CROP PROTECTION PROGRAMME

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

R8205 (ZA0522)

FINAL TECHNICAL REPORT

1st September 2002 – 31 December 2004

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31 January 2005

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
[(R8205) Crop Protection Programme]

Executive Summary

Pigeonpea is a major pulse crop of the dry tropics mainly cultivated under marginal conditions by millions of resource poor farmers in the Indian subcontinent. The crop is used in multipurpose ways and plays an important role in food security, balanced diet and is important in allowing the poor access to food and employment. Pigeonpea seed is the major dietary protein source for an estimated 1.1 billion people in the subcontinent. Sterility mosaic disease (SMD), endemic in the subcontinent is a major constraint on pigeonpea production and the economic well being of the poor farmers. Cultivation of broad-based SMD resistant varieties is most feasible and eco-friendly way to manage the disease. To achieve this, this project was undertaken to characterise the agent of SMD and its isolates and develop sensitive diagnostic tools and to select and supply broad-based SMD-resistant genotypes to the farmers and NARES.

- PPSMV characterisation has led to the development of diagnostic tools and identification of its biotypes, which were categorized into virulent and highly virulent isolates. Three PPSMV isolates were bio-chemically characterized and ELISA and RT-PCR based diagnostic tools developed. SMD epidemiology studied and critical factors contributing to inoculum survival during off seasons identified. Physiological affects of SMD on host plants, flowering and crop yield determined.

- An efficient SMD screening strategy for the precise selection of broad-based resistant sources was established. This method resulted in selection of six pigeonpea varieties (ICP7035, ICPL 87051, 99050, 96053, 96058 and 96061); six breeding lines (ICPL 83015, 93087, 93183, 93184, 95020 and 95024); and fifteen wild pigeonpea accessions (ICP 15164, 15615, 15626, 15684, 15688, 15700, 15701, 15725, 15734, 15736, 15737, 15740, 15924, 15925 and 15926), for on-farm evaluation and utilization in breeding programmes. ICP7035 has been approved for pre-release and ICPL 96058 and 96053 is being evaluated on-station for release to the farmers. Seed of these promising pigeonpea varieties were multiplied and supplied for farmer cultivation and to NARES as foundation and breeders seed.

- Training courses organized to NARES in virus detection and resistance screening methods, and to farmers in integrated management of SMD, wilt and pod borer. Two village-level seed production systems established for sustainable seed production of ICP7035 and ICPL96058. Field days were organized to increase awareness on SMD and to popularise SMD resistant varieties.

The project outputs led to more productive, efficient and eco-friendly systems for the management of SMD. This is contributing to the substantial increase in pigeonpea production, benefiting farmers nutritionally from consumption of the high-protein grain, and economically from sales of the high-value fresh peas and dry grain, thereby enhancing livelihoods of marginal farmers in the SMD endemic areas. Furthermore, pigeonpea cultivation increases soil fertility, leading to sustainability of farming systems. Additionally, the methods developed and information on PPSMV is of significant scientific contribution to the field of virology. Transfer of developed technology and products to NARES and farmers were attained through participatory research, training courses, on-farm trials and release of SMD resistant pigeonpea varieties through national programmes. These activities would sustain the research outputs ex-ante and facilitate further research on SMD and contributed to the development of elite pigeonpea cultivars. The project outputs resulted in several publications and attracted merit awards.

Background

The crop

Pigeonpea (*Cajanus cajan*) is a very important pulse crop in marginal farming systems adopted by smallholder farmers in the Indian subcontinent. Pigeonpea can be grown under wide climatic conditions and inter-cropped with any crop with no negative impact on the main crop. Its seed is the dietary protein source for an estimated 1.1 billion people, most of whom are poor and vegetarian, and cannot afford animal-based protein diets. Additionally, it contributes to fodder and fuel wood, a scarce commodity in dry tropics. Pigeonpea farming requires minimal external inputs, such as fertilisers and irrigation. It has tolerance to high temperatures and the deep extensive root system allows the plant to sustain drought conditions making it most suitable for cultivation in arid zones. It ameliorates soil by fixing atmospheric nitrogen and mobilises soil-bound phosphorous benefiting both the pigeonpea crop and subsequent crops in rotation. For this reason, pigeonpea is most popular and is the major pulse crop of the tropics and subtropics and is currently cultivated on 5.25 m ha globally, with nearly 90% of it being grown in subcontinent.

Pigeonpea plays an important role in livelihood, because every part of the plant provides economic returns to the subsistence farmer (Figure B1). This crop is a major source of income where surpluses are traded in both local and commercial markets. It is mainly cultivated for human food. The nutritious pigeonpea seed, which contains nearly 30% protein, is the major dietary protein source for millions of poor living in rural and urban populations in the subcontinent. It can contribute to adequate supplies of much needed protein for a balanced diet and have the immense potential to narrow the gap between per capita requirements and availability of dietary protein. Research during the last 3 decades, have developed several high yielding short and medium duration types that can fit into many cropping systems, including cereal based ones. This has turned pigeonpea into a high value crop, leading to increases in the area of cultivation from 2.18 m ha in 1950-51 to 5.25 m ha in 2002-03. Despite the increase in area of production, its productivity over the decades has not changed (Figure B2). This is mainly because of the susceptibility of this crop to biotic (sterility mosaic disease, wilt and pod borer) and abiotic (water logging, salinity and drought) stresses, the narrow genetic base of the available varieties, and the unavailability of quality seeds for farmers.

Economic importance of major biotic problems of pigeonpea

Among various biotic stresses, Helicoverpa pod borer (yield loss of worth US$ 310 million globally, estimated in 1993); sterility mosaic disease (yield loss of worth US$282 million in India and Nepal, estimated in 1993); and fusarium wilt (yield loss of worth US$ 76 million in India and eastern Africa, estimated in 1993) are most important. SMD is not lethal to pigeonpea and appears innoxious in the fields and disguise from detection, but it inhibits flowering rendering plants sterile leading to 40-90% yield loss. This disease occurs with regularity, with an annual incidence range between 10-100%. SMD alone is responsible for annual grain loss of c. 10% of total net production (valued over US$ 300 million).

Sterility mosaic disease

Sterility mosaic disease (SMD), first described in 1931, is a major disease limiting the pigeonpea production in the Indian subcontinent (Figure B3). The SMD causal agent is spread by the arthropod mite vector, *Aceria cajani* (Acari: Eriophyidae). The disease is characterized by sterility (complete or partial cessation of flower production), mosaic symptoms on leaves, excessive vegetative growth, severe stunting and reduction in leaf size. SMD symptoms vary depending on the pigeonpea genotype and are categorized into three types: (i) severe mosaic and sterility; (ii) mild mosaic with partial sterility; and (iii) chlorotic ring spots without any noticeable sterility. Yield losses caused in most genotypes by SMD occurrence during early in the season can lead to >90% crop loss. Annual losses due to SMD exceed US$300 million in India and Nepal alone. The SMD causal agent is not known but graft transmission experiments showed that it was an infectious agent. Under

natural conditions the eriophyid mite, *Aceria cajani* Channabasavanna (Acari: Arthropoda), transmits the agent. This mite is highly host-specific and is restricted to pigeonpea and a few of its wild relatives. The poor understanding of SMD and the lack of knowledge on the SMD causal agent have hindered research efforts to control this disease.

Vital breakthrough

Studies to determine if mite bio-diversity was responsible for the variation in host-plant resistance, using advanced molecular biological methods and DNA fingerprinting techniques indicated that there is no detectable biodiversity in the mite vector and that the variability in resistance observed at different locations was probably due to the occurrence of different SM pathogen strains and their interaction with the host (DFID Project R6407H). In simultaneous attempts, breakthrough was achieved in identification of the SMD causal agent, the *Pigeonpea sterility mosaic virus* (PPSMV). Subsequently PPSMV was characterized from the SMD-affected pigeonpea plants from Patancheru location, Inida (DFID Project R7452). This revealed that PPSMV is a novel virus with properties unrelated to any characterized virus (see Figure B4).

Studies on PPSMV

- Purified PPSMV preparations (Patancheru isolate) from SMD-affected pigeonpea plants contained highly flexuous filamentous virus-like particles (VLPs) of 3-10 nm in width and of undefined lengths.
- Purified VLP preparations from virus-infected pigeonpea and *Nicotiana benthamiana* had a buoyant density in cesium chloride of 1.22 to 1.23 g cc^{-1} and contained a major virus-specific protein species of c. 32 kDa and 5-7 segmented RNA species of about 6.8 to 1.1 kb, amounting to total genome size of about 16 kb.
- The sequence of some complementary DNA clones to RNA from purified VLP preparations had no significant matches in database searches. Two oligonucleotide primers derived from one such sequence, when used in RT-PCR assays, amplified a product of 321 bp specifically from SMD-affected pigeonpea plants.
- The purified PPSMV preparations from infected pigeonpea contained large amounts of the 52 kDa host protein. Consequently, the polyclonal antiserum produced in a rabbit against these preparations gave high ELISA values to healthy pigeonpea sap in ELISA. However, by cross-absorbing this antiserum with healthy pigeonpea leaf sap (1:10 w/v) the background reaction could be eliminated without affecting the detection of PPSMV.
- The polyclonal antibodies detected the virus using ELISA, and the virus-specific 32 kDa protein in Western immunoblotting (WIB). In such assays, the virus was detected consistently in all SMD-affected pigeonpea plant samples from several different locations in India, but not in samples from symptom-free pigeonpea plants from the same locations. In experimental studies, all pigeonpea plants inoculated with viruliferous *A. cajani* and those plants graft-inoculated with SMD-affected tissue, were infected with the virus as assessed by ELISA and WIB, but not any uninfected pigeonpea plants.
- The transmission characteristics of PPSMV to pigeonpea (*Cajanus cajan*) by its eriophyid mite vector, *Aceria cajani* were studied. The transmission efficiency of single *A. cajani* was up to 53% but was 100% when >5 mites per plant were used. *A. cajani* acquired PPSMV after a minimum acquisition access period (AAP) of 15 min and inoculated virus after a minimum inoculation access period (IAP) of 90 min. No latent period was observed and mites retained virus for up to 13 h. None of the mites that developed from eggs taken from PPSMV-infected leaves transmitted the virus indicating that it is not transmitted transovarially.
- Natural host range of PPSMV includes pigeonpea and few of its wild relatives only. Host range studies using a various herbaceous, crop and weed species by mechanical sap inoculation and viruliferous mite vectors revealed that PPSMV can be transmitted to French bean (*Phaseolus vulgaris*) with mite vector, and to *Nicotiana benthamiana* and N. *clevelandii* by mechanical inoculation. No other host was found infected. However, mechanical transmission to pigeonpea is not possible.

- Ultrastructural studies of symptom-bearing leaves of two pigeonpea cultivars, (ICP8863 and ICP2376) and *N. benthamiana* infected with PPSMV, detected quasi-spherical, membrane bound bodies (MBBs) of c. 100-150 nm and amorphous electron-dense material (EDM). These structures were distributed singly or in groups, in the cytoplasm of all cells, except those in conductive tissues. Fibrous inclusions (FIs), composed of randomly dispersed fibrils with electron lucent areas, were present in the cytoplasm of palisade cells and rarely in mesophyll cells of the two pigeonpea cultivars but were not detected in infected *N. benthamiana* plants. In the PPSMV-infected pigeonpea and *N. benthamiana,* immuno-gold labelling, using antiserum to PPSMV, specifically labelled the MBBs and associated EDM, but not the FIs. The MBBs and associated inclusions are 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.
- PPSMV has some properties similar to virus species in the genera Tospovirus and Tenuivirus and with the eriophyid mite-transmitted High plains virus (HPV) but is distinct from these and from all other characterized viruses. The combination of novel properties shown by PPSMV and HPV suggest that they may constitute species in a new genus of plant viruses.
- Evidence based on host-differentials indicated the occurrence of several PPSMV isolates differing in their virulence.

Virus characterization and development of sensitive methods for its detection and the differentiation of its biotypes led to the establishment of eco-friendly methods for the selection of durable SMD resistant sources. This facilitated for the first time, the precise screening of various pigeonpea accessions (cultivated, wild and breeding lines). *[For more details on PPSMV please refer to FTR of R7452 project; Kumar et al., 2003 (Phytopathology); Jones et al., 2004 (Plant disease) listed in the outputs].*

Project (R8205) objectives

To benefit from the vital scientific breakthroughs achieved on SMD, this project (DFID Project R8025) was undertaken to:

- Identify high yielding pigeonpea genotypes possessing broad-based resistance to SMD and also fusarium wilt.
- Evaluate SMD resistance in pigeonpea genotypes at various agro-ecological locations in farmer-participatory trials.
- Further characterise PPSMV and assess its bio-diversity in SMD-endemic areas to distinguish the strains of the virus and their distribution in the subcontinent, which is necessary for the selection of appropriate cultivars.
- Integrate developed technology into the national pigeonpea improvement programs through partnerships and training for sustaining project outputs.
- Multiply seed of promising resistant cultivars for supply to poor farmers in SMD-endemic regions and release of varieties through national programmes.

The economic impact of SMD and other biotic problems on pigeonpea production and the need for pigeonpea varieties with broad-based resistance to SMD to stabilize pigeonpea production has been highlighted in several peer reviewed publications and reports from national programmes. Furthermore, pigeonpea demand projection indicates an increase of about 30% by year 2010. To meet this demand average yields of pigeonpea have to be increased from present 650-700 kg/ha to 800-825 kg/ha. This task of enhanced production can be achieved by varieties resistant to diseases and pests.

Figure B1: Pigeonpea – all in one

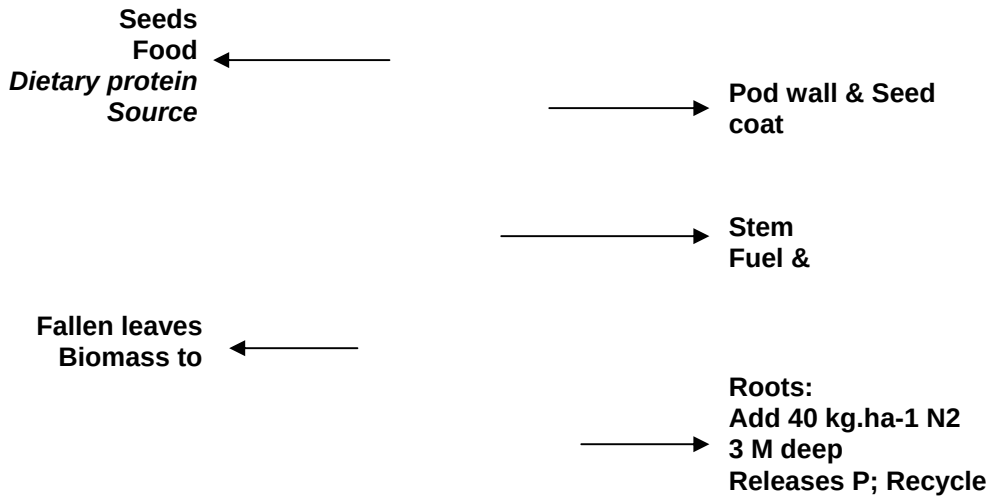
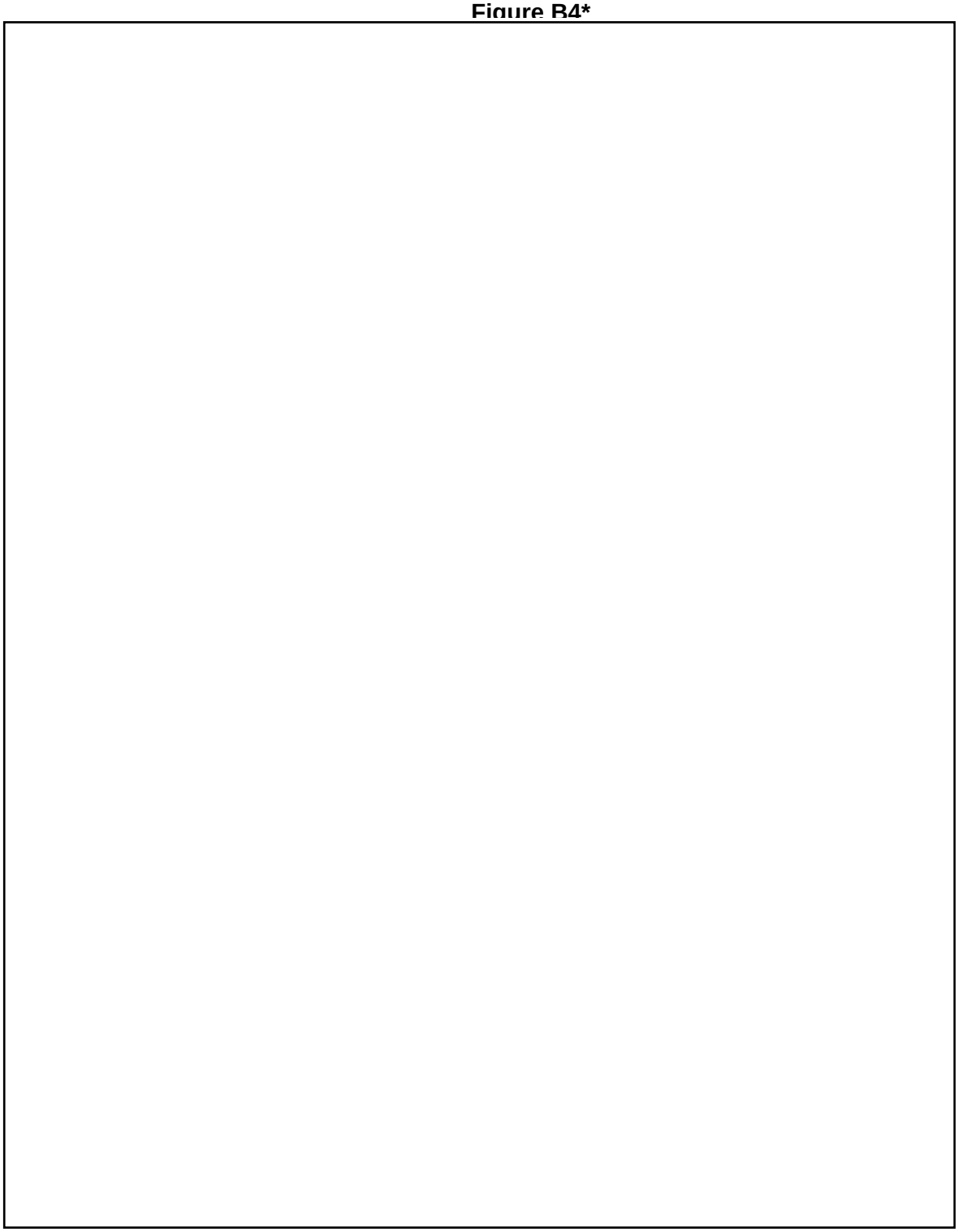


Figure B2: Production and productivity of pigeonpea since 1955 and 2000 (global). Note that production increase is proportional to increase in area, not due to productivity gains.

Figure B3: SMD-affected (left) and healthy pigeonpea (right) at maturity. Note lack of flowers on infected plants despite normal vegetative growth. Inset, SMD vector, *Aceria cajani*

Figure B4*



(a). Electron micrographs of purified preparations of PPSMV stained with uranyl acetate, pH 3.5.
(b). Resolution of proteins from purified PPSMV preparations in 12% denaturing polyacrylamide gel (1) and Western imuunoblots (2). Polyacrylamide gel was stained with silver and Western blot was probed with PPSMV antiserum. Lanes, pH, healthy pigeonpea; pI, PPSMV-infected pigeonpea; M, marker. PPSMV 32 kDa protein indicated with arrows. The 52 kDa host protein indicated with arrowheads.
(c). Resolution of RNA species from purified PPSMV preparations electrophoresed under non-denaturing conditions in 1% agarose gel. Gels stained with ethidium bromide.
(d). Resolution of RT-PCR products in 1% agarose gel. Lanes contain the PCR products from templates of total RNA extracts from PPSMV-infected pigeonpea (1) and *N. benthamiana* (3); total RNA from healthy pigeonpea (2) and *N. benthamiana* (4); and nucleic acid from purified PPSMV (5); lane M, marker.
(e). Immuno-gold labelled (IGL) sections of PPSMV-infected pigeonpea showing membrane bound-bodies (MBBs) and specific labelling of these bodies with PPSMV antiserum.

**Figure reproduced from FTR of R7452 project*

Project Purpose

The purpose of the project is to increase food security and improve livelihoods of poor farmers living in marginal farming systems by enhancing pigeonpea production through cultivation of elite SMD resistant pigeonpea varieties and the eco-friendly management of major biotic problems of pigeonpea.

This was achieved by the characterisation of the SMD causal agent and its biotypes and the development of diagnostic tools. These were utilized in selection and evaluation of genotypes to identify broad-based resistance to SMD and they were simultaneously evaluated for fusarium wilt. Through on-farm trials seed of SMD and wilt disease resistant varieties were made available to the needy farmers. Through grass-root level NGOs farmers were trained in integrated management of pod borer using bio-pesticides and judicial application of chemical sprays.

Pigeonpea varieties with durable resistance to SMD, combined with integrated management of fusarium wilt and pod borer would stabilise pigeonpea production and enhance yield potential at no extra cost to the farmers. Thus benefiting resource poor farmers living in marginal areas in the subcontinent, where pigeonpea is a major subsistence crop and women are involved in post-harvest processing and marketing. Scientists in NARES and NGOs, benefit from the technology developed in this project in handling a labile virus, and to develop high-yielding pigeonpea cultivars thereby providing a foundation for continued future progress.

Research Activities

A1.1. Screening wild and cultivated pigeonpea accessions, and breeding lines for broad-based SMD resistance against various PPSMV isolates

PPSMV culture maintenance:

- The test genotypes were evaluated for resistance against PPSMV isolates at Patancheru (P), Bangalore (B), Coimbatore (C), Gulbarga (G) and Varanasi (V), India. PPSMV cultures in each location were obtained from naturally infected pigeonpea plants several years ago and maintained subsequently in PPSMV-susceptible pigeonpea cultivars at research stations. Virus and mite cultures were maintained by periodically replacing old plants with young seedlings. PPSMV is not transmitted mechanically sap inoculation. Viruliferous mite vectors were used for the virus transmission by following the leaf-stapling technique.

Screening for SMD resistance:

- The evaluation of various wild and cultivated pigeonpea genotypes was based on the inoculation of plants with viruliferous mites, followed by testing of selected promising lines by petiole grafting to determine the specifics of resistance.

- Evaluations were done in greenhouses or on-station open fields, at locations where the respective isolates are endemic. These were: ICRISAT, Patancheru, for isolate P; the Department of Plant Pathology, University of Agricultural Sciences, Bangalore, for isolate B; and the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, for isolate C. In addition genotypes were also evaluated on-station at Agriculture Research Station (ARS), Gulbarga; ARS, Bidar and Banaras Hindu University, Varanasi. Evaluations were done in batches from 2002-04. The size of on-station/on-farm experimental plots ranged from 0.1 to 0.5 ha. Field operations followed for on-station trials were as per the package of practises of local research stations. Trials were maintained under rainfed conditions. In case of extreme drought, protective irrigation was given. No plant protection measures followed to control SMD or fusarium wilt. Depending on the pod borer incidence 0 to 3 chemical sprays were given in the experimental stations to manage the pest.

- As PPSMV is not transmissible by mechanical inoculation of sap, viruliferous *Aceria cajani* were used for virus inoculation to 12-20 day-old pigeonpea seedlings following the leaf-stapling technique. In this leaflets from SMD-affected plants infested with mites were stapled onto primary leaves of healthy seedlings. Mites from the source leaf migrate onto the test seedling to feed, and virus transmission takes place while feeding. For on-station field evaluation, test genotypes were evaluated in SMD nursery, where in infector-rows of SMD-susceptible genotype was established within the field. Infector rows were sowed 20 days in advance. These plants were inoculated with mites for establishing mite and virus cultures. These plants act as virus source for the test genotypes sown at later stage.

