Broad-based resistance to pigeonpea sterility mosaic disease in wild relatives of pigeonpea (*Cajanus*: Phaseoleae)

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Summary

Sterility mosaic disease (SMD), an important biotic constraint on pigeonpea (*Cajanus cajan*) in the Indian subcontinent, is caused by Pigeonpea sterility mosaic virus (PPSMV) transmitted by the eriophyid mite, *Aceria cajani*. Distinct PPSMV isolates occur in different geographical regions and broad-based resistance to all these isolates is scarce in cultivated pigeonpea germplasm. Wild relatives of pigeonpea, which are known to possess resistance to several pests and diseases, were evaluated for broad-based SMD resistance. One hundred and fifteen wild *Cajanus* accessions from six species (*C. albicans, C. platycarpus, C. cajanifolius, C. lineatus, C. scarabaeoides* and *C. sericeus*) were evaluated against three PPSMV isolates prevailing in peninsular India. Evaluations were done under greenhouse conditions in endemic locations of each isolate through mite-mediated virus inoculation. Fifteen accessions showed resistance to all three isolates: ICP 15614, 15615, 15626, 15684, 15688, 15700, 15701, 15725, 15734, 15736, 15737, 15740, 15924, 15925 and 15926. Most of the wild accessions did not support mite multiplication. The majority of the accessions resistant to PPSMV following inoculations with viruliferous mites were susceptible by graft inoculation, suggesting that vector resistance is conferring resistance to infection with PPSMV. The 15 accessions identified as being resistant to infection to all three virus isolates tested are cross compatible with pigeonpea by traditional breeding. They are therefore useful for exploitation in breeding programmes to increase both the level of SMD resistance and to diversify its genetic base in the cultivated pigeonpea gene pool.

Key words: Sterility mosaic disease, virus, eriophyid mite, resistance, mite resistance, wild species, *Cajanus cajan*

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millspaugh) is the principal legume crop of subsistence farming systems in the semi-arid tropics of Asia and is cultivated for its protein-rich seed. Over 90% of the world's pigeonpea is produced in India, Myanmar and Nepal (Saxena, 2000). Sterility mosaic (SMD), the most damaging disease of pigeonpea responsible for yield losses worth over US$300 million per annum, is endemic in all the pigeonpea-growing areas of south Asia. SMD-affected plants show characteristic mosaic symptoms on leaves with reduced or no flowering (sterility) (for review see Jones *et al*., 2004). SMD symptoms depend on the pigeonpea genotype and are categorized into three symptom types with genotypes that show: severe mosaic (SM) and sterility; (ii) mild mosaic (MM) with partial sterility; and (iii) chlorotic ring spots (RS) without any noticeable sterility. The causal agent of SMD has recently been characterised and identified as a distinct virus, named Pigeonpea sterility mosaic virus (PPSMV) (Kumar *et al*., 2002, 2003). The virus is transmitted in a semi-persistent manner by the eriophyid mite, *Aceria cajani* Channabasavanna (Acari: Arthropoda) (Kulkarni *et al*., 2002). This mite is highly host-specific and dependent on pigeonpea during all stages of its life cycle. Mites inhabit the lower surface of leaflets but their feeding causes no obvious damage to the host.

Management of SMD through the cultivation of resistant varieties is the most viable and cost-effective option for small-scale farmers. Numerous efforts have been invested in the identification and development of SMD resistant varieties (Nene & Reddy, 1976*a;* Nene *et al.,* 1981). Screening of the germplasm repository at ICRISAT, which holds about 13 000 accessions of cultivated pigeonpea (*C. cajan*), identified 326 accessions with field resistance to SMD (Nene *et al*., 1981; Remanandan, 1990). However, PPSMV occurs as various geographical

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isolates, consequently performance of various resistant genotypes differed across locations. For instance, some pigeonpea cultivars resistant to PPSMV in central India are highly susceptible to the disease when grown in southern peninsular and northern India (Reddy *et al.,* 1993). At least five PPSMV isolates were suspected to occur in India alone, and three distinct isolates have recently been characterised, *viz*., Patancheru (P), Bangalore (B) and Coimbatore (C), each in peninsular India (Reddy *et al*., 1993). Most of the SMD-resistant accessions were resistant to isolate P. Resistance to other isolates or broad-based resistance was scarce in cultivated germplasm (Nene *et al*., 1989). This has necessitated a search for alternative sources of broad-based durable resistance to SMD.

Wild relatives of pigeonpea were shown to possess many agronomically desirable traits, including resistance to SMD and other diseases (Saxena *et al*., 1990; Kulkarni *et al*., 2003*b*). The genus *Cajanus* consists of 32 species, of which only *C. cajan* is cultivated, the others being wild species. The ICRISAT gene bank holds 213 accessions of 20 wild *Cajanus* species (Kameswara-Rao *et al*., 2003). This paper reports the greenhouse evaluation of 115 accessions of six wild *Cajanus* species (*C. albicans*, *C. cajanifolius*, *C. lineatus*, *C. platycarpus*, *C. scarabaeoides* and *C. sericeus*), compatible for inter-specific hybridization with pigeonpea, for resistance to three isolates of PPSMV occurring in peninsular India.

