

Original Research Article

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Studies on the Molecular Variability of Sterility Mosaic Disease of Pigeonpea in Tamil Nadu State, India

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ABSTRACT

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Sterility mosaic disease (SMD) is one of the major biotic problems for pigeonpea production in the Indian subcontinent. A systematic survey and comprehensive study was carried out to determine the incidence of *Pigeonpea sterility mosaic virus* (PPSMV) and molecular variability among disease causing agents in Tamil Nadu state of southern India during the 2019 Kharif season. The results indicate there was a variation in SMD incidence among the districts. In Tamil Nadu SMD incidence was observed ranging from 3.6% to 21.3%. In this study we analysed the SMD infected samples from 12 locations of major pigeonpea growing regions of Tamil Nadu state. All isolates contain both PPSMV-1 and PPSMV-2 as mixed infection none of the sample was positive for PPSMV-I or PPSMV-2 alone. Overall our study provides evidence for variability and increase in disease incidence.

Introduction

Pigeonpea (*Cajanus cajan*, L.) is a major pulse crop grown in over 5 million ha with an annual production of more than 3 million tonnes. Nearly 90 % of its cultivation takes place in India and Nepal, followed by Africa (6%), the Caribbean (2%) and other Southeast Asian countries. *Pigeonpea sterility mosaic virus* (PPSMV), a species of the genus *Emaravirus*, is the causal agent of sterility mosaic disease (SMD) of pigeonpea [*Cajanus cajan* (L.) Millsp], and transmitted by mites

(*Aceria cajani*) (Mitra *et al.*, 1931; Elbeaino *et al.*, 2014; Jones *et al.*, 2004; Kumar *et al.* 2000). SMD is characterized by stunted and bushy plants, leaves of reduced size with chlorotic rings or mosaic symptoms, and partial or complete cessation of flower production (*i.e.* sterility). SMD has variable symptomatology, within which three major syndromes are recognized severe mosaic and sterility (partial or complete cessation of flower production), mild mosaic with partial sterility and chlorotic ring spots with no apparent sterility. Infected perennial and

volunteer plants serve as a source for both the virus and its vector mites which play an important role in the disease cycle (Kulkarniet al.2002).

Two emaravirus species, *Pigeonpea sterility mosaic emaravirus 1* (PPSMV-1) and *Pigeonpea sterility mosaic emaravirus 2* (PPSMV-2) are associated with sterility mosaic disease (SMD) of pigeonpea contains negative-sense dsRNA genomes. PPSMV-1 and PPSMV-2 were reported to contain six genomic RNA segments. The largest segment, RNA-1 is 7022 nt in length codes for RNA-dependent RNA polymerase RNA-2, with a sequence length of 2223 nt, codes for glycoproteins RNA-3, with a sequence length of 1442 nt, codes for nucleocapsid protein RNA-4, with a sequence length of 1563 nt, codes for movement protein and RNA-5, with a sequence length of 1689 nt, codes for p5 and sequence length of 1094 nt, codes for p6 these protein has unknown function (Elbeaino et al., 2013,2014,2015).The first four RNA segments of PPSMV-2 share higher sequence similarity with *Fig mosaic virus*(FMV) than with PPSMV-1 (Patil et al., 2015).

To develop any virus management strategies, it is important to understand the population structure of the viruses, for which sequence variability study is essential. In this study, we investigated the current variability of PPSMV isolates present in Tamil Nadu state, India. We analyzed RNA-4 of PPSMV in SMD infected leaves collected from 12 locations of Tamil Nadu state.

Materials and Methods

Selection of locations

For data collection, the details on major pigeonpea growing locations in Tamil Nadu was obtained from different Agricultural Research Stations, Krishi Vignan Kendra and

State Agricultural Universities across Tamil Nadu and surveyed for SMD incidence.

Survey and disease incidence

Major Pigeonpea growing districts of the Tamil Nadu state were surveyed for the occurrence of SMD during *Kharif*2019 the Pigeonpea crop was in the middle of the maturity and flowering stage (30-45 Days After Sowing- DAS). Infected leaf samples were showing characteristic symptoms like severe mosaic, ring spot, reduction in leaf size and stunted growth.

