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Second Coordinators' Meeting on Peanut Stripe Virus

International Crops Research Institute for the Semi-Arid Tropics

Abstract

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Peanut stripe virus disease of groundnut has spread rapidly in many groundnut-producing countries, and causes economically significant crop losses. Groundnuts are an important oilseeds crop in many regions of the developing world, as well as a major protein source. Plant quarantine strategies are a critical factor in containing this seed-transmitted virus because plant breeders frequently exchange germplasm, often between countries. In this publication, scientists review research and the country-specific situation of peanut stripe disease in China, India, Indonesia, Japan, Philippines, Thailand, and the USA. In addition, the Peanut Collaborative Research Support Program (Peanut CRSP), USA, and a microcomputer virus data base are described. Six technical papers cover seed-borne legume viruses, control of tropical legume viruses, comparison of peanut stripe virus isolates, purification and serological relationships of peanut stripe virus, the molecular basis for potyvirus serology, and use of high-performance liquid chromatographic peptide profiling of coat protein digests. Recommendations are made for further action to control the spread of this disease, to estimate crop losses, and to formulate management strategies.

Résumé

Référence: ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). 1989. Deuxième réunion des coordonnateurs sur le peanut stripe virus, 1-4 août 1989, Centre ICRISAT, Patancheru, A.P. 502 324, Inde : ICRISAT.

La propagation du peanut stripe virus (PStV) — une maladie de l'arachide très importante au plan économique — a été rapide dans plusieurs pays producteurs d'arachide. L'arachide est une importante culture oléagineuse ainsi qu'une grande source de protéines dans les régions en développement. Les mesures préventives de la quarantaine végétale sont essentielles pour le contrôle de ce virus transmis par les semences vu les échanges fréquents des ressources génétiques parmi les sélectionneurs et entre les pays. Dans cette publication, les chercheurs passent en revue les recherches conduites sur le PStV dans de nombreux pays, à savoir l'Inde, l'Indonésie, le Japon, les Philippines, la Thaïlande et les Etats-Unis. Le Programme américain d'appui à la recherche collaborative sur l'arachide (Peanut CRSP) et une base de données sur le virus utilisable sur micro-ordinateur sont décrits. Les divers thèmes couverts par les six communications techniques sont : virus des légumineuses transmis par les semences; lutte contre les virus des légumineuses tropicales; comparaison entre les isolats du PStV; base moléculaire de la sérologie des potyvirus; analyse par chromatographie liquide haute performance (HPLC) des peptides issus de la digestion des protéines virales. Des mesures futures visant à maîtriser cette maladie, à évaluer des pertes culturales ainsi qu'à élaborer des stratégies de gestion sont proposées.

Cover: An electron micrograph of a purified peanut stripe virus preparation.

Summary Proceedings of the Second Coordinators' Meeting on Peanut Stripe Virus

1-4 Aug 1989

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ICRISAT

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Contents

Introduction

Objectives of the Meeting		1
Welcome Address	Y.L. Nene	1
AGLN and PStV	D.G. Faris and C.L.L. Gowda	2

Summaries of Papers

Country Papers

Keynote Address: A virus data base for aiding plant pathologists	A. Gibbs, A.A. Brunt, C. Buchen-Osmond, K. Crabtree, and G. McLean	4
Research on peanut stripe virus supported by the Peanut CRSP	J.W. Demski	5
Groundnut virus research in China	Xu Zeyong, Zhang Zong Xi, Chen Kunrong, and Chen Jinxen	7
Peanut stripe virus research in India	R.D.V.J. Prasada Rao, A.S. Reddy, S.K. Chakrabarty, K.S. Sastry, D.V.R. Reddy, Ram Nath, and J.P. Moss	8

Research on peanut stripe virus in Indonesia	Nasir Saleh, K.J. Middleton, Y. Baliadi, N. Horn, and D.V.R. Reddy	9
Screening for peanut stripe virus resistance of ICRISAT peanut collection at Maros, Indonesia	W. Wakman, A. Hasanuddin, and Ansar	10
Yield loss of groundnut due to peanut stripe virus	W. Wakman, S. Pakki, and A. Hasanuddin	11
Research on peanut stripe virus in Japan	T. Senboku	11
Groundnut virus research in the Philippines	M.P. Natural, F.L. Mangaban, and L.D. Valencia	12
Groundnut virus research in Thailand	Sopone Wongkaew	12

Technical Papers

Keynote Address: Seed-borne legume viruses: importance, detection and management	R.I. Hamilton	14
Keynote Address: Tropical legume viruses and their control	A.A. Brunt	15
Comparison of peanut stripe virus isolates using symptomatology on particular hosts and serology	Sopone Wongkaew and M. Dollet	16

Purification and serological relationships of peanut stripe virus	P. Sreenivasulu, J.W. Demski, and D.V.R. Reddy	17
Molecular basis for potyvirus serology	D.D. Shukla and C.W. Ward	18
Use of high-performance liquid chromatographic peptide profiling of coat protein digests to compare strains of peanut stripe virus with related and unrelated potyviruses	M.M. McKern, O.W. Barnett, H.J. Vetten, J. Dijkstra, L.A. Whittaker, and D.D. Shukla	19

Recommendations

Detection and identification of viruses (seed movement and quarantine) 20

Epidemiology (vectors, transmission, yield-loss studies, and surveys) 20

Groundnut viruses and their control 21

Regional activities 21

Participants 23

Introduction

Objectives of the Meeting

To share existing knowledge on PStV and other viruses of groundnut, to determine the problems still requiring answers, and to recommend future collaboration to overcome high-priority problems by:

- identifying progress on the recommendations of the First Coordinators' Meeting on PStV held in June 1987;
- learning about the present knowledge available on PStV and other groundnut viruses;
- finding the yield losses and other problems caused by PStV and other groundnut viruses; and
- listing the priorities for future research on groundnut viruses, research needs, responsibilities for the research, collaborative links, and necessary funding.

Welcome Address

Y.L.Nene

On behalf of ICRISAT, I would like to welcome you to this Second Peanut Stripe Coordinators' Meeting. It is gratifying to see participants representing 10 countries and 3 international organizations.

Some of you may be aware that although ICRISAT was established in 1972, groundnut (or peanut) was added to the mandate in 1976 because this crop is so important to the small farmers of the semi-arid tropics for both food and cash. In the past 13 years our groundnut scientists have made strong contributions to the improvement of groundnut for SAT conditions.

As you know, if a crop is to be improved, germplasm must move between countries, and there is potential danger of introducing new pathogens into a country. This working group was formed mainly out of the fear of introducing peanut stripe virus into new geographical areas.

