CROP PROTECTION PROGRAMME

Characterisation of the Causal Virus of Pigeonpea Sterility Mosaic Disease: A Step Towards Attaining Sustainability of Pigeonpea Production in the Indian subcontinent

R No 7452 (ZA No. 0321)

FINAL TECHNICAL REPORT

1 September 1999 - 31 August 2002

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12 September 2002

"This publication is an output from a research project funded by the United Kingdom Department for International Development for the benefit of developing countries. The views expressed are not necessarily those of DFID."
[R7452; Crop Protection Programme]

Executive Summary

The Indian subcontinent is the world's most important pigeonpea-growing region with 82% of global cultivation confined to India. Pigeonpea is used in multipurpose ways and plays an important role in food security, balanced diet and source of income to millions of poor farmers living in marginal farming systems. Pigeonpea sterility mosaic disease (PSMD), endemic in the subcontinent can cause >90% yield reduction. It is a major constraint on crop yield and economic well being of poor farmers. Cultivation of broad-based durable resistant cultivars is essential to mitigate losses due to PSMD to benefit these smallholders. To achieve this, this project was undertaken to characterise Pigeonpea sterility mosaic virus (PPSMV) - the agent of PSMD, develop versatile and sensitive diagnostic tools for its detection, and to select and supply broad-based PSMD-resistant genotypes for utilisation in breeding programmes and to farmers for cultivation. PPSMV characterisation led to the development of diagnostic tools for the detection of virus and identification of its biotypes. This knowledge was utilised to establish an efficient PSMD screening strategy for the precise selection of broad-based resistance sources. This technology was used in a participatory manner with NARS to evaluate several cultivated and wild pigeonpea accessions for PSMD resistance under various agro-ecological conditions. This identified several genotypes in cultivated and wild pigeonpea possessing resistance to more than one PPSMV biotype, thus demonstrating the potential of the technologies developed in this project. The seed of the most promising genotypes were tested on-farm in the farmer participatory trials. Pathways were established for the dissemination of the developed technology and products to the NARS, NGOs and farmers through participatory research, training courses and farmer participatory trials. These activities led to the identification of high yielding pigeonpea cultivars with broad-based resistance to PSMD and the supply of seed to needy farmers. This provides the basis for the sustaining and stabilising of pigeonpea production with a consequent increase in income to smallholders, contributing to poverty alleviation. This project has unravelled the identity of the PSMD causal agent, a seven decade-old mystery. The methodologies developed and the virus characterisation are significant scientific contributions to the field of virology which aids our understanding of several eriophyid-mite borne disease of undefined aetiology, such as rose rosette and fig mosaic. Most importantly, it contributed to the development of technologies for monitoring the disease and the development of broad-based resistance sources for eco-friendly management of this very serious disease in the subcontinent. The project outputs benefit resource poor farmers living in marginal areas in the Indian subcontinent and rural households, where pigeonpea is a major subsistence crop and where women are involved in post-harvest processing and marketing.

Background

Pigeonpea sterility mosaic disease (PSMD), considered as the '*Green Plague of Pigeonpea*', is one of the major biotic factors limiting pigeonpea production in the Indian subcontinent. This disease, which was first described in 1931, affects flower production rendering the plant sterile. Yield losses caused in most genotypes by PSMD infection early in the season can lead to >90% crop loss. Annual losses estimated in 1993 are valued at US$282 million in India and Nepal alone, which is equal to the combined losses due to pod-borer (*Helicoverpa* spp) and *Fusarium* wilt. Considerable progress has been made in controlling wilt and pod-borer problems through cultivation of wilt resistant cultivars and IPM, respectively. The poor understanding of PSMD and the lack of knowledge on its causal agent have hindered research efforts to control this disease.

Although the occurrence of PSMD was first recorded in 1931, its aetiology remained as a mystery. The agent was shown to be transmitted by the eriophyid mite, *Aceria cajani* and by grafting but not by mechanical inoculation of sap extracts. Previous efforts to identify the causal agent of the disease at several laboratories in India and at ICRISAT were unsuccessful. However, convincing evidence was provided to show that a fungus, bacterium, viroid or phytoplasma was not involved in PSMD aetiology and that it was not caused by mite toxaemia. The PSMD agent was suspected to be a virus.

Efforts to identify sources of resistance to PSMD have been successful. A leaf stapling technique was used for laboratory and field screening. Spreader rows in between test rows were used to screen large numbers of plants for disease resistance. Scoring was based on visual symptoms. A number of genotypes were shown to possess field resistance to PSMD but this was later found to be largely location specific. The resistance mechanism(s) are not fully understood, but in some lines it was attributed to resistance to the mite vector. More recently, breakdown in resistance was attributed either to the presence of different *A. cajani* biotypes, different species of *Aceria* mites, or to the occurrence of different biotypes of the causal agent. Based on the reaction of seven differential pigeonpea genotypes in nine locations in India, the existence of five distinct biotypes of the causal agent was reported.

Assessment of PSMD-variants based on symptoms alone is complicated by the fact that symptoms are governed by many biotic and abiotic factors. In studies at ICRISAT, host reaction to PSMD was shown to be governed by more than one gene and was assumed to be “strain specific”. Pigeonpea is a partially cross-pollinated crop and, in addition to environmental factors, genotypic variability induced as a result of cross-pollination plays an important role in symptomatology. Thus, variability in the pathogen, mite vector, germplasm and the environment, and mixed infection with other pathogens, may all contribute to variability in symptom expression.

In the DFID project (R6407H), variation within and between *A. cajani* populations in PSMD-endemic locations of Asia was addressed by analysing the nuclear ribosomal RNA encoding genes and associated transcribed spacers, collectively known as ribosomal DNA (rDNA). No significant variation in rDNA was demonstrated in analyses of *A. cajani* populations from various locations in India, Nepal and Myanmar. Work on the identification of the causal agent of PSMD was also initiated simultaneously. A tombusvirus was isolated from some PSMD-affected plants and characterised. Extensive tests conducted on many healthy and diseased pigeonpea plants have shown that this tombusvirus is not consistently associated with infected plants and a few apparently healthy looking plants also contained the virus. No symptoms have developed in pigeonpea plants inoculated mechanically with purified preparations of this virus and the virus was confined to inoculated leaves only. These studies suggest strongly that the tombusvirus is not associated specifically with PSMD.

Various protocols reported for the identification of mite-transmitted viruses were tested for the isolation of the causal agent of PSMD. Using a modified procedure for isolating *Peach mosaic virus* and Maize stripe tenuivirus, a highly flexuous filamentous shaped virus was isolated from PSMD-affected pigeonpea plants. Partially purified preparations of this virus contained a 32 kDa protein and 5-8 RNA species ranging in size from 9.0 kb to 1.5 kb. This specific protein and the associated RNA species were present consistently in all of 32 PSMD-affected samples collected from four different locations in South India, but not in any of 30 comparable healthy pigeonpea samples. The same virus was also isolated from PSMD-affected pigeonpea samples inoculated previously with mites (*A. cajani*) at the two-leaf stage and maintained subsequently in a growth chamber. The consistent isolation of this virus-like particle from PSMD-affected plants, especially from laboratory maintained cultures, and the similarity of its properties with another mite transmitted virus reported recently from maize, suggested that this virus from pigeonpea is probably the causal agent of PSMD.