The locations are field of TNAU, Devarayapuram and Thondamuthur villages of Coimbatore district. Elur, Perumpallipatti and Kandipalayam villages of Namakkal district Attaiyampatti, Papparapatti and Minnakal villages of Salem district Arachi and Keelakkunuppatti villages of Trichy district. It was represented in Table 1. All these leaf samples were immediately brought to TNAU Coimbatore and snap frozen in liquid nitrogen and stored in a -80°C freezer for further use. The disease incidence of every field was calculated by counting the number of infected plants from 500 randomly selected plants and per cent SMD incidence was calculated by using the formula

$$\% \text{ SMD incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100.$$

Inoculum maintenance by the leaf-stapling technique

Aceria cajani mites were observed under Stereo zoom binocular microscope to confirm each infected leaf has sufficient mite population to transmit the virus particles. Then the mites were inoculated into the susceptible variety ICP8863 at two leaf stage through leaf-stapling technique. The pots were covered by insect proof cage. Symptom

development was observed in 10 to 15 days. It was represented in Fig. 2.

Total RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

Leaf sample and total RNA extraction

Pigeonpea leaves exhibiting typical SMD symptoms were collected from 12 locations in Tamil Nadu state. 100 mg leaf tissue of symptomatic and healthy pigeonpea was used for RNA extraction, by grinding in liquid nitrogen to a fine powder. Total RNA was extracted from leaf powder using Trizol method. The RNA purity and quality were assessed using a spectrophotometer (NanoDrop 8000, Thermo-Fisher Scientific) and stored at -20°C .

Reverse transcription- polymerase chain reaction (RT- PCR)

RT-PCR was performed as per the protocol suggested by Elbeaino *et al.*, (2015). The total RNA was reverse transcribed using a cDNA synthesis kit (Verso cDNA Synthesis kit, Thermo Fisher Scientific India) with RT Random primers and other components following the manufacturer's instruction. PCR was performed using Eppendorf – Master Cycler nexus gradient S (Eppendorf, A G, Hamburg, Germany) in a 20 μl reaction mixture containing 10 μl of Hot Start Green Master Mix (2X), 1.0 μl of each forward and reverse primer of RNA-4, 2.0 μl of cDNA and rest of the PCR water.

The PCR mixture tube was incubated by one cycle of denaturation at 94°C for 4 min, followed by 35 cycles at 92°C for 30 seconds, $45-65^{\circ}\text{C}$ for 30 seconds and 72°C for 30 seconds. The final extension was at 72°C for 7 min. The amplification products were resolved in a 1% TBE agarose gel, visualized and documented by a Gel-doc system.

Specific primers used for RNA-4 amplification was represented in Table 1.1kb DNA ladder was used as a marker. The PCR amplified fragments of the isolates yielded band of around 400bp.

Results and Discussion

Prevalence of SMD in Pigeonpea in Tamilnadu

Data on the survey conducted for the occurrence of pigeonpea sterility mosaic disease (SMD) during *Kharif* 2019 in major pigeonpea growing regions of Tamil Nadu state was summarized in Table 2 and represented in Fig. 1. In the surveyed areas, farmers were cultivating pigeonpea crop under rainfed conditions as sole and intercropped with other pulses and cereals.

In Tamil Nadu, the Pigeonpea was cultivated both as sole crop and inter-cropped with groundnut, pearl millet, sorghum, field bean, horse gram, and finger millet. SMD incidence was observed with varying range from 3.6% to 21.3%. Among the four districts and 12 locations surveyed for SMD prevalence, the least disease incidence (3.6%) was observed in Perumpallipatti village Namakkal district, whereas, Trichy, Salem and Coimbatore districts recorded with disease incidences of 4.4, 6.2 and 7.3% respectively. The highest SMD incidence was recorded in Thondamuthur village Coimbatore district (21.3%). However, in previous studies, the highest incidence (37.50%) of SMD was observed in the Pudukkottai district (Kannaiyan *et al.*, 1984), a range of 0–100% incidences across Tamil Nadu (Ramakrishnan and Kandaswamy1972). (8.77%) of SMD was incidence observed in the Coimbatore district (Sayiprathap *et al.*, 2020).

