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BIOLOGY, ETIOLOGY AND MANAGEMENT OF PIGEONPEA STERILITY MOSAIC DISEASE

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ABSTRACT

Sterility mosaic (SMD) is the most damaging disease of pigeonpea (*Cajanus cajan*) in the Indian subcontinent. The disease appears to be native to pigeonpea growing countries of Asia, and has not been recorded elsewhere. The disease was known since 1930s, but its causal agent, *Pigeonpea sterility mosaic virus* (PPSMV), vectored by an eriophyid mite, *Aceria cajani,* was characterized recently. Serological- and nucleic acid-based diagnostic tools were developed for the virus detection. The virus has novel properties with similarities in transmission and cytopathology with the eriophyid mite-transmitted High Plains virus and the agents of unidentified etiology associated with rose rosette, fig mosaic, thistle mosaic, wheat spot chlorosis and yellow ringspot of budwood. The virus occurs as several geographically distinct isolates and host-plant resistance to the highly virulent isolates are scarce. Knowledge on properties and distribution of various PPSMV isolates,

its relationships with other viruses and SMD epidemiology is limited. However, recent breakthroughs made on the identification, detection and transmission of PPSMV are presenting opportunities for new initiatives to study these aspects enabling the development of broad-based durable resistant cultivars to combat this major disease of pigeonpea.

INTRODUCTION

Pigeonpea (*Cajanus cajan* L. Millspaugh) is a very important pulse crop in India, and is also popular in southern and eastern Africa and Central America (74). Globally it is cultivated in 4.36 m ha, with a production of over 3.2 million tonnes (http://www.faostat.fao.org). Nearly 90% of the global pigeonpea cultivation is confined to India, Myanmar and Nepal. It is primarily grown for its seed, which contains 20-30% protein and is the principal source of dietary protein for an estimated 1.1 billion people (13,63). Sterility mosaic disease (SMD) first described in 1931 from Bihar State, India (40), is the major constraints on pigeonpea production in the Indian subcontinent. The disease is responsible for annual grain loss of worth over US$300 million. SMD was subsequently described from other pigeonpea growing countries in Asia (Bangladesh, Nepal, Thailand, Myanmar, Sri Lanka and China), but is not known to occur outside Asia (7,58). The SMD inhibits flower production and renders plants sterile (Fig. 1), but the disease is not lethal to the host and affected-plants survive normally in the fields. The disease is also regarded as *'green plague'*

Fig. 1. SMD-affected pigeonpea (circled) and healthy plants in the fields. Note pods on healthy plants, but due to lack of flowering infected plant is non-bearing.

because affected plants remain green due to excessive vegetative growth and under congenial condition disease spreads rapidly leading to severe epidemics. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has assessed the economic importance of various biotic problems of pigeonpea and reported that SMD causes greater yield loss than any other disease affecting pigeonpea in India (22).

Despite extensive efforts, the SMD causal agent has remained elusive to identification and characterization over many decades (16,43,70). Involvement of a pathogen in SMD etiology was shown by graft transmission studies (5) and subsequently the vector of SMD agent was identified as an eriophyid mite, *Aceria cajani* Channabasavanna (Acari: Arthropoda) (65) (Fig. 2). Various studies for SMD etiology have ruled out the involvement of a bacterium, fungi or phytoplasma (16). The invariable association of vector mites with diseased plants led to a speculation that the disease could be due to mite toxemia but this was excluded by experiments using SMD-agent-free mite colonies (16,24). In the absence of other likely causes, and on the basis of symptoms and mode of transmission, the SMD agent was suspected to be a virus (35, 43, 70). Recently, using a new purifi-cation procedure, a breakthrough was achieved in identifying the SMD causal agent, a virus named *Pigeonpea sterility mosaic virus* (PPSMV), leading to studies on its transmission, diversity and development of methods for its detection and improved screening methods (20,31). This article presents a review on the intricacies of SMD, recent breakthroughs and strategies for sustainable management of this disease.