Detection of PPSMV in test plants:

- Disease incidence was recorded based on symptoms and ELISA for virus detection. A stereo-binocular microscope was used to record mite numbers on 5 young trifoliate leaves collected randomly from 5 plants of each accession at 60-70 dpi.

- To determine the type of resistance, most promising accessions that were resistant following mite inoculation were evaluated by graft inoculation using mite-free, PPSMV-infected, pigeonpea-petioles as scions (petiole-graft inoculation method). For this purpose, seeds of test accessions were sown in plastic pots and 25-35 day-old plants were used for graft inoculation. Test plants were maintained in mite-proof cages.

Observations on symptom type and percent disease incidence were recorded at 30 and 60 days post grafting.

ELISA for PPSMV detection:

- Polyclonal antibodies raised to PPSMV isolate P, which detects all the virus isolates studied (Table A4.2), were used to assay the test plants by double antibody sandwich (DAS)-ELISA, as per the method described in Kumar et al. (2002; Methods manual). From symptomatic plants, only young leaflets showing clear symptoms were selected but, from apparently healthy plants, young leaflets were chosen from at least three branches and pooled. Test leaves were extracted in phosphate-buffered saline (1:10 w/v), and 100 μl of this extract was loaded into wells of ELISA plates pre-coated with PPSMV polyclonal antibodies at 1:10,000 dilution. Penicillinase (PNC)-labelled PPSMV IgGs at 1:1,500 dilution were cross-adsorbed with healthy pigeonpea sap extract (1:10 w/v) for 45 min at room temperature to eliminate cross reaction to healthy antibodies. These were used in ELISA to detect trapped antigen. Sodium penicillin G was used at 0.05mg/ml in 0.015% (w/v) bromothymol blue buffer, pH 7.4. Optical density values at 620nm (A_{620}) were measured in an ELISA plate reader. Readings were considered to be virus positive if the absorbance values of a sample differed three-fold from those given by the virus-free control samples.

Genotypes evaluated

- *Accessions of wild Cajanus species:* Seeds of 115 accessions of 6 wild *Cajanus* species were obtained from the gene bank of ICRISAT (Table O1.1). Seeds were scarified by slicing the seed coat with a scalpel blade, treated with Thiram at 30 mg/10 g seed and sown in 21cm diameter plastic pots filled with Alfisoil in an insect-proof greenhouse. Pigeonpea cultivars ICP 8863, TTB-7 and Vamban-1 were used as virus susceptible controls, and ICP 7035 as the virus resistant control. The wild *Cajanus* species were evaluated for resistance against PPSMV isolates P, B and C. This evaluation was done in batches during 2002-04.

- *Pigeonpea breeding lines:* Thirty-eight pigeonpea breeding lines developed at ICRISAT using broad-based SMD resistant variety, ICP7035, as one of the parents, were evaluated on-station for resistance against P and C isolates during 2003-2005 (Tables O1.4 – O1.6). All these lines have short to medium duration maturity (100-170 days). These lines were evaluated on-station during 2003-04. Seed of uninfected plants from each line were pooled and tested again in 2004-05. Promising accessions selected were evaluated under greenhouse and lines that were resistant to SMD were transplanted in the field. These plants were bagged for selfing.

- *Cultivated pigeonpea accessions:* Forty-three different pigeonpea varieties were evaluated against three PPSMV isolates, P, B, C and V (Tables O1.7 to O1.10). Of these 22 were developed at ICRISAT and rest were from national programmes. Evaluations were done under greenhouse conditions and also on-station.

A1.2. Working group meetings and training courses to NARES NGOs and farmers

- Workshops and training courses were held at ICRISAT and NARES centres during the inception of the project and also during the course of project, involving all partners and stakeholders from NARES centres, NGOs and also farmers (see Figure A3.1 and Table C3 for list of various events organized).

A1.3. Evaluation of selected pigeonpea genotypes at various agro-ecological locations in NARS centres and on-farm.

- Forty-three pigeonpea genotypes were evaluated initially at four locations in Andhra Pradesh, Karnataka and Tamil Nadu states (Tables O1.7 - O1.10). From this, 5 genotypes were selected for further on-farm evaluation: ICP7035, ICPL 96058, 96053, 99050 and 87051. Agronomic traits of these lines were evaluated previously. In this study these lines were evaluated for SMD resistance.

- On-station trials of three new medium duration SMD and wilt resistant pigeonpea lines (ICPL 87051, 99050, 96061) were initiated in 13 locations in India (Ahmadabad, Gujarat; Bangalore, Karnataka; Warangal, Andhra Pradesh; Khargone, Madhya Pradesh; Dadnapur, Maharastra; Pusa, Bihar; Mahyco, Jalna; Rahuri, Maharashtra; Coimbatore, Tamil Nadu; two locations in Ranchi, Jarkhanda; Varanasi, Uttar Pradesh). These genotypes were evaluated in a common SMD and wilt sick nurseries being maintained at respective locations.

- Data from on-station trials conducted at ICRISAT indicated that these medium-duration lines performed well and out yielded the check ICPL 87119, a released variety being cultivated in central India. Of these, ICPL 96061 recorded 2.46 t ha-^{1} yield, nearly 30% higher than ICPL 87119 (1.72 t ha-^{1}). Data from other locations and that of ICP96053 and ICPL96058 are awaited. ICPL96061 will be tested on-farm in this cropping season.

- ICP7035 was selected for release in Karnataka state. This variety was evaluated on-station and on-farm for three years. This variety was evaluated as per the "Varietal Release Committee" procedures. ICP7035 has been approved for pre-release on-farm evaluation in Zone-5 agro-eco region in Karnataka state. The final release proposal was submitted to Zonal Research and Extension Advisory Committee (ZREAC). The committee has provisionally approved the release of ICP7035 in zone-5 region of Karnataka state, India.

A2.1. Extended farmer-managed trials of promising sources in SMD-endemic areas with emphasis on data on economic traits

- On-farm trials of broad-based resistant genotypes ICP7035, ICPL96058 and ICPL96053 were organised in SMD endemic locations in India. These trials were regularly monitored through NARES, NGOs, extension department and ICRISAT. Field trials of selected genotypes were organized during 2002-03, 2003-04 and 2004-05 rainy seasons. ICRISAT supplied seeds to the farmers. Farmers managed crop as per their regular practise.

- *Trials during 2002-03:*
  - ***Regional trial 1:*** In the year 2002-03 three genotypes (ICPL 96058, 96053 and ICP7035) were evaluated in farmers fields in SMD-endemic locations in Karnataka state. These varieties were managed as per the regular practice of concern farmers. Seed was supplied by the ICRISAT and the trials were monitored by the NARES and ICRISAT.
    - ICPL96058 was evaluated in three farmers fields at Dabarabad, Gulbarga Taluka (0.4 ha); Doginala, Aland Taluk (0.4 ha) and Kollur, Afzalpur Taluka (0.4 ha). ICPL 96053 was evaluated in farmers fields at Kollur, Afzalput Taluka (0.4 ha)
    - In these areas PPSMV-P isolate and wilt are endemic. The ICPL 96058 and 96053 is resistant to both the pathogens. Local variety ICP8863 (SMD susceptible, but wilt resistant high yielding variety) was used as control.
    - The year 2002-03 was a drought year. Due to severe water crisis crops suffered. The SMD incidence was between 5-80%. However, no SMD incidence was observed on ICPL 96058 and ICPL 96053.
    - The average yield of ICPL 96058 was 2450 kg.ha^{-1}, whereas control yields were 2190 kg.ha^{-1}. The average yield of ICPL 96053 was 1650 kg.ha^{-1}, whereas control was 1770 kg.ha^{-1}.

- Farmers were satisfied with the performance and seed quality, but ICPL 96058 and 96053 mature 2-3 weeks later than local variety, ICP8863. Due to delayed maturity (200-220 days), this variety was recommended for cultivation in deep black soils.

o ***Regional trial 2:*** ICP7035 was evaluated in 6 farmers' fields in Bangalore District Karnataka state. Adishaktihalli, Ramnagar Taluk; Chennappagowdadoddi, Ramnagar Taluk; Balagipadi, Kolar district; Nagamongali, Mandya district; Tiptur, Thumkur district; Kathalgae, Dhawangere district.
- These villages are SMD prone and PPSMV-B isolate (severe strain) was prevalent, for which there was no known resistance. Local variety TTB-7 (SMD susceptible, but wilt resistant high yielding variety) was used as control.
- It was a drought year and SMD incidence was very high (>80%). Under these conditions ICP7035 performed well. No SMD incidence was observed on the crop.
- Farmers were satisfied with the ICP7035 performance. Several farmers adopted this variety from these trials through farmer-to-farmer seed exchange.
- Average yield of ICP7035 was 1375 kg.ha^{-1}, where as TTB-7 was 1273 kg.ha^{-1}

- *Trials during 2003-04*
  - On-farm trials of the SMD-resistant genotype ICP7035 and of ICPL96058 and 96053, were conducted in 2003-04 under 9 broad-regional trails. Seed (270 kg of ICP7035 and 285 kg of ICPL96058) was supplied to farmers. In addition, seed of the local grown genotype, ICPL87119, ICP8863, LRG-30 and TTB-7 were also supplied to use as control checks to assess the relative performance of varieties (disease incidence, yield and quality of produce).
  - Overall, 244 trials in farmers' fields were conducted in major pigeonpea growing regions, where SMD is endemic. According to the local conditions and SMD strain, pigeonpea varieties were supplied to farmers to cultivate as per their usual practice. Farmers were supplied information on disease recognition, measures to control disease and cropping practises for enhanced production. Simultaneously, information is also supplied to control the pod borer pest.
  - Sowings were made in central India at regular period, whereas it was delayed in southern India due to the delayed monsoon. In some locations crop was affected due to lack of moisture at later stages of the crop growth.
  - Despite this, overall performance of the crop was satisfactory. In one location (3 trials) in Mahboonagar District, pre-mature flower dropping was observed in ICPL96058. Precise reasons for this were not known. Unusual low temperatures of 11 – 9 ^{0}C prevailed in this region, this might have had some affect on flower dropping. The overall incidence of SMD was low (0-30% incidence). In all trials new genotypes performed superiorly (up to 15% yield increase or on-par with control checks). Farmers were satisfied with the performance of ICP7035 and ICPL96058.
  - ***Regional trial 1:***
    State Karnataka; Districts Gulbarga and Aland; Trials 6; Total area: 3.6 ha
    Locations: Kamalapur, Dabarabad, Kollur, Aland, Kotaga and Marepally
    SMD and wilt resistant varieties ICPL96058; 0.25 ac Asha (ICPL87119)
    Local variety (as control): Maruti (ICP8863)
  - ***Regional trial 2:***
    State: Andhra Pradesh; District: Chittoor; Trials 7; Total area: 4.6 ha:
    Locations: Rompicherla, Penumur, B. Kotha Kota, Cheeku Chettu Palli, Gollapalli, (B. Kothakata M), Kandlamadugu (B. Kothakota M) and RARS, ANGRAU, Tirupati.
    SMD and wild resistant varieties: ICPL9058; ICP7035; ICPL96053;
    Local varieties (as control) LRG-30; TTB-7
  - ***Regional trial 3:***
    State: Andhra Pradesh: District: Mahaboonagar; Trials: 92; Total area 21 ha

Locations: Thodellagadda thanda, Venkatraopet, Metalkunta, Thurati and Singaraju palli
SMD and wilt resistant variety: ICPL96058
Local variety (as control): LRG-30 and Maruthi (ICP8863)

- ***Regional trail 4:***
  State: Karnataka; District: Bangalore and Tumkur; Trials 4; Total area 1.6 ha
  Locations: Magadi, Nelamangala, Doddaballapur and Tumkur
  SMD resistant variety: ICP7035
  Local variety (as control): TTB-7
- ***Regional trail 5:***
  State: Karnataka; District: Bangalore; Trials 19; Total area 3.4 ha
  Locations: Bangalore (Urban), Bangalore North, Bangalore South, Anekal, Bangalore (Rural), Tumkur, Gubbi and Cheluru
  SMD resistant variety: ICP7035
  Local variety (as control): TTB-7
- ***Regional trail 6:***
  State: Karnataka; District: Bangalore; Trials 1; Total area 10 ha
  Locations: Doddaganganwadi village, Ramanagar taluk
  SMD resistant variety: ICP7035
  Local variety (as control): TTB-7
- ***Regional trail 7:***
  State: Karnataka; Districts: Tumkur, Gulbarga, Bidar, Chitradurga, Mysore, Bangalore; Trials 16; Area 3.2 ha
  Locations: Chimmanachoda post (Chincholi Taluk, Gulbarga); Malenahalli (Shimoga); Maastikatte; Dodda Agrahaara (Shira Taluk, Tumkur); Harapanahalli (TalukDavangere); Hundimala (Hunsur Taluk, Mysore); Mudigere (Chikmaglur); Bidar; Hundimala (Hunsur Taluk, Mysore); Katihalli (Siddapura, Chitradurga); Hundimala Grama (Bilikere Hobli, Hunsur Taluk, Mysore); Anche (Bilikere Hobli, Hunsur Taluk, Mysore);Govindarajanagar (Bangalore); Chikkagangawadi (Doddagangawadi post, Kootgal hobli, Ramnagara); Chikkagangawadi (Doddagangawadi post Kootgal hobli, Ramnagara); Chikkagangawadi (Doddagangawadi post, Kootgal hobli, Ramnagara)
  SMD resistant variety: ICP7035
  Local variety (as control): TTB-7
- ***Regional trail 8:***
  State: Uttar Pradesh; Location: Varanasi; Trials: 2; Total area 1.2 ha.
  SMD resistant variety: ICP7035
  Control: ICP8863
- ***Regional trail 9:***
  State: Andhra Pradesh; District Mahaboonagar; Trails 97; Total area 20 ha
  Locations: Kasturipalli (Kodangal mandal) and Botlavoni thanda (Bomraspeta mandal), Mahaboonagar
  SMD and wilt resistant varieties: ICPL96058 and ICP7035
  Control: ICP8863

- *Trials during 2004-05*
  - In the year 2004-05, a total of 86 on-farm trials were organized in Andhra Pradesh and Karnataka.
  - All these trials were taken up during June-July-August ’04, depending on the rainfall. In some locations, following initial rains, a long dry spell followed that has affected the crop growth. However, late rains in September have rescued most of these trials. Test varieties are performing well. Crop is being harvested now. A detailed report on the on-farm trials will be submitted later.
  - ***Regional trial 1***
    Thirty-five ICP7035 on-farm trials in Zone-5 agro-ecological regions of Karnataka state are being organized by the Karnataka State Extension Department (KSDA),

University Extension Education Unit, Directorate of Extension and University of Agriculture Sciences Bangalore.

- ***Regional trial 2***
  Forty-six on-farm trials of ICPL96058 are being organized in the Kasturipalli and Indhanoor villages in Mahabubnagar district of Andhra Pradesh.
- ***Regional trial 3***
  Seven on-farm trials of ICPL96058 and ICPL96053 are being organized in Gulbarga and Bidar districts of Karnataka.
- ***Regional trial 4***
  Forty-five on-farm trials of ICPL96058 and ICP7035 are being organized in the Northern regions of Andhra Pradesh.
- ***Regional trial 5***
  Sixty on-farm trials of ICP7035 (28 trials), ICPL96058 (16 trials), ICPL87051 (16 trials) are being organized in Telangana region of Andhra Pradesh state
- ***Regional trial 6***
  Seven large-scale on-farm trials organized for the multiplication of IPC7035 and ICPL96058 seed for distribution to the farmers

A2.2. Multiplication of high-yielding pigeonpea genotypes with broad-based SMD-resistance and germplasm lines for distribution to farmers and breeders

- ICPL96058 and ICP7035 genotypes were multiplied for seed purpose in 3 ha and 1.5 ha, respectively, in Mahyco Pvt. Ltd. farms at Rudrur, Nizamabad District, AP, during 2002-03. Four hundred and fifty kg of ICP7035 and 1750 kg of ICPL96058 seed was obtained from this trial (yield after stringent quality maintenance). The seed produced was used for supplying to farmer's and state extension departments for adoption and further multiplication.

- ICPL 96058 and ICP7035, was multiplied in 0.32 ha and 0.5 ha at ICRISAT during 2002-03, and 740 kg and 300 kg of seed, respectively, was obtained from this seed multiplication trial. This was supplied to farmers and NARES in India, Nepal and also China.

- ICP7035 is being multiplied in 2.5 ha at ICRISAT for seed purpose. Standard crop management practises were followed. The fields were regularly monitored and off-types were removed to maintain the pure-stock. This trial is expected to yield 2,000 kg of seed, which will be distributed to the farmers and other stakeholders.

- Pigeonpea breeding lines: ICPL 11719(A), 11719(B), 14454, 14456, 14719, 14834(A), 14834(B), 16313, 16326(A), 16326(B), 16327 is being multiplied at ICRISAT (2004-05 season) for seed purpose. The plants were grown in glasshouse conditions and inoculated with PPSMV to eliminate susceptible lines. Healthy plants were transplanted in the field and they were bagged to prevent open pollination. This seed will be used for yield evaluation and supply to NARES breeders.

- Seed-villages for multiplication of pigeonpea seed at Thodaylagadda and Kasturipalli in Mahboonagar District, were established with NGO support. In this, a network of farmers multiplies pigeonpea genotypes for seed purpose. Standard crop management practises were implemented and isolation distance were maintained. The seed produced was collected and sold at nominal price to the local farmers. This ensures sustainability of seed production. ICP7035, ICPL96058, ICP8863, ICPL87115 are being multiplied under this scheme. National Watershed Development Programme, based at University of Agriculture Sciences, Bangalore, also undertook seed multiplication of ICP7035 for supply to farmers in watershed areas.

- Free seed vouchers of ICP7035 were distributed to farmers and extension officers during farmers' field days, to reclaim it for free seed during cropping seasons from NARES and ICRISAT (Figure A2.1).

A3. Dissemination and integration of technology into national pigeonpea improvement programmes

- This was achieved through collaborations and training courses (See Figure A3.1). Three SMD working group meetings: (i) At ICRISAT, Patancheru; (ii) Sri Venkateswara University, Tirupati; and (iii) Agriculture Research Station, Gulbarga, were organized during September-2002, April-2003, and May-2004, respectively. One international Pigeonpea Scientist meeting was organized at ICRISAT, Patancheru. Participants for these events included all the collaborating partners and other stakeholders from national and international programmes. The annual working group meetings were conducted to finalise work plans and review project progress. In these meetings, locations for genotype evaluation, locations for on-farm testing, data collection and its analysis, identification of any socio-economic constraints for uptake by farmers, status of seed distribution and requirements were evaluated and research priorities were identified.

- Various training courses were organized to scientists from NARES and NGOs and also to the farmers (see Tables C3 and C6 for details). These courses on SMD management were tailored to suite specific needs of targeted regions.

  Training to NARES and NGOs:
  - Students, researchers and extension officers from NARES and NGOs were given advanced training in virus disease management. These training courses were organized as one-day or 10-day events. In 10-day training course, to the selected personnel, hands-on training was provided in diagnosis of plant virus diseases, management of virus diseases and selection of host-plant resistance and seed multiplication. Special reference was made to the management of SMD. The training courses combined specific lectures from experts, laboratory and field demonstrations and hands-on training in application of various techniques. Detailed laboratory manuals with complete experimental details were prepared and supplied to the participants. Trainees were supplied with requisite diagnostic reagents for their end-use and also the seed of promising genotypes for evaluation and further perpetuation. The one-day training courses primarily consist of specific-lecture on disease diagnosis and management and laboratory and field demonstrations.

  Training to farmers:
  - For training farmers, emphasis was placed on integrated management of SMD, wilt (Fusarium wilt, endemic in the subcontinent) and pod borer (*Helicoverpa armigera*). Rational for this approach was, in the field these three biotic problems occur regularly. To sustain the yield gains attained through management of SMD, it is essential to manage other two problems, and vice-versa.
  - Our advocacy on management of SMD and wilt was through cultivation of resistant genotypes, and for pod borer management, a combination of traditional methods and judicious application of chemical sprays. The training module for on-farm implementation is given in the Figure A3.2. Application of trichoderma for management of wilt was optional, and implemented in only few farms. Pest management module promulgated was developed at ICRISAT IPM unit. In this project, this technology was adopted for promotion under 'Integrated Disease and Pest Management (IDPM)'. Information and training provided to farmers in post-harvest seed storage to protect seed from storage pests, and decorticating of seeds.
  - Farmers were given training in IDPM, and in preparation of neem-based products, chilli-garlic paste and Nuclear polyhedrosis virus (NPV) extracts for pest control

(details of these methods were described elsewhere and also in Chari et al. 2004). Training courses were organized as one-day events, at Agriculture Research Stations, NGO training halls, and on-farm during field demonstration visits. In these events farmers were given lectures in local language. Demonstrated the affects of SMD on pigeonpea and performance of resistant varieties. A small number of progressive farmers were selected and given intensive training in preparation of bio-pesticides using neem seed kernels (NSK) and chilli-garlic paste. This will be mixed with water and sprayed on crops. Farmer field days were organized to demonstrate on-farm performance of pigeonpea varieties to the farmers. All the farmers training programmes and field days were organized in collaboration with NGOs and State Extension Education Units. For such events information bulletins were prepared in local language and supplied to the farmers.

- Various products resulted from this project were disseminated to the collaborators and other stakeholders (listed in Tables C4, C5 and C6). Diagnostic reagents (polyclonal antibodies to PPSMV and oligonucleotide primers) were supplied to the NARES and to others upon request. Seed of promising varieties were made available to NARES, NGOs and farmers through on-station and on-farm trials. Some of these lines were integrated into national programmes through varietal release programmes (e.g. ICP7035, ICPL 96058 and ICPL96053). ICRISAT (antibodies, oligonucleotide primers and seeds) and NARES and NGO collaborators (seeds) will continue to supply limited quantities of these products upon request.

A4.1. Further characterisation of Pigeonpea sterility mosaic virus (PPSMV)

This study is in continuation of the work initiated in earlier DFID project (R7452; please see background notes for details).

Modifications to virus purification protocol

- The PPSMV cultures maintained in pigeonpea at ICRISAT (Patancheru isolate) were used for further characterisation of the virus. Minor modifications made to the previous described purification protocol (Kumar et al., 2003, Phytopathology) by altering the concentration of the detergent (Triton X-100) to reduce host contaminants were not effective. PPSMV purification remained as a difficult task due to the unusual nature of 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.

  A new experimental host, *Phaseolous vulgaris* var Topcrop (French bean), was used for culturing PPSMV using vector mites by leaf-stapling method. Although percent infection was low (30-60%), preparations had less host contaminants. The purified virus was used for producing polyclonal antibodies and also for characterizing viral nucleic acid.

Further characterisation of PPSMV genome

- A new cDNA library was constructed to RNA from purified PPSMV-P isolate preparations. cDNA was constructed by Gobbler and Hoffman method using random hexamers. Final cDNA product was cloned into pZeroKan or pGEM-T vectors, and transformed in *E. coli* Top 10 cells. cDNA library was screened for inserts and clones with >400 bp inserts were sequenced. Over 130 clones were screened for inserts and sequenced them. Sequences of most of the clones corresponded to host genome, especially, rRNAs (data not shown). Sequences of only 12 cDNA clones do not have any matches in the database searches (clone names listed in Table A4.1). The sequences of these clones were compared with each other. These revealed that all except four clones carry non-overlapping sequences. The clones C2 and C24, and B14 and B35 have overlapping sequences. The total length of the inserts present in the 12 clones amount to about 8 kbp. The non-redundant cDNA sequences obtained amount to about 30% of the total PPSMV genome. By northern hybridisation specificity of some of these clones were

confirmed. For instance cd1.1 corresponds to RNA-5, whereas C2 corresponds to RNA-2 of the PPSMV genome. Remaining clones are yet to be characterized. Due to lack of any homologies in the database searches, functions of the sequenced genes could not inferred. To understand functional analysis of PPSMV genes, obtaining full-length cDNAs for the individual segments of PPSMV RNA is essential. This data would also be useful to understand the gene functions and for comparative studies with other PPSMV isolates. Poor cDNA turnover and difficulties in obtaining sufficient quantities PPSMV RNA preparations were the main difficulties for this work.

