Broad-Based Resistance to Pigeonpea Sterility Mosaic Disease in Accessions of *Cajanus scarabaeoides* (L.) Benth

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Abstract

Sterility mosaic disease (SMD) is the most important biotic constraint on pigeonpea production in the Indian subcontinent. It is caused by Pigeonpea sterility mosaic virus (PPSMV) that is transmitted by the eriophyid mite, Aceria cajani *(Acari: Arthropoda). Several SMD resistant/tolerant pigeonpea genotypes identified showed resistance to a mild strain of PPSMV (Patancheru isolate) but are highly susceptible to a severe strain of PPSMV (Bangalore isolate), and also to major pigeonpea fungal pathogens and pests. Wild relatives of pigeonpea have been shown to possess high levels of resistance to several of these biotic constraints. Sixty accessions of* Cajanus scarabaeoides *were screened against mild and severe strains of PPSMV. Accessions were evaluated for resistance by the 'leaf-stapling' method, followed by the testing of promising accessions by 'petiole grafting'. Test plants were monitored for mites and assayed for PPSMV infection by double antibody sandwich-ELISA. Of the 60 accessions evaluated, 21 (ICP15684, 15688, 15692, 15695, 15697, 15699, 15700, 15701, 15702, 15703, 15707, 15712, 15725, 15726, 15728, 15734, 15736, 15737, 15739, 15740 and 15741) were resistant to both the mild and the severe strains of PPSMV. These can be exploited in breeding programs to increase the levels and to diversify the genetic base of resistance to SMD in the cultivated gene pool.*

Keywords: Pigeonpea, sterility mosaic disease, resistance, *Cajanus scarabaeoides*

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is a principal legume crop grown for its protein-enriched seed in the Indian subcontinent. Sterility mosaic disease (SMD) is the most important biotic constraint on pigeonpea production in the subcontinent (Singh *et al.*, 1999). SMD occurs regularly, sometimes leading to severe epidemics resulting in near 100 % crop loss. Estimated annual losses due to SMD exceed US$293 million in India and Nepal alone. SMD is caused by Pigeonpea sterility mosaic virus (PPSMV) transmitted by the eriophyid mite, *Aceria cajani* (Acari: Arthropoda) (Kulkarni *et al.*, 2002; Kumar *et al.*, 2002 a,b).

Growing SMD resistant cultivars is the most viable and ecofriendly option to manage this disease. However, progress was hampered due to an impasse in identifying the SMD causal agent and the lack of diagnostic tools to detect it (Nene, 1995). Despite this, ICRISAT in collaboration with the Indian Council of Agriculture Research (ICAR) have identified several SMD resistant/ tolerant pigeonpea genotypes (Reddy *et al.*, 1998). The majority of these genotypes showed resistance to a mild strain of PPSMV (Patancheru isolate) but were highly susceptible to a severe strain of PPSMV (Bangalore isolate) and most of these genotypes are also susceptible to major pigeonpea fungal pathogens (*Fusarium* wilt and *Phytophthora* blight) and pests (*Helicoverpa armigera*). For sustainable pigeonpea production it is essential to develop broad-based SMD resistant cultivars containing resistance to these additional major biotic constraints.

Adequate levels of resistance are scarce in the cultivated pigeonpea gene pool. Wild relatives of pigeonpea have been shown to possess high levels of resistance to several of the pigeonpea biotic constraints (Remanandan, 1981). The global pigeonpea germplasm collection at ICRISAT has 271 accessions of 47 wild species related to the genus *Cajanus* (Remanandan *et al.*, 1988). Among these, *Cajanus scarabaeoides* (L.) Benth. is ubiquitous and predominantly distributed in Asia and Australia. It is placed in the secondary gene pool and is cross-compatible with cultivated pigeonpea via traditional breeding or introgression through backcrossing methods. At present, ICRISAT maintains 77

accessions collected from various geo-ecological regions. Some of these accessions have been assessed for various agronomic traits and for resistance to biotic constraints (Saxena *et al.*, 1990). This study reports evaluation of 60 accessions of *C. scarabaeoides* against a mild and severe strain of PPSMV and discusses the opportunities for their possible utilization in breeding programs to develop multiple disease resistant pigeonpea cultivars.

Materials and methods

Screening of *C. scarabaeoides* accessions

Screening against a mild and severe strain of PPSMV was done at endemic locations of the respective isolates, i.e. ICRISAT, Patancheru for the mild strain and the Dept. of Plant Pathology, Hebbal, University of Agricultural Sciences, Bangalore, Karnataka for the severe strain.