Materials and Methods

Seed material, PPSMV isolates and mite cultures

Seeds of 115 accessions of six wild *Cajanus* species were obtained from the gene bank of ICRISAT, India (Table 1). Seeds were scarified by slicing the seed coat with a scalpel blade, treated with Thiram at 30 mg/10 g seed and sown in 21 cm diameter plastic pots filled with Alfisoil in an insect-proof greenhouse. Due to limited seed availability a few accessions could not be tested against all three PPSMV isolates. Pigeonpea cultivars ICP 8863, TTB-7 and Vamban-1 were used as virus susceptible controls, and ICP 7035 as the virus resistant control. As PPSMV is not transmissible by mechanical inoculation of sap, viruliferous mites were used for virus inoculation to 12–20 day-old pigeonpea seedlings following the leaf-stapling technique described by Nene & Reddy (1976*b*). Briefly, leaflets from SMD-affected plants infested with mites (minimum five mites leaflet^{-1}) were stapled onto primary leaves of healthy seedlings. Mites from the stapled leaf migrate onto the test seedling to feed and in feeding transmit the virus. The wild *Cajanus* species were evaluated for resistance against PPSMV isolates P, B and C, which were obtained from naturally infected pigeonpea plants several years ago and maintained subsequently in PPSMV-susceptible pigeonpea cultivars at research stations within the endemic regions where these isolates occur. Virus and mite cultures were maintained by periodically replacing old plants with young seedlings.

Screening of wild Cajanus *accessions by mite inoculation and grafting*

The evaluation of wild *Cajanus* species was based on the inoculation of plants with viruliferous mites, followed by testing of selected promising lines by petiole grafting to determine the specifics of resistance. This was done in a greenhouse at locations where the respective isolates are endemic. These were: ICRISAT, Patancheru, for isolate P; the Department of Plant Pathology, University of Agricultural Sciences, Bangalore, for isolate B; and the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, for isolate C. Due to space constraints in the greenhouse, evaluations were done in batches from 2001 to 2003. The 12–20 day-old seedlings of the test accessions, together with the controls, were inoculated with viruliferous *A. cajani* by the leaf-stapling method. Based on symptoms, disease incidence was recorded at 30, 60 and 90 days post inoculation (dpi). Using a stereo-binocular microscope, mite numbers were recorded on five young trifoliate leaves collected randomly from five plants of each accession at 60–70 dpi. To determine the type of resistance, accessions that were resistant following mite inoculation were evaluated by graft inoculation using mite-free, PPSMV-infected, pigeonpea-petioles as scions as described by Reddy *et al*. (2002). For this purpose, seeds of test accessions were sown in plastic pots and 25–35 day-old plants were used for graft inoculation. Test plants were maintained in mite-proof cages. Observations on symptom type and percent disease incidence were recorded at 30 and 60 days post grafting.

Detection of PPSMV in test plants

Polyclonal antibodies raised to PPSMV isolate P, which detects all the three virus isolates studied, were used to assay all test plants for PPSMV by double antibody sandwich (DAS)-ELISA as described by Kumar *et al*. (2004). From symptomatic plants, only young leaflets showing clear symptoms were selected but, from apparently healthy plants, young leaflets were chosen from at least three branches and pooled. Test leaves were extracted in phosphate-buffered saline (1:10 w/v), and 100 µL of this extract was loaded into wells of ELISA plates pre-coated with PPSMV polyclonal antibodies at 1:10 000 dilution. Penicillinase (PNC)-labelled PPSMV IgGs was used at 1:1500 dilution to detect trapped antigen. Sodium penicillin G was used at 0.05 mg mL^{-1} in 0.015%

(w/v) bromothymol blue buffer, pH 7.4. Optical density values at 620 nm (A_{620}) were measured in an ELISA plate reader. Readings were considered to be virus positive if the absorbance values of a sample differed three-fold from those given by the virus-free control samples.

Results

Response to mite-mediated inoculation with PPSMV

The complete data for the evaluation of wild *Cajanus* accessions by mite inoculation with PPSMV isolates are given in Table 1. The susceptible controls (ICP 8863, Vamban-1 and TTB-7) at each of the three locations resulted in 91–100% infection and plants developed typical severe SMD symptoms 12–20 dpi, confirming the efficiency and reliability of the inoculation method (Table 1). PPSMV-infected wild *Cajanus* plants developed systemic severe mosaic or mild mosaic symptoms 20–30 dpi. Only a few plants of ICP 15620, 15625 and 15627 infected with isolate B showed ring spot symptoms (Table 1). Some accessions that initially showed mild mosaic symptoms on a few leaves developed severe mosaic at later stages of growth (e.g. ICP 15664). In general, infected plants showed a common symptom response to all three isolates (e.g. ICP 15622) but a few accessions showed different symptoms when infected with different virus isolates (e.g. ICP 15641) (Table 1). Symptoms in infected plants persisted throughout the observation period. In DAS-ELISA, only symptomatic plants tested positive for PPSMV and all asymptomatic plants were negative (data not shown). The A_{620} of leaf samples ranged between 0.08 to 0.28 for those with severe mosaic, 0.3 to 0.69 for those with mild mosaic, and > 0.9 for symptomless leaf samples (data not shown).

Of the 115 accessions studied, 83 were tested against all three virus isolates and of these, 48 were uninfected with one or more isolate. Of the 32 accessions tested against only two isolates (largely isolates P and B), 17 were uninfected with one, or both, isolate (Table 1). Thus, of the 115 accessions tested, 65 (56%) were uninfected with one or more virus isolate but only eight accessions (ICP 15614, 15615, 15626, 15924, 15926 15700, 15701, 15734) were uninfected with all three isolates (Tables 1 and 2). However, eight accessions of *C. scarabaeoides* (ICP 15695, 15702, 15703, 15707, 15712, 15726, 15728, 15739) were uninfected with isolates P and B but were not tested against isolate C (Table 1), so these may be further sources of broad-based resistance to infection. In some accessions, only one or two plants were infected with virus despite the high inoculum pressure (Tables 1 and 2). Therefore, those accessions with up to 12% infection incidence were also considered as (partially) resistant to infection; all accessions with > 13% infection were considered susceptible (Tables 1 and 2). Of the six accessions evaluated, most resistant accessions were of *C. scarabaeoides* and *C. albicans,* with decreasing numbers of *C. lineatus, C. cajanifolius, C. sericeus* and *C. platycarpus* respectively, but all 15 accessions considered resistant to infection to all three virus isolates were within *C. albicans* and *C. scarabaeoides* (Table 2).