This current DFID project has sought to characterise the PSMD agent, develop versatile and sensitive diagnostic tools for its detection, and to develop broad-based PSMD-resistant genotypes for utilisation in breeding programmes and to supply to farmers. Achievement of these objectives would provide versatile diagnostic tools to detect the PSMD agent and the identification of high yielding pigeonpea cultivars with broad-based resistance to PSMD. These would provide the basis for the sustaining and stabilising of pigeonpea production with a consequent increase in income to smallholders.

Project Purpose

The purpose of the project was to attain sustainable pigeonpea production in the Indian subcontinent through the development of cultivars with broad-based resistance to PSMD. This was

to be achieved by the characterisation of the PSMD causal agent and the development of diagnostic tools for its detection. These would be utilized in the selection and evaluation of pigeonpea genotypes with resistance to PSMD and its biotypes. The most promising cultivars with broad-based resistance would be selected for utilization in breeding programmes and for supplying to farmers. Genotypes possessing multiple resistance to PSMD and to wilt would also be identified. This information on PPSMV should lead to the development of strategies for the efficient management of PSMD, wilt and pod borer - the three most damaging problems affecting pigeonpea. Additionally, pigeonpea cultivation improves the soil and augments the income and nutrition of smallholders under low input agriculture.

Research Activities

1. Characterisation of the causal virus of Pigeonpea sterility mosaic disease.

- The PSMD cultures maintained at ICRISAT (Patancheru isolate) were used for virus characterisation. Modifications were made to the preliminary protocol in order to maximise virus yield and reduce host contaminants. The new purification protocol significantly improved the purity of virus preparations and can be completed in one working day. However, PPSMV purification remained difficult due to the unusual nature of the virus, to its instability *in vitro* and to its association with host components. The interfering host material affected subsequent enzymatic reactions used for analysis of viral proteins and nucleic acids. These factors have hindered complete virus characterisation.
- **PPSMV properties:** Purified preparations from PSMD-affected plants contained highly flexuous filamentous virus-like particles (VLPs) of 3-10 nm in width and of undefined lengths (Fig 1a). These preparations had a buoyant density of 1.22-1.23 g/cc in caesium chloride and contained a major virus-specific protein of c. 32 kDa and up to seven RNA species of size c. 6.8 kb (R1), 2.7 kb (R2), 2.1 kb (R3), 1.6 kb (R4), 1.4 kb (R5), 1.2 kb (R6) and 1.1 kb (R7) (Fig 1b,1c). Purified preparations also contained a 52 kDa host-derived protein. Methods to eliminate the 52 kDa host protein were unsuccessful.
- Some complementary DNA clones were constructed to viral RNA using random primers. Sequences of these cDNA clones detected no matches in database searches, indicating that they are novel. Two oligonucleotide primers, SM-1 and SM-2, derived from a *cd1.1* clone sequence were used to amplify a 321 bp product in reverse transcription-PCR (RT-PCR), for virus detection (Fig 1d).
- A proteomics approach using matrix assisted laser desorption and ionization-time of flight (MALDI-ToF) - mass spectrometry was used to identify the 32 kDa PPSMV-specific protein and the 54 kDa host-derived protein. This confirmed that the 52 kDa host-derived protein was the large chain of ribulose-1, 5-bisphosphate carboxylase (RuBisCo). Whereas the PPSMV 32 kDa protein found no matches, suggesting the novelty of PPSMV.
- Polyclonal antibodies were produced to PPSMV VLP preparations in a New Zealand White rabbit. The optimum working dilution of these antibodies was 1:5,000 in direct antigen coating (DAC)-ELISA; 1:15,000 for double antibody sandwich (DAS)-ELISA; and 1:10,000 for Western immunoblotting. Antibodies detected the PPSMV 32 kDa protein in infected plants by enzyme-linked immunosorbent assay (ELISA), and in Western immunoblotting (Fig 1b) (detailed in Output 2).
- Purified PPSMV VLP preparations were not infectious, but PPSMV was transmitted experimentally by mechanical inoculation from fresh leaf sap extracts of PSMD-affected pigeonpea to *Nicotiana benthamiana, N. clevelandii* and *French bean* (Fig 2). Virus infectivity was short lived in plant sap and transmission frequency was low. Virus transmission to French bean was also achieved using viruliferous mites. This is the first report of mechanical transmission of PPSMV and it infecting species outside the genus *Cajanus*. This finding is a breakthrough of considerable practical importance in understanding the nature of eriophyid mite-transmitted agents of unknown aetiology (see below).

- Ultrastructural studies of symptom-bearing leaves of pigeonpea (cv. ICP8863 and ICP2376) infected with viruliferous *A. cajani*, and *N. benthamiana* infected by mechanical inoculation, detected quasi-spherical, membrane bound bodies (MBBs) of c. 100-150 nm (Fig 1e). *In situ* immuno-gold labelling experiments using PPSMV antiserum specifically labelled the MBBs indicating that these structures contained the PPSMV 32 kDa protein (Fig 1e)**.** The MBBs were similar in appearance to those reported for plants infected with the eriophyid mite-transmitted High plains virus and the agents of unidentified aetiology associated with rose rosette, fig mosaic, thistle mosaic, wheat spot chlorosis and yellow ringspot of budwood, each of which is transmitted by eriophyid mites. The detection of MBBs in PPSMV-infected *N. benthamiana*, that are indistinguishable in structure and specific labelling from those found in infected pigeonpea, confirms the mechanical transmission of PPSMV. This is a significant breakthrough because several decades of work on eriophyid mite-transmitted agents of unknown aetiology have suggested that these agents are not transmitted mechanically and the nature of MBBs identified in affected plants in early 1970s were undetermined. Our study has shown that the MBBs are pathogen derived and contains nucleoprotein, indicating that they are virus-like structures, similar to that of tospoviruses.
- The transmission characteristics of PPSMV to pigeonpea by its vector, *Aceria cajani* were determined. The transmission efficiency of a single *A. cajani* was up to 53% but >5 mites per plant were required for 100% infection. *A. cajani* acquired PPSMV after a minimum acquisition access period (AAP) of 15 min and inoculated the virus after a minimum inoculation access period (IAP) of 90 min. No latent period was observed. *A. cajani* retained the virus for up to 13 h. Mites that developed from eggs, from PPSMV-infected leaves, did not transmit the virus. Therefore, the virus is not transmitted transovarially. The data suggest a semi-persistent mode of transmission of PPSMV by *A. cajani*. This is the first detailed study of an eriophyid mite-transmitted virus using single mites. The information obtained will be vital to understand PSMD epidemiology and control.
- The properties of PPSMV indicate that it is a previously undescribed virus with an unusual combination of properties. The PPSMV has some morpho-physical similarities to members in the genera *Tospovirus* and *Tenuivirus.* PPSMV shows most similarity with the recently described High plains virus (HPV) of wheat and corn and other eriophyid mite-transmitted agents (rose rosette and fig mosaic) that produce membrane bound-bodies in infected cells. Therefore, we conclude that PPSMV may constitute a species under a new plant virus genus.
- PPSMV biodiversity was assessed using a set of pigeonpea genotypes, ICP2376, 7035, 8862, 8863, 10976, 10984 and 11146. These were tested by infecting with the PPSMV isolates at Patancheru and Bangalore. Based on host reaction, the PPSMV isolate at Bangalore was shown to be different from that at Patancheru (Table 1). For instance, ICP2376, showed chlorotic ringspots (localised infection) to the Patancheru isolate, whereas it developed severe mosaic to the Bangalore isolate. Our study revealed that the majority of pigeonpea genotypes tested at Bangalore showed severe mosaic symptoms and a high PSMD incidence (detailed in Output 3). Similar tests in this and previous studies at other locations in India suggest that there are basically two types of PPSMV strains prevailing in PSMD endemic locations, a mild strain (at Patancheru and Gulbarga) and a severe strain (at Bangalore and Coimbatore). This information was most essential to evaluate genotypes for broad-based resistance.
- The severe strain of PPSMV from Bangalore was purified and compared with that of the milder strain at Patancheru. No differences were noted in the sizes and numbers of protein and nucleic acid species, in serological reactions and in the sequence of one RNA-4. Further virus characterisation is essential to determine the characteristics that can distinguish these virus strains.