I would like to suggest that this group consider expanding its activities to work on additional virus problems of regional importance. Today there are certainly many more trained virologists in all Asian countries. In many developing countries the policy makers and administrators find it difficult to finance basic research, but funds are usually available to support applied virology research. This situation will change over time, but, in the meanwhile, meetings such as this one are extremely useful for

scientists from developing countries to exchange information with scientists from laboratories with excellent basic virology research facilities.

There is excellent cooperative work of a similar nature being conducted through the International Working Group on Legume Viruses (IWGLV). This group has more than 100 members in over 30 countries, including developing countries, and the working relationships are excellent. I know from first-hand experience that members are very willing to help each other, and I am wondering if the peanut stripe coordinators could be a subgroup of IWGLV.

Once again let me extend a hearty welcome to you, along with best wishes for a successful meeting.

AGLN and PStV

D.G. Faris and C.L.L. Gowda

Networks are becoming increasingly popular in agricultural research all over the world. A research network is a group of scientists or institutions linked by a common interest, working dependently or interdependently on an identified, shared problem. All research networks have five basic components: membership of scientists, institutions, and research groups that make up the body of the network; research to answer the problems for which the network was established; coordination to organize and harmonize the activities of the network; communication to provide the links among members; and assets or resources shared among the members, with external funding if needed.

The Coordination Unit of the Asian Grain Legumes Network (AGLN) is a part of the Legumes Program at ICRISAT. AGLN was established in 1986 as a result of meetings in 1983 and 1985 which recommended that ICRISAT assist the national programs in Asia through a network to include chickpea, pigeonpea,and groundnut, the mandate legume crops of ICRISAT.

AGLN activities are based on a formal memorandum of understanding (MOU) between ICRISAT and each AGLN country. We now have MOUs with 10 Asian countries. A country coordinator is nominated by the concerned government or agency to be the contact person and link between the country and the AGLN Coordination Unit. A work plan of collaborative research and other network activities s prepared in a joint meeting between the AGLN staff and local scientists in each country. The work plan is reviewed every 1 or 2 years to evaluate progess and make plans for further research activities. The Asian Development Bank is providing funds to support AGLN activities and strengthen collaborative research with Bangladesh, Burma, Nepal, and Sri Lanka. The Australian Centre for International Agricultural Research (ACIAR), the Australian International Development Assistance Bank

(AIDAB), FAO, Peanut Collaborative Research Support Program (Peanut CRSP), and IDRC have provided funds for specific activities associated with the AGLN. Using this framework the Coordination Unit facilitates contacts between AGLN scientists in each AGLN country and scientists at ICRISAT.

Another objective of ICRISAT is to coordinate or link activities among research groups in Asia working on AGLN crops. One way that this is done is by organizing workshops and meetings for scientists from many countries working on common problems to exchange and share ideas, information, and material, and develop collaborative research plans. One such meeting organized in 1987 was the First Peanut Stripe Virus (PStV) Coordinators' Meeting at Malang, Indonesia. The meeting was cosponsored by the Agency for Agricultural Research and Development (AARD), ACIAR, FAO, Peanut CRSP, IDRC, and the Dutch ATA Project at Malang. The scientists at that meeting discussed and exchanged knowledge existing on PStV, made plans for future research, and developed a series of recommendations. A working group on PStV was formed to carry out these recommendations. The AGLN Coordination Unit was given responsibility to coordinate the activities of the working group. Members of this working group include the PStV coordinators in each country, and other scientists working on PStV. The working group successfully implemented all the recommendations made at the first meeting. This record is a splendid example of collaborative research and action helping to solve a problem—which if left unchecked could cause widespread yield reductions. The Coordination Unit of the AGLN would like to thank all those who helped to make this progress possible, and hopes that the present momentum will continue.

Summaries of Papers

Country Papers

Keynote Address: A Virus Database for Aiding Plant Pathologists

A.J. Gibbs, A.A. Brunt, C. Buchen-Osmond, K. Crabtree, and G. McLean

There are two sides to the identification of any object. First, observing its character, and second, comparing the observations with stored information about known objects. Most discussion of virus identification has been dominated by the observations, but there is an increasing realization of the importance of efficient data collation and management.

One source of data for comparing the characteristics of described and newly isolated viruses is the CMI/AAB Descriptions of Plant Viruses. These are loose-leaf descriptions of around 400 viruses.

We have for some years been developing a complementary source, a computer database of information provided by working virologists. This project is the VIDE (Virus Identification Data Exchange) project (Boswell et al. 1986; Watson et al. In press), which uses the DELTA (DEscription Language for TAxonomy) database system (Dallwitz 1980). The DELTA system is designed to handle all forms of taxonomic information in a user-friendly and flexible way. The data may then be used in various ways, for example, key-generating or interactive identification programs, and automatic typesetting or microfiche-producing facilities.

Information on over 500 characters is currently sought for each virus by questionnaire from the relevant expert, and is stored in the database; over two-thirds of the information is on the susceptibility of a range of commonly used test plant species. The remainder represent the wide range of other characters used for virus identification. In many laboratories, workers rely on simple tests such as the resistance of the infectivity of sap from infected plants to ageing, heat, etc., and on the symptoms produced in indicator plants. By contrast, those in well-equipped laboratories rely on electron microscopy and serological tests.

The database includes host range information even though many virologists probably believe that host range data are not a reliable guide to virus identification. In practice, host range studies are still an important component of plant virus diagnosis in many laboratories and, with standardization, they could become more useful.

In 1988 the VIDE project published Viruses of Plants in Australia (Buchen-Osmond et al. 1988). We recently produced a microfiche edition (free, while stocks last) of the complete database, and have started to distribute floppy disks of the data complete

with an interactive key program (INTKEY) for use in IBM-compatible microcomputers using the MS-DOS operating system and at least 512K RAM. Later this year, the Commonwealth Agricultural Bureaux International plans to publish a VIDE book, Viruses of Tropical Plants, and in 1990 we hope to have a computer/book version of the entire database.

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Research on Peanut Stripe Virus Supported by the Peanut CRSP

J.W. Demski

The Collaborative Research Support Programs (CRSP), funded by the United States Congress to address food needs and assist research for developing countries, are a product of and administered by the U.S. Agency for International Development. Numerous U.S. universities participate in the programs, which are coordinated through the Board for International Food and Agricultural Development. The programs are conducted collaboratively by the U.S. universities and host institutions in developing countries. Initially the virus project was designed to support research on groundnut rosette disease, which is an economically important groundnut disease in Africa. After the discovery of peanut stripe virus (PStV), most of the Peanut CRSP virus project research efforts in the U.S. were directed to PStV. In 1988, research efforts on groundnut rosette in Nigeria were phased down, and cooperative research linkages were established with Khon Kaen University in Thailand and the University of the Philippines at Los Baños. Part of the research in Thailand and in the Philippines will be directed to PStV.

Research on PStV funded by Peanut CRSP includes the isolation of PStV and its separation from other viruses such as PMV; purification and production of antisera;

the development of diagnostic aids; identification of principal aphid vectors; work on seed transmission of PStV in groundnut, soybean, and cowpea; and determination of serological relationships of PStV.

A portion of the funds to the host countries can be used to support travel by host-country scientists to meetings and workshops in order to disseminate research results, become acquainted with the latest developments in their disciplines, and establish cooperative links with scientists in other countries. Additionally, Peanut CRSP provides funds for graduate students to carry on a research program that will lead to increased food production in their home countries. The Peanut CRSP virus project has supported several graduate students.

To promote cooperative linkages, Peanut CRSP funds supported two visiting scientists. Dr D.V.R. Reddy, ICRISAT, was supported with laboratory supplies, bench fees, a technician, and U.S. travel funds for 6 months while he was a visiting professor at the Georgia Experiment Station in 1983. Dr Reddy's research efforts were of tremendous benefit in helping to prevent the spread of PStV into commercial groundnut production in the USA. Much of the early information on PStV characterization and virus relationships also resulted from this cooperative research effort.

Dr P. Sreenivasulu from S.V. University, India, was supported by the Peanut CRSP for 18 months as a visiting professor at the Georgia Experiment Station in 1987-88. His efforts led to:

- production of tomato spotted wilt virus (TSWV) antiserum for use against a U.S. groundnut TSWV isolate, and determination of serological relationships with other TSWV isolates from other countries;
- definition of the relationship of PStV to other potyviruses by host range and serological tests;
- work on the effectiveness of single feeding versus sequential feeding of aphids on groundnut plants infected with PStV and/or PMV;
- identification of a virus related to PStV from weeds in groundnut fields; and
- compilation of a review article on all viruses that infect groundnuts naturally or have been shown to infect them by artificial means.

This information has contributed substantially to our knowledge of groundnut viruses and is relevant to groundnut virologists worldwide.

The Peanut CRSP also supports scientific meetings, primarily by providing travel funds for scientists from host countries and U.S. cooperators. The Peanut Stripe Virus Coordinators' Meetings held in 1987 and 1989 are examples.

The Peanut CRSP is funded until 30 Jun 1990. Critical reviews are currently under way to evaluate the 7 years of Peanut CRSP operations. These reviews and subsequent recommendations will determine if a 5-year extension will be granted that would begin on 1 July 1990. If the Peanut CRSP is extended, support is expected for supplies and some laboratory equipment in Thailand and the Philippines, travel for cooperating scientists, and training for graduate students and/or technicians. Specific areas of study in host countries would include:

- surveys of groundnut fields for viruses and their incidence,
- critical identification of groundnut viruses,
- survey for alternate hosts of groundnut viruses,

- establishment of virus-free groundnut seed stock,
- determination of geographical locations where virus-free seeds can be produced,
- identification of principal vectors of viruses,
- epidemiology studies, and
- continued search for resistant germplasm.

Research in the USA would:
- initiate a new program to incorporate resistance to PStV and PMV from 'other' groundnut types such as *Arachis glabrata* and *A. chacoense* into the commercial groundnut types of *A. hypogaea*,
- study the effect of multiple virus infection of groundnut, and
- continue research programs on the different groundnut viruses, but increase efforts on TSWV.

Groundnut Virus Research in China

Xu Zeyong, Zhang Zong Xi, Chen Kunrong, and Chen Jinxen

In the People's Republic of China groundnuts are grown mainly in north, in Shandong, Hebei, Henan, Liaoning, Jiangsu, Anhui, and Hubei provinces. Although virus diseases on groundnut were first reported in 1939, their economic importance was not realized until the early 1970s when frequent outbreaks were recorded. In 1976 and 1986, severe epidemics of virus diseases in the major groundnut growing areas substantially reduced groundnut yields. Currently, peanut stripe virus (PStV), which was previously reported as peanut mild mottle, cucumber mosaic (CMV), peanut stunt (PSV), and tomato spotted wilt viruses (TSWV), are all considered to be economically important. Mixed infections are common in middle- and late-season crops. PStV appears to be the most widely distributed of all groundnut viruses. CMV and PSV are restricted to Hebei, Liaoning, Shandong, Jiangsu, and Henan provinces, and Beijing. In southern China, PStV has been detected, and in Guangdong and Guangxi provinces TSWV is economically important.

Experiments on the epidemiology of PStV were conducted in Wuhan and Beijing provinces. Seed appears to be the primary source of inoculum. In seed-detection tests by ELISA, PStV was detected in the embryo. Testa were found to be a poor source for detecting PStV. Several groundnut cultivars grown in China were evaluated for seed transmission frequency of PStV, and the majority varied from 1.0 to 3.8%. Three spanish types showed seed transmission rates of up to 8%. Plants mechanically inoculated at early growth stages showed high seed transmission frequency. Shriveled and small seeds carried a higher proportion of PStV compared with healthy-looking seed. Of 987 groundnut genotypes tested, none was found to be resistant to PStV. All

the genotypes were also evaluated for seed transmission. F87-158 and F87-157 genotypes, which were earlier shown not to transmit peanut mottle virus, showed negligible seed transmission of PStV.

CMV was found to be widely distributed in China, seed was the primary source of inoculum. By utilizing virus-free seed, CMV could be effectively controlled.

Research on peanut viruses is mainly conducted in the Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences, in Wuhan. Future work on peanut viruses will concentrate on surveys, development of specific methods for identifying individual viruses, identification of principal vectors, germplasm screening under both field and laboratory conditions, and identification of genotypes which do not transmit viruses through seed. Epidemiological studies will be restricted to economically important viruses. The ultimate aim is to develop integrated management practices for economically important groundnut viruses.

Peanut Stripe Virus Research in India

R.D.V.J. Prasada Rao, A.S. Reddy, S.K. Chakrabarty, K.S. Sastry, D.V.R. Reddy, Ram Nath, and J.P. Moss

Peanut stripe virus (PStV) was first observed in India in 1987 on some groundnut entries being evaluated in a coordinated rainy-season trial at several regional research stations. The disease was observed at 6 of 12 research stations surveyed. Efforts were made to contain the spread of the disease by destroying the trial at all 35 locations. In 1988 surveys were conducted at various research centers, and wherever PStV-infected plants were observed they were destroyed. Since the virus was observed only on entries that originated from one location, it was recommended that the seed from this location should be checked for PStV prior to use. Currently, it is not known if PStV has spread to farmers' fields in India.

The virus was identified based on typical symptoms on *Arachis hypogaea*: host range, particle morphology, and serological affinities. PStV maintained in groundnut was used for purification. A polyclonal antiserum of high titer was produced.

None of 59 commonly grown groundnut cultivars was found to be resistant to PStV in laboratory tests. However, of 54 accessions of wild *Arachis* spp tested by mechanical sap inoculations, ICG 4983, ICG 8215, ICG 8958, ICG 8973, ICG 11558, ICG 11560, ICG 11562, and ICG 12168 showed resistance to PStV. These eight accessions also showed resistance after exposure to viruliferous aphids. Nevertheless, when they were graft-inoculated, only ICG 11558 could not be infected with PStV, indicating that it is immune to PStV.

Seed transmission tests were carried out with five groundnut cultivars under greenhouse conditions. Transmission frequency varied from 29% for Robut 33-1 to 12% for JL 24.

Ten seeds from each of all groundnut germplasm lines introduced into India were tested for seed-borne viruses of quarantine importance by DAC-ELISA, followed by grow-out and infectivity tests. In case of a positive reaction, individual seeds from each lot were tested by ELISA. The seeds that gave positive reaction to PMV or PStV were eliminated from grow-out tests. Any plant that showed abnormal or virus-like symptoms in grow-out tests was detained and only healthy looking plants were released to be grown in the postentry quarantine isolation field (PEQIF) located at ICRISAT Center. Ultimately only seed harvested from healthy-looking plants in the PEQIF was released to consignees.

During 1988, of 410 groundnut seeds tested from the germplasm lines imported from Myanmar, one seed contained PStV. This was also tested in grow-out tests and the presence of PStV was confirmed. This appears to be the first record of the occurrence of PStV in Myanmar.

Research on Peanut Stripe Virus in Indonesia

Nasir Saleh, K.J. Middleton, Y. Baliadi, N. Horn, and D.V.R. Reddy

Peanut stripe virus (PStV) is an economically important virus disease of groundnut in Indonesia. Although only recently identified, it is presumed that PStV has been infecting groundnut for many years in Indonesia. Since there is a high incidence of PStV in Muneng, this location was chosen to screen groundnut genotypes for resistance to the disease. Over two seasons, 5400 *Arachis hypogaea* genotypes obtained from ICRISAT were screened under high PStV incidence. None was resistant to PStV, but 14 genotypes showed only late infection, and those will again be evaluated during the 1989 season. Of 100 interspecific hybrids, 23 showed less than 50% PStV incidence. Seed from uninfected plants of the 23 genotypes, and additional interspecific derivatives, are currently being tested during the 1989 season.

Since PStV has been frequently isolated from soybean grown in farmers' fields, several soybean accessions were screened in the greenhouse for resistance to PStV. Ten-day-old plants were sap-inoculated with PStV. Those which did not show disease symptoms were tested by ELISA and by infectivity assays on *Chenopodium amaranticolor*. Of 270 accessions tested, 32 were found to be resistant.

Extensive laboratory tests were conducted on transmission of PStV through seed of the Gajah groundnut cultivar. Transmission varied from 0.5% to 4.0% depending on the age of the plants at the time of inoculation. Field-infected Gajah and Kelinci cultivars showed seed transmission frequencies of 0.3% and 0.6% respectively. *Aphis craccivora*, *A. glycines*, and *Hysteroneura setariae* were found to transmit PStV very efficiently. *Rophalosiphum maidis* was found to be an inefficient PStV vector.

Yield losses of groundnuts due to PStV were estimated in two different trials. In one trial conducted at Muneng in 1988, the cultivar Gajah was sown on a 25-m^2 block with a plant population of 14 400. One thousand randomly chosen plants were scored for PStV at weekly intervals. Harvested pods were pooled depending on the age at which the mother plants were infected. Yield losses varied depending on how long after sowing the plants were infected: 2 weeks, 50%; between 3 and 8 weeks, over 25%; and between 9 and 10 weeks, 14%.

In the second experiment conducted at Jambegede during the 1988-89 season, the cultivar Gajah was sown on four 20-m^2 blocks with a plant population of 5000 in each block. Each block was sown with a border crop of maize to minimize aphid movement between blocks. In each block, randomly chosen plants were mechanically sap-inoculated to give 1, 5, or 10% PStV incidence, and one block was left as an noninoculated control. PStV incidence in each block was recorded at 2-week intervals. After harvest, pods from all infected plants in each block were pooled according to the age of plants when infected. Yield loss varied from 10 to 40% depending on the age at which the plants were infected. No yield losses were observed with plants whicl. first showed symptoms 10 weeks after planting. It is evident that PStV has the potential to cause severe yield losses of groundnuts.

Limited tests on various management practices indicated that sowing only healthy seed can substantially reduce PStV incidence. Rogueing of infected plants, controlling weeds, and application of aphicides did not significantly reduce PStV incidence.

Screening for Peanut Stripe Virus Resistance of ICRISAT Peanut Collection at Maros, Indonesia

Wasmo Wakman, A. Hasanuddin, and Ansar

Field evaluation of 1959 groundnut genotypes from ICRISAT for resistance to peanut stripe virus (PStV) was conducted at Maros during August and November 1988. The Gajah cultivar was used as a control. Infected Gajah plants were transplanted into two rows surrounding the entire field 2 weeks prior to sowing the test genotypes. *Aphis craccivora* colonies were released onto the infected plants. Sixty seeds of each genotype were sown in a single row and, for every 20 rows, one row was sown with Gajah. Plants were scored for PStV infection at monthly intervals.

One, two, and three months after planting, one or more plants of 16%, 89%, and 100% of the genotypes were infected, respectively. Three months after planting, infection rates of plants were 1-5% of 4 genotypes, 6-25% of 70 genotypes, 26-50% of 196 genotypes, 51-75% of 245 genotypes, and 76-100% of 1444 genotypes. Thus the majority of genotypes were highly susceptible. Genotypes that showed less than 10% infection will be retested next season.

Yield Loss of Groundnut Due to Peanut Stripe Virus

Wasmo Wakman, S. Pakki, and A. Hasanuddin

Groundnut yield losses due to peanut stripe virus (PStV) were estimated on the Bontobili Experimental Farm in South Sulawesi, Indonesia, during the 1988-89 season. The cultivar Gajah was sown on three (A, B, and C) 252-m plots. One week after sowing, 30 PStV-infected groundnut plants in plastic pots with numerous *Aphis craccivora* were distributed evenly in plot A, and 15 similarly infected plants in plot B. No aphids were introduced in plot C. Five hundred randomly chosen plants were observed weekly in each block for PStV symptoms. Symptoms of PStV appeared 4 weeks after planting in plots A and B, and 6 weeks after planting in plot C. The number of infected plants increased gradually, and by the 10th week after planting, all 500 of the observed plants were infected in plots A and B. In plot C, 75% of the plants were infected. By harvest all plants in plot C were infected. Harvests from infected plants were grouped depending on the age at which they were infected. Plants infected 1 month after planting in both blocks A and B showed over 50% yield loss. Plants infected at 6 and 9 weeks in all blocks showed over 28% and 15% yield losses, respectively. The results indicate that PStV has the potential to cause severe losses in yields of groundnuts.