This drastic reduction in the incidence over the past few years could be due to growing of resistant cultivars and adoption of

prophylactic measures such as removal of ratoon crop and spraying of insecticides to control the disease/vector in the early stage (Sayiprathap *et al.*, 2020). But this study

showed increase in disease incidence compare to previous reports. The reason for disease increase was may be molecular variability of the two strains.

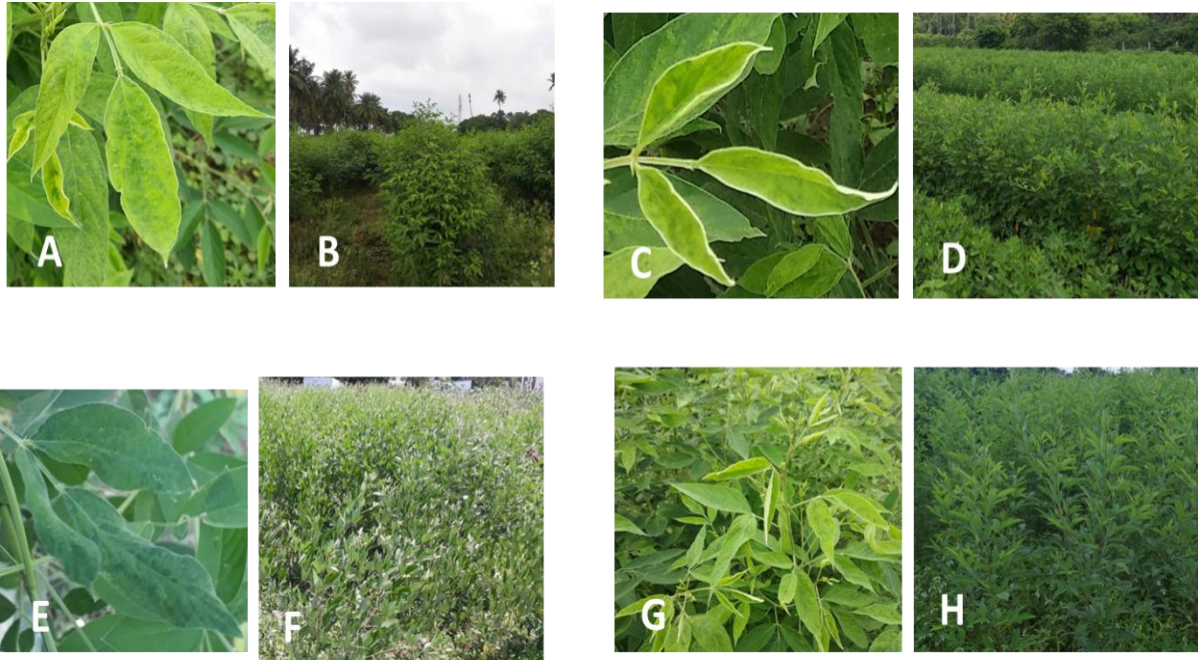
Table.1 Specific primers of RNA-4 used for variability analysis

S. No	CODE	PRIMER	AMPLICON SIZE
1.	PPSMV-1	F- CACCTGCACGTTTTCTATTTTTN	400 bp
		R- TTAGGTAGCTTCACCAATTTTTN	
2.	PPSMV-2	F- CACCATGATGCCTAGCACCTCN	400 bp
		R- TTA CTGACTTCACCTATTAC	

Table.2 Survey of PPSMV infected leafs in Coimbatore, Namakkal, Salem and Trichy districts for assessing disease incidence