Oligonucleotide primers SM-1 and SM-2 developed to amplified a 321 bp region (corresponding to RNA-5 of P isolate; accession no AJ439561) were used to amplify the target region from the viral RNA isolated from the B and C isolates. These were cloned into pGEM-T vector and sequenced and compared with P isolate (accession no AJ439561). The sequences of amplified parts of B and P isolates were similar. Whereas four nucleotide differences were found in C isolate (Figure A4.3), which were exploited to design another set of primers SMs-1 and 2 for specific detection of C isolate by RT-PCR.

PPSMV Isolates and their reaction on differential pigeonpea genotypes

- Severity of PPSMV isolates was studied by inoculating various isolates on to a set of differential pigeonpea genotypes (ICP2376, 7035, 8862, 8863, ICPL 10976, 10984 and 11146). The leaf-stapling technique was used for virus inoculations. PPSMV isolates from Patancheru (P), Andhra Pradesh state; Coimbatore (C), Tamil Nadu; Bangalore (B), Gulbarga (G) and Dharwad (D) Karnataka state; Varanasi (V), Uttar Pradesh state, India; and Nepalgunj (N), Nepal; were tested at respective locations. Plants were monitored for symptoms and virus infection by DAS-ELISA.

  The PPSMV isolates from C, B, V and N induced severe mosaic symptoms on all but ICP8862 and ICP7035 genotypes (Table A4.3). These isolates induced mild mosaic symptoms on ICP8862, and ICP7035 remained uninfected. The reaction of pigeonpea genotypes to P, G and D where also similar, except that ICP8862 was uninfected, and the ICP2376 showed localized chlorotic ringspots on inoculated leaves. From previous and current resistance screening work, it was know that C, B, V and N isolates can overcome resistance in most genotypes that offer resistance to P, G and D isolates (Table A4.3). Therefore, the resistance breaking isolates were regarded as “highly virulent” types. The resistance breaking isolates induced sever mosaic symptoms on ICP2376 (Table A4.3), whereas on the same genotype, P, G and D isolates induced chlorotic ringspot symptoms. This observation demonstrated that the genotype ICP2376 is useful to identify resistance breaking “highly virulent isolates”.

Purification and properties of PPSMV-C and B isolates

- The C isolate was purified from the SMD-affected pigeonpea cultures. The virus preparations contained 5-7 segments of RNA and a major virus-specific protein of 35 kDa (Figure A4.1). The RNA profile of C isolate was similar to that of P isolate (see Figure B4). The denatured 35 kDa protein of purified preparations was eluted from the 12% SDS-PAGE gels and immunized to a rabbit. The polyclonal antiserum and IgG fractions from it were tested for virus detection by direct antigen coating (DAC) (using Alakalinephosphatase detection system) and DAS-ELISA (using pencillinase detection system). There was no reaction either with healthy or infected leaf samples tested in ELISA (data not shown). In Westerns the same antibodies detected the virus specific 35 kDa protein when used at 1:500 dilution, but only weak reaction was noticed. To further boost the antibody titer, recently the same rabbit was given booster injections with purified PPSMV-C preparations. The animal will be bled for antibodies and assayed for virus detection.

  The diversity of the mite vector in the C isolate prevalent region was assessed using rDNA marker analysis using PCR-RFLP as described in Kumar et al. (2001, Annals of Applied Biology). This study revealed that rDNA profiles of *A. cajani* in C isolate regions are indistinguishable from those tested from the P isolate region and elsewhere in India (Figure A4.2).

The RNA-5 of C isolate was amplified using SM-1/SM-2 oligonucleotide primers designed earlier (Kumar et al., 2003, Phytopathology). They were cloned and sequenced. The sequences of these clones were identical to P isolate, except for 4 nucleotides in C isolate (Figure A4.3). These single nucleotide differences were consistently detected in sequences from 7 individual clones, indicating they were not due to *Taq* polymerase errors. Based on the 4 single nucleotide differences, a subset RT-PCR was developed for distinguishing C and P isolates. A new primer set, SMs1/SMs2, was designed for this purpose and used for PPSMV detection by RT-PCR in conjugation with SM-1/SM-2 primers (Figure A4.4). In this assay, SM-1/SM-2 primer set amplified target of 321 bp from all PPSMV infected tissues. Whereas the SMs1/SMs2 primers amplified a 164 bp product from C isolate only (Figure A4.4).

Transmission of the PPSMV-C isolate by *A. cajani* was studied using the methods described for PPSMV-P in Kulkarni et al. (2003, Plant Disease). The acquisition access period (AAP) and inoculation access period (IAP) of *A. cajani* were 10 min and 1 h, respectively. Mites when given APP periods of >15 h always resulted in 100% virus transmission. Four hours of pre-acquisition and post-acquisition starvation reduced the AAP and IAP to 5 min and 30 min respectively. Individual mites were able to transmit PPSMV, but only 42 per cent of the pigeonpea plants were infected. Plants inoculated with >10 mites always resulted in 100 per cent transmission. Observations on the mite population showed that the mites preferred SMD infected plants and their survival was poor on healthy plants. PPSMV was not transmitted transovarially to the next generation through eggs of viruliferous mites.

The PPSMV-C infected plant tissues were fixed, sectioned using microtome and they were stained with lead citrate and uranyl acetate as described in Kumar et al., 2002 (Annals of Applied Biology). Immuno-gold labelling studies (IGL) were performed using PPSMV-P antibodies. The infected cells revealed membrane bound bodies (MBBs) similar to those detected in PPSMV-P infected cells (Figure B4 and Figure A4.6). However, in IGL experiments, labelling of PPSMV-P antibodies was poor.

PPSMV-B isolate

- The PPSMV – Bangalore (B) isolate was purified. The preparations have a 32 kDa protein and RNA profile similar to that of P isolate (Figure B4). Polyclonal antiserum was produced against the 32 kDa nucleoprotein purified from 12% PAGE gels (Figure A4.5). The antibodies failed to detect the virus in DAC or DAS-ELISA. However, they detected the virus-specific protein in Western immunoassays (Figure A4.5). Further injections with purified PPSMV-B preparations will be given to boost the titer.

  Ultracytopathology of B isolate infected tissues revealed membrane bound bodies (MBBs), fibrous inclusions and electron dense material, similar to those observed in P isolate tissues (see Figure B4). In addition to these structures, crystalline arrays of rod-shaped structures were found in the infected cells, which were not observed in C or P isolate infected tissues (Figure A4.6). In IGL experiments using PPSMV-P antibodies, MBBs were specifically labelled.

A4.2. Surveys for SMD epidemiology

- *Survey in Northern Karnataka*

  About 80% of the total pigeonpea production in Karnataka state is confined to Bidar and Gulbarga districts. This region was regarded as “pigeonpea bowl” of Karnataka. SMD is a major problem in this area, and the disease was occurring in severe proportion every year. To understand the reasons for high disease incidence, surveys were conducted. SMD was assessed based on symptoms and random testing of plants by DAS-ELISA to confirm virus infection (Figure A4.7). A wilt resistant variety, ICP8863 (Maruthi), is extensively cultivated in this region (over 80% of area), which is highly susceptible to SMD. About 30-80% incidence was recorded in almost all farmers’ fields (Table A4.3). The reasons for increased epidemics of SMD in recent years could be due to the continuous cultivation of SMD-susceptible varieties over large areas, as a sole crop year

after year in the same fields. Additionally, farmers practise of leaving stubbles in the fields and cultivation of perennial pigeonpea within the sugarcane fields allows virus and mites to perpetuate during off season. They act as volunteer inoculum sources for new pigeonpea crop sown in the following season. Farmers in SMD hot spots were advocated to uproot stubbles and to cultivate SMD and wilt resistant varieties to reduce the SMD incidence in the fields.

- *Survey in southern Karnataka*
  Six districts in Karnataka state (Bangalore Rural, Tumkur, Hassan, Mysore Mandya and Chamrajnagar), and Chittoor district in Andhra Pradesh state, were surveyed and samples were collected from 40 different locations. Pigeonpea was observed in almost all villages in the surveyed districts. Most of the cultivars grown were medium to long duration types. TTB-7 and Hy3c are the predominant varieties. Samples were collected from infected plants and analysed by DAS-ELISA. SMD incidence was highest in Bangalore rural district (incidence between 40-90%) (Table A4.4). Whereas in Tumkur, SMD incidence was between 30-60%. SMD incidence was low (5-40%) in Mandya, Hassan, Mysore and Chamrajnagar districts. In these four districts majority of the fields were free from infection. SMD symptoms ranged from mild to severe. In areas of low disease incidence, plants showed mild mosaic symptoms on few branches. Interestingly, in some fields, only one or two branches of 4-5 plants showed mosaic symptoms and rest of the crop was free from infection.

- *Survey in Tamil Nadu*
  The PPSMV biodiversity within Tamil Nadu state was studied using differential pigeonpea genotypes (ICP 10976, ICP 2376, BDN-1, ICP 8862, C-11, ICP 8862, ICP 11164, Purple-1, ICP 7035 and LRG-30). Leaf samples from SMD-affected pigeonpea were collected from major pigeonpea growing areas of Tamil Nadu *viz*., Coimbatore, Namakkal, Vellore, Pudukttai, Dharmapuri and Tirunelveli, and the samples were inoculated to differential genotypes by leaf-stapling method. Plants were observed for symptoms and tested for virus by DAS-ELISA. The differential genotypes inoculated with various isolates in Tamil Nadu showed uniform reaction suggesting that these isolates may be similar to C isolate, at least in terms of virulence (Table A4.5).

A4.3. Studies to understand resistance mechanisms

Leaf morpho-metrics

- On most of the SMD-resistant pigeonpea genotypes mites were not found. However, several such genotypes could be infected by graft inoculation, indicating genotype susceptibility to the virus. This indicates that SMD-resistant genotypes may be resistant to vector, due to physical barrier or non-preference to feeding. To understand the role of physical barriers, such as cell wall, thickness of lower-leaf cuticle and epidermal cell wall, length and number of leaf hairs on susceptible and resistant genotypes were studied.

  Healthy leaves of SMD resistant (ICP 7035, H4-3C) and susceptible (ICP 8863, TTB-7) cultivars of pigeonpea were grown in glasshouse. Young, middle and old leaves were collected from 2-month old plants from same position from 5 test plants of each genotype. The leaf bits measuring 1 cm x 0.5cm were cut from the central portion of the leaf and embedded and fixed in resin, and thin sections were made using microtome. Using ocular microscope, thickness of leaf epidermis, cuticle and number of leaf hairs per sq cm^{-1} were determined. Leaf and the cuticle thickness of the SMD resistant genotypes are 3 to 8 times greater to that of susceptible genotypes. The length of leaf hairs and their density on the lower epidermis in resistant genotypes were low compared to susceptible genotypes (Table A4.5). The thick cuticle and epidermal cell wall may complicate feeding of mite feeding as they have short stylets of 1-2 μm. In addition, low density of leaf hairs may not provide adequate protection required for mites to withstand wind currents. Thus these two features probably play an important role in preventing mite colonization, multiplication on the resistant cultivars.

Movement of virus in the host plants

- Following inoculation with viruliferous *Aceria cajani*, the susceptible pigeonpea genotypes produce systemic severe or mild mosaic symptoms. However, very few genotypes have shown chlorotic rings spot (RS) symptoms, but such plants show no sterility and such genotypes were regarded as tolerant types (e.g. ICP2376). Studies were conducted to understand possible reasons for tolerant reaction. Two sets of ICP2376 plants were inoculated with PPSMV-P (Patancheru isolate) by the leaf-stapling technique. One set of plants was sprayed with acaricide to eliminate mites 48 hrs after inoculation. Plants sprayed with acaricide showed RS symptoms only on inoculated leaves, whereas unsprayed plants showed RS symptoms on new growth also. Careful observations revealed that virus multiplies in only those cells that were inoculated with virus by the mite vectors. Spread of mites within the plant was responsible for appearance of systemic RS symptoms, but not due to long distance spread of the virus. This was confirmed by testing, petioles, stems and leaf lamina for PPSMV by DAS-ELISA. The virus was detected only in leaf lamina, indicating that PPSMV do not enter into the phloem tissue, as virus was not detected in the petiole or stem fractions. Graft inoculation on ICP2376 did not result in infection, further suggesting that systemic spread of virus was lacking. However, this mechanism was found to be PPSMV isolate specific and so far was associated with PPSMV isolates such as P, G and D. ICP2376 when inoculated with PPSMV-B, C or V isolates, developed sever systemic mosaic symptoms, indicating that highly virulent PPSMV isolates have an ability to overcome tolerant reaction and therefore systemic spread within the host. Coincidentally, the isolates that were able to spread systemically in ICP2376, can also overcome the virus resistance in genotypes that offered resistance to P, G or D isolates. Thus, this interaction between the PPSMV isolate and ICP2376 can be exploited for the identification of “highly virulent” isolates.

A4.4. Studies on physiological affects of SMD

SMD-affect on pigeonpea seed and germination

- To understand the affect of PPSMV on flowers and seeds, samples collected from infected plants were analyzed for virus. Petals, sepals, anthers, ovaries, seed coats, pod coats and cotyledons were tested separately for PPSMV by DAS-ELISA. PPSMV was detected in all the floral parts, seed coats, pod walls, but not in the cotyledons. Grow-out test was performed with seeds collected from the infected plants. Based on the quality and size, seed from SMD-affected plants were sorted into three groups: (i) apparently normal, (ii) misshapen and pale seeds, and (iii) highly shrivelled seed (Figure A4.7). In grow-out tests, shrivelled seed has poor germination and vigour (60 - 70% germination), but plants were free from the virus (Figure A4.7). In general, up to 50% reduction in dry-weight of seed was observed from infected plants (Table A4.7). Although virus was not found in the seeds, but seed produced have quality damage, which accounted to reduction in weight as well as the germination. This is the first study that showed the SMD affect on quality, quantity and germination of the seed.
-

SMD-affect on plant physiology

- In healthy pigeonpea, at maturity axillary buds modify into floral structure. Whereas in SMD-affected plants axillary buds modify into leafy structures, contributing to the excessive vegetative growth. When plants were infected at early stage of the crop growth, entire plant developed SMD symptoms and the virus concentration was high in such plants. In case of late infections, symptoms were confined to fewer branches. Crop loss due to SMD in terms of biomass, yield and revenue at various levels of infection was studied in ICP8863 (Table A4.8). This indicated that maximum plant height (155.8 cm) and shoot dry weight (171.5g) was recorded in healthy plants. In case of plants that showed 2–4 symptomatic branches, plant height was 126 cm and shoot dry weight was 105.5g (20% and 39% reduction, respectively). Plants with 4-8 symptomatic branches

measured 116.9 cm in height and with a shoot dry weight of 86.15g. When infection occurred in entire plant, plant ht reduced to 100.5 cm and shoot dry wt. was 60.6 g.

The number of pods/plant (163.7), pod dry wt. (75 g), yield/plant (63.2 g) was recorded in healthy plants. In case of infected plants with 2–4 branches, pods per plant were 115.1; pod dry wt. was 41.4 g; and yield was 35.9 g. When infection occurred in 4-8 branches pods per plant, pod dry wt. and yield g/plant was 57.9, 22.3 g and 18.5 g, respectively. These values were least in case of plants with more than 12 and above branches infected (pods/plant 3.0, pod dry wt 1.3g and yield g/plant 0.8). The maximum loss in yield was observed in 12 and above branches infected plants (97.5%) followed by 8-12 branches infected plants (81.5%) and it was least when infection was in 2-4 branches per plant (26.6%).

Table A4.1. New cDNA clones developed to PPSMV-P isolate

Data base searches	No of clones	cDNA Clone name (kbp)*
No match	11 (22.9)	C2 (1.2), C4 (>0.5) C21 (>0.6), C24 (>1) C37 (>0.6), C38 (>1) C42 (>0.6), C85 (>1) B14 (>0.8), B35 (>0.8) F39 (0.7), F59 (>1),
PPSMV cd1.1 clone	1 (2)	F60

*Values in parenthesis are insert size

Table 4.2 Comparison of PPSMV polyclonals in detecting three PPSMV isolates by DAS-ELISA

Antibody[1] coating (dilution)	Sample	Isolate					
		ICRISAT		Bangalore		Coimbatore	
		1:10	1:100	1:10	1:100	1:10	1:100
1:5,000	Expt –1	0.168	0.208	0.156	0.336	0.170	0.256
	Expt –2	0.168	0.205	0.164	0.346	0.177	0.236
1:10,000	Expt –1	0.163	0.221	0.176	0.347	0.172	0.277
	Expt –2	0.163	0.234	0.190	0.466	0.165	0.316
Average		0.166	0.217	0.171	0.374	0.171	0.271
SD		0.002	0.013	0.014	0.061	0.004	0.034

OD of healthy pigeonpea 1.058 (Average from 8 wells); buffer control 1.578 (Average from 5 wells)
[1]PPSMV-P polyclonal antibodies; Detecting antibody: PPSMV-P IgG-labelled with pencillinase enzyme
OD readings measured at 620 nm. (Note: In penicillinase assay, OD_{620nm} values of virus positive samples will be lower than negative samples)

Table A4.3. Reaction of various PPSMV isolates on differential genotypes

Genotype	Patancheru (P)	Gulbarga (G)	Dharwad (D)	Bangalore (B)	Coimbatore (C)	Varanasi (V)	Nepalgunj (N)
ICP2376	**RS**	**RS**	**RS**	**SM**	**SM**	**SM**	**SM**
ICP7035	NS	NS	NS	NS	NS	NS	NS
ICP8862	NS	NS	NS	**MM**	**MM**	nt	nt
ICP8863	SM	SM	SM	SM	SM	SM	SM
ICP10976	MM	MM	MM	nt	SM	nt	nt
ICP11164	MM	MM	MM	SM	SM	SM	nt
BDN-1	SM	SM	SM	SM	nt	SM	nt
C-11	*SM*	*SM*	*SM*	SM	*SM*	-	nt
LRG 30	SM	SM	SM	SM	SM	SM	nt
Purple-1	SM	SM	SM	SM	SM	SM	nt

RS - Ring spot, MM - Mild Mosaic, SM - Severe Mosaic, NS - No Symptoms, nt = not tested.
Differential genotype reaction indicated in bold letters.

Table 4.4. Mean incidence of SMD in northern districts of Karnataka during 2000-01 and 2001-02[1]

District	Taluk	Area surveyed (ha)	% SMD incidence[2] 2000-01	% SMD incidence[2] 2000-02	Mean
Gulbarga	Gulbarga	766	20.5	24.2	*22.35*
	Aland	322	12.0	40.9	*26.45*
	Chincholi	161	48.0	58.2	*53.10*
	Afzalpur	129	12.0	14.1	*13.05*
	Mean	*344.5*	*23.12*	*34.35*	*28.73*
Bidar	Humnabad	242	42.0	53.2	*47.60*
	Bhalki	161	48.0	56.5	*52.25*
	Bidar	262	52.2	60.3	*56.25*
	Basavakalyan	153	40.3	42.3	*41.30*
	Mean	*204.5*	*45.6*	*53.0*	*49.35*

1. *SMD incidence was based on symptoms. Random samples were tested for PPSMV by DAS-ELISA Nearly 80% of the surveyed field contained Maruti variety; rest were local varieties (cultivar information unknown).*
2. *Incidence given is the average from each district.*

Table A4.5. Biodiversity study to assess the variation of PPSMV isoalte in Tamil Nadu during 2002-03

Genotype	Symptom type					
	Coimbatore (C)	Namakkal	Vellore	Pudukottai	Dharmapuri	Tirunelveli
ICP 10976	sm	sm	sm	sm	sm	sm
ICP 2376	sm	sm	sm	sm	sm	sm
BDN-1	sm	sm	sm	sm	sm	sm
ICP 8862	mm	mm	mm	mm	mm	mm
C-11	sm	sm	sm	sm	sm	sm
ICP 8863	sm	sm	sm	sm	sm	sm
ICP 11164	sm	sm	sm	sm	sm	sm
Purple 1	mm	mm	mm	mm	mm	mm
ICP 7035	ns	ns	ns	ns	ns	ns
LRG 30	sm	sm	sm	sm	sm	sm

sm – severe mosaic; ns – no symptoms; mm-mild mosaic

Table A4.6: Morpho-histological characters of PPSMV resistant and susceptible pigeonpea cultivars

Genotype	Total leaf thickness (μm)			Thickness of epidermis (μm)			Thickness of epidermis cuticle (μm)			Length of leaf hairs (μm)			Number of hairs per 10 μm leaf section		
	Young	Middle	Old	Young	Middle	Old	Young	Middle	Old	Young	Middle	Old	Young	Middle	Old
Resistant															
HY 3C	119.84	194.31	232.83	10.27	13.69	17.12	1.49	3.21	4.92	152.37	171.20	181.47	271.40	90.60	46.4
ICP 7035	141.24	228.55	274.78	11.13	17.98	21.40	1.92	5.35	7.06	136.1	153.22	159.22	123.40	80.60	38.8
Mean	130.54	211.43	253.81	10.70	15.83	19.26	1.70	4.28	5.99	144.23	162.21	170.34	197.40	171.20	42.60
Susceptible															
ICP 8863	78.75	162.64	219.99	7.70	9.42	11.98	0.21	1.07	2.35	184.04	186.61	253.38	305.40	217.40	156.40
TTB 7	102.74	163.56	229.41	8.56	12.48	15.34	0.64	2.60	3.53	155.79	178.90	231.96	563.00	350.40	184.60
Mean	90.74	163.10	224.70	8.13	10.95	13.66	0.42	1.83	2.94	169.91	182.75	242.67	434.20	283.90	170.50

Table A4.7. Affect of SMD on dry seed weight

Expt No.	100 g seed weight		% Reduction in seed weight
	Seed from Healthy plants	Seed from SMD-affected plants	
1	11.86	6.82	42.50
2	12.00	6.13	48.97
3	11.92	7.80	34.60
Average	11.92 (0.07)	6.90 (0.83)	42.11

ICP8863 (Maruthi) variety used; Plants were infected at 2 leaf stage (12-14 days).

Table A.4.8. Affect of SMD on plant growth and yield

Parameter	Healthy	SMD-affected plants Number of infected branches		
		2-4	4-8	>12
Plant Height (cm)	155.8	126	116.9	100.5
Shoot dry weight (g)	171.5	105.5	86.15	60.6
Pods / plant (no)	163.7	115.1	57.9	3.0
Pod dry weight (g)	75	41.4	22.3	1.3
Yield / plant 9g)	63.2	35.9	18.5	0.8
Reduction in yield (%)	0	26.6%	-	81.5%

Average from ten plant observations from ICP8863 taken at maturity from field grown plants in 2002 at Gulbarga, Karnataka state

Figure A2.1. Free seed voucher for 1 kg ICP7035

Figure A3.1. Various means followed for transferring technology and capacity building of to relevant NARE

Figure A3.2. Scheme for integrated management of diseases and pests (IDPM)

Management of SMD and Wilt

- Cultivation of common SMD and wilt disease resistant varieties: *Asha ICPL87119; ICPL96058; ICPL96053; ICP7035*
- Removal of volunteer SMD inoculum sources
- 5 kg *Trichoderma viridie* mixed with 125 kg farm-yard manure ha^{-1} to control wilt (*Fusarium udum)*

Management of insect pests

- Deep summer ploughing to control pest and to conserve soil moisture
- Random planting of maize and sorghum to attract insectivorous birds
- Mechanical shaking of plants to dislodge *Helicoverpa* larvae
- Spraying with:
  - Neem seed kernal (NSKE) @ 5% (w/v) to control insect pests
  - Nuclear polyhedrosis virus (NPV) @ 500 $L.E.ha^{-1}$ to control *Helicoverpa*
  - Neem oil @ 3% (v/v) to control *Helicoverpa*
- Pheromone traps [10 per ha] for monitoring *Helicoverpa* population
- Bird Perches @ 50 ha^{-1} to attract insectivorous birds

Figure A4.1. Western-immunoblot analysis of purified PPSMV preparations of PPSMV P and C isolates.

Proteins were resolved in 12% SDS-PAGE and probed with PPSMV-P polyclonal antibodies. Positive reactions were detected chromogenically using BCIP/NBT substrate. Note that 32 or 35 kDa protein is absent in purified preparations made from healthy pigeonpea (Lane H). The high molecular weight protein (c. 50 kDa) in health preparations is Rubisco Large Subunit.

Figure A4.2. Fingerprint of rDNA of *A. cajani* from

1 2 3 4 5 6 7 8

10% PAGE gel
(Stained with ethidium bromide)

Lane-1 Marker VIII (Roche)
Lane-2 Clone 17 (ICR) C-Caj2
Lane-3 Coim-3 (C-MB;ICR) C-caj2
Lane-4 Coim-2 mites Caj2-C
Lane-5 Coim-1 Caj2-C
Lane-6 Clone N (ICR Caj1-E) Caj3-E
Lane-7 Coim-3 Caj3-E
Lane-8 Coim-2 Caj3-E

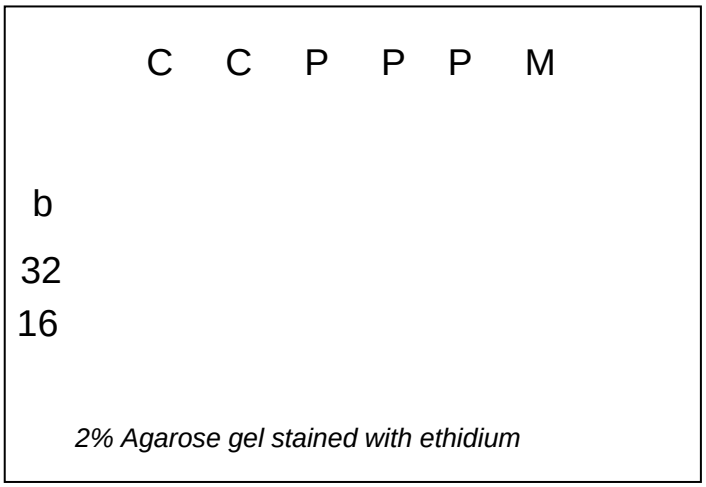
ICR: Clones of A. cajani from Patancheru
Coim: rDNA of mites from Coimbatore
DNA in lanes 2-5 were amplified with C-Caj-2 primer set
DNA in lanes 6-9 were amplified with Caj3-E primer set and digested with Taq I enzyme

Figure A4.3. Nucleotide sequence of RNA-5 of PPPSMV P and C isolates amplified by RT-PCR using SM-1 and SM-2 primers

```
P  ACATAGTTCA ATCCTTGACT GCGAAAATGA ATAAGTTTCA TGGCCTTGAA 50
C  ACATAGTTCA ATCCTTGACT GCGAAAATGA ATAAGTTTCA TGGCCTTGAA
       SM-1>
P  ACATCGACAT TCAAGGATGC AGTTGCTGTT GTTGGCTTGG AGAATATTAA
```

A

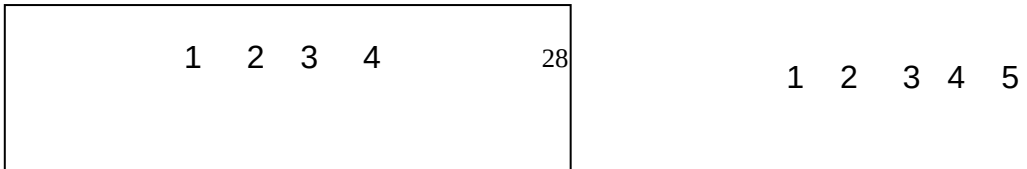
Figure A4.4. Subset RT-PCR to distinguish P and C isolates of PPSMV using SM-1/2 and SMs1/2 oligonucleotide primer sets



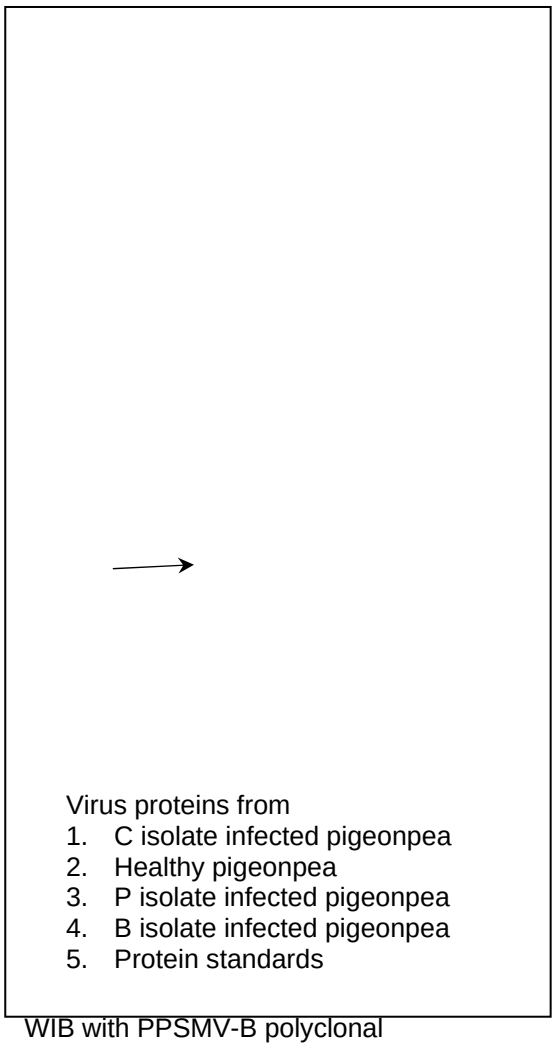
C = Coimbatore isolate; P = Patancheru isolate;
M = DNA molecular weight standard

SM-1/2 primer set result in 321 bp product in all isolates
SMs-1/2 primer amplifies 164 bp product from RNA of PPSMV-C isolate

Figure A4.5. Western immunoblot analysis of gel eluted 32 kDa protein of PPSMV-B isolate



PPSMV protein →



Virus proteins from
1. C isolate infected pigeonpea
2. Healthy pigeonpea
3. P isolate infected pigeonpea
4. B isolate infected pigeonpea
5. Protein standards

WIB with PPSMV-P polyclonal antibodies. Note that this antibodies produced against whole preparation also recognizes host proteins. Positions of 29 kDa and 45 kDa protein marker bands indicated.

WIB with PPSMV-B polyclonal antibodies produced against eluted 32 kDa protein. Note that antibodies did not recognize proteins in healthy preparations. Multiple bands could be due to protein degradation. Positions of 29 kDa and 45 kDa protein marker bands indicated.

Figure A4.6. Ultracytopathology of PPSMV P, B and C infected pigeonpea

Transmission of electron micrographs. Pigeonpea tissues were fixed and embedded in resin. Ultra-sections were made using microtome, stained with uranyl acetate and lead-citrate and observed under EM

(A) PPSMV-P isolate infected tissues showing the Membrane bound bodies (MBBs), Fibrous Inclusions (FI). Inset, electron dense material (EDMs), gold labelled with PPSMV-IgG.

(B) PPSMV-B isolate infected pigeonpea tissue showing MBBs and crystalline arrays of rod-shaped structures. Note that this section was stained with PPSMV-P antibodies and labelled with gold-labelled antibodies (IGL)

(C) PPSMV – C isolate infected pigeonpea tissue. MMBs indicated with arrows.

Note that in IGL experiments antibodies specifically labelled MBBs and EDMs, but not Fis. (Kumar et al., 2002, Annals of Applied Biology)

Figure A4.6. SMD endemic locations (indicated with stars) in Northern Karnataka

At endemic locations SMD occurs annually with incidence between 30-80%

Figure A4.7. Affect of SMD on pigeonpea seeds and germination

A = Seed from ICP8863 health plants
B = Seed from SMD-affected ICP8863 plants (sever infection)
C = Seed from SMD-affected ICP8863 plants (mild infection)
Note the reduction in size and malformed seed growth in B and C; poor germination and vigour of seedlings from B and near normal growth in case of C.

Outputs

O1. Identification of sources of SMD resistance with underlying priority traits in accessions of cultivated and wild pigeonpea germplasm collections and their availability for pigeonpea breading programs:

Screening of wild and cultivated pigeonpea accessions against three PPSMV isolates [P, B C] resulted in the identification of several accessions possessing resistance to P isolate and a few to B and C isolates. Reactions of various lines screened were summarised in the Tables O1.1 to O1.10. Several SMD resistant accessions in various genes pools were identified for farmer cultivation and for utilization in NARES breeding programmes.

Broad-based SMD resistance in wild Cajanus species (Tables O1.1 – O1.3):

- Of 115 accessions of 6 wild *Cajanus* species (*C. albicans*, *C. cajanifolius*, *C. lineatus*, *C. platycarpus*, *C. scarabaeoides* and *C. sericeus*) screed for resistance to three isolates of PPSMV, 65 (56%) were uninfected with one or more virus isolate. (Tables O1.1 and O1.2). Eight accessions of *C. scarabaeoides* (ICP 15695, 15702, 15703, 15707, 15712, 15726, 15728, 15739) were uninfected with isolates P and B but were not tested against isolate C due to lack of sufficient seed (Table O1.1), so these may be further sources of broad-based resistance to infection. The majority of the SMD resistant accessions were resistant to mite inoculation of virus (Table O1.1).

- Of the 15 accessions of wild *Cajanus* that showed resistance to all the three isolates, eight (ICP 15614, 15615, 15626, 15924, 15926 of *C. albicans,* and ICP 15700, 15701, 15734 of *C. scarabaeoides)* were identified as potential sources of strong broad-based SMD resistance (Tables O1.1 and O1.2). 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 are transferable to pigeonpea by conventional breeding programmes.

- Six of the SMD resistant *C. scarabaeoides* accessions were identified earlier to contain resistance to wilt, pod borer and other pests (Table O1.3). They can be used as parents in interspecies breeding for multiple disease resistant varieties to mitigate losses to SMD, wilt and pod borer, all of which seriously affect pigeonpea cultivation.

- This is the first comprehensive evaluation of wild Cajanus accessions against different PPSMV isolates and that has identified sources of broad-based resistance to the disease that can be transferred to *C. cajan*. These accessions have immense value in developing multiple disease resistant pigeonpea varieties.

SMD resistance in short-medium duration breeding lines (Tables O1.4 – O1.6):

- Screening of plant populations from 38 breeding lines derived from ICP7035 (broad-based SMD resistant donor) against B and P isolates of PPSMV resulted in identification of 11 promising lines (Tables O1.4 to O1.6). They were retested in 2005 for further selections. These lines have uniform phenotypic characters and this trial will e advanced for evaluation of yield parameters.

- This screening trial has led to the identification of SMD resistant breeding lines with short to medium duration maturity (90-150 days) that escape terminal drought, frequently encountered in rain-fed agro-eco systems. These lines have resistance to B and P isolates of PPSMV and tolerance to fusarium wilt. Further selections are being made from these promising lines. Seed is being multiplied for on-farm evaluation of the breeding lines and also for supply to NARES.

Broad-based SMD resistance in pigeonpea accessions (Tables O1.7 - O1.10):

- Forty-three different pigeonpea varieties were evaluated against three PPSMV isolates (P, B and C) (Tables O1.7 to O1.10). Of these 22 were developed at ICRISAT and rest were from national programmes. Most of these varieties are susceptible to PPSMV at one or more locations.

- Three new promising SMD resistant varieties, viz., ICPL99050, ICPL87051, ICPL96061, were identified. These varieties have good resistance to P, and moderate resistance to C and B isolates.

- Pigeonpea variety ICP7035 has absolute resistance to PPSMV isolates at all locations tested (Patancheru, Bangalore, Coimbatore, Gulbarga, Bidar and Varanasi) and is suitable for both seed and vegetable purpose. A pigeonpea variety bred by Indian national programmes, MAL-19, was also resistant against all PPSMV isolates tested (Patancheru, Bangalore, Coimbatore, Gulbarga and Varanasi).

- The screening trials during 2002-04 led to the identification of several new resistant sources in landraces and breeding lines. ICP7035 was approved for release in Zone-5 region of Karnataka state. ICPL87051, 99050, 96053, 96058 and 96061 are being evaluated on-farm. These lines were streamlined for release purpose. Through on-farm and on-station trials seed of promising varieties were supplied to farmers and NARES for adoption.

O2. Selection of several high-yielding pigeonpea cultivars with broad-based resistance to SMD and other biotic constraints suitable for cultivation by poor farmers:

- Of various pigeonpea varieties evaluated, pigeonpea varieties ICP7035 (broad-based SMD resistance and tolerant to fusarium wilt), ICPL 96058, 96053, 87051 and 99050 (resistant to PPSMV P isolate and fusarium wilt) were selected for on-farm evaluation and release.

- ICP7035 and ICPL96058 were tested in large number of farmers' fields and relative merits of this variety were studied (Tables O2.1. to O2.6). Both these varieties were well accepted for the farmers, however, due to late maturity of ICPL96058 (2-3 weeks later than local varieties), this variety is recommended for cultivation in deep-black soils. ICPL96058 is a high yielder. This variety is being cultivated by the farmers in the central peninsular India, where PPSMV P isolate and fusarium wilt are endemic. This variety has been incorporated into national IVT programme through Agriculture Research Station, Gulbarga.

- The ICP7035 has been evaluated for 5 years in the farmers' fields in SMD-endemic areas of southern Karnataka (Table O2.1-O2.6). This variety is a boon to SMD resistance due to its durability and broad-based resistance to the disease. ICP7035 was evaluated in the SMD endemic zones where B and C isolates are prevalent. This variety was also evaluated in the states of Andhra Pradesh, Madhya Pradesh, Chattisgarh and Uttar Pradesh, India; and Nepalgunj, Nepal. This variety severs dual needs of the farmers, i.e. it can be used as vegetable and for grain purpose. ICRISAT and University of Agriculture Sciences, Bangalore, India, has proposed to release this variety in Karnataka. This variety was evaluated as per the formalities of varietial release procedures during 2000-04 seasons. Data was verified and approved by the university committees, and Zonal Research and Extension Advisory Council (ZREAC) and provisional approval for release of ICP7035 was given.

- The nutritional quality parameters were assessed for the ICP7035 and SMD susceptible cultivar TTB-7 (Tables O2.7-O2.8). The parameters like moisture, crude protein, crude fat, crude and dietary fibre, starch, dry matter, carbohydrates, followed by minerals and trace elements which includes calcium, copper, iron, magnesium, manganese and phosphorous were estimated. The methods followed for the nutritional quality assessment were as per the Bureau of Indian Standards and this was done at Pristine Laboratories (Bangalore, India). There were no significant differences in the moisture, crude fat, acid insoluble ash and dry matter content between ICP7035 and TTB-7. But there were significant differences in the crude protein, crude fibre, dietary fibre, starch, total ash, metabolizable energy and carbohydrates. ICP7035 has significantly higher amounts of starch, total ash, carbohydrates and metabolizable energy. In contrast TTB-7 contained significantly higher amounts of crude protein, crude fibre and dietary fibre respectively. Regarding minerals and trace elements viz., copper, iron and magnesium is concerned there were no significant differences between ICP7035 and TTB-7. But ICP7035 contained significantly higher amounts of calcium, manganese and phosphorus than TTB-7.

O3. Integration of new SMD resistance screening tools and technologies to NARES and NGOs to enhance the efficacy of pigeonpea breeding/improvement programmes

- Dissemination pathways were established by partnerships with stakeholders. Technologies developed were transferred to the beneficiaries through partnerships with targeted national institutes, organisation of training courses and working group meeting for the NARES, and organisation of farmer-participatory trials to evaluate genotypes with broad-based resistance. Direct partnerships with stakeholders allowed testing methodologies simultaneously and their feedback was instrumental in achieving the targets (Table C2; Figures A3.1 and C1).

- Sufficient quantities of diagnostic tools (PPSMV polyclonal antibodies, PPSMV-pencillinase IgG conjugates and oligonucleotide primers) and seed material of promising genotypes and differential cultivars were distributed to the NARES involved in SMD research (see Tables C4 and C5). Seed of promising genotypes, especially ICP7035 and ICPL96058 and breeding lines, were multiplied for distribution to NARES, and farmers in the SMD endemic areas. Improved pigeonpea seed material was supplied to NARES at free of cost and seed material can be further used in their research programmes.

- Farmers were given training in Integrated Disease and Pest Management (IDPM) and post-harvest seed processing to prevent seed contamination with storage pests, and value-addition to the crop through sale of dehusked seeds, which is expected to add additional income to farmers.

- Training to NARES (scientists, graduate and postgraduate students) in virus detection methods, resistance screening and disease management would contribute to capacity building and sustainability of knowledge and further continuation research on SMD.

- A laboratory manual incorporating detailed descriptions of various experimental procedures and techniques were published and supplied to the NARES and also placed in “Learning Systems” page in ICRISAT website (see Table C1). All scientific outcomes on virus characterization studies and descriptions of various pigeonpea genotypes screened for SMD resistance were published in various international and national journals, on-line articles and in conferences for wider disseminate of the project outputs (Table C1). This would not only contribute to the sustainability of knowledge generated, but would be the gate-way for vital information on diseases resistant varieties and contribute to the further research on this most economically important problem.

O4. Additional information on the biochemical characteristics of PPSMV and its biotypes

- Seven PPSMV isolates were studied. PPSMV isolates from Coimbatore (C) and Bangalore (B) were purified and its major properties determined. Whereas the severity of PPSMV isolates from Varanasi (V) (Uttar Pradesh), Nepalgunj (N) (Nepal), Dharwad (D) and Gulbarga (G) (Karnataka) were determined using differential host genotypes. This study has indicated that B, C, V and N isolates are highly virulent, and can overcome resistance selected against P, D and G isolates. This information is useful for selection of disease resistant genotypes.

- About 30% of PPSMV-P genome was sequenced. These sequences have no matches in the database. This has impeded deriving benefit from 'comparative genomics' to assess the functional aspects of the sequenced genome. Based on single nucleotide differences in RNA-5 sequence of P and C isolates, SMs-1 and SMs-2 primers were developed to distinguish these two isolates by RT-PCR.

- Studies on three PPSMV isolates indicated complexity of various PPSMV isolates occurring in the subcontinent. The biological properties (reaction on differential host range and severity) of C were similar to those of B, but the nucleoprotein sizes of these two isolates were distinct (35 kDa for C and 32 kDa for B). The B and P isolates have similar sized nucleoprotein (32 kDa), but these two isolates have different phenotypic reaction on the differential genotypes (Figure O4.1; Table A4.3). Further studies based on genomic comparisons are necessary for proper understanding of the diversity of various isolates. The differential pigeonpea genotypes are useful indicators to determine the severity of the isolates.

- Surveys for disease epidemiology indicated existence of several PPSMV isolates in the Indian subcontinent, and that three isolates were prevalent in peninsular Indian alone. These isolates have distinct biological and physical properties. Despite the continuous cultivation of pigeonpea, the various PPSMV isolates seems to have maintained distinctiveness. For instance in Karnataka state, P isolate is prevalent in Northern region and B isolate is prevalent in Southern region. The B isolates is highly virulent and can overcome resistance selected against P isolate. There are no geographic barriers preventing isolate spread and despite continuum in pigeonpea cultivation within the state, the reaction on genotypes was uniform (confirmed using differential genotypes) over several years, indicating probable separation of isolates.

- Mites were seldom found on resistant varieties at any location. Testing of resistant genotypes by graft inoculation showed that most of the resistant genotypes were infectible by this method, suggesting that SMD resistance in most genotypes could be due to mite resistance. Studies on leaf morphometrics indicated cell wall thickness in resistant varieties was greater than in susceptible varieties, which might be acting as barrier for mites to feed, and thus poor vector survival and consequently resistance to SMD.

- Certain physical and physiological affects of PPSMV on pigeonpea were determined to assess the disease impact on plant growth and yield (Table A4.7 and A4.8). In a susceptible genotype virus invades all parts of the plant, but not cotyledons. Although seeds were free of PPSMV, seed from infected plants were poor in quality with up to 50% reduction in dry weight. Such seed has poor germination rate and vigour.

Table O1.1. Responses of accessions of six *Cajanus* species inoculated with three distinct *Pigeonpea sterility mosaic virus* (PPSMV) isolates using viruliferous *Aceria cajani*

	Patancheru (P)			Bangalore (B)			Coimbatore (C)		
†ICP No.	N	PI	SYT	N	PI	SYT	N	PI	SYT
C. albicans									
15614	14	0	NS	6	0	NS	8	0	NS
15615	18	0	NS	17	0	NS	25	0	NS
15616	20	0	NS	21	33	MM	18	17	MM
15617	22	0	NS	21	57	MM	9	22	SM
15618	17	0	NS	24	63	MM	17	0	NS
15619	19	0	NS	12	25	MM	28	25	MM
15620 [SRI]	21	0	NS	20	40	RS	19	16	MM
15621	20	0	NS	24	42	MM	17	0	NS
15622	12	33	MM	24	46	MM	16	13	MM
15623	15	47	MM	21	24	MM	23	17	MM
15624	12	75	MM	24	17	MM	16	44	SM
15625	15	0	NS	22	32	RS	12	0	NS
15626	19	0	NS	21	0	NS	20	0	NS
15627	12	8	MM	20	45	RS	25	24	SM
15628	10	30	MM	19	32	SM	21	14	MM
15924 [SRI]	16	0	NS	24	0	NS	22	0	NS
15925 [SRI]	34	3	MM	25	0	NS	18	0	NS
15926 [SRI]	22	0	NS	20	0	NS	13	0	NS
15927 [SRI]	23	0	NS	25	52	MM	19	0	NS
C. cajanifolius									
15629	9	11	MM	16	44	SM	18	0	NS
15630	11	27	SM	9	33	SM	18	17	MM-SM
15631	16	6	SM	38	39	MM	17	29	SM
15632	16	19	SM	37	38	MM	19	0	NS
C. lineatus									
15641	25	28	SM	17	0	NS	18	17	MM
15642	19	42	SM	12	33	SM	13	31	SM
15643	16	31	SM	15	0	NS	20	10	SM
15644	9	0	NS	13	62	SM	21	0	NS
15645	12	25	MM	13	70	MM	19	21	MM-SM
15646	14	43	SM	15	40	MM	17	35	MM-SM
15647	10	20	MM	5	60	SM	19	11	MM
15648	15	0	NS	7	57	SM	13	0	NS
15649	12	8	SM	13	0	MM	21	0	NS
15650	15	13	SM	13	0	MM	13	31	MM
C. platycarpus									
15661	16	63	SM	17	71	MM	22	36	SM
15662	14	79	SM	15	73	SM	25	52	SM
15663	16	100	SM	12	59	SM	10	30	MM
15664	22	46	MM-SM	13	77	SM	23	48	MM-SM
15665	26	65	MM-SM	15	53	MM	25	0	NS
15666	21	57	MM-SM	14	64	MM	14	14	MM
15667	23	65	MM-SM	20	45	MM	24	33	SM
15668	24	71	MM-SM	19	63	SM	17	29	SM
15669	30	73	MM-SM	19	53	MM	11	18	SM
15670	24	50	SM	21	0	NS	29	28	MM-SM
15671	11	91	MM	20	0	NS	13	15	MM
15672	12	25	MM	16	44	SM	21	19	MM
15673	12	33	MM	18	44	SM	19	32	MM
15921	27	63	SM	17	47	MM	19	16	SM
16144	17	59	SM	23	48	MM	16	13	MM
16145	26	69	SM	21	71	SM	26	35	SM
16146	26	54	SM	18	78	MM	22	0	NS
C. scarabaeoides									
15683*	16	100	MM-SM	ng	-	-	9	78	SM

15684	28	4	MM	24	0	NS	14	7	MM
15685	26	54	MM	14	0	NS	20	35	MM
15686	27	93	MM-SM	26	8	SM	24	88	SM
15687*	20	40	MM-SM	16	13	SM	ng	-	-
15688	33	3	MM	9	11	SM	18	11	SM
15689	25	64	MM-SM	34	21	SM	29	45	SM
15690	26	58	MM-SM	27	33	SM	24	46	SM
15691*	24	58	MM-SM	20	25	SM	ng	-	-
15692*	20	15	MM-SM	22	5	SM	ng	-	-
15693	34	65	SM	22	9	SM	27	56	SM
15694 [SRI]*	27	37	MM-SM	22	18	SM	ng	-	-
15695 [SRI]*	21	5	SM	37	3	SM	ng	-	-
15696 [MYA]	26	35	MM	33	12	SM	24	13	SM
15697*	21	0	NS	14	14	MM	ng	-	-
15698*	21	43	SM	21	43	SM	ng	-	-
15699*	26	12	SM	15	13	SM	ng	-	-
15700	16	0	NS	17	0	NS	13	0	NS
15701	22	0	NS	18	0	NS	25	0	NS
15702*	21	0	NS	24	8	SM	ng	-	-
15703*	25	8	MM-SM	7	0	NS	ng	-	-
15704*	29	14	SM	19	16	SM	ng	-	-
15705*	19	16	MM-SM	19	5	SM	ng	-	-
15706*	29	21	SM	23	9	SM	ng	-	-
15707*	22	5	MM	23	0	NS	ng	-	-
15708*	23	0	NS	23	0	MM	ng	-	-
15709*	15	0	NS	12	0	MM	ng	-	-
15710	30	87	SM	15	27	SM	19	53	SM
15711	27	59	MM	20	5	SM	22	14	SM
15712*	10	0	NS	17	0	NS	nt	-	-
15713*	16	13	MM-SM	24	21	SM	nt	-	-
15716	10	80	MM	32	0	NS	14	21	MM
15717*	15	13	MM	21	19	SM	nt	-	-
15718	26	81	SM	19	21	SM	21	33	SM
15719	12	83	MM-SM	10	30	SM	27	22	SM
15720 [PHIL]	11	91	SM	18	22	SM	16	19	SM
15721 [PHIL]	18	83	MM-SM	32	6	MM-SM	17	53	MM-SM
15722	19	95	MM	33	3	SM	22	14	SM
15723	39	80	SM	26	39	SM	29	38	SM
15724	41	83	MM	27	0	NS	18	33	MM
15725	20	5	MM	16	0	NS	16	0	NS
15726*	24	0	NS	26	0	MM	nt	-	-
15727	32	69	MM-SM	34	12	SM	22	23	MM-SM
15728*	20	0	NS	25	0	NS	nt	-	-
15729	26	27	MM-SM	26	3	SM	9	33	SM
15730*	17	12	SM	20	30	SM	nt	-	-
15731	31	65	SM	12	42	SM	23	65	SM
15732*	26	23	MM	9	33	SM	nt	-	-
15733	28	68	MM-SM	14	7	MM	18	38	MM
15734 [AUS]	23	0	NS	10	0	NS	19	0	NS
15735 [AUS]	14	100	MM-SM	14	14	SM	22	23	SM
15736 [FIJI]	26	4	MM	7	0	NS	15	0	NS
15737 [FIJI]	35	6	MM	11	9	MM	19	11	MM
15738	41	83	SM	9	33	SM	26	27	SM
15739*	20	5	MM	12	0	NS	nt	-	-
15740	21	5	MM	15	0	NS	22	0	NS
15741[unknown]	25	4	MM	12	8	MM	14	14	MM
15742 [AUS]*	22	9	MM	16	75	SM	nt	-	-
15743 [AUS]	23	0	NS	15	27	MM	17	6	MM
15744 [AUS]*	22	18	MM	13	46	SM	nt	-	-
15922*	nt	-	-	21	52	MM	15	13	SM
C. sericeus									

15760*	nt	-	-	17	35	MM	17	0	NS
15761*	nt	-	-	19	53	MM	21	24	SM
15762*	nt	-	-	19	37	MM	16	17	MM-SM
15763 [AUS]*	nt	-	-	18	33	MM	27	27	SM
Controls									
ICP 8863	30	100	SM	21	91	SM	12	100	SM
Vamban – 1	nt	-	-	nt	-	-	30	100	SM
TTB – 7	nt	-	-	20	95	SM	nt	-	-
ICP 7035	20	0	NS	20	0	NS	20	0	NS

†All accessions were of Indian origin, except those indicated as: AUS = Australia; MYA = Myanmar; PHIL = Philippines; SRI = Sri Lanka; the origin of 15741 is unknown. *Accessions not tested against all PPSMV isolates.

N = number of plants tested; PI = percent infected plants determined by DAS-ELISA; SYT = Symptom type: SM = severe mosaic; MM = mild mosaic; RS = chlorotic ringspots; MM-SM = initial mild mosaic followed by severe mosaic; NS = no symptoms; ng = poor germination; nt = not tested

Table O1.2. Accessions within six *Cajanus* species resistant to more than one *Pigeonpea sterility mosaic virus* isolate following inoculation using viruliferous *Aceria cajani*

Species	Resistant to *(ICP No.)*			
	P+B+C	**P+C**	**P+B**	**B+C**
C. albicans [19/20]†	15614, 15615, 15626, 15924, *15925*, 15926	15618, 15621, 15625, 15927	-	-
C. cajanifolius [4/5]†	-	*15629*	-	
C. lineatus [10/10] †	-	15644, 15648, *15649*	-	*15643*
C. platycarpus [17/17]†	-	-	-	-
C. scarabaeoides [61/102]†*	*15684, 15688*, 15700, 15701, *15703, 15725*, 15734, *15736*, *15737, 15740*	15697, *15743*	15695, *15702, 15707*, 15712, 15728, 15739, *15741*	*15705*
C. sericeus [4/4]†*	-	-	-	-

Total no. in the ICRISAT genebank; * = not all accessions tested at all the locations. Accessions with up to 12% infection incidence are indicated in italics (see Table 1 for more details);
P = Patancheru; B = Bangalore; C = Coimbatore

Table O1.3. Accessions of *Cajanus scarabaeoides* with multiple disease resistance

ICP No.	Resistant to:	
	PPSMV isolate*	**Other pathogens and pests**[†]
15684	P, B, C	Fusarium wilt, *Helicoverpa armigera* larvae, Immune to pod fly damage
15688, 15725	P, B, C	Fusarium wilt
15695	P, B	Fusarium wilt, Oviposition non-preference, Immune to pod fly, *Helicoverpa armigera* larvae, cyst nematode Resistant to pod damage by *H. armigera* and pod wasp
15712	P, B	Fusarium wilt
15726	P, B	oviposition non-preference, *H. armigera* larvae

*See Table O1.1 for more details (P = Patancheru; B = Bangalore; and C = Coimbatore)

Table O1.4. Responses of accessions of ICP7035 breeding lines inoculated with Bangalore (B) isolate of *Pigeonpea sterility mosaic virus* (PPSMV) using viruliferous *Aceria cajani*

Sl. No.	Accession no.	2003-04		2004-05	
		No. Infected/ Plant population	Incidence (%)	No. Infected/ Plant population	Incidence (%)
1.	ICP11632 (ICPL178)	22/35	63	16/62	25
2.	ICP11719 (ICPL269)	18/34	53	8/43	18
3.	ICP14399 (ICPL83008)	39/51	76	27/100	27
4.	ICP14404 (ICPL83015)	33/41	85	15/62	24
5.	ICP14410 (ICPL83024)	45/53	85	19/60	31
6.	ICP14454 (ICPL85050)	31/39	79	13/76	17
7.	ICP14456 (ICPL85054)	20/38	52	25/42	59
8.	ICP14478 (ICPL86021)	21/34	62	28/87	32
9.	ICP14480 (ICPL86023)	31/71	43	nt	nt
10.	ICP14487 (ICPL86030)	34/41	83	18/46	39
11	ICP14719 (ICPL87113)	29/40	72	42/55	76
12	ICP14834 (ICPL89007)	21/36	58	35/320	29
13	ICP16160 (ICPL90002)	30/49	61	68/90	75
14	ICP16161 (ICPL90004)	27/39	69	57/88	65
15	ICP16165 (ICPL90010)	43/62	69	37/120	31
16	ICP16166 (ICPL90011)	18/27	66	7/81	28
17	ICP16167 (ICPL90016)	18/31	58	18/24	75
18	ICP16169 (ICPL90020)	28/46	61	30/103	29
19	ICP16171 (ICPL90023)	22/34	64	70/80	87
20	ICP16202 (ICPL91019)	25/37	67	39/80	49
21	ICP16206 (ICPL91026)	38/45	84	18/49	37
22	ICP16208 (ICPL91031)	31/44	70	20/49	41
23	ICP16224 (ICPL92030)	27/46	59	17/81	21
24	ICP16242 (ICPL92049)	33/47	70	81/105	77
25	ICP16281 (ICPL93072)	22/42	52	45/135	33
26	ICP16293 (ICPL83084)	38/52	73	50/164	30
27	ICP16294 (ICPL93085)	28/39	72	15/64	23
28	ICP16296 (ICPL93087)	7/38	18	30/63	47
29	ICP16297 (ICPL93088)	25/45	55	15/67	22
30	ICP16298 (ICPL93089)	25/46	54	54/140	38
31	ICP16313 (ICPL93104)	21/39	54	55/95	57
32	ICP16322 (ICPL93180)	26/36	72	85/126	67
33	ICP16325 (ICPL93183)	26/48	54	20/54	37
34	ICP16326 (ICPL93184)	43/45	95	14/44	32
35	ICP16327 (ICPL93185)	33/34	97	46/70	66
36	ICPL95020	23/36	63	29/60	48
37	ICPL95024	26/27	96	22/48	46
38	ICPL95029	21/25	84	14/69	20

Table O1.5. Features of promising ICP7035 breeding lines selected from 2004-05 trial**

	ICP No.	SMD Infection (%)	Days to maturity	*Average height (cm)	*Average no. of branches	*Average no. of seeds /pod (range)
1	11632	25	130	114	5	4-5
2	11719	18	135	115	6.5	4
3	14404	24	140	95	4	4
4	14478	32	165	90	3.5	4
5	16165	31	115	97	4	4-5
6	16166	28	125	165	6	4-5
7	16169	30	123	90	3.5	3-4
8	16293	30	108	107	6	4
9	16294	23	123	127	5.5	4-5
10	95029[#]	20	120	96	3	4-5
11	14399	27	110	101	4	4

**From 10 plants. [#]ICPL No. **(for details refer to Table O1.4.)*

Table O1.6. ICP7035 breeding lines evaluated at Patancheru during 2004-05

	Genotype ICP No.	Pedigree	Days to flowering	Days to maturity	Plant height (cm)
1	11719 (A)*	ICPX-740174-27-1-B-B*H1-HB-HB	88	135	115
2	11719 (B)*	ICPX-740174-27-1-B-B*H1-HB-HB	88	135	115
3	14454	ICPX-780326-HB-H10-H1-HB-HB-HB	82	139	155
4	14456	ICPX-780327-HB- HB-H5-H6-H1'-HB-HB	81	140	180
5	14719	ICPX-780329-HB-H13-HB-H1-HB-HB-HB	79	119	160
6	14834 (A)*	ICPX-810099-HB-SB*-HB-HB-HB-HB	85	108	190
7	14834 (B)*	ICPX-810099-HB-SB*-HB-HB-HB-HB	85	108	190
8	16313	ICPX-870023-HB-H38-HB-B-B	82	125	90
9	16326 (A)*	ICPX-840018-HB-SWB-13-WSMB-WSMB-WSMB	91	142	175
10	16326 (B)*	ICPX-840018-HB-SWB-13-WSMB-WSMB-WSMB	91	142	175
11	16327	ICPX-860043-HB-SWB-10-WSM2-WSMB-WSMB	80	130	143

All lines have maturity period of 100-169 days with non-determinate growth habit. This lines are specifically selected for evaluation in Central India.
**Seed of lines that have different phenotypes during evaluation in 2003-04 were selected separately and designated as A and B*

Table O1.7. Evaluation of promising pigeonpea varieties against C and P isolates of PPSMV under high inoculum pressure in greenhouse during 2002-03

Genotype accession No.	Patancheru isolate		Coimbatore isolate	
	Number infected / No tested (% infection)	Symptom type	Number infected / No tested (% infection)	Symptom type
ICPL 99050	5/41 (12)	MM	7/12 (58)	SM
ICPL 87119	37/38 (97)	SM	8/10 (80)	SM
ICPL 96061	3/45 (7)	MM-SM	5/11 (45)	SM
ICPL 96053	39/43 (90)	SM	4/8 (50)	SM
ICPL 96058	43/43 (100)	SM	5/11 (45)	SM
ICPL 87	39/40 (97)	SM	8/12 (67)	SM
ICPL 87051	1/40 (2)	RS-MM	5/15 (33)	MM

SM = severe mosaic; mm = mild mosaic; ns = no symptoms

Table O1.8. Various pigeonpea genotypes evaluated against Coimbatore (C) PPSMV under high inoculum pressure in greenhouse during 2002-03

	Accession No.	Plants infected/tested (%infection)	Symptom type		Accession No.	Plants infected/tested (%infection)	Symptom type
1	ICPL99050	9/15 (60)	MS	9	CORG 9407	6/14 (43)	SM
2	ICPL99046	7/13 (54)	MM	10	CO 6	7/9 (78)	SM
3	MAL-19	0/9 (0)	NS	11	CORG9701	5/10 (50)	SM
4	ICPL96048	6/12 (50)	SM	12	ICPL87	8/12 (67)	SM
5	ICPL99048	6/14 (43)	SM	13	CORG 9904	4/7 (57)	SM
6	CO 5	8/11 (73)	SM		***Controls***		
7	APK-1	3/1/3 (26)	MM		ICP8863	12/12 (100)	SM
8	VBN-1	5/9 (56)	SM		ICP7035	0/15 (0)	NS

SM = severe mosaic; mm = mild mosaic; ns = no symptoms;

Table O1.9. Evaluation of pigeonpea breeding lines

against PPSMV Bangalore (B) isolate in 2003-04

	Genotype accession No.	SMD incidence (%)	Symptom type
1	ICPL99050	33	SM
2	ICPL96061	61	SM
3	ICPL96047	60	SM
4	ICPL96058	87	RS-SM
5	ICPL9303	59	SM
6	ICPL88047xICP1381	72	SM
7	ICPL88047xICP13831	85	SM
8	GUPH1126-9-2 (ms) x ICPL87159	83	SM
9	GUPH 1126-29-1 (ms) x ICPL87159	80	SM
10	GUPH 1126-29 (ms) x ICPL87159	87	SM
11	GUPH 1126-47-1 (ms) x ICPL87159	63	SM
12	GUPH 1126-21-1	77	SM
13	IPH487-79-4 (ms) x ICPL87159	87	SM
14	IPH 487-78-2 (F)x ICPL 87159	77	SM
	Susceptible control		
	TTB-7	96	SM

SM = sever mosaic; RS-SM = Initial chlorotic ringspots, later turn to severe mosaic

Table O1.10. On-farm evaluation of high yielding pigeonpea lines to SMD in Bidar, and for fusarium wilt in Gulbarga, Karnataka during 2004-05

	Genotype accession No.	SMD (%)	Wilt (%)		Genotype accession No.	SMD (%)	Wilt (%)
1	2008-1	13.33	2.51	11	WRP-1	16.67	2.60
2	GPS-041	3.33	23.43	12	GS-1	13.33	81.48
3	P-9-12	20.00	8.88	14	ICPL96058	0.00	2.60
4	P-9-27	23.33	21.53	15	ICPL96053	0.00	3.80
5	P-9-14	30.00	42.16	16	ICPL87051	0.00	0.00
6	2009-1	30.00	50.76	17	ICPL87119	3.33	2.35
7	2001-4	36.67	7.77	19	TS-3	0.00	14.71
8	WRP-230-1-1	20.00	14.77		***Controls***		
9	WRP-266	16.67	6.22		ICP8863	36.67	2.75
10	GPS-2003	10.00	3.24		ICP7035	0.00	7.61

Table O2.1. Performance of pigeonpea variety ICP 7035 in the on-station trials conducted during 2003-2004 at UAS, Bangalore

Green pod yield (kg/ha)

Genotypes	Row spacing		Mean of Genotypes
	45 cm	60 cm	
BRG 1	9263	7831	8546
ICP 7035	6289	7153	6721
HY 3C	7188	7101	7144
Mean of Row spacing	7579	7361	-
	Genotypes	**Row Spacing**	**Interaction**
CD @ 5%	1081	NS	NS
CV (%)	13.6	13.6	13.6

Grain yield (kg/ha)

Genotypes	Row spacing		Mean of Genotypes
	45 cm	60 cm	
BRG 1	1968	1796	1882
ICP 7035	1824	1671	1747
HY 3C	1736	1817	1776
Mean of Row spacing	1843	1761	-
	Genotypes	**Row Spacing**	**Interaction**
CD @ 5%	NS	NS	NS
CV (%)	13.3	13.3	13.3

Random block design
Note that the trials were conducted under disease free situation. Control varieties BRG-1 and Hy3C sometimes resulted in high yields, but they are susceptible to SMD, especially to the B isolate in Southern Karnataka. This impedes yielding ability of these varieties, whereas ICP7035 yield remained unchanged even during SMD outbreaks.

Table O2.2. Performance of ICP 7035 in various on-farm trials during 2002-04

Sl. No.	Location	No. of Trials	Area (Hectares)	SMD incidence (%)	Seed Yield (Kg/ha)	
					ICP 7035	TTB 7
A. Extension Education Unit						
1.	At UAS, Bangalore	3	0.02	T1 = 0 T2 = 0	1700	1225
B. KSDA						
1.	Bangalore Urban District	3	0.05	T1 = 0 T2 = 3	404	440
2.	Bangalore Rural District	12	0.02	T1 = 1% T2 = 20	680	470
3.	Tumkur District	4	0.02	T1 = 0 T2 = 3	1800	1175
					1146	827.50
	Average Grain Yield					

TTB-7 local variety, susceptible to SMD

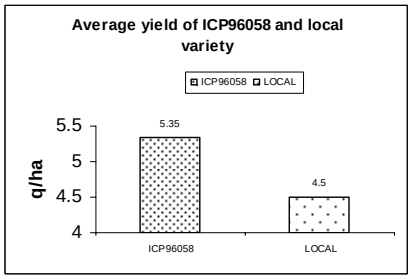
Table O2.3. Performance of ICP 7035 in the on farm trials conducted during 2003-2004

Sl. No.	Location	Green pod yield (kg/ha)		Grain yield (kg/ha)	
		ICP7035	**TTB-7**	**ICP7035**	**TTB-7**
I. EXTENSION EDUCATION UNIT					
1.	Tumkur	-	-	1200	800
2.	Magadi	-	-	2200	1100
3.	Nelmanagala	-	-	1700	1775
II.	Bangalore Urban District				
4.	Bangalore North	-	-	396.4	320.0
5.	Bangalore South	-	-	408.4	680.6
6.	Anekal	-	-	407.2	319.4
III. BANGALORE RURAL DISTRICT					
A. Kanakapura Taluk					
7.	Hukunda	-	-	600	750
8.	Byregowdanadoddi	-	-	700	800
9.	Satanur	-	-	550	600
B. Ramanagaram Taluk					
10.	Dalimba	700	600	-	-
11.	Kutgal	250	150	50	100
12.	Kutgal	300	200	100	100
13	Kasaba	950	1075	1000	1000
14	Bidadi	960	1060	860	900
C. Magadi Taluk					
15	Kallentepalya	-	-	1125	625
16	Jodukatte	420	160	-	-
17	Kalyanpura	650	770	250	300
18	Virupapura	-	-	1085	608
IV	Tumkur District				
D. Tumkur Taluk					
19	Janivranahalli	-	-	2000	3000
20	Hanumanthapura	-	-	1750	2750
C.	Gubbi Taluk				
21	Adugondanahalli	-	-	925	525
22	CN pura	-	-	525	425

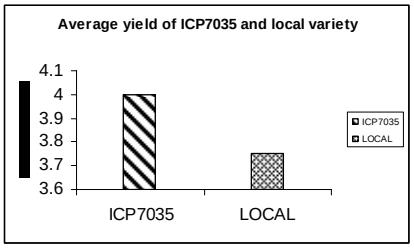
- = not tested; TTB-7 is the local variety susceptible to SMD. Trials conducted as per the farmer normal practices.

Table O2.4. Performance of pigeonpea varieties ICP7035 and ICPL96058 in farmer fields in Mahaboobnagar, Andhra Pradesh (Todellagadda and Polishettipally tanda) during 2003-04

Particulars	ICP-96058	Local variety (Check)
1. Cost of cultivation	Rs 4037/ha	Rs 4200/ha
2. Average yield	5.35 q/ha	4.5 q / ha
3. Average Net income	Rs 4790/ha	Rs 3225/ha

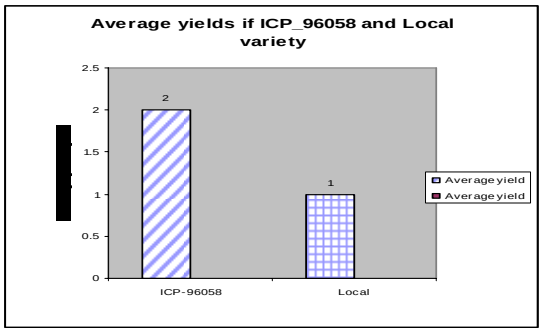


Particulars	ICPL-7035	Local variety (Check)
1. Cost of cultivation	Rs. 1625 / ha	Rs 2312 / ha
2. Seed yield	4 q/ha	3.75 q / ha
3. Average Net income	Rs.4975 / ha	Rs.3875/ ha



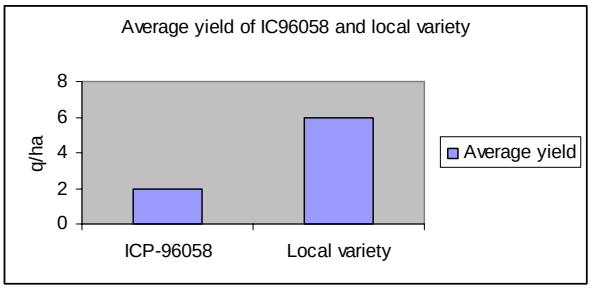
Performance of ICPL96058 and ICP7035 at Thurati, Warangal District, AP

Particulars	ICP-96058	Local variety (Check)
1. Cost of cultivation	Rs 1400/ha	Rs1270/ ha
2. Average yield/ha	2 q/ha	1 q/ha
3. Average Net income/ha	Rs.2200	Rs.530



5. Performance of ICP7035 in Venkat raopet, Andhra Pradesh

Particulars	ICP-96058	Local variety (Check)
1. Cost of cultivation	Rs.1825	Rs.2225
2. Average yield/ha	2q/ha	6q/ha
3. Average Net income/ha	Rs.1175	Rs.6775



The yield performance of ICP-96058 at Navajyothi Medak district is below average yields than local variety. This was because of severe flower drop during winter months.

Table O2.5. Performance of high yielding pigeonpea varieties ICPL 96058 and 96053 resistant to SMD and wilt in farmer fields during kharif 2003-04 in Gulbarga District, Karnataka, India

Villages	Farmers	Varieties	Area (in ha.)	Spacing (ft (b/w rows)	No of Irrigation	Plot Yield (kg)	Yield in kg/ha	Date of sowing	Date of harvest	Duration (Days)
Aland Taluk										
1. Aland	Surjit K. Patil	ICPL 96058	0.4	3	0	240	600	12.7.03	15.1.04	186
Gulbarga Taluk		ICPL 87119*	0.1	3	0	54	540		04.1.04	178
		ICPL 8863*	0.1	3	0	48	480		25.12.03	173
2. Kollur	Madhava Rao	ICPL 96053	0.25	3	1	390	1577	8.7.03	20.1.04	188
	Guttedar	ICPL 87119*	0.1	3	1	146	1608		14.1.04	182
		ICPL 8863*	0.1	3	1	118	1056		30.12.04	170
3. Dabarabad	Siddramappa	ICPL 96058	0.4	4	2	900	2250	7.7.03	10.1.04	186
	Gadalegao	ICPL 87119*	0.1	4	2	200	2000		31.12.03	176
		ICPL 8863*	0.1	4	2	150	1500		20.12.03	172
4. Kamalapur	Ramesh	ICPL 96058	0.2	3	0	275	1314	28.6.03	9.1.04	195
Chincholi taluk	S. Shetty	ICPL 87119*	0.15	3	0	140	965		9.1.04	195
		ICPL 8863*	0.11	3	0	90	807		20.12.04	175
5. Marepally	Vijayakumar	ICPL 96058	0.4	5	2	525	1312	2.7.03	5.2.04	196
	Desai	ICPL 87119*	0.1	5	2	135	1350		5.2.05	196
		ICPL 8863*	0.1	5	2	98	980		9.1.04	170
6. Kotega	Rajappa	ICPL 96053	0.4	4	2	820	2050	04.7.03	15.2.04	225
	Shiva	ICPL 87119*	0.1	4	2	230	2300	04.7.03	02.2.04	212
	Sharanappa	ICPL 8863*	0.1	4	2	190	1900	04.7.03	14.1.04	194

**Local varieties grown as controls*

Table O2.6. Performance and economics of pigeonpea verity ICPL 96058 in Kasturipalli Village during Kharif – 2003-04

Sl. No.	Name of the Farmer	Demonstration field in acres	Land preparation exp.	Bio-Pesticides	Shaking Method	Yield per Ac	Total Yield Rs.	Net Profit Rs.
1	S. Mogulappa S/o. Pakirappa	2 Ac.	1,000.00	1,100.00	4 times 240.00	7 qtls.	14,700.00	12,360.00
2	Sawari Ashappa	1 Ac.	Crop damaged					
3	Golla Ashappa	1/2 Ac.	250.00	80.00	2 times 120.00	1.5 qtls.	3,150.00	2,700.00
4	Dasari Bhimappa	2 Ac.	500.00	600.00	3 times 180.00	5 qtls.	10,500.00	9,220.00
5	Buthpur Sayappa	1 Ac.	300.00	80.00	2 times 120.00	2 bags	4,200.00	3,700.00
6	Kuruva Mallappa	1 Ac.	500.00	200.00	4 times 240.00	4 bags	8,400.00	7,460.00
7	Kuruva Sharnappa	1 1/2 Ac.	750.00	300.00	5 times 300.00	5 qtls.	10,500.00	9,150.00
8	Mala Narsimhulu	1 Ac.	250.00	No crop due to flooding after sowing				
9	Mala Ashamma	1 Ac	300.00	80.00	3 times 180.00	2 bags	4,200.00	3,640.00
10	Yashodamma	1 Ac.	600.00	280.00	4 times 240.00	2.5 qtls.	5,250.00	4,130.00
11	M.D. Gouse	1 Ac.	600.00	200.00	4 times 240.00	3 bags	6,300.00	5,260.00
12	Kuruva Sayappa	1 Ac.	500.00	300.00	4 times 240.00	2.5 bags	5,250.00	4,210.00
13	D. Mohan S/o. Ashappa	1/2 Ac.	300.00	150.00	3 times 180.00	1.5 bag	3,150.00	2,520.00
14	Papamma/Anantaiah	1 Ac.	500.00	250.00	3 times 180.00	3 bags	6,300.00	5,370.00
15	Emambee/Shaboddin	1 Ac.	600.00	300.00	5 times 300.00	4 bags	8,400.00	7,200.00
16	Sakali Ashamma	2 Ac.	800.00	500.00	6 times 360.00	6 qtls.	12,600.00	10,940.00
17	Harijan Narsamma	1/2 Ac.	No crop due to flooding after sowing					
18	Sakali Balamma	4 Ac.		4,000.00	5 times 300.00	-	10,500.00	6,200.00
19	Sakali Venkatappa S/o. Narsappa	1 Ac	600.00	250.00	4 times 240.00	4 bags	8,400.00	7,310.00
20	Golla Mogulappa/ Ashappa	2 Ac.	800.00	500.00	4 times 240.00	7 bags	14,700.00	13,160.00
21	Shabhoddin	1 Ac.	500.00	300.00	3 times 180.00	3 bags	6,300.00	5,320.00
22	Jeengala Satyamma	1 Ac.	600.00	300.00	4 times 240.00	3 bags	6,300.00	5,160.00
23	Harijan Buggappa	1 Ac.	500.00	300.00	3 times 180.00	4 bags	8,400.00	7,420.00
24	Dasari Hanumamma	2 Ac	600.00	300.00	4 times 240.00	8 bags	16,800.00	15,660.00

25	Bhimappa/ Manikyappa	1 Ac.	500.00	250.00	3 times 180.00	4 bags	8,400.00	7,470.00
26	Yella Reddy/ Asi Reddy	1 Ac.	600.00	500.00	3 times 180.00	4 bags	8,400.00	7,120.00
27	Venkat Reddy/ Bhim Reddy	1 Ac.	600-00	300.00	4 times 240.00	5 bags	10,500.00	9,360.00
28	Venkatamma/ Bhim Reddy	1 Ac.	500-00	250.00	3 times 180.00	2 bags	4,200.00	3,270.00
29	Geengal Ashanna	1 Ac.	500-00	300.00	4 times 240.00	4 bags	8,400.00	7,360.00
30	Golla Dasappa	1 Ac.	600-00	300.00	2 times 120.00	3 bags	6,300.00	5,280.00
31	Kasimbee	2 Ac.	800-00	600.00	4 times 240.00	7.5 bags	15,750.00	14,110.00
32	Kuruva Basappa	1 Ac.	600-00	300.00	4 times 240.00	3 bags	6,300.00	5,160.00
33	Anpa Ashanna	1 Ac.	550-00	300.00	4 times 240.00	6 bags	12,600.00	11,510.00
34	Veera Reddy	2 Ac.	1000-00	600.00	4 times 240.00	8 bags	16,800.00	14,960.00
35	Bhim Reddy/ Narsi Reddy	2 Ac.	1000-00	600.00	5 times 300.00	8 bags	16,800.00	14,900.00
36	Syfantehaja	1 Ac.	400-00	260.00	3 times 180.00	3 bags	6,300.00	5,460.00
37	Geengala Ananthaiah	1 Ac.	600-00	300.00	4 times 240.00	4 bags	8,400.00	7,260.00
38	Kuruva Chandrappa	1 Ac.	600-00	350.00	5 times 300.00	5 bags	10,500.00	9,250.00
39	Mala Sayanna	1/2 Ac.	300-00	150.00	3 times 180.00	1 bag	2,100.00	1,470.00
40	Bhich Reddy	1 Ac.	600-00	350.00	5 times 300.00	4 bags	8,400.00	7,150.00
41	Anupa Basanthu	1 Ac.	500-00	300.00	4 times 240.00	3 bags	6,300.00	5,260.00
42	Harijan Narsappa	1 Ac.	500-00	Crop damaged				
43	Harijan Anjilamma	1 Ac.	350-00	Crop damaged				
44	K. Basanth Reddy	1 Ac.	600-00	400.00	5 times 300.00	5 bags	10,500.00	9,200.00
45	Dasari Narsamma	1 Ac.	400-00	Crop damaged				
46	Kuruva Mallappa	1 Ac.	600-00	300.00	5 times 300.00	4 bags	8,400.00	7,200.00
47	Chandra Reddy	1 Ac.	600-00	250.00	5 times 300.00	4 bags	8,400.00	7,250.00
48	Venkatesh/Ashappa	1/2 Ac.	300-00	250.00	3 times 180.00	1 bag	2,100.00	1,370.00
49	Bal Reddy	1 Ac.	650-00	350.00	4 times 240.00	4 bags	8,400.00	7,160.00
50	Ismail sab	1 Ac.	1000-00	1,000.00	-	-		
51	Kummari Savitramma	1/2 Ac.	300-00	180.00	2 times 120.00	1.5 bags	3,150.00	2,550.00
52	Kanakappa/ Ashappa	1 Ac.	No crop due to flooding after sowing					

53	Harijan Hashanamma	1/2 Ac.	Red soil mixed crop damaged (due to flooding after sowing)					
54	Harijan Ashappa	1/2 Ac.	Crop damaged					
55	Kurva Chennaiah	1 Ac.	500.00	300.00	4 times 240.00	4 bags	8,400.00	7,360.00
56	Gangireddy/ Shivareddy	1 Ac.	550.00	600.00	3 times 180.00	4 bags	8,400.00	7,070.00
57	Kurva Nagappa/Ashappa	1 Ac.	600.00	300.00	4 times 240.00	3 bags	6,300.00	5,160.00
58	Mala Buggappa/ Narsappa	1 Ac.	No crop due to flooding after sowing					
59	S.Ramanamma W/o. Ashanna	1 Ac.	600.00	300.00	4 times 240.00	3.5 bags	7,350.00	6,210.00
60	Mohan Reddy/ Bhim Reddy	1 Ac.	600.00	500.00	3 times 180.00	5 bags	10,500.00	9,220.00
61	Chinna Chandrappa/ Mallappa	1 Ac.	500.00	550.00	4 times 240.00	3 bags	6,300.00	5,010.00
62	Manikappa/Bichappa	1 Ac.	600.00	400.00	4 times 240.00	3 bags	6,300.00	5,060.00
63	Golla Chinna Ashanna/ Govindappa	1 Ac.	600.00	350.00	3 times 180.00	3 bags	6,300.00	5,170.00
64	Anpu Ashappa/ Sandappa	1 Ac.	No crop due to flooding after sowing					
65	Golla Ashappa/ Sandappa	1 Ac.	No crop due to flooding after sowing					
66	Narsi Reddy/ Bal Reddy	1 Ac.	600.00	400.00	4 times 240.00	4 bags	8,400.00	7,160.00

**One bag = 100 kg seeds (approximately)*

Table O2.7. Nutritional quality of grain of pigeonpea varieties TTB 7 and ICP7035

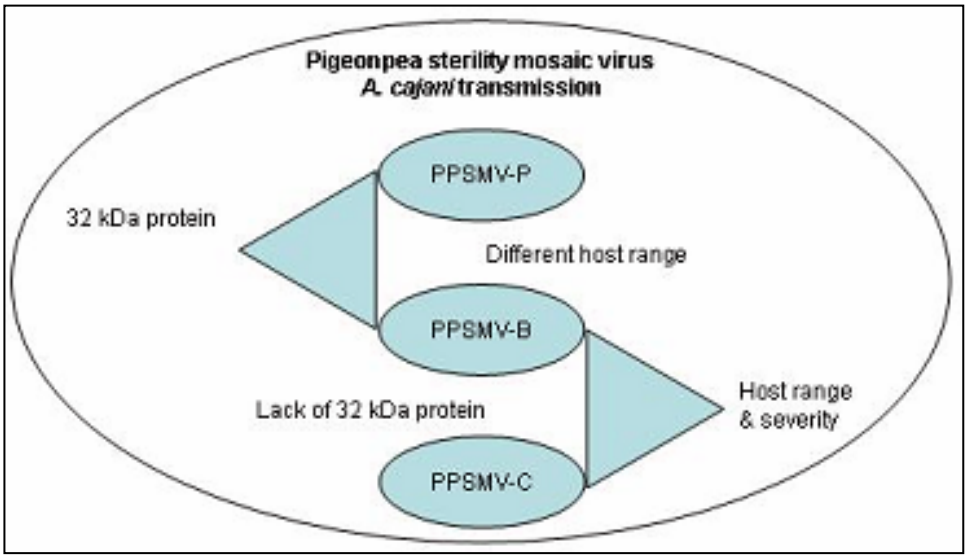
Sl. No.	*Parameters*	TTB 7	ICP 7035
1.	Moisture (%)	8.10	8.00
2.	Crude Fat (%)	1.00	1.10
3.	Crude Protein (%)	23.60	19.60
4.	Crude Fibre (%)	10.10	8.10
5.	Dietary Fibre (%)	32.88	27.41
6.	Starch (%)	30.04	33.43
7.	Total Ash (%)	3.87	4.22
8.	Acid Insoluble Ash (%)	0.03	0.03
9.	Metabolizable Energy (K.cals/100g)	316.60	324.30
10.	Dry Matter (%)	91.90	92.00
11.	Carbohydrates (%)	63.40	67.10
12.	Minerals and Trace elements (mg / 100 g of seed)		
	a. Calcium (Ca)	470.00	510.00
	b. Copper (Cu)	2.00	2.00
	c. Iron (Fe)	4.00	4.00
	d. Magnesium (Mg)	280.00	280.00
	e. Manganese (Mn)	1.00	3.00
	f. Phosphorous (P)	350.00	380.00
	Sem = 1.44; CD = 4.33; CV = 5.94		

TableO2.8: Nutritional qualities of ICP7035 vegetable pods

	Parameters	TTB 7	ICP 7035
1	Crude protein	8.56%	8.61%
2	Crude fibre	3.03%	3.00%
3	Dietary fibre	11.85%	11.93%
4	Crude fat	0.14%	0.11%
5	Moisture	62.52%	62.36%
6	Total ash	1.45%	1.39%
7	Acid insoluble ash	0.02%	0.03%
8	Metabolizable energy	132.7Kcal/100g	133.55Kcal/100g
9	Carbohydrates	27.33%	27.53%
10	Dry matter	37.48%	37.64%
11	Starch	18.54%	18.19%
12	Calcium	0.15%	0.15%
13	Phosphorous	0.13%	0.11%
14	Copper	1mg/100g	2mg/100g
15	Iron	4mg/100g	4mg/100g
16	Magnesium	0.11%	0.12%
17	Manganese	2mg/100g	1.5mg/100g

Nutritional parameters were estimated at Pristine Laboratories (approved by Govt. of India), Sahakarnagar Post, Bangalore 560 092, India

Figure O4.1. Relationships of three PPSMV isolates: Patancheru (P), Bangalore (B) and Coimbatore (C)



Contribution of Outputs to Developmental Impact

Outputs linking developmental goals

The goal of the project is to attain sustainable pigeonpea production by mitigating losses due to SMD through the cultivation of broad-based SMD resistant varieties thereby contributing to the sustainability of pigeonpea production system and increased economic returns to the farmers contributing to the poverty alleviation. This was achieved through this project outputs. Further information on PPSMV isolates and diagnostic tools contributed to the development of efficient resistance screening methods for the precise selection of broad-based resistant sources. This technology was used in a participatory manner with NARES to evaluate several cultivated and wild pigeonpea genotypes at various agro-ecological regions (Output 1). This led to the identification of promising genotypes suitable for cultivation in different agro-ecological zones (Output 2). For long-term impact of the outputs, the technology and the products of the project were disseminated to the NARES, NGOs and farmers through participatory research, training courses and on-farm trials (Output 3). This contributes to the capacity building of the national systems and stabilises the 'seed production and distribution systems' to deliver quality seeds to farmers. Further information on isolates of PPSMV and its affects on plant physiology (Output 4), revealed the diverse nature of the various isolates prevailing in the subcontinent, their geographic distribution and contributed to the development of bioassays, serological and nucleic acid-based tools which are useful for their identification of isolates, to determine isolate virulence, and for precise selection of durable resistant sources. This way the outputs of the project were interlinked and worked in a cascading manner leading one output to subsequent output, ultimately leading to the achievement of the project goal (This is depicted schematically in Figure C1).

Outputs delivered

This project has generated several outputs in the form of scientific publications and disseminated information and technologies developed in the project. Seed of elite disease resistant varieties were supplied to the farmers and for utilization in breeding programmes by NARES. Contributed to the capacity building through collaborations and training courses (the various outputs are listed in the Tables C1-C6; Figures C2-C3).

***Sustainability of outputs* ex-ante**

This project addressed a priority constraint on pigeonpea production in the Indian subcontinent. Pigeonpea is cultivated mainly by subsistence farmers in rainfed farming systems. Demand for pigeonpea is increasing every year. In addition, the crop is being exploited for non-traditional uses, such as for soil conservation and fodder and is also cultivated in cereal-based cropping system. This has further increased the value of the crop. SMD, fusarium wilt and pod borer (*Helicoverpa armigera*) are the endemic problems in the subcontinent. Research institutes, especially ICRISAT and the Indian Council of Agriculture Research (ICAR), have pigeonpea in their mandate with a research agenda supported from core and external agencies for the application of the technologies developed in this project to continue research for sustainable management of SMD and other biotic problems. These institutes have extensive networks (such as Cereals and Legumes Asia Network – CLAN of ICRISAT) and MoUs for dissemination of technology, information and varieties to NARES and farmers in the subcontinent. This will enable to achieve the goal of improving the availability of food, better nutrition and increased income to marginal farmers.

NARS (GKVK, Bangalore; Agricultural Research Station, Gulbarga) and NGO organisations (CWS, Hyderabad) working at grass root level in SMD and wilt endemic areas are involved in the project. These organizations have specific research programs for pigeonpea improvement, seed multiplication for distribution to farmers and to support the farmers' cause by providing on-station and village-level training programmes. State extension agencies are involved in varietal release and seed multiplication. Upon approval of release of a variety, these agencies would undertake regular seed production for supply to farmers.

ICRISAT invested in disease resistant hybrid pigeonpea development programmes with private seed sector agencies for developing hybrid pigeonpea. ICP7035, a broad-based SMD resistant variety, is being used as one of the parents in generating hybrids. This partnership will continue to utilize SMD resistance screening technologies and contribute to the production of elite hybrids for farmers.

Seed companies are interested in multiplying elite pigeonpea varieties. Ms JK Seed Corporation has undertaken commercial multiplication of ICP7035 selected from this project.

ICRISAT will continue to invest on research on SMD and other biotic problems to enhance pigeonpea production. Based on outputs from this project, a research proposal has been developed and submitted to funding agencies to continue key research and extension activities at ICRISAT and collaborating national institutes to further gain from these achievements.

The end product of the project are seed-based, which is easy to disseminate and simple to adopt by farmers at no additional cost. Therefore is sustainable.

Figure C1: Pictorial representation of R8205 activities and outputs contribution to developmental impact

LIST OF VARIOUS OUTPUTS & ACHIEVEMENTS

Table C1. PUBLICATIONS

Peer reviewed publications

1 KULKARNI, N.K., KUMAR, P.L., MUNIYAPPA, V., JONES, A.T. and REDDY, D.V.R. **2002**. Studies on the host range of Pigeonpea sterility mosaic virus. *Journal of Mycology and Plant Pathology*. 32: 141-145.

2 KULKARNI, N.K., REDDY, A.S., KUMAR, P.L., VIJAYNARASIMHA, J., RANGASWAMY, K.T., REDDY, L.J., SAXENA, K.B., JONES, A.T. and REDDY, D.V.R. **2002**. Broad-based resistance to Pigeonpea sterility mosaic disease in the accessions of *Cajanus scarabaeoides*. *Indian Journal of Plant Protection* 31: 6-11.

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

4 KUMAR, P.L. and MARTELLI, G.P. **2003**. Pothos latent virus. Association of Applied Biologists Descriptions of Plant Viruses. *Association of Applied Biologist*, CD-ROM Publication. UK.

5 BHATTACHARJEE, R., KUMAR, P. L., KOLESNIKOVA-ALLEN, M. and CHHABRA, A. K. **2003**. Plant biotechnology towards food security. pp. 1-11. In: *Enhancing Production and Food Value of Plants: Genetic Options Vol. 1* (eds. BEHL, and CHHABRA, A.K.). CSK, HPKV, Palampur, India.

6 REDDY, S.V. and KUMAR, P.L. **2004**. Transmission and properties of a new luteovirus associated with chickpea stunt disease in India. *Current Science* 86:1157-1161.

7 MAKKOUK, K.M., KUMARI, S.G., HUGHES, J.D'A., MUNIYAPPA, V. and KULKARNI, N.K. 2003. In *Virus and Virus-like Diseases of major Crops in Developing Countries*. Eds. G. Loebenstein and G. Thottappilly. Kluwer Academic Publishers. pp447-475

8 KUMAR, P.L. **2004**. Aceria cajani: Alien Invasive Species (datasheet). CABI Crop Protection Compendium. CABI-Wallingford, England. CD-ROM Publication.

9 JONES, A.T., KUMAR, P.L., SAXENA, K.B., KULKARNI, N.K., MUNIYAPPA, V. AND WALIYAR, F. **2004**. Sterility mosaic disease - the "green plague" of pigeonpea: advances in understanding the etiology, transmission and control of a major virus disease. *Plant Disease* 88:436-445.

10 DHARMARAJ, P.S., NARAYANA, Y.D., KUMAR, P.L., WALIYAR, F. and JONES, A.T. **2004**. Pigeonpea sterility mosaic disease: an emerging problem in northern Karnataka. *International Chickpea and Pigeonpea Newsletter* 11: 47-49.

11 KUMAR, P.L., LATHA, T.K., KULKARNI, N.K., RAGHAVENDRA, K., SAXENA, K.B., WALIYAR, F., RANGASWAMY, K.T., MUNIYAPPA, V., SABITHA DORISWAY and JONES, A.T. **2005**. Broad-based resistance to pigeonpea sterility mosaic disease in wild relatives of pigeonpea (Cajanus: Phaseoleae). *Annals of Applied Biology* (Accepted)

Methods manuals

1 KUMAR, P.L. JONES, A.T. and REDDY, D.V.R. **2002**. *Pigeonpea sterility mosaic virus: Detection and screening for resistance*. Methods manual. ICRISAT, Patancheru 502 324, India, 65pp

2 KUMAR, P.L. JONES, A.T. and WALIYAR, F. (Eds). **2004**. *Serological and Nucleic Acid Based Methods for the Detection of Plant Viruses*: Methods manual. ICRISAT, Patancheru 502 324, India, 120pp (also on CD-ROM)

Presentations in conferences

1 KUMAR, P.L., JONES, A.T., KULKARNI, N.K., MUNIYAPPA, V., RANGASWAMY, K.T., SREENIVASULU, P., SAXENA, K.B. and REDDY, D.V.R. **2002**. Towards sustainable management of Pigeonpea sterility mosaic disease. In abstracts Asian Congress of Mycology and Plant Pathology. October 1-4, 2002, University of Mysore, Mysore, India, pp14. (Abstract)

2 KULKARNI, N.K., REDDY, A.S., KUMAR, P.L., VIJAYNARASIMHA, J., RANGASWAMY, K.T., REDDY, L.J., SAXENA, K.B., JONES, A.T. and REDDY, D.V.R. **2002**. Broad-based resistance to Pigeonpea sterility mosaic disease in the accessions of Cajanus scarabaeoides. In Abstracts National Seminar on Resources Management in Plant Protection During the Twenty-first Century. NBPGR, Rajendranagar, Hyderabad, AP, India, pp58. (Abstract) [Also In Resources Management in Plant Protection Vol 2. Eds. SARATH BABU *et al*. Plant Protection Association of India, Rajendranagar, Hyderabad AP, India]

4 VIJAYNARASIMHA, J., RANGASWAMY, K.T., PRAMEELA, H.A. and MUNIYAPPA, V. **2002**. Mechanism of resistance to sterility mosaic virus disease in pigeonpea genotype ICP7035. In abstracts of a meeting on Plant Disease Scenario In Southern India, University of Agriculture Sciences, Bangalore 560065, India, 23-24. (Abstract)

5 KUMAR, P.L. JONES, A.T. LATHA, T.K.S., MUNIYAPPA, V. and WALIYAR, F. **2003**. Preliminary characterisation of three isolates of Pigeonpea sterility mosaic virus (PPSMV). In International Conference on Advances in Plant Virology 2003. September 20-22, 2003. CIRAD, Montpellier, France.