Research on Peanut Stripe Virus in Japan

Toshihiro Senboku

The occurrence of peanut stripe virus (PStV) in Japan was reported in 1988. The virus was isolated from groundnut plants showing mild mottle and vein banding. A single lesion isolate was obtained by serial transfers on *Chenopodium amaranticolor*. Subsequently the virus was maintained *in Phaseolus vulgaris* cv. Yamashiro Kurosando. Filamentous particles of about 750 nm long were detected in crude and purified preparations. In thin sections of infected groundnut and *P. vulgaris* plants, pinwheel or ring-type inclusion bodies were observed. Purified virus reacted strongly with antisera to groundnut chlorotic ring mottle and PStV. Surveys for PStV occurrence in groundnut fields were conducted in Chiba and Ibaraki prefectures, which are the main groundnut-production centers in Japan. PStV was detected in both prefectures, but the details of the distribution of PStV in other areas remain to be determined. Additionally, it is essential to detect PStV in groundnut seed stocks in Japan by using ELISA and dot blot hybridization. Monoclonal antibodies will soon be produced for PStV and they are expected to distinguish various isolates. Details of methods employed will be described.

Groundnut Virus Research in the Philippines

M.P. Natural, F.L. Mangaban, and L.D. Valencia

Isolates of peanut stripe virus (PStV) present in the Philippines reacted with PStV antisera from three different sources. All 107 groundnut genotypes tested by mechanical sap inoculations were susceptible to PStV, which could be transmitted by several aphid species, including *Aphis craccivora*, *A. gossypii*, *A. citricola*, *Myzus persicae*, and *Rophalosiphum maidis*. Seed transmission varied from 0 to 33%, depending on the cultivar. PStV could be mechanically transmissible to 7 of 23 species. Systemic infection was obtained in *Crotolaria incana* L., *Desmodium triflorum* (L.) D.C., *Glycine max* (L.) Merr., *Vigna radiata* (L.) Wilczek, and *Vigna unquiculata* Walp. Local chlorotic lesions were obtained on *Chenopodium amaranticolor* and *C. quinoa*. The thermal inactivation point was found to be 65 °C for 10 min, dilution end-point was 10-3 to 10-4, and longevity in vitro in crude sap was 1 day stored at room temperature (25-30 °C) and 3 days stored in a refrigerator (0-5 °C).

The effect of PStV on growth and yield was variable. In trials conducted during the 1988 dry season on cv UPL Pn4, plants showed significant reductions in plant height, pod numbers per plant, 100-seed mass, and pod and seed yields, especially in plants infected within 10 days of sowing. However, in trials conducted during the 1988 dry season on cvs UPL Pn2, UPL Pn4, and UPL Pn6, no significant yield reductions were noticed. We are conducting additional experiments on losses due to PStV.

Groundnut Virus Research in Thailand

Sopone Wongkaew

Research on groundnut viruses in Thailand has been conducted mostly by Khon Kaen University and the Department of Agriculture, with the main emphasis on peanut stripe virus (PStV) since it was identified in 1985. Early studies focused on the symptomatology of different variants, their seed transmission frequencies, and alternate hosts. From 1987 to 1989 the effect of PStV on yield under field conditions was also assessed. Results varied from year to year and depended on the cultivars tested. When the infection started very early, the chlorotic ring isolate could reduce pod yield up to 42% in all cultivars.

Various virus purification methods were tried, and the combined purification methods for black eye cowpea mosaic (BICMV) and PStV were successful. Cowpea cultivar KC 84 R was found to be the most suitable PStV propagation host. An

antiserum prepared from a chlorotic ring isolate, and antisera of peanut chlorotic ring mottle virus (PCRMV), PStV, and BICMV reacted strongly with various PStV variants collected in Thailand. Tests on seeds from different sources indicated that as much as 16.5% of foundation seed produced by certain research stations contained PStV, while 3% of groundnut seed produced by contracted farmers of the National Seed Production Scheme contained PStV. Insecticide sprays reduced PStV incidence considerably, and studies on alternate hosts using serological and aphid transmission tests revealed that *Indigofera amoena*, *Peuralia phaseoloides*, *Stylosanthes capitata*, *S. scraba*, and *Vigna unguiculata* can act as alternate hosts for PStV under field conditions.

Peanut yellow spot virus was also studied in detail. The local isolate was found to be serologically related to the Indian isolates, but not to tomato spotted wilt virus. The virus is widespread and in farmers' fields can infect up to 80% of the plants. It has a wide host range, but produces mostly localized symptoms. It can be transmitted by a *Scirtothrips* sp.

Bud necrosis caused by tomato spotted wilt virus occurred in epidemic proportions in farmers' fields in some locations in Thailand. The incidence of this disease has been increasing each year, especially on dry-season crops.

Technical Papers

Keynote Address: Seed-Borne Legume Viruses: Importance, Detection, and Management

R.I. Hamilton

About 30 viruses are known to be seed-transmitted in food legumes (e.g., bean, broad bean, cowpea, lentil, pea, peanut, and soybean). Twenty-three (64%) of the 34 recognized groups and families of plant viruses are seed-transmitted to a wide range of crops and, of these seed-transmitted categories, 12 contain viruses transmitted by seed in legumes.

Seed transmission is the culmination of a complex interaction between two genetic systems (host and virus), as modified by environmental conditions. The majority of viruses are seed-transmitted via infected embryos (embryo-borne virus), following zygote formation by gametes derived from infected megaspore and microspore mother cells. Infected seed coats may be a source of virus for transmission to seedlings (e.g., sunn-hemp mosaic virus in bean and cowpea).

Several consequences follow seed infection. Infected seed allows perennation of those viruses which would not survive outside the host. Such seeds are the major means of long-distance transmission. They may be the primary source of inoculum in the planted crop with consequent spread within the crop and to other species by vectors. Contamination of germplasm and foundation seed stock can adversely affect crop improvement programs, especially those involving germplasm which is moved internationally. Direct injury, i.e., failure to realize potential yield, is another consequence.

Viruses can be detected in seeds by a direct method (grow-out test) and by several indirect methods (serological or cDNA). The grow-out test is often the method of choice in developing countries, but correct identification of the viruses should be confirmed by serological tests (Saleh et al. 1989). Indirect methods for large-scale testing of seed extracts include ELISA (Bharathan et al. 1984), immuno dot blot (Lange and Heide 1986), immunosorbent electron microscopy (Hamilton and Nichols 1978), and cDNA probes (Bijaisoradat and Kuhn 1988). Group-specific monoclonal antibodies may be useful, especially in quarantine facilities. The relationship between the quantity of detected antigen or nucleic acid and transmission to seedlings must be determined if indirect methods are to be reliable alternatives to the grow-out test. Adequate sample size, especially in seed certification schemes, must also be employed (Geng et al. 1983, Maury et al. 1987).

Transmission of viruses by legume seeds poses certain problems in disease management. Contaminated germplasm must be discarded or reclaimed; compliance with recently drafted guidelines for the safe and rapid international movement of legume

germplasm (Bos et al. In press) will materially reduce shipment and accession of infected germplasm. Virus-free mother plants must be employed in breeding programs, and adequate testing of foundation and certified seed is advisable. Development and application of strain-specific monoclonal antibodies may facilitate screening for disease resistance. Genetic resistance to virus infection, seed transmission, and vector(s) attained by conventional plant breeding may be supplemented by transformation of plants with genes of viral and other origins.

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Keynote Address: Tropical Legume Viruses and Their Control

A.A. Brunt

Rational methods for controlling tropical legume viruses, like those used for minimizing virus-induced losses of other crops, are dependent upon thorough knowledge of not only the epidemiology and properties of the viruses, but also the ecology of the vectors, crops, and cultivated and uncultivated hosts of both viruses and vectors. Such

information has been used with partial success to control some tropical legume viruses transmitted by aphids, thrips, whiteflies, and the plasmodiophorid fungus *Polymyxa graminis*.

Because numerous and taxonomically diverse viruses infect tropical legume crops, control is also largely dependent upon the rapid and correct identification of viruses, strains, and pathotypes. With viruses such as groundnut rosette, identification of pathogenic satellite RNA is also necessary. A range of conventional and more recently developed procedures have been used successfully for the diagnosis of peanut stripe, peanut mottle, tomato spotted wilt, peanut clump, groundnut rosette complex, and other viruses. Sensitive methods for detecting viruliferous vectors also now permit vector populations to be monitored, and thereby assess risks of crop infection.

Because many legume viruses are seed-borne, the elimination of this primary and randomly distributed source of infection is important to minimize secondary virus spread. Sensitive assay procedures (including various types of ELISA, immunosorbent electron microscopy, radioimmunoassay, cDNA probes, etc.) greatly facilitate the production of virus-free seed stocks of some legume crops. Secondary virus spread can be minimized by chemical and nonchemical methods.

Virus-induced crop losses can also be minimized by modifying cultural practices. Thus, levels of infection by groundnut rosette, tomato spotted wilt, and other viruses can be decreased, with concomitant yield increases, by closer plant spacing, judicious choice of planting date, and the use of mulches and physical or plant barriers.

The most practical procedure at present for disease control, however, is the production and use of genotypes that are immune or have high levels of resistance to the viruses and/or vectors. The initial successes at ICRISAT in identifying groundnut genotypes with resistance to viruses (tomato spotted wilt, peanut mottle, and peanut stripe viruses) and vectors (notably *Aphis craccivora* and thrips), and previous experience with other crops elsewhere, should encourage further efforts to identify and incorporate genes for their resistance into high-yielding cultivars of legume crops. In the longer term, genetic engineering techniques may facilitate the production of transgenic plants with high levels of resistance to viruses and vectors. The use of biological control agents might also be a viable and environmentally safe alternative to the chemical control of vectors.

Comparison of Peanut Stripe Virus Isolates Using Symptomatology on Particular Hosts and Serology

Sopone Wongkaew and M. Dollet

Twenty-four collections of peanut stripe virus from seven different countries (Myanmar, India, Indonesia, Philippines, China, Thailand, and USA) were compared under

similar conditions at the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Montpellier, France. Four of the collections from Thailand had been maintained in Japan since 1972 and were sent to France for this particular study. By using disease reactions on a set of groundnut genotypes and on other diagnostic hosts, the collections could be grouped into eight isolates. mild mottle, blotch, stripe, blotch-stripe, blotch-CP- N, chlorotic ring-mottle, chlorotic line-pattern, and necrotic types. Similarities were noted among each of eight isolates regardless of their origin. Serological tests indicated that peanut chlorotic-ring mottle was an isolate of peanut stripe virus (PStV). Groundnut eye spot virus (GEV) was considered to be a distinct potyvirus. Serological reactions of single lesion isolates, produced on *Chenopodium amaranticolor* utilizing different polyclonal PStV antisera and those of other related viruses. correlated well with the grouping based on reactions on diagnostic hosts. Similar tests using antigens from groundnut were less effective in differentiating the strains. PStV has apparently been present in Thailand since 1972 because viruses collected at that time have now been identified as PStV, but they may have been misidentified earlier as peanut mottle virus.

Purification and Serological Relationships of Peanut Stripe Virus (PStV)

P. Sreenivasulu, J.W. Demski, and D.V.R. Reddy

Peanut stripe virus (PStV) was successfully purified using the basic procedure developed for the purification of peanut mottle virus. The ultraviolet absorption spectrum of the purified virus had a shoulder at 290 nm while the A260/280 and A260/245 ratios were 1.25 and 1.18, respectively. Virus yields were 40-60 mg for *Lupinus albus* leaves. In indirect ELISA tests, PStV and peanut mild mottle virus (PMMV) reacted strongly with antisera to blackeye cowpea mosaic (BICMV), soybean mosaic (SMV), watermelon mosaic 2 (WMV 2), pea seed-borne mosaic (PSBMV), and peanut chlorotic ring mottle (PCRMV) viruses. PStV and PMMV reacted weakly with peanut green mosaic (PGMV), WMV-2 (sesame isolate) tobacco etch, and zucchini yellow mosaic (ZYMV) virus antisera. They failed to react with antisera to WMV-1, bean yellow mosaic, or bean common mosaic viruses. Although PStV and PMMV antisera also reacted with several potyviruses, results were not conclusive because the titer of PMMV antiserum was very low.

Molecular Basis for Potyvirus Serology

D.D. Shukla and C.W. Ward

The potyvirus group is by far the largest of the 34 currently recognized plant virus groups. Much of its taxonomy is complex, inconsistent, and confused. Among the various properties used in the past to identify and classify members of this group, serological tests appear to offer the most practical method to establish the identity of a new isolate. However, serological relationships among distinct members of the group are extremely complex.