Fig. 2. *Aceria cajani*, the vector of *Pigeonpea sterility mosaic virus*

SYMPTOMOLOGY AND IMPACT ON PRODUCTION

PPSMV causes a wide array of symptoms on pigeonpea. These include various patterns of mosaic on leaves, small and misshapen leaves, proliferation of axilliary shoots, stunting and complete or partial cessation of flower production (sterility) (20,58). The precise symptoms of SMD infection depend on the plant genotype, which are of three kinds: genotypes that show (i) systemic severe mosaic and sterility; (ii) systemic mild mosaic and partial sterility; and (iii) localized chlorotic ring spots and no sterility. In susceptible cultivars, early infection (<45 day-old plants) show characteristic disease symptoms and near complete cessation of flowering whereas late infections (>45 day-old plants) result in symptoms on only a few branches or on parts of some branches and only partial sterility. When such infected plants are pruned (ratooned), newly emerging shoots show clear symptoms and complete cessation of flowering. The incidence of asymptomatic infections in fields are common and sometimes much higher than the disease incidence based on visible foliar symptoms (PL Kumar and YD Narayana, unpublished data). Pod number, seed size and seed yield per plant can be greatly decreased by PPSMV infection and these effects have been correlated with the time at which initial symptoms appear. Plants infected at an early stage results in a 95-100% loss in yield (22,60), whilst losses from late infection depend on the level of infection (i.e. number of affected branches /plant) and range from 26 to 97% (9,22). Seeds from partially affected plants are discoloured and shrivelled with about 10-40% reduction in dry weight. In addition, PPSMV infection exacerbates powdery mildew (*Oidiopsis taurica*) (56) infection and infestation by spider mites (*Schizotetranychus cajani*) (71) compounding the damage.

SMD ETIOLOGY AND DETECTION

The SMD causal virus was first isolated from infected pigeonpea plants from Patancheru (P), Andhra Pradesh, India. Purified PPSMV preparations of this isolate contained aggregates of highly flexuous, irregularly branched, filamentous virus-like particles (VLPs) of 8 to 11 nm diameter and of undetermined length, resembling in appearance particles of tenuiviruses (12) (Fig 3A). The purified virus preparations contained a

major protein of 32 kDa and up to 6 segmented RNA species of size 1.1-6.8 kb. The virus was isolated consistently from all SMD-affected plant samples collected from different locations of peninsular India and from SMD-affected pigeonpea samples infected by graft inoculation, and by infective mites (*A. cajani*) (30,34,36). The nucleotide sequence of some cDNA clones constructed to PPSMV-RNA and the monoisotopic masses of the 32 kDa nucleoprotein, showed no similarity with these viruses, or with any other organisms in databases (21,34).

Fig. 3 A. Electron micrographs of the flexuous filamentous virus-like particles of *Pigeonpea sterility mosaic virus* (PPSMV) in a preparation purified from infected pigeonpea. VLPs are stained with uranyl acetate pH3.5. **B.** Cytopathology of PPSMV-infected pigeonpea cells showing membrane bound bodies (MBB; indicated with arrows) and fibrous inclusions (FI; indicated with arrowheads). **C.** Immuno-gold labelling of PPSMV polyclonal antibodies to MBBs in PPSMV-infected pigeonpea cells. **D.** FIs in an SMD-affected cells. Ch, chloroplast; na, nucleus; va, vacuole.

Polyclonal antibodies to PPSMV VLP preparations produced in a rabbit were very effective in detecting PPSMV in plant tissues by double antibody sandwich (DAS) ELISA (31,34). PPSMV was detected by ELISA in all SMD-affected pigeonpea plants infected either experimentally by *A. cajani,* and by

grafting, or naturally in the field at several different locations in India, and in infected accessions of wild *Cajanus* species. Furthermore, it was detected in various pigeonpea genotypes showing the different symptom forms of the disease, and from *Nicotiana benthamiana* and *N. clevelandii* infected by mechanical inoculation. Leaves from hundreds of healthy or uninoculated pigeonpea plants were negative in ELISA (25,34). The virus-specific 32 kDa protein was detected in extracts of groups of vector mites by Western immuno-blotting. Taken together, these data demonstrated the complete and specific association of PPSMV with SMD, and provide very strong circumstantial evidence that PPSMV is the causal agent of the disease, ending the search for one of the most elusive plant pathogens. Unequivocal evidence that PPSMV is the causal agent depends on fulfilling Koch's postulates but several technical difficulties prevent this, including the unstable nature of the virus and the difficulty of infecting pigeonpea by mechanical inoculation.

Although the purified PPSMV VLP preparations were not infective to plants, PPSMV was transmitted experimentally by mechanical inoculation of fresh leaf sap extracts of SMD-affected pigeonpea to *N. benthamiana* and *N. clevelandii*, but not to pigeonpea. However, it was not possible to transmit the agent from infected *Nicotiana* species to pigeonpea by mechanical inoculation of sap. Systemically infected leaves of the *Nicotiana* species developed mild chlorosis and some necrotic spots (30,33,34).

