonpea hybrid using genetic male-sterile lines poses problems of seed purity as fertile plants from the plots must be rouged out. The alternative has been to look for cytoplasmic-genic male-sterile lines, which has been successfully exploited in several crops and has overcome many of the disadvantages associated with the ge-

netic male-sterile systems. Attempts to obtain cytoplasmic male sterility in spontaneous mutants in the world pigeonpea collection have been unsuccessful (Reddy et al. 1978). Wide hybridization involving conventional backcrossing of *Cajanus sericeus* and *C. cajan* with multiple genome transfer led to the development of cytoplasmic-genic male sterility in pigeonpea (Ariyanayagam et al. 1995).

Cytoplasmic male-sterility (CMS) is a maternally inherited trait with the plant remaining female fertile while the pollen formation is abnormal. CMS is manifested as a result of the incompatibility of the nuclear-mitochondrial interaction and the changes are mainly encoded in the mitochondrial genome (Londsdale et al. 1988). Restriction fragment length polymorphism (RFLP) of mitochondrial DNA (mtDNA) has been used as an efficient tool to look at the rearrangements in the mtDNA of male-sterile lines in many crop species (Londsdale et al. 1988). The technique has also been used to examine the differences between the male sterile and their counterpart maintainer lines and for classification of the different cytoplasmic systems (Rajeshwari et al. 1994). In the present study we have carried out RFLP analysis of total genomic DNA from the different male parents of *Cajanus* species, the wild species *C. sericeus* used as the female parent in the development of a cytoplasmic male-sterility system in pigeonpea, and the putative CMS progenies.

The first cross for developing a cytoplasmic-genic system of male-sterility was made between *C. sericeus* accession EC 121208, as the female parent, and *C. cajan* line ICPX 880227, as the male parent. Transfer of the nuclear genome of pigeonpea into *C. sericeus* cytoplasm was effected through successive genomic transfer stages (GTS) with different male parents of pigeonpea instead of the conventional backcrossing as described by Ariyanayagam et al. (1995). We selected the male-sterile lines at the fifth or sixth genetic transfer stage (GTS 5/6) and the progenies selected were 7-1 (*C.sericeus* × ICPX 880227 × 90035 × 85030 × 85030 × 85030) × 85030 (corresponding to an average pollen sterility of 85%), 12-3 (*C. sericeus* × ICPX 880227 × 90035 × 85030 × 85030) × 85030 (70% average pollen sterility), and 33-1 (*C.sericeus* × ICPX 880227 × 90035 × 85030 × 90035) × 85010 (corresponding to an average pollen sterility of 75%). ICPL 87 was used as the control for the male parents and the two genetic male-sterile lines MS Prabhat and QMS 1 having different male sterility genes were included for comparison.

DNA was extracted from pigeonpea leaves as described by Murray and Thompson (1980) with some modifications. The maize mtDNA specific clones were those described by Sujata et al. (1994). Total genomic DNA (about 10 µg) was digested with 30 units of the restriction enzymes *EcoR*I, *Hind*III, and *EcoR*V according to the manufacturer's protocols, and the fragments were separated on 0.8% agarose gels by electrophoresis in TBE buffer (0.089 M Tris-Borate 0.002 M EDTA, pH 8.0). The DNA fragments were transferred onto Nylon membrane (Amersham, UK) by vacuum transfer. Random primed labeling method of Feinberg and Vogelstein (1983) was used for the preparation of ^{32}P-labeled probes.

Southern blot hybridization of *EcoR*I-digested total genomic DNA when probed with the maize mtDNA specific *atp*6 clone revealed identical patterns for *C. sericeus* and the three putative CMS progenies obtained after the multiple genome transfer of pigeonpea lines (Fig. 1 lanes 4–7) and were distinct from all the other male parents used in the crosses and the genetic male-sterile lines studied. However, pigeonpea lines (*C. cajan*) and the genetic male-sterile lines also differed among themselves. Three of the male parental lines used in the crosses (Fig. 1 lanes 8–10) showed identical hybridization patterns whereas ICPL 85010 (Fig. 1 lane 11) differed from the others. The genetic male-sterile lines, MS Prabhat (Fig. 1 lane 2) differed from the genetic male-sterile line QMS 1 (Fig. 1 lane 3) and other male parental lines in the hybridization patterns. This difference can easily be explained, since the gene contributing to male sterility in MS Prabhat and QMS has been shown to be different (Saxena et al. 1986). ICPL 85010 showed a pattern (Fig. 1 lane 11) similar to MS Prabhat (Fig. 1 lane 2) though there are some differences in the faintly hybridizing bands.

