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## MECHANISM OF RESISTANCE TO *ACERIA* *CAJANI* IN PIGEONPEA\*

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### ABSTRACT

Leaf anatomy was studied in relation to resistance to *Aceria cajani*, the vector of SM pathogen in seven lines of pigeonpea (*Cajanus cajan*). The leaf cuticle and epidermal cell wall were 50-100% thicker in SM-resistant lines than in susceptible lines. In the resistant lines cuticle thickness was 3.79  $\mu\text{m}$  in ICP 7035 and 3.03  $\mu\text{m}$  in ICP 8862. In the susceptible lines it was 1.89  $\mu\text{m}$  in C 11, 1.52  $\mu\text{m}$  in BDN 1, and 2.27  $\mu\text{m}$  in ICP 8863. The cuticle thickness in the moderately resistant lines ICP 2376 (1.89  $\mu\text{m}$ ) and ICP 10976 (2.27  $\mu\text{m}$ ) was similar to that of the susceptible lines. When measured the stylet length of the eriophyid mite vector, *Aceria cajani* was less than leaf cuticle thickness of the resistant lines. Resistance is therefore attributed to the thick cuticle of resistant lines through which the mite vectors cannot penetrate into the living epidermal cells to transmit the SM pathogen.

### INTRODUCTION

Sterility mosaic (SM) is the most important disease of pigeonpea [*Cajanus* L.) Millsp.] in India, Nepal, and Myanmar (Kannaiyan *et al.*, 1984). The annual loss to SM in India during 1975-80 have been estimated at US\$ 74 million (Kar *et al.*, 1984). Recent reports indicate that SM incidence in India is increasing (Zote *et al.*, 1991). The causal agent of the disease has yet to be determined, but the pathogen is known to be transmitted by an eriophyid mite *Aceria cajani* Channabasavanna (Seth, 1962).

Several pigeonpea lines resistant to SM have been identified (Nene and Reddy, 1976; Nene *et al.*, 1989). These resistant lines seldom supported continued mite multiplication (Reddy and Nene, 1980). An SM-resistant line was found to have thicker leaves than susceptible lines (Prameela *et al.*, 1990). Sheila *et al.*, (1988) studied

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the morphology and biology of the mite vector using a scanning microscope. The eriophyid mite vector *A. cajani* is a tiny creature with piercing and sucking mouth parts that sucks sap through its slender stylet (Reddy *et al.*, 1990). Because it is so small, the stylet of the mite may not be able to pierce through the relatively thick cuticle of the leaves into the living epidermal cells to transmit the SM pathogen. The thick cuticle of the leaves might therefore be responsible for the inability of the mites to multiply on resistant plants. In order to test this hypothesis, the thickness of the leaf cuticle in a set of SM-resistant, moderately resistant, and susceptible pigeonpea lines, and the length of the stylet of the mite vector *A. cajani* were measured.

## MATERIALS AND METHODS

### Light Microscopy of Leaf Sections

Two SM-resistant (ICP 7035, ICP 8862), two moderately resistant (ICP 2376, ICP 10976) and three susceptible pigeonpea lines (C 11, BDN 1, and ICP 8863) were sown in an alfisol field at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Center, Patancheru on 15 July 1992. Leaves were collected from 2-month-old plants on 15 September 1992 and fixed in 3% glutaraldehyde in 0.2 M phosphate buffer. The leaf samples were then washed in 0.1 M phosphate buffer and post fixed in 2% osmium tetroxide for 4 h. The fixed samples were washed 3-4 times in double distilled water with 45 min in each change, dehydrated in graded series of acetone (30-100%) with 45 min in each grade, infiltrated in Spurr:acetone (1:1) for 1 h, and embedded in blocks filled with Spurr (Reddy *et al.*, 1991). After 48-72 h polymerization, the blocks were trimmed and used for sectioning. Transverse sections of the leaves were obtained with Ultracut Reichert Jung Ultramicrotome and stained with 1% toluidine blue. Micrograph of the leaf sections were taken under 66.6 x 66.6% magnification using an Olympus BH-2 microscope, 4x magnified prints were obtained, and cuticle (and epidermal cell wall) thickness was measured.