Ultrastructural studies of PPSMV-infected pigeonpea, and *N. benthamiana* [and recently French bean (*Phaseolus vulgaris* var. Topcrop) (PL Kumar, unpublished data)] plants identified 100-150 nm quasi-spherical membrane bound-bodies (MBBs) and fibrous inclusions (FIs) (30) (Fig. 3B,D). The MBBs were labelled *in situ* specifically with antiserum to PPSMV, indicating that they contain the PPSMV-specific 32 kDa antigen (Fig. 3C). The FIs found in PPSMV-infected cells are possibly a non-structural inclusion protein of PPSMV (30). In leaf sap extracts and in purified preparations of PPSMV, no structures comparable to MBBs were detected (30) but the MBBs were heavily labelled with PPSMV antiserum to purified VLPs. It is therefore possible that these particles are released from ruptured MBBs during the purification process.

However, attempts to detect MBBs in purified PPSMV preparations made without the use of detergents and organic solvents were not successful. It is likely that MBBs might represent complete particle of PPSMV (similar to that of tospovirus particles) and VLPs found in the purified preparations could be the ribonucleoprotein particles, but this needs to be confirmed by isolation and purification of intact MBBs.

The properties of PPSMV indicate that it is a previously undescribed virus with an unusual combination of properties. In the size and appearance of its VLPs and the number and sizes of its protein and RNA components, it is similar to viruses in the genus *Tenuivirus* (12). However, all tenuiviruses are phloem limited, transmitted by *Delphacid* plant-hoppers and infect plant species in the Poeaceae (12). PPSMV cytopathology resembles that of tospoviruses and tenuiviruses. Although tenuiviruses do not produce cellular inclusion bodies that resemble the MBBs found in PPSMV-infected cells their non-structural protein inclusions (NCP) in infected cells resemble the FIs of PPSMV (10,11,30). The filamentous VLPs of PPSMV resemble the nucleoprotein particles of *Tomato spotted wilt virus* (TSWV) and *Peanut bud necrosis virus* (PBNV) and the MBBs of PPSMV are similar to, though larger than, those of TSWV (23). However, serological tests failed to detect any relationship of PPSMV to *Maize stripe virus*-sorghum strain (MSpV-Sg) and PBNV a tenuivirus and tospovirus respectively, that are endemic in the Indian subcontinent (49,54). Furthermore, tospoviruses and tenuiviruses are transmitted in a persistent and often propagative manner by their insect vectors (75), PPSMV is transmitted by an eriophyid mite in a semi-persistent manner (24,55). Moreover, the nucleotide sequence of c. 6 kb of PPSMV-RNA and the monoisotopic masses of the 32 kDa nucleoprotein, show no similarity with these viruses, or with any other organisms in databases (34; P. L. Kumar and A. T. Jones, unpublished data).

PPSMV shows most similarity with High plains virus (HPV) reported on corn and wheat in North America and Australia (19). Each virus is transmitted by eriophyid mites, has 4-7 RNA species, a virus-specific 32 kDa protein, membrane bound-bodies of similar size and morphology, and is mechanically transmitted with difficulty in sap extracts but not in purified preparations (1,19,30,38,39). However, no serological relationship was

detected between these two viruses (34). MBBs similar to those detected in PPSMV- and HPV-infected plants are also detected in plants affected with other eriophyid mite-transmitted agents that cause fig mosaic, wheat spot mosaic, thistle mosaic and rose rosette diseases (2,4,15,17). These agents, together with PPSMV and HPV, probably represent species in a new genus of plant viruses.