2. Development of precise diagnostic tools for detection of PPSMV and differentiation of its biotypes.

- Purified PPSMV preparations from infected pigeonpea contained a 52 kDa host protein. Consequently, the polyclonal antiserum produced against these preparations also reacted with healthy pigeonpea sap. By cross-absorbing the antiserum with healthy pigeonpea leaf sap, the non-specific reaction was minimized without affecting significantly the detection of PPSMV (Tables 2.1, 2.2).
- Initially for virus detection, the direct antigen coating (DAC)-ELISA method was developed. ELISA plates were coated with leaf sap extracts and PPSMV polyclonal antibodies, diluted 1:5,000, and were used for virus detection. Anti-rabbit alkaline phosphatase (ALP)-labelled antibodies were used for detecting the bound PPSMV antibodies. Absorbance values taken at 405 nm (A_{405nm}) values greater than 2× those of healthy controls were considered as PPSMV positive (Table 2.1). However, longer incubation periods in DAC-ELISA resulted in background reaction, thus affecting the sensitivity of the assay.
- To increase the sensitivity of the assay and to render it cost-effective, double antibody sandwich (DAS)-ELISA using the penicillinase (PNC) detection system was developed. In DAS-ELISA, a 1:15,000 dilution of PPSMV polyclonal antibodies was used to coat ELISA plates followed by the addition of the test sap extract. The trapped antigen was detected by the addition of penicillinase (PNC)-labelled PPSMV-specific immuno-gamaglobulins (IgGs). The positive reaction was detected by adding sodium-penicillin-G substrate in bromothymol blue (BTB) buffer. The reactions were read in an ELISA plate reader fitted with a 620nm filter. Up to >8 fold difference in absorbance values were noted between healthy and infected reactions (Table 2.2). In this system, reactions can also be detected visually, as in positive reactions the blue colour of the PNC substrate turns to orange-yellow and in negative reactions the blue colour of the substrate remains unchanged (Fig 3). For PPSMV detection, the PNC-based DAS-ELISA offered greater sensitivity levels and negligible background. The PNC-based assay is inexpensive and the required chemicals are readily available in developing countries. Results can be recorded visually. Therefore this assay was adopted for the detection of PPSMV in plants.
- Oligonucleotide primers, SM-1 and SM-2 were used to detect PPSMV by RT-PCR. The primer pair amplified a 321 bp product corresponding to the RNA-4 segment of the PPSMV genome from total RNA extracts from virus-infected samples (Fig 1d). In addition to this, immuno-capture RT-PCR (IC-RT-PCR) was developed. In this the virus was first trapped from crude plant extract using PPSMV polyclonal antibodies, trapped virus particles were then disrupted by heat treatment to release viral RNA, which was then amplified by the SM-1 and SM-2 primers. The IC-RT-PCR circumvents the need for a separate RNA isolation from test material and is cost effective and highly specific.
- Assessment of virus infection by ELISA indicated that, different PSMD-affected pigeonpea genotypes showed markedly different PPSMV concentrations (Tables 2.3, 2.4). ELISA for PPSMV also detected the virus in the experimental hosts (*N. benthamiana*, *N. clevelandii* and *Phaseolus vulgaris*) and in extracts of groups (>20) of *A. cajani* from PSMD-affected pigeonpea. No reaction was detected with leaf sap from pigeonpea affected with *Mungbean yellow mosaic virus*, *Fusarium* wilt, powdery mildew and in *N. benthamiana* plants infected with *Raspberry bushy dwarf virus* (RBDV) and *Tobacco mosaic virus* (TMV), indicating the specificity of the PPSMV antibodies.
- PPSMV antibodies and RT-PCR primers SM-1 and SM-2, detected the virus in all PSMD-affected plants tested in various locations from India, indicating that these assays are broadly specific. Due to the apparent lack of major differences in the properties of mild and severe strains of PPSMV, isolate specific detection tools were not developed.
- For PPSMV strain identification, a bioassay using ten pigeonpea genotypes that showed differential reactions to the two strains were established. Based on the host reaction, PPSMV isolate at various locations could be identified as mild or severe. Although this assay takes 3-4 weeks to complete, in conjunction with detection tools, it provided an accurate identification of PPSMV isolates.

3. Identification and evaluation of genotypes with broad-based resistance to more than one biotype of Pigeonpea sterility mosaic virus (PPSMV)