Observations for mites

Of the 110, 114 and 89 accessions inoculated at locations P, B and C, mites were not found on 53 (48%), 48 (42%) and 28 (31%) accessions, respectively (Table 1) and the majority of these accessions were resistant to mite inoculation of virus (Table 1). Of the remaining accessions, mite numbers were very low (usually between 1 and 7) compared to the susceptible controls and comprised accessions that were both infected and uninfected with PPSMV (Table 1). More than 10 mites were observed on only 11 accessions and all of these accessions became infected with PPSMV. With the exceptions of ICP 15614 (two mites at location P) and ICP 15671 and 15924 (one mite each at location B), mites were not detected on accessions with no infected plants. One to three mites, mostly confined to symptomatic leaves of plants, were observed on accessions that had 12% or fewer incidences of infected plants (Table 1). Overall, mites were not found on 11 of the 83 accessions evaluated at all three locations: ICP 15615, 15626 and 15926 of *C. albicans*; ICP 15649 of *C. lineatus*; and ICP 15685, 15700, 15701, 15725, 15734, 15740 and 15741 of *C. scarabaeoides* but three of these (ICP 15649, 15685 and 15741), were infected with PPSMV at one of the three locations (Table 1).

On some accessions, there was variation in mite colonisation between the three locations and this may be due to the different environmental conditions and times of the assay. Additionally, infection with PPSMV can influence mite multiplication on *Cajanus* plants (Kulkarni *et al.*, 2002). However, overall mite numbers on these wild accessions were too low to draw any meaningful conclusions.

Response to graft-inoculation with PPSMV

Twenty-four and eight accessions that were uninfected with PPSMV following inoculation with viruliferous mites were evaluated by graft inoculation with isolates C and P respectively and the results are given in Table 3. It shows that graft-inoculation of the susceptible controls ICP 8863 and Vamban-1 resulted in over 82% infection and infected plants developed typical SMD symptoms, confirming the relative reliability of the technique (Table 3). All graft-inoculated wild species accessions were infected with PPSMV except ICP 15614 inoculated

Table 1. *Responses of accessions of six Cajanus species inoculated with three distinct Pigeonpea sterility mosaic virus (PPSMV) isolates using viruliferous* Aceria cajani

		Patancheru (P) isolate				Bangalore (B) isolate				Coimbatore (C) isolate			
†**ICP No.**	§Resistance to PPSMV isolate	N	PI	M	SYT	N	PI	M	SYT	N	PI	M	SYT
C. albicans													
15614	**P B C**	14	0	2	NS	6	0	0	NS	8	0	0	NS
15615	**P B C**	18	0	0	NS	17	0	0	NS	25	0	0	NS
15616	**P - -**	20	0	0	NS	21	33	2	MM	18	17	2	MM
15617	**P - -**	22	0	0	NS	21	57	2	MM	9	22	4	SM
15618	**P - C**	17	0	0	NS	24	63	2	MM	17	0	0	NS
15619	**P - -**	19	0	0	NS	12	25	3	MM	28	25	3	MM
15620 [SRI]	**P - -**	21	0	0	NS	20	40	2	RS	19	16	2	MM
15621	**P - C**	20	0	0	NS	24	42	2	MM	17	0	0	NS
15622	- - -	12	33	0	MM	24	46	1	MM	16	13	1	MM
15623	- - -	15	47	1	MM	21	24	3	MM	23	17	2	MM
15624	- - -	12	75	3	MM	24	17	1	MM	16	44	5	SM
15625	**P - C**	15	0	0	NS	22	32	1	RS	12	0	0	NS
15626	**P B C**	19	0	0	NS	21	0	0	NS	20	0	0	NS
15627	*P* - -	12	8	0	MM	20	45	0	RS	25	24	4	SM
15628	- - -	10	30	0	MM	19	32	3	SM	21	14	2	MM
15924 [SRI]	**P B C**	16	0	0	NS	24	0	1	NS	22	0	0	NS
15925 [SRI]	*P* **B C**	34	3	0	MM	25	0	1	NS	18	0	0	NS
15926 [SRI]	**P B C**	22	0	0	NS	20	0	0	NS	13	0	0	NS
15927 [SRI]	**P - C**	23	0	0	NS	25	52	2	MM	19	0	0	NS
C. cajanifolius													
15629	*P* - **C**	9	11	0	MM	16	44	4	SM	18	0	0	NS
15630	- - -	11	27	2	SM	9	33	4	SM	18	17	0	MM-SM
15631	*P* - -	16	6	0	SM	38	39	3	MM	17	29	2	SM
15632	**- - C**	16	19	2	SM	37	38	2	MM	19	0	0	NS
C. lineatus													
15641	- **B** -	25	28	3	SM	17	0	0	NS	18	17	0	MM
15642	- - -	19	42	2	SM	12	33	3	SM	13	31	7	SM
15643	- **B** *C*	16	31	1	SM	15	0	0	NS	20	10	2	SM
15644	**P - C**	9	0	0	NS	13	62	5	SM	21	0	0	NS
15645	**- - -**	12	25	0	MM	13	70	3	MM	19	21	1	MM-SM
15646	**- - -**	14	43	6	SM	15	40	2	MM	17	35	2	MM-SM
15647	**- -** *C*	10	20	0	MM	5	60	2	SM	19	11	3	MM
15648	**P - C**	15	0	0	NS	7	57	1	SM	13	0	0	NS
15649	*P* - **C**	12	8	0	SM	13	0	0	MM	21	0	0	NS
15650	- - -	15	13	0	SM	13	0	0	MM	13	31	2	MM
C. platycarpus													
15661	- - -	16	63	12	SM	17	71	1	MM	22	36	3	SM
15662	- - -	14	79	7	SM	15	73	2	SM	25	52	7	SM
15663	- - -	16	100	16	SM	12	59	3	SM	10	30	1	MM
15664	- - -	22	46	5	MM-SM	13	77	2	SM	23	48	4	MM-SM
15665	**- - C**	26	65	3	MM-SM	15	53	0	MM	25	0	0	NS
15666	- - -	21	57	6	MM-SM	14	64	0	MM	14	14	3	MM
15667	- - -	23	65	11	MM-SM	20	45	1	MM	24	33	8	SM
15668	- - -	24	71	9	MM-SM	19	63	3	SM	17	29	5	SM
15669	- - -	30	73	21	MM-SM	19	53	2	MM	11	18	9	SM
15670	**- B -**	24	50	7	SM	21	0	0	NS	29	28	3	MM-SM
15671	**- B -**	11	91	5	MM	20	0	1	NS	13	15	2	MM
15672	- - -	12	25	0	MM	16	44	2	SM	21	19	1	MM
15673	- - -	12	33	1	MM	18	44	0	SM	19	32	2	MM
15921	- - -	27	63	5	SM	17	47	2	MM	19	16	3	SM
16144	- - -	17	59	3	SM	23	48	3	MM	16	13	3	MM
16145	- - -	26	69	11	SM	21	71	3	SM	26	35	7	SM
16146	- - **C**	26	54	5	SM	18	78	2	MM	22	0	0	NS