The three progenies of multiple cross genome transfer of pigeonpea lines showed hybridization patterns identical to that of *C. sericeus* when *Hind*III-digested total genomic DNA was probed with the maize mtDNA specific *atp*9 clone (Fig. 2 lanes 4–7). The three male parental lines ICPX 880227, ICPL 95030, and ICPL 90035, and the genetic male-sterile line QMS 1 (Fig. 2 lanes 8–10 and 3) showed identical hybridization patterns. The two genetic male-sterile lines, MS Prabhat and QMS 1 (Fig. 1 lanes 2 and 3) differed from each other in their hybridization patterns. The results obtained with this enzyme-probe combination were similar to those obtained with *EcoR*I-digested DNA except that ICPL 87 could be distinguished from all other male parental lines by the presence of the additional 5.5 kb fragment which is absent in all other lines. The data fur-

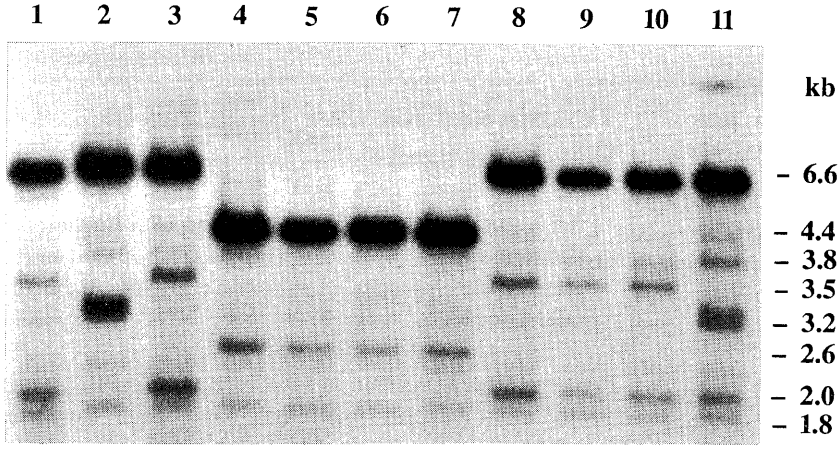

Figure 1. Southern blot hybridization of genomic DNA from the different pigeonpea lines digested with *Eco*RI, and probed with maize mtDNA specific *atp6* clone.

Lane
1 ICPL 87
2 MS Prabhat
3 QMS-1
4 CMS 7-1
5 CMS 12-3
6 CMS 33-1
7 *C. sericeus*
8 ICPX 880227
9 ICPL 85030
10 ICPL 90035
11 ICPL 85010

Fragment sizes are indicated in kilobases (kb).