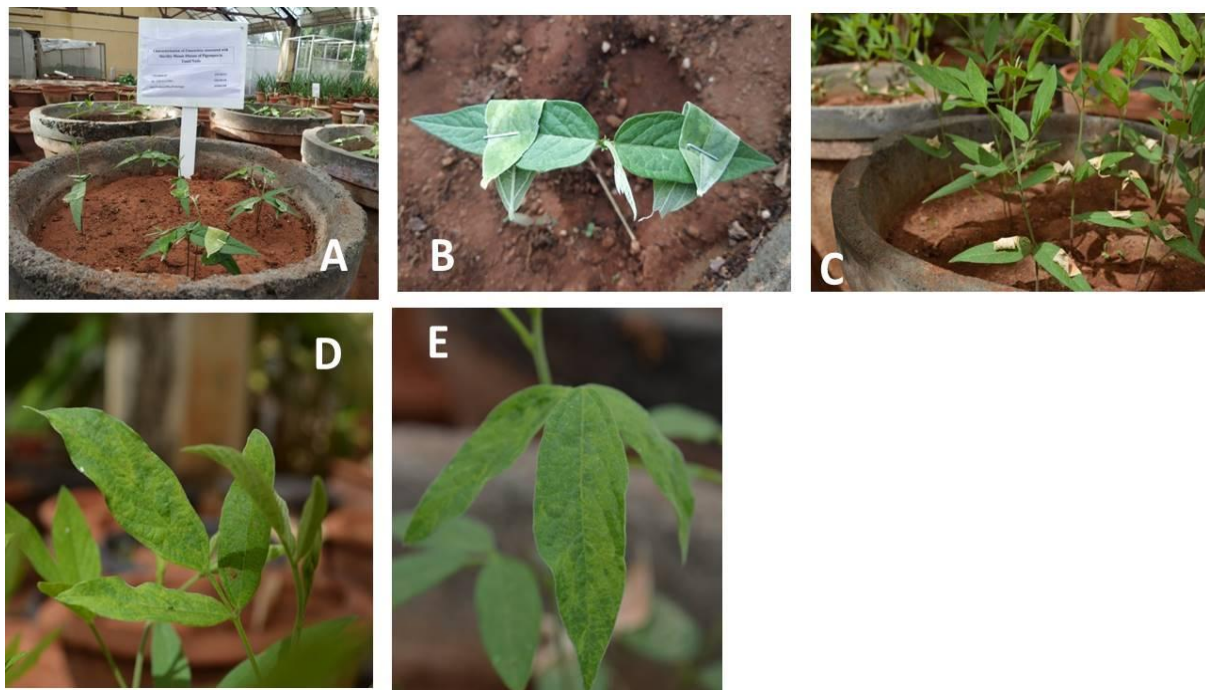
S.No	Location	Code	Latitude/ Longitude	PPSMV type	PDI(%)	Variety	Symptoms
1.	Coimbatore Field of TNAU	C1	11.0123° N / 76.9355° E	Mixed infection	13.5	Co-5, Co-8	Severe mosaic, complete sterility
	Devarayapuram	C2	10.9946° N / 76.8124° E	Mixed infection	7.3	Co-1, Co (Rg)-7	Mosaic, partial sterility
	Thondamuthur	C3	10.9899° N / 76.8409° E	Mixed infection	21.3	Co-1, Co (Rg)-7	Severe mosaic, complete sterility
2.	Namakkal Perumpallipatti	N1	10.9945°N / 76.9248°E	Mixed infection	3.6	Co-6, Vamban-2,	Mosaic, partial sterility
	Kandipalaiyam	N2	11.2704° N / 77.9467° E	Mixed infection	11.5	Co-6, LRG-41, BRG-1,2	Severe mosaic, complete sterility
	Elur	N3	11.3506° N / 78.1089° E	Mixed infection	12.4	Co-6, Vamban-2,	Mosaic, partial sterility
3.	Salem Attayampatti	S1	11.5355° N / 78.0566° E	Mixed infection	4.4	Vamban-2, Co-6,LGR-41	Mosaic, partial sterility
	Papparapatti	S2	11.5246° N / 78.0623° E	Mixed infection	8.5	Vamban-2, Co-6,LGR-41	Severe mosaic, complete sterility
	Minnakkal	S3	11.5353° N / 78.0837° E	Mixed infection	17.8	Vamban-2, Co-6,	Severe mosaic, complete sterility
4.	Trichy Arachi A	T1	11.1017° N / 78.4787° E	Mixed infection	6.2	Vamban-1,2, Vamban (Rg)-3, Co-6	Mosaic, partial sterility
	Keelakkunuppatti	T2	11.1243° N / 78.6473° E	Mixed infection	13.8	Vamban-1,2, Co-6	Severe mosaic, complete sterility
	Arachi B	T3	11.0896° N / 78.4794° E	Mixed infection	15.5	Vamban-1,2, Co-6	Severe mosaic, complete sterility