- A new method was established for rapid screening of pigeonpea genotypes and to evaluate the 'type' of resistance offered by the test plants. Plants were raised in growth chambers and inoculated at the two-leaf stage with mites by the leaf-stapling technique. Plants were monitored for disease symptoms and tested for PPSMV by DAS-ELISA. Resistant genotypes (asymptomatic and ELISA negative) are tested again by graft inoculation. As PPSMV is not mechanically transmissible to pigeonpea, graft transmission tests were made to confirm its resistance to virus. For this purpose, a more efficient graft inoculation method was developed. Genotypes were also monitored for mite populations. Large scale testing of genotypes was performed in the field. Individual plants were inoculated with mites using the leaf-stapling method and plants were monitored as above. The complete resistance-screening scheme is depicted in Fig 4.
- New technologies developed for PSMD resistance screening were used for testing a range of pigeonpea germplasm currently held at ICRISAT following on international agreement with FAO. These include cultivated and wild *Cajanus* species and also breeding lines from crosses between wild and cultivated short duration pigeonpea genotypes.
    - Of the 138 cultivated genotypes tested against the Patancheru isolate of PPSMV, 51 were resistant to PSMD (Table 3.1). Several of these lines also possess resistance to *Fusarium* wilt. These lines will be tested against the Bangalore isolate.
    - Of the 28 medium duration advanced breeding lines developed at ICRISAT, 17 were resistant (<10% PSMD incidence) to PPSMV mild strain at Patancheru, whereas only one line (ICPL96061) was resistant to PPSMV severe strain at Bangalore (Table 3.2). Seed of ICPL96061 is being multiplied for evaluation at other locations in India.
    - Of the 62 wild pigeonpea accessions tested, 24 were resistant to the mild strain of PPSMV and 32 were resistant to the severe strain (Table 3.3). Some of these wild accessions also possessed resistance to *Fusarium* wilt and pod borer infestation. These accessions are compatible for breeding with cultivated genotypes for developing broad-based multiple resistant pigeonpea genotypes with high yield potential. An accession of *Cajanus scarabeoideous* was used for crossing with a cultivated elite short duration pigeonpea cv. Pant A2. The progeny of crosses are now at the F2 stage. Several of them posses PSMD resistance and have the phenotype of cultivated pigeonpea. This work is continuing.
- High yielding and PSMD-resistant genotypes selected in these trials will be evaluated further at multi-locations to assess their performance under different agro-ecological conditions.
- One genotype, ICP7035, possessing broad-based PSMD resistance was tested along with a local variety TTB7 in three different farmers' fields at Ramnagar taluk in Bangalore district, during July '01-Feb '02. Genotype ICP7035 was completely free from PSMD infection and TTB7 showed 50-60% incidence. PSMD incidence in Ramnagar taluk ranged between 65-100%, in the year 2001-2002. ICP7035 withstood PPSMV severe strain infection in the prevailing epidemic conditions (Table 3.4). This genotype will be evaluated throughout India and Nepal.
- Resistance screening in this study revealed for the first time that there are genotypes that are (i) resistant to PPSMV and mites (ICP7035), (ii) resistant to mites, but not to PPSMV (ICP15688) (iii) resistant to PPSMV, but not to mites (ICP8113) and (iv) susceptible to PPSMV and mites (ICP8863). Using a combination of ELISA, mite transmission by leaf-stapling and transmission by grafting, it was possible to determine in 4 to 5 weeks, the precise nature of the genotype reaction to PSMD. This information is vital for developing broad-based resistance to PSMD.
- Work is in progress to study the inheritance of PSMD resistance using three susceptible (HY3C, TTB7 and ICP8863) and three resistant genotypes (ICP7035, ICP10976 and ICP6686) against the severe strain of PPSMV. The output from this study will be very useful in planning further breeding programmes for pigeonpea improvement.

4. Transference of technology to the relevant NARS and NGO's

- Direct partnerships with two NARS centres, UAS, Bangalore and SVU, Tirupati, was established. The technology developed was disseminated to these centres.
- Partnership was established with national programmes for the evaluation of promising pigeonpea genotypes by collaborating with the 'All India Coordinated Research Project on Pigeonpea (AICRPP)' of the Indian Institute of Pulses Research (India Council of Agricultural Research Organisation). Nominated staff from AICRPP were trained in the application of PSMD screening technologies at ICRISAT in June-July.
- A training course entitled, "Methods for the detection of Pigeonpea sterility mosaic virus and screening for sterility mosaic disease resistance" was run at the Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bangalore, India. The main objectives were: 1. To disseminate techniques for PPSMV detection to scientists in NARS; 2. To acquaint participants with screening techniques for the identification of broad-based resistance to PSMD; and 3. To provide information on selected economically important plant viruses in India, highlighting options for their management.
- Eleven candidates were nominated by the national systems for the course (Fig 5), from the University of Agricultural Sciences (UAS), Dharwad, Karnataka State; UAS, Bangalore, Karnataka State; Agricultural Research Station, Gulbarga, Karnataka State; ANGRAU, Hyderabad, Andhra Pradesh State; Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu State; and Sri Venkateswara University (SVU), Tirupati, Andhra Pradesh State.
- A course manual was prepared which described the main techniques. Techniques to screen cultivated as well as wild pigeonpea were also described. In the training course wherever possible locally made chemicals and reagents were used. The successful organisation of a training course in the NARS centre demonstrated the applicability of the technology in developing countries.
- Another training course was planned on, "PPSMV detection and screening for sterility mosaic disease resistance", at ICRISAT, Patancheru 502 324, India in August 2002. National centres of India (Northern States) and Nepal were asked to participate.
- Three NARS scientists received two months intensive training from May '02 at ICRISAT in all aspects of pigeonpea crop improvement.
- A stakeholders meeting was planned at ICRISAT in August to chart plans to maximise the uptake pathways in order to reap ample benefit from the project outputs, and to discuss the future agenda.
- Information obtained in this project has been published in peer-reviewed journals and presented at scientific meetings. Information brochures describing procedure summaries of important methods were produced and distributed to relevant NARS. Technical information was also placed in the public domains (Internet) for easy access to scientists and NARS.
- Promising seed material was supplied to the NARS for testing at their centres. In a pilot study, ICP7035, a genotype with broad-based resistance to PSMD, was tested in three different farmers' fields (Fig 6). Plans were made to evaluate ICP7035 seed material in farmers' fields at various other locations in India.

5. Supply of diagnostic tools and multiplication of seed material of promising PSMD-resistant genotypes

- PPSMV polyclonal antiserum and SM-1 and SM-2 primers were supplied to stakeholders. Personnel were trained in the application of DAS-ELISA for PPSMV detection and diagnostic tools and seed material were supplied to several scientists in NARS.
- Differential pigeonpea genotypes were supplied to NARS for evaluation of PPSMV strains present at their location.
- Plans were made to multiply seed of promising pigeonpea genotypes to NARS for evaluation and utilisation in their breeding programmes.

- Pigeonpea cv. ICP7035 (which possess broad-based resistance to PSMD) seed was supplied to NARS and to the farmers for “on farm testing”. ICP7035 seed is being produced in large quantities for distribution to farmers for “on farm evaluation” at various PSMD hot spot locations in India for evaluation during the 2001-02 sowing season.

Table 1: Reaction of pigeonpea host differentials to the Patancheru (mild) and Bangalore (severe) strains of PPSMV.

Genotype	Patancheru			Bangalore		
	[1]SMD incidence (%)	Symptom type	Mites/ leaf[2]	[1]SMD incidence (%)	Symptom Type	Mites/ leaf[2]
ICP2376*	93	RS	40	100	SM	23
C-11	55	*SM*	91	88	SM	36
ICP11164	7	RS + SM	72	88	SM	46
ICP8862*	0.0	NS	9	75	MM	2
Purple-1	14	SM	28	88	SM	23
ICP7035	0.0	NS	0	0.0	NS	0
ICP10976	14	RS + SM	59	41	MM	6
LRG 30	93	SM	57	85	SM	32
ICP8863	98	SM	96	93	SM	49
BDN-1	94	SM	79	100	SM	28

RS *- Ring spot,* ***MM*** *- Mild Mosaic,* ***SM*** *- Severe Mosaic,* ***NS*** *- No Symptoms*
[1]Plants were assessed for PPSMV by DAS-ELISA
[2]Mean of five replications
**Genotypes that showed differential reaction.*

Table 2.1. DAC-ELISA for the detection of PPSMV in pigeonpea sap extracts.