Analysis for virus variation: Our studies have shown that variation in PPSMV isolates is contributing to the variation in host plant resistance across regions in the sub-continent (28,59). To assess virus variation a set of differential pigeonpea genotypes were planted at six different field locations in India (Table 1; Fig. 4). Genotypes inoculated with PPSMV isolates present in pigeonpea at Patancheru, Andhra Pradesh (P), Dharwad (D) and Gulbarga, Karnataka, showed similar phenotypic reactions. PPSMV isolates present in pigeonpea at Bangalore, Karnataka (B), Coimbatore, Tamil Nadu (C) and Varanasi, Uttar Pradesh (V) showed similar symptoms in genotypes but they differed from those caused by isolates P, D and G (Table 1). Virus isolates at B, C and V infect or induce severe symptoms on pigeonpea genotypes that were uninfected or produced only mild symptoms when infected with isolates P, D and G. This indicates that isolates B, C and V overcome the resistance to isolates P, D and G,

Table 1. Symptom response of pigeonpea genotypes to infection with virulent and highly virulent isolates of Pigeonpea sterility mosaic virus (PPSMV) occurring in India

Genotype	Virulent isolates			Highly virulent isolates*		
	Patancheru (P)	Gulbarga (D)	Dharwad (G)	Bangalore (B)	Coimbatore (C)	Varanasi (V)
ICP 2376	RS	RS	RS	SM	SM	SM
ICP 7035	NS	NS	NS	NS	NS	NS
ICP 8862	NS	NS	NS	MM	MM	MM
ICP 8863	SM	SM	SM	SM	SM	SM

SM = severe mosaic; MM = mild mosaic; RS = chlorotic ring spots

and this observation is reflected in the fact that most of the resistance sources selected against P isolate were highly susceptible to isolates occurring in northern and southern India (43,59). Based on these observations, PPSMV isolates seem to be of two main types: B-type isolates, that have the ability to overcome resistance to P-type isolates (29). Available information suggests that P-type isolates are localized in south-central and central India (Fig 4). Identification of PPSMV isolate as P or B types can be done using differential pigeonpea genotypes (Table 1).

Fig. 4. Location of various PPSMV isolates analysed in India, and probable distribution of virulent (VI) and highly virulent (HVI) PPSMV isolates

Following biological characterization, biochemical characterization of isolates B and C showed that they were indistinguishable serologically in ELISA with P antibodies. The properties of purified virus preparations of B and P isolates are similar, however the cytopathology of B isolate infected cells showed that in addition to membrane bound-bodies, fibrous incusion and electron dense material, crystalline aggregates were detected (PL Kumar and AT Jones, unpublished data). Isolate C was distinguished from isolates B and P in reacting weakly with antiserum to isolate P (29). The protein specifically associated with VLP preparations of isolate C had a size of c. 35 kDa, and not 32 kDa as with isolates P and B. Recently, we found that in the sequence of RNA-5, single nucleotide differences of isolate C distinguished it from isolates P and B. Furthermore, immuno-gold labelled P antibodies poorly labelled MMBs of C isolate, suggesting differences at the genomic and serological level between isolates P and C

(PL Kumar and TKS Latha, unpublished data). Further studies on the nucleotide sequence of the genome of the various PPSMV isolates is necessary to identify those sequences contributing to biodiversity. Once this is obtained, the sequence of the genome regions that differ can be used to develop sensitive nucleic acid-based methods, such as reverse transcription (RT)-PCR, to detect and differentiate PPSMV isolates. Recently, a sub-set RT-PCR has been developed based on single nucleotide differences between isolates P and C to distinguishing the two isolates (PL Kumar and TKS Latha, unpublished data).

TRANSMISSION AND VECTOR BIOLOGY

PPSMV is transmitted naturally by the eriophyid mite *Aceria cajani* Channabasavanna (Acari: Arthropoda). PPSMV is not transmitted through pollen or seed, or by nematodes, dodder or soil-borne fungi (16,20,70). Experimental transmission from pigeonpea to pigeonpea is possible by grafting (16,53), but transmission by mechanical inoculation of sap was unsuccessful (16,33). For routine experimental transmission 'leaf-stapling' is used - a leaflet from an SMD-affected and *A. cajani*-infested plant is stapled onto healthy pigeonpea seedling, facilitating the migration of viruliferous mites from the source leaflet to the test plant and virus transmission by mite feeding (44,55).

The mite vector is highly host-specific and obligately dependant on pigeonpea in all-active stages of its life cycle. Adult *A. cajani* measure 200-250 µm and completes its life cycle, comprising egg (30 x 40 µm) and two nymphal stages, in about 2 weeks (46). Mites inhabit the lower surface of leaflets and are found predominantly on symptomatic leaves of PPSMV-infected plants (28,37,42,57). Their numbers are very high on young and symptomatic leaves compared to old symptomatic leaves. Their feeding causes no obvious damage to the host plant. Once established on PPSMV-susceptible genotypes, mites can multiply to high densities within a few weeks. Their dispersal is passive, assisted mainly by wind currents.

The "floating leaflet" method developed to generate non-viruliferous mite cultures facilitated studies on mite-virus relationships (24). Single *A. cajani* transmit PPSMV, but the maximum transmission achieved with single mites was about 50% (24). *A. cajani* required a minimum 15 min

virus acquisition access period and a 90 min virus inoculation access period. These periods were decreased to 10 min and 60 min respectively, when mites were starved prior to feeding. The mite vector can retain PPSMV for up to 6 h when feeding and for more than 13 h without access to a host. Viruliferous mites lose the ability to transmit PPSMV after feeding for 2-10 h on healthy plants and there is no apparent latent period and no evidence for transovarial transmission (24,45,46). Taken together these data on PPSMV transmission by *A. cajani* indicate that transmission is of a semi-persistent type (24), similar to the transmission of other eriophyid-mite transmitted agents like *Peach mosaic virus, Ryegrass mosaic virus* and *Wheat streak mosaic virus* (17,41,47). Multiplication of *A. cajani* on PPSMV-susceptible pigeonpea hosts is very high compared to those on healthy plants, confirming earlier observations that mites prefer infected plants (42,57).

A. cajani biodiversity was assessed using a polymerase chain reaction (PCR)-based rDNA analysis technique that was shown previously to be rapid and sensitive in the unambiguous identification of morphologically closely related mite species (14,32). Scanning electron microscopy was also used to study morphological features of *A. cajani* from India, Nepal and Myanmar based on the first description of *A. cajani* by Channabasavanna (6). These analyses of mite populations obtained from SMD-affected plants from several locations in India, Nepal and Myanmar indicated that there was no significant variation in rDNA regions, or in the morphological features studied by scanning electron microscopy (28). These results suggest that *A. cajani* on pigeonpea across the Indian sub-continent constituted one population and that no other *Aceria* species and probably no *A. cajani* biotypes are involved in PPSMV transmission. Recent studies on isolate PPSMV-C demonstrated that the transmission properties and virus-vector interactions were very similar to those observed for isolate P (37), indicating that *A. cajani* can transmit PPSMV across all locations with similar efficiency, further corroborating the evidence from rDNA marker study. These results confirm further that that the variation observed in SMD-resistance in pigeonpea genotypes across the Indian sub-continent is not influenced by the mite vector, but is due to the variation in the virus isolates (28).

HOST RANGE OF PPSMV AND *A. CAJANI*

In the field, pigeonpea and its wild relatives were naturally infected with PPSMV and supported *A. cajani* multiplication. Evaluation of several crops and weed species within and adjoining SMD-affected pigeonpea fields for PPSMV and vector mites revealed mites on *Hibisucs penduliformis*, but they are free from PPSMV (25). Under experimental transmission using viruliferous mites on a range of crop and weed species, only *Phaseolus vulgaris* were infected with PPSMV by vector mites, but mites survived for only about 4 h. By mechanical inoculation of infective sap, PPSMV was transmitted to *N. benthamiana and N. clevelandii*, but not to pigeonpea or *P. vulgaris* (33). Although PPSMV is able to infect plants outside the genus *Cajanus*, because mites are highly host specific to species in the genus *Cajanus* only cultivated and wild accessions of pigeonpea have the potential to serve as sources of PPSMV under field conditions. Some weed species, such as *H. penduliformis*, *Oxalis circulata* and *Canavis sativus* may act as a refuge for mite survival in transit, and may therefore aid the spread of SMD (25,69). Natural infection of *P. vulgaris* by PPSMV was not observed.

EPIDEMIOLOGY OF SMD

The SMD pathosystem consists of the virus, mite vector and *Cajanus* genotype. The dynamics of SMD pathosystem are influenced by many abiotic and biotic factors, diverse agriculture systems and environmental conditions. Information on off-season survival of virus and mite vector, their spread during the cropping season from crop to crop, and within the crop and variation in disease incidence in a region is critical for understanding disease ecology. This is a challenging task considering that the crop is mainly grown in marginal farming systems with divergent cropping practises. SMD occurs in every year in almost all the pigeonpea growing regions in India, but disease incidence in each region and seasons varies widely. There are several conflicting reports on the influence of climatic conditions (8,57,69). Crops grown under irrigation or in near the other irrigated fields are reported as being most vulnerable to early infection (9,48).

The disease is not seed borne and it has to be introduced into the field by the mite vector. SMD is a polycyclic disease because infected plant

serve as a source for initiating subsequent spread in the field. The diseased plants left in the fields after harvest, plants on the field banks, kitchen gardens, perennial pigeonpea and wild relatives of pigeonpea are regarded as primary inoculum sources from which potentially viruliferous mites move onto emerging pigeonpea (9). The common source of inoculum in rainfed pigeonpea agriculture is the stubble left in the field after harvesting. Those near to water sources such as canals and wells, or in shade, maintain their foliage and harbour mites as well as virus and, following early rains, they start growing and serve as inoculum to nearby crops. Such early primary infection provides a good opportunity for repeating cycles of infection to occur in the region leading to widespread incidence. Disease incidence in a season within and among fields depends on the proximity to an inoculum source, plant age, cultivar type, climatic factors and mite populations.

For the cause of the reappearance of the disease in areas where one pigeonpea crop is followed by a wide temporal gap, and where volunteer pigeonpea are not common is unknown. However, in such regions it is suspected that wild *Cajanus* species play major role in disease spread. Often pigeonpea cultivation in the subcontinent overlaps in different regions and various cultivars with different maturity periods are grown and in some regions pigeonpea is grown as a perennial. Mites from such plants, assisted by wind currents, may spread long distances and they may serve as inoculum sources in areas where pigeonpea is cultivated after a long gap. This is clearly an area for further investigation.

MANAGEMENT OF SMD

Several methods have been investigated to reduce SMD incidence by using pesticides to delay the onset of infection and disease spread, control through cropping management practices and host-plant resistance. A number of organophosphorous chemicals were used either for seed dressing (Carbofuran, Aldicarb), soil application (Carbofuran and Temik 10G) or as foliar sprays (Oxythioquinox, Kelthane, Dinocap, Monocrotophos, Tedion, Metasystox) to control the spread of mite vectors and to minimize the spread of the disease (16,52). Correct timing and dosage are critical for effective control of the vector populations. However, as this is an expensive option, subsistence farmers seldom use it to control SMD.

Several studies to determine the affect of sowing date, plant density, spacing, intercropping with millets, border and inter-cropping with millets and fibre crops, found no significant effect on SMD incidence (3,18,50,68,76). Destruction of sources of SMD inoculum prior to the cropping season can reduce SMD incidence/and or delays the early onset of the disease thereby reduces the disease impact. However, practices like these are seldom followed due to the preoccupation of farmers with other revenue generating activities, lack of resources and labour constraints in marginal farming conditions where the crop is predominantly grown. Disease management through host-plant resistance has received maximum emphasis, as it requires no special expertise on the part of growers.

Host plant resistance: Sources of resistance to SMD was first identified in pigeonpea land race Sabour 2E in India. Subsequently several disease resistant and tolerant lines were identified (43,50). Concerted efforts for identifying sources of resistance were initiated at ICRISAT in 1975 (43,44). Over 13,015 pigeonpea accessions available from the global pigeonpea germplasm collection at ICRISAT were screened for SMD resistance and 326 resistant lines, 97 tolerant lines identified (43). The resistant lines selected were evaluated at different locations in India. Although all the genotypes selected for multi-location trials performed well at Patancheru, and surrounding regions, only 10 genotypes were found to be resistant across all trial locations in India. Our studies have shown that this variation is due to the occurrence of isolates of PPSMV that interact differently with different plant genotypes (20). Most of the lines selected conferred resistance to type P isolates, very few lines were found to be resistant against all known PPSMV isolates in India (for example ICP7035, ICP7867, ICP10976, ICP10977 offered broad-based resistance to the disease). In recent years, research programs have focused on the development of high yielding genotypes with combined resistance to PPSMV and *Fusarium* wilt, as both these diseases are endemic in the subcontinent. However, due to the narrow genetic base of resistance in cultivated germplasm, very few pigeonpea lines were found to possess resistance to both diseases (e.g. ICPL87119, ICPL8363, ICPL8362, ICPL96058, and ICPL96053). Moreover, most of the multiple disease resistant lines were resistant to only PPSMV type P isolates.