cont...

Table 1 cont...

†ICP No.	§Resistance to PPSMV isolate	Patancheru (P) isolate				Bangalore (B) isolate				Coimbatore (C) isolate			
		N	PI	M	SYT	N	PI	M	SYT	N	PI	M	SYT
C. scarabaeoides													
15683*	**- . -**	16	100	3	MM-SM	ng	-	-	-	9	78	4	SM
15684	*P* **B** *C*	28	4	2	MM	24	0	0	NS	14	7	2	MM
15685	**- B -**	26	54	0	MM	14	0	0	NS	20	35	0	MM
15686	- *B* -	27	93	4	MM-SM	26	8	3	SM	24	88	6	SM
15687*	- - .	20	40	3	MM-SM	16	13	0	SM	ng	-	-	-
15688	*P B C*	33	3	0	MM	9	11	2	SM	18	11	3	SM
15689	- - -	25	64	2	MM-SM	34	21	2	SM	29	45	4	SM
15690	- - -	26	58	15	MM-SM	27	33	1	SM	24	46	7	SM
15691*	**- - .**	24	58	2	MM-SM	20	25	2	SM	ng	-	-	-
15692*	- *B* .	20	15	2	MM-SM	22	5	2	SM	ng	-	-	-
15693	- *B* -	34	65	3	SM	22	9	1	SM	27	56	2	SM
15694 [SRI]*	- - .	27	37	2	MM-SM	22	18	1	SM	ng	-	-	-
15695 [SRI]*	*P B* .	21	5	0	SM	37	3	0	SM	ng	-	-	-
15696 [MYA]	- - -	26	35	0	MM	33	12	2	SM	24	13	1	SM
15697*	**P -** .	21	0	0	NS	14	14	3	MM	ng	-	-	-
15698*	- - .	21	43	4	SM	21	43	1	SM	ng	-	-	-
15699*	- - .	26	12	2	SM	15	13	1	SM	ng	-	-	-
15700	**P B C**	16	0	0	NS	17	0	0	NS	13	0	0	NS
15701	**P B C**	22	0	0	NS	18	0	0	NS	25	0	0	NS
15702*	**P** *B* .	21	0	0	NS	24	8	0	SM	ng	-	-	-
15703*	*P* **B** .	25	8	0	MM-SM	7	0	0	NS	ng	-	-	-
15704*	**- - .**	29	14	0	SM	19	16	0	SM	ng	-	-	-
15705*	- *B* .	19	16	1	MM-SM	19	5	0	SM	ng	-	-	-
15706*	- *B* .	29	21	3	SM	23	9	1	SM	ng	-	-	-
15707*	*P* **B** .	22	5	0	MM	23	0	0	NS	ng	-	-	-
15708*	**P -** .	23	0	0	NS	23	0	0	MM	ng	-	-	-
15709*	**P -** .	15	0	0	NS	12	0	0	MM	ng	-	-	-
15710	- - -	30	87	5	SM	15	27	2	SM	19	53	6	SM
15711	- *B* -	27	59	0	MM	20	5	0	SM	22	14	1	SM
15712*	**P B** .	10	0	0	NS	17	0	0	NS	nt	-	-	-
15713*	- - .	16	13	2	MM-SM	24	21	4	SM	nt	-	-	-
15716	**- B -**	10	80	9	MM	32	0	0	NS	14	21	3	MM
15717*	- - .	15	13	0	MM	21	19	1	SM	nt	-	-	-
15718	- - -	26	81	18	SM	19	21	2	SM	21	33	5	SM
15719	- - -	12	83	6	MM-SM	10	30	2	SM	27	22	4	SM
15720 [PHIL]	- - -	11	91	14	SM	18	22	3	SM	16	19	7	SM
15721 [PHIL]	- *B* -	18	83	2	MM-SM	32	6	3	MM-SM	17	53	3	MM-SM
15722	- *B* -	19	95	0	MM	33	3	0	SM	22	14	1	SM
15723	- - -	39	80	14	SM	26	39	4	SM	29	38	9	SM
15724	**- B -**	41	83	4	MM	27	0	0	NS	18	33	3	MM
15725	*P* **B C**	20	5	0	MM	16	0	0	NS	16	0	0	NS
15726*	**P** *B* .	24	0	0	NS	26	0	0	MM	nt	-	-	-
15727	- - -	32	69	7	MM-SM	34	12	2	SM	22	23	4	MM-SM
15728*	**P B** .	20	0	0	NS	25	0	0	NS	nt	-	-	-
15729	**-** *B* **-**	26	27	8	MM-SM	26	3	0	SM	9	33	5	SM
15730*	**- - .**	17	12	3	SM	20	30	2	SM	nt	-	-	-
15731	**- - -**	31	65	3	SM	12	42	3	SM	23	65	2	SM
15732*	**- - .**	26	23	1	MM	9	33	2	SM	nt	-	-	-
15733	- *B* -	28	68	20	MM-SM	14	7	2	MM	18	38	8	MM
15734 [AUS]	**P B C**	23	0	0	NS	10	0	0	NS	19	0	0	NS
15735 [AUS]	- - -	14	100	0	MM-SM	14	14	0	SM	22	23	2	SM
15736 [FIJI]	*P* **B C**	26	4	2	MM	7	0	0	NS	15	0	0	NS
15737 [FIJI]	*P B C*	35	6	0	MM	11	9	0	MM	19	11	1	MM
15738	- - -	41	83	9	SM	9	33	1	SM	26	27	3	SM

cont...

Table 1 cont...

†ICP No.	§Resistance to PPSMV isolate	Patancheru (P) isolate				Bangalore (B) isolate				Coimbatore (C) isolate			
		N	PI	M	SYT	N	PI	M	SYT	N	PI	M	SYT
15739*	*P* **B** .	20	5	0	MM	12	0	0	NS	nt	-	-	-
15740	*P* **B C**	21	5	0	MM	15	0	0	NS	22	0	0	NS
15741[unknown]	*P B* -	25	4	0	MM	12	8	0	MM	14	14	0	MM
15742 [AUS]*	*P* - .	22	9	0	MM	16	75	1	SM	nt	-	-	-
15743 [AUS]	**P** - *C*	23	0	0	NS	15	27	0	MM	17	6	2	MM
15744 [AUS]*	- - .	22	18	1	MM	13	46	0	SM	nt	-	-	-
15922*	. - -	nt	-	-	-	21	52	0	MM	15	13	1	SM
C. sericeus													
15760*	. **- C**	nt	-	-	-	17	35	0	MM	17	0	0	NS
15761*	. - -	nt	-	-	-	19	53	0	MM	21	24	8	SM
15762*	. - -	nt	-	-	-	19	37	1	MM	16	17	56	MM-SM
15763 [AUS]*	. - -	nt	-	-	-	18	33	0	MM	27	27	7	SM
Controls: *C. cajan*													
ICP 8863	- - -	30	100	27	SM	21	91	19	SM	12	100	32	SM
Vamban – 1	. . -	nt	-	-	-	nt	-	-	-	30	100	14	SM
TTB – 7	. - .	nt	-	-	-	20	95	18	SM	nt	-	-	-
ICP 7035	- - -	20	0	0	NS	20	0	0	NS	20	0	0	NS

†All accessions were of Indian origin, except those indicated as: AUS = Australia; MYA = Myanmar; PHIL = Philippines; SRI = Sri Lanka; the origin of 15741 is unknown.
§Accession reaction to PPSMV isolates. Accessions with no infected plants is given in bold; accessions with 12% or less infected plants are italicised; *Accessions not tested against all PPSMV isolates. – = infected; . = not tested.
N = number of plants tested; PI = percent infected plants determined by DAS-ELISA; M = Mean numbers of mites per trifoliate leaf; SYT = Symptom type: SM = severe mosaic; MM = mild mosaic; RS = chlorotic ringspots; MM-SM = initial mild mosaic followed by severe mosaic; NS = no symptoms; ng = poor germination; nt = not tested

Table 2. *Accessions within six* Cajanus *species resistant to more than one Pigeonpea sterility mosaic virus isolate following inoculation using viruliferous* Aceria cajani

Species	P+B+C	P+C	P+B	B+C
C. albicans [19/20]†	15614, 15615, 15626, 15924, *15925*, 15926	15618, 15621, 15625, 15927	-	-
C. cajanifolius [4/5]†	-	*15629*	-	
C. lineatus [10/10] †	-	15644, 15648, *15649*	-	*15643*
C. platycarpus [17/17]†	-	-	-	-
C. scarabaeoides [61/102]†*	*15684, 15688*, 15700, 15701, *15703, 15725*, 15734, *15736*, *15737, 15740*	15697, *15743*	15695, *15702, 15707*, 15712, 15728, 15739, *15741*	*15705*
C. sericeus [4/4]†*	-	-	-	-

†No. of accessions evaluated / total no. in the ICRISAT genebank; * = not all accessions tested at all the locations. Accessions with up to 12% infection incidence are indicated in italics (see Table 1 for more details)
P = Patancheru; B = Bangalore; C = Coimbatore

with isolate P and ICP 15615 inoculated with isolate C. The incidence of infected plants in the other accessions ranged from 6% to 82% and all infected plants showed only mild mosaic symptoms and were PPSMV-positive in ELISA (Table 3). However, in some accessions such as ICP 15927 and 15700, only one or two plants became infected (Table 3).