Antiserum dilution	*Not cross-absorbed*			Cross-absorbed[1]		
	Healthy	Infected	Ratio[2]	Healthy	Infected	Ratio[2]
1:5,000	1.01	1.33	1.3	0.07	0.31	4.3
1:10,000	0.78	1.53	2.0	0.04	0.16	4.5
1:15,000	0.89	1.58	1.8	0.03	0.12	3.7

[1] Antiserum absorbed with 10 mg/ml healthy pigeonpea leaf material.
[2] Ratio of A_{405nm} readings of PPSMV-infected and healthy pigeonpea leaves.

Table 2.2. DAS-ELISA for the detection of PPSMV in pigeonpea sap extracts.

Antiserum dilution for coating plates	*Not cross-absorbed*			Cross-absorbed[1]		
	Healthy	Infected	Ratio[2]	Healthy	Infected	Ratio[2]
1:5,000	0.701	0.133	5.2	1.095	0.055	19.9
1:10,000	0.899	0.110	8.1	1.097	0.055	19.9
1:15,000	0.887	0.104	8.5	1.118	0.054	20.7

[1]PPSMV rabbit-IgGs cross-absorbed with 10 mg/ml healthy pigeonpea leaves.
[2] Ratio of A_{620} readings of healthy and PPSMV infected pigeonpea leaves.

Table 2.3. Detection of PPSMV by DAC-ELISA in different pigeonpea genotypes inoculated with the Patancheru strain of PPSMV.

Pigeonpea genotype	Symptoms	A_{405} readings
ICP2376	chlorotic ring spots	0.34
ICP7035	none	0.27
ICP8136	none	0.21
ICP8113	none	0.23
ICP15225	none	0.03
ICPL85010	none	0.29
ICPL87119-13	mild mosaic	0.45
ICPL87119-14	none	0.28
ICPL99092	mild mosaic	0.65
BND-1	mild mosaic	0.48
Purple-1	severe mosaic	0.88
HPL24	mild mosaic	0.55
HRG-30	mild mosaic	0.40
ICPW15685	mild mosaic	0.71
ICP8863 (40 days post inoculation (dpi))	severe mosaic	2.5
ICP8863 (>75 dpi)	mosaic on new growth	1.3
ICP8863 (>140 dpi)	none	0.13
ICP8863 (new growth after ratooning)	severe mosaic	1.94
ICP8863	severe mosaic	1.97
ICP8863 (healthy control)	none	0.14
ICP8863 (healthy control)	none	0.17
ICP8863	mosaic	1.68

Table 2.4. Detection of PPSMV by DAC-ELISA in different pigeonpea genotypes inoculated with the Patancheru strain of PPSMV.

Location	A_{620}	PPSMV
1. ICP2376 (GKVK)	0.055	Positive
2. V-118 (GKVK)	1.015	Negative
3. 118 No (ICRISAT) (NS)	0.875	Negative
4. 114 (ICRISAT) (S)	0.075	Positive
5. V-138 (GKVK)	0.062	Positive
6. 104 (ICRISAT) (S)	0.073	Positive
7. V-132 (GKVK)	0.066	Positive
8. 104 (ICRISAT) (NS)	0.895	Negative
9. ICP2376 (GKVK)	0.056	Positive
10. V-105 (GKVK)	0.055	Positive
11. 106 (GKVK)	0.846	Negative
12. V-114 (GKVK)	0.920	Negative
13. V-106 (ICRISAT) (NS)	0.750	Negative
14. V-106 (ICRISAT) (S)	0.072	Positive
15. ICP7035 (GKVK)	1.120	Negative
16. TTB-7 (GKVK) (NS)	1.001	Negative
17. V-106 graft inoculated (GKVK)	0.121	Positive
18. V-104 (GKVK)	0.057	Positive
19. 114 (ICRISAT) (NS)	0.785	Negative
20. TTB-7 (GKVK) (S)	0.062	Positive
ICP8863 (Infected)	0.055	Positive
ICP8863 (Infected)	0.054	Positive
ICP7035 (Healthy)	0.984	Negative
ICP7035 (Healthy)	1.04	Negative

S = Symptoms; NS = No symptoms

Table 3.1: List of sterility mosaic disease (SMD) resistant pigeonpea genotypes identified by screening cultivated pigeonpea genotypes in the ICRISAT germplasm collection at Patancheru, during kharif 2001-2002.

Rahuri Material		
Accession No.	***Symptom Type***	**SMD incidence (%)**
PT-1037 (W)	NS	4.3
PT8208-1 (R)	NS	6.5
SMD and Fusarium common resistant screening trial		
ICP 6630	NS	0
ICP 9177	MS/RS	0
ICP 11438	NS	0
ICP 9576	MM	1.3
ICP 6974	NS/MM	0
ICPL 93179	RS	0
AWR 74/16	NS	0
ICP 12756	NS	3.4
ICP 12759	NS	0
KPBR 80-2-4	MM/NS	0
ICP 12730	NS	0
ICPL 93184	NS	0
ICP 8610	NS	0
PR5149	NS	0
ICP 14819	NS	0
ICH 732	NS	0
KPBR 80-2-2-1	NS	2.6
ICP 11436	NS	0
ICPL 85063	NS	0
ICP 11047	NS	0
ICPL 342	NS	0
ICP 6997	NS	0
ICP 12290	NS/RS	0
ICP 12755	NS	0
ICP 10984	NS/RS	3.2
ICP 8094	RS	0
ICP 12749	NS	0
ICP 14826	NS	3.2
ICP 9145	RS	0
ICP 8862	NS	2.7
SMD screening trial		
ICP 11206	NS	1.4
ICPL 93177	NS	0
ICP 11824	NS	3.1
ICP 11040	NS/RS	0
ICP 9689	NS/RS	0
ICP 11049	RS	0
ICP 10986	NS	0
ICP 11136	RS	9.1
ICP 11810	RS	0
ICP 11791	NS	4.3
ICP 9174	NS	0
AWR 74/16	NS	0
ICP 11812	RS	0
ICP 11845	NS/RS	14.3
KWR 1	NS	0
ICP 118838	NS	0
ICP 6953	RS	3.7
ICPL 94062	RS	0
ICP 2719	NS	0

RS *- Ring spot,* ***MM*** *- Mild Mosaic,* ***SM*** *- Severe Mosaic,* ***NS*** *- No Symptoms*
[1]Plants were assessed for PPSMV by DAS-ELISA
Genotypes that showed <10% infection were considered as PPSMV resistant.

Table 3.2: Evaluation of advanced medium duration pigeonpea breeding lines for PSMD resistance at ICRISAT, Patancheru and at Bangalore during kharif 2000-2001.