Adequate levels of SMD resistance are scarce in the cultivated pigeonpea gene pool but wild relatives of pigeonpea have been shown to posses high levels of resistance to several biotic constraints (62). The global pigeonpea germplasm collection at ICRISAT has over 270 accessions of 47 wild species related to the genus *Cajanus*. Of these 115 accessions of 6 wild *Cajanus* species, *C. albicans*, *C. cajanifolius*, *C. lineatus*, *C. platycarpus*, *C. scarabaeoides* and *C. sericeus*, were screed for resistance to PPSMV isolates at Patancheru, Bangalore and Coimbatore. Fifteen accessions, ICP 15164, 15615, 15626, 15684, 15688, 15700, 15701, 15725, 15734, 15736, 15737, 15740, 15924, 15925 and 15926 were identified to contain broad-based SMD resistance and no symptoms or mites were noted on these plants (27). In addition, some of these accessions also possessed resistance to pest damage, cyst nematode and wilt (26). Apart from *C. platycarpus*, the species tested were from the secondary gene pool, which are inter-fertile by traditional breeding. Therefore, the resistance in these accessions is transferable to pigeonpea by conventional breeding programmes.

Efficient screening methods for virus resistance: A method was developed for rapid screening of pigeonpea genotypes under laboratory conditions. Pigeonpea plants raised in growth chambers were inoculated at the 2-leaf stage with viruliferous mites by stapling SMD-affected pigeonpea leaves containing mites onto leaves of test plants (44). About 2-3 weeks later, plants were assessed for disease symptoms on leaves and tested for PPSMV by DAS-ELISA. Genotypes resistant to mite inoculation were assessed for virus resistance by "petiole grafting" with mite-free SMD-infected pigeonpea material to identify their resistance to the virus. Screening in this way demonstrated that SMD resistant genotypes were either (i) resistant to PPSMV only, (ii) resistant to mites only or, (iii) resistant to both PPSMV and mites. It proved possible to determine the nature of the resistance to SMD in individual pigeonpea genotypes within 6-8 weeks. To maximize the effectiveness of resistance, all resistant material needs to be critically evaluated for performance against various PPSMV strains in different environments to identify genetically stable resistance.

*Mechanisms and inheritance of resistance to SMD***:** Our studies have indicated that SMD resistance in some genotypes is due to immunity to PPSMV and in others to resistance to *A. cajani*, and in a few others to

resistance to both organisms. With regard to mite resistance, it is known that some SMD-resistant genotypes have a thicker leaf cuticle and epidermal cell wall than those of mite-susceptible genotypes (61). Conceivably, the thick cuticle prevents the short mite stylet reaching epidermal cells preventing feeding altogether. However, a complicating factor in determining the precise nature of the resistance mechanism is that the reproduction of *A. cajani* is much greater on PPSMV-infected plants than on healthy plants of the same genotype (24,42,57). There seems to be a beneficial relationship between the vector mite and the virus it transmits, and this may explain why mites are rarely found on PPSMV-resistant pigeonpea genotypes. Studies on genetic inheritance is limited and complicated owning to the occurrence of various SMD strains and a wide array of pigeonpea lines with different kinds of resistance. Earlier studies indicated that susceptibility to SMD is dominant over tolerance and that resistance and disease response to SMD infection is under the control of independent non-allelic genes (64,66,67,72,73). However, our finding of several distinct resistance mechanisms to SMD infection, the occurrence of various PPSMV isolates, the close relationship between mite numbers and PPSMV infection, and the wide array of pigeonpea lines, makes the interpretation of these genetical studies of resistance more difficult.

CONCLUSION

Recent advances in understand SMD etilogy and progress towards developing broad-based SMD resistant and identification of SMD resistance in wild *Cajanus* species, some of which are also resistant to *Fusarium* wilt and pod borer, is a major step towards an integrated approach to manage SMD, wilt and pod borer problems. However, much need to be understood about PPSMV isolates, its relationships with other viruses and its genome properties. Characterization of various PPSMV isolates to identify the basis for the differences is essential to develop diagnostic tools for the precise identification of the virus isolates to conduct comprehensive surveys to assess the prevalence of various PPSMV isolates and their impact on pigeonpea cultivars.

Future strategy for breeding for resistance should focus on developing broad-based resistance combined with disease resistance to pests and

wilt. Information on virus isolates, resistant sources in wild pigeonpea accessions, improved resistance screening, all can play a major role in enhancing the efficiency of this selection. Selected genotypes should be evaluated on-farm in different production systems for assessing their performance, stability and effectiveness of resistance in different zones. Recently, a very promising SMD-resistant pigeonpea genotype, ICP7035, which has broad-based resistance to SMD and is tolerant to wilt, has been released for cultivation in India (51).

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