Evaluation of Pigeonpea Genotypes for Resistance to *Pigeonpea Sterility Mosaic Virus* - B Isolate

K M Nagaraj, Chikkadevaiah, V Muniyappa, K T Rangaswamy and P Lava Kumar†

University of Agricultural Sciences, GKVK, Bangalore - 560 065, Karnataka, India.
†International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru - 502 324, Andhra Pradesh, India.

Abstract

Pigeonpea genotypes (89) were evaluated for resistance to Pigeonpea sterility mosaic virus *Bangalore isolate (PPSMV-B). Of these, three genotypes, ICP 7035, MAL 14 and MAL 19, were found resistant, and two genotypes, ICP 6997 and ICP 8862, were tolerant to PPSMV-B. All the resistant lines tested negative to virus in enzyme-linked immunosorbent assay (ELISA) using PPSMV polyclonal antiserum. The resistant lines can be used in breeding programme for developing PPSMV-resistant high yielding cultivars.*

Keywords: *Cajanus cajan,* host resistance, eriophyid mite, sterility mosaic, PPSMV

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is one of the most important grain legumes predominantly grown in the semi-arid tropics of India, contributing to >80% of the total world production (FAO, 2005). However, the productivity of pigeonpea in India is much lower than the potential yields of 2,000-2,500 kg ha^{-1} (Dhar, 2000). Of various causes that limit pigeonpea yield, sterility mosaic disease (SMD) is the most damaging disease recognized in all the pigeonpea growing countries of Asia (Kumar *et al*., 2004a). It is caused by *Pigeonpea sterility mosaic virus* (PPSMV) and is transmitted by an eriophyid mite, *Aceria cajani* (Acari: Arthropoda) (Kumar *et al*., 2003). SMD-affected plants show mosaic and mottling symptoms on leaves, with severely reduced or no flowering (sterile). SMD symptoms depend on the pigeonpea genotype, are categorized into three types: genotypes that show severe mosaic (SM) and sterility; mild mosaic (MM) with partial sterility; and chlorotic ring spots (RS) without any noticeable sterility. Susceptible cultivars that produce SM symptoms infected early in the growth stage (i.e., <45 day-old plants) result in >90% yield losses (Jones *et al*., 2004).

SMD management through acaricidal sprays to control the vector mite is not considered economically viable and eco-friendly. Systematic resistance breeding was initiated at International Crops Research Institute for the Semi Arid Tropics (ICRISAT), Patancheru, India during 1975 and several cultivars with field resistance / tolerance to SMD were identified (Nene *et al.,* 1981b). However, the task of developing resistant varieties has become complicated in view of the occurrence of geographical isolates of PPSMV (Kumar *et al*., 2004b). The pigeonpea genotypes, which were reported to be resistant to SMD at one location, were found to be susceptible at other locations (Amin *et al.,* 1993). A comprehensive study over a period of four consecutive years, using a set of seven differentials, at nine different locations in India, revealed the occurrence of five different variants of the SMD pathogen in India (Reddy *et al.,* 1993). Recent studies based on bio-chemical characterization of PPSMV indicated that PPSMV isolates at Patancheru (P), Bangalore (B) and Coimbatore (C) are distinct from each other (Kumar *et al*., 2005a), and host resistance to PPSMV is scarce in the germplasm. Moreover, a few genotypes that showed resistance to P isolate succumbed to infection against B and C isolates, indicating that these isolates have an ability to overcome resistance selected against P isolate (Reddy *et al*., 1993; Jones *et al*., 2004). Hence, in the present study pigeonpea genotypes were evaluated to identify sources of resistance to PPSMV-B isolate.

Materials and methods

Seeds of 89 pigeonpea genotypes, obtained from ICRISAT, Patancheru and Indian Institute of Pulse Research (IIPR), Kanpur, were evaluated against PPSMV–B isolate by planting in SMD screening nursery during *Kharif* (rainy season) 2001. Pigeonpea cultivars, TTB 7 and ICP 8863 were used as susceptible controls. Each genotype was planted in two replicated rows of five-meter length, with 25 plants per row. The susceptible check ICP 8863 was planted after every two-test entries. As PPSMV-B is not transmissible by mechanical inoculation, viruliferous mites

were used for inoculation of all the test plants at two-leaf stage (14-20 days after germination) (Nene and Reddy, 1976). In this, mite-infested leaflets obtained from SMD-affected plants (maintained at University of Agriculture Sciences, GKVK, Bangalore) were stapled to the leaves of test plants. Mites from the stapled leaf migrate onto the test seedling resulting in virus transmission.

The test entries were graded as resistant, tolerant or susceptible based on per cent disease incidence and symptom type at 75 DAS as per the rating scale given by Nene *et al.* (1981a) and Gupta *et al.* (1988) with minor modifications (Table 1). Selected test entries were evaluated for PPSMV-B by double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA) using the antibodies produced to PPSMV Patancheru isolate that can detect all the PPSMV isolates, as described by Kumar *et al*. (2004b). Briefly, young leaflets from symptomatic and apparently healthy plants were extracted in phosphate-buffered saline (1:10 w/v), and 100 µl of this 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 1:1,500 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. Absorbance values at 620nm (A_{620}) were measured in an ELISA plate reader (Multiskan, Labsystems) and readings were considered to be virus positive if the absorbance values of a sample were three-folds or more than those given by the virus-free control samples. Observations on symptom type and severity were recorded at 15 days intervals upto 75 DAS, by which time 100% incidence was recorded in susceptible controls and all the plants showed severe symptoms.

Results and discussion

The test genotypes were classified as resistant, moderately resistant, tolerant, moderately susceptible and susceptible based on the criteria given in Table 1. Recent developments following the identification of SMD causal agent have paved a way for accurate monitoring of PPSMV incidence and precise identification of resistant sources (Jones *et al*., 2004). Susceptible controls, ICP 8863, and TTB-7 showed 100% infection and they developed typical SMD symptoms in 14-20 days post inoculation (dpi) confirming the reliability of virus inoculation method (Table 2).

Majority of the test entries developed severe mosaic symptoms within 25-30 dpi, with 40-100% incidence (Table 2). Only ICP 7035, MAL 14 and MAL 19, did not show any symptoms and were negative for virus in DAS-ELISA and classified as resistant. Earlier reports by Reddy and Nene (1980), Singh *et al*. (1989), Amin *et al*. (1993) and Rangaswamy *et al.* (1997) indicated that ICP 7035 showed resistance to SMD at different locations in India, which shows its broad-based resistance to various isolates of SMD. The genotypes ICP 6997 and ICP 8862 showed chlorotic ring spot symptoms, with per cent incidence of 50.9 and 63.0, respectively and classified as tolerant (Table 2). In DAS-ELISA only symptomatic regions tested positive and non-symptomatic areas were negative to the virus. Recent studies have shown that systemic movement of the virus from inoculated leaves was absent in genotypes exhibiting chlorotic ringspots, and such symptoms were mostly confined to the site of mite inoculation indicating that symptoms were due to localized infection, and such genotypes show normal flowering pattern (P L Kumar, Personal communication). Apparent systemic symptoms observed on such genotypes were due to multiple inoculations by the vector mites. ICP10976, MAL 10, BSMR-736 showed mild mosaic symptoms with <20% incidence and they were classified as moderately resistant; MAL 12, MAL 13, Bahar and KSMR-33 showed severe mosaic symptoms but incidence was <20%, and they were classified as moderately susceptible; and rest of the 77

Table 1. Rating scale for screening pigeonpea genotypes against sterility mosaic disease

Rating	Genotype reaction to SMD	Category
1	No symptoms on any plant, and no sterility	Resistant
3	Severe mosaic symptoms on <10% plants or mild mosaic symptoms on <20% of the plants, without any noticeable stunting; and recovery of infected plants, with partial sterility	Moderately resistant
5	Ring spot symptoms on a few/all plants, and no sterility	Tolerant
7	Severe mosaic on 10-20% of the plants or mild mosaic symptoms on most plants, without any noticeable stunting, and partial sterility	Moderately susceptible
9	Severe mosaic on >20% plants with severe stunting and near complete sterility	Susceptible

*Sterility = inhibition of flowering

Table 2. Response of pigeonpea genotypes inoculated with PPSMV-B isolates using *Aceria cajani*

Sl. No.	Genotype identity	Per cent incidence	Symptom type	Reaction
1	Bahar	11.7	SM	MS
2	BSMR-736	18.3	MM	MR
3	DPPA 85-14	90.0	SM	S
4	GUPH 1126-1	94.4	SM	S
5	GUPH 1126-9-2	97.3	SM	S
6	GUPH 1126-29-1	89.9	SM	S
7	GUPH 1126-29-2	96.9	SM	S
8	GUPH 1126-29-5	89.1	SM	S
9	GUPH 1126-47	89.7	SM	S
10	GUPH 1126-47-1	78.0	SM	S
11	GUPH 1126-47-2	92.1	SM	S
12	ICP 1206	67.1	SM	S
13	ICP 1207	22.7	SM	S
14	ICP 2376	87.0	SM	S
15	ICP 2668	81.6	SM	S
16	ICP 6997	50.9	RS	T
17	ICP 7035	0.0	NS	R
18	ICP 7039	94.0	SM	S
19	ICP 7550	94.6	SM	S
20	ICP 7867	95.9	SM	S
21	ICP 8087	93.4	SM	S
22	ICP 8094	74.0	SM	S
23	ICP 8362	81.8	SM	S
24	ICP 8610	100.0	SM	S
25	ICP 8860	85.0	SM	S
26	ICP 8862	63.0	RS	T
27	ICP 8869	81.8	SM	S
28	ICP 10976	13.7	MM	MR
29	ICP 10977	14.3	SM	S
30	ICP 10979	14.6	SM	S
31	ICP 10983	74.1	SM	S
32	ICP 11049	72.6	SM	S
33	ICP 11204	74.0	SM	S
34	ICP 11207	73.8	SM	S
35	ICP 11231	98.6	SM	S
36	ICP 11297	93.0	SM	S
37	ICP 12947	92.9	SM	S
38	ICP 13914	85.5	SM	S
39	ICP 14035	55.1	SM	S
40	ICP 14198	90.8	SM	S
41	ICP 14217	93.8	SM	S
42	ICP 14271	91.7	SM	S
43	ICP 14298	78.3	SM	S
44	ICP 14410	100.0	SM	S
45	ICP 14415	94.6	SM	S
46	ICP 14503	91.0	SM	S
47	ICP 14513	51.1	SM	S
48	ICP 14514	23.9	SM	S
49	ICP 14523	64.9	SM	S
50	ICP 14566	90.8	SM	S
51	ICP 14652	82.4	SM	S
52	ICP 14722	53.7	SM	S
53	ICP 14751	65.6	SM	S
54	ICP 14757	82.1	SM	S
55	ICP 14813	84.6	SM	S
56	ICP 14819	89.5	SM	S
57	ICP 14827	88.5	SM	S
58	ICP 15052	84.6	SM	S
59	ICP 16255	85.7	SM	S
60	ICP 16273	78.1	SM	S
61	ICP 16274	75.9	SM	S
62	ICP 16275	84.6	SM	S
63	ICP 16276	75.0	SM	S
64	ICP 87119	86.4	SM	S
65	ICP 93001	34.9	SM	S
66	ICPL 93003	73.2	SM	S
67	ICPL 96047	66.0	SM	S
68	ICPL 96053	70.0	SM	S
69	ICPL 96057	89.8	SM	S
70	ICPL 96061	62.8	SM	S
71	ICPL 99048	82.9	SM	S
72	ICPL 99051	100.0	SM	S
73	ICPL 99054	91.7	SM	S
74	ICPL 99055	96.2	SM	S
75	ICPX 900148-SMB	92.9	SM	S
76	IPH-487-75-1	73.2	SM	S
77	KPL-43	48.2	SM	S
78	KPL-272	92.3	SM	S
79	KSMR-33	13.5	SM	MS
80	MAL 10	17.7	M M	M R

Continued

Table 2. continued

81	MAL 12	14.5	SM	MS
82	MAL 13	13.7	SM	MS
83	MAL 14	0.0	NS	R
84	MAL 15	21.4	SM	S
85	MAL 18	23.9	SM	S
86	MAL 19	0.0	NS	R
87	PI-397430	85.3	SM	S
88	PR-5149	75.8	SM	S
89	PWS-1	89.5	SM	S
Susceptible controls				
	TTB-7	100.00	SM	S
	ICP 8863	100.00	SM	S

SM = Severe mosaic; RS = Ring spot; MM = Mild mosaic; NS = No symptoms; S = Susceptible; R = Resistant; MR = Moderately resistant; T = Tolerant; MS = Moderately susceptible

genotypes showed severe mosaic symptoms with >20% incidence and were classified as susceptible (Table 2). The results showed that nearly 87% of the genotypes evaluated were susceptible to PPSMV-B isolate. Evaluation of wild *Cajanus* species for SMD resistance indicated that fewer genotypes were resistant to PPSMV-B isolate (Kumar *et al*., 2005b). Genotypes, ICP 14410, ICP 8610 and ICPL 99051 showed 100% incidence. In DAS-ELISA, the leaf samples collected from genotypes, which exhibited mild mosaic (MAL 10, ICP 10976 and BSMR 736), chlorotic ring spots (ICP 6997 and ICP 8862) reacted positive to virus and all the asymptomatic plants and also resistant genotypes (ICP 7035, MAL 14 and MAL 19) tested negative. Broad-based resistance to PPSMV in ICP7035 has been confirmed by various studies (Reddy *et al*., 1993). The genotypes MAL 14 and MAL 19 needs to be further evaluated against other PPSMV isolates in India.

Owing to its resistance to PPSMV-B, superior agronomic performance in multilocational trials, and its use for vegetable as well as grain purpose, ICP7035 has been released for cultivation in Zone-5 region of Karnataka state (Rangaswamy *et al*., 2005). This genotype is also being used in several breeding programmes as PPSMV-B resistance donor. Similarly, resistant and tolerant varieties identified in this study can be exploited for cultivation in SMD endemic areas and also in resistance breeding programmes to mitigate losses against SMD.

Acknowledgements

The first author is grateful to IIPR, Kanpur, India, for providing the seed material of some genotypes evaluated in this study. The work presented in this document is partly supported from a project (No. R8205) funded by the Crop Protection Program, Department for International Development (DFID), United Kingdom, for the benefit of developing countries. The views expressed are not necessarily those of DFID.

References

Amin, K. S., Reddy, M. V., Nene, Y. L., Raju, T. N., Shukla,P., Zote, K. K., Arjunan, G., Bendre, J. N., Rathi, Y. P. S., Sinha, B. K., Gupta, R. P., Anil Kumar, T. B., Chauhan, V. B., Singh, G., Jha, D. K. and Gangadharan, K. 1993. Multilocational evaluation of pigeonpea for broad based resistance to sterility mosaic disease in India. *Indian Journal of Agriculture Sciences* **63 :** 542-546.

Dhar, V. 2000. Seven decades of research on sterility mosaic of pigeonpea. *Indian Journal of Pulses Research* **13 :** 1-10.