Genotype	Patancheru			Bangalore		
	[1]SMD incidence (%)	Symptom type	[2]Mites/ leaf	[1]SMD incidence (%)	Symptom Type	[2]Mites/ leaf
ICPL 93001	18	RS	46	89	SM	37
ICPL 93003	8	*RS+SM*	3	88	SM	6
ICPL 87051	9	RS+SM	17	100	SM	26
ICPL 96047	16	RS+SM	79	100	SM	4
ICPL 96048	10	RS*	48	88	SM*	6
ICPL 99044	1	RS*	22	93	SM	36
ICPL 96053	9	RS*	89	91	SM	41
ICPL 96061	5	RS*	27	15	MM*	5
ICPL 99046	3	RS*	12	83	SM	8
ICPL 99047	8	RS+SM	31	57	SM	28
ICPL 99048	7	RS+SM	10	82	SM	41
ICPL 99049	7	RS+SM	10	78	SM	77
ICPL 99050	5	RS+SM	10	81	SM	21
ICPL 99051	3	RS*	8	64	SM	16
ICPL 99054	7	RS+SM	9	82	SM	48
ICPL 99055	6	RS+SM	6	88	SM	39
ICPL 96058	35	RS+SM	27	69	SM	82
ICPL 87119	60	RS+SM	84	88	SM	7
ICPL 99086	15	RS+SM	6	78	RS+SM*	23
ICPL 99087	11	RS*	48	68	MM+SM*	14
ICPL 99092	10	RS-SM	50	63	MM*	17
ICPL 99093	5	RS+SM	36	72	SM	5
ICPL 99096	13	RS+SM	10	94	SM	24
ICPL 99097	7	RS+SM	4	98	SM	17
ICPL 99098	9	RS+SM	70	100	SM	4
ICPL 99100	6	RS+SM	2	91	SM	8
ICPL 99101	10	RS+SM	2	87	SM	10
ICPL 99102	16	RS+SM	11	37	SM	27

RS *- Ring spot,* ***MM*** *- Mild Mosaic,* ***SM*** *- Severe Mosaic,* ***NS*** *- No Symptoms*
Plants were assessed for PPSMV by DAS-ELISA
[2]Mean of five replications
Genotypes that showed <10% infection were considered as PPSMV resistant.

Table 3.3: Screening of wild pigeonpea genotypes against the Patancheru (mild) and Bangalore (severe) PPSMV strains.

Genotype	*Patancheru*			*Bangalore*		
	[1]SMD incidence (%)	Symptom type	Mites/ Leaf [2]	[1]SMD incidence (%)	Symptom type	Mites/ Leaf[2]
ICP15614	0	NS	2	0	NS	0
ICP15650	67	SM	0	NT	-	-
ICP15683	100	MM-SM	3	0	NS	0
ICP15684	4	MM	2	0	NS	0
ICP15685	54	MM	0	0	NS	0
ICP15686	93	MM-SM	4	8	SM	3
ICP15687	40	MM-SM	3	13	SM	0
ICP15688	3	MM	0	11	SM	2
ICP15689	64	MM-SM	2	21	SM	2
ICP15690	58	MM-SM	15	33	SM	1
ICP15691	58	MM-SM	2	25	SM	2
ICP15692	15	MM-SM	2	5	SM	2
ICP15693	65	SM	3	9	SM	1
ICP15694	37	MM-SM	2	18	SM	1
ICP15695	5	SM	0	3	SM	0
ICP15696	35	MM	0	12	SM	2
ICP15697	0	NS	0	14	MM	3
ICP15698	43	SM	4	43	SM	1
ICP15699	12	SM	2	13	SM	1
ICP15700	0	NS	0	0	NS	0
ICP15701	0	NS	0	0	NS	0
ICP15702	0	NS	0	8	SM	0
ICP15703	8	MM-SM	0	0	NS	0
ICP15704	14	SM	0	16	SM	0
ICP15705	16	MM-SM	1	5	SM	0
ICP15706	21	SM	3	9	SM	1
ICP15707	5	MM	0	0	NS	0
ICP15708	0	NS	0	26	MM	0
ICP15709	0	NS	0	33	MM	0
ICP15710	87	SM	5	27	SM	2
ICP15711	59	MM	0	5	SM	0
ICP15712	0	NS	0	0	NS	0
ICP15713	13	MM-SM	2	21	SM	4
ICP15716	80	MM	9	0	NS	0
ICP15717	13	MM	0	19	SM	1
ICP15718	81	SM	18	21	SM	2
ICP15719	83	MM-SM	6	30	SM	2
ICP15720	91	SM	14	22	SM	3
ICP15721	83	MM-SM	2	6	MM-SM	3
ICP15722	95	MM	0	3	SM	0
ICP15723	79	SM	14	39	SM	4
ICP15724	83	MM	4	0	NS	0
ICP15725	5	MM	0	0	NS	0
ICP15726	0	NS	0	4	MM	0
ICP15727	69	MM-SM	7	12	SM	2
ICP15728	0	NS	0	0	NS	0
ICP15729	27	MM-SM	8	3	SM	0
ICP15730	12	SM	3	30	SM	2
ICP15731	65	SM	3	42	SM	3
ICP15732	23	MM	1	33	SM	2

ICP15733	68	MM-SM	20	7	MM	2
ICP15734	0	NS	0	0	NS	0
ICP15735	100	MM-SM	0	14	SM	0
ICP15736	4	MM	2	0	NS	0
ICP15737	6	MM	0	9	MM	0
ICP15738	83	SM	9	33	SM	1
ICP15739	5	MM	0	0	NS	0
ICP15740	5	MM	0	0	NS	0
ICP15741	4	MM	0	8	MM	0
ICP15742	9	MM	0	75	SM	1
ICP15743	0	NS	0	27	MM	0
ICP15744	18	MM	1	46	SM	0

RS *- Ring spot,* ***MM*** *- Mild Mosaic,* ***SM*** *- Severe Mosaic,* ***NS*** *- No Symptoms, NT Not tested*
[1]Plants were assessed for PPSMV by DAS-ELISA
[2]Mean of five replications
Genotypes that showed <10% infection were considered as PPSMV resistant.

Table 3.4: On-farm testing of sterility mosaic disease (SMD) resistant pigeonpea genotype ICP7035 in Bangalore district, Karnataka, in the growing season 2001-2002.

Farmer's name and location[1]	Area sown (in guntas)		Plants tested		SMD incidence[2] (%)		Mite count[3]
	ICP7035	TTB-7	ICP7035	TTB-7	ICP7035	TTB-7	
Lingaiah ***Beere Gowdana Doddi village*** Ramanagar taluk	3	3	2592	3146	0	50.6	5.7
Papanna ***Chennegowdana Doddi village*** Ramanagar taluk	3	3	1890	2841	0	57.7	4.2
Hemagiriyaiah Adishaktihalli village Ramanagara taluk	***2.5***	***2.5***	***1855***	***2288***	***0***	***52.6***	***3.7***
Other farmers' field							
Field 1	-	***2 ha***	-	-	-	***90.1***	-
Field 2	-	***1 ha***	-	-	-	***70.8***	-
Field 3	-	1/2 ha	-	-	-	65.5	-

Date of sowing: 1, 2 on 20.7.2001 and 3 on 31.7.2001
Date of final observation: 2.12.2001
[1]PPSMV severe strain prevail in this region
[2]Plants assessed for PPSMV infection by DAS-ELISA
[3]Mean of 25 leaflets

Outputs

- The isolation and characterisation of PPSMV have resolved the aetiology of PSMD. The characterisation of PPSMV revealed that it is a novel virus with an unusual combination of properties. This information is a significant scientific contribution to the field of virology and is useful in understanding several other eriophyid mite-transmitted diseases of undefined aetiology.