### Scanning Electron Microscopy of *Aceria cajani*

Adult mites maintained on SM-susceptible pigeonpea line ICP 8863 were fixed overnight in 3% glutaraldehyde in 0.2 M phosphate buffer, washed in 0.1 M phosphate buffer and then post fixed in 2% osmium tetroxide for 4 h. The samples were then washed in distilled water, and dehydrated in a graded ethanol series (30-100%) with 45 min in each change. Samples were kept in 100% ethanol for 1 h before critical point drying (CPD 750). After drying, the samples were mounted on aluminium stubs and coated with 200 nm layer of gold in an Emscope FD 500 Sputter Coater. Micrographs of the anterior portions of the mites with protracted stylets were taken under a JEOL JSM 35 CF Scanning Electron Microscope. The stylet lengths of *A. cajani* were determined from the micrographs.

## RESULTS AND DISCUSSION

Cuticle (epidermal cell wall) thickness of the upper surfaces of the pigeonpea leaves showed significant ( $P=0.01$ ) differences (Table 1; Fig. 1). The mean cuticle thickness of the resistant lines (3.03 and 3.79  $\mu\text{m}$ ) was about 50-100% higher than that of the susceptible lines (1.52, 1.89, and 2.27  $\mu\text{m}$ ). However, the cuticle thickness of the moderately resistant lines was similar (1.89 and 2.27  $\mu\text{m}$ ) to that of the susceptible lines. The average length of the stylet of eight *A. cajani* adults was 2.03  $\mu\text{m}$  (0.44 to 2.75  $\mu\text{m}$ ) (Fig. 2). The stylet length of *A. cajani* was more than the cuticle thickness of the SM-susceptible and moderately resistant lines but less than that of the resistant lines. The variation in the length of the stylets could be due to the degree of their protraction during fixation or natural variation in the mite population.

Pigeonpea lines such as ICP 7035 and ICP 8862, which are resistant to SM, do not show any apparent symptoms of the disease (Fig. 3). Neither do they support continued multiplication of the mite vector *A. cajani* (Reddy and Nene, 1980). Thus back inoculations with mites from resistant lines to susceptible lines were not meaningful. In moderately resistant lines such as ICP 2376 and ICP 10976, the symptoms of SM appear as ring spots with a green island in their center surrounded by a chlorotic halo (Fig. 3). These lines support mite multiplication. Back inoculations with mites from plants with ring spot symptoms to susceptible lines result in severe mosaic symptoms indicating that these are SM symptoms (Reddy and Nene, 1979). Green and light green mosaic mottle symptoms appear on leaves of susceptible lines infected with SM. These lines also support mite multiplication and back inoculations are successful.

From the results of the present study it is clear that the mite vector *A. cajani* is not able to feed on the leaves of SM-resistant pigeonpea lines, because although their stylets are able to pierce the cuticle they do not succeed in reaching living epidermal cells. The mites are thus unable to transmit the pathogen to the SM-resistant lines. Their inability to reach the epidermis could also be the reason why mites do not multiply on SM-resistant lines (Reddy and Nene, 1980). In lines moderately resistant and susceptible to SM, the mites are able to both feed and transmit the pathogen as their stylets are long enough to pierce through the leaf cuticle and reach the epidermal cells. Thus it is clear that SM resistance in the two resistant lines tested is because their cuticle is thicker than the stylets of the mites. It is possible that in addition to cuticle thickness, some other factors may also be involved in mite resistance. We have not seen references to any work on the mechanism of resistance in plants to eriophyid mites. Our observations on leaf hairs of SM-resistant and susceptible genotypes did not reveal any major differences between them. The SM pathogen is not sap-transmissible (Reddy *et al.*, 1990). Whether the lines tested here are resistant to the SM pathogen or not can only be determined when an alternate method of SM inoculation to mite transmission is developed. If such lines again show resistance to the SM

Table 1: Leaf cuticle thickness in pigeonpea genotypes with different reactions<sup>1</sup> to the sterility mosaic (SM) pathogen.

Cultivar	Cuticle thickness <sup>2</sup>
<b>Resistant</b>	
ICP 7035	3.79 ± 0.097
ICP 8862	3.03 ± 0.087
<b>Moderately resistant</b>	
ICP 2376	1.89 ± 0.039
ICP 10976	2.27 ± 0.032
<b>Susceptible</b>	
CH	1.89 ± 0.037
BDN 1	1.52 ± 0.037
ICP 8863	2.27 ± 0.037
CV (%)	20.2
SE	± 0.055
CD (0.1%)	1.057

1. Resistant = no apparent SM symptoms; moderately resistant = ring spot symptoms; susceptible = severe mosaic symptoms.
2. Mean of five measurements.

