Biodiversity of Mite Vector of *Pigeonpea sterility mosaic virus* in Tamil Nadu, India

T K S Latha, P Lava Kumar, Sabitha Doraiswamy[$] and F Waliyar

International Crops Research Institute for the Semi - Arid Tropics (ICRISAT), Patancheru - 502 324, Andhra Pradesh, India.
$ Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore - 641 003, Tamil Nadu, India.

Sterility mosaic disease (SMD) is the most economically important disease of pigeonpea (*Cajanus cajan* L.) in the Indian subcontinent (Kumar *et al.*, 2004). The SMD-affected plants show severe stunting and mosaic symptoms on leaves with complete or partial cessation of flowering (sterility). The disease is caused by the *Pigeonpea sterility mosaic virus* (PPSMV), transmitted by an eriophyid mite, *Aceria cajani* (Acari: Arthropod) (Kumar *et al.*, 2003). PPSMV occurs as several geographically distinct pathotypes, which differ in their severity on pigeonpea genotypes (Reddy *et al.*, 1993; Kumar *et al.*, 2004). Based on differential host range studies, the isolate prevailing in Coimbatore region of Tamil Nadu was regarded as a distinct pathotype (referred as C isolate), which has an ability to overcome host resistance selected against the PPSMV Patancheru (P) isolate. Recently, PPSMV-C isolate was characterized and shown to have properties distinct from that of P isolate (Latha, 2003). In this study, the diversity in mite populations vectoring PPSMV-C was analyzed by assessing variability in nucleotide sequence of the ribosomal DNA (rDNA) internal transcribed spacers (ITS) and associated rDNA genes by restriction analysis and comparison of the profiles with the reference clones that contain inserts of rDNA ITS regions from Patancheru, Andhra Pradesh. This rDNA-based method was shown to be highly useful for assessing diversity in eriophyid species (Kumar *et al.*, 1999).

Individual mites from PPSMV-C infected pigeonpea leaves were collected using a fine needle and placed in a 1.5 ml tube containing 0.01% Decon (BDH, UK) in phosphate buffered saline. Mite DNA was isolated and rDNA was amplified with oligonucleotide primers, Caj-3, E, C and Caj-2 (Table 1), as described by Kumar *et al.* (2001). The PCR products were digested with *Taq* I (Promega) restriction enzyme as per the manufacturer instructions, and the digested products were separated in a 10% polyacrylamide gel in tris-glycine buffer, pH 8.3. The gel was stained with ethidium bromide and visualized under UV transilluminator.

The primers Caj-2 + C amplified about 280 bp region corresponding to the 3' end of the 18S gene and a part of ITS-1 and Caj-3 + E amplified about 1060 bp region corresponding to the 3' end of ITS-1/5.8 S/ITS-2 and the 5' end of the 28S gene in all the samples tested. No variation was observed in the size of the PCR products amplified from the mite samples of Coimbatore and the products from the reference plasmid clones (data not shown). There was no restriction site polymorphism in digests using *Taq* I (Figure. 1), indicating *A. cajani* prevailing in Coimbatore is similar to *A. cajani* in other SMD regions in the Indian subcontinent. In an earlier study (Kumar *et al.*, 2001) also, no variation was found in the mite samples from India, Nepal and Myanmar. Thus, this study indicates that *A. cajani* is involved in the transmission of a distinct PPSMV isolate prevailing in Coimbatore region, and eliminates the role of vector in variability observed in host resistance.

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Table 1. Properties of the PCR primers used for rDNA amplifications

Primer	Sequence (5 → 3)	Amplification direction	Anneling site
Caj-2	TTCCACACTGATATGGTAGTCGC	Upstream	ITS-1
C	GACCAACTAAAAGTCGTAACAAG	Downstream	18S rRNA
Caj-3	ACTACCATATCAGTGTGGAAGCGCG	Downstream	ITS-1
E	CAACTTTCCCTCACGGTACTTG	Upstream	28S rRNA

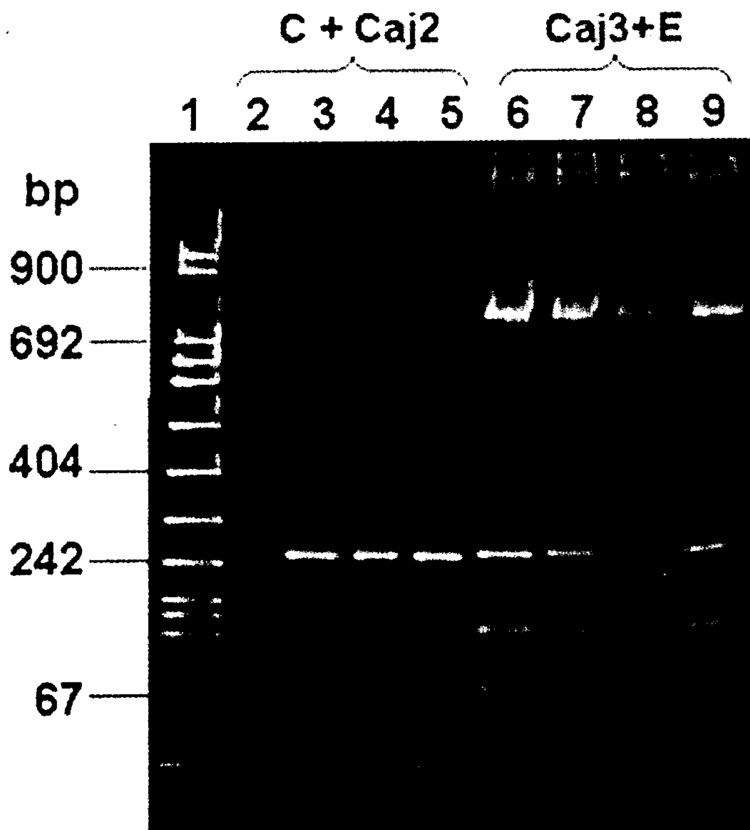

Figure 1. RFLP patterns obtained after digesting with *Taq* I of *Aceria cajani* rDNA from SMD-affected pigeonpea from Coimbatore (Tamil Nadu) (Lanes 4, 5, 7, 8 and 9) and Patancheru (Andhra Pradesh) (Lanes 2, 3, 6). Lane 1: DNA size standards (Roche, Marker VIII). Oligonucleotide primers (C, Caj2 and Caj3) used for the amplification is indicated.

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