6 KUMAR, P.L., JONES, A.T., SAXENA, K.B., KULKARNI, N.K., MUNIYAPPA, V., RANGASWAMY, K.T., DHARMARAJ, P., NARAYANA, Y.D., SREENIVASULU, P and WALIYAR, F. **2003**. Combating the pigeonpea sterility mosaic disease for sustainable pigeonpea production and diversification. National Symposium on Pulses for Crop Diversification and Natural Resources Management. 20-22 Dec 03, Kanpur, India. (Abstract)

7 JONES, A.T., WALIYAR, F. and KUMAR, P.L. 2003. Overview of DFID funded research on sterility mosaic disease. In: International Meeting on Combating Biotic Stresses of Pigeonpea, 13-14 November **2003**. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, AP. India.

8 KUMAR, P.L., JONES, A.T. and WALIYAR, F. **2003**. Understanding sterility mosaic disease. In: International Meeting on Combating Biotic Stresses of Pigeonpea 13-14 November 2003. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, AP. India

9 KULKARNI, N.K., KUMAR, P.L., MUNIYAPPA, V., JONES, A.T. and REDDY, D.V.R. **2003**. Studies on pigeonpea sterility mosaic disease; transmission, virus vector relationships and identification of resistant sources. In Plant Pathogens Diversity in Relation to Plant Health, Osmania University, Hyderabad 500 007, AP, India. Abstract in Indian Phytopathology 56: 308. ***[Winner Prof. M. J. Narasimhan Merit Academic Award 2003 Competition organized by the Indian Phytopathological Society]***

10 BHATTACHARJEE, R., HASH, C.T., and KUMAR, P.L. **2003**. Application of DNA markers in conservation of plant genetic resources. pp. 183. In: Proceedings of National Seminar on Advances in Genetics and Plant Breeding - Impact of DNA Revolution, October 30-31, 2003. University of Agricultural Sciences, Dharwad, Karnataka, India: Indian Society of Genetics and Plant Breeding. (Abstract)

11 KUMAR, P.L., JONES, A.T., KIMMINS, F. and WALIYAR, F **2004**. An example of DFID supported natural resource research from the Crop Protection Programme: Sterility Mosaic Disease in Pigeonpea - An Overview. 2004. In Summaries of "Teach-In" Meeting Presentations at Department for International Development (DFID) to Sir David King. January 28, 2004, DFID, Palace Street, London, UK. ***[By invitation]***

12 CHARI, M.S., RAJASHEKAR, G., VAGMARE, G., RAGHUNATH, T.A.V.S KUMAR, P.L., SAXENA, K.B., WALIYAR, F. and JONES, A. T. **2004**. Village-level implementation of eco-friendly disease management strategies for sustainable pigeonpea production. National Seminar on Resource Management for Sustainable Agriculture. 28-29 Jan 2004, Baptala, India. The Andhra Agriculture Journal 50: 482-483. ***[Winner best poster presentation award].***

13 PRAMOD BABU, K., NARAYANA, Y.D., KUMAR, P.L. and DHARMARAJ P.S. **2004**. Assessment of pigeonpea crop loss due to sterility mosaic disease in Gulbarga and Bidar districts of North Karnataka. In abstracts National Symposium on Crop Surveillance, Disease Forecasting and Management, February 19-21, 2004, Division of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi - 110 012, India. pp55. (Abstract)

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BSc thesis

BINITA DAS. **2005**. An account on viruses infecting ICRISAT mandate crops. St Francis Degree College for Women, Hyderabad, India, pp42.

MSc thesis

1 RAGHAVENDRA, K. **2003**. Characterization of Bangalore Isolate of Pigeonpea Sterility Mosaic Virus and Screening of Pigeonpea Genotypes for Disease Resistance. University of Agricultural Sciences, Bangalore, India. 130pp

2 DIVA, P. **2004**. Molecular characterisation of pigeonpea sterility mosaic virus isolates. University of agriculture Sciences, Bangalore 560 065, India, pp102.

PhD thesis

1 LATHA, T.K.S. **2003**. Studies on Coimbatore isolate of Pigeonpea sterility mosaic virus. Tamil Nadu Agriculture University, Coimbatore, India. 180pp.

Information brochures

1 MUNIYAPPA, V. and KUMAR, P.L. **2002**. Measures to control sterility mosaic disease of pigeonpea. University of Agricultural Sciences, Bangalore, India,.4pp. (in Kannada language) **(1000 copies)**

2 Kimmins, F., Ward, A., Jones, A.T., Waliyar, F and Kumar, P.L. **2002**. Brochure on Controlling the green plague of pigeonpea. Department for International Development (DFID) Crop Protection Programme. Kent, UK.

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6 KUMAR, P.L. **2004**. ICP7035: A dual-purpose pigeonpea variety with broad-based resistance to sterility mosaic disease. Information Broacher for Pigeonpea Scientists Meeting at ICRISAT, 9 December 2004, ICRISAT, Patancheru, India. 1pp. (50 copies)

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1 KUMAR, P.L. **2004**. From sterility to fertility. SAT Trends 41. http://www.icrisat.org/

2 KUMAR, P.L. **2004**. Tackling Asia's pigeonpea plague. New Agriculturist On-line. July 2004. http://www.new-agri.co.uk/

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1 Jones, A.T., Waliyar, F and Kumar P.L. **2003**. Solution to a mite(y) problem. Department for International Development (DFID) Crop Protection Programme Annual Report for 2002-2003. pp1.21

2 CSA. **2004**. Life Sustains Life. Annual report 2003-04, Center for Sustainable Agriculture, Secunderabad 500017, AP, India. 66pp.

Extension Documents

	Type	Details
1	Public fair	SCOTTISH CROP RESEARCH INSTITUTE. **2003**: International Collaboration and Training: Plants with Resistance to Stress. At Invergowrie Charity Fair, 31 May 2003, Invergowrie, Scotland. UK

		(Poster)
2	Seed vouchers	Free Seed vouchers. **2003**. Pigeonpea variety ICP7035 free seed vouchers (for 300g seed) supplied to farmers and extension workers.
3	Seed vouchers	Free Seed vouchers. **2004**. Pigeonpea variety ICP7035 free seed vouchers (for 1 kg seed) supplied to farmers and extension workers.
4	Video CD	ETV Video. **2004**. Video on "pigeonpea sterility mosaic disease, ICP7035 – a durable SMD resistant variety and integrated pest management" in Kanada language. Produced by University of Agriculture Sciences, Bangalore, Extension Education Unit and ICRISAT, in association with ETV Network.
5	Article in news paper	DHARMARAJ, P. **2004**. Information on sterility mosaic disease and its control. *Vijaya Karnataka*, 5 June 2004. (Kanada language)
6	Article in news paper	DHARMARAJ, P. **2004**. Information on sterility mosaic disease and its control. *Kanada Prabha*, 9 June 2004. (Kanada language)
7	Radio broadcast	KUMAR, P. L. **2004**. Tackling Asia's pigeonpea plague (transcript of an interview). *WREN Media: World Radio for the Environment*. February 2004
8	Poster	KUMAR, P.L. **2004**. Virology @ ICRISAT. Vision-2010, colloquium cum walk-through exhibition, 14 December 04, ICRISAT, Patancheru 502 324, AP, India.
9	Book	Kumar, P.L. and Jones, A.T. **2004**. Green Gold. In Positive Developments B. Siderman-Wolter (Ed). NR International, Kent, UK. p51. (Runner Up Poster at "Positive Developments" Photo Exhibition at Eden Park, England, UK)
10	Poster	UAS-Bangalore. **2004**.Pigeonpea Sterility Mosaic Disease Resistant Variety ICP 7035 and Its Cultivation Practices. Displayed during farmers field day on 29 November 2004 at Kuchangi Village, Tumkur District, Karnataka, India. (In English and Kanada languages)

News Reports

	Type	Details
1	International Newsletter	Awards for DFID-funded research on pigeonpea sterility mosaic. *International Chickpea and Pigeonpea Newsletter* 10: 3. **2003**
2	News Paper report	ANONYMOUS. 13.6.**2003**. SMD resistant high-yielding pigeonpea seed village at Thodellagadda, Mahboobnagar District, AP. *Andhra Joythi*
3	Newspaper report	ANONYMOUS, 1 January **2003**: Pigeonpea: Progress towards management of sterility mosaic disease. *Praja Vani* (Premier Kannada daily news paper)
4	Newsletter	Outbreak of SMD, *Krishi Vigayana Kendra Newsletter*, 1 June **2004**.

5	In house newsletter	ICRISAT **2004**. Demonstration of SMD resistant variety. *ICRISAT Happenings* No 1130. p3.
6	Science and Environment magazine	ANONYMOUS **2004**. Pigeonpea now smiles*. Down to Earth - Science and Environment Fortnightly* (Dec 15, 2004). 24-25
7	Press release	ASHOK, B.S. **2004**. ICRISAT scientist wins CGIAR award in Mexico. *Financial Express*. (8 Nov 2004)
8	Press release	ANONYMOUS. **2004**. Pigeonpea ICP7035 Farmers Field Day. In *Sogadu* – Kannada language newspaper. (27 Nov 04)
9	Press release	ANONYMOUS. **2004**. In the CG Science Awards. New*Agriculturist On line*. http://www.new-agri.co.uk/ (November 2004, issue)
10	Press release	ANONYMOUS. **2004**. ICRISAT bags two awards. *Business Standard*. Daily News Paper. (3 Nov 2004)
11	Press release	ANONYMOUS. **2004**. ICRISAT bags two awards. *The Hindu – National Daily*. (4 Nov 2004)
12	Press release	ANONYMOUS. **2004**. CGIAR Announces 2004 Science Awards - Recognition goes to food, fisheries and livestock researchers battling global hunger and poverty. *CGIAR online*. http://www.cgiar.org
13	Press release	ANONYMOUS. **2004**. ICRISAT wins awards at Mexico. *ICRISAT online*. http://www.cgiar.org
14	Press release	ANONYMOUS. **2004**. ICRISAT bags two awards. *The New Indian Express*. (3 Nov 2004)
15	Press release	ANONYMOUS. **2004**. Awards to ICRISAT. *Eenadu* Telugu Daily. (Vernacular news paper) (3 Nov 2004)
16	In-house Newsletter	ICRISAT. **2004**. The SMD working group meets again. *ICRISAT Happenings* No. 1102.

Internal Reports

	Type	Details
1	Internal report	KUMAR, P.L. September 14, **2002**. Report on SMD training course at ICRISAT, Patancheru, India.
2	Field visit report	KUMAR, P.L. September 13, **2002**. Report on-farm trials of ICPL960053, ICPL96058 and ICPL87119, in Gulbarga and Bidar districts
3	Farmers field day report	KUMAR, P.L. December 31, **2002**. Report on Farmers Field Day to demonstrate SMD and broad-based SMD resistant pigeonpea cultivar ICP7035.
4	Meeting report	KUMAR, P.L. October 8, **2002**. 1. Report on paper presentation in the Asian Congress of Mycology and Plant Pathology (ACMPP), University of Mysore, Mysore, Karnataka, India and meeting at

		University of Agriculture Sciences, Bangalore, Karnataka, India, to discuss on-farm trials and project management.
5	Internal report	KUMAR, P.L. and JONES, A.T. November **2002**, Report on Dr AT Jones Visit to ICRISAT
6	Work plan report	KUMAR, P.L., JONES, A.T. and WALIYAR, F. November **2002**. Project activities and work plans of R8025 (ZA0522) project
7	Field visit report	KUMAR, P.L. December 4, **2002**. Report on-farm trials of pigeonpea varieties ICPL960053 and ICPL96058 in Gulbarga district and SMD incidence in Gulbarga district, Karnataka
8	Field visit report	KUMAR, P.L. December 26, **2002**. Report on video graph of SM-affected farms and on-farm trials for preparing a video on SMD
9	Farmers Training	KUMAR, P. L. **2003**. Summary Report Integrated management of biotic problems of pigeonpea: Training course to farmers. 25 November 2003, Agriculture Research Station, Gulbarga, India. 3pp
10	Internal report	KUMAR, P. L. **2003**. Report of DFID R8025 review and work-planning meeting at SV University, Tirupati.
11	Internal Report	WALIYAR, F. **2003**. Tour Report Summary: Participation in Farmers Field Day at Ramanagar Taluk, Bangalore.
12	Internal Report	JONES, A.T. **2003**. Tour Report Summary of a visit to ICRISAT, India, to participate in project review meeting and International pigeonpea scientist meeting. 3pp.
13	Internal Report	RANGASWAMY, K. T. **2003**. Tour Report Summary of a visit to the Scottish Crop Research Institute (SCRI), Dundee, UK.
14	Internal report	KUMAR, P. L. **2003**. Summary of review meeting on GKVK activities.
15	Internal report	KUMAR, P.L. **2004**. Report on DFID Teach-In meeting at London. 3 pp.
16	Training report	DIVYA, P. **2004**. Surveys for the geographic distribution of Pigeonpea sterility mosaic Virus isolates in Karnataka: Report on methods for the detection of PPSMV isolates. 20 pp
17	Farmers Field Day	MUNIYAPPA, V., KUMAR, P.L., WALIYAR, F., SAXENA K. B. and JONES. A.T. **2004**. Proceedings summary Demonstration of On-farm Performance of ICP7035: A Broad-Based Sterility Mosaic Disease Resistant Pigeonpea Variety for Vegetable and Seed Purpose. Farmers Day at Doddagangavadi Watershed Area Ramanagara Taluk, Bangalore District, Karnataka State December 16, 2003. 14pp
18	Internal Report	RAGUNATH, T.A.V.S and KUMAR, P.L. **2004**. Report of a visit to on-farm trials at Todallagada, Mahboonagar District, AP. 3pp.
19	Farmers Field Day	MUNIYAPPA, V., KUMAR, P.L., WALIYAR, F., SAXENA K. B. and JONES. A.T. **2004**. Proceedings summary Demonstration of On-farm

		Performance of ICP7035: A Broad-Based Sterility Mosaic Disease Resistant Pigeonpea Variety for Vegetable and Seed Purpose. Farmers Day at Doddagangavadi Watershed Area Ramanagara Taluk, Bangalore District, Karnataka State December 16, 2003. 14pp
20	Training course report	KUMAR, P.L. WALIYAR, F. and GOWDA, C.L.L. **2004**. Technical Report on CPP-DFID sponsored virology-training course at ICRISAT. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India pp21.
21	Internal report	KUMAR, P.L. **2004**. Summary of working group meeting at Agriculture Research Station, Gulbarga. Tour Report Summary 18.5. 2004. 2pp.
22	Internal report	KUMAR, P.L. **2004**. Summary of survey for SMD in Southern Karnataka. Tour Report Summary 9 September 2004. 5pp.
23	Internal report	KUMAR, P.L. **2004**. Tour report on UAS-Dharwad and ICRISAT Farmers day, at UAS-Dharwad. 2pp
24	Internal report	KUMAR, P.L. **2004**. Tour report on CGIAR AGM-2004 and CGIAR Science Awards at Mexico City, Mexico. 3pp.
25	Internal report	KUMAR, P.L. **2004**. Tour report on farmers field demonstrating ICP7035 and IPM training to farmers in Kuchangi village, Tumkur Taluk, Karnataka, India. 5pp.

Publications in Preparation / Planned

	Type	Details
1	Review article	KUMAR, P.L., JONES, A.T. and WALIYAR, F. **2004**. Pigeonpea sterility mosaic disease. *Annual Review of Plant Pathology* Vol. III. (by invitation).
2	Conference presentation	KUMAR, P.L., NARAYAN, Y.D., MUNIYAPPA, V., RANGASWAMY, K.T., JONES, A.T., WALIYAR, F. and KIMMINS, F. **2005**. Epidemiology and management of pigeonpea sterility mosaic disease, for presentation in the *IX International Plant Virus Epidemiology Symposium* in Lima, Peru on April 4-7, 2005.
3	Journal article	KUMAR, P.L., JONES, A.T., LATHA, T.K.S., and WALIYAR, F. Improved detection of Pigeonpea sterility mosaic virus by DAS-ELISA using penicillinase reporter system.
4	Journal article	VIJAYANARASIMHA, J., RANGASWAMY, K.T., KUMAR, P.L., MUNIYAPPA, V., WALIYAR, F and JONES, A.T. Mechanism of resistance to Pigeonpea sterility mosaic virus and its vector, *Aceria cajani* in pigeonpea genotypes.
5	Journal article	MUNIYAPPA, V., RANGASWAMY, K.T., KUMAR, P.L., SAXENA, K.B., GOWDA, B.G., PANDURANGIAHA, K., WALIYAR, F. and JONES, A.T. ICP 7035: A broad-based sterility mosaic disease resistant pigeonpea variety for seed and vegetable purpose.

6	Journal article	LATHA, T.K.S., KUMAR, P.L., JONES, A.T. and SABITHA DORISWAMY. Biochemical characterization of Pigeonpea sterility mosaic virus -Coimbatore isolate
7	Book chapter	KUMAR, P.L. and JONES, A.T. Properties of Pigeonpea sterility mosaic virus In Molecular Diagnosis of Plant Viruses (Ed. RAO, G.P.). Studium Press, LLC, P.O. Box 722200-Houstan, Texas 77272, USA.
8	Information Bulletin	KUMAR, P.L., JONES, A.T., SAXENA, K.B., WALIYAR, F. Sterility mosaic disease. ICRISAT, Patancheru 502 324, AP, India.

Table C2. EXTENSION ACTIVITIES

	Event	Details
1	Farmers field day	Farmers day to demonstrate affects of sterility mosaic disease on pigeonpea crop, and performance of broad-based SMD resistant pigeonpea genotype ICP7035 in farmers fields at Channegowdanadoddi and Adishakti Halli, Ram Nagar Taluk, Bangalore District, Karnataka. December 29, **2002**. (ICRISAT-DFID-University of Agricultural Sciences, Bangalore-Karnataka State Extension Department, joint event). **(130 farmers)**
2	Special lecture and demonstration in training progarmmes	KUMAR, P.L. **2002**. Selection for Virus Disease Resistance. In "Training course on Pigeonpea Improvement with Special Emphasis on CMS Based Hybrid Breeding Technology". 16 to 18 September 2002, ICRISAT, Patancheru, India. **(12 participants)**
3	Special lecture and demonstration in training progarmmes	KUMAR, P.L. **2002**. Sterility Mosaic Disease: Management Options. In "ICRISAT and Rice Wheat Consortium (RWC) for the Indo-Gangetic Plains Training Course on Integrated Management of Pigeonpea and Chickpea Pests & Diseases". 3 -6 December 2002, ICRISAT, Patancheru, India. **(12 participants)**
4	Working group meeting	Ist Working group meeting on "Combating Sterility Mosaic Disease – A step Towards Sustainable Pigeonpea Production", 5 September **2002**, ICRISAT, Patancheru 502 324, AP, India. **(25 participants)**
5	Working group meeting	IInd Working group meeting on 'combating pigeonpea sterility mosaic disease for sustainable pigeonpea production', 3-4 April **2003**, Sri Venkateswara University, Tirupati 517502, AP, India. **(30 participants)**
6	Training to farmers	Training programme on "IPM and IDM methods to manage pigeonpea biotic problems", 12 June **2003**, Thodellagadda, Mahboobnagar District, AP **(50 farmers)**
7	Training course to Farmers	Training Programme for farmers on management of SMD, wilt and Pod borer, held on 27 November **2003**, at Agricultural Research Station, Aland Gulbarga in association with International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. **(50 farmers)**
8	Special lecture and demonstration in training progarmmes	KUMAR, P.L. **2003**. Diseases of Pigeonpea. In Training Course on Pigeonpea Cultivation to Delegation from China. 4-13 March 2003, ICRISAT, Patancheru, India**. (40 participants)**
9	International meeting	International Meeting on "Combating Biotic Stresses in Pigeonpea" 13-14 November **2003**, ICRISAT, Patancheru, India. **(30 participants)**
10	Working	IIIrd Working group meeting on "Building on Strengths for Maximizing

	group meeting	the Research Impact", 17 May 2004, Agriculture Research Station, Gulbarga, Karnataka State, India. **(40 participants)**
11	Awareness meeting	Awareness meeting for farmers on SMD and IPM of pod borer, March and April **2004**, in Kasturipalli and Indanur villages of Kodangal Mandal, Mahbbonagar District, AP, India. **(250 farmers)**
12	Training in seed-village to farmers	Training to farmers on management of seed-village programmes, 15 April **2004**, PEACE, Bhuvangir, Nalgonda District, AP, India **(25 participants)**
13	Training to field coordinators	Training to field coordinators on IPM, IDM and crop management, 17 July **2004**, Center for Sustainable Agriculture, Taranaka, Hyderabad, India **(20 Participants).**
14	Training course to NARES	Serological and Nucleic Acid Based Methods for the Detection of Plant Viruses, 12-20 April **2004**, ICRISAT, Patancheru 502 324, India. **(9 scientists from NARS)**
15	Farmers training	Training to farmers in "Eco-friendly management of SMD, wilt and pod borer", March and April **2004**, Indanur villages of Kodangal Mandal, Mahbbonagar District, AP, India. **(60 farmers)**
16	Farmers field day	Field day to demonstrate on-farm performance of SMD resistant varieties, 30 November **2004**, Pyalamaddi village, Mahbbonagar District, AP, India. **(60 farmers)**
17	Farmers field day	Field to demonstrate SMD resistant pigeonpea genotypes ICPL 96058, ICPL 96053 and BSMR 736. 27 December **2004** at Bhammanahalli village, Aland taluka, Gulbarga District, Karnataka, India. **(80 farmers)**
18	Farmers field day	Farmers day to demonstrate performance of pigeonpea variety ICP7035 and training in integrated crop management practices. Sponsored by Crop Protection Programme, DFID, UK. November 29, **2004**. Kuchangi Village, Tumkur District, Karnataka State, India. **(350 farmers)**

Table C3. MERIT AWARDS

	Type	Details
1	Individual award	KUMAR, P.L. *Millennium ICRISAT Science Award – 2002, "Promising Young Scientist".* Presented by ICRISAT, Patancheru, India, presented in December **2002**.
2	Individual award	KUMAR, P.L. *Sri Vererapaneni Narasimham Memorial Gold Medal for Best Research Worker in the Plant Pathology – 2001*. Presented by Acharaya NG Ranga Agriculture University, Hyderabad, presented in March **2003**.
3	Individual award	KULKARNI, N.K. *Prof. M. J. Narasimhan Merit Academic Award* **2003**, For presenting a paper titled "Studies on pigeonpea sterility mosaic disease; transmission, virus vector relationships and identification of resistant sources" by N.K. Kulkarni, P. Lava Kumar, V. Muniyappa, A.T. Jones and D.V.R. Reddy, in Plant Pathogens Diversity in Relation to Plant Health, Osmania University, Hyderabad, India
4	Team award	CHARI, M.S. et al. *Best Poster Award of Andhra Agriculture Union Golden Jubilee Celebrations*. "Village-level implementation of eco-friendly IPM and IDM methods for sustainable pigeonpea production" was rated as best poster In National Seminar on Resource Management for Sustainable Agriculture, January 28-30, **2004**, Agriculture College, Bapatla, India
5	Individual award	KUMAR, P.L. *ICRISAT Loyalty Award – 2004*. On the occasion of completing 5 years employment with ICRISAT. Presented by ICRISAT Patancheru, 14 December **2004**
6	Individual award	KUMAR, P.L. *CGIAR Science Award for Promising Young Scientist – 2004*. Presented on 27th October 2004 by Dr Ian Johnson, Chairman CGIAR, during AGM **2004** at Mexico City, Mexico
7	Individual award	KUMAR, P.L. *Millennium ICRISAT Science Award – 2004*, "Promising Young Scientist". Presented by ICRISAT, Patancheru, India, presented in December **2004**.