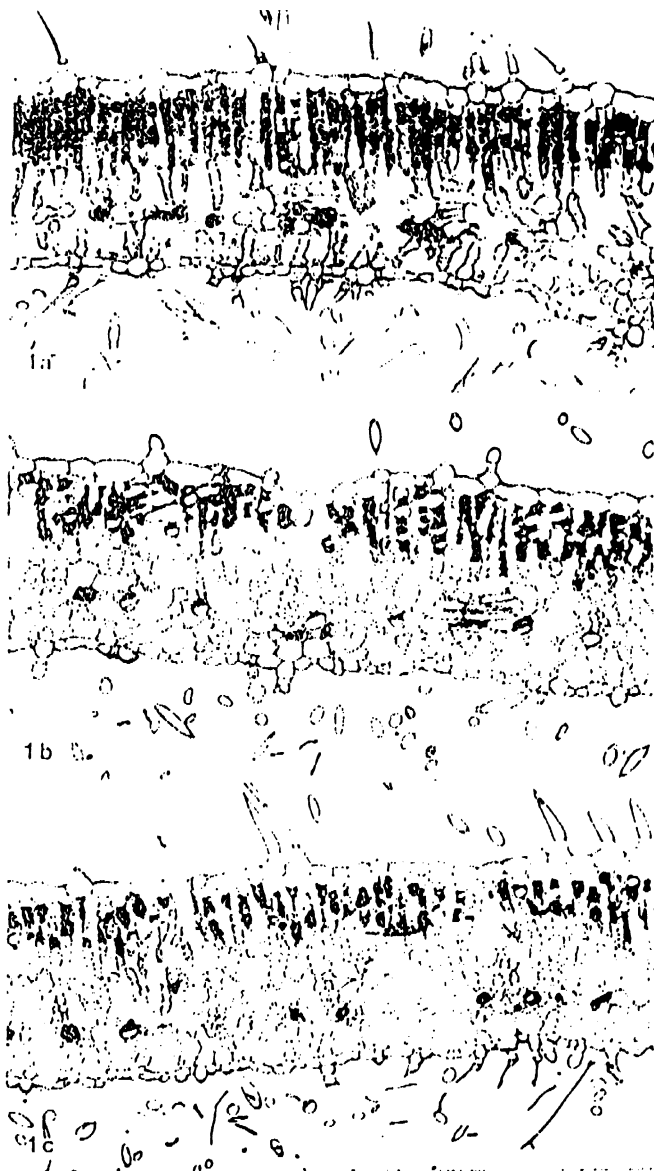


Fig. 1 : Transverse sections (X260) of leaves of pigeonpea sterility mosaic (a) resistant (ICP 7035) (b) moderately-resistant (ICP 2376), and (c) susceptible (C 11) cultivars showing differences in cuticle and cell wall thickness.

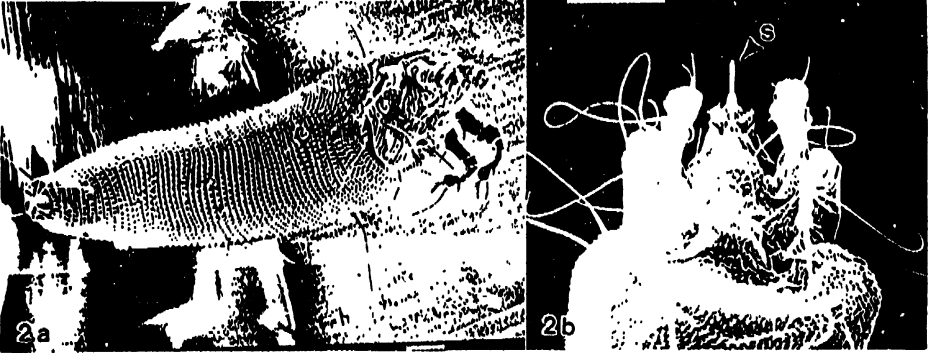


Fig. 2 : Scanning electron micrographs of (a) adult eriophyid mite vector *Aceria cajani* (ventral view) and (b) anterior end of mite showing stylet (s) (bar represents 10  $\mu$ m).

Fig. 3 : Leaves of pigeonpea cultivars showing different reactions to sterility mosaic infection : resistance (no symptoms left), moderate resistance (ring spots) (middle), and susceptibility (mosaic symptoms) (right).