Our results from structural studies of potyvirus coat proteins demonstrated that:

- Distinct members of the group possess a sequence homology of 38-71% with major differences in length and sequence of their N-termini, whereas strains of individual viruses exhibit a sequence homology of greater than 90% and have N- terminal sequences that are very similar.
- The N- and C-termini of the coat proteins are located on the particle surface. These termini can easily be removed from the intact particles by mild enzyme treatment, and their removal does not affect the infectivity and morphology of virus particles.
- The N-terminus is the only large region in the entire coat protein of a potyvirus that is virus-specific, and therefore antibodies directed to this region should recognize all strains of that potyvirus.
- Distinct members possess extensive sequence homology in the core region of their coat proteins, and antibodies directed to this region should, therefore, recognize most, if not all, potyviruses.

Our immunochemical analysis of native virus particles, core particles (minus N- and C-termini), dissociated core proteins, and overlapping synthetic octapeptides covering the entire coat proteins of potyviruses showed that:

- N-terminus is the most immunodominant region in the entire coat protein,
- early bleed responses are generally restricted to the N- terminal region and are virus-specific, and
- core-targeted antibodies are produced after successive prolonged immunizations, and antisera containing core-targeted antibodies recognize other distinct potyviruses.

These results have established for the first time the molecular basis for potyvirus serology, explained many of the problems currently associated with the conventional serology of potyviruses, and led to the design and use of novel approaches for the accurate identification and classification of potyviruses.

Use of High-Performance Liquid Chromatographic Peptide Profiling of Coat Protein Digests to Compare Strains of Peanut Stripe Virus with Related and Unrelated Potyviruses

N.M. McKern, O.W. Barnett, H.J. Vetten, J. Dijkstra, L.A. Whittaker, and D.D. Shukla

Previous serological comparisons of three potyvirus isolates from soybean (PM, PN, and 74) originating in Taiwan and azuki bean mosaic virus (AzBMV) from Japan suggested that they were closely related to each other and to an isolate of peanut stripe virus (PStV) from the USA. The host range and symptomatology of the soybean isolates and AzBMV also closely resembled PStV; however only PStV infected peanuts. The taxonomic status of the soybean isolates and AzBMV is therefore uncertain at present.

We compared the HPLC profiles of coat protein tryptic digests from PM, PN, 74, AzBMV, PStV-blotch, PStV-stripe, and PStV-mild mottle. We also compared these profiles with those from legume-infecting potyviruses known to be biologically and serologically related to PStV: blackeye cowpea mosaic virus (BICMV), clover yellow vein virus (CYVV), soybean mosaic virus (SMV), bean yellow mosaic virus (BYMV). They were also compared with the unrelated nonlegume-infecting potyviruses potato virus Y (PVY) and tobacco etch virus (TEV).

Our results show that the three soybean isolates, PM, PN and 74, and AzBMV, PStV-blotch, PStV-stripe, and PStV-mild mottle have very similar peptide profiles, suggesting that they all could be strains of the one virus (PStV). The peptide profiles of the two BICMV (Type and W) strains showed definite similarity with profiles of the PStV strains, indicating a close structural relationship between these two viruses. In contrast, the peptide profiles of BYMV, CYVV, SMV, PVY, and TEV were substantially different from those of the PStV strains. We recently demonstrated that HPLC tryptic peptide profiles of coat proteins from distinct potyviruses are considerably different, but those from the different strains of the same virus are very similar.

Recommendations

Detection and Identification of Viruses (Seed Movement and Quarantine)

1. An antiserum, specific for peanut stripe virus, should be produced at ICRISAT and made available to groundnut virologists in Asia and Oceania as an aid to diagnose and identify PStV. Identification of symptom variants of PStV should be made by mechanical inoculation of a standard list of diagnostic hosts and confirmed by serological tests using the PStV specific antiserum.
2. An information bulletin on diagnosis and identification of groundnut virus diseases, complete with appropriate color photographs, should be prepared by ICRISAT for distribution in Asia and Oceania.
3. The ELISA method for detecting PStV (use of the PStV-specific antiserum on bulk samples of 10-25 seeds) should be adopted as the standard method for detecting seed-borne groundnut viruses.
4. Groundnut seed, imported for research purposes, should be tested for all known seed-transmitted viruses by a grow-out test and/or ELISA in quarantine facilities of the importing country. Only seed from virus-free mother plants shall be entered in the germplasm collection as an accession for use in crop improvement schemes.
5. Imported groundnut seed intended for commercial purposes should be tested by ELISA for seed-transmitted viruses known to occur in the country of origin. An adequate sample size in relation to established procedures should be used.
6. Attention should be paid to the possible introduction of plant species or cultivars containing genes susceptible to virus diseases in the country of origin, but which are not known to occur in the species present in the importing country.
7. The exporting country should ensure that seed lots are free from seed-transmitted groundnut viruses.

Epidemiology (Vectors, Transmission, Yield-Loss Studies, and Surveys)

1. Aphid specimens for identification should be sent to Dr J.W. Demski, Professor, Department of Plant Pathology, Georgia Experiment Station, Griffin, GA 30223, USA. The aphids (20 winged and 20 apterous) should be in a vial containing 95% alcohol.
2. Use of yellow pan traps for aphid trapping during the growing season is advised.
3. Testing seven aphid species (*Aphis craccivora, A. gossypii, A. citricola, A. glycine, Myzus persicae, Rhopalosiphum maidis, Hysteronura setariae*) for vector transmission and efficiency studies is recommended.
4. Alternate hosts for the virus and vector should be sought, and the efficiency of

various hosts as a source of inoculum for principal aphid vectors should be investigated.
5. The frequency of seed transmission in local groundnut cultivars and a standard cultivar (Tainan-9) should be determined. Dr S. Wongkaew would be able to supply Tainan-9 seed. Determination of seed transmission in plants that were mechanically sap-inoculated, aphid-inoculated, and plants infected through seed-borne inoculum is recommended.
6. Multiyield loss experiments: select an area which had low PStV incidence in the previous year. Select nine 10 × 10 m plots with 20-m spacing between each plot. Grow maize as a barrier crop between the plots. Spacing within and between rows is 30 cm. Inoculate 100 plants at the first true leaf stage. Tag the infected plants and also tag those infected naturally. Obtain yield data from the mechanically infected plants and from the same number of healthy plants. Final detailed plans will be sent by Mr K.J. Middleton, who will be able to help with data analysis.
7. Surveys should be conducted to determine the geographical distribution and importance of groundnut virus diseases. Scientists should adopt appropriate survey procedures to detect groundnut virus diseases and their locations, and to determine their incidence. Wherever applicable, surveys should be conducted in wet- and dry-season crops over 2 years. Surveys during the pod filling stage (before leaf drop) are encouraged. Dr J.W. Demski will send a procedure for conducting surveys.