Seeds of 60 accessions of *C. scarabaeoides* were obtained from the Gene Bank, ICRISAT, Patancheru (Tables 1 and 2), India. Accessions 15734, 15735, 15741, 15742 and 15743 were of Australian origin; 15696 was from Myanmar; 15720 and 15721 from the Philippines; 15694 and 15695 were from Sri Lanka; and the rest from India. Seeds were scarified by slicing the seed coat with a scalpel blade, treated with fungicide, Thiram 75 % WP (Sudama Chemtech P. Ltd, Gujarat, India) and sown in plastic pots. Test plants were maintained in an insect proof glasshouse maintained at 28-30° C during the day and at 18-20 °C at night period, with a 70% relative humidity.

Test plants were inoculated with viruliferous *A. cajani* cultures at the two-leaf stage following the leaf-stapling technique (Nene and Reddy, 1976). Pigeonpea cultivars, ICP8863 and TTB7 were used as susceptible checks. Observations on disease symptoms and percent disease incidence were recorded after 30 and 60 days post inoculation (dpi), whereas observations on the number of mites per trifoliate leaf were recorded after 60 dpi. Test plants were assessed for PPSMV by DAS-ELISA as described by Kumar *et al.* (2002c) (detailed below). Accessions that showed no or low disease (<15 %) incidence at Patancheru and Bangalore were evaluated further by petiole graft inoculation using vector-free PPSMV-infected pigeonpea tissue (Reddy *et al.*, 2002). Observations were recorded 30 dpi and plants were assayed for PPSMV by DAS-ELISA.

Assessment for PPSMV infection

Every test plant was assayed for PPSMV infection by looking for PPSMV symptoms and by DAS-ELISA 30 dpi, using anti-PPSMV rabbit polyclonal antibodies as described previously (Kumar *et al.*, 2002c). One hundred mg of leaf tissue per test plant was extracted in 1 ml phosphate buffered saline containing 2 % polyvinyl pyrrolidone (mol. wt 40,000), 0.2 % ovalbumin and 0.02 % Tween-20, and 100 µl of this extract placed in wells of ELISA plates (Nunc, Denmark) pre-coated with PPSMV immuno-γ-globulins (IgGs) at 1:10,000 dilution. Penicillinase (PNC)-labelled PPSMV IgGs was used at 1:1,500 to detect the trapped antigen. Sodium penicillin G was used at 0.05 $mg.ml^{-1}$ in substrate buffer (0.015% (wt/vol) bromothymol blue in 5 mM NaOH, pH 7.2) and readings made at 620nm in a Multiscan Plus ELISA plate reader (Labsystems, Helsinki, Finland). Absorbance readings at 620 nm of >3 times those of healthy sample were considered PPSMV positive. An accession was considered as negative for PPSMV if the disease incidence is less than 15 %.

Results and discussion

Identification of the SMD causal agent and the development of tests to detect it paved the way for the development of improved resistance screening methods for the precise selection of SMD resistance (Kumar *et al.*, 2002c). It allowed the screening of *C. scarabaeoides* accessions for identification of durable resistance both to PPSMV and to its vector in order to improve the genetic base of multiple resistance in pigeonpea genotypes. The evaluation of these lines was based on the inoculation of plants with infective mites, followed by testing of promising lines by graft inoculation. These experiments were done under glasshouse conditions at high inoculum pressure.

PPSMV is not transmissible to pigeonpea by mechanical sap inoculation. Therefore, viruliferous mite vectors were used for virus transmission. Control pigeonpea plants (ICP8863 and TTB7), inoculated by the leaf stapling method developed typical SMD symptoms, indicating that the mites were viruliferous. PPSMV infection in these control plants was confirmed by DAS-ELISA.

Germination of *C. scarabaeoides* seed was not uniform and was between 30 to 60 %. Out of 60 *C. scarabaeoides* accessions tested by leaf stapling, 21 were resistant to mild (Patancheru isolate) and severe (Bangalore isolate) PPSMV isolates (Table 1). One to three plants of some of these accessions (e.g. 15737, 15699) showed mild to severe mosaic symptoms and plants of some accessions (e.g. 15700, 15728) did not show any symptoms at both locations (Table 1). In DAS-ELISA only symptomatic plants tested positive for PPSMV and all the asymptomatic plants were PPSMV negative. The SMD resistant accessions did not support mite multiplication (0-2 mites/leaf; Table 1). In order to confirm whether resistance showed by the particular accession was to the vector or to PPSMV, the accessions that were resistant to PPSMV at ICRISAT and Bangalore (ICP15684, 15688,