FAO 2005. FAOSTAT Data. http://www.faostat.fao.org/.

Gupta, R. P., Singh, D. P. and Katiyar, R. P. 1988. Response of pigeonpea lines/germplasm to sterility mosaic. *International Pigeonpea Newsletter* **7 :** 32-34.

Jones, A. T., Kumar, P. L., Saxena, K. B., Kulkarni, N. K., Muniyappa, V. and 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.

Kumar, P. L., Jones, A. T. and Reddy, D. V. R. 2003. A novel mite-transmitted virus with a divided RNA genome closely associated with pigeonpea sterility mosaic disease. *Phytopathology* **93 :** 81-91.

Kumar, P. L., Jones, A. T. and Waliyar, F. 2004a. Biology, etiology and management of pigeonpea sterility mosaic disease. *Annual Review of Plant Pathology* **3 :** 77-100.

Kumar, P. L., Jones, A. T. and Waliyar, F. (Eds). 2004b. Serological and nucleic acid based methods for the detection of plant viruses: methods manual, ICRISAT, Patancheru - 502 324, Andhra Pradesh, India, 120pp.

Kumar, P. L., Latha, T. K. S., Chauhan, V. B., Aparna, V. and Waliyar, F. 2005a. Studies on Pigeonpea sterility mosaic virus isolates in India. In Abstracts 2nd Asian Congress of Plant Pathology 25-28 June 2005, National University of Singapore, Singapore. p 85-86.

Kumar, P. L., Latha, T. K., Kulkarni, N. K., Raghavendra, K., Saxena, K. B., Waliyar, F., Rangaswamy, K. T., Muniyappa, V., Sabitha Doriswamy and Jones, A. T. 2005b. Broad-based resistance to pigeonpea sterility mosaic disease in wild relatives of pigeonpea (*Cajanus:* Phaseoleae). *Annals of Applied Biology* **146 :** 371-379.

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

Nene, Y. L., Kannaiyan, J. and Reddy, M. V. 1981a. Pigeonpea diseases: Resistance screening technique. *Information Bulletin* **4**: ICRISAT, Patancheru, Andhra Pradesh, India. pp. 14.

Nene, Y. L., Kannaiyan, J. and Reddy, M. V. l981b. Resistance to major pigeonpea diseases. In: *Proceedings of International Workshop on Pigeonpea*. Dec. 15-16, 1980. ICRISAT, Patancheru, India, pp.121-128.

Rangaswamy, K. T., Sulladmath, V. V. and Byregowda, M. 1997. Reaction of certain pigeonpea entries and varieties to sterility mosaic disease. In: A*bst. Symposium on Economically Important Diseases of Crop Plants*, IPS Southern chapter, Univ. Agric. Sci., Bangalore, Dec. 18 -20, pp. 74.

Rangaswamy, K. T., Muniyappa, V., Kumar, P. L., Saxena, K. B., Byregowda, M., Raghavendra, N., Pandurangaiah, K., Kumar, R. V., Waliyar, F. and Jones, A. T. 2005. ICP 7035 - A sterility mosaic resistant vegetable and grain purpose pigeonpea variety for Karnataka state of India. *International Chickpea and Pigeonpea Newsletter* **12 :** 42-44.

Reddy, M. V. and Nene, Y. L. 1980. Estimation of yield loss due to sterility mosaic. In: *Proceedings of International Workshop on Pigeonpea* 15-19, Dec. 1980. ICRISAT center, India Vol. 2, Patancheru, A.P. pp. 305-312.

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

Singh, R. P., Sharada Joshi, Shrestha, D., Karna, R. S. L. and Bharati, M. P. 1989. Pigeonpea germplasm resistant to sterility mosaic identified in Nepal. *International Pigeonpea Newsletter* **9 :** 21-22.

Received and revised : 30-05-06, 17-11-06 Accepted : 23-11-06