Figure C2. Merit awards in recognition to the outstanding achievements of the CPP-DFID R8205 project.

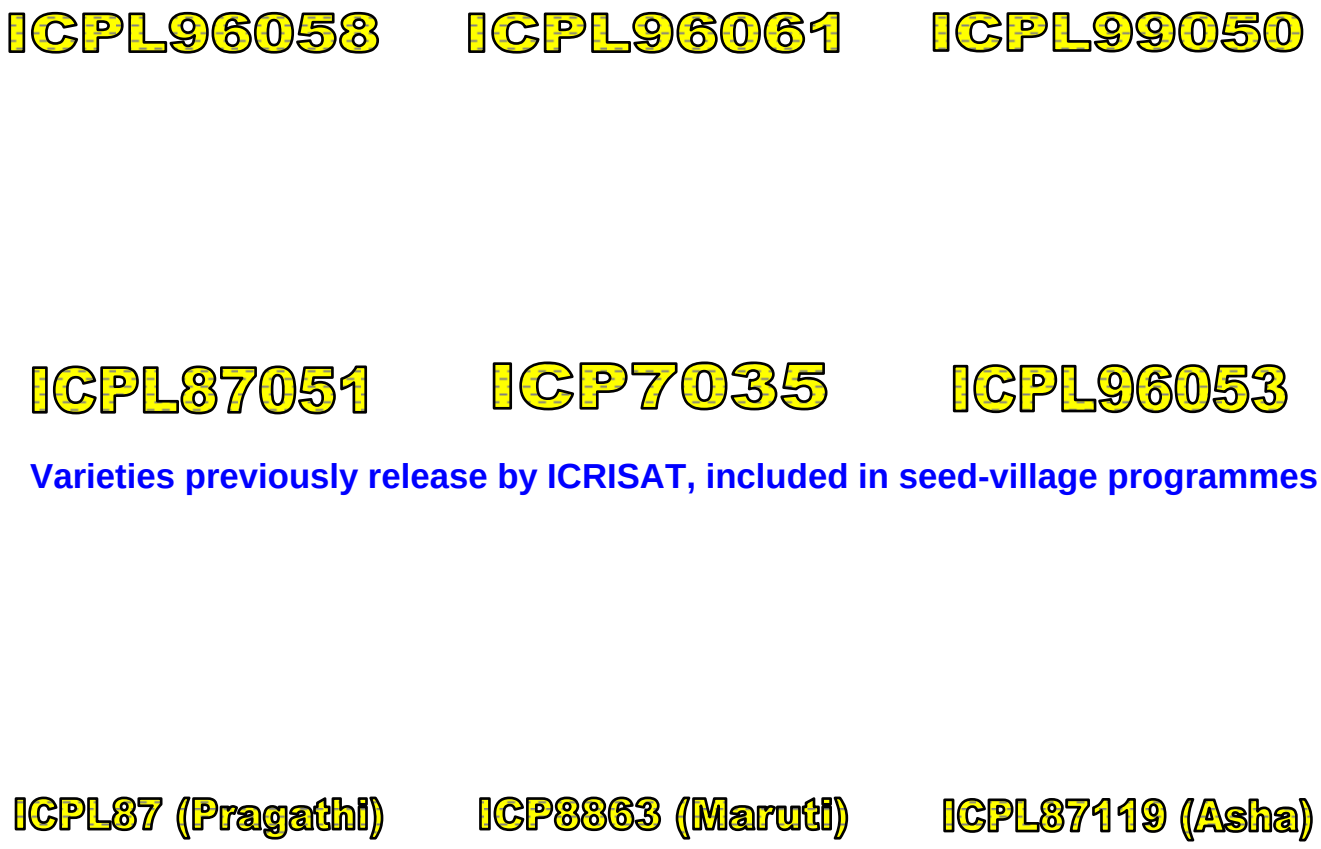
Note: Please see Table C3 for more details about awards

Table C4. PROMISING SMD-RESISTANT PIGEONPEA (WILD AND CULTIVATED) GENOTYPES

	Genotype	Advantages	Status of dissemination
		Pigeonpea varieties	
1	ICP7035	Broad-based SMD resistance and tolerance to wilt. Useful as both vegetable and seed purpose. Medium duration.	Approved for release in Karnataka. This variety is being grown in Southern Karnataka, Northern Andhra, Central India and Uttar Pradesh. Seeds available for farmers and NARES
2	ICPL96058	Durable SMD resistance to P isolate of PPSMV. Resistant to wilt. Seed purpose. Medium duration.	Advanced pre-release on-farm trials in Northern Karnataka and Andhra Pradesh states. Seeds available for farmers and NARES
3	ICPL96053	Durable SMD resistance to P isolate of PPSMV. Resistant to wilt. Seed purpose. Medium duration.	Advanced pre-release on-farm trials in Northern Karnataka and Andhra Pradesh states. Seeds available for farmers and NARES
4	ICPL99050	Durable SMD resistance to P isolate of PPSMV. Resistant to wilt. Seed purpose. Medium duration.	Pre-release on-farm trials in Northern Karnataka and Andhra Pradesh states. Seeds available for farmers and NARES
5	ICPL96061	Durable SMD resistance to P isolate of PPSMV. Resistant to wilt. Seed purpose. Medium duration.	Pre-release on-farm trials in Northern Karnataka and Andhra Pradesh states. Seeds available for farmers and NARES
6	ICPL87051	Durable SMD resistance to P isolate of PPSMV. Resistant to wilt. Seed purpose. Medium duration.	Pre-release on-farm trials in Northern Karnataka and Andhra Pradesh states. Seeds available for farmers and NARES
		Pigeonpea breeding lines	
1 2 3 4 5	ICPL83015 ICPL93087 ICPL93183 ICPL93184 ICPL95020	• Developed from ICP7035 (broad-based SMD resistance) as one of the parents to develop short-medium duration SMD resistant	• On-station evaluation and seed multiplication for on-farm and multi-locational evaluation.

6	ICPL95024	varieties to overcome terminal drought. • These lines are resistant to PPSMV B and P isolates, • Matures in 110-150 days and suitable for seed purpose.	• Limited seed available for supply to NARES.
		Wild Cajanus accessions	
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15	ICP15164 ICP15615 ICP15626 ICP15684 ICP15688 ICP15700 ICP15701 ICP15725 ICP15734 ICP15736 ICP15737 ICP15740 ICP15924 ICP15925 ICP15926	• All these genotypes are broad-based resistance to three PPSMV isolates (P, B and C). • ICP15684 is immune to wilt, pod borer damage and pod fly. • ICP15688 is resistant to wilt. • All these lines belong to *C. scarabaeoides* and *C. albicans* species of secondary genepool and compatible for conventional breeding programmes. • Very useful for breeding multiple disease resistant varieties and also to broaden the genetic base of resistance.	• Limited seed available to supply to NARES upon request.

Figure C3: Promising SMD and wilt resistant pigeonpea varieties selected for promotion in SMD endemic areas.



ICP 7035:** Broad-based SMD resistance and wilt tolerance. Suitable for cultivation allover India. Suitable for vegetable and also for seed purpose. *For release by UAS, Bangalore, ICRISAT and KSDA.*

ICPL 96058, 96053:** SMD resistance (P isolate) and wilt resistance. On-station and on-farm trials conducted. Suitable for central India. On-farm trials for for farmer and NARES adoption

ICPL 96061, 99050, 87051**: SMD (P isolate) and wilt resistance. On station trials and limited on-farm trials. Suitable for central India
(**All the above varieties are medium duration)*

Varieties released previously by ICRISAT:
ICP8863: Wilt resistance**
ICPL87119: wilt and SMD** resistance (P isolate)
ICPL87: Short duration determinate type**.

**Included for seed multiplication in seed-village programmes

Table C5. DIAGNOSTIC REAGENTS

	Type of reagents	Dissemination status
1	Polyclonal antibodies (rabbit) to PPSMV-Coimbatore isolate	• Diagnostic reagents supplied to all partners and also to other stakeholders and researchers upon request. • Sufficient quantities of antibodies and oligonucleotide primers available to supply upon request.
2	Polyclonal antibodies (rabbit) to PPSMV-Bangalore isolate	
3	Polyclonal antibodies (rabbit) to PPSMV-Patancheru isolate (from preparations made from French bean host)	
4	Subset RT-PCR oligonucleotide primers ss1 and ss2 for PPSMV-C isolate detection	

Table C6. HUMAN RESOURCE DEVELOPMENT

	Target audience	Method of training
1	**Farmers**	• Trained in IPM and IDM to control SMD and wilt, through cultivation of SMD resistant varieties, and pod borer through bio-pesticides using locally available plant products. • Trained in managing seed-village programmes to multiple seed of promising varieties. • Farmers awareness on SMD and disease resistance varieties enhanced through exposure visits, field days and farmers schools • Seed of pigeonpea varieties approved for release (ICP7035) and pre-release on-farm evaluation (ICPL 87051, 99050, 96053, 96058 and 96061) supplied for evaluation and adoption.
2	**NGOs**	• Intermediary and grass-root level NGOs trained in SMD diagnosis and management • Seed of pigeonpea varieties approved for release (ICP7035) and pre-release on-farm evaluation (ICPL96058, 96053, 87051) supplied for evaluation and local adoption.
3	**NARES & Students**	• Scientists (14 from Indian NARS) and postgraduate students (20 from Indian institutes) trained in SMD diagnosis, virus detection methods and virus purification. • Project work lead to PhD thesis (1 student from TNAU, Coimbatore) and three MSc thesis (2 students from UAS, Bangalore) • Supplied diagnostic reagents and seed of promising varieties for utilization in regional pigeonpea improvement programmes.

Biometricians Signature

Not applicable

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

Signature:	-
Name (typed):	-
Position:	-
Date:	-

APPENDIX

Photographs of various activities of R8205 project

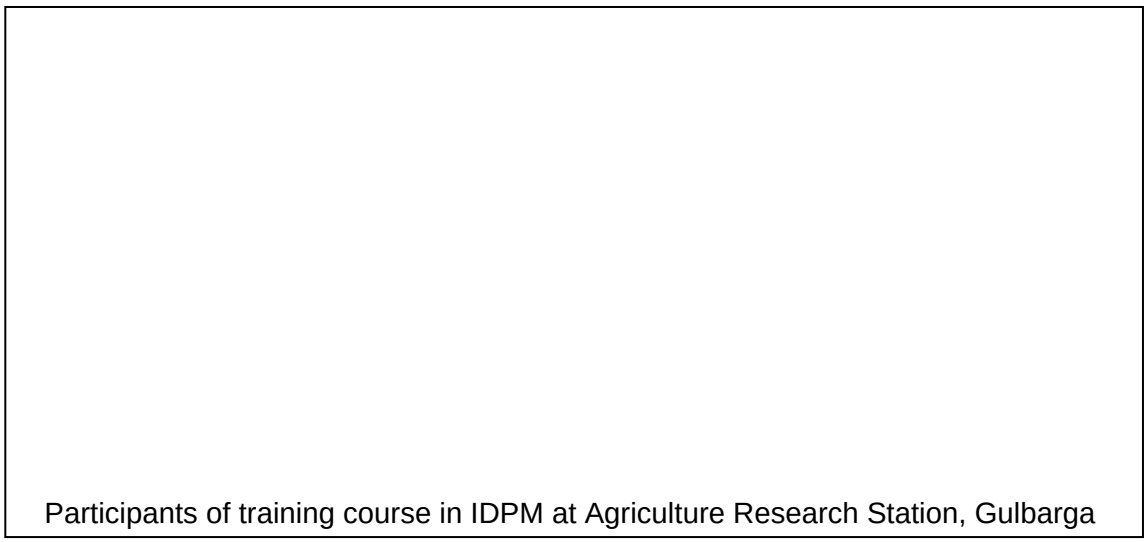
Farmers in field demonstrations listening to IDPM lecture

Women farmer demonstrating preparation of bio-pesticide (Neem seed kernel extract and chilli-garlic paste)

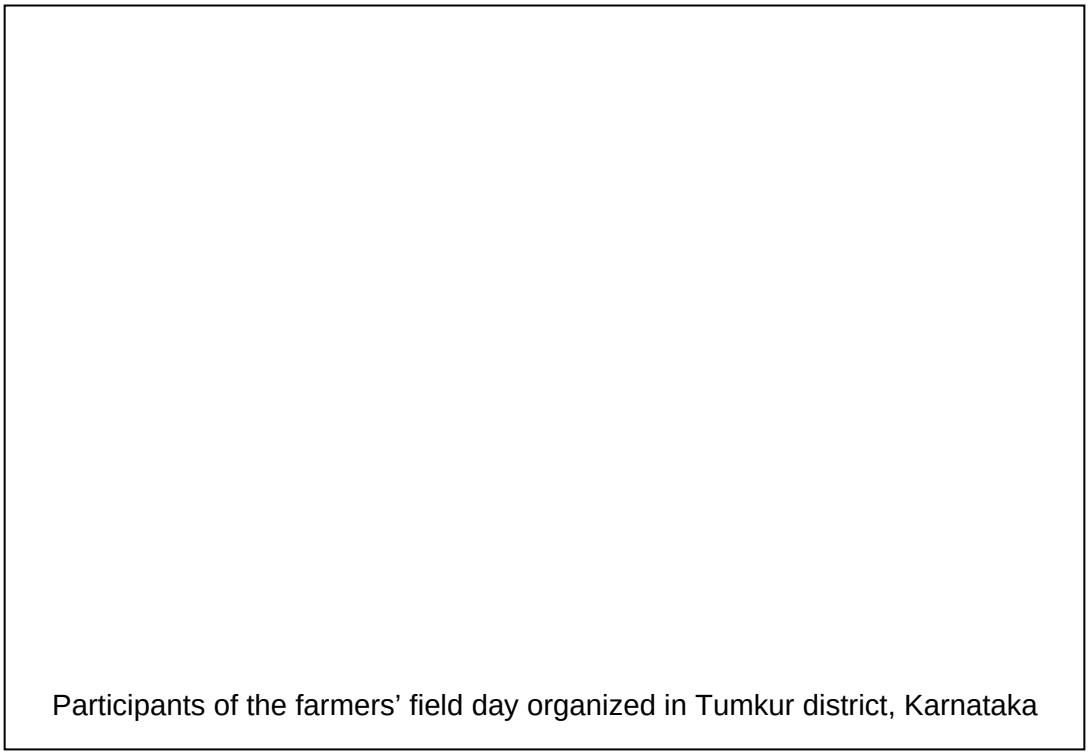
Farmers visiting ICRISAT stall in ICRISAT-UAS, Dharwad Field day

Women farmers demonstrating innovative sweets and savouries prepared with ICP7035 seeds

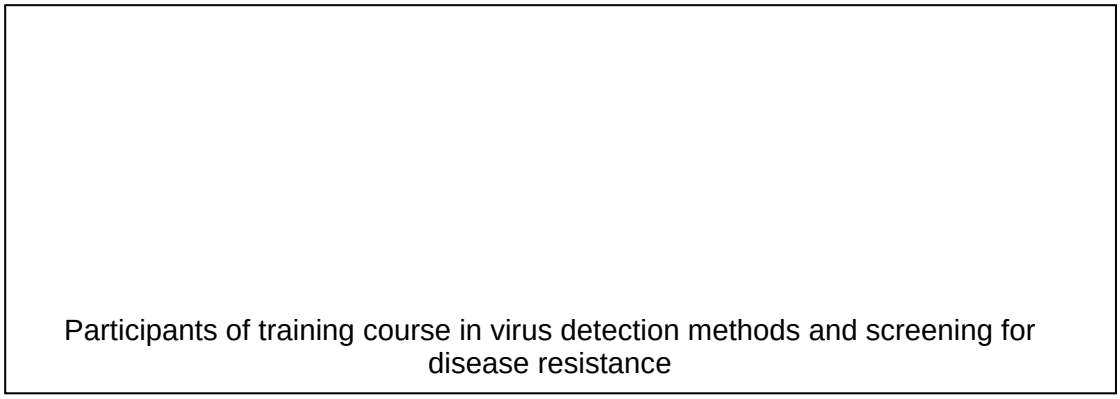
Banish SMD with ICP7035:
Happy farmer holding SMD-affected plants in front of ICP7035 fields



Participants of training course in IDPM at Agriculture Research Station, Gulbarga



Participants of the farmers' field day organized in Tumkur district, Karnataka

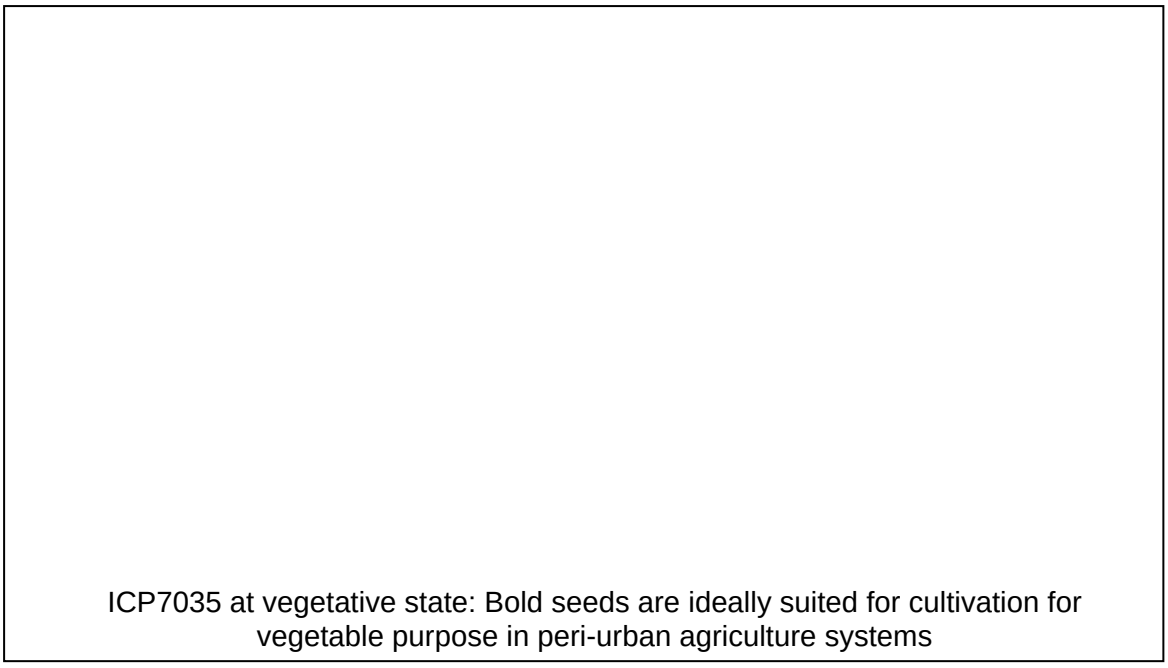


Participants of training course in virus detection methods and screening for disease resistance

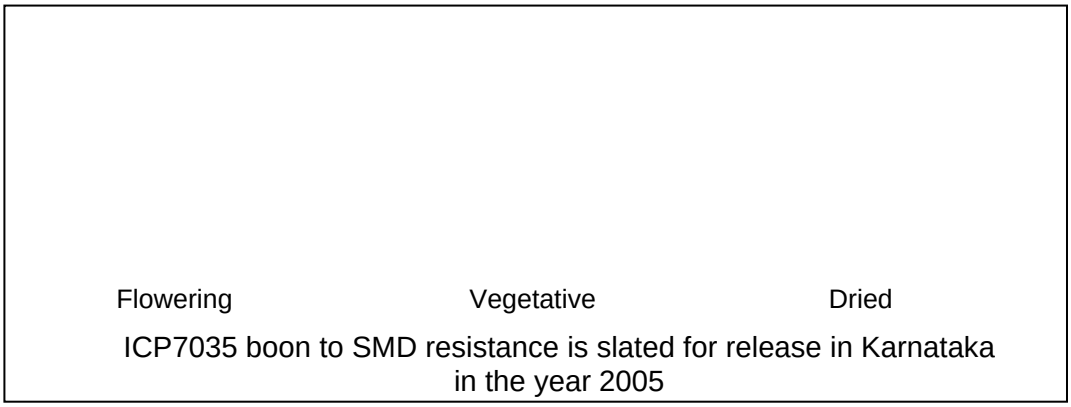
Participants of DFID-ICRISAT International Pigeonpea Scientist Meeting held in November 2003

NARES scientists in the pigeonpea demonstration field making selections on new varieties bread at ICRISAT

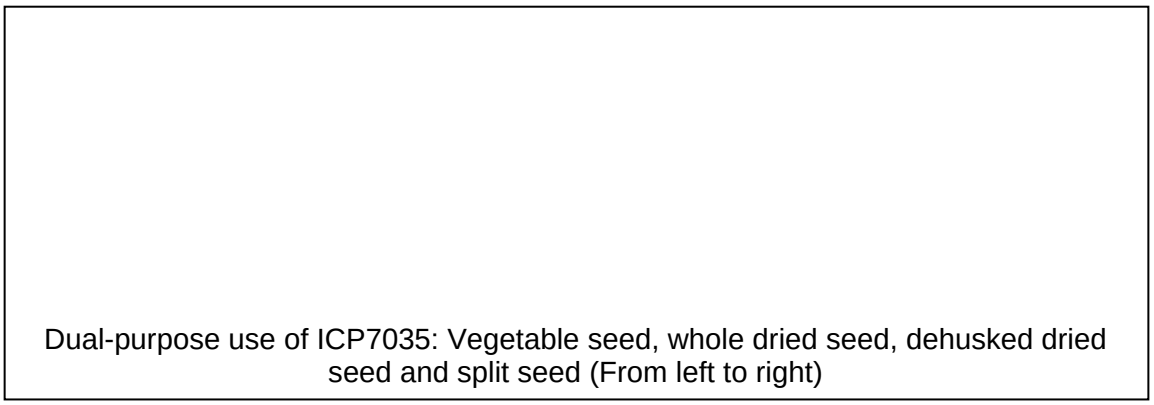
Village-level training: Farmers listening to lecture on SMD management



ICP7035 at vegetative state: Bold seeds are ideally suited for cultivation for vegetable purpose in peri-urban agriculture systems



ICP7035 boon to SMD resistance is slated for release in Karnataka in the year 2005



Dual-purpose use of ICP7035: Vegetable seed, whole dried seed, dehusked dried seed and split seed (From left to right)

ICPL96058: SMD and fusarium wilt resistant medium duration high yielding variety for central India. It is suitable for cultivation in deep-black soils. It is suitable for grain purpose.

Seed multiplication of ICP7035 breeding lines at ICRISAT. Selected breeding lines were transplanted in the field and at flowering stage were bagged to prevent out-crossing.