- Knowledge gained on PPSMV led to the development of diagnostic tools necessary for its detection and have laid the foundation for further research to differentiate biotypes.

- Information on virus properties and the development of diagnostic tools allowed the project to devise strategies for selecting broad-based PSMD resistant sources and to understand the epidemiology of this serious disease of pigeonpea in the Indian sub-continent.

- Serological and nucleic acid-based assays for the unambiguous diagnosis of PSMD were developed. DAS-ELISA using the PNC detection system is sensitive, cost-effective and most suitable for application in developing countries. Biological assays using differential pigeonpea genotypes was standardised for the identification of PPSMV strains. These tools are essential to the identification of broad-based resistant sources.

- Diagnostic tools and efficient virus inoculation methods have made it possible to evaluate precisely broad-based PSMD resistance. Several cultivated pigeonpea genotypes evaluated possess resistance to the mild strain of PPSMV and fewer genotypes showed resistance to both mild and severe strains. One such genotype, ICP7035, possessing broad-based PSMD resistance and economic traits, was selected for release to the farmers for adoption. Broad-based resistance in several wild *Cajanus* accessions, some of which also possess resistance to *Fusarium* wilt and pod-borer were identified. These accessions are now being utilised in breeding programmes to develop high yielding pigeonpea genotypes with broad-based resistance to PSMD, wilt and pod-borer problems.

- Dissemination pathways were established by partnerships with stakeholders. The developed technologies from this project were transferred to the beneficiaries through partnerships with targeted national institutes, organisation of training courses for the NARS, and organisation of farmer-participatory trials to evaluate genotypes with broad-based resistance.

- Sufficient quantities of diagnostic tools (antibodies and oligonucleotide primers) and seed material of promising genotypes and differential cultivars were distributed to the NARS involved in PSMD research. Seed of promising genotypes, especially ICP7035, are being multiplied for distribution to plant breeders in NARS and to poor farmers in PSMD endemic areas.

Contribution of Outputs to developmental impact

The goal of the project was to develop technologies for sustainable pigeonpea production through the development of pigeonpea cultivars with broad-based resistance to PSMD.

PPSMV characterisation (Output 1) led to the development of diagnostic tools for the detection of virus and the identification of biotypes (Output 2). This knowledge was utilised to establish an efficient PSMD screening strategy for the precise selection of broad-based resistance sources (Output 3). This technology was used in a participatory manner with NARS to evaluate several cultivated and wild pigeonpea accessions for PSMD resistance at various agro-ecological conditions (Outputs 3 and 4). This led to the identification of several genotypes in cultivated and wild pigeonpea possessing resistance to more than one PPSMV strain (Output 3), thus demonstrating the potential of the technologies developed in this project. The seed of the promising genotypes were tested on-farm in farmers' participatory trials (Outputs 4 and 5). Pathways were established for the dissemination of the developed technology and products to the NARS, NGOs and farmers through participatory research, training courses and farmer participatory trials (Outputs 4 and 5). Thus the outputs of the project worked in a cascading manner leading from one output to another, ultimately leading to the achievement of the project goal. This is depicted schematically in Fig 7.

For effective utilisation of these recently developed technologies and the dissemination of promising seed material for cultivation by farmers, further work in collaboration with national programs and extension workers, is essential to:

- Further evaluate several pigeonpea genotypes which are resistant to mild and/or severe PPSMV strains and to other biotic stresses such as wilt;
- Selection of promising genotypes suitable for various agro-ecological conditions in the sub-continent for adoption by local farmers;
- Test several lines present in the global germplasm for broad-based resistance;
- Bulk up seed of promising sources for cultivation by farmers;
- Obtain further information on PPSMV to distinguish the strains of this virus and their distribution in the subcontinent. This is necessary for the selection of appropriate cultivars.

Publications:

1	Peer-reviewed journal	KUMAR, P.L., JONES, A.T., FENTON, B., SREENIVASULU, P. and REDDY, D.V.R. (1998). Isolation of a virus associated with sterility mosaic disease of pigeonpea (*Cajanus cajan* (L.) Millsp). Indian Journal of Plant Protection 26:164-166.
2	Abstracts of Scientific Meeting	KUMAR, P.L., JONES, A.T., SREENIVASULU, P. and REDDY, D.V.R. (1999). Unfolding the mystery of the causal agent of pigeonpea sterility mosaic disease. Abstracts of the International Conference on Life Sciences in the Next Millennium, University of Hyderabad, India. pp. 65-66.
3	Abstracts of Scientific Meeting	KUMAR, P.L., JONES, A.T., SREENIVASULU, P. and REDDY, D.V.R. (1999). Characterisation of a virus associated with sterility mosaic of pigeon pea. Abstracts of the XIth International Congress of Virology, 9-13 August 1999, Sydney, Australia, pp. 90.
4	Book chapter	JONES, A.T. Eriophyid mite-transmitted viruses and virus-like agents of plants. In: Biotic interactions in plant-pathogen interactions. Jeger, M.J. and Spence, N. (Eds). CABI, UK.
5	Peer-reviewed journal	KUMAR, P.L., JONES, A.T., SREENIVASULU, P. and REDDY, D.V.R. (2000) Breakthrough in the identification of the causal virus of pigeonpea sterility mosaic disease. Journal of Mycology and Plant Pathology 30: 249.
6	Peer-reviewed journal	KUMAR, P.L., JONES, A.T., SREENIVASULU, P., FENTON, B. and REDDY, D.V.R. (2001) Characterization of a virus from pigeonpea with affinities to species in the genus *Aureusvirus*, family *Tombusviridae*. Plant Disease 85: 208-215.
7	Peer-reviewed journal	KUMAR, P.L., FENTON, B., DUNCAN, G., JONES, A.T., SREENIVASULU, P. and 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.
8	Conference abstracts	KUMAR, P.L., JONES, A.T., DUNCAN, G., ROBERTS, I.M. and REDDY, D.V.R. (2001). Characterization of a novel mite-transmitted virus associated with pigeonpea sterility mosaic disease. *Phytopathology* 91:S51.
9	Peer-reviewed journal	KUMAR, P.L., DUNCAN, G., ROBERTS, I.M., JONES, A.T., and 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.
10	Peer-reviewed journal	KUMAR, P. L., JONES, A.T. and REDDY, D.V.R. (2002). Mechanical transmission of Pigeonpea sterility mosaic virus. Journal of Mycology and Plant Pathology 32:88-89.
11	Peer-reviewed journal	REDDY, A.S., KULKARNI, N.K., KUMAR, P.L., JONES, A.T., MUNIYAPPA, V. and REDDY, D.V.R. (2002). A new graft inoculation method for screening resistance to Pigeonpea sterility mosaic virus. International Chickpea and Pigeonpea Newsletter 9.44-46.
12	Peer-reviewed journal	KUMAR, P.L., JONES, A.T. and REDDY, D.V.R. (2002). A novel mite-transmitted virus with a divided RNA genome closely associated with pigeonpea sterility mosaic disease 92: (accepted for publication).
13	Peer-reviewed journal	KULKARNI, N.K., KUMAR, P.L., MUNIYAPPA, V., JONES, A.T. and REDDY, D.V.R. (2002). Transmission of Pigeonpea sterility mosaic

virus by the eriophyid mite, *Aceria caja*ni (Acari: Arthropoda). Plant Disease 86: (in press).