Table 1. Resistance to SMD in *C. scarabaeoides* accessions

Accession ICP No	Patancheru isolate			Bangalore isolate			Resistance to other biotic problems[3]
	Infected/ tested [1]	Symptoms	Mites/ leaf [2]	Infected/ tested [1]	Symptoms	Mites/ leaf [2]	
15684	1/28	mm	2	0/24	ns	0	Resistance to *H. armigera* larvae Immue to pod fly damage Resistant to pod damage by *H. armigera*
15688	1/33	mm	0	1/9	sm	2	Resistant to *Fusarium* wilt
15692	3/20	mm/sm	2	1/22	sm	2	Resistant to *Fusarium* wilt Immue to pod fly damage Resistant to pod damage by *H. armigera*
15695	1/21	sm	0	1/37	sm	0	Oviposition non-preference Resistance to *H. armigera* larvae Resistant to *Fusarium* wilt Resistant to cyst nematode Immue to pod fly damage Resistant to pod damage by *H. armigera* and pod wasp
15697	0/21	ns	0	2/14	mm	3	Resistant to *Fusarium* wilt
15699	3/26	sm	2	2/15	sm	1	
15700	0/16	ns	0	0/17	ns	0	
15701	0/22	ns	0	0/18	ns	0	
15702	0/21	ns	0	2/24	sm	0	
15703	2/25	mm-sm	0	0/7	ns	0	
15707	1/22	mm	0	0/23	ns	0	
15712	0/12	ns	0	0/17	ns	0	Resistant to cyst nematode
15725	1/20	mm	0	0/16	ns	0	Resistant to *Fusarium* wilt
15726	0/24	ns	0	1/26	mm	0	Oviposition non-preference Resistance to *H. armigera* larvae
15728	0/20	ns	0	0/25	ns	0	
15734	0/23	ns	0	0/10	ns	0	
15736	1/26	mm	2	0/7	ns	0	
15737	2/35	mm	0	1/11	mm	0	
15739	1/20	mm	0	0/12	ns	0	
15740	1/21	mm	0	0/15	ns	0	
15741	1/25	mm	0	1/12	mm	0	

ns = no symptoms, mm = mild mosaic, sm = severe mosaic,
[1]All plants tested by DAS-ELISA, [2]Mean count from five leaves/plant, [3]Saxena *et al.*, 1990 and Sharma, 2000

15700, 15701, 15725, 15736, 15737, 15740) were tested by graft inoculation with the PPSMV Patancheru isolate (Table 3). About 6-36 % of the plants per accession inoculated by grafting developed mild mosaic symptoms (Table 3). It was noteworthy that, following mite inoculation, only 0-11 % of these accessions showed mild mosaic symptoms (Table 3). This suggests that the increased levels of resistance observed when plants were inoculated with mites could be due to antixenosis or antibiosis of plants to the mite vector. This is further supported by the observation that some accessions (e.g. ICP15722, 15735, 15685, 15696, 15703, 15704, 15711, 15717 and 15735) that showed mosaic symptoms and > 25 % incidence contained no mites (Table 2). By contrast, some of the susceptible accessions contained 4-18 mites/ leaf (e.g. ICP15690, 15718; Table 2).

Accessions ICP15708, 15709, 15742 and 15743 were resistant only to the Patancheru isolate (Table 2), and accessions ICP15683, 15685, 15686, 15687, 15693, 15705, 15706, 15711, 15716, 15722, 15724, 15729 and 15733 were resistant to only

Table 2. Reaction of *C. scarabaeoides* accessions against PPSMV at ICRISAT and at Bangalore