Groundnut Viruses and Their Control

1. The production of virus-free seed for farmers' use should be a long-term goal. The search for areas suitable for the production of virus-free seed should be continued.
2. With the advice and collaboration of agronomists, further information should be acquired on possible use of mixed cropping and intercropping to reduce the incidence of PStV.
3. For financial, health, and environmental reasons, attempts to control PStV with chemical applications should be discouraged.
4. Screening for resistance to virus and/or vector should be continued on a large scale. Further screening of genotypes for nonseed transmission should be encouraged.

Regional Activities

1. Future research on groundnut viruses should continue to be coordinated by the Asian Grain Legume Network (AGLN) of ICRISAT. The group will coordinate research on peanut stripe virus and other economically important groundnut viruses in the Asia-Pacific Region.
2. A training workshop should be held on "Identification of Economically Important Groundnut Viruses in Asia", preferably in the Philippines in 1990 or early 1991, and a second one in Thailand preceding the Third Coordinators' Meeting on Peanut Stripe Virus. Various international agencies should be approached for funds. Participants

should be chosen from groundnut-growing countries of the Asia-Pacific region. ICRISAT will coordinate this activity.

3. A research bulletin on peanut stripe virus should be produced by the end of 1990 by ICRISAT. Dr J.W. Demski will coordinate its production.

4. The following scientists will coordinate research on groundnut viruses. Since the group has expanded to include several scientists from outside the region, it will be known as the Working Group on Asia-Pacific Groundnut Viruses.

Australia	Mr K.J. Middleton, Dr D.D. Shukla, Dr A.J. Gibbs
China	Mr Xu Zeyong
India	Dr R.D.V.J. Prasada Rao, Dr P. Sreenivasulu, Dr A. Varma, Dr Ram Nath
Indonesia	Dr N. Saleh, Dr A. Hasanuddin
Japan	Dr T. Senboku
Malaysia	Dr Ong
Philippines	Dr M. Natural, Dr N. Bajet
Thailand	Dr S. Wongkaew, Dr D. Choopanya
USA	Dr J.W. Demski, Dr J. Sherwood
ICRISAT	Dr D.V.R. Reddy
Canada	Dr R.I. Hamilton
France	Dr M. Dollet
UK	Dr A.A. Brunt

5. The third meeting of Working Group on Asia-Pacific Groundnut Viruses should be held in 1992, preferably in Thailand, to review the progress made by various groups and to plan future research on groundnut viruses in the Asia-Pacific region.

Participants

From Selected Countries

Australia

Dr A.J. Gibbs
Plant Molecular Biology Group
Australian National University
Box 475
Canberra City, ACT 2601

Dr D.D. Shukla
Principal Research Scientist
CSIRO, Division of Biotechnology
Parkville, 343 Royal Parade
Melbourne 3052

Canada

Dr R.I. Hamilton
Head, Virology Department
Research Station
Agriculture Canada
6660 N.W. Marine Drive
Vancouver, BC, V6T 1X2

China

Dr Xu Zeyong
Associate Professor
Oil Crops Research Institute
Chinese Academy of
Agricultural Sciences
430062 Wuchang
Wuhan, Hubei Province

France

Dr J.M. Dollet
Laboratoire de Virologie
CIRAD-IRHO
Avenue du val de Montferrand
BP 5035
34032 Montpellier

India

Mr Ram Nath
Head
Division of Plant Quarantine
NBPGR, Pusa Campus
New Delhi 110 012

Dr R.D.V.J. Prasada Rao
NBPGR
Regional Research Station
Rajendranagar
Hyderabad 500 030

Dr M.V.R. Prasad
Principal Scientist
ICAR
Directorate of Oilseeds Research
Rajendranagar
Hyderabad 500 030

Dr P. Srinivasulu
Reader in Plant Virology
Virology Department
S.V. University
Tirupati 517 502

Dr Anupam Varma
Head
Department of Plant Pathology
IARI (Pusa)
New Delhi 110 012

Dr K.S. Vara Prasad
NBPGR
Regional Plant Quarantine Station
Rajendranagar
Hyderabad 500 030

Dr V.V. Chenulu
Professor of Plant Pathology,
IARI (Retd)
204, Dwaraka Apartments
Punjagutta
Hyderabad 500 482

Indonesia

Dr Nasir Saleh
Virologist
Malang Research Institute for
Food Crops (MARIF)
Jln Raya Kendalpayak
PO Box 66
Malang 65101, East Java

Japan

Dr Toshihiro Senboku
National Institute of
Agro-environment Science
Tsukuba, Ibaraki 305

Philippines

Dr (Ms) M.P. Natural
Plant Pathologist
Institute of Plant Breeding
University of the Phillipines at
Los Baños
College of Agriculture
Laguna 4031

Thailand

Dr Sopone Wongkaew
Department of Plant Pathology
Faculty of Agriculture
Khon Kaen University
Khon Kaen 40002

Ms Sumitra Kantrong
Research Assistant
Department of Plant Pathology
Faculty of Agriculture
Khon Kaen University
Khon Kaen 40002

United Kingdom

Dr A.A. Brunt
Head, Plant Pathology Section
Institute of Horticultural Research
Worthing Road, Littlehampton
West Sussex, NB17 6LP

From Donor Agencies

Peanut CRSP

Dr J.W. Demski
Professor
The University of Georgia
College of Agriculture
Georgia Experiment Station
Griffin, Georgia 30223-1797

ACIAR

Dr K.J. Middleton
Senior Plant Pathologist
Department of Primary Industries
J. Bjelke-Petersen Research Station
P.O. Box 23, Kingaroy
Queensland 4610

From ICRISAT

J.J. Abraham
Editor, Information Services

S.L. Dwivedi
Plant Breeder, Legumes

D.G. Faris
Principal Coordinator,
Asian Grain Legumes Network (AGLN)

C.L.L. Gowda
Senior Legumes Breeder, AGLN

B.C.G. Gunasekera
Adviser to Director General
for Donor Relations

N.M. Horn
Asst. Principal Virologist, Legumes

N.C. Joshi
Chief Plant Quarantine Officer

D. McDonald
Acting Program Director, Legumes

M.H. Mengesha
Principal Germplasm Botanist
and Program Leader, Genetic
Resources Unit

J.P. Moss
Principal Cytogeneticist

Y.L. Nene
Deputy Director General

D.L. Oswalt
Principal Training Officer
and Program Leader

M. Pimbert
Principal Entomologist, Legumes

A.S. Ratna
Research Associate, Legumes

A.S. Reddy
Research Associate, Legumes

D.V.R. Reddy
Principal Plant Virologist, Legumes

L.J. Reddy
Plant Breeder, Legumes

W.A. Sacks
Postdoctoral Fellow, Legumes

B. Srinivasa Rao
Research Associate, Legumes

M.R. Sudarshana
Research Associate, Legumes

J.A. Wightman
Principal Entomologist, Legumes

J.B. Wills
Head, Information Services