Fig.1



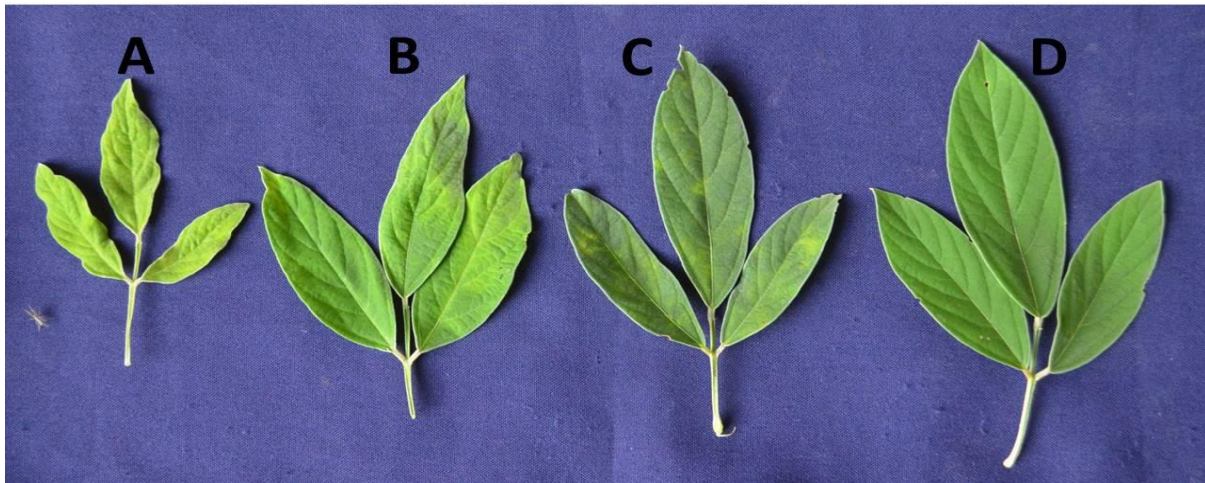
Collection of SMD infected leaf samples A,B – samples collected from Coimbatore, C,D - samples collected from Namakkal, E,F - samples collected from Salem, G,H - samples collected from Trichy