Discussion

This is the first comprehensive evaluation of wild *Cajanus* accessions against different PPSMV isolates and that has identified sources of broad-based resistance to the disease that can be transferred to *C. cajan*. Recent developments on the identification of the SMD causal agent have paved the way for this study (Jones *et al*., 2004), especially the development of ELISA for PPSMV detection and petiole graft inoculation that facilitated the testing of young plants to identify resistance to the virus.

Accessions of the six wild *Cajanus* species showed variable reaction to the three PPSMV isolates when inoculated with mites (Table 1). A significant number were uninfected with one or more isolate and a few more had less than 12% infected plants, despite the exposure to a high inoculum pressure at the seedling stage (Table 1). Although only a few accessions were assayed by graft inoculation with different

Table 3. *Evaluation of accessions within six* Cajanus *species by graft-inoculation with Pigeonpea sterility mosaic virus isolates Patancheru (P) and Coimbatore (C)*

ICP No.†	No. infected / no. tested (symptom type)	
	C isolate	P isolate
C. albicans		
15614	2/10 (MM)	0/13
15615	0/7 (NS)	nt
15618	1/5 (MM)	nt
15621	4/9 (MM)	nt
15625	3/6 (MM)	nt
15626	3/7 (MM)	nt
15924	1/5 (MM)	nt
15925	3/8 (MM)	nt
15926	4/5 (MM)	nt
15927	1/7 (MM)	nt
C. cajanifolius		
15629	1/9 (MM)	nt
15632	5/9 (MM)	nt
C. lineatus		
15644	1/4 (MM)	nt
15648	2/6 (MM)	nt
15649	4/7 (MM)	nt
C. platycarpus		
15665	4/9 (MM)	nt
16146	2/8 (MM)	nt
C. scarabaeoides		
15684	nt	5/15 (MM)
15688	nt	6/13 (MM)
15700	1/5 (MM)	2/31 (MM)
15701	5/9 (MM)	4/24 (MM)
15725	1/5 (MM)	nt
15734	6/9 (MM)	nt
15736	4/8 (MM)	5/21 (MM)
15737	nt	7/28 (MM)
15740	2/6 (MM)	7/24 (MM)
C. sericeus		
15760	2/4 (MM)	nt
Controls		
ICP8863 (susceptible)	17/20 (SM)	14/17 (SM)
ICP7035 (resistant)	nt	1/25 (MM)
Vamban – 1	nt	nt

NS = no symptoms; RS = chlorotic ring spots; MM = mild mosaic; nt = not tested
†All accessions included are resistant to isolates P and C by mite inoculation (see Table 1 for details)

virus isolates, all but two of them became infected with virus (Table 3), suggesting that the observed resistance to virus infection by mite inoculation is probably due to vector resistance rather than resistance to PPSMV. Indeed, compared to controls, very low mite numbers were found on most of the accessions, including several accessions that are very susceptible to graft inoculation with the virus (Tables 1, 3). If effective mite resistance is operating in some accessions, then it would be expected to provide resistance to mite inoculation of all PPSMV isolates. Possibly this is the explanation for the observed resistance to all three PPSMV isolates in some accessions. However, another explanation for observed host resistance in some mite-inoculated accessions could be due to inhibition of cell-to-cell or long distance virus movement through phloem, and graft-inoculation could have overcome this type of resistance (Valkonen, 2001), but further work is required to clearly understand the resistance mechanism to SMD in wild *Cajanus* species.

The lack of mites on some accessions that became infected with PPSMV indicates that mites had fed for at least 60–90 min, the minimum inoculation access period for PPSMV transmission (Kulkarni *et al*., 2002), but they may not have fed for much longer and did not multiply on these plants (e.g. 15672, 15696; Table 1). A similar situation was observed following inoculation of *A. cajani* to French bean (*Phaseolus vulgaris*), which resulted in PPSMV transmission, but the mites did not multiply on this host (Kulkarni *et al.*, 2003*a*). It is known that many plant properties can influence resistance to virus vectors and thereby provide partial or very effective control of the viruses they transmit (reviewed by Jones, 1987, 1998). Possibly the plant chemistry (Dodia *et al*., 1996) or its physical features contributed to the poor multiplication of mites on plants of these accessions. It is noteworthy that trichomes are dense on the lower leaf surface of *C. cajan* and provide a vital microclimate for mite survival and multiplication (J Vijayanarasimha, K T Rangaswamy and P L Kumar, unpublished) whereas trichomes were very sparse on the lower leaf surface of wild *Cajanus* species (data not shown). Possibly this physical factor may have contributed to the poor survival of mites on

Table 4. *Accessions of* Cajanus scarabaeoides *with multiple disease resistance*

ICP No.	PPSMV isolate*	Resistant to: Other pathogens and pests†
15684	P, B, C	Fusarium wilt, *Helicoverpa armigera* larvae, immune to pod fly damage
15688, 15725	P, B, C	Fusarium wilt
15695	P, B	Fusarium wilt, Oviposition non-preference, immune to pod fly, *Helicoverpa armigera* larvae, cyst nematode. Resistant to pod damage by *H. armigera* and pod wasp
15712	P, B	Fusarium wilt
15726	P, B	Oviposition non-preference, *H. armigera* larvae

*See Table 1 for more details (P = Patancheru; B = Bangalore; and C = Coimbatore)
†Dodia *et al.,* 1996; Saxena *et al.,* 1990 ; Sharma, 1995; Shanower *et al.,* 1997

these species. Additionally, it has been shown that in some SMD resistant cultivated pigeonpea genotypes a thicker leaf cuticle and epidermal cell wall prevents the mite stylet from reaching epidermal cells (Reddy *et al*., 1995). Possibly this mechanism may account for the fact that no infection occurred and mites were not found on some wild *Cajanus* accessions that were susceptible to the virus by graft inoculation (Tables 1, 3).