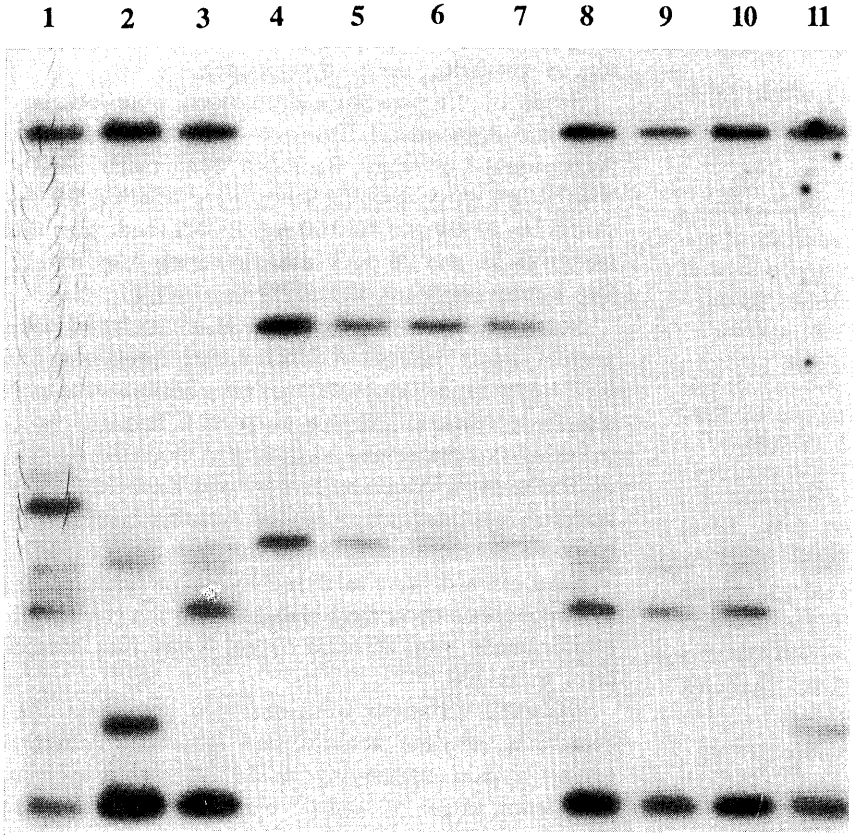

Figure 2. Southern blot hybridization of genomic DNA from the different pigeonpea lines digested with *Hind*III, and probed with maize mtDNA specific *atp9* clone.

Lane
1 ICPL 87
2 MS Prabhat
3 QMS-1
4 CMS 7-1
5 CMS 12-3
6 CMS 33-1
7 *C. sericeus*
8 ICPX 880227
9 ICPL 85030
10 ICPL 90035
11 ICPL 85010

Fragment sizes are indicated in kilobases (kb).

ther indicate that even within the cultivated *Cajanus* species there can be variation in the mitochondrial genome though the reasons for this difference are not clear.

The results presented here suggest that the crosses between the wild species *C. sericeus* and the cultivated species *C. cajan* produced male-sterile lines having the mitochondria of the latter. The differences in the restriction fragment patterns among the various pigeonpea cultivars could be the result of inter- and intramolecular rearrangements within the mitochondrial genome, which is a common phenomenon in higher plant mitochondria (Levings and Brown 1989). This cytoplasmic-genic male sterility system differs from the genetic male sterility system as evidenced by the hybridization patterns and the field observations made. Studies on anther morphology also clearly indicate the differences between the cytoplasmic male-sterile lines and the genetic male-sterile lines.

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Crop Quality/Utilization

Trypsin and Amylase Inhibitors in Pigeonpea Seeds

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Pigeonpea seeds contain at least nine trypsin inhibitors, seven chymotrypsin inhibitors, and four amylase isoinhibitors (Giri 1994). These inhibitors have being extensively studied as antinutritional factors, and as potential defences against pests. In this communication, we report the synthesis of protease and amylase inhibitors in developing seeds of pigeonpea.

Seeds of 12 pigeonpea accessions were obtained from the Agricultural Research Station, Marathwada Agricultural University, Badnapur, Maharashtra, India. Two stages of developing seeds were selected for the study: 10–20 mg and 80–100 mg. BDN2 seeds gain this weight in 11 and 28 days after flowering respectively. Dry mature seeds constituted the third stage.

Seed proteins were extracted (from defatted seed powder) in six volumes of distilled water containing 1% PVP (polyvinylpolypyrrolidone, w/v). Protein was estimated according to Lowry et al. (1951). Trypsin inhibitor assay was performed according to modified method of Kunitz as described by Pichare and Kachole (1992). Amylase and amylase inhibitor activity assays were based on Bernfeld's method for amylase assay (1955).

Seed extracts were analyzed by polyacrylamide gel electrophoresis in vertical slab gel. and the trypsin inhibitor bands were detected by gel X-ray film contact print technique.

Marginal variations were observed in protein concentration, amylase activity, and inhibitory activities against trypsin and amylase in the different accessions at different stages of seed development (Table 1). Detectable activity of these inhibitor proteins was found only in the third developmental stage. Protein levels in stage 2 appear to be lower than those in stage 1 and 3.

Mature seeds of several pigeonpea accessions had no significant differences in trypsin and chymotrypsin inhibitor activities and the profiles of the inhibitors. Singh et al. (1984) reported the presence of protease inhibitors in green (age not specified) and mature seeds of nine pigeonpea cultivars. Trypsin inhibitor activity was