Fig.2



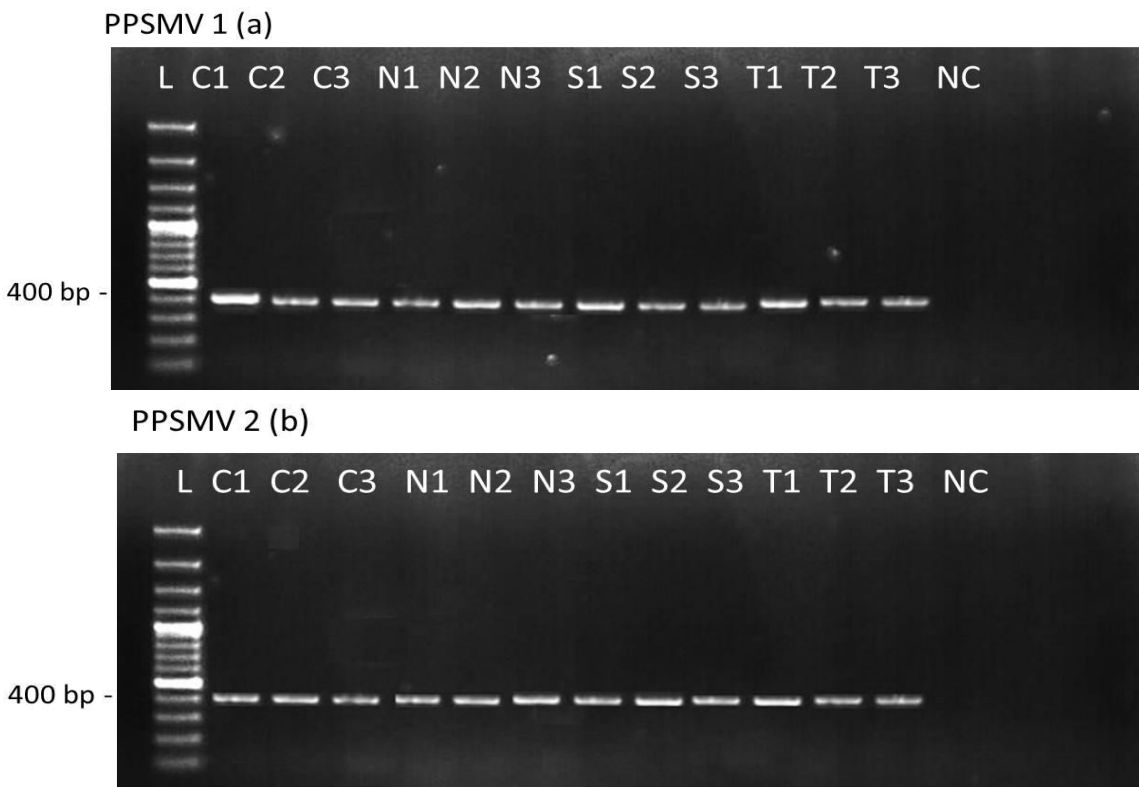
Inoculum maintenance by leaf stapling A - pigeonpea plants with two leaf stage, B - mite inoculation, C -10 days after inoculation, D,E - leaves with mosaic and leaf crinkle

Fig.3



Sterility mosaic virus affected pigeonpea plants exhibiting different kind of SMD symptoms, **A** - Leaf crinkle with severe mosaic, **B** - Mild mosaic, **C** - chlorotic ring spots and **D** - Healthy leaves

Fig.4



L – 100 bp lader NC – negative control

RT-PCR amplification of RNA-4 segment for detection of PPSMV 1(a) and PPSMV 2,(b) in SMD affected pigeonpea samples. Lane L 100 bp DNA ladder; lane C1 - C3 Coimbatore isolates, lane N1 - N3Namakkal isolates, lane S1 - S3 Salem isolates, T1 - T3Trichy isolates, NC – Healthy plant sample as Negative control

Symptom variability of sterility mosaic disease (SMD) of pigeonpea in field and glass house conditions

SMD infected plants were identified based on symptoms viz., yellow mosaic, chlorotic ring spots on leaves, reduced leaf size, severe stunting, or more vegetative growth. Severity was depending on infection age and variety of the crop. Inoculation of viruliferous mites in 30 to 45 DAS leads to severe mosaic, inoculation after 45 DAS leads to Mild mosaic and inoculation in reproductive stage leads to chlorotic ring spots on leaves. It was represented in Fig. 2 and 3.

RT-PCR for detection of PPSMV-1 and PPSMV-2

RT-PCR was done for all 12 isolates by using specific primers for RNA-4. All 12 isolates of Tamil Nadu had Both PPSMV-1 and PPSMV-2 as mixed infection. Pati *et al.*, 2015 has reported the presence of PPSMV-2 alone in Coimbatore, but Sayiprathap *et al.*, 2020 has reported both PPSMV-1 and PPSMV-2 present in Coimbatore. It was represented in Fig. 4.

It was proved by our work. Mixed infection in Coimbatore locations could be due to transfer of virus by its mite vector, the eriophyid mite (Sayiprathap *et al.*, 2020) (Dipshikha *et al.*, 2013).

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