Of the 27 accessions graft inoculated with two PPSMV isolates, only two, ICP 15614 and 15615, were found to be resistant. However, ICP 15614 was resistant to isolate P but not isolate C and ICP 15615 was tested against only isolate C (Table 3). Whilst in some other graft-inoculated accessions only a small number of plants became infected (Table 3), it is not known if this is due to resistance to virus inoculation and/or multiplication and/or invasion, or a reflection of difficulty in grafting the very slender stems of these wild accessions.

The variable reaction of some accessions to the three PPSMV isolates in symptom type and in infection incidence may indicate the involvement of different genetic determinants in the host reaction to inoculation and infection. In this connection, it is noteworthy that PPSMV resistance in *C. cajan* is isolate dependent and regarded as polygenic with susceptibility dominant over resistance, and with resistance and disease response to SMD infection controlled by independent non-allelic genes (Sharma *et al.,* 1984; Srinivas *et al*., 1997*a,b*). A similar genetic mechanism may be operating in wild *Cajanus* species. However, it is to be noted that each isolate was tested in different geographic locations and it is possible that different growth conditions at each locality may also have influence on host resistance. Testing of accessions against all the three virus isolates under the same conditions is necessary because of variation in host resistance across locations.

There was some variability in mite colonization on a few accessions when tested at the three different locations. Multiplication of *A. cajani* on cultivated pigeonpea has been shown to be much greater on PPSMV-infected plants than on healthy plants of the same genotype (Reddy & Nene, 1980; Muniyappa & Nangia, 1982; Kulkarni *et al*., 2002) but this cannot explain all the differences. Furthermore, a variation in *A. cajani* biotypes at these locations also seems unlikely because earlier studies using DNA markers ruled out this possibility (Kumar *et al*., 2001). Because of the very low numbers of mites on the accessions in these locations no firm conclusions can be drawn on the reasons for the variability.

Of the 15 accessions of wild *Cajanus* that showed resistance to all the three isolates by mite inoculation, eight (ICP 15614, 15615, 15626, 15924, 15926 of *C. albicans,* and ICP 15700, 15701, 15734 of *C. scarabaeoides;* Tables 1 and 2) were identified as potential sources of strong broad-based SMD resistance because all plants of these accessions failed to become infected with any of three PPSMV isolates. On five of these accessions no mites were recorded on plants (Table 1). In addition, six of these resistant *C. scarabaeoides* accessions were identified earlier to contain resistance to other pests and pathogens (Table 4) (Dodia *et al.,* 1996; Saxena *et al.,* 1990, Sharma, 1995; Shanower *et al.,* 1997). With their resistance to these other biotic constraints, their use as parents in interspecies breeding for broad-based SMD resistance may provide multiple disease resistant varieties to mitigate losses to SMD, wilt and pod borer, all of which seriously affect pigeonpea cultivation. Apart from *C. platycarpus,* the species tested for resistance were from the secondary gene pool, which are inter-fertile by traditional breeding methods and have been successfully crossed with *C. cajan* (Pundir and Singh, 1987). Therefore, the resistance in these accessions can be transferred simply to pigeonpea.

Further research is focused on the identification and distribution of PPSMV isolates in other SMD endemic regions in India to further evaluate these promising accessions, and to identify the genetic components controlling resistance to various PPSMV isolates in order to enhance the breeding for protection against this major virus disease.

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References

Dodia D A, Patel J A, Patel S I, Dhulia F K, Tikka S B S. 1996. Antibiotic effect of pigeonpea wild relatives on *Heliothis armigera*. *International Chickpea and Pigeonpea Newsletter* **3**:100–101.

Jones A T. 1987. Control of virus infection in crop plants through vector resistance: a review of achievements, prospects and problems. *Annals of Applied Biology* **111**:745–772.

Jones A T. 1998. Control of virus infection in crop plants through breeding plants for vector resistance. In *Plant Virus Disease Control*, pp. 41–55. Eds A Hadidi, R K Khetarpal and H Koganezawa, H. St Paul, MN :APS Press.

Jones A T, Kumar P L, Saxena K B, Kulkarni N K, Muniyappa V, Waliyar F. 2004. Sterility mosaic disease – the "green plague" of pigeonpea: advances in understanding the etiology, transmission and control of a major virus disease. *Plant Disease* **88**:436–445.

Kameswara-Rao N K, Reddy L J, Bramel P J. 2003. Potential of wild species for genetic enhancement of some semi-arid food crops. *Genetic Resources and Crop Evolution* **50**:707–721.

Kulkarni N K, Kumar P L, Muniyappa V, Jones A T, Reddy D V R. 2002. Transmission of Pigeonpea sterility mosaic virus by the eriophyid mite, *Aceria cajani* (Acari: Arthropoda). *Plant Disease* **86**:1297–1302.

Kulkarni N K, Kumar P L, Muniyappa V, Jones A T, Reddy D V R. 2003*a*. Studies on host range of Pigeonpea sterility mosaic virus. *Journal of Mycology and Plant Pathology* **33**:141–145.

Kulkarni N K, Reddy A S, Kumar P L, Vijaynarasimha J, Rangaswamy K T, Reddy L J, Saxena K B, Jones A T, Reddy D V R. 2003*b*. Broad-based resistance to Pigeonpea sterility mosaic disease in the accessions of *Cajanus scarabaeoides*. *Indian Journal of Plant Protection* **31**:6–11.

Kumar P L, Fenton B, Duncan G H, Jones A T, Sreenivasulu P, Reddy D V R. 2001. Assessment of variation in *Aceria cajani* (Acari: Eriophyidae) using analysis of nuclear rDNA ITS regions and scanning electron microscopy: implications for the variability observed in host plant resistance to pigeonpea sterility mosaic disease. *Annals of Applied Biology* **139**:61–73.

Kumar P L, Duncan G H, Roberts I M, Jones A T, Reddy D V R. 2002. Cytopathology of Pigeonpea sterility mosaic virus in pigeonpea and *Nicotiana benthamiana*: similarities with those of eriophyid mite-borne agents of undefined aetiology. *Annals of Applied Biology* **140**:87–96.

Kumar P L, Jones A T, Reddy D V R. 2003. A novel mite-transmitted virus with a divided RNA genome closely associated with pigeonpea sterility mosaic disease. *Phytopathology* **93**:71–81.

Kumar P L, Jones A T, Waliyar F. (Eds). 2004. *Serological and nucleic acid based methods for the detection of plant viruses: methods manual*. Patancheru 502 324, A.P. India: ICRISAT.

Muniyappa V, Nangia N. 1982. Pigeonpea cultivars and selections for resistance to sterility mosaic in relation to prevalence of eriophyid mite *Aceria cajani* Channabasavanna. *Tropical Grain Legume Bulletin* **25**:28–30.

Nene Y L, Reddy M V. 1976*a*. Screening for resistance to sterility mosaic of pigeonpea. *Plant Disease Reporter* **60**:1034–1036.

Nene Y L, Reddy M V. 1976*b*. A new technique to screen pigeonpea for resistance to sterility mosaic. *Tropical Grain Legume Bulletin* **5**:23–24.

Nene Y L, Kannaiyan J, Reddy M V, Remanandan P. 1981. *Sources of resistance to selected pigeonpea diseases*. Pulse Pathology Progress Report 16. Patancheru 502324, AP, India: ICRISAT.

Nene Y L, Reddy M V, Beniwal S P S, Mahmood M, Zote K K, Singh R N, Sivaprakasam K. 1989. Multilocational testing of pigeonpea for broad-based resistance to sterility mosaic in India. *Indian Phytopathology* **42**:444–448.

Pundir R P S, Singh R B. 1987. Possibility of genetic improvement of pigeonpea [*Cajanus cajan* (L.) Millsp.] utilising wild gene sources. *Euphytica* **36**:33–37.

Reddy A S, Kulkarni N K, Kumar P L, Jones A T, Muniyappa V, Reddy D V R. 2002. Improved graft inoculation method for screening for resistance to Pigeonpea sterility mosaic virus. *International Chickpea Pigeonpea Newsletter* **9**:44–46.

Reddy M V, Nene Y L. 1980. Influence of sterility mosaic resistant pigeonpeas on multiplication of the mite vector. *Indian Phytopathology* **33**:61–63.

Reddy M V, Raju T N, Nene Y L, Ghanekar A M, Amin K S, Arjunan G, Astaputre J V, Sinha B K, Reddy S V, Gupta R P, Gangadharan K. 1993. Variability in sterility mosaic pathogen in pigeonpea in India. *Indian Phytopathology* **46**:206–212.

Reddy M V, Sheila V K, Murthy A K, Padma P. 1995. Mechanism of resistance to *Aceria cajani* in pigeonpea. *International Journal of Tropical Plant Disease* **13**:51–57.

Remanandan P. 1990. Pigeonpea: genetic resources. In *The Pigeonpea*, pp. 89–115. Eds Y L Nene, S D Hall and V K Sheila. Wallingford, UK: CAB International.

Saxena K B. 2000. Pigeonpea. In *Plant Breeding: Theory and Techniques*, pp. 82–111. Ed. S K Gupta. Jodhpur, India: Agrobios.

Saxena K B, Singh L, Reddy M V, Singh U, Lateef S S, Sharma S B, Remanandan P. 1990. Intra-species variation in *Atylosia scarabaeoides* (L.) Benth., a wild relative of pigeonpea (*Cajanus cajan* (L.) Millsp.). *Euphytica* **49**:185–191.

Shanower T G, Yoshida M, Peter A J. 1997. Survival, growth, fecundity and behavior of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on pigeonpea and two *Cajanus* species. *Journal of Economic Entomology* **90**:837–840.

Sharma S B. 1995. Resistance to *Rotylenchulus reniformis, Heterodera cajani* and *Meloidogyne javanica* in accessions of *Cajanus platycarpus*. *Plant Disease* **79**:1033–1035.

Sharma D, Gupta S C, Rai G S, Reddy M V. 1984. Inheritance of resistance to sterility mosaic disease in pigeonpea. *Indian Journal of Genetics and Plant Breeding* **44**:84–90.

Srinivas T, Reddy M V, Jain K C, Reddy M S S. 1997*a*. Inheritance of resistance to two isolates of sterility mosaic pathogen in pigeonpea (*Cajanus cajan* (L.) Millsp.). *Euphytica* **97**:45–52.

Srinivas T, Reddy M V, Jain K C, Reddy M S S. 1997*b*. Studies on inheritance of resistance and allelic relationships for strain-2 of pigeonpea sterility mosaic pathogen. *Annals of Applied Biology* **130**:105–110.

Valkonen J P T. 2001. Plant resistance to infection with viruses. In *Encyclopaedia of Life Sciences* (www.els.net). Basingstoke, UK: Macmillan Reference Limited/ Nature Scientific American.

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