Accession	Patancheru isolate			Bangalore isolate		
ICP No	Infected/tested [1]	Symptoms	Mites/ leaf [2]	Infected/ tested [1]	Symptoms	Mites/ leaf [2]
Accessions resistant at Patancheru isolate						
15708	0/23	ns	0	6/23	mm	0
5709	0/15	ns	0	4/12	mm	0
15742	2/22	mm	0	12/16	sm	1
15743	0/23	ns	0	4/15	mm	0
Accessions resistant to Bangalore isolate						
15683	16/16	mm-sm	3	0/3	ns	0
15685	14/26	mm	0	0/14	ns	0
15686	25/27	mm-sm	4	2/26	sm	3
15687	8/20	mm-sm	3	2/16	sm	0
15693	22/34	sm	3	2/22	sm	1
15705	3/19	mm-sm	1	1/19	sm	0
15706	6/29	sm	3	2/23	sm	1
15711	16/27	mm	0	1/20	sm	0
15716	8/20	mm	0	0/32	ns	0
15722	18/19	mm	0	1/33	sm	0
15724	34/41	mm	4	0/27	ns	0
15729	7/26	mm-sm	8	1/36	sm	0
15733	19/28	mm-sm	20	1/14	mm	2
Accessions susceptible at Bangalore and Patancheru						
15689	16/25	mm-sm	2	7/34	sm	2
15690	15/26	mm-sm	15	9/27	sm	1
15691	14/24	mm-sm	2	5/20	sm	2
15694	10/27	mm-sm	2	4/22	sm	1
15696	9/26	mm	0	4/33	sm	2
15698	9/21	sm	4	9/21	sm	1
15704	4/29	sm	0	3/19	sm	0
15710	26/30	sm	5	4/15	sm	2
15713	2/16	mm-sm	2	5/24	sm	4
15717	2/15	mm	0	4/21	sm	1
15718	21/26	sm	18	4/19	sm	2
15719	10/12	mm-sm	6	3/10	sm	2
15720	10/11	sm	14	4/18	sm	3
15721	15/18	mm-sm	2	2/32	mm-sm	3
15723	31/39	sm	14	10/26	sm	4
15727	22/32	mm-sm	7	4/34	sm	2
15730	2/17	sm	3	6/20	sm	2
15731	20/31	sm	3	5/12	sm	3
15732	6/26	mm	1	3/9	sm	2
15735	14/14	mm-sm	0	2/14	sm	0
15738	34/41	sm	9	3/9	sm	1
15744	4/22	mm	1	6/13	sm	0
Controls						
8863	47/48	sm	18	19/21	sm	11
TTB7	nt	-	-	15/16	sm	6

rs = ringspot, mm = mild mosaic, sm = severe mosaic, ns = no symptoms, nt = not tested
[1]All plants tested by DAS-ELISA, [2]Mean of five replications

Table 3. Reaction of *C. scarabaeoides* accessions to graft inoculation with the Patancheru isolate of PPSMV

Accession ICP No	Infected/ tested (% infection)[1]	Symptoms	Accession reaction to mite inoculation[2]	
			Patancheru isolate	Bangalore isolate
15684	5/15 (33)	mm	3.5	0.0
15688	6/13 (46)	mm	3.0	11.0
15700	2/31 (6)	mm	0.0	0.0
15701	4/24 (16)	mm	0.0	0.0
15725	1/10 (10)	mm	4.0	0.0
15736	5/21 (23)	mm	3.8	0.0
15737	7/28 (25)	mm	5.7	9.0
15740	7/24 (29)	mm	9.0	0.0
Controls				
7035	1/25 (4)	mm	0.0	5.0
TTB7	14/16 (87.5)	sm	nt	93.7
8863	14/17 (82.3)	sm	98.0	90.4

rs = ringspot, mm = mild mosaic, sm = severe mosaic, ns = no symptoms, nt = not tested
[1]All plants tested by DAS-ELISA; [2] Table 1

the Bangalore isolate (Table 2). Accessions ICP15700, 15701, 15712, 15728 and 15734 were resistant to both the Bangalore and Patancheru PPSMV isolates and no symptoms or mites were noted on these plants. Accessions 15684, 15688, 15692, 15695, 15697, 15712, 15725 and 15726 in addition to possessing broad-based SMD resistance, are also resistant to pest damage, cyst nematode and wilt (Table 1).

Thus, sources of broad-based SMD resistance were identified in *C. scarabaeoides* accessions and can be used for incorporating broad-based SMD resistance into existing cultivated pigeonpea. This, and previous studies (Saxena *et al.*, 1990; Sharma, 2000), demonstrate that there is great variability in *C. scarabaeoides* accessions in resistance to biotic constraints but selection of appropriate accessions is necessary for utilizing it in breeding programs. The accession(s) identified as potential parents for inter-generic hybridization are: ICP15700, 15701, 15712, 15728 and 15734 for broad-based SMD resistance; ICP15695 for broad-based SMD resistance, and resistance to *Fusarium* wilt, cyst nematode, *H. armigera* larvae, pod fly and pod wasp; ICP15692 for resistance to wilt, pod borer and pod fly. These accessions can be exploited for crossing with commercial cultivars to develop pigeonpea varieties with adequate levels of multiple resistance to enhance pigeonpea production in the subcontinent.

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