Internal reports:

1	Internal report	Project activities and work-plan meeting. November 6, 1999. ICRISAT, Patancheru 502 324, India.
2	Internal report	1st Annual meeting. August 25, 2000. ICRISAT, Patancheru 502 324, India.
3	Internal report	Training course and 2nd annual meeting; January 3-12, 2002. Department of Plant Pathology, University of Agricultural Sciences, Bangalore, India.
4	Interim technical report	Jones, A.T., Kumar, P.L. and Reddy, D.V.R. (2002). Characterisation of the Causal Virus of Pigeonpea Sterility Mosaic Disease: A Step Towards Attaining Sustainability of Pigeonpea Production in the Indian subcontinent. 41 pp
5	MSc thesis	TIRUMALA-KUMAR. (2000). Studies on pigeonpea sterility mosaic disease and its management. University of Agricultural Sciences, Bangalore, India. MSc Thesis. 110pp
6	PhD thesis	KULKARNI, N.K. (2002). Studies on pigeonpea sterility mosaic disease: transmission, virus-vector relationships and identification of resistant sources. ICRISAT, Patancheru and University of Agricultural Sciences, Bangalore, India. PhD Thesis. 155 pp.
7	MSc thesis	VIJAYA-NARASIMHA, J. (2002). Field and molecular evaluation of pigeonpea genotypes for SMD resistance. University of Agricultural Sciences, Bangalore, India. MSc Thesis. (In preparation)
8	PhD thesis	NAGARAJ, K.M. Inheritance of resistance to Pigeonpea sterility mosaic virus in pigeonpea. (Work is still progressing)

Other dissemination of results, technology transfer activities etc:

1	Conference presentation	KUMAR, P.L., JONES, A.T. and REDDY, D.V.R. (2002). Properties of a novel eriophyid mite-transmitted virus from pigeonpea. In International Conference on Advances in Plant Virology. April 17-19, 2002. Homerton College, Cambridge, England, United Kingdom.
2	Conference presentation	KUMAR, P.L. (2000). A novel virus associated with pigeonpea sterility mosaic disease in India. In Association of Applied Biologist Conference on Advances in Plant Virology. September 20-22, 2000. Dundee University, Dundee, Scotland.
3	Electronic media	KUMAR, P.L., JONES, A.T. and REDDY, D.V.R. (2000) Sterility mosaic: The green plague of pigeonpea. Breakthrough in identification of the causal agent and strategies for sustainable pigeonpea production. Research brief for ICRISAT GREP Website. <www.icrisat.org>.
4	Information leaflet (also in internet)	KUMAR, P.L., REDDY, D.V.R. and JONES, A.T. (2000) ELISA for the detection of pigeonpea sterility mosaic virus. Procedure Summary. ICRISAT, 4pp. (also in www.icrisat.org)
5	Newspaper article	MARSH, J. (2000) Scot's discovery may end Third World famine. *Metro Scotland, Edinburgh 10 May.* (News magazine article)
6	Newspaper article	NICHOLSON, M. (2000) Scots in disease breakthrough. *Financial Times* 10 May. (Newspaper article)
7	Newspaper article	ANONYMOUS. (2000) Crop disease breakthrough. *Evening Telegraph – Dundee* 9 May. (Newspaper article)
8	Newspaper article	ANONYMOUS. (2000) *Scottish Farmer, Glasgow* 13 May. (Newspaper article)
9	Newspaper article	ANONYMOUS. (2000) Culprit caught. *Times Higher Educational Supplement, London* 12 May. (Newspaper article)

10	Newspaper article	AGRICULTURAL CORRESPONDENT. (2000) Combating pigeonpea mosaic disease. *The Hindu* 13 July. (Newspaper article)
11	Fact sheet	Rooting out sterility mosaic disease of pigeonpea. The United Kingdom and ICRISAT. *ICRISAT Information Flyer*, 4pp.
12	Newsletter	Causal agent of sterility mosaic disease identified. (2001) International Chickpea and Pigeonpea Newsletter.
13	Training course manual	KUMAR, P.L. JONES, A.T. and REDDY, D.V.R. (2002). Methods for the detection of Pigeonpea sterility mosaic virus and screening for SMD resistance – training course manual. ICRISAT Publication, Patancheru 502324, India, 47pp.
14	Training course manual	KUMAR, P.L. JONES, A.T. and REDDY, D.V.R. (2002). Methods for the detection of Pigeonpea sterility mosaic virus and screening for SMD resistance – training course manual. Version 2. ICRISAT Publication, Patancheru 502324, India, 65pp.
15	Information leaflet (also in internet)	KUMAR, P.L., JONES, A.T. and REDDY, D.V.R. (2002). Selection of durable resistance to pigeonpea sterility mosaic disease – Procedure Summary brochure. ICRISAT Publication, Patancheru 502324, India. (also in www.icrisat.org).
16	Training course	Organised a training course entitled **"***Methods for the detection of Pigeonpea sterility mosaic virus and screening for sterility mosaic disease resistance***"** at Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bangalore, India.
17	Training course (Planned)	Planned to organise training course on "PPSMV detection and screening for sterility mosaic disease resistance", in June 2002, at ICRISAT, Patancheru 502 324, India.
18	Seed and diagnostic tools	PPSMV diagnostic reagents and promising pigeonpea seed material distributed to NARS for utilisation in breeding programmes.
19	Seed	Pigeonpea cv. ICP7035 (possess broad-based resistance to SMD) seed material supplied to NARS and farmers for "on farm testing"

Biometricians Signature

The projects named biometrician must sign off the Final Technical Report before it is submitted to CPP. This can either be done by the projects named biometrician signing in the space provided below, or by a letter or email from the named biometrician accompanying the Final Technical Report submitted to CPP. (Please note that NR International reserves the right to retain the final quarter's payment pending NR International's receipt and approval of the Final Technical Report, duly signed by the project's biometrician)

I confirm that the biometric issues have been adequately addressed in the Final Technical Report:

Signature:
Name (typed